

Pawpaw: An Underutilized Tree with Potential

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Key words: annonaceous acetogenins, clonal propagation, dichogamy, storage

Abstract

Pawpaw [*Asimina triloba* (L.) Dunal] is an endemic North American tree that is gaining commercial interest because it produces desirable, nutritious fruit for human consumption. The plant evolved in the forest understory and can grow well in shaded and sunny environments. As a native tree, there are few arthropod pests or diseases, thus, the trees require few pesticide inputs. The acetogenins synthesized by the tree can be used as natural pesticides or in cancer therapy treatment. Pawpaw is primarily propagated by seed in nurseries, and cultivars are grafted via the whip-and-tongue and chip bud methods. However, there are several challenges that limit wider adoption of pawpaw production in North America including the lack of an adequate tissue culture protocol for clonal propagation, poor understanding of fruit physiology, and lack of rootstocks for commercial production. Modern biotechnology serves as viable option for overcoming these challenges and accelerating the development of pawpaw for commercial production.

The genus *Asimina* is the only temperate climate representative of the tropical family Annonaceae which includes 107 genera (Callaway, 1993). Native trees are generally found in the understory of a forest (Young and Yavitt, 1987) and are naturally suckering. The tree grows in a pyramidal shape in an open orchard setting to between 4-6 m in height at maturity. Vegetative buds are narrow and pointed (Pomper and Layne, 2005). Mature pawpaw leaves are large; approximately 15-30 cm long and 10 to 15 cm wide (Bailey, 1960; Layne, 1996; Pomper and Layne 2005). Simple and elongate, the leaves are alternating and ovate oblong in shape (Pomper and Layne, 2005). They are lush, dark-green and looping at maturity, and will transition to a gold, then rusty yellow color as they senesce (Layne, 1996).

Vegetative and flower buds occur at different points along the stem with the flower buds located basipetally (Pomper and Layne, 2005). Pawpaw flower buds, unlike vegetative buds, are round and covered with a dark pubescence (Pomper and Layne, 2005). The flower buds are produced in the late summer and early autumn on current growth and then

overwinter in a relatively undifferentiated condition (Lampton, 1957).

Flowers

Pawpaw has perfect and solitary flowers (Lagrange and Tramer, 1985). A long peduncle, around 4 cm, develops from the axils of prominent leaf scars and connects the maroon flower, width of 2-3 cm, to the stem (Layne, 1996; Kral, 1960). A pawpaw flower has a calyx approximately 8-12 mm in size accompanied by three triangular sepals and outer petals 1.5-2.5 cm long (Kral, 1960). Mature flowers have an outer and inner layer of three maroon-colored, three lobed petals. The inner petals are smaller and have a higher concentration of nectar relative to the outer petals (Layne, 1996). The flower has a globular androecium, and a gynoeceium composed of three to seven carpels with seven to ten ovules in each flower (Lampton, 1957; Pomper and Layne, 2005; Willson and Schemske, 1980). As a result, each flower yields three to seven fruited clusters, but up to nine fruited clusters have been noted (Pomper and Layne, 2005).

Pawpaw flowers exhibit dichogamy where the pistil and stamen of the flower develop

at different times, and more specifically they portray protogynous dichogamy as the pistil develops before the stamen. The flower bud opens fifteen days before anther dehiscence (when pollen becomes available), showing the stigmas (Losada et al., 2017).

Depending upon the cultivar, bloom duration lasts between 23-36 days (Pomper et al., 2008). Similarly, the bloom period lasts between 21-28 days in Transylvania (Romania) and southern Spain from April-May (Ferrer-Blanco et al., 2022; Szilagyi et al., 2016). ‘Wells’ and ‘Middletown’ had a later flowering period relative to the other cultivars studied, which could be advantageous when considering the risk of late spring frost (Pomper et al., 2008).

Pollination

Most pawpaw cultivars are thought to be self-incompatible; however, some cultivars like ‘Sunflower’ are thought to be self-fertile (Pomper et al., 2008). In the natural environment, stands of pawpaw may appear to be unproductive but it is possible they are all the same clone, hence self-infertile. Flower petals originally appear green but transition to maroon five days before anther dehiscence. During bloom, the flowers emit a fetid aroma which is thought to attract flies, beetles, and nocturnal insects as pollinators (Ferrer-Blanco et al., 2022; Losada et al., 2017; Pomper and Layne, 2005). Goodrich et al. (2023) found that pawpaw employs floral mimicry of fermenting aromas. The volatile profile produced by pawpaw flowers acetoin, 2,3-butanediol and ethanol, overlap with the volatile profile generated by decaying *Magnolia* × *soulangeana* floral tissues, fermenting mulberry (*Morus alba*) and sap and fungivore dung (Goodrich et al., 2023). Saunders (2012) suggested that the longer stigmatic receptivity of pawpaw flowers (an aspect of pawpaw’s unusually long flowering period) might be an adaptive consequence of low pollinator visitation rates.

