PROPAGATION OF SAND PLUM (*PRUNUS ANGUSTIFOLIA*) MARSH.: AN EXCITING START TO DOMESTICATION

By

ELIZABETH ANN MCMAHON

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Horticulture

Texas A&M University

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Thesis Approved:

Dr. Bruce Dunn

Thesis Adviser

Dr. Eric Stafne

Dr. Karen R. Hickman

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Sand plums (*Prunus angustifolia* Marsh.) hold potential for further development as fruit bearing trees. Propagation information is lacking however. The objective of the first experiment was to evaluate different rootstocks for their effects on sand plum accessions, to demonstrate initial differences between sand plum accessions and to preform preliminary detection of incompatibility if possible. Experiments were performed in the field and in the greenhouse. Chip budding, t-budding and cleft grafting was used in the field and chip budding in the greenhouse experiment. Rootstocks 'Lovell', 'St. Julien A', and myrobalan were used at two field sites, and rootstocks 'Lovell', 'Nemaguard', st. julien and 'Myrobalan 29 C' were used in the greenhouse experiment. Field trees were monitored for bud survival. T-budding, chip budding and cleft grafting had poor graft take in the field. Bud survival and height were measured in the greenhouse experiment. Greenhouse chip budding resulted in 66% percent overall bud survival. Accession 44 had significantly less bud survival out of all accessions and control. 'Myrobalan 29 C' rootstock had significantly reduced height when only surviving buds were used. The objective of the second experiment was to determine which concentration of IBA and which season was the most appropriate to initiate high rooting success of sand plum. Three seasons (spring, summer, fall) and five IBA concentrations (0, 100, 1,000, 3,000 and 7,000 ppm) were used. Rooting percentage, root quality and presence or absence of callusing was measured. Experiment 2 cuttings in spring and summer seasons with higher (3,000 and 7,000 ppm) concentrations of IBA resulted in more rooting than cuttings with lower IBA rates. Root quality was not affected by IBA concentration or season. The objective of the third experiment was to determine the best stratification and scarification methods for sand plum seed. Seeds were stratified for 0, 30 and 60 days and scarified by the creation of a small hole in the top of the endocarp. Scarification did not produce any significant results. The 60 days stratification period had significantly highest germination. Highest germination was 60 days cold unscarified seeds at 31%.

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CHAPTER I

INTRODUCTION

Native *Prunus* (L.) species hold great potential for propagation and further development. Possible uses include food products derived from the fruit, restoration and windbreak capabilities, as well as ornamental and wood purposes. Additionally, many of these species have been incorporated to create resistant rootstocks for commercial plum (*Prunus domestica* L.), peach (*Prunus persica* L.), and apricot (*Prunus armeniaca* L.) varieties.

The genus *Prunus* is in the Rosaceae family. Characteristics of the genus *Prunus* include five petals and sepals and fruit that contains a stony endocarp (Rehder, 1940). The genus *Prunus* can be further divided into several sections. Commercial fruits, such as peaches and apricots, exist in the sections *Amygdalus* and *Armeniaca*, respectively, while native sand plums and other motte forming plums exist in the section *Prunocerasus*. Eighteen species of *Prunus* exist in the natural lands of Oklahoma (USDA NRCS, 2013). Of these species, *Prunus mahaleb* (L.*)* and *Prunus persica* (L.) Batsch are introduced. Sixteen native species remain. Of these species, the low shrub spreading forms can be difficult to distinguish vegetatively. Plum species are known to overlap in regions, leading to hybrid populations which can make identification difficult. Table 1 details identifying characteristics of Oklahoma native plums.

Sand Plum (Prunus angustifolia Marsh.*)*

Sand plums, also known as Chickasaw plum, Cherokee plum, or Sandhill plum (*P. angustifolia*), are native fruit-producing shrubs or small trees in Oklahoma. Uses of sand plums include making jams and jellies from the fruit and include cover for birds such as bobwhite quail and the lesser prairie chicken (USDA NRCS, 2011a; USDA NRCS, 2011b). Raccoons and coyotes consume the fruit (Cooper et al., 2010).

Sand plums naturally form clonal clump communities, also known as mottes. The plant ranges in height from 1.5 to 7.6 m, depending upon soil and water conditions (Row and Geyer, 2010). Leaves are bright green and have serrated edges that have tiny orange dots on each serration. The leaves have a slick feeling to the touch. This characteristic can help distinguish it from Oklahoma Plum (*Prunus gracilis* Engelm. & A. Gray*)*, which looks very similar but has leaves with a fuzzy texture. The bark initially is a deep reddish brown color that turns ash gray as the branch ages. Flowers typically appear March-April and are arranged in clusters. These flowers are a brilliant white and may have a faint fragrance. They usually are no bigger than 1.57 cm across (Row and Geyer, 2010). Flowering will last for a couple of weeks and either red or yellow fruit will form afterward. Both colors of fruit occur in the same areas of Oklahoma. Ripening of the fruit occurs from June to early August. Fruit size can range from 0.6-2.54 cm. Plants may be thorny.

Pest and Disease Problems

Sand plums can contract similar diseases as peaches and plums. Of these diseases, brown rot and black knot have been noted to have the most obvious effects upon wild populations. Black knot *(Dibotryon morbosum)* is a fungal disease (Fig. 1A). It kills trees slowly, working its way through plants twig by twig. It has not been observed to be common in wild *P. angustifolia* populations. Brown rot (*Monilia fructicola*) is evident in the late summer by the appearance of soft brown patches on the fruit. White speckles may be present on these brown patches. During the remainder of the year infected trees produce cankers and gummy sap (Fig. 1B). Cankers may

girdle and kill young trees, and will shorten the lifespan of sand plums. Cytospora canker (*Leucostoma cincta (*Fr ex. Fr.) Hohn) may also cause gummy sap to occur. Other diseases include bacterial leaf spot (*Xanthomonas pruni*) (Fig. 1C) and fireblight (*Erwinia amylovora*) (Fig. 1D) (Row and Geyer, 2010). Fruit production may be decreased by plum curculios (*Conotrachelus nenuphar* Herbst.). Plum curculios burrow into the fruit, damaging it as well as leaving openings for pathogens such as brown rot. Other problematic insects include fall webworms (*Hyphantria cunea* Drury.) and grasshoppers (Suborder Caelifera) that will consume foliage, flowers, and fruit.

Cultural Methods

Sand plums prefer sandy soil that is not strongly alkaline (Roy and Geyer, 2010). Though sand plums grow closely in their native setting, bigger and healthier trees will result if trees are well spaced. No official spacing recommendation exists, but it is suggested to have 3-3.7 m between plants for orchard settings and 0.6-1 m apart if thicket settings are desired (Cooper et al., 2010; USDA NRCS, 2011a). Orchard fertilization recommendations are dependent upon soil characteristics, but generally 453.6 g of 10-10-10 of fertilizer per tree should be spread for the first year, with an increase of 453.6 g every year until the third year (McCraw et al., 2006). Pruning should be used to remove undesirable branches and to decrease fruit load. Overloading the tree with fruit will stress the tree, eventually killing it. Suckering is a concern for this species. Graham (2002) reported an average of 70 suckers per year when *P. angustifolia* was used as a rootstock.

Commercial Availability

Varieties of sand plums such as ‘Rainbow’, ‘Guthrie’, and ‘Chisholm’ exist and are commercially available from commercial and USDA nurseries. Both ‘Rainbow’ and ‘Chisholm’ can be used for fruit production, as well as for providing cover for bobwhite quail (USDA-NRCS, 2011a). Cultivar ‘Guthrie’ is primarily used for fruit production (Creech, 2010). Unpublished information reports it to have more disease resistance than commonly found sand plums and to

not sucker. Identification of 'Guthrie' as *P. angustifolia* could be erroneous, the cultivar being *Prunus rivularis* Scheele instead. According to Shaw and Small (2004), *P. rivularis* along with *Prunus munsoniana* W. Wight and Hendrick and *Prunus hortulana* L.H. Bailey could be easily confused with *P. angustifolia*. *Prunus rivularis* does not sucker and the leaves are similar in appearance to sand plum. Hybrids of *P. angustifolia* and commercial Japanese plum *Prunus salicina* Lindl., such as 'Bruce', exist as well.

Difficulties arise in obtaining sand plum trees. Sand plums are available at some nurseries and finding trees similar to local species can be difficult. Digging up trees is an option, but may be restricted or difficult. An experiment by West et al., (2012) showed that coppiced and left intact root transplants had disappointing success. Intact transplants only had up to 20% survival after four years and coppiced transplants had less than 40% survival after four years (West et al., 2012). However, the bare root sand plums obtained from the nursery showed 80% survival. Using *P. angustifolia* with pre-established roots is far superior than using directly planted root cuttings (West et al., 2012). Sand plums can be grown using grafts, cuttings, or from seed. These methods may be more successful than transplanting. However, information on length of cold stratification period, graft suitability, and cutting procedures are conflicting or non-existent for this species. The overall objective of this thesis was to clarify these procedures and provide basic propagation information on this plant.

TABLES AND FIGURES

Table 1.1. Native *Prunus* species in Oklahoma and their characteristics

Common Name	**Scientific Name**	**Distinguishing Trait**
American plum	*Prunus americana*	short-med. height tree, slick wide leaves, hairy twigs
Sand plum	*Prunus angustifolia*	shrub-short tree, long and slender slick leaves that fold along midrib
Oklahoma plum	*Prunus gracilis*	shrub-short tree, fuzzy leaves with small marble size fruit
Hortulan plum	*Prunus hortulana*	short-med. tree, Eastern Oklahoma, glossy egg shaped leaf with triangular tip
Mexican plum	*Prunus mexicana*	short-med. tree, drooping slick wide leaves, hairless twigs
Wild goose plum	*Prunus munsoniana*	long and slender leaf, Eastern Oklahoma.
Creek plum	*Prunus rivularis*	short tree, long leaves with broad triangular tips. Rare in Oklahoma.
Black cherry	*Prunus serotina*	short-med. height tree, small black fruit, wide slick leaves
Chokecherry	*Prunus virginiana*	short-med. height tree, small dark red fruit, wide slick leaves

Figure 1.1 Visual indicators of sand plum (*Prunus angustifolia* Marsh.) diseases. Photographs taken by Elizabeth McMahon. **1A** Black knot on limbs of emerging sand plum. **1B** Gummosis on sand plum branch, a symptom of brown rot or cytospora canker is gummosis. **1C** Bacterial leaf spot on sand plum**. 1D** Suspected fireblight symptoms on sand plum.

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CHAPTER II

GRAFTING OF SAND PLUM

INTRODUCTION

Grafts are an important part of the fruit production industry. The ability to graft has allowed many different cultivars to be grown in areas where they would otherwise be unsuited, whether by disease susceptibility or soil characteristics. The first step in a graft is to select a functioning scion that has desirable traits. The scion (top part of a graft) is placed into tight contact with the rootstock (bottom half of graft). The cambium layers (xylem and phloem) of the scion and rootstock are aligned and parenchyma cells are produced, creating callus tissue that connects the two parts. Cambial cells differentiate from this mass and the xylem and phloem cells are repaired (Hartmann et al., 2011; Pina and Errea, 2005). Grafting can be performed in a number of ways, including cleft grafting and chip and t-budding (Fig. 2.1). T-budding is when a t shaped slit is made into the bark of the rootstock, and a freshly cut bud is slid in. The wound is then tied tightly. T-budding can only be done when the vascular cambium is actively growing (Hartmann et al., 2011). This is called 'slipping'. Chip budding consists of slicing out a chip from the rootstock, then inserting a similar sized chip with bud into the open wound, while aligning the cambium layers to match on either sides of the wound. Chip budding does not require the bark to be slipping. This method is thought to be preferable to t-budding due to quicker healing and straighter trees (Patrick, 1992 as cited in Hartmann et al., 2011). Cleft grafting is used for bigger trees. The top of the tree is removed, and a chisel or knife is driven into

the live stump. Slant edged scions are aligned with the rootstock cambium. The chisel is removed and the cut portions are sealed with wax or pruning sealant and wrapped tightly. Cleft grafting is usually performed just before trees break bud, and uses dormant material.

Incompatibility may occur between graft unions of *Prunus* species. Incompatibility can be defined as when a rootstock and a scion are unable to form a union. For *Prunus* species, incompatibility may be detected within two months or years later (Herrero 1951,1968; Lapins 1958; Guerriero et al., 1985; Moing and Carde 1988; Zarrouck et al., 2006). The causes of incompatibility vary. Callus formation and differentiation not occurring, insufficient cellular recognition, inability to metabolize prunasin, and disease can also cause incompatibility in *Prunus* (Mosse, 1962, as cited in Errea et al., 1994).

