

EVALUATION OF DIFFERENT PROPAGATION METHODS (BUDDING,  
GRAFTING AND CUTTINGS) FOR PECAN

A Thesis

by

CASSANDRA JO WARREN

Submitted to the Office of Graduate and Professional Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Leonardo Lombardini
Committee Members,	Mengmeng Gu
	W. Todd Watson
Head of Department,	Daniel R. Lineberger

December 2015

Major Subject: Horticulture

Copyright 2015 Cassandra Jo Warren

## ABSTRACT

Pecan [*Carya illinoensis* (Wangenh.) K. Koch] is an economically important nut tree native to Texas which is cultivated throughout much of the southern United States.

Pecan trees are slow growing with a long period of juvenility, therefore asexual propagation through vegetative means is used to speed up the process as well as aiding in maintaining desirable characteristics through cloning. Current propagation methods for pecan typically require seedlings be at least two or three years old before they can be budded or grafted. One additional year or two may be required if budding or grafting is performed and unsuccessful.

Studies on the propagation methods of T-budding, cuttings and a new method, the V-graft, were conducted on pecan seedlings. These methods are used on one- or two-year-old seedlings. T-budding and V-graft methods were performed testing different wrapping materials and foil coverings. Softwood pecan cuttings were collected and subjected to a common method for cutting propagation and the Ellepot system, as well as two different hormone concentrations. To support the findings of the budding and grafting studies, a callus study was also conducted in which pecan rootstocks were wounded, covered with the different wrapping treatments used in the T-budding and V-grafting studies, and placed in a controlled environment ideal for callus formation.

The results supported that neither softwood cuttings nor T-budding are appropriate for propagation of pecan seedlings. V-grafting is a newly introduced method for pecan, and had some success. Since this is a newer method not yet reported to have

high success, it is recommended that more grafts be conducted to observe what the outcome could be on a commercial level.

## ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Leonardo Lombardini, and members, Dr. W. Todd Watson and Dr. Mengmeng Gu for their guidance and support throughout this process. Thanks are also due to Dr. George Ray McEachern and Mr. Monte Nesbitt for their assistance and direction of ideas for the project. Thanks also to my fellow graduate students for their friendship and encouragement.

Funding for this project was provided by USDA-NIFA-SCRI #003357, the Salopek Foundation, and Mr. Wylie Hatcher.

Finally, I would like to thank my parents for encouraging me to pursue anything I set my mind to and pushing me to go ahead and continue my academic career, as well as my husband, Jared, for encouraging me during this time. The love and support they provided has been invaluable.

## TABLE OF CONTENTS

	Page
ABSTRACT .....	ii
ACKNOWLEDGEMENTS .....	iv
TABLE OF CONTENTS .....	v
LIST OF FIGURES .....	vii
LIST OF TABLES .....	ix
CHAPTER I INTRODUCTION AND LITERATURE REVIEW .....	1
1.1 Introduction .....	1
1.2 Propagation methods .....	2
1.2.1. T-budding .....	2
1.2.2 Cuttings .....	3
1.2.3 Grafting .....	6
1.3 Propagation factors.....	7
1.3.1 Temperature.....	7
1.3.2 Materials .....	8
1.4 Summary and objectives .....	9
CHAPTER II MATERIALS AND METHODS .....	10
2.1 T-budding experiments .....	10
2.2 Cuttings experiment .....	12
2.3 V-grafting experiments .....	14
2.4 Image analysis and histology .....	16
2.5 Callus study .....	18
2.6 Statistical analysis .....	21
CHAPTER III RESULTS .....	22
3.1 T-budding .....	22
3.2 Cuttings .....	23
3.3 V-grafting .....	24
3.4 Callus development .....	26
3.5 Image analysis and histology .....	29
CHAPTER IV DISCUSSION AND CONCLUSION .....	32

