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Cutting and Seed Propagation of Chickasaw Plum (*Prunus angustifolia*)

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*Chickasaw plum (*Prunus angustifolia *Marsh.) has the potential to be planted for windbreaks, wildlife, and for human consumption; however, reliable propagation protocols need to be established. Propagation of plants by seed using mechanical scarification treatment and stratification as well as stem cuttings using selected rooting hormone rates for Chickasaw plum were investigated. Seeds and stem cuttings of previous year's growth were collected in Payne County, Oklahoma. Seed stratification treatments included 0, 30, or 60 d at 3°C. Mechanical scarification employed tip removal from seeds with a nail clipper. Stem cuttings were treated with five IBA rates (0, 100, 1000, 3000, and 7000 mg·L^{-1}) in factorial combination with three collection times (May, August, and October). Scarifying the seed coat did not affect germination percentage. Non-scarified seeds stratified 60 d at 3°C had the highest germination at 31%. The time of year for cutting selection and amount of IBA rooting hormone applied was critical for rooting success. Rooting success was greatest at the highest IBA concentration when cuttings were harvested in May or August (44% and 49%), respectively.*

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KEYWORDS *Rosaceae, sand plum, native, small fruit, dormancy, adventitious rooting, auxin, season*

INTRODUCTION

Chickasaw plum also known as sand plum, Cherokee plum, or sandhill plum, is a native fruit-producing shrub or small tree in Oklahoma. Uses include fruit jams and jellies, cover for birds and other wildlife, and as windbreaks (Okie, 1987; USDA Natural Resources Conservation Service, 2011). Chickasaw plums naturally form clonal clump communities, also known as mottes. Plants range in height from 0.91 to 7.6 m, depending on genotype, soil, and water conditions (Row and Geyer, 2010). Leaves are bright green with serrated edges that are tipped by tiny orange dots and feel slick to the touch. This characteristic distinguishes it from Oklahoma plum (*P. gracilis* Engelm. & A. Gray), which looks similar but has pubescent leaves. Chickasaw plum bark is a reddish brown and turns ash gray as the branch ages. Plants may have pointed branches. Flowers are arranged in clusters that typically bloom from March through April in Oklahoma depending on genotype and location. Flowers are vivid white, may have a faint fragrance, and are usually no bigger than 1.57 cm across (Row and Geyer, 2010). Flowering lasts for about 2 weeks. Fruit are red or yellow in color and ripen from June to early August. Fruit size ranges from 0.6 to 2.54 cm.

Chickasaw plum plants are scarce since only a few specialty nurseries offer this species. Transplanting field-dug plants is often unsuccessful. West et al. (2012) reported that coppiced and left-intact root transplants had less than 40% survival rates after 4 years. Another method of propagate Chickasaw plum is by seeds. Seeds provide a method for replicating a large number of plants in a short amount of time. For many *Prunus* L. species, seed treatments are generally needed for germination to occur (Grisez et al., 2008). Stratification, one type of pretreatment, is the exposure of seeds to certain temperatures in an aerated and moist medium (Hartmann et al., 2011). These treatments appear to be species specific. For example, black cherry (*P. serotina* Ehrh.) failed to germinate without exposure to cold stratification (Esen et al., 2007), while American plum (*P. americana* Marsh.) required at least 210 d stratification to yield 71% germination (Giersbach and Crocker, 1932). The Knox City Texas United States Department of Agriculture (USDA) Natural Resources Conservation Service (NRCS) Plant Materials Center holds Chickasaw plum seeds in stratification for 60 d at 2 to 9°C and achieves 60% germination (Esquivel, 2001).

Germination of *Prunus* seeds is restricted by a seed coat and thick endocarp, which contains a plant growth regulator that decreases germination (Chen et al., 2007). Several mechanical and chemical treatments have been used to crack, remove, or soften the endocarp of *Prunus* species with varying

success (Grisez et al., 2008). Removal of the endocarp increased germination in American plum (*P. americana*), almond (*P. dulcis* (Mill) D.A. Webb), sweet cherry (*P. avium* L.), sour cherry (*P. cerasus* L.), peach (*P. persica* (L.) Batsch), and blackthorn (*P. spinosa* L.) but no advantage for bullace plum (*P. domestica* L.) was seen (Grisez et al., 2008). While Chickasaw plums can be germinated by seed, but this will not produce true-to-type genotypes. Propagation by grafting or cuttings does produce clones, and in comparison to grafting, cuttings may be easier, cheaper, and more successful for Chickasaw plum.