Because of incompatibility, careful selection of rootstock is essential. Myrobalan rootstock is derived from *Prunus cerasifera* (Ehrh.). It includes many cultivars that vary in disease resistance and compatibility. Suckering may be a problem for some cultivars (Gryzb and Sitarek, 1998). Although myrobalan clones vary in vigor, none have been reported to be dwarfing, being classified with high to very high growth vigor (Renaud and Salessess, 1994; Gryzb and Sitarek, 1998; Botu et al., 2002; Dimitrova and Marinov, 2002). Peaches have been reported to be compatible and incompatible with myrobalan clones (Moing and Carde, 1988). Myrobalan rootstock cultivars '29C', '2', 'GF 3-1', and 'P 1079' showed incompatibility when grafted with the nectarine cultivar 'Summergrand' (Grzyb and Sitarek, 1998). No significant differences were found between rootstocks of myrobalan and other rootstocks when grafted with 'Stanley' peach scions, but some significant differences were found when grafted with other peach cultivars (Hrotko et al., 2002). St. Julien rootstock is derived from *Prunus insititia* (N.). This rootstock is reported to have a low number of suckers (Werheim, 1990). It is cold hardy and can be used with peaches (Reighard, 1994; Reighard and Loreti, 2008). It may produce a semi-dwarfing effect when used as a rootstock (Wertheim, 1990; Wertheim and Kemp, 1998; Botu et al., 2002; Hrotko et al., 2002). 'St Julien A' was found to be susceptible to *Pseudomonas*

syringae and to have a low fruiting, when grafted to 'Redglobe' peach (Reighard et al., 1997). In contrast, 'St. Julien GF 655/2' was found to have significantly higher fruiting than other cultivars and graft combinations, except with 'Stanley' peach (Hrotko et al., 2002). 'Lovell' rootstock is derived from peach (*Prunus persica* L.). In comparison to other rootstocks such a 'Siberian C' and '881-2AC-2', 'Lovell' was hardier with fluctuating temperatures and had better survival (Yadava and Doud, 1989). 'Lovell' is reported to have a medium life span on peach tree short life sites and to be susceptible to root knot nematode and ring nematode *Criconemella xenoplax* ((Raski) Luc and Raski) (Okie et al., 1994). 'Nemaguard' *(P. persica* × *P. davidiana)*, as suggested by the name, has excellent resistance to nematodes which include southern rootknot nematode (*Meloidogyne incognita*,) peanut rootknot nematode (*M. arenaria*) and Javense rootknot nematode (*M. javanica)*, but not pine cyst nematode (*M. floridensis*) or ring nematode (Reighard and Loreti, 2008). 'Nemaguard' produced significantly higher yields than 'Lovell' and sand plum (*Prunus angustifolia* Marsh.) in a rootstock trial test using 'Harvester' peach (Graham, 2002). 'Nemaguard' has high vigor and suckering and may reduce cold hardness in scions (Reighard and Loreti, 2008).

Sand plum has been used to create hybrids for fruit production such as 'Bruce', 'Robusto', 'Segundo' and 'Six Weeks' plum (Byrne, 1989). Reighard and Loreti (2008) noted that use of sand plum as a rootstock produced a dwarfing effect in peach but this was not found in Graham (2002). Okie (1987) also listed a dwarfing effect, but for Japanese plum instead. As a rootstock, sand plum had comparable success to 'Nemaguard' (Graham, 2002). In the Graham (2002) experiment, 'Harvester' peach was budded onto *P. angustifolia*, along with other rootstocks such as 'Lovell', 'St. Julien A', and American wild types. The peach scion was found to be very vigorous on *P. angustifolia*, having a significantly higher tree circumference area than six of the other rootstocks (Graham, 2002). Fruit size did not appear to be affected but it produced a large number of suckers, 70 per tree per year (Graham, 2002). Suckering in native

settings leads to formation of sand plum mottes, clumps of genetically identical plants in one location.

Sand plum was reported to be an incompatible rootstock for the 'Herald' prune (Detjen, 1920). Graham (2002) recorded that 25% of the trees died from incompatibility with the peach and sand plum grafts. This does not indicate a limitation if sand plum were used as the scion instead of the rootstock. For example, when 'Marianna' plum scions were grafted onto peach rootstock they were compatible, but when peach scions were grafted onto 'Marianna' rootstock incompatibility resulted (Heuser, 1972).

No published information exists for the graft suitability of sand plum upon peach and plum rootstocks, though *P. persica* and *Prunus hortulana* L.H. Bailey were found to be compatible with sand plum scions in trial experiments (Reid, personal communication). The objective of this experiment was to evaluate different rootstocks for their effects on sand plum cultivars, to demonstrate initial differences between sand plum cultivars and to preform preliminary detections of incompatibility if possible.

METHODS AND MATERIALS

Field Study Site

Perkins

Plot was located at Cimarron Valley Field Station, Perkins, OK. Soil pH was 6.0 and soil series was Teller loam. Elevation was 295 m and coordinates were 35°59'52.05" N, 97°02'38.99" W (Google Earth, Google INC., Mountain View, CA). Site was a former peach orchard. Plot was disked and plowed in late 2011, and Glystar Plus glyphosate (Albaugh, INC., Ankeny, IO) was applied to remove bermudagrass cover. There were 10 rows of 15 trees, with 4.3 m spacing between trees and rows 6.1 m apart.

Trees were planted in raised beds on March 2, 2012. Three rootstocks of 50 trees each were planted: 'Lovell' (*P. persica*), myrobalan (*P. cerasifera*) and 'St. Julien A' (*P. insititia*). Myrobalan and 'Saint Julien A' were received from Carlton plants (Dayton, OR) and 'Lovell'

was received from Ripley County Farms LLC (Doniphan, MO). Rootstock plantings were completely randomized across the field site. Three trees were lost and replaced with rootstock from the greenhouse. Rootstock and excessive shoots above the middle of trunk were pruned away and top of the tree was removed twice to restrict size. Fertilizer was applied at the rate of 454 g of 19-19-19 (Agrinutrients INC., Catoosa, OK) per tree on April 18, 2012. Application method consisted of applying the fertilizer around the rootstock base. Trees were watered as needed and irrigation was provided via pressure compensated drip lines at a rate of 3.8 L a minute (Toro, Bloomington, MN). Trees were not treated with fungicides nor were insecticides used on the trees at this site. Glyphosate was used for weed reduction on rows but was only applied occasionally.

Crescent

Plot was located on land owned by Eddy Fenton, north of Crescent, OK. Soil pH was 6.1 and soil series is Renfrow silt loam. Elevation was 318 meters and site coordinates were 35^{o} 59'41.94" N, 97^{o} 33'56.26" W (Google Earth, Google INC., Mountain View, CA). Former use of site was for grape vines. Field design was completely random. There were 11 rows of 15 trees, except for the last row which had 10 trees. Rows were 6.1 m apart and rootstock was 4.3 m apart. Three rootstocks of 50 trees each were planted: 'Lovell' (*P. persica*), myrobalan (*P. cerasifera*) and 'St. Julien A' (*P. insistia*). Myrobalan and 'Saint Julien A' were received from Carlton plants (Dayton, OR) and 'Lovell' was received from Ripley County Farms (Doniphan, MO).

Rootstock was planted on February 29, 2012. One tree was replanted from stock taken from the greenhouse. Fertilizer was applied at the rate of 454 g of 19-19-19 (Agrinutrients INC, Catoosa, OK) per tree on April 18, 2012. Application method consisted of applying the fertilizer around the rootstock base, but dispersal of fertilizer was reduced by owner. The spaces in-between the rows were occasionally mowed and no weed control occurred at this site. Irrigation was by poly drip tubing (DIG, Vista, CA) and was applied occasionally.

T-budding Experiment

Trees were budded May 8-9, 2012 at Perkins and May 10-11, 2012 at Crescent using the t-budding method. Rootstock and budwood combinations were completely randomized. Neither trees nor budwood were dormant. Budwood consisted of accessions 31, 45, 17, 39, and 32 for Perkins and accessions 16, 34, 44, 28, and 18 for Crescent (Table 2.1). Buds were at least one year old or older. Grafts were wrapped with parafilm tape after budding completion and observed weekly for signs of emergence. Bud survival was recorded in June 2012.

Chip Budding Experiment 1

Rootstock used for the experiment were the same trees used in t-budding experiment. Trees were budded June 18-19, 2012 at Perkins and June 20-22, 2012 at Crescent. New buds were placed above the scars of the old ones on the main trunk. Neither trees nor budwood was dormant. Method of grafting was chip budding. Budwood used included accessions 31, 21, 'Loring' peach, 41, and 15 for Perkins and accessions 16, 29, 44, 'Intrepid' peach, and 17 for Crescent (Table 2.1). Buds were at least one year old or older. Buds were wrapped with rubber bands after being encased with parafilm tape. Bud survival was recorded weekly.

Chip Budding Experiment 2

Rootstock used in the experiment were the same trees used in first chip budding experiment. Perkins site was grafted on October 22-26, 2012. Method of grafting was chip budding. Budwood was dormant for this experiment, but rootstocks were not. Budwood used included accessions 7, 23, 43, 16, and 'Harvester' peach (Table 2.1). Sand plum buds were at least one year old or older, peach buds were from current and past season's growth. Trees were grafted between 10 am–3 pm and relative humidity did not fall past 40% during the grafting. Bud survival was recorded weekly. Experiment was considered concluded on December 18, 2012.

Greenhouse Grafting

On February 25, 2013, 160 trees, 40 trees per rootstock type, were planted in 2.45 L pots at the Oklahoma Department of Horticulture and Landscape Architecture Research Greenhouses,

Stillwater, OK. Spacing was staggered 1.22x1.22 m (4x4 ft) with 10 blocks. Scion and rootstock combinations were blocked by bench location in order to reduce greenhouse light and temperature variability. Rootstock consisted of 'Lovell' (*P. persica*), 'Nemaguard' (*P. persica* × *P. davidiana*), 'Myrobalan 29 C' (*P. cerasifera*) and st. julien (*P. insistia*). 'Lovell' and 'Nemaguard' were received from Fowler nurseries (Newcastle, CA). St. Julien was received from Lawyer nurseries (Plains, MT) and 'Myrobalan 29 C' was received from Carlton plants (Dayton, OR). Budwood consisted of accessions 16, 29, 44, and 'Intrepid' peach as a control (Table 2.1). Buds were at least one year old or older. Blocks 1, 2, and 3 were grafted February 26, 2013, blocks 4, 5, and 6 were grafted February 27, 2013, blocks 7 and 8 were grafted February 28, 2013 and blocks 9 and 10 were grafted on March 1, 2013. Grafting method was chip budding. Buds were wrapped with parafilm buddy tape (Aglis & CO, Yame City, Fukuoka, Japan) and tied with a rubber band. Grafting knives were sterilized between each graft with a dip in 50% diluted bleach solution, then two separate rinses in tap water. Trees were placed on drip irrigation but were hand watered when dry. Fertilizer was administered March 20, 2013 at 12 g per tree of Scott's Osmocote 3-4 month mix (Charleston, SC). Trees were staked to prevent horizontal growth. Fertilizer was applied again at 8 g per tree of Scott's Osmocote 3-4 month mix on May 17, 2013. Bud emergence records began March 5, 2013. Height measurements began April 5, 2013 and were taken every seven days until May 31, 2013. Height data with bud survival accounted for and bud survival not accounted for was analyzed using SAS 9.3 (SAS Institute Inc., Cary, NJ) LSMEANS in PROC GLIMMIX with a normal distribution. Bud survival data was analyzed using PROC GLIMMIX with a binary distribution.

Cleft Grafting

Sand plum accessions 28, 15 and 'Redhaven' peach were cleft grafted onto 60 trees at Crescent on March 6-8, 2013. One hundred forty rootstock at Perkins were cleft grafted March 11-13, 2013. Accessions at Perkins consisted of 36 and 29 (grafted March 11, 2013), 'Harvester' peach (grafted March 12, 2013), and 16 and 34 (grafted March 13, 2013) (Table 2.1). Cleft graft

method consisted of removing tops of trees and leaving a 30 cm tall stump. Circumference of stump varied by tree and location. Knife blade was tapped into stump about 2.54 cm deep. With another knife, two halves of the stump were pried apart and one slant edged scion was inserted so that it aligned with the outer cambium of the stump (Fig. 2.1C). Grafts were wrapped with vinyl tape to add pressure to graft. Cut surfaces were then painted with Valspar exterior untinted paint (Minneapolis, MN) to retain moisture and to protect trees from disease. Trees were monitored weekly for bud break.

RESULTS AND DISCUSSION

T-budding Experiment

Initial bud survival was 10%, as only 16 buds out of the 150 took at both locations. Buds did not emerge by June 2012 and as of January 2013 none of these buds remained. Buds visually appeared to be being forced out of t-cuts. Weather conditions and technique may have contributed to the low success.

Chip Budding Experiments 1 and 2

For the first chip budding experiment, the success rate was similar to the t-budding experiment. Three buds at Perkins and six buds at Crescent locations were thought to be viable, but had not emerged a month later. Buds being forcibly ejected from the plant was reduced due to the presence of rubber bands. Buds showed no signs of life, and the Perkins field was re-grafted under different weather circumstances and with dormant budwood in a second chip budding experiment. During the first three weeks more success was seen in this experiment than in the first chip budding experiment. By the conclusion of the experiment in December, 38 out of 150 buds were thought to have survived. However, as of May 2013, none of these buds ever produced leaves.

The second chip budding experiment was thought to be more successful because grafting took place under more hospitable conditions. In an experiment by Eiseman and Thomas (1987), they tried chip budding and t-budding from February-April in New Zealand. Their highest

successful take (96%) was obtained when temperatures were 12-23^{o}C. Temperature ranges from 10-21^{o}C provided a decent take as well, being 78% and 71%, respectively. However when they chip budded at 8-20^{o}C, graft take was only 6%. For apple, callus cells do not form below freezing, and growth of cells ceases above 32^{o}C (Hartmann et al., 2011). At Perkins, temperatures ranged from 1-29^{o}C (Fig. 2.2A), suggesting that in October temperatures may have been too cold for budding. Also auxin levels, which promote callusing, are lower later in the year as well and this might have slowed the bud take (Lachaud, 1989). The first chip budding experiment was performed at temperatures of 22-33^{o}C, (Fig. 2.2A), which were too warm for budding. Chip budding of walnut and apple in India resulted in low successes during June and July (Gautam, 1990; Dimri et al., 2005). Locations were usually windy at times of grafting. Wind was not discussed in Eiseman and Thomas's paper, but is listed in Hartmann et al. (2011) as a factor that will cause buds to dry out quicker, thus decreasing their chances of survival.