REFERENCES.....	36
-----------------	----

## LIST OF FIGURES

	Page
Fig. 1. Representation of V-grafting method. Dark green represents the scion, light green represents the rootstock. ‘A’ represents the rootstock, ‘B’ and ‘C’ represent the side and cross-section of the V cut, respectively. ‘D’ represents the corresponding cut on the scion, ‘E’ and ‘F’ show how the two pieces fit together, and ‘G’ represents the final graft with wrapping material. ....	7
Fig. 2. Illustration of experimental setup for the February callus experiment. Brown rectangle represents the callus box viewed from above. Blue circles represent placement of HOBO sensors as they were for the experiment. ....	19
Fig. 3. Illustration of experimental setup for the April callus experiment. Brown rectangle represents the callus box viewed from above. Blue circles represent placement of HOBO sensors as they were for the experiment. This time, individual units were a treatment, and remained in containers (black boxes). ....	21
Fig. 4. Results from the April T-budding experiment conducted on 1-year-old potted ‘Elliott’ pecan seedlings. Frequency of take indicates the number of buds that still had green wood 3 weeks after budding occurred, and frequency of success refers to the number of buds which grew when forced after being checked at 3 weeks. ....	22
Fig. 5. Survival rates of V-grafts conducted on ‘Choctaw’ pecan seedlings in the orchard from both years. After grafts were performed, union was wrapped in three different tapes: Buddy tape (B), electrical tape (E) or Poly tape (P). ....	24
Fig. 6. Average monthly temperatures and monthly rainfall data collected during V-grafting of ‘Choctaw’ pecan seedlings at the TAMU orchard in both 2014 and 2015, from February to August. ....	25
Fig. 7. Daily rainfall received from February to May in both 2014 and 2015 at Easterwood Field weather station, located at lat. 30.58917°, long. - 96.36472° approximately 9 km east of the research site. ....	26
Fig. 8. Average areas of callus measured from wounded stem sections on ‘Elliot’ pecan seedlings from February 2015. Treatment numbers indicate the following treatments: 1- Control, 2- Buddy tape, 3- poly tape, 4- electrical tape. ....	27

Fig. 9. Average areas of callus measured from wounded stem sections on ‘Elliot’ pecan seedlings from April 2015. Treatment numbers indicate the following treatments: 1- Control, 2- Buddy tape, 3- poly tape, 4- electrical tape. ....	28
Fig. 10. Percent of wounded area covered with callus on ‘Elliot’ pecan seedlings. ‘Callus area’ refers to the percentage of callus tissue formed over a wounded area on the stem. Treatment numbers indicate the following treatments: 1- Control, 2- Buddy tape, 3- poly tape, 4- electrical tape. ....	29
Fig. 11. Image of a successful bud union and growth (taken at 20x magnification) of pecan budded seedlings. Letters A, B and C indicate the scion, callus bridge formation, and rootstock tissues, respectively. ....	30
Fig. 12. Image of a successful bud union with unsuccessful growth (taken at 10x magnification) of pecan budded seedlings. Letters A, B and C indicate the scion, callus bridge location, and rootstock tissues, respectively. ....	31



## LIST OF TABLES

	Page
Table 1. Factors applied to T-budded trees in experiment 1. Each combination of factors led to a total of 24 treatments, replicated 5 times for 120 trees total. For bud size, 'Large' refers to scion buds of 1.5 cm or larger, and 'Small' refers to scion buds smaller than 1.5 cm. ....	12
Table 2. List of the five treatments corresponding to those buds from the April T-budding experiment conducted on 1-year-old potted 'Elliott' pecan seedlings which grew out successfully.....	23

# CHAPTER I

## INTRODUCTION AND LITERATURE REVIEW

### 1.1 Introduction

Pecan [*Carya illinoensis* (Wangenh.) K. Koch] is an economically important nut tree species native to Texas which is cultivated throughout much of the southeastern U.S., from Texas to Florida (Thompson and Grauke, 1991). Pecan trees are slow growing with a long period of juvenility, therefore asexual propagation through vegetative means is used to speed up the process. Vegetative propagation also aids in maintaining desirable phenotypic and genotypic characteristics through cloning. The method of propagation by taking cuttings of a tree and allowing it to root and grow as a clone of the mother plant has been practiced since ancient times, but commercially for at least a century (Geneve, 2001; Preece, 2003). The commercially important crops of cherry, grape, and apple are examples of a few species which are routinely grafted on rootstocks that are clonally propagated by cuttings for uniformity in desirable characteristics, such as disease resistance, salinity tolerance or dwarfing (Hartmann et al., 2011). Several other important species, such as peach, almond and citrus are budded and/or grafted as another means of clonal vegetative propagation. The pecan industry would benefit from having clonal rootstock with the potential to possess and propagate these characteristics, including dwarfing. Much of this propagation is done by set standard grafting or budding methods. These current types of propagation, however, delay the time it takes to produce trees in the nursery, as 3 years are needed to get the