Unlike in the wild where fruit development is pollen-limited, in cultivated settings,

high fruit set has been observed (Layne, 1996; Willson and Schemske, 1980). Hand-pollination has been shown to increase fruit set in wild settings but may be time consuming for commercial growers. Finding ways to attract flies and beetles to the pawpaw planting might be the most successful method in improving pollination. Intercropping plants to attract some of the most common pawpaw pollinators such as calyptrate flies (Diptera) (Goodrich et al., 2023) might be a potential avenue to pursue.

Fruits

Pawpaw produces the largest, edible fruits of any tree species native to North America. Pawpaw cultivars are typically larger than fruit found in the wild. The pawpaw fruit is oblong-cylindrical in shape and is a true berry. They are typically 3 to 15 cm long and 3 to 10 cm wide (Layne, 1996; Pomper and Layne, 2005). Brannan et al. (2021) later found the average weight of a pawpaw among 16 cultivars to be 194 g, ranging between 122 g to 292 g. When ripe, the skin ranges from green to brownish black; the flesh color can range from a creamy white through bright yellow to shades of orange (Layne, 1996). Two rows of large seeds are embedded in the flesh and can range from 12 to 20 in number (Pomper and Layne, 2005). The flavor of the pawpaw fruit has been likened to mango, banana, and pineapple (Duffrin et al., 2009). Roark (2023) identified diacetyl, acetaldehyde, lactones, acids, furanones, floral alcohol compounds, and vanillin as the major compounds associated with pawpaw flavor by using gas chromatography – mass spectrometry (GC-MS) and gas chromatography olfactometry (GC-O). McGrath and Karahadian (1994) previously identified ethyl ester compounds in pawpaw via Tenax GC traps and gas chromatography olfactometry (GC-O). It is now understood that pawpaw’s unique flavor is the result of the presence of esters combined with the novel compounds identified by Roark (2023).

Pawpaw fruit forms in clusters from a single pollinated flower; there are typically 3-10

pawpaw fruit in cluster (Ferrer-Blanco et al., 2022; Pomper et al., 2008). Generally, pawpaw seedlings take longer to produce fruit relative to clonal cultivars. Seedlings flower when they reach a height of 1.8 m but may take 5 to 8 years after they are planted to set fruit (Pomper and Layne, 2005). Clonal cultivars typically flower after the third year of planting, but often fail to set fruit (Merwin et al., 2003; Pomper and Layne, 2005; Pomper et al., 2003b). Among all cultivars, the harvest period for pawpaw is from late August through October (Archbold et al., 2003).

Pawpaw fruit is climacteric (McGrath and Karahadian, 1994; Peterson, 1991) as peaks in ethylene and respiration were identified in softening fruit (Archbold et al., 2003). However, fruit harvested immature did not ripen off the tree, even when treated with the commercial ethylene product ethephon at 1000 mg·L⁻¹ (Archbold et al. 2003). Without refrigeration, the shelf-life of pawpaw may be between 2-3 days (Layne, 1996; McGrath and Karahadian, 1994), or 5-7 days for fruit just beginning to soften (Archbold et al., 2003; Pomper and Layne, 2005). Softer fruit should be stored immediately after harvest at 4°C (Pomper and Layne, 2005). When kept at 4°C, pawpaw can be stored for 4 weeks before developing signs of chilling injury that presents as black discoloration from an increase in polyphenol oxidase activity, rapid loss of firmness, and the development of off-flavor volatile compounds (Galli, 2007; Galli et al., 2009; Archbold et al., 2003). 1-methocyclopropane applications after harvest did not significantly improve the shelf life of 'ShenandoahTM', 'Susquehanna[®]', 'Pennsylvania-Golden' or 'Allegheny[®]' stored at 4°C (Erich and Al Shoffe, unpublished data).

Based off 52 cultivars, the average ripe pawpaw fruit on average weighs between 72-244 g (Greenawalt, 2016). Similarly, Brannan et al. (2021) found the average weight of a pawpaw among 16 cultivars to be 194 g, ranging between 122 g to 292 g. The pH of the fruit ranged from 5.4-6.3 and it was determined that fruits with a higher pH had an