For all grafts to be successful, the cambium must be aligned (Hartmann et al., 2011; Garner, 2013). In this experiment, because of the size of the buds being cut from the budwood and the size of the rootstock trunk, it was impossible to appropriately align the cambium on both sides of the graft. In this experiment, the buds instead were aligned with the outer edge, not the inner cambium (Fig. 2.6). Lacking the close contact with the vascular system the buds dried out and died. Chip budding may have been more successful if it had taken place during tree planting. Trees at this time would have been of more appropriate size for the buds that were being used and the surge of growth and auxin may have contributed positively to bud survival. Gautam (1990) and Dimri et al. (2005) respectively found that with walnut and apple budding late February and March produced the highest successes for chip budding.

Cleft Grafting

As of May 24, 2013, 10 out 140 grafts had emerged but only four were still alive at Perkins. None of the Crescent grafts showed any success. Emergence was present in all three rootstocks. None of the 'Harvester' peach added as controls broke bud. Stems remained alive,

but buds appeared dead and inactive. Scion wood that emerged would grow quickly but die suddenly with black leaves, possibly indicating that the graft connection was not sufficient to support growth. Several cleft grafts were knocked out of their places by animal damage, and several more were knocked out due to weed eater damage.

Lack of emergence could be due to weather. Unlike previous years, highs in March 2013 remained low, and there were many cloudy days (Fig. 2.2B, Fig. 2.4B). This could have delayed rootstock growth, with the grafted scions suffering from low flow of resources. Technique and disease could also to be considered in decreasing the success of the buds. Exudate and gummosis was seen oozing from some of the graft unions, but was not present on all, and therefore was not considered the significant factor for graft failure on the majority of the grafts. Improper alignment could also be a factor as well. Proper alignment is necessary for the success of the graft (Hartmann et al., 2011; Gardner, 2013). Grafts were aligned with the outer cambium, instead of the inner cambium (Fig. 2.6) and this may have led to decreased success.

Greenhouse Grafting Experiment

Overall budding success was 65% for this experiment. Accessions and rootstocks showed significant effects ($P<0.05$) in means calculated from only surviving buds and in means calculated from both surviving and non-surviving buds (adjusted against height). The interaction of rootstock and accession was not significant for both sets of means. A non-significant interaction means that the rootstocks affected each scion equally, and the accessions affected each rootstock equally. For the heights of the surviving buds, accession 16 showed significantly greater height on all rootstocks than any other accession (Table 2.2). Accession 44 had the lowest bud survival, and this decreased its height mean when the data was adjusted against survivability. Accession 29 showed a significant decrease in height when only the surviving buds were counted (Table 2.2). With only surviving buds, rootstock ‘Myrobalan 29 C’ had significantly less height than any other rootstock (Table 2.2). When all buds both surviving and not surviving were counted, st. julien had significantly less height than all other rootstocks except ‘Myrobalan 29 C’.

Peach grafted onto ‘Myrobalan 29 C’ shows symptoms of yellowing of leaves and a decrease in vigor, killing the trees by starvation within 1-2 years (Reighard and Loreti, 2008). ‘Myrobalan 29 C’ is not a dwarfing rootstock, yet all accessions grafted on it had significantly lower heights than the other rootstocks when only surviving buds were counted. Even st. julien, which was not recognized to be a dwarfing rootstock in this experiment, was not found to have a significant decrease in height. The decrease in st. julien’s height when both surviving and non-surviving buds are used is accredited to its low bud survival. Moing and Carde (1988) showed decreased height of incompatible myrobalan grafted with peach within 60 days after grafting. These significantly lower heights in this experiment could be the beginnings of incompatibility. Signs of nitrogen deficiency, which too indicate incompatibility, were detected in the plants, but this was determined to be a nonspecific response and diminished in most plants after the addition of fertilizer.

Budbreak began as early as March 5, 2013. Specific accessions did not significantly break bud before other accessions. Accession 29, ‘Intrepid’ peach and accession 16 had significantly higher bud survival than accession 44. Rootstock ‘Myrobalan 29 C’ had the highest average bud survival take, but was not significantly different from ‘Lovell’ and ‘Nemaguard’. St. Julien had significantly lower bud survival than ‘Myrobalan 29 C’ and ‘Lovell’. Poor graft success could be an indicator of graft incompatibility (Kishore and Randhawa, 1983). However, incompatible grafts such as peach on ‘Myrobalan 29 C’ and ‘Reliable’ apricot on peach rootstock can have successful grafts but show incompatibility later (Lapins, 1958). It is not thought in this experiment that the low grafting success demonstrated incompatibility.

CONCLUSION

All field experiments had low success rates, never reaching higher than 10% at experiment conclusion and with observations approximately a year later revealing no successful field buds. Rates of success within greenhouse were greatly increased, with an overall average of 66% success rate of bud take. The field experiments and greenhouse experiments cannot be

compared due to different accessions being used, but the controlled climate plus use of dormant material were probably the factors that increased the survival rate of the greenhouse grafts. Because the material in the greenhouse was smaller, alignment of bud to rootstock was easier. Appropriately sized rootstock is suggested for grafting. Rootstock 'Myrobalan 29 C' is not suggested for use, but 'Lovell', 'Nemaguard' and st. julien are recommended.

Table 2.1 List of sand plum (*Prunus angustifolia*) accessions and approximate flowering times.

Accession	Location	Flowering	Unusual Characteristics
7	Payne County[z]	Middle[v]	None
15	Lake McMurtry[y]	Very Late	Low fruiting
16	Lake McMurtry	Very Late	Low fruiting
17	Lake McMurtry	Early	Profuse flowering, tall trees
18	Lake McMurtry	Middle/Late	Tall trees, less suckering
21	Lake McMurtry	Early	Profuse flowering
23	Lake McMurtry	Middle	Profuse flowering and fruit
28	Crescent[x]	Early	Fruit excellent taste, very thorny[u]
29	Crescent	Middle	None
31	Crescent	Early	Low fruiting
32	Crescent	Early	None
34	Crescent	Middle	None
36	OSU Range Area[w]	Late	Yellow fruit
39	OSU Range Area	Middle	None
41	OSU Range Area	Early	Profuse flowering, heavy insect damage
43	OSU Range Area	Middle	Very thorny
44	OSU Range Area	Early/Middle	None
45	OSU Range Area	Late	Low flowering

[z] Coyle Rd., Payne County, OK
[y] Lake McMurtry, Stillwater, OK
[x] County roads on and near county line of Kingfisher and Logan County, near Crescent, OK
[w] Sherraton pasture, OSU Range Area, Payne County, OK
[v] Flowering times: Early-greater than 65% open before 3/16/12 and more than 50% flowers open before 3/27/2013. Middle- 65-40% flowers open at 3/16/12 and 20-50% flowers open 3/27/13. Late-less than 40% flowers open 3/16/12 and flowers barely opening 3/27/13. Very Late-no flowers open 3/27/13 and less than 30% open 3/16/12.
[u] As reported by Leon Cook, Crescent, OK

Table 2.2 Percent survival and grafting means for each accession by rootstock for greenhouse grafting experiment.

Budwood[z]	Variables	Lovell	Nemaguard	Myrobylan 29 C	St. Julien
16					
	% Survival	80	50	80	60
	Mean Height Adj.[y](cm) (SD)[x]	55 (39)	47 (40.9)	46.4 (42.5)	40.3 (43.9)
	Mean Height(cm) (SD)	78.6 (10.8)	78.33 (7.7)	77.3 (19.6)	80.6 (16.5)
29					
	% Survival	90	90	100	70
	Mean Height Adj.(cm) (SD)	48 (20.7)	55.5 (20.9)	52.6 (9.9)	37.1 (33.1)
	Mean Height(cm) (SD)	53.33 (12.7)	61.7 (7.8)	52.6 (9.9)	61.8 (12.1)
44					
	% Survival	50	40	60	10
	Mean Height Adj.(cm) (SD)	29.9 (39.3)	23.1 (30.5)	25.7 (27.6)	7.4 (23.4)
	Mean Height(cm) (SD)	74.8 (12.7)	57.8 (11.2)	51.4 (8.1)	74[w]
Intrepid					
	% Survival	70	90	70	50
	Mean Height Adj.(cm) (SD)	55.8 (29.7)	69.4 (25.6)	41.8 (31.2)	34.5 (38.4)
	Mean Height(cm) (SD)	69.7 (5.2)	77.1 (8.4)	59.7 (14.7)	69 (18.5)

[z] Numbers in budwood column are accessions. See Table 2.1 for details on accessions.
[y] Mean height adjusted against bud survival. Both surviving and non-surviving heights were counted for this measurement.
[x] SD stands for standard deviation from the mean
[w] Variable n=1. All other means were at least n=4.

Figure 2.1 Three different grafting methods. Photographs taken by Elizabeth McMahon. **2A** is t-bud of a sand plum (*Prunus angustifolia*) on a myrobalan (*Prunus cerifera*) rootstock before the bud is wrapped. **2B** is chip bud of 'Intrepid' peach (*Prunus persica*) on peach rootstock. Graft had budding tape just removed. **2C** is cleft graft of sand plum on myrobalan rootstock before wrapping and painting.

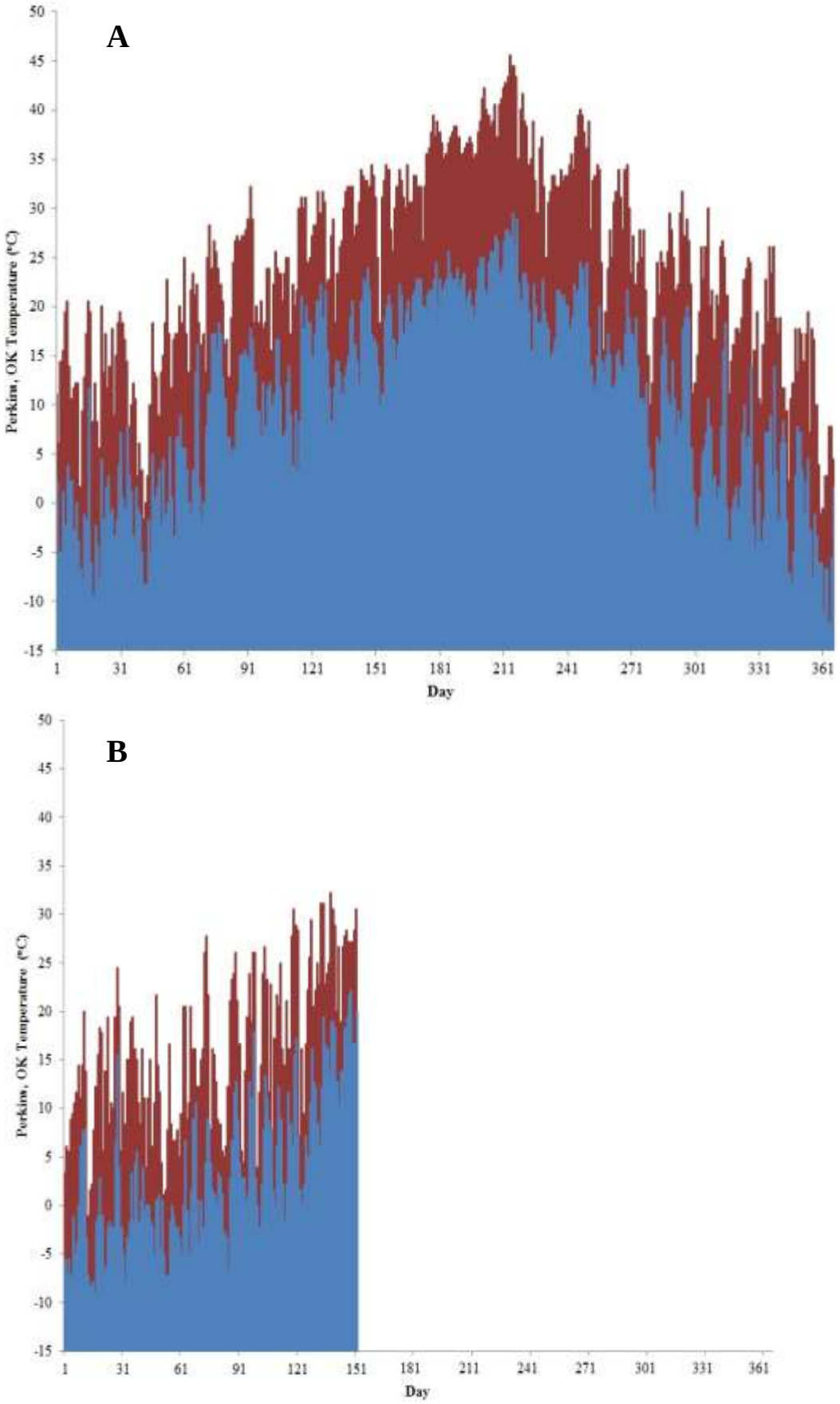


Figure 2.2 Temperature maximums (red) and minimums (blue) for Perkins, Oklahoma. Every 30 days is approximately a separate month. **2A** 2012. **2B** 2013 until May.