For cutting propagation, auxin is often used to facilitate greater and quicker rooting (Nordstrom et al., 1991). Although plant growth regulating chemicals, such as auxin, that promote rooting are produced naturally, application can assist root development. Indolebutyric acid (IBA) is a commonly used plant growth regulator to stimulate rooting. Optimum concentration to promote rapid, abundant rooting varies among species. Indolebutyric acid is a natural plant auxin that can be made synthetically (Hartmann et al., 2011). Too little IBA can decrease rooting and too much can be phytotoxic. *Prunus* species and cultivars vary in the optimum IBA concentration to promote rooted cuttings (Tsipouridis et al., 2003). For example, concentrations of IBA for successful rooting of semi-hardwood cuttings ranged from 50 to 2000 $mg{\cdot}L^{-1}$ (Chauhan and Maheshwari, 1970; Sulusoglu and Cavusoglu, 2010; Tsipouridis et al., 2003). Loreti and Morini (2008) suggest IBA concentrations from 1000 to 3000 $mg{\cdot}L^{-1}$ for the rooting of hardwood peach (*P. persica*) cuttings. Other experiments have similar findings. In an experiment using hardwood wild cherry (*P. avium* 'Gisela') cuttings, greater rooting occurred at 1000 $mg{\cdot}L^{-1}$ IBA than at 0, 2000, 4000, or 6000 $mg{\cdot}L^{-1}$ (Exadaktylou et al., 2009). Concentrations of 2000 and 0 $mg{\cdot}L^{-1}$ resulted in excellent rooting, but concentrations higher than 2000 $mg{\cdot}L^{-1}$ decreased rooting greatly (Exadaktylou et al., 2009). Rooting percentage decreased with increasing IBA concentrations in Azorean cherry (*P. azorica* (Hort. ex Mouillef.) Rivas Mart., Lousã, Fern. Prieto, E. Dias, J.C. Costa, and C. Aguiar) with rooting percentage of 28% at 7000 $mg{\cdot}L^{-1}$ IBA compared to 70% at 2500 $mg{\cdot}L^{-1}$ IBA for semi-hardwood cuttings (Moreira et al., 2009). However, rooting percentage can increase with increasing IBA concentration. Rooting was lower at 1000 $mg{\cdot}L^{-1}$ IBA than it was for 5000 or 10000 $mg{\cdot}L^{-1}$ IBA for hardwood peach cuttings (Couvillon et al., 1986).

The time of year when *Prunus* cuttings are taken affects rooting success. Generally, hardwood cuttings have greater rooting between October and January (Lovell and White, 1986). Softwood and semi-hardwood cuttings typically root well when collected in the summer (July–August) (Couvillon et al., 1986; Loreti and Morini, 2008; Sharma and Aier, 1989). Specific information for Chickasaw plum cutting and seed propagation is limited. The objectives of this experiment were to (1) determine what stratification period provides

the best germination and whether scarification affects the stratification period required for Chickasaw plum germination and (2) determine the concentration of IBA that is the most effective at initiating high rooting of hardwood Chickasaw plum cuttings at selected collection dates.

MATERIALS AND METHODS

Experiment 1

In June 2012, fruit was collected from one Chickasaw plum motte in Payne County, OK. Seeds were cleaned by hand de-pulping and sorted using a float test (Tinus, 1978) and any floating seeds were discarded. The experimental design involved 2 scarification treatments (scarified or unscarified) × 3 stratification treatments (0, 30, and 60 d at 3°C). Scarified seed consisted of removing approximately 1 cm of one end of the endocarp using a nail clipper and thus exposing the inner seed. All seeds were planted 2.5 cm deep in 90-cell trays containing a Redi-earth plug and seeding mix (Sun Gro Horticulture, Bellevue, WA, USA). Trays were covered with plastic bags to retain moisture and placed into a cooler (International Cold Storage Inc., Wichita, KS, USA) on 13 June 2012. After the designated stratification period, trays were moved to a greenhouse under natural photoperiods with the temperature set at 18°C/21°C day/night with a photosynthetic photon flux density (PPFD) range of 600 to 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 1200 HR. Germination, defined as seeds sprouting above soil layer, was recorded weekly for 8 weeks. Experimental design was a randomized complete block with six seed treatments. Treatments were replicated three times with 50 subsamples (seeds) per treatment. Data were analyzed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA) with PROC MIXED LSMEANS and PROC MEANS.