increased susceptibility to browning as a low pH regulates the activity of polyphenol oxidase (Brannan et al., 2021). The soluble solids concentration of mature pawpaw fruits has been reported to be between 18.2-26.1 °Brix (Brannan et al., 2021; McGrath and Karahadian, 1994). The cultivars 'Estill' and 'SAB Overleese' exhibited some of the greatest SSC in the fruits that were measured at 24.7 °Brix and 26.1 °Brix respectively (Brannan et al. 2021). Archbold and Pomper, (2003) and McGrath and Karahadian, (1994) found that fruit firmness declines during pawpaw senescence, and Brannan et al. (2021) recorded pawpaw firmness as between 0.391 kg·N⁻¹ in 'Estill' and 0.727 kg·N⁻¹ in 'ShenandoahTM'. The hardness of ripe pawpaw fruit is between that of a ripe mango and a ripe banana (Adainoo et al., 2023b). Of the sixteen cultivars measured, the skin color range for all the cultivars exhibited a* (greenness) values of -6.0 to -14.0, b* (yellowness) values between 37.0, and 49.3 hue angles ranging from 99.0 to 109.1 (Brannan et al., 2021). Adainoo et al. (2023b) measured values of 0.9 +/- 4.9 for a*, 27.8 +/- 5.7 for b* and 87.4 +/- 9.1 for hue angles of wild pawpaw fruit. This suggests that humans have been selecting pawpaw fruit that is less green, more yellow, and has a brighter complexion than what is found in the wildtypes.

Pawpaw seeds contain a class of cytotoxic compounds known as acetogenins, which, if ingested by humans, can lead to indigestion (Layne, 1996). Unripe pawpaw fruit pulp has been shown to have higher concentrations of acetogenins compared to ripe fruit pulp (Nam et al., 2018).

Pawpaw plants contain many nutrients and vitamins that may aid human health. Adainoo et al. (2023b) reported that unripe pawpaw fruit has a higher concentration of dietary fiber relative to ripe pawpaw fruit, but Peterson et al. (1982) found that ripe pawpaw fruit contains a higher concentration of dietary fiber relative to banana or orange. Galli (2007) found pawpaw fruit contained sucrose at 66 ± 6 mg×g⁻¹ FW, fructose at 9 ± 2 mg×g⁻¹ FW, and glucose at 5 ± 2 mg×g⁻¹ FW, like the val-

ues reported by Peterson et al. (1982) which were $60 \text{ mg} \times \text{g}^{-1}$ FW sucrose, $13 \text{ mg} \times \text{g}^{-1}$ FW fructose and $18 \text{ mg} \times \text{g}^{-1}$ FW glucose. Pawpaw fruit contain over 16 times the concentration of free sugars than what has been measured in the leaves, twigs, and roots (Nam et al., 2018). Relative to the other parts of the plant, the roots contain the greatest concentration of malic and citric acid (Nam et al., 2018). Acetic acid was the most abundant organic acid found in ripe fruit (Adainoo et al., 2023a; Nam et al., 2018; Pande and Akoh, 2010; Park et al., 2022). Peterson et al. (1982) reported that pawpaw was rich in amino acids, exceeding apple and equaling banana and orange. Additionally, fruit contained a high concentration of potassium, far more than an apple or orange (Nam et al., 2018; Peterson et al., 1982). Pawpaw fruit also has a greater total fat content than banana, apple, and orange and is high in vitamin A (Peterson et al., 1982).

Pawpaw fruit has a high phenolic content, carotenoid content, and antioxidant capacity. Phenolic antioxidants are important for human health because they scavenge free radicals and prevent damage to cellular components (Kobayashi et al., 2008; Sun et al., 2002). Carotenoids have been linked with lower rates of heart disease and cancer in people which consume products in which they are in a high concentration (Galli, 2007). Pawpaw is also rich in procyanidins which are excellent antioxidants for health (Brannan et al., 2015). Compared with other fruits, the phenolic content and antioxidant capacity were equivalent or even superior to other common fruits such as lemon, peach, orange, banana, pear, pineapple, and grapefruit (Kobayashi et al., 2008; Sun et al., 2002). The phenolic content and antioxidant content was also similar to some blueberry cultivars (Kobayashi et al., 2008). The carotenoid content of pawpaw is about 80% higher than that of banana and grapefruit, 70% higher than strawberry, and 40% higher than apple, orange, and mandarin (Galli et al., 2007). Pawpaw is especially rich in procyanidins (condensed tannins) which

serve as antioxidants in the human diet (Brannan et al., 2015). Procyanidins also serve as the substrate for the polyphenol oxidase enzyme and the brown color it produces via enzymatic browning (Brannan et al., 2015).

As pawpaw shelf life remains a major barrier to commercial production, cultivar selection will have to balance which cultivars that contain high procyanidin concentration for nutraceutical benefits and those with a low procyanidin concentration for long-term storage (Brannan et al., 2015).