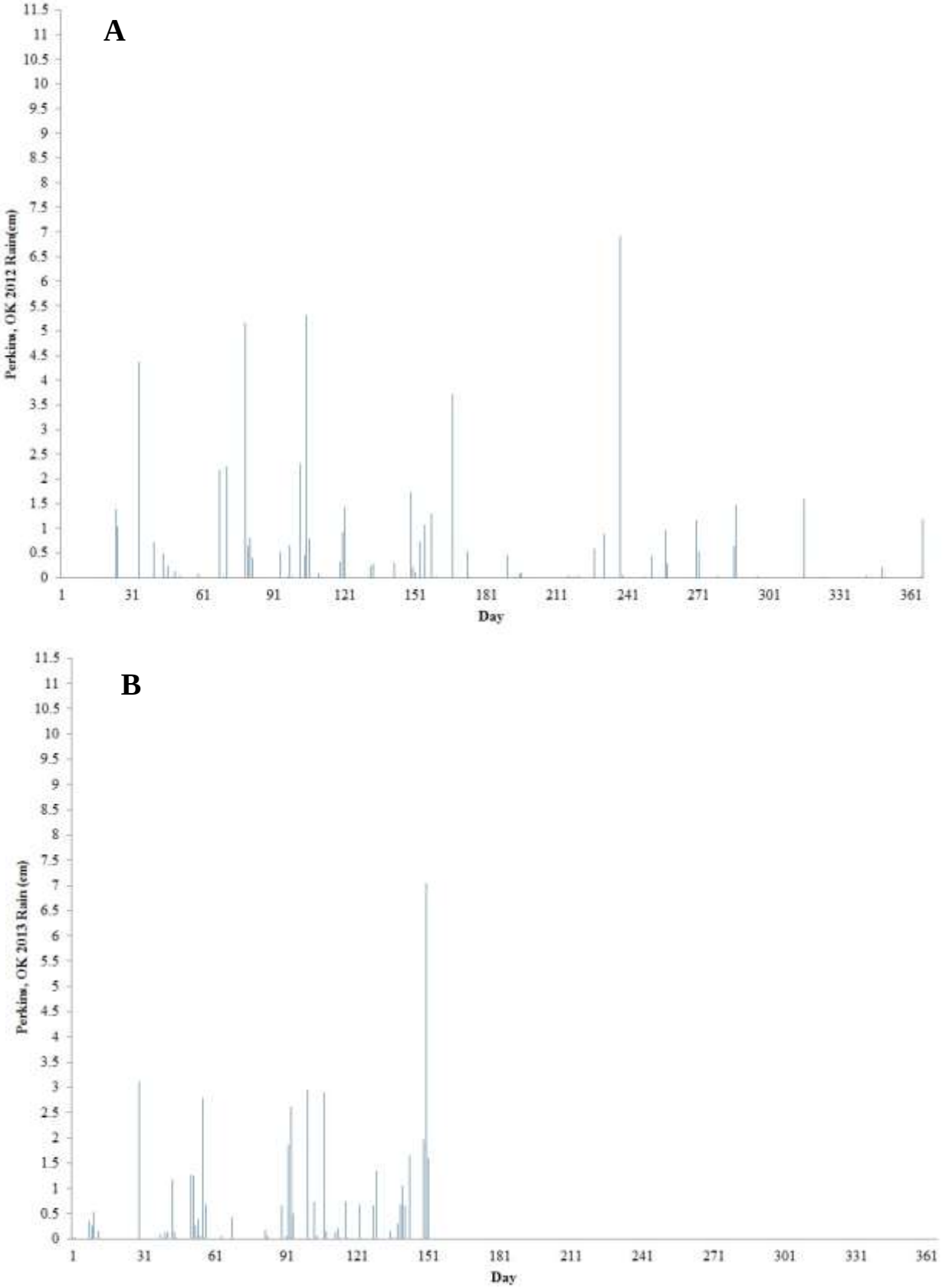
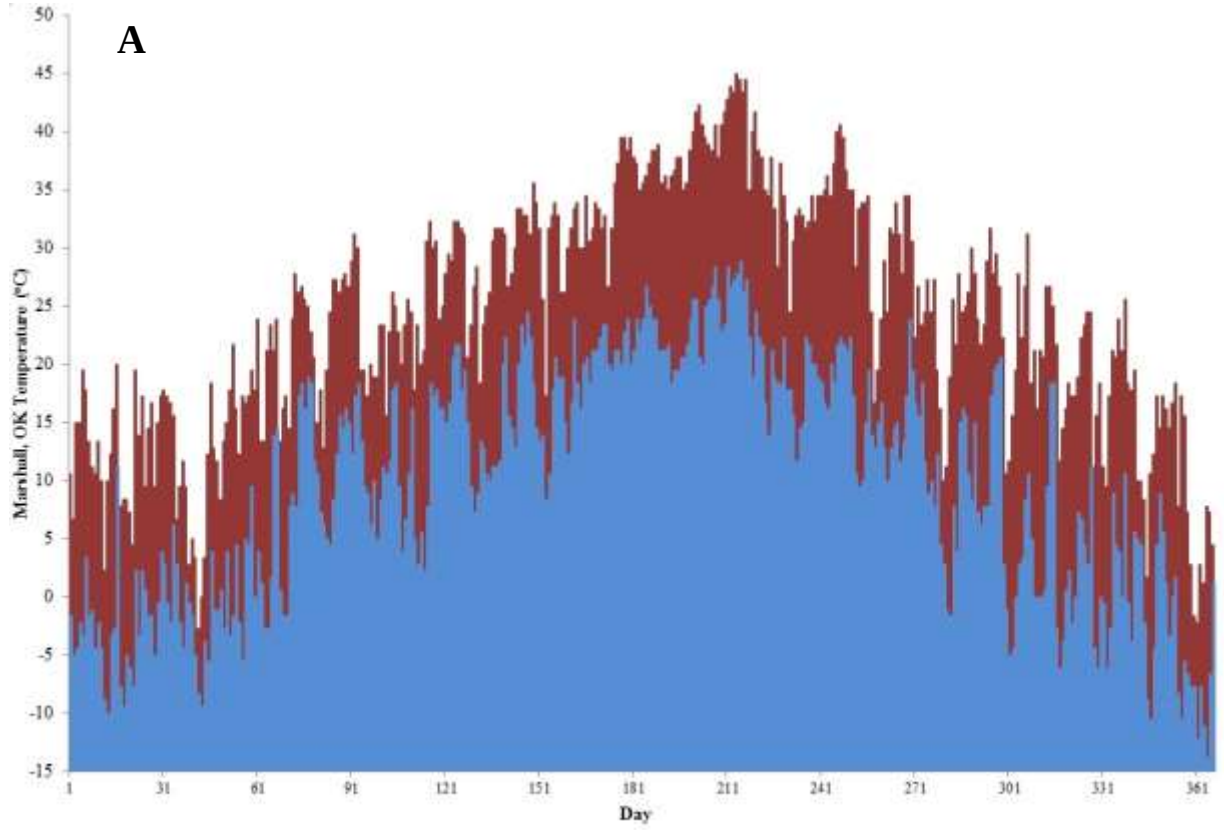


Figure 2.3 Rain in cm for Perkins, Oklahoma. Every 30 days is approximately a separate month. **2A** 2012. **2B** 2013 until May.



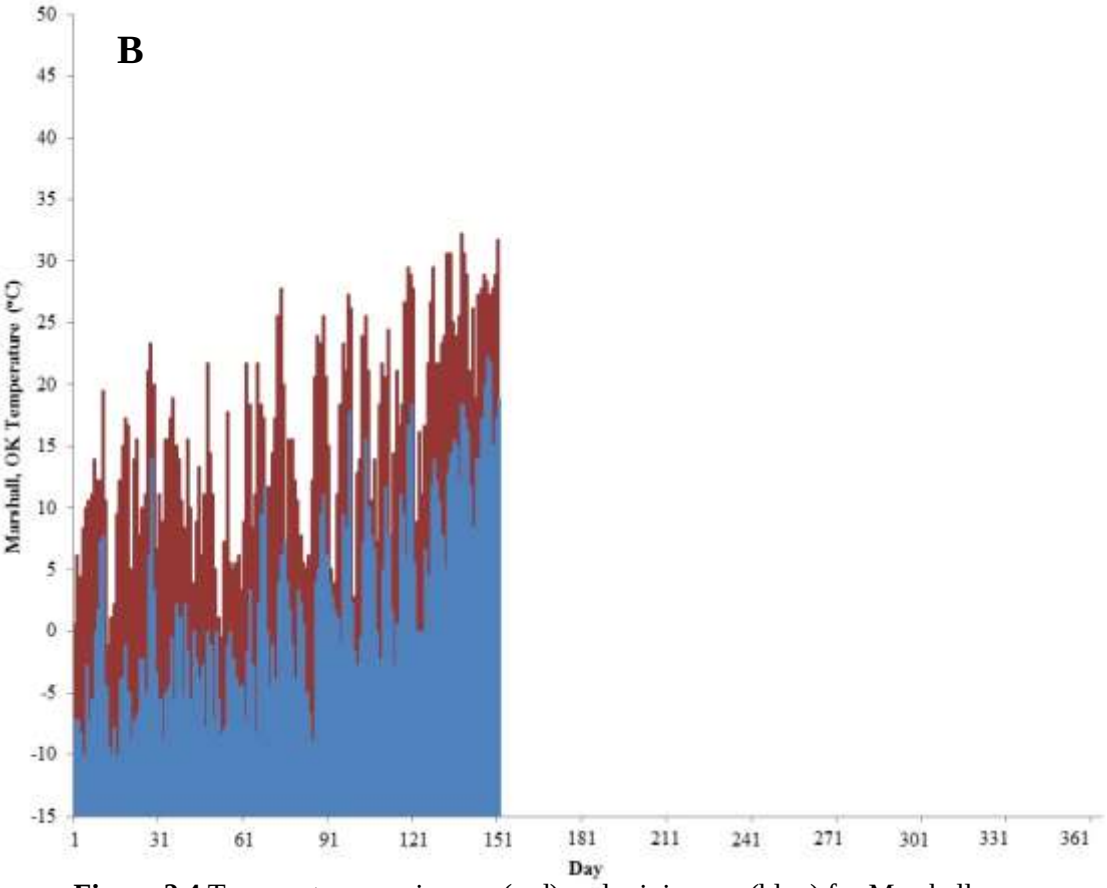


Figure 2.4 Temperature maximums (red) and minimums (blue) for Marshall, Oklahoma. Marshall, Oklahoma was the closest weather station to the Crescent field site. Every 30 days is approximately a separate month. **2A** 2012. **2B** 2013 until May.

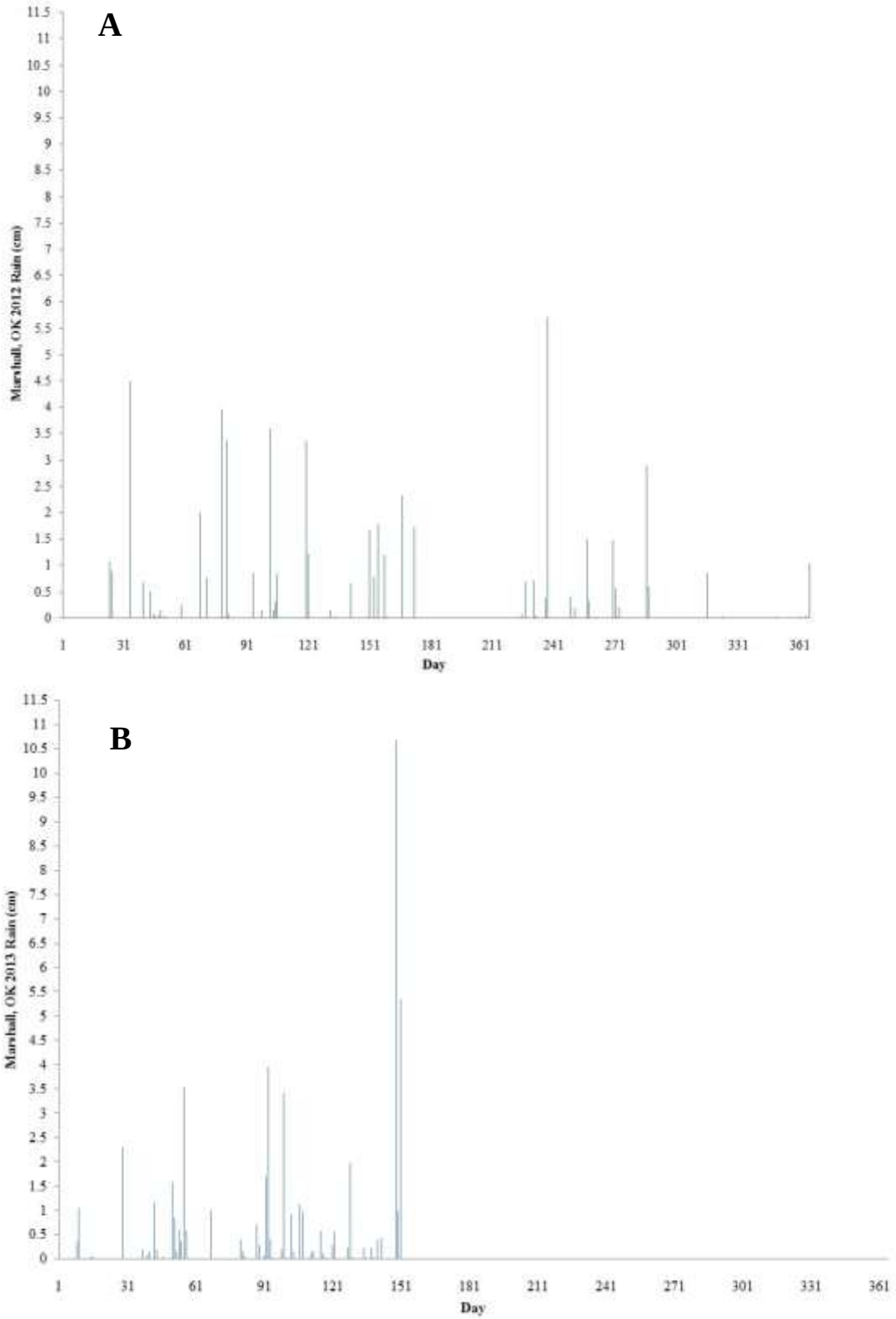


Figure 2.5 Rain in cm for Marshall, Oklahoma. Marshall, Oklahoma was the closest weather station to the Crescent field site. Every 30 days is approximately a separate month. **2A** 2012. **2B** 2013 until May.