Experiment 2

Stem cuttings of the previous year's growth were collected from three locations in Payne County, OK. Fifteen cuttings were dipped into IBA solution at approximately 5-cm depth for 10 s. Hardwood cuttings of ~8.9 cm were placed into pre-moistened 90-cell styrofoam (similar to those produced by Dubois Agrinovation, Saint-Remi, Canada) trays that contained 50:50 (v/v) perlite and vermiculite. Rooting hormone treatments consisted of 0, 100, 1000, 3000, and 7000 $mg \cdot L^{-1}$ IBA (Hortus IBA 20% water soluble salts, Hortus USA, New York City, NY, USA) diluted in tap water. The IBA solutions were prepared independently for each season treatment. Cuttings were collected 21–23 May 2012, 7–9 Aug. 2012, and 16–17 Oct. 2012 and stored at 3°C in a trash bag for 1 d until treatment application. One to two leaves were present on all cuttings except for the October collection.

Intermittent mist was set at 20 s every 4 min. Cuttings were removed from the mist bench after 52 d. Rooting percentage and callusing were recorded. Successful rooting percentage was defined as presence of a root without using magnification. Successful callusing was defined as presence of callusing visible to the unaided eye. Experimental design was a randomized complete block consisting of five IBA treatments. Treatments were replicated 5 times with 15 subsamples (cuttings) per treatment. Rooting percentage and callusing was analyzed using JMP 10 (SAS Institute Inc., Cary, NC, USA) with Fit Model Procedure. Data between rooting percentage and callusing was correlated using PROC CORR.

RESULTS AND DISCUSSION

Experiment 1

Scarification did not affect seed germination. Germination was significantly greater ($P < 0.01$) for the 60-d stratification treatment (31% unscarified, 25% scarified) when compared to the 0 (0%) and 30 d (1% scarified, 0% unscarified) treatments (Fig. 1). Germination was apparent 2 weeks after seeds were removed from stratification for the 60 d cold stratification treatment. There was little additional germination after the 3rd or 4th week after transfer to the greenhouse.

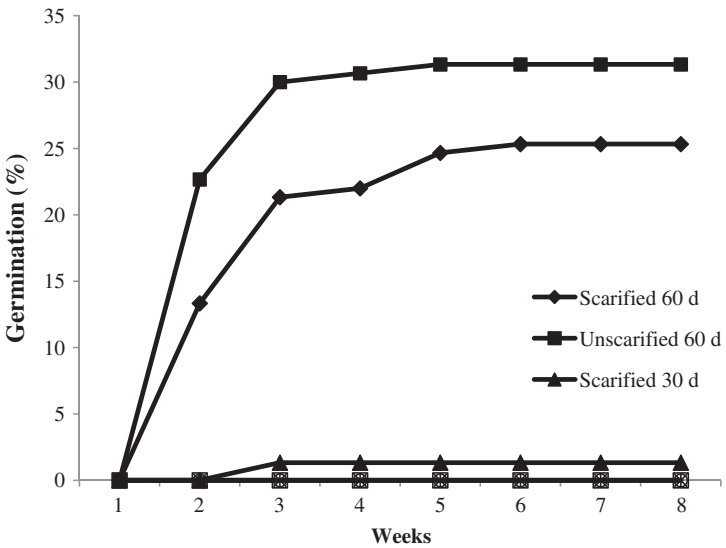

FIGURE 1 Cumulative germination percentages for Chickasaw plum (*Prunus angustifolia*) seeds ($n = 150$) after primary dormancy relieving treatments. Scarified seed consisted of removing approximately 1 cm of one end of the endocarp using a nail clipper. Stratification consisted of placing a seed in moist media at 3°C for 0, 30, and 60 d in a cooler.

Scarifying the endocarp did not significantly increase germination percentage or rate. A possible explanation is that an insufficient amount of the endocarp was removed. For peach seeds, the entire endocarp and seedcoat were removed to achieve high germination rates after only 15 d (Mehanna and Martin 1985). Chen et al. (2007), working with bellflower cherry (*P. punctata* Lam.), found that removing the endocarp resulted in 3% germination after 6 d and 21% after 27 d, but removing the endocarp and seedcoat increased germination to more than 60% after just 5 d. They concluded that the seedcoat had germination-inhibiting growth regulators, such as abscisic acid (ABA). Thus, scarification may need to include disruption of the endocarp as well as the seedcoat.