tree to grow before any sort of grafting can be done (McEachern, 2007) which are followed by 1-2 additional years for grafting/budding establishment in the nursery before trees can be sold. Also, pecan rootstocks are all seedlings, i.e., they all derive from individual open-pollinated seeds, which can cause variations in growth and performance of the tree overall. Consequently, these seedling rootstocks are not genetically uniform, which also means there are currently no defined traits for rootstock tolerances to major issues such as salinity, cotton root rot and improvement in zinc uptake (Youssef et al., 1993).

## **1.2 Propagation methods**

### *1.2.1. T-budding*

One method of vegetative propagation that would show potential benefit to pecan production for speeding up the process is the T-budding system. T-budding is a grafting method that is commonly used on peach, rose and citrus. This method is chosen for these crops because it is easy to perform and because it can be done on younger stock, which allows for quicker time out of the nursery. The method and technique of T-budding is also much quicker to perform, another reason why it is preferred by those who need to produce budded crops in large numbers, and common among rose and citrus producers (Alexander and Lewis, 1998). While this would not address the issue of uniformity in rootstock, it would allow for nurserymen to sell their trees to orchard growers much quicker (by about 1-2 years) than they would by the use of traditional grafting methods of four-flap graft, inlay bark graft or patch budding. Growers in central Illinois reported

success using T-budding methods on the walnut, a relative of pecan (Gerardi, 1954). One grower in particular stated best results were obtained when bud of about 2.5 cm in length was used. This same individual also stressed the importance of leaving a slight space between wrapping, to allow for oxygen exchange in the bud so that it will not drown in its sap (Gerardi, 1954). A modification of the T-budding method has been reported by one individual to have success on pecan. Before insertion of the shield-shaped bud into the T-shaped incision, a V-shaped chisel is made on the lower portion of the bud. This method allows for a better fit of the bud into the T incision (Reed, 1926).

### *1.2.2 Cuttings*

Propagation of pecan by cuttings does have potential and has been done with some success. Experiments in rooting of young, tender softwood cuttings have been explored since the early part of the 20<sup>th</sup> century. One experiment in particular looked at the benefits of indolebutyric acid (IBA) and naphthaleneacetic acid (NAA) applied to pecan softwood cuttings (Gossard, 1944). This was a very early study in pecan research, but it did discover that IBA and NAA enhanced the rooting ability of softwood pecan cuttings (Gossard, 1944). Smith and Chiu (1980) reported a 71% success with juvenile softwood cuttings rooted in a 0.5% K-IBA solution. In this same study, repeated experiments often found the juvenile softwood cuttings to root more successfully than adult semi-hardwood cuttings (Smith and Chiu, 1980). These successes, if expanded upon, could have significant positive impacts on the pecan industry by speeding up the time from the nursery to the orchard as well as potential for clonal rootstocks. Another

benefit offered by young, softwood cuttings is the ability to leave some foliage attached, which provides stored food reserves in leaves to developing roots (Taylor and Odom, 1969). The early study by Gossard also reported that foliage retention was a benefit. However, the transplanting process is key when it comes to the survival of these delicate cuttings (McEachern, 1973). Pecan roots are sensitive, particularly the new, young roots on cuttings which are easily broken during the transplant process.

Another benefit of taking softwood cuttings is the benefit of juvenility associated with latent shoot growth. These young, juvenile shoots have retained characteristics of their original form when initial growth occurred. This includes the ability to form adventitious roots and a lack of sclerenchyma tissue fibers in the stem tissue which can become a physical barrier to root development (Griffin and Bassuk, 1996). This is beneficial, because an adult tree of value can be cut back and encouraged to produce latent, juvenile shoots from the stump that will grow without heavy lignification.