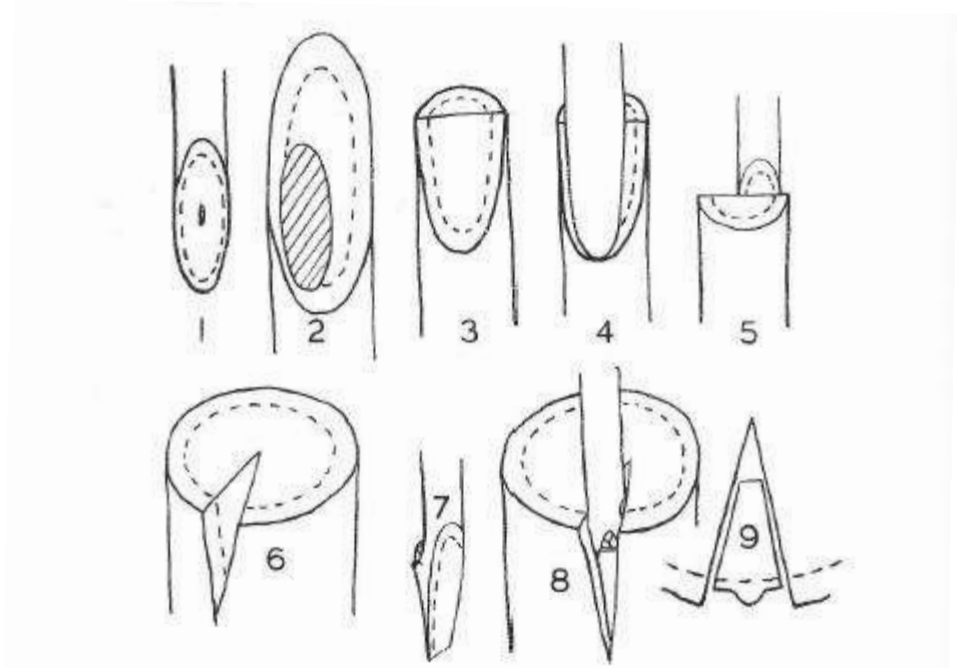


Fig. 4 Examples of cambial contact

1. Scion with thin rind. Cambium (dotted line) close to the outside of the rind. 2. Stock with thick rind. 3. Stock prepared to achieve good apical and basal contact with scion cambium. 4. Scion applied to stock. Note good contact at base and matching of inner rind (cambium) rather than outer rind (bark). 5. Good cambial contact at top of stock. 6. Large stock with thick rind prepared for this scion. 7. Scion with thin rind. 8. Stock and scion fitted. Note parts of stock rind outside the scion. 9. Cross-section. Note alignment of cambia and unmatched barks.

Figure 2.6 Illustration of alignment of scion to rootstock. Reprinted from *The Grafter's Handbook* copyright 2013 by R.J. Garner et. al. with permission by Chelsea Green Publishing (www.chelseagreen.com).

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CHAPTER III

CUTTING PROPAGATION

INTRODUCTION

Sand plum (*Prunus angustifolia* Marsh.) is a versatile species of many uses including windbreaks, wildlife and ornamental plantings and as a source of fruit (Row and Geyer, 2010; Okie, 1987). This shrub to small tree is native to the Southern United States and provides a potentially untapped market for Made-in-Oklahoma foods. While sand plums can be germinated by seed, this method will not produce true to type genotypes. Propagation by grafting and cuttings does produce clones, and in comparison to grafting, use of cuttings has the potential to be easier, cheaper, and more successful for sand plum. Many factors, including use of rooting hormones and seasonality of when cuttings are taken, can affect rooting percentage, root quality, and callusing of cuttings (Chauhan and Maheshwari, 1970; Couvillon, 1985; Sharma and Aier, 1989; Jawanda et al., 1991; Loreti and Morini, 2008; Exadaktyloua et al., 2009; Moreira et al., 2009; Sulusoglu and Cavusoglu, 2010; Hartmann et al., 2011).

Indolebutyric acid (IBA) is a synthetic auxin. Application of IBA stimulates the natural auxins in the plant which assist in root development. Too little hormone can decrease rooting and too much can result in tissue death. Loreti and Morini (2008) found that when using softwood peach (*Prunus persica* (L.) Stokes) cuttings, concentrations of IBA above 3,000 ppm led to death of tissue if alcohol was included in the IBA solution. The difficulty in rooting cuttings can be

in determining the amount of IBA necessary for sufficient rooting percentage and good quality cuttings. *Prunus* L. species and even cultivars show great variability for ideal IBA concentrations (Tsipouridis et al., 2003). For example, hardwood cuttings of peach 'Professor Black' rooted at 100% while cuttings of 'Early Coronet' rooted at 55% at the same IBA rates (Bartolini et al., 1979 as cited in Couvillon, 1985).

The amount of IBA necessary can be dependent on the type of cutting. Softwood cuttings generally require the least amount of IBA. Softwood cuttings lack woody tissue, are soft and flexible, and wilt easily. Semi-hardwood cuttings are not as flexible but are still relatively soft and have a partial woody consistency (Loreti and Morini, 2008). For semi-hardwood sub-terminal and terminal cuttings, 50 ppm IBA provided the best rooting (Chauhan and Maheshwari, 1970), while 100 ppm provided the best rooting for plum apical and basal cuttings (Jawanda et al., 1991). An experiment using cherry laurel (*Prunus laurocerasus* (L.)) semi-hard wood cuttings found that 2,000 ppm was the best treatment (Sulusoglu and Cavusoglu, 2010).

Hardwood cuttings are firm, possess woody tissue, and are not as limited by loss of moisture. Hardwood cuttings have similar recommendations as semi-hard wood cuttings. In an experiment using wild cherry (*Prunus avium* (L.) L.) 'Gisela' cuttings, the best rooting was 1,000 ppm IBA (Exadaktyloua et al.,2009). Loreti and Morini (2008) suggest IBA concentrations from 1,000 to 3,000 ppm for the rooting of hardwood peach cuttings. Rooting percentage may decrease with an increase in IBA concentration (Moreira et al., 2009). In Moreira et al. (2009) rooting percentage means were 28% at 7,000 ppm IBA compared to 70% at 2,500 ppm IBA. Rooting mean percentage may also increase with increasing IBA concentration. Rooting was lower at 1,000 ppm IBA than it was for 5,000 or 10,000 ppm IBA for peach cuttings (Couvillon et al., 1986).

Time of year when peach cuttings are taken can affect root formation (Loreti and Morini, 2008). Softwood and semi-hard wood plum and peach cuttings taken in summer (July-August) had good rooting (Couvillon et al., 1986; Sharma and Aier, 1989; Loreti and Morini, 2008). Other

experiments have found that winter collection is preferable (Chauhan and Maheshwari, 1970). Semi-hardwood cuttings for peach 'Sunred' and 'Fertilia' taken in January rooted the best (Bartolini and Briccoli-Bati, 1976, as cited in Couvillon, 1985). Hardwood cuttings typically respond better to winter collection. Hardwood cuttings of peach cut from October to January generally root better according to Loreti and Morini (2008).

Due to the great variation of *Prunus* cuttings in regards to IBA concentration and season of collection, the objectives of this experiment were to determine what concentration of IBA and which season is the most appropriate to initiate high rooting success of sand plum (*P. angustifolia).*

MATERIALS AND METHODS

Experiment 1

Cuttings were taken from a motte located near Crescent, Oklahoma. One to two leaves were present on each cutting. Cuttings varied in stem thickness and age. Cutting length ranged from approximately 5.5-8 cm. Seasonality treatments consisted of two different seasons, late summer (August 29- September 1, 2011) and fall (October 24-26, 2011). The IBA treatments consisted of 0, 100, 1,000, 2,000, 4,000, and 8,000 ppm. The IBA was prepared using Hortus IBA 20% water soluble salts (Hortus USA, New York City, NY). No alcohol was used for the preparation of the IBA solution. The IBA was prepared once for each season treatment and refrigerated between sticking cuttings. Three repetitions of 45 cuttings were taken per treatment combination.

Cuttings were held in IBA solution for 10 seconds at a depth of about 5 cm. Randomization was by treatment in styrofoam block cells (similar to those produced by Dubois Agrinovation, Saint-Remi, Canada). Media was a 50/50 perlite and vermiculite (Sun Gro Horticulture, Bellevue, WA) mix and was pre-wetted before cuttings were stuck. Cuttings were placed under mist for 42 days at 20 seconds of mist every four minutes. After 42 days, the cuttings were harvested. Rooting percentage and presence or absence of callusing was recorded.

Results were analyzed using SAS 9.3 (SAS Institute Inc., Cary, NJ) PROC GLIMMIX LSMEANS with a normal distribution.

Experiment 2

Cuttings were collected from three different locations, labeled A, B, and C. Location A was a motte located near the entry road to Lake McMurtry, Oklahoma and locations B and C were from separate mottes at the Oklahoma State University Range Research Station, Payne County, Oklahoma. Cutting length was approximately 8.9 cm and cuttings varied in stem thickness and age. Cuttings were taken from sections that were not current season's growth.

The IBA treatments consisted of 0, 100, 1,000, 3,000, and 7,000 ppm IBA prepared from Hortus IBA 20% water soluble salts and tap water. No alcohol was used for the preparation of the IBA solution. The IBA was prepared once for each season treatment and refrigerated at 3^{o} C between sticking cuttings. Season treatments consisted of cuttings collected in late spring (May 21-23, 2012), late summer (August 7-9, 2012) and late fall (October 16-17, 2012). One to two leaves were present on all cuttings except for the late fall collection.

Experiment consisted of five reps of 15 cuttings per treatment combination. If sticking of cutting lasted longer than one day, unprocessed cuttings were sprayed with water and placed into a refrigerator (International Cold Storage Inc, Wichita, KS) at 3^{o} C. Fifteen cuttings were dipped into IBA solution at an approximately equal depth and held in solution for 10 seconds. Cuttings were placed into pre-moistened 90 cell styrofoam trays that contained a 50/50 perlite and vermiculite mix. Cuttings were randomized by treatment. Mist settings were set for the mist to come on every four minutes and stay on for 20 seconds, except July 7-9, 2012 when a fuse shorted out. Cuttings were removed after 52 days. Survival, rooting percentage, callusing and root quality were recorded. Root quality was based upon a scale of quality factors (Fig. 3.1). A cutting received a quality rating of one if it had produced a root but the root had died. Quality ratings of two went to cuttings that had a singular root with no branching. Cutting quality ratings of three went to cuttings that had two or more roots present but no branching from roots. Cutting

quality ratings of four went to cuttings had had two or greater roots and branching of the roots. Cutting quality ratings of five went to cuttings that had five or greater roots present. Results were analyzed using SAS 9.3 (SAS Institute Inc., Cary, NJ) PROC GLIMMIX LSMEANS with a normal distribution.

RESULTS AND DICUSSION

Experiment 1

Season and IBA

With the summer root cuttings the 8,000 ppm IBA treatment produced significantly greater rooting percentages than all other IBA rates except for 4,000 ppm IBA. Couvillon et al. (1986) did not find any significant differences between IBA rates of 5,000 and 10,000 ppm. Both rates produced the highest rooting percentages for peach 'Harvester' (Couvillon et al., 1986). Concentration of IBA had a significant effect in summer, but it did not have a significant effect in fall, and therefore did not have a significant effect on rooting percentage (Table 3.1).

Season when cuttings were taken was shown to be significantly different ($P<0.005$) in regards to rooting percentage. Overall rooting was rare in the fall season. This is a stark contrast to the majority of the literature. Loreti and Morini (2008) demonstrated that hardwood peach cuttings cut from October to January generally root better. Chauhan and Maheshwari (1970) demonstrated this as well.

Despite the lack of rooting, cuttings were not dead. The majority of un-rooted cuttings were still alive at the conclusion of both treatments. Heavy callusing was evident and callusing means were greater than rooting percentages. Cuttings in late summer had significantly more callusing (73%) than cuttings in the fall (55%). Use of IBA induced more callusing than the control except at 100 ppm IBA (Fig 3.2). Callus formation can be a precursor to root formation. A possible reason for the low rooting in fall, despite the cuttings still being alive, was that the cuttings did not receive enough time for roots to develop.

Experiment 2

Root quality

The IBA treatment did not show a significant effect on root quality among rooted cuttings. This differs from several experiments in the literature. Couvillon et al. (1986) with 'Harvester' peach cuttings found higher quality with IBA rates of 5,000 and 10,000 ppm than with IBA rates of 1,000 and 0 ppm IBA. Plum cuttings with 3,000 ppm IBA produced the most roots per cutting in Sharma and Aier (1989). The lack of response in this experiment could be due to the absence of a wounding treatment. Couvillon et al. (1986) found that with hardwood peach cuttings a wounding treatment greatly improved root quality, and also led to an increase in rooting percentage. Another factor could be the low rooting in certain treatments. Neither season nor the interaction of IBA and season produced significant results on root quality.