Pawpaw's promise as a commercial fruit crop also derives from the plant's production of annonaceous acetogenins that have strong medicinal and pesticidal properties (McLaughlin, 2008; Ratnyake et al., 1993). Rupprecht et al. (1990), identified the compound asimicin along with 49 other annonaceous acetogenins in the bark and seeds of pawpaw (McLaughlin et al. 2008). Three compounds—bullatacin, bulletin, and bullanin—have high potencies against human solid tumor cell lines in vitro, and the acetogenins as a whole class of compounds are promising in the treatment of both nonresistant and multi-drug resistant (MDR) types of tumors (Pomper and Layne, 2005; McLaughlin et al., 2008; Zhao et al., 1994). The essential oils generated from pawpaw leaves also have demonstrated anticancer capabilities against breast and lung cancer cell lines (Farag, 2009; Nam et al., 2018).

As an insecticide, annonaceous acetogenins disrupt cellular respiration which are highly effective against fifteen documented species of arthropods and nematodes (Sampson et al., 2003). It is also harder for pests to develop resistance to botanically derived insecticides relative those produced synthetically, because they have a larger pesticidal spectrum and do not target one pest specifically (Arnason et al., 1989; Pomper and Layne, 2005). These compounds ward off larger herbivores, as well. Slater and Anderson (2014) found that only pawpaw species density increased relative to the other tree species in Illinois, an area overpopulated with deer during the time of their

study. Male deer can sometimes rub trees with their antlers to break branches but will generally not eat twigs due to the high concentration of annonaceous acetogenins (Ratnyake et al., 1992). Acetogenins also serve as potent tool in combatting the parasite *Hemonchus contortus*, a nematode that infects sheep, goats, and other animals (McLaughlin, 2008). The concentrations of three of the major bioactive acetogenins, asimicin, bullatacin, and triloban peak concurrently between May and June, and the pawpaw shoot biomass is collected for commercial purposes in May (Gu et al., 1999; McLaughlin, 2008). Unlike the fruit, pawpaw twigs can later be harvested, dried, ground and stored for later extraction (Johnson et al., 1996; Pomper and Layne, 2005).

Seedling Care and Management

The pawpaw seed is quite large and averages about 2.8 cm in length and 1.5 cm in width (Geneve et al., 2003). Within the seed, there is a small, rudimentary embryo imbedded in a large ruminant endosperm (Finneseth, 2000; Finneseth et al., 1997). Embedded within the persistent endosperm is a small embryo that is dicotyledonous and apical (Finneseth et al. 1997). Pawpaw also exhibits hypogeal, belowground, germination (Finneseth et al. 1997; Layne, 1996).

Pawpaw seeds can be collected from the fruit when the flesh is overripe after several days of fermentation in water (Geneve et al., 2003; Hartmann et al., 2002; Layne, 1996). When removed from the fruit, pawpaw seeds typically have a moisture content of approximately 37% (Geneve et al., 2003). As the moisture content decreases below 25%, approximately half of the seeds lose viability. Seeds with a moisture content between 5-15% are rendered completely unviable. Thus, growers need to ensure that the seeds remain moist during germination.

The temperature at which the seeds are stored plays a significant role in determining their viability. Finneseth et al. (1998) determined that 5°C is the optimum temperature

for pawpaw seed storage for two seasons as the seeds retained 52-74% viability depending on the seed lot; however, the germination was reduced to 25% after a third year of storage (Geneve et al. 2003). Seeds stored at warmer temperatures fared worse relative to the seeds stored at 5°C lost 40-65% viability after 28 weeks in storage and no seeds survived through a year (Finneseth et al., 1998; Geneve et al., 2003). Storing seeds at too cold of a temperature will damage them, as well. Pomper et al. (2000) reported that seeds stored below -15°C became unviable because the embryo was damaged.

Pawpaw seeds also exhibit morphophysiological dormancy (Finneseth et al. 1998; Geneve et al. 2003; Pomper and Layne, 2005). They must undergo cold stratification between 60 and 120 days at 5°C to satisfy an endogenous physiological dormancy before germination (Dirr and Heuser 1987; Finneseth et al. 1998; Pomper and Layne, 2005; Young and Young 1992). After fulfilling the stratification requirement, it takes approximately seven weeks for the pawpaw to reach 50% germination (Finneseth et al. 1998) and the greatest germination rate (84 to 90%) comes after 100 days of stratification (Finneseth et al., 1998; Pomper and Layne, 2005). Another report suggested seedling emergence occurs 45 to 90 days after planting (Callaway, 1993). After surpassing the physiological dormancy, pawpaw seedlings will still exhibit morphological dormancy (Finneseth et al., 1998; Pomper and Layne, 2005). Morphological dormancy is described as the seed containing either a rudimentary or linear embryo that is not fully developed at the time the seed is mature and occupies less than one-half of the seed cavity (Baskin and Baskin, 1998; Nikolaeva, 1977). When the stratified seeds are moved to warm conditions, the cotyledons and the radicle begin to grow at nearly comparable rates until radicle emergence (Finneseth et al., 1998). The radicle and hypocotyl continue to thicken to form a taproot, and their emergence was identified between days 12 and 27 after planting, respectively (Pom-