Many studies have also been done on different types of cuttings: softwood, semi-hardwood and hardwood. The physiological differences between hardwood and softwood cuttings have an impact on callusing and rooting percentage, based on the varied reports of successes and failures with both types of cuttings (Allan et al., 1980; Preece et al., 2002; Taylor and Odom, 1969). IBA concentration and temperature had varying effects also. High levels of IBA (5,000 and 10,000 ppm) were reported to have the best effect on increasing root number, which correlates with survival on softwood cuttings (Whatley et al., 1966). Mature hardwood cuttings did root with some success also using high levels of IBA; however, these were much more susceptible to transplant

shock and needed to be carefully timed for transplant just as root tips were beginning to emerge (Allan et al., 1980). A study on the attempt to root pecan hardwood cuttings using lower IBA concentrations (200-500 ppm) reported that anatomical factors inhibited adventitious roots from forming. Pith parenchyma is where rooting in pecans occurs; however, once the root initials reached the xylem, hard resinous tissue prevents roots from moving much further. This same study also reported that internal biochemical factors have a role in preventing root growth (Wally et al., 1980). The authors (Wally et al.) reference a study by Taylor and Odom (1970) in which a compound similar to juglone (present in walnut) was discovered in pecan leaves, and was found to have some root-inhibitory properties. Another genus difficult to root by cuttings, the oak, has been reported to have some success when cuttings from juvenile softwood stump sprouts are taken and subjected to at least 8,000 ppm IBA (Hare, 1977).

The majority of the literature reviewed contains studies heavily focused on the initial rooting of cuttings, but not the transplant and continued growth after establishment (Whatley et al., 1966). McEachern (1973) reported the use of peat pots which facilitated easier transplanting (McEachern, 1973). Ellepots™ are a relatively new growing system for young plants which allow for air-pruning of roots and ease of transplanting by the use of degradable, mesh-like paper encasing growth media. Little research was found on the use of the system, but one study found that the use of Ellepots in cuttings of *Elaeagnus x ebbingei* did improve root growth and branching of shoots (Maguire and Harun, 2007). Personal communications with nurserymen and a manager at Knox Nursery led to the hypothesis that this system could offer good potential to

assist in rooting of pecan cuttings, as the nursery industry does commonly use Ellepots for rooting of young cuttings of species such as rose, geranium, and eucalyptus, among other types of propagation of seeds and tissue culture acclimatization.

### *1.2.3 Grafting*

Another method which has potential for propagation of young pecan trees is the V-grafting method (Fig. 1). Similar to the cleft graft, this is a method which was introduced by a pecan grower residing in central Texas. The method has shown 90% graft success on pecan and other fruit trees such as apple and pear (R. Schutze, pers. comm.). Electrical tape and a foil covering is used with the V-grafting method on trees that are 1 or 2 years old. Success of the method on a large scale is unknown; however, the cuts made allow for long sections of the cambium to contact on both the scion and rootstock. Cambial contact is a critical step in any successful graft, so that a union can develop rapidly (Hartmann et al., 2011), and this method appears to allow for a large amount of cambial contact compared to other budding and grafting methods performed on young trees. Further observations of the method with the materials used by Schutze and other commonly used grafting materials are necessary to determine the potential of V-grafting. However, this method could allow for a larger tree to be produced at a younger age, compared to those which are patch budded.

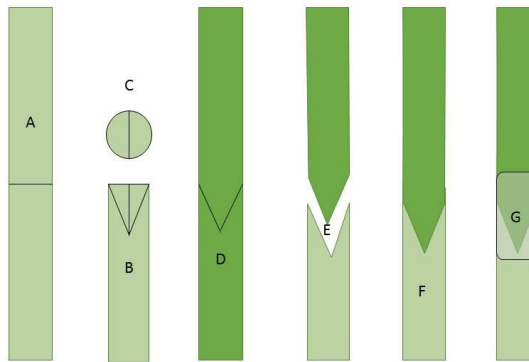


Fig. 1. Representation of V-grafting method. Dark green represents the scion, light green represents the rootstock. 'A' represents the rootstock, 'B' and 'C' represent the side and cross-section of the V cut, respectively. 'D' represents the corresponding cut on the scion, 'E' and 'F' show how the two pieces fit together, and 'G' represents the final graft with wrapping material.