Rooting percentage

Indole-3-butyric acid as a main factor was significant while season as a main factor was not significant. The interaction of the two was significant (Table 3.3). For the spring season, 3,000 and 7,000 ppm IBA produced significantly highest rooting. This was not seen in the summer season, which exhibited a sharp drop in rooting after 7,000 ppm IBA. For the fall season, low rooting was seen for all IBA concentrations.

Generally for *Prunus* species 1,000 ppm to 3,000 ppm IBA are satisfactory for rooting cuttings (Chauhan and Maheshwari, 1970; Exadaktyloua et al., 2009; Sulusoglu and Cavusoglu, 2010). Moriera et al. (2009) even showed a decline in rooting at higher IBA levels for azorean cherry (*Prunus azorica* (hort. ex Mouill.) Rivas Mart., Lousã, Fern. Prieto, E. Dias, J.C. Costa & C. Aguiar). This is not indicative of all experiments. Couvillon et al. (1986) had rooting percentages for hardwood peach cuttings that were not significantly different at 5,000 or 10,000 ppm IBA, but were significantly greater than IBA rates of 0, 1,000, and 15,000 (though one cultivar, 'Redhaven', showed 80% rooting at 15,000 ppm and 50% rooting at 1,000 ppm).

It could be considered contrary that higher concentrations of IBA result in greater 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, the plant produced auxin, decreases as root primordia form (Blakesley, 1993). The hormones IAA and IBA are not persistent in plants. Internal levels of applied IAA decreased from 558 to 32.1 ng/g and IBA levels decreased from 147.8 to 20.5 ng/g after two days in 'Gisela' cherry (Stefancic et al., 2005). Blakesley (1993) suggests that higher levels of auxin are more important in inducing root induction, but once root primordia form lower levels enable better root growth. This is necessary for if the 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 induction of rooting. This could be indicative of why the higher IBA concentrations had better rooting in the spring and summer seasons for the higher IBA rates.

A variety of months and seasons have been shown to be preferable for rooting *Prunus* cuttings. Why winter and fall months produced preferential rooting was not described in those experiments, however one possible reason was suggested by a cutting experiment using douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). Roberts and Fuchigami (2006) took cuttings every month except for April and May for three years. The experiment found that cuttings taken in October and September had low rooting results. Supplemental auxin was used in the experiment and did not increase rooting during those specific months. The experiment found a correlation between low rooting and higher bud dormancy in douglas fir. This effect is not limited to douglas fir. Wavy leaved fig tree (*Ficus infectoria* Wild.) cuttings were reported to have higher rooting when cambial activity was greater, but not when cambial activity was low, which follows auxin levels (Anand and Heberlein, 1975). Dormancy and low internal auxin levels could have decreased rooting of sand plum in this experiment for the fall treatment.

The cuttings in this experiment in the fall treatment were not necessarily dead however. Callusing in experiment 2 was not significant for the main effects by themselves, but for the interaction of season and IBA it was significant. The greatest callusing was seen in summer 3,000 and 1,000 ppm IBA and the least callusing in spring 3,000 and 7,000 ppm IBA (Table 3.4).

The elevated presence of callusing could be a possible explanation for the low success rates in certain seasons. Callusing is an avenue for the formation of adventitious root primorida but it could also delay or cease root formation (Stefancic et al., 2005). Though this value could not be quantified, it was noted in the experiment that higher concentrations of IBA had greater callusing per cutting. Excess IBA with thick callusing could have contributed to slow root growth during the summer and fall seasons. The literature shows mixed results on this subject. For *P. avium* 'Gisela', concentrations of IBA above 2,000 ppm decreased callusing (Exadaktylou et al., 2009). 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 cells were very compact and the roots could not break through (Cormack, 1965). Considering that visually the cuttings with higher IBA concentrations had heavier callusing, but also better rooting percentages in spring and for the 7,000 ppm IBA concentration in summer, it could be concluded that heavy callusing did not impede the development of roots in the spring season.

Experiment 1 vs. Experiment 2

For both experiments, response to higher IBA treatments was similar, as were responses to season treatments. Fall cutting collection was shown to not be as beneficial as cutting collection in the spring or summer. Rooting percentages were higher in experiment 2 than experiment 1. Two main changes were made to experiment 2 as a result of experiment 1. The

length of time the cuttings were allowed to root was extended from 42 days to 52 days and the length of the cuttings was approximately doubled.

Tsipouris et al. (2003) demonstrated the effect of cutting length on rooting percent. Cuttings of peach at 10 cm had only 46% rooting. Cuttings at 20 cm had 74% rooting, but cuttings at 25 cm had decreased rooting (52%), so longer cuttings were not necessarily better. Longer cuttings may be better due to more possible carbohydrate content, though Bartolini et al. (2000) reported that cuttings with higher carbohydrate levels did not necessarily root better than cuttings with lower carbohydrate levels. Exadaktylou et al. (2009) also found no correlation between starch levels and rooting percentage. This may indicate that cuttings with thicker stems are not necessarily better either. Cuttings that had a thickness of 6-8 mm compared to cuttings that had a thickness of 9-11 mm were significantly different at rates of 1000 ppm IBA by as much as 23 %, but were not significantly different at IBA concentrations of 2000, 4000 and 6000 ppm. Cuttings with diameters 12-14 mm did not root at all in Exadaktylou et al. (2009)'s experiment. Cutting diameters in experiments 1 and 2 ranged from 4-10 mm but diameters were usually 6 mm with some exceptions.

Another factor contributing to the success of experiment 2 was the additional 12 days. Cuttings typically have a lag phase between the first anatomical event and the cutting being removed from the source plant (Lovell and White, 1986). In the first experiment, the absolute minimum amount of days required for rooting found in the literature review was used. For cuttings, several other experiments had periods as long as 62 days for successful rooting, and for some treatments of hardwood cuttings 200 days were used (Sharma and Aier, 1989).

CONCLUSION

With such great variance within IBA concentrations and seasonality of when cuttings are taken between all *Prunus* species, it suggests that there could be another major factor not accounted for that affects cutting rooting success. This possible factor could be juvenility, which

was not tested in this experiment due to the lack of material. The interaction of IBA and season was shown to have significant effects on rooting percentage in this experiment. Recommendations for sand plum cuttings are to use IBA concentrations that are at least as high as 7,000 ppm.

TABLES AND FIGURES

Table 3.1 Percent rooting means for sand plum (*Prunus angustifolia*) experiment 1.

Season	IBA (ppm)[z]	Mean[y]
Summer	0	1.11 bc[x]
	100	1.11 bc
	1000	0.00 c
	2000	0.00 c
	4000	7.22 ab
	8000	12.22 a
Fall	0	0.00 c
	100	0.00 c
	1000	0.00 c
	2000	0.00 c
	4000	0.00 c
	8000	1.11 bc
IBA		ns[w]
Season		**
IBA*Season		*

[z] Cuttings dipped in Hortus 20% water soluble salts for 10 seconds.
[y] Percent mean (n=45) separated by time of cutting and IBA treatment
[x] Means with different letters are significantly different from each other at $P<0.05$.
[w] ns stands for not significant, * stands for P<0.05, ** stands for P<0.01 and *** stands for P<0.001.

Table 3.2 Callusing means by season and IBA for experiment 1.

Season	IBA (ppm)[z]	Mean[y]
Summer	0	39.44 e[x]
	100	61.67 bdc
	1000	81.11 ba
	2000	93.89 a
	4000	85.56 a
	8000	76.67 bac
Fall		
	0	46.11 ed
	100	44.44 ed
	1000	48.89 ed
	2000	57.78 edc
	4000	56.11 edc
	8000	80.56 ba
IBA		***[w]
Season		***
IBA*Season		*

[z] Cuttings dipped in Hortus 20% water soluble salts for 10 seconds.

[y] Percent mean (n=45) separated by time of cutting and IBA treatment

[x] Means with difference in letters indicates significant differences for interaction at P<0.05.

[w] ns stands for not significant, * stands for P<0.05, ** stands for P<0.01 and *** stands for P<0.001

Table 3.3 Percent rooting means for experiment 2. Sand plum (*Prunus angustifolia*) cuttings separated by season and IBA treatment.

Season	IBA (ppm)[z]				
	0	100	1000	3000	7000
Spring	2.22 de	6.22 de[y]	19.11 c	35.11 b	44.44 a
Summer	0.44 e	1.33 de	5.77 de	4.89 de	48.89 a
Fall	0.00 e	0.00 e	0.00 e	1.33 de	2.67 de
IBA		***[x]			
Season		n.s.			
IBA*Season		***			

[z] Cuttings dipped in Hortus 20% water soluble salts for 10 seconds.
[y] Percent mean of five repetitions of 15 cuttings each. Means with difference in letters indicates significant differences between interaction of season and IBA at $P<0.05$.
[x] ns stands for not significant, * stands for P<0.05, ** stands for P<0.01 and *** stands for P<0.001

Table 3.4 Callusing means by season and IBA concentration for sand plum (*Prunus angustifolia*) in experiment 2.

Season	IBA (ppm)[z]				
	0	100	1000	3000	7000
Spring	32.44 abc	29.78 bc[y]	26.67 d	20.00 d	14.22 d
Summer	49.33 ab	50.67 ab	56.44 a	57.33 a	32.44 bc
Fall	27.11 d	38.67 abc	35.11 abc	35.11 abc	42.20 abc
IBA			ns[x]		
Season			ns		
IBA*Season			**		

[z] Cuttings dipped in Hortus 20% water soluble salts for 10 seconds.
[y] Percent mean of five repetitions of 15 cuttings each. Means with difference in letters indicates significant differences between interaction of season and IBA at $P<0.05$.
[x] ns stands for not significant, * stands for P<0.05, ** stands for P<0.01 and *** stands for P<0.001

Figure 3.1 Quality designations of rooting. Cuttings were treated with IBA ranges 0 to7,000 ppm and gathered in spring, summer, and winter from three different locations.

1	Root dead. No branching on root, one root present and less than 7.6 cm in length
2	Root singular, no branching on roots, one root present
3	Root singular, long and mostly white color. No branching on roots. Two or more roots present.
4	Root branching present. Two or greater number of roots and mostly white color
5	Five or greater roots present. Roots mostly white color. Branching may be present or absent.

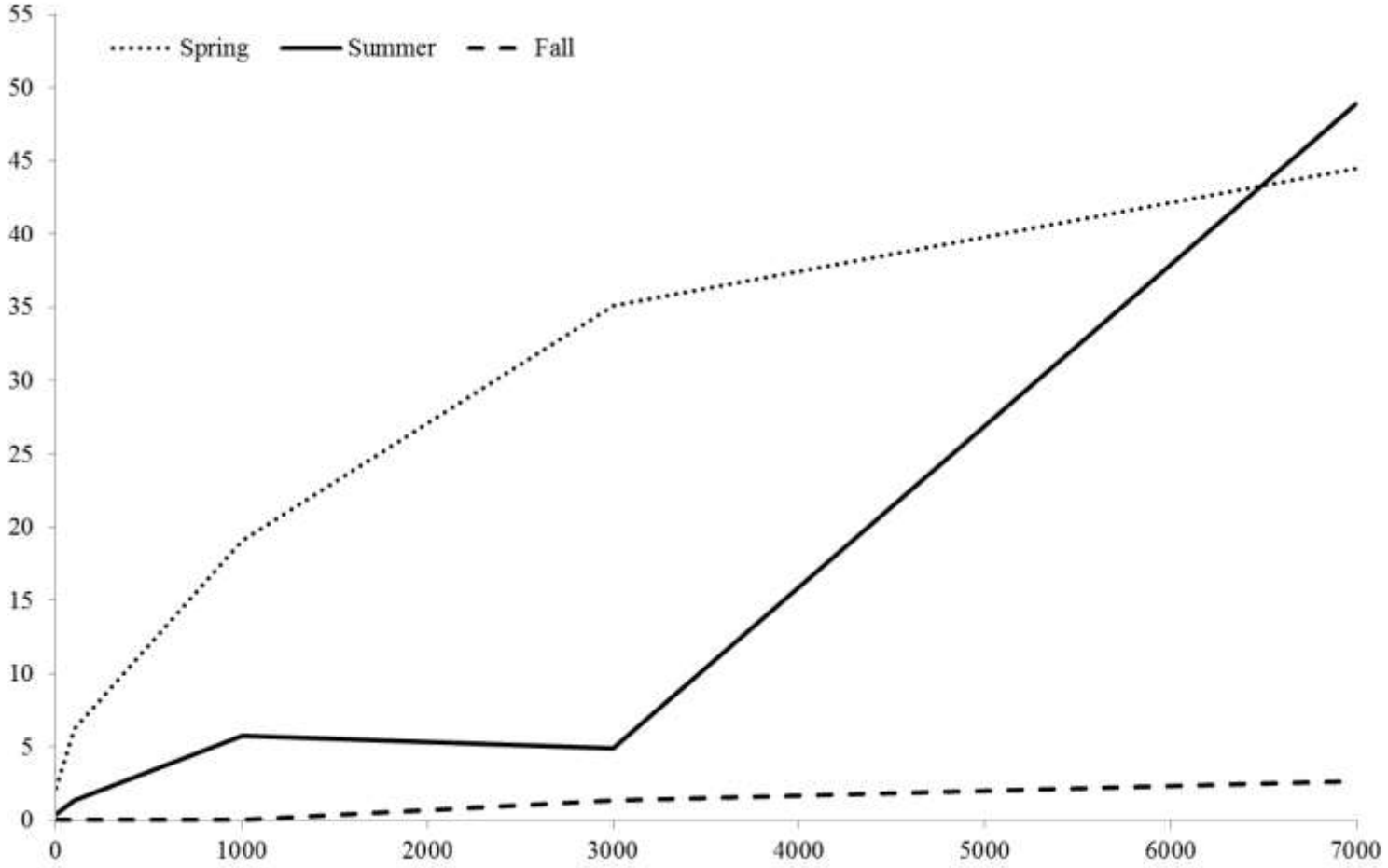

Figure 3.2 Rooting percent means for three seasons over IBA ranges for sand plum (*Prunus angustifolia*) in experiment 2.