per and Layne, 2005; Finneseth et al., 1998). After 45 days, the epicotyl emerges from the growing substrate and the taproot averages 15 cm in length and represents approximately 75% of the dry mass of the seedling (Pomper and Layne, 2005). Peterson (1991) reported similar growth rates as he identified radicle and epicotyl emergence at 18 and 64 days, respectively. The cotyledons are thought to be haustorial and translocate storage material from the endosperm to the growing embryo and shed from the endosperm once the epicotyl begins to elongate (Finneseth et al., 2000; Geneve et al., 2003). The cotyledons do not emerge from the seed during germination (Baskin and Baskin, 1998).

Currently, many nurseries propagate pawpaw from seeds (Crabtree, 2004; Pomper et al., 2002a; Pomper et al., 2002b; Pomper et al., 2002c; Pomper et al., 2003a). The seed are commonly planted into tall containers to allow the taproot enough soil volume to develop (Layne, 1996). Larger root systems are considered ideal for field establishment (Pomper et al., 2003a). Roottrainers (Spencer-Lemaire Industries Limited, Edmonton, Alta., Canada) that are $5.1 \times 6.35 \times 25.4$ cm with a volume of 737.4 cm³ or similar sized containers, have been used to successfully produce pawpaw seedling (Pomper et al., 2002a; Pomper et al., 2002b; Pomper et al., 2002c). Seedlings grown in Roottrainers (Spencer-Lemaire Industries Limited, Edmonton, Alta., Canada) are then transplanted when the plant has developed its first ten leaves or else the pawpaw may produce a terminal bud and cease growth for that season (Pomper et al., 2003a). Treepots (Stueweand Sons, Inc., Corvallis, Ore.)—deep containers used to produce pawpaw from seed—of 3.8 to 7.6 L (1 to 2 gal) are also well-suited for pawpaw production; in a greenhouse experiment, stratified pawpaw seedlings produced in tall 3.6 L Treepots had a greater root mass relative to seeds produced in shorter Treepots of the same volume (Pomper et al., 2003a).

Pomper et al. (2002b) determined that a well-aerated substrate, such as Pro-Mix BX (Pre-

mier Horticulture, Inc., Red Hill, Pa.) with a high sphagnum peat moss component (> 75% by volume), cation exchange capacity, and water-holding capacity can be used to effectively grow pawpaw seedlings. Osmocote (Miracle-Gro®, Marysville, OH) — a slow-release fertilizer—represents one of the most effective and preferred sources of nutrients for developing seedlings. Calcium nitrate was also recommended at rate of 500 mg·L⁻¹ three months after sowing to prevent nitrogen deficiency (Pomper et al., 2003c).

Bottom heating, a technique accomplished by providing heating pads underneath tree pots, improves pawpaw seedling germination and growth (Pomper et al., 2003a). Bottom heating at 32°C promoted germination nine days earlier compared to ambient temperature (Pomper et al., 2002b; Pomper et al., 2003a). Seedlings subjected to bottom heat also exhibited increased leaf number, plant height, whole-plant leaf area, shoot dry weight, root dry weight, lateral root dry weight, and total plant dry weight, and a lower root:shoot ratio (Pomper et al., 2002b; Pomper et al., 2003a). Young pawpaw trees appear to be sensitive to ultraviolet (UV) light, which could hinder their establishment (Peterson, 1991; Pomper et al., 2002a; Pomper et al., 2003a). Low (28%) to moderate (51%) shading of pawpaw trees will improve their establishment when grown outdoors; using polypropylene shade fabric has been shown to increase chlorophyll a and b content, leaf number, total leaf area, and total plant dry weight when compared to the non-shaded seedlings. If the plants are produced on a gravel pad, then shade cloth with a higher light interception percentage was shown to improve seedling growth (Pomper et al., 2002a; Pomper et al., 2003a). Pomper et al. (2002a) found that pawpaw seedlings that were grown in a greenhouse were subject to 60% less UV irradiance than field produced seedlings and did not exhibit symptoms of sunburn. They reported that seedlings subjected to shade treatments of 33% had greater total and average leaf area, and greater shoot dry weight than the unshad-

ed seedlings in the greenhouse (Pomper et al., 2002a). Sun scorch is not common on mature pawpaw trees.