### 1.3 Propagation factors

#### 1.3.1 Temperature

Temperature is an important factor to be considered where propagation by cuttings, budding and grafting is concerned. Another important nut crop, the pistachio, was studied using different budding methods during the long summer growing season in Turkey (Arpaci et al., 1997). T-buds were most successful when performed in June, in temperatures that averaged 31°C annually (Arpaci et al., 1997). In Iran, a research team compared callusing and bud take percentage in environmentally controlled greenhouse conditions to standard field conditions in walnut (Ebrahimi et al., 2006). Temperature and humidity were determined to have a distinct effect on callus formation and bud take in both patch and shield (another name for the T-bud method) budded trees, with more



callusing and survival in the greenhouse budded trees (Ebrahimi et al., 2006). It is well known in the horticultural community that warm temperatures are needed for callus to form on cuttings. A study was carried out in China on the rooting of pecan cuttings in a hot bed. Temperatures between 20 °C and 25 °C were found to be most favorable for callus formation (Ichikawa, 1958). For pecan, it is known that temperatures of 23°C to 27°C are best for the formation of callus in graft unions (Brison, 1986).

### *1.3.2 Materials*

Current research on grafting studies often includes studying the effects of coverings and tapes for the graft union, in combination with other factors such as method, temperature, and so on. Rarely do these studies observe the effect of the covering only, leaving the question of why there are varying tapes and wraps but no knowledge of any difference between them. One author reported some difference between film-forming liquid coatings on grafted pecans (McFadden, 1962). McFadden also reported that certain properties of films, such as regulation of gas exchange and light transmission, may have an effect on formation of the graft union. Buddy tape is one such material that is known for its air and moisture permeability (Nishihira, 2013). Communications with nurserymen confirm that for high-volume production, tapes such as Buddy tape, or plastic poly tape are preferred over liquid coatings (T. Britt, pers.comm.); however, cost and availability is often an issue. In 2013, the wholesale price of one roll of Buddy tape was \$15.35, and the price at retail was on average \$23-25 per roll (Nishihira, 2013). One roll has 571 pieces per roll, which corresponds to 571

buds or grafts, or double the number for smaller buddings if only a half strip is used (Nishihira, 2013). One author reported some differences among readily available grafting tapes and wraps in shoot length of grafted apples (Singha, 1990). This study included grafting tape, masking tape, electrical tape, duct tape and polyethylene strips. All wrappings had successful graft unions; however, masking tape produced the smallest shoot lengths (Singha, 1990). These ‘readily available’ tapes are all priced at less than \$5 per roll at common hardware chains such as Lowe’s®, Home Depot®, etc., which is reasonable for small growers and smaller nurseries to pay.

#### **1.4 Summary and objectives**

Pecan is a slow growing tree with an increase in demand, and alternative propagation methods that could speed up time to production would greatly benefit the industry. This thesis aims to achieve the following objectives: to determine an alternative method for quicker, simpler propagation of pecan, as well as effectiveness and efficiency. For T-budding, the best combination of wrapping tape, T orientation and bud size will be observed, while the V-grafting method will be tested with factors of wrapping and foil cover combinations, as well as time of year. For cuttings, the main factor to be studied is the benefit of air pruning of roots to enhance cuttings offered by Ellepots for pecan propagation, as well as the application of bottom heat. Callus production under different wrapping materials will also be observed.

## CHAPTER II

### MATERIALS AND METHODS

#### 2.1 T-budding experiments

Two T-budding experiments were conducted in a greenhouse on the main Texas A&M University campus. The temperature in the greenhouse was regulated with a fan and pad cooling system. Temperature readings were collected using temperature/light data loggers (HOBO Pendant®, Onset Computer Corp., Bourne, MA), and readings were taken every 15 minutes. Average greenhouse temperature was 26 °C day/23 °C night. Rootstocks used were ‘Elliott’ seedlings planted in January 2012 in a Texas Pecan Special medium (Vital Earth/Carl Pool Products, Gladewater, TX) in 5-L containers (Stuewe and Sons, Inc., Tangent, OR). First, a factorial experiment using a completely randomized design was conducted in April 2013. Bud wood was collected at the TAMU Orchard (F.M. 50, west of College Station, TX) in February 2013 from 1-year growth of dormant ‘Apalachee’ trees grafted in 2010. Four factors were assessed: tape type, ‘T’ position, bud size and insulation. Types of tapes used were: poly budding tape (also referred to as simply ‘poly tape’, A.M. Leonard, Piqua, OH), Buddy Tape (25 mm × 40 mm perforations Buddy Tape, Shigyo Company, Truckee, CA) and both, with Buddy tape underneath poly tape. ‘T’ position was either inverted or upright ‘T’. Buds were divided in two classes, based on their size: large (2-2.5 cm in length) or small (1.3- 1.5 cm in length). The ‘insulation’ treatment consisted in either covering the wrapped bud with 10.16 × 10.16 cm piece of reflective bubble wrap material (Reflectix, Inc., Markleville, IN) and securing with two large paperclips, or no covering. This lead to a