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CHAPTER IV

SEED PROPAGATION

INTRODUCTION

Use of seeds in propagation can provide a method for replicating a large number of specimens in a short amount of time. Sand plum (*Prunus angustifolia* Marsh.), a native shrub to small tree, can produce profuse amounts of fruit, depending on lateness of frost and plum curculio (*Conotrachelus nenuphar* Herbst.) damage. Though generation of new trees inside the motte is primarily by suckering, seeds can spread and create new mottes. Mottes serve as a fruit source for wildlife and humans, as well as cover for native wildlife species such as bobwhite quail (USDA NRCS, 2011a; USDA NRCS, 2011b). Like many *Prunus* L. species, pretreatment of seed is generally needed in order for germination to occur. Stratification, one type of pretreatment, is the exposure of seeds to set temperatures in an aerated and moist medium (Hartmann et al., 2011).

Stratification provides the chance for seeds to imbibe moisture, which promotes cell expansion and growth. Requirements for cold stratification periods are species specific in the *Prunus* genera. For some *Prunus* species such as wild cherry (*Prunus avium* (L.) L.), cold stratification is essential for germination to occur (Finch-Savage et al., 2002). Black cherry (*Prunus serotina* Ehrh.) had 0% germination without a cold stratification treatment (Esen et al., 2009). Other *Prunus* species may not need a stratification period. Plum (*Prunus domestica* L.) cultivars had higher than 50% germination despite no cold treatment (Hjeltnes and Nornes, 2007).

Red stinkwood (*Prunus africana* (Hook.f.) Kalkman) had at least 75% germination without a cold stratification treatment (Sacande et al., 2004).

An addition of a warm stratification treatment to a cold stratification treatment may substantially improve germination. Warm moist stratification can occur before or after the cold stratification period and exposes seeds to a range of temperatures (15^{o}C-25^{o}C). Seeds may require warm stratification because they are immature or underdeveloped (Finch-Savage et al., 2002). This treatment allows the seed embryos to grow to the necessary size. In an experiment by Chen et al., (2007) on bellflower cherry (*Prunus campanulata* Maxim.), germination was significantly increased from 70% (8 weeks cold stratification) to 99% with the addition of a four week warm treatment. In Esen et al., (2009) a 90 day cold stratification treatment resulted in 39% germination in black cherry. With 120 days cold stratification, the highest germination was only 39%, however with the addition of a 20 day warm stratification period to the 90 day cold stratification period, germination increased to 91%. With fluctuating warm and cold temperatures wild cherry germination was greater than 97%, despite the seed being over 15 years old (Bujarska-Borkowska and Chmielarz, 2010). A warm stratification pretreatment for this species before cold stratification improved seed germination from 25% to 85% (Finch-Savage et al., 2002).

The seed of *Prunus* species is restricted by a thick endocarp. This can decrease radicle emergence as well as leach germination inhibitors (Mehanna and Martin, 1985). Mechanical scarification can reduce this problem. Damaging or removing the endocarp can provide significant increases in germination and decreases in the time required for germination to occur. For example, in an experiment where peach endocarps had been removed, Martinez-Gomez and Dicenta (2001) found germination as early as one week. Seeds with endocarps present germinated in two weeks. In Chen et al. (2007) experiment with bellflower cherry, seeds with their endocarps removed had 25% germination within 21 days. Germination was not observed during the 21 day time period in the seeds that retained their endocarp. For almond (*Prunus*

dulcis (Mill.) D.A. Webb), 50% germination occurred in seeds with endocarps removed as early as four weeks, while seeds with endocarps had germination above 50% beginning week six (Garcia-Gusano et al., 2004). Mehanna and Martin (1985) tested a variety of treatments with peach cultivars 'Nemaguard' and 'Halford'. With both the endocarp and seed coats removed, 100% germination was seen with 'Nemaguard' and 90% germination with 'Halford' after only 15 days. If the seed coat remained attached, 'Nemaguard' and 'Halford' had only 20% and 0% germination, respectively. This experiment also tested puncturing the seed coat and found that germination did not increase above 37% for both cultivars. Removing a section of the seed coat increased germination to at least 85%.

The Knox City Texas NRCS Plant Materials Center holds sand plum seeds in cold stratification for 60 days and achieves 60% germination by this method (Esquivel, 2001). Conversely, the United States Forestry Service recommends for various *Prunus* wild-types to use a 30 day heat treatment and 30 to 45 day cold stratification period before planting (Huffman, 1996). Scarification has been suggested to increase the speed of germination. Based on previous findings, it is purposed that if a lesser period of cold stratification could be used in combination with a mechanical treatment, germination could be increased and new trees could be grown quicker. However, there are not any published protocols in the literature for sand plum. With an increase in desire to know more about this species for commercial purposes, the objectives of this research were to determine what stratification period provides the best germination and to determine if scarification will shorten the germination time of sand plum.

MATERIALS AND METHODS

Experiment 1

Experiment 1 was conducted on August 5, 2011 and consisted of five seeds per treatment. Seeds were obtained from native sand plum populations in Crescent, Oklahoma. Pretreatments consisted of seeds collected in 2011 and dried at room temperature, seeds heated to a boil then dried at 21^{o}C-38^{o}C, seeds dried to temperatures of 21^{o}C-38^{o}C without pulp being removed, and

seeds extracted from frozen fruit collected in 2010. A tetrazolium test using 0.5 g triphenyltetrazolium chloride (TTC) in 500 ml water confirmed the majority of seeds were still viable. Seed treatments consisted of soaking seeds for 0, 10, 20, or 40 minutes in sulfuric acid. Seeds were then gently washed under running water for less than a minute and placed in quart size Ziploc plastic bags filled half way with moist Metro-Mix 702 (Sun Gro Horticulture, Bellevue, WA). For each treatment, bags were exposed to either 30 days or 60 days cold stratification treatments in a cooler (Powers INC., Warminister, PA) at 10^{o}C. Freezing was not considered a stratification treatment for the seeds extracted from the frozen fruit due to the presence of the exocarp. After cold stratification, seeds were planted in 13.3 x 10.5 x 5.3 cm jiffy trays and checked once a week for germination.

Experiment 2

Fruit was collected from one native sand plum motte at Lake McMurtry, Oklahoma in June 2012. Experiment took place June 12-14, 2012. Mechanical treatment consisted of clipping the end of the seed and removing approximately 1 cm of the endocarp using a nail clipper, thus exposing the inner seed (Fig. 4.3). Control consisted of no mechanical or chemical damage. Seeds were cleaned by hand de-pulping to ensure no prior mechanical damage. Seeds at this time were tested using a float test and all seeds that floated were removed from the experiment. Stratification periods were 0, 30 and 60 days cold stratification. Seeds were planted 2.54 cm deep in 90 cell trays containing Redi-earth plug and seeding mix (Sun Gro Horticulture, Bellevue, WA). Trays were placed into a refrigerator (International Cold Storage INC., Witchita, KS) at 3^{o}C, and were covered with plastic bags to retain moisture. Trays were checked weekly and were removed after stratification period. Germination was recorded weekly after trays had been removed. The experiment was arranged in split plot design with mechanical treatment as the main plot and month as the split plot. There were three replications of each trial with each replication having 50 seeds each. Data were analyzed using SAS 9.3 (SAS Institute INC., Cary, NJ) PROC MIXED LSMEANS and PROC MEANS.

RESULTS AND DISCUSSION

Experiment 1

Germination was absent or low within treatments except for the 0 minute sulfuric acid 60 days cold stratification treatment (2011 dried seeds). Only one out of five seeds germinated during the 30 days stratification treatment. Four out of the five seeds germinated in the 0 minute sulfuric acid 60 days cold stratification treatment (2011 dried seed). Only one out of the five seeds planted in the 60 days 20 minute sulfuric acid treatment germinated (2011 dried seeds). The acid scarification did not promote germination of sand plum seeds in this experiment. Although the results of this trial experiment were too low to be analyzed for significance, only one seed out of 60 germinated in the acid 60 days cold stratification treatments. Four out of five germinated in the 60 days cold stratification treatment without acid scarification (2011 dried seed). Acid scarification does not promote germination of other *Prunus* species either. Phyartl et al. (2009) had black cherry seed with zero percent germination after acid scarification using 65% sulfuric acid and a 120 day cold stratification treatment. The control which was the same except for the lack of acid scarification had 34% germination (Phartyal et al., 2009). Ghayyad et al., (2010) soaked mahaleb cherry (*Prunus mahaleb* (L.)) seeds in sulfuric acid without a stratification treatment and none of the seeds germinated. However, seeds treated with sulfuric acid and a stratification treatment did germinate. Wild Himalayan cherry (*Prunus cerasoides* (D.Don.)) seeds showed no germination after a hot water and sulfuric acid treatment (Tewari et al., 2011). Phartyal et al. (2009) indicated that seeds rotted after acid scarification treatment. Rotting of seed was observed in this trial experiment as well.

Pretreatment of seeds was shown to have an effect on sand plum seed germination. Seed that was adversely treated (frozen, excessively heated or fermented) had less germination than seed that was dried and kept in cool temperatures. Wild cherry seeds can tolerate freezing, but it is suggested that they be dried first (Chmielarz, 2009). Seeds in this experiment were frozen with the fruit still attached, so no drying of the seed occurred. Grisez et al. (2008) indicated that

treatments that decrease germination include fermenting or holding the seeds at warm temperatures for an extended period of time (Grisez et al., 2008). This trial experiment confirmed those results for sand plum. Thus with germination mostly absent with acid treatment, acid scarification was not pursued in the second experiment.

Experiment 2

Germination for 30 days cold stratified seeds began 30 days after removal from cold treatment. Germination was never higher than 0.7% for the 30 days cold treatment. Instances of germination in the 30 days cold stratification treatment came from seeds that had been scarified. Scarified seeds did not have significantly greater germination than unscarified seeds for the 30 days treatment. Germination for 60 days cold stratified seeds began approximately three weeks after removal from cold treatment. Peak germination was reached after five weeks (Fig. 4.1). Germination was significantly greater for the 60 days cold stratification treatment (P <0.01) when compared to the 0 and 30 day treatments (Fig. 4.2, Table 4.1). Unscarified seeds showed the highest germination at 31% with 60 days cold stratification (Table 4.1). The interaction between month and mechanical treatments was not found to be significant (Table 4.1). Germination was lower than expected for the 60 days treatment. Explanations for the low germination include the possibility that sand plum seeds may require a warm stratification treatment in addition to a cold stratification treatment. Many *Prunus* species including black cherry, bellflower cherry and wild cherry require a warm stratification treatment (Finch-Savage et al., 2002; Chen et al., 2007; Esen et al., 2009) and it is not unlikely that sand plum would as well.

Another possibility is that the cold stratification treatment was not long enough. American plum (*Prunus americana* Marsh.), a species that is closer phylogenetically to sand plum (*P. angustifolia)* than any of the previously mentioned species, is recommended to be held in cold stratification for at least 160 days for good germination (Morrison, 2007). In an experiment by Giersbach and Crocker (1932) using the same species, 54% germination only occurred after five months at 10^{o}C. The highest germination at four months was only 4%.

Germination continued to increase as months increased, reaching its peak at seven months (Giersbach and Crocker, 1932).

Damaging the endocarp did not provide a significant response in increasing germination percentage or rate. A possible explanation is that not enough of the seed endocarp was removed. In Mehanna and Martin (1985) experiment using peach seeds, the endocarp and seed coat were fully removed in order to achieve high germination rates after only 15 days. Garcia-Gusano et al. (2004) and Chen et al. (2007) removed the endocarp in their experiments as well. In this experiment it was thought that partial removal of the endocarp would be sufficient to trigger increased germination percentage and rate. However, because most of the seed endocarp remained, any inhibitor hormones remained as well. Yet removal of the endocarp may not be enough to trigger germination. Chen et al. (2007) examined this with the addition or absence of gibberellic acid (GA) or abscisic acid (ABA) to seeds of bellflower cherry. In their experiment, with the removal of the endocarp and no stratification period the highest germination was only 21%. This contradicts Mehanna and Martin (1985), who obtained at least 90% germination with removal of the seed endocarp. When Chen et al. (2007) tried a warm and cold stratification period, the highest germination rate reported was 98% and occurred after 16 weeks. Stratification appears to have an effect, even more so than the application of GA; however, 52% germination occurred after six days with the application of 50 mM fluridone without cold stratification. Fluridone inhibits ABA synthesis (Yoshioka, 1998). Bellflower cherry seed coats contain a high concentration of ABA. If removing the source of ABA was the only barrier, the seeds without the endocarp should have had higher germination and additional chemicals should not have been necessary to increase germination. Abscisic acid and GA have similar chemical steps for the terpenoid pathway in plant synthesis (Crozier et al., 2000). As a result, ABA is gradually replaced with GA under stratification treatments (Chen et al., 2007). Without this replacement, the presence or absence of ABA in the seed matters little. However, Mehanna and Martin (1985) did not add any additional GA or other chemicals, and only removed the seed coat and endocarp

and still obtained significantly high germination at 100% and 90% for ‘Nemaguard’ and ‘Halford’, respectively. The only conclusion with these conflicting results is that the hormonal effect is species specific.