Cupric hydroxide [$\text{Cu}(\text{OH})_2$] applications have been shown to promote a more fibrous root system by causing lateral branching further back on the root leading to a lower root:shoot ratio (Arnold and Struve, 1993; Pomper et al., 2002a). The change in the root to shoot ratio results from an alteration in dry matter partitioning and the more vertical distribution of the root system in the container (Arnold and Struve, 1993; Pomper et al., 2002a). $\text{Cu}(\text{OH})_2$ is most commonly applied as a wet paint to the inner walls of tree pots. Applying $\text{Cu}(\text{OH})_2$ to the walls of the Rootainers (0.7 L) at a concentration of $100 \text{ g} \cdot \text{L}^{-1}$ reduced total and lateral root dry weight in non-shaded seedlings and caused chlorosis and reduction of the chlorophyll levels (Pomper et al., 2002a). Interestingly, $\text{Cu}(\text{OH})_2$ stimulated seedling lateral root dry weight when applied in larger (8 L) containers (Pomper et al., 2002a).

Clonal Propagation

Clonal propagation allows for superior genotypes to be produced that are true-to-type, contain desirable traits such as high yields, fruit quality, pest resistance, and/or low vigor for dwarfing purposes (Crabtree, 2004). Currently, pawpaw cultivars with superior fruit characteristics are propagated asexually by grafting and budding on to seedling rootstocks (Geneve et al., 2003; Layne, 1996; Pomper and Layne, 2005). Some grafting methods used on pawpaw include whip-and-tongue, cleft, bark inlay, and chip budding (Layne, 1996). Out of those methods, chip budding appears to have a higher success rate (Pomper et al., 2009). Budwood is obtained in March, after the chilling requirement has been met (Crabtree, 2004). Seedling rootstocks are sown in greenhouses in February and are ready to use for grafting in July (Crabtree, 2004). Chip budding is most successful when the seedling rootstock is at least 0.5 cm in diameter and is actively

growing (Pomper and Layne, 2005). If these conditions are met, bud take can exceed 90% (Pomper and Layne, 2005). However, grafting methods may be somewhat cultivar dependent. Behrends et al. (2019) found that the whip-and tongue-grafting was the most successful for 'KSU AtwoodTM', 'KSU ChappelleTM' and 'Hi7-1' with a 96% success rate, while chip budding only had a 54% success rate across the same cultivars.

Unlike other commercial fruit species, such as apple, stone fruit, and grape, there is a paucity of clonal rootstocks available for pawpaw production. The genetic diversity of rootstocks could contribute to high rates of suckering and variation in scion growth observed in the regional pawpaw trial at Kentucky State University (Pomper et al., 2009). When evaluating K8-2 and 'Sunflower' rootstock on the performance of 'Sunflower' and 'SusquehannaTM' seedling, Pomper et al. (2009) found that removing all leaves from the rootstock decreased the ability for the bud to take. Conversely, the bud take was improved when 6-8 leaves were present on the rootstock, but the scion growth rate (measured by the number of leaves on the scion) was slower relative to scions growing on leafless rootstocks (Pomper et al., 2009). When 6-8 leaves were removed four weeks after harvest, budding success was higher on K8-2 rootstock relative to 'Sunflower' rootstock (Pomper et al., 2009). For optimal performance, it is advised for nursery producers to retain 6-8 leaves when budding trees and then remove the leaves by cutting back the rootstock to about 30 cm above the chip bud about 6 weeks after grafting to maximize budbreak (Pomper et al., 2009).

Stem propagation has a low success rate and does not appear to be a viable commercial practice (Geneve et al., 2003). Finneseth (1997) found that only one stem cutting out of 1,200 (~0.83%) from 5-year-old mature pawpaw trees produced adventitious roots. Finneseth (1997) also attempted to use the rooting indole-3-butyric acid (IBA) at concentrations ranging from 0 to 80,000 ppm on

seven-month-old pawpaw trees. Applications of IBA above 40,000 ppm caused complete necrosis of the cuttings, and the highest success rate (16%) was observed at 10,000 ppm (Crabtree, 2004; Finneseth, 1997). Applications of IBA to a five-year old mature tree was also deemed unsuccessful, with only one cutting out of 5,100 (~0.2%) rooted successfully (Crabtree, 2004; Finneseth, 1997). Geneve et al. (2003) discovered that pawpaw seedlings less than 2 months old have a strong tendency to produce roots when treated with 50 mM (10,000 ppm) of IBA; 75% of the cuttings averaged two roots. However, cuttings taken from pawpaw seedlings at 7 months largely failed to produce cuttings as less than 10% of cuttings rooted when treated with the same concentration of IBA.