Germination was lower than expected for the 60-d stratification treatment, suggesting that either a warm, moist treatment may be needed before stratification, or that the stratification treatment was not long enough. Many *Prunus* species require a warm, moist treatment before stratification (Chen et al., 2007; Esen et al., 2007; Grisez et al., 2008), and it is likely that Chickasaw plum would as well. For example, germination of black cherry increased from 39% after 120 d stratification to 91% germination when seeds were exposed to a 20-d warm, moist treatment before stratification (Esen et al., 2007). American plum (*P. americana*), a species that is closer phylogenetically to Chickasaw plum than any of the previously mentioned species (Lee and Wen, 2001), germination increased from 54% to 72% when stratification duration was increased from 150 to 210 d (Giersbach and Crocker, 1932). Our results showed that slight disruption to the endocarp of Chickasaw plum did not improve germination compared with control seeds. Future research should focus on the effect of removing the entire endocarp alone or in conjunction with the seedcoat, longer stratification durations, or adding a warm, moist treatment prior to stratification.

Experiment 2

Cuttings collected in May or August had a similar positive response in rooting with increasing IBA rates (0 to 7000 mg·L^{-1}) (Figs. 2 and 3). Cuttings collected in October responded differently than those in May and August, but rooting still increased with increasing IBA rates (0 to 7000 mg·L^{-1}) (Fig. 4). Generally, for species of *Prunus*, concentrations of 1000 to 3000 mg·L^{-1} IBA are satisfactory for rooting cuttings (Chauhan and Maheshwari, 1970; Exadaktylou et al., 2009; Sulusoglu and Cavusoglu, 2010). Moriera et al. (2009) showed a decline in rooting at IBA concentrations past 2500 mg·L^{-1} for Azorean cherry. This was not observed in this experiment, as results were more similar to Couvillon et al. (1986), who reported greater rooting percentages for hardwood peach cuttings at 5000 or 10,000 mg·L^{-1} IBA than concentrations of 0, 1000, and 15,000 mg·L^{-1} IBA. Although one cultivar, Redhaven, had 80% rooting at 15,000 mg·L^{-1} and 50% rooting at

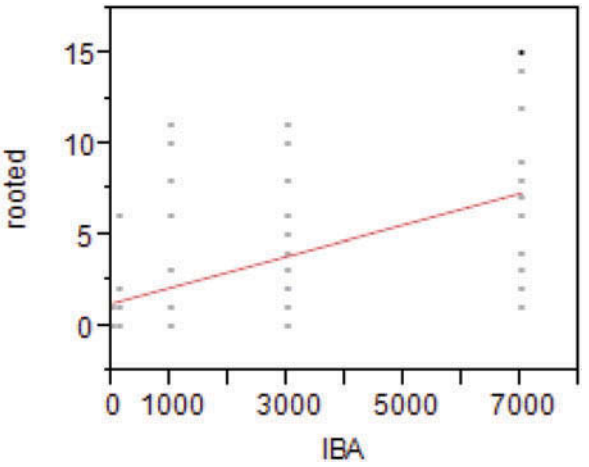

FIGURE 2 Regression of rooted Chickasaw plum stem cuttings (n = 45) taken from the previous year's growth collected in May with five Hortus IBA treatments of 0, 100, 1000, 3000, and 7000 mg·L^{-1}. R^2 = 0.32.

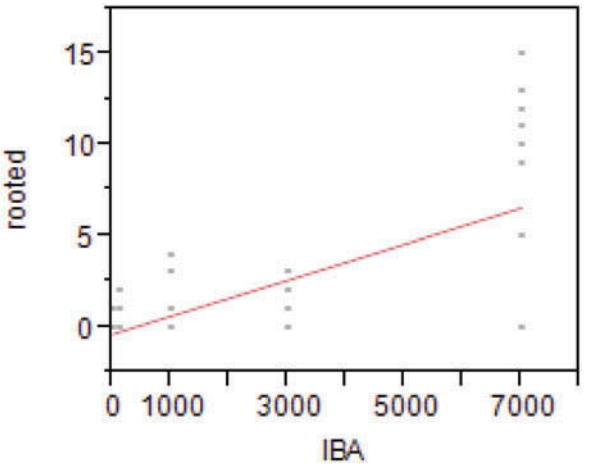

FIGURE 3 Regression of rooted Chickasaw plum stem cuttings (n = 45) taken from the previous year's growth collected in August with five Hortus IBA treatments of 0, 100, 1000, 3000, and 7000 mg·L^{-1}. R^2 = 0.46.