combination of 24 different treatments with 5 repetitions each yielding 120 trees total (Table 1). Buds were checked 3-4 weeks after budding for viability and nicked with a slight incision on the bud shield to check for green (alive) wood. The trees with buds determined to still be alive were cut back about 5 cm above the top of the 'T' to force those buds to grow. Average temperature during the experiment was 25.8 °C day/22.5 °C night. Trees were watered daily during the grafting experiment, and sprayed twice for aphids and spider mites with M-Pede® (Dow AgroSciences, LLC, Indianapolis, IN). Trees remained in the greenhouse until May 2014, after which they were moved to a concrete and gravel pad between greenhouses.

A second factorial experiment using a completely randomized design was conducted in June 2013 in the same greenhouse used for the T-budding experiment conducted in April 2013 and described above. This experiment was performed with the best combination of factors found in T-budding experiment in April 2013. These treatments were uniformly applied along with two new factors: growth stage (dormant vs. current season growth) of bud wood, and presence or removal of the wood of the scion shield. This removal of wood served to test if strictly cambial contact is enough for callus formation. Treatment 18 from experiment 1 was used (Table 1). Dormant bud wood was the same as used in April, current season bud wood was collected from growing branches of the same block of 'Apalachee' trees in the Texas A&M Orchard.

Table 1. Factors applied to T-budded trees in experiment 1. Each combination of factors led to a total of 24 treatments, replicated 5 times for 120 trees total. For bud size, ‘Large’ refers to scion buds of 1.5 cm or larger, and ‘Small’ refers to scion buds smaller than 1.5 cm.

Treatment number	Wrapping type	T incision	Bud size	Insulation
1	Poly tape	Inverted	Large	Yes
2	Poly tape	Inverted	Large	No
3	Poly tape	Inverted	Small	Yes
4	Poly tape	Inverted	Small	No
5	Poly tape	Upright	Large	Yes
6	Poly tape	Upright	Large	No
7	Poly tape	Upright	Small	Yes
8	Poly tape	Upright	Small	No
9	Buddy tape	Inverted	Large	Yes
10	Buddy tape	Inverted	Large	No
11	Buddy tape	Inverted	Small	Yes
12	Buddy tape	Inverted	Small	No
13	Buddy tape	Upright	Large	Yes
14	Buddy tape	Upright	Large	No
15	Buddy tape	Upright	Small	Yes
16	Buddy tape	Upright	Small	Yes
17	Buddy and poly tape	Inverted	Large	Yes
18	Buddy and poly tape	Inverted	Large	No
19	Buddy and poly tape	Inverted	Small	Yes
20	Buddy and poly tape	Inverted	Small	No
21	Buddy and poly tape	Upright	Large	Yes
22	Buddy and poly tape	Upright	Large	No
23	Buddy and poly tape	Upright	Small	Yes
24	Buddy and poly tape	Upright	Small	No

Trees were watered daily during the experiment, and were treated at the same time as experiment 1 for aphids and spider mites. Trees remained in the greenhouse until June 2014.

## 2.2 Cuttings experiment

The cuttings experiment was conducted in a different greenhouse located on the west side of the TAMU campus. Average temperature in this greenhouse was 24 °C day/21 °C night. This greenhouse has a fan and pad cooling system for temperature