The use of *Agrobacterium rhizogenes* has also been utilized to initiate root formation on softwood stem cuttings of 'Mitchell' (Ayala-Silva et al., 2007). The treatments consisted of two strains of *Agrobacterium rhizogenes*, MSU-1 (A4 wild type) and MT232 (TR105 mutant), IBA at 20,000 mg·L⁻¹ and an untreated control. Both strains promoted root formation, unlike the other two treatments, and MSU-1 promoted rooting more than MT-232 (Ayala-Silva et al., 2007). Similarly, Ayala-Silva et al. (2007) observed only cuttings from seedlings were responsive to *A. rhizogenes* treatment, and that juvenility was an important factor in a successful transformation.

Given that pawpaw is a naturally sucker-ing species, both in the wild and in orchard settings, the plant forms adventitious shoots from roots which are clones of the parent (Geneve et al., 2003). In fact, ensuring the survivorship of new stems is the main ecological role in the clonal growth of pawpaw (Hosaka et al., 2005). Root cuttings might serve a suitable purpose given that shoots derived adventitiously from roots retain a juvenile character and could serve as a source for stem cuttings or explants for tissue culture (Geneve et al., 2003; Hackett, 1985; Pomper and Layne, 2005). Finneseth (1997) analyzed the rela-

tionship between root diameter and stem cuttings and found that 65% of root pieces 5 mm in diameter or greater produced adventitious shoots, but no shoots were formed on root cuttings less than 5 mm in diameter. On average, the responding roots produced 2.5 buds and 1.1 elongating shoots (Finneseth, 1997; Pomper and Layne, 2005). The buds were visible on the root pieces 12 weeks after planting and shoot elongation was evident after 16 weeks (Finneseth, 1997; Pomper and Layne, 2005). The roots were dusted with Captan fungicide and planted horizontally (about 5 cm deep) in flats filled with perlite and vermiculite (1:1 vol/vol) and grown outside in 22°C (Finneseth, 1997; Geneve et al., 2003). Stooling, or mound layering, has also been attempted in the field as another method to propagate pawpaw plants (Pomper and Layne, 2005). However, various levels of IBA application (0, 3,000, or 6,000 mg·L⁻¹) applied on either girdled or non-girdled trunks resulted in the formation of only two shoots out of 80 treated trees (Pomper and Layne, 2005). Preliminary experiments with root microcuttings of pawpaw subject to IBA treatment have also been unsuccessful (Geneve et al., 2003).

Tissue Culture

While using seedling explants, Finneseth (1997) demonstrated that nodal explants respond more favorably than apical sections for the establishment of cultures (Geneve et al., 2003). Finneseth (2000) tried propagating nodal explants of seedling, mature, and rejuvenated (shoots developing on root pieces from mature plants as mentioned in the root cuttings section above) sources was attempted using the MS (Murashige and Skoog, 1962) medium supplemented with 10 µM 6-benzyladenine (BA), plus 0.1 µM thidiazuron. The seedling explants (that were 12 weeks old) established at 100% and developed faster than the other explants; none of the mature explants survived longer than 12 months in culture (Finneseth et al., 2000; Geneve et al., 2003). One accession (A10-11) developed from a rejuvenated explant showed continu-

ous growth and shoot production during subculturing (Geneve et al., 2003) and remained in culture for three years after its establishment. When storing pawpaw in tissue culture over the long term either MS or Woody Plant Medium (Lloyd and McCown, 1981) with 8.9 μM BA and 2.7 μM naphthalene acetic acid (NAA), 3.0 % sucrose, and 0.7% agar work optimally (Geneve et al., 2003). Newlines in culture should be maintained at 16 h photoperiod while and 20 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ of light provided by cool white, fluorescent bulbs at a room temperature of 25°C (Geneve et al., 2003).

To generate single stem explants with shoots from the three-year old A10-11 accession line, a medium consisting of 9.8 μM IBA plus 5.4 μM NAA in combination with BA ranging from (0 to 20 μM) was used (Geneve et al., 2003). Initial explants elongated but did not form shoots after 8 weeks in culture (J. Egilla, unpublished data). The shoots were then subcultured to the same medium and after 9 weeks the cultures treated with 15 to 20 μM had the greatest number of shoots per culture and the 15 μM had the most vigorous shoot growth (Geneve et al., 2003; Pomper and Layne, 2005). The experiment indicates that pawpaw can retain morphogenetic potential for an extended period in culture (Geneve et al., 2003; Pomper and Layne, 2005).

More recent work examined the effects of plant-growth regulator-free media to initiate shoot and root growth. PGR-free media subject to gibberellic acid (GA_3) treatment boosted shoot growth (Geneve et al., 2007). The greatest shoot elongation (49% increase) was found using a combination of plant-growth regulator-free medium and active charcoal (in either the agar medium alone or in a 3-day liquid overlay containing activate charcoal) (Geneve et al., 2007). Shoots that were produced on PGR-free media containing charcoal and then subculture to either PGR media or PGR-free media were able to initiate shoots as well (Geneve et al., 2007). Microshoots subject to high auxin, etiolation and activated charcoal all failed to root on the

PGR-free media (Geneve et al., 2007).