10,000 mg·L^{-1} (Couvillon et al., 1986). Concentrations of IBA greater than 3000 mg·L^{-1} would not be expected to result in more rooting, because it has been suggested that auxin is not the limiting factor for species that are difficult to root (Davies and Hartmann, 1988). This reasoning is that IAA, an endogenous auxin, decreases as root primordia form (Blakesley, 1993). The hormones IAA and IBA are not persistent in plants. Internal concentrations of applied IAA decreased from 558 to 32.1 ng·g and IBA concentrations

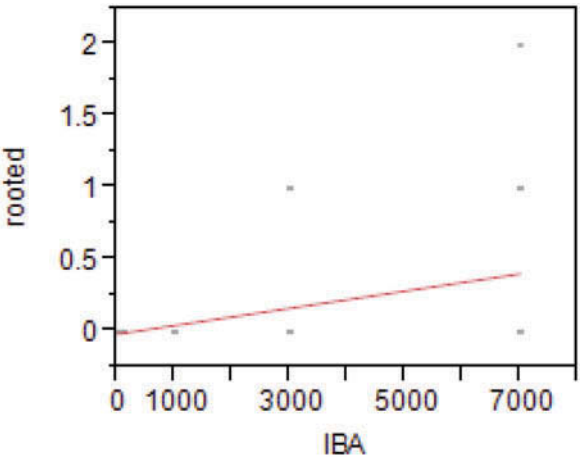

FIGURE 4 Regression of rooted Chickasaw plum stem cuttings ($n = 45$) taken from the previous year's growth collected in October with five Hortus IBA treatments of 0, 100, 1000, 3000, and 7000 mg·L^{-1}. $R^2 = 0.19$.

decreased from 147.8 to 20.5 ng·g after 2 days in 'Gisela' cherry (Stefancic et al., 2005). Blakesley (1993) suggested that higher concentrations of auxin are more important in inducing root induction, but once root primordia form lower concentrations enable better root growth. If high amounts of auxin remained, it could inhibit root growth (Jarvis, 1986). Since older plants have lower amounts of auxin (Haffner et al., 1991), a higher concentration of IBA could produce greater rooting. This could explain why higher IBA concentrations resulted in better rooting in May and August.

Fall and winter months are recommended for rooting cuttings of *Prunus* (Loreti and Morini, 2008). Hardwood cuttings rooted up to 71% when taken in late November (Exadaktylou et al., 2009). The lack of rooting in October in the present study could be explained by bud dormancy or that different species root differently at various growth stages. Roberts and Fuchigami (1973) took Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) cuttings every month except for April and May for 3 years. Cuttings taken in September and October had low rooting. Supplemental auxin was used in the experiment and did not increase rooting during those specific months. Their experiment found a correlation between low rooting and bud endodormancy. Anand and Heberlein (1975) reported wavy leaved fig tree (*Ficus infectoria* Wild.) cuttings had better rooting when cambial activity and auxin concentrations were greater. Dick and Leakey (2006) found that hardwood cuttings taken from adult wild cherry wood in June had poor rooting, while hardwood cuttings taken from juvenile wood in June had approximately 63% rooting success.

Callus formation decreased as IBA concentration increased for May ($P < 0.05$) (data not shown). Indolebutyric acid rates for cuttings taken

in August and October were not related to callus formation. Indolebutyric acid concentration did not affect callusing. The correlation between rooting and callusing was highly significant ($P < 0.0001$) (data not shown). As rooting increased, callusing decreased. Callusing is an avenue for the formation of adventitious root primorida, but can also delay or cease root formation (Stefancic et al., 2005). Mackenzie et al. (1986) found that heavy callusing within the wound was necessary for roots to form, though the authors and Spethmann and Hamzah (1988) noted that callusing was not always related to root initiation. Adventitious roots may originate near the wound, from callus and from in situ roots (Lovell and White, 1986). However, for *Populus balsamifera* L., callus mass at the base restricted initiated roots, because the callus cells were very compact and the roots could not break through (Cormack, 1965), and thus callusing may have impacted the rooting of the cuttings. From these results, 3000 and 7000 $mg{\cdot}L^{-1}$ IBA concentrations should be used on plum cuttings using the previous season's growth collected in May or August.

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