regulation. Temperature readings were collected using temperature/light data loggers (HOBO Pendant®, Onset Computer Corp., Bourne, MA), and readings were taken every 15 minutes. Softwood cuttings of ‘Elliott’ pecan trees grown in the greenhouse and approximately 15 cm long were taken in mid-December 2013. These were from the same group of ‘Elliott’ trees grown in the greenhouse and used for the T-bud experiment as described in 2.1. The trees in the greenhouse were cut back to encourage latent buds to force out; these buds formed during initial plant growth which still had juvenile characteristics (Preece et al., 2002). Three variable factors were applied: pot tray type, IBA/NAA (Dip ‘N Grow, Dip ‘N Grow, Inc., Clackamas, OR) concentration and heat. Cuttings were stuck in 50% peat, 50% perlite in either the Ellepot trays (40 count Woody Special trays, 5 cm × 9 cm, The Ellepot Store, Knox Nursery, Winter Garden, FL) or plug trays (720562C, PL-38-Star-DP, T.O. Plastics, Clearwater, MN). Pots in both trays are approximately 5 cm × 9 cm. IBA/NAA to induce root formation was applied in concentrations of either 5,000 ppm or 10,000 ppm. Constant bottom heat was applied at 23 °C using heated propagation mats (RHM 2110, 53.34cm x 3.048m, Redi-Heat Propagation Mat, 120 volt, 400 watt capability, Phytotronics Inc., Earth City, MO) as another treatment to one tray of Ellepots and one tray of plugs. The corresponding treatment to bottom heat was unheated (no mats underneath) Ellepot and plug trays. Cuttings were set under intermittent mist for 6 s every 6 min. Mist duration and intervals between mists were adjusted as necessary with careful observation to moisture of cutting bed. After 1 week of experiment start date, mist duration was lowered to 4 s. At 3 weeks after the experiment start date, mist interval was changed to mist for 6 s every 10 min,

due to excess moisture causing some cuttings to rot and some algae buildup on the mist bench. The cuttings were removed and observed for rooting immediately following the 90-d period. Checkups occurred at least biweekly to record measurements of viability and ensure mist system is working properly. Temperature/light data loggers (HOBO Pendant®, Onset Computer Corp., Bourne, MA) were used on heat mats and unheated areas of the bench to monitor temperature.

### **2.3 V-grafting experiments**

V-grafting experiments were performed in 2014 in both greenhouse and field conditions and in 2015 only in field conditions. Two V-grafting experiments were conducted in greenhouse on the main Texas A&M University campus. Average greenhouse temperature was 26 °C day/23 °C night. The greenhouse used a fan and pad cooling system for temperature regulation. Temperature readings were collected using temperature/light data loggers (HOBO Pendant®, Onset Computer Corp., Bourne, MA), and readings were taken every 15 minutes. Grafting was also performed three times in a seedling row at the orchard in 2014. The remainder of the V-grafting experiments was conducted at the Texas A&M University pecan research orchard (lat. 30°31'N, long. 96°24'W, elevation 67 m), located west of College Station, TX. The section of the orchard used is located on Weswood silty clay loam soil (0 to 1% slopes, fine-silty, mixed, superactive, thermic Udifluventic Haplustepts) (NRCS, 2014). Throughout the study, trees were irrigated using drip tape every 1 to 2 weeks from May through October as needed. Tree rows were maintained vegetation-free using a glyphosate-based

herbicide and alleys were left vegetated, primarily by bermudagrass to reduce erosion. All V-grafting experiments used ‘Nacono’ scion wood, collected from the same orchard location as ‘Apalachee’ described in 2.1. Rootstocks used for the greenhouse experiments were ‘Elliott’ seedlings, planted at the same time as those described in 2.1 from the T-budding experiment. Rootstocks used for the orchard experiments were ‘Choctaw’ seedlings, planted in a row at the TAMU orchard.

The first greenhouse grafting was conducted on 27 March 2014. Treatment was the grafting method: Whip and Tongue graft, and V-graft. For this initial experiment, the covering used was electrical tape with a foil covering, which is what the grower who developed this method used. There were 15 trees for each treatment and 30 trees total. A second greenhouse grafting occurred on 9 May 2014. This time only the V-grafting method was used to replicate the following orchard experiment in a controlled environment. Treatment was the following wrapping materials: Buddy tape, electrical tape, and poly tape. An interaction of foil was added, so there were six treatments total. There were 4 trees per treatment, and 24 trees total.

Orchard V-grafting was performed three separate times in 2014, and twice in 2015. For 2014, all three grafting periods used wrapping treatments same as the previous greenhouse experiment. In 2015, the two grafting periods used the same wrappings as 2014, but no foil interaction. Both years used ‘Choctaw’ seedling rootstock and ‘Nacono’ scions.



## **2.4 Image analysis and histology**

Images for the T-budding experiments were taken using a Zeiss Axiophot (Carl Zeiss Microscopy, LLC, Thornwood, NY) and captured with a Nikon DXM 1200 digital color camera using Nikon ACT-1 software (Nikon Corporation, Melville, NY). Computer image analysis was performed using ImageJ software (ImageJ, U.S. National Institutes of Health, Bethesda, MD).