Contamination is problematic for tissue culture pawpaw explants. Bellini et al. (2002) prepared explants subjected to five disinfection treatments consisting of immersion for 2 to 5 minutes in 1.0%, 2.0%, 2.5%, 3.5%, and 5.0% sodium hypochlorite. Among the cultivars that were tested ('Davis', 'Sunflower', 'Overleese', 'Prima 1216', and 'Prolific') only 'Davis' treated with 5% sodium hypochlorite for 2 minutes proved to be free of microbial growth (Bellini et al., 2002). Future research on pawpaw should focus on developing a protocol for tissue culture to allow for faster thruput clonal propagation.

Outlook for Pawpaw Production

Pawpaw has potential for agricultural production and as an ornamental tree. The plant is stress tolerant, shade tolerant, and resistant to most pests and diseases. The fruit has a high market value and can serve as a lucrative specialty crop for growers. Consumer interest in pawpaw is very high as reflected by market demand and social media postings. However, the difficulty of producing pawpaw in tissue culture combined with the lack of clonal rootstock propagation makes it difficult to generate pawpaw trees for commercial production. Seedling trees are readily available, but often of poor fruit quality. Clonal trees can be difficult and expensive to obtain. Another challenge facing producers is that the fruit easily bruises and has a short shelf life, making it pawpaw difficult to harvest, transport, store, and sell. The skin of ripe fruit may turn black within a few days of harvest.

Genetic improvement of pawpaw has centered around traditional breeding methods which may take decades to yield results. As described by Frost (2023), categorizing cultivar traits with genomic groupings is difficult with the currently available information. Sequencing the pawpaw genome will permit greater potential for the genetic advancement of the tree. As traits are mapped to specific chromosomal loci, breeders and physiologists can work together to generate mutants

and screen new progeny with higher success rates. Genetic editing of cultivars like K8-2, which already possess potential to serve as a viable rootstock, with modern tools like CRISPR/Cas-9 can accelerate the development of the genotype as a rootstock. Yeast one-hybrid assays and RNA interference (RNAi) technology may be employed to understand protein-protein interactions and knockdown the expression of specific genes in the fruit's physiology respectively. These tools might be useful in elongating the shelf life of pawpaw as senescence triggers major changes in gene expression. Identifying the key genes involved in controlling senescence could aid plant breeders when developing cultivars with slower ripening times.

The vast amount of untapped genetic diversity of wild pawpaw populations (Wyatt et al., 2021) bodes well for pawpaw production over the long term. Pawpaw has a large range spanning southern Ontario to the Florida panhandle; Huang et al. (2000) concluded that marginal populations within the natural range are more likely to capture the rare alleles responsible for their differentiation from other populations. Thus, accessions from the extreme ends of the range can be identified to allow breeders and growers to develop resilient cultivars that can withstand the effects of climate change.

To summarize, pawpaw producers face several challenges that limit the growth of the industry. First, the low availability of superior clonal trees is a major impediment, but this could be addressed by developing tissue culture propagation techniques. Second, the fruit has a short shelf-life which limits market acceptability and commercialization. The pawpaw fruit has atypical physiology so attention to techniques to prolong storage and lessen bruising would improve market potential. A third limitation is the lack of available rootstocks to control height and prevent disease. Developing dwarfing rootstocks that are easy to asexually propagate is a long-term venture but could greatly contribute to the development of high-density orchard systems that

have been adapted for many temperate fruit species.

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About the Front Cover Image:

While the Passiflora family has over 600 known species, only a few are considered to be of commercial quality for fruit production. The primary species is *Passiflora edulis*, the passionfruit. This species has two varieties, *P. edulis* var. *edulis* and *P. edulis* var. *flavicarpa*. The former is generally purple in color whereas the latter is yellow. The purple fruit is usually smaller, but sweeter. The yellow fruit is larger but more acidic and is commonly used in juice production rather than fresh consumption. Intraspecific hybrids of these are also popular. Most *Passiflora* species originate in the tropical regions of Central and South America; however, a few, such as *Passiflora incarnata*, grow in temperate or subtropical areas. In countries where passionfruit is native or grown widely, consumption is common, with uses including fresh market, juices, dried, and teas. Passionfruit production in the U.S. is small and consumers are generally unfamiliar with the fruit, but interest is growing. Presently, attempts are being made to expand production outside of traditional regions like California, Florida, Hawaii, and Puerto Rico. Identification of cold tolerant selections and incorporation of new interspecific hybrids may lead to new areas of passionfruit within the US and other non-traditional growing regions.

Photo and description by Eric Stafne, Ph.D., Mississippi State University