The fact that a decrease in germination rate was not obtained by this experiment may not be detrimental. Quickly generating trees from seeds by removing the endocarp or other methods may not produce high quality trees. Hartmann et al., (2011) details two examples that show that although germination may be obtained earlier, the quality of the resultant plant may not be desirable. Martinez-Gomez and Dicenta (2001) found a decrease in growth in seedlings that germinated early. These included peach seeds that retained their endocarp, but germinated within two weeks (Martinez-Gomez and Diecenta, 2001). Longer periods of stratification are not necessarily better either. In Martinez-Gomez and Dicenta (2001), longer periods of stratification decreased later growth at rates similar to that of the seeds that had germinated quickly. Longer stratification times may also increase seed rot (Giersbach and Crocker, 1932).

Other decreases in germination can be accredited to insect damage or seed size. Insect damage was observed to be common for seeds, though damaged seed was not used in the experiment. Most insect damage was caused by the plum curculio (Fig. 4.3B). Esen et al. (2007) thought seed size may be a factor contributing to the variances between seed viability and germination in their experiment. The authors noted that the seeds of black cherry that were of American origin were bigger than seeds of other origins. Bigger seeds might have had more endosperm stored, which might give them more energy for germination, which would result in greater germination. Due to the previous two years drought in Oklahoma, the sand plum seeds may not have filled enough, resulting in weak seed and poor viability. Germination viability of seed can be decreased if seeds are held at warm temperatures for long periods of time without moisture (Grisez et al., 2008). Fenner and Thompson (2005) state that viability is prolonged in seeds if stored under cool dry conditions. They also mention that even in these conditions, seeds will still lose viability over time because of natural aging. Why seeds lose viability is not known

though there are contributing factors that likely include loss of moisture, lowering of lipid content, deterioration of seed coat and genetic problems (chromosome aberrations)(Fenner and Thompson, 2005).

CONCLUSION

For *Prunus* species in general, low germination could almost be considered the norm. In an experiment by Takos and Efthimiou (2003) they found that *Prunus spinosa* L. germinated very poorly but still had high viability percentages. Esen et al. (2007) had seeds from a variety of worldwide locations and elevations. All seed tested with 100% viability but germination from United States seed sources ranged from 7.8% to 90.5% for a 20 day warm, 90 day cold period. The best recommendation is to use the length of cold period from where the seed was collected as the time needed for stratification, in order to obtain the highest natural germination if seed germination methods are unknown. Further questions include if a warm and cold stratification treatment, longer stratification periods, complete removal of endocarps, and if use of hormones would have beneficial effects upon the germination of sand plums. Recommendations for growers at this present time include stratifying seeds for at least 60 days before planting and leaving the endocarp undamaged.

Table 4.1 Comparison of mechanical scarification treatments and varying stratification times as percent means for sand plum (*Prunus angustifolia).*

Treatment	Stratification (days)[z]		
	0	30	60
Unscarified	0 Ab[y]	0 Ab	31.3 Aa
Scarified	0 Ab	1.33 Ab	25.3 Aa
Scarification*Stratification	ns[x]	ns	ns

[z] At 3°C

[y] Differences in letters are significant by $P < 0.05$ using an LSMEANS test. Uppercase letters indicate significant differences by rows. Lowercase letters indicate significant differences by columns.

[x] ns stands for not significant.

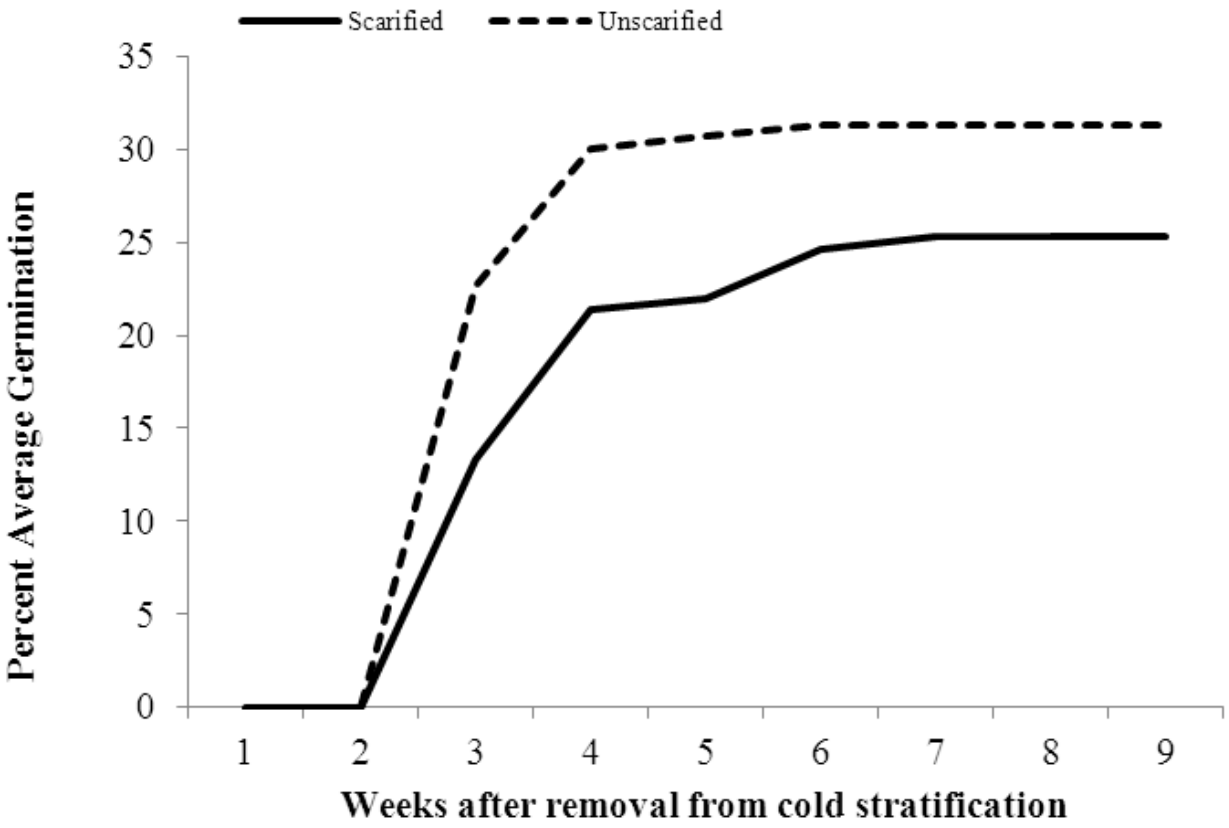

Figure 4.1 Germination rates of sand plum (*Prunus angustifolia)* scarified and unscarified seed after removal from 60 days cold stratification.

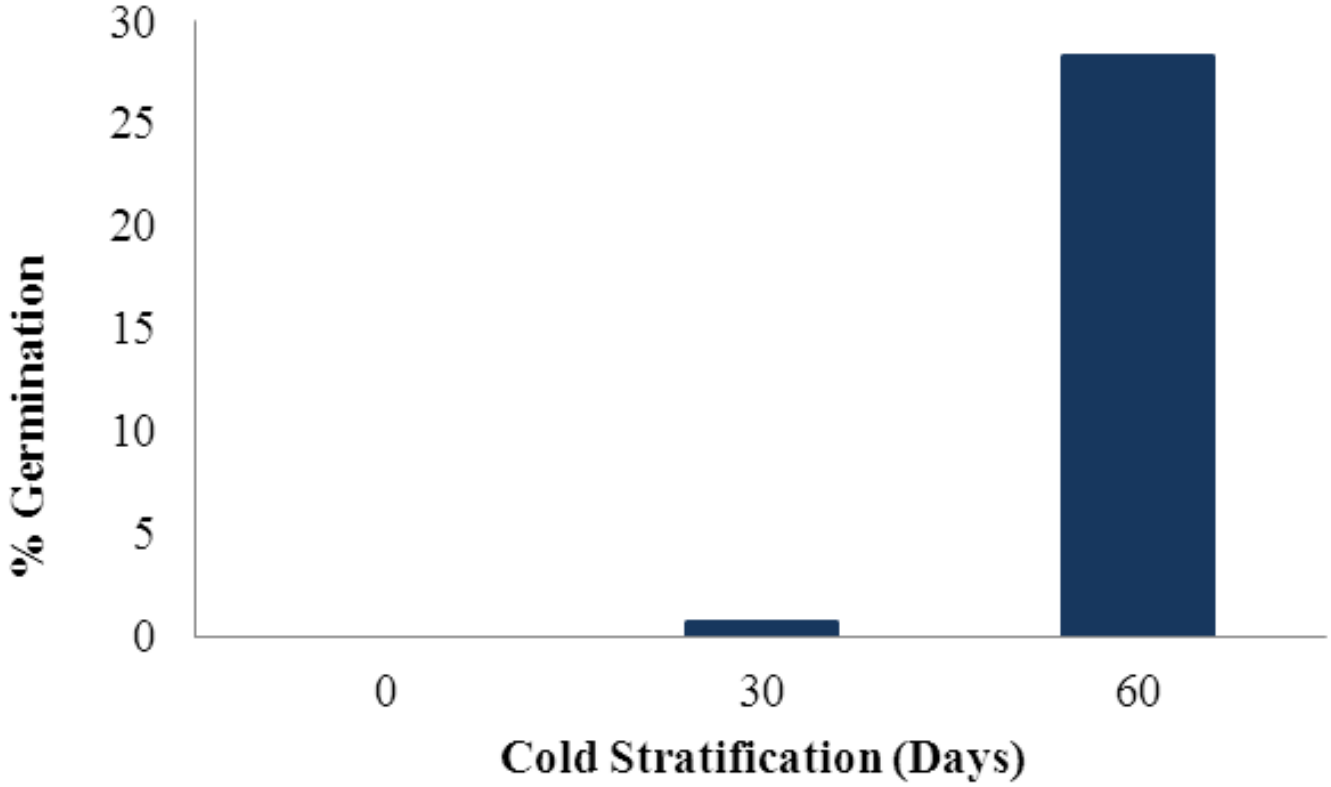

Figure 4.2 Percent germination of cold stratified sand plum (*Prunus angustifolia)* regardless of scarification treatment.

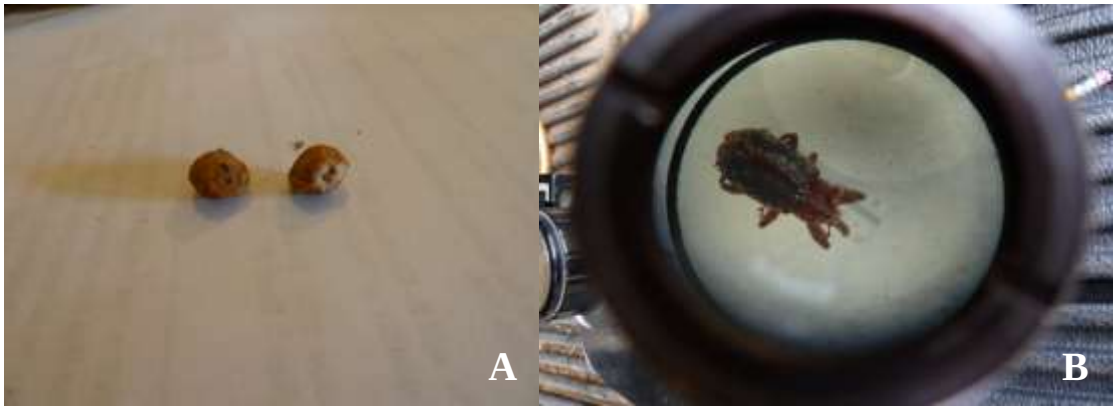

Figure 4.3 A Unscarified seed (left) compared to scarified seed (right). **B** Plum curculio in microscope at scope magnification. (Magnification 10 X 1.25). Photographs taken by Elizabeth McMahon.

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VITA

Elizabeth Ann McMahon

Candidate for the Degree of

Master of Science

Thesis: PROPAGATION OF SAND PLUM (*PRUNUS ANGUSTIFOLIA*) MARSH.: AN EXCITING START TO DOMESTICATION

Major Field: Horticulture

Biographical:

Education:

Completed the requirements for the Master of Science in Horticulture at Oklahoma State University, Stillwater, Oklahoma in July, 2013.

Completed the requirements for the Bachelor of Science in Rangeland Management and Horticulture at Texas A&M University, College Station, TX in 2010.

Experience:

Research Assistant, Department of Horticulture, Oklahoma State University, Stillwater, OK, August 2011-July 2013.

ACE Nursery Intern, National Park Service, Grand Canyon, AZ, February 2011-July 2011.

Botany Field Technician, Center for Environmental Management of Military Lands, Fort Polk, LA, May 2010-August 2010.

Student Worker, Department of Ecosystem Science and Management, Texas A&M University, College Station, TX, May 2009-August 2009.

Professional Memberships:

American Society for Horticultural Science
Oklahoma Native Plant Society