Histology was performed on all bud samples prior to image analysis. Samples were first fixed individually in small glass jars in 10 mL Trump's fixative (McDowell and Trump, 1976) for each bud. Bud samples were allowed to sit in the fixative for one week. At the end of the week, there was still evidence of air in the samples. Samples were then placed with their fixative containers in a low-voltage microwave-assisted vacuum (Pelco BioWave™ 34700, Ted Pella, Inc., Redding, CA) for 30 min to ensure full infiltration of the fixative and complete removal of air bubbles. Fixative was then discarded from sample jars using a pipette to gently remove all possible solution from the buds. Samples were rinsed with distilled water three times. After rinsing, bud samples were then treated with a 10% ethylenediamine solution for wood softening. The solvent used was distilled water. Each jar received 10 mL of the ethylenediamine solution and was allowed to incubate in a 55 °C oven (LabLine® L-C oven, Lab-Line Instruments, Inc., Melrose Park, IL) for 3 d. After this period, the ethylenediamine solution was discarded and samples were rinsed with 10 mL distilled water in the microwave for 1 min at 150W. After this, a series of graded ethanol rinses, for 5 minutes each at 150W occurred to prepare the samples for storage and further processing. Three

rinses occurred at 30%, 50% and then 70% ethanol. After the last ethanol rinse, samples were then stored in fresh 70% ethanol for storage and shaking overnight. Once shaking was complete, the samples were replaced with fresh 70% ethanol and transported to the histology laboratory on the TAMU campus. The laboratory was responsible for paraffin infiltration and embedding of the samples.

After receiving the completed samples from the histology lab, sample cross sectioning and slide mounting of sections took place. Slides were prepped with a light swab of Mayer's Egg Albumin (Ruzin, 1999) and dried overnight. For sectioning of the samples, a rotary microtome was used (American Optical Spencer 820 Microtome, Buffalo, NY) with a 120 mm sharp steel blade (Reichert Technologies, Reichert, Inc., Depew, NY) to make 15- $\mu$ m sections. Prior to sectioning, slides were placed on a slide warmer at 40 °C with several drops of water on top of the slide. This was done to allow ribbons to stretch and adhere easily to the albumin when placed on the slide. Bud samples were sectioned into ribbons of longitudinal cross sections, one section per slide, patted dry with a tissue and allowed to dry overnight. After slides were dry, a staining procedure was used similar to one used by Graham and Trentham (Graham and Trentham, 1998). Xylene was used instead of MicroClear® for three changes (MicroClear® was not available), and a graded ethanol rinse was used instead of isopropanol. Following these rinse steps, slides were laid flat and allowed to dry overnight. The staining method used was the same as Graham and Trentham's, with the exception of Bismarck brown Y (Graham and Trentham, 1998). Once stained slides were completely dry, coverslips were applied using Preservaslide (Taylor Scientific, St.

Louis, MO) and allowed to dry overnight. Slides were observed under brightfield conditions under a Zeiss Axiophot (Carl Zeiss Microscopy, LLC). All images were captured using a digital camera (DXM 1200, Nikon Corp.) and with color image software (ACT-1, Nikon Corp.). Image analysis was performed on ImageJ software.

## **2.5 Callus study**

As a follow-up to the grafting studies, a study was conducted using several different wrapping materials to observe if there were any significant differences in callus growth between the different materials. Two experiments were conducted in February and April 2015.

For the February experiment, dormant 'Elliott' seedlings were used. These were from the same group of seedlings as used for greenhouse grafting in 2.2 and 2.4. Approximately 100 dormant seedlings were removed from their containers on 7 February 2015 and prepared for cooler storage, to maintain the dormant stage. Soil was removed from the root ball, and rinsed off with RO (reverse osmosis) water. The tops and roots were trimmed, leaving an approximate 20 cm stem, with an average 8.7 mm diameter. After trimming, seedlings were buried in a large plastic bucket in moist cedar shavings, covered with a large black trash bag, and stored in the cooler for one week until it was time to conduct the experiment. Three wrapping materials were used: Buddy Tape, poly budding tape, and electrical tape (3/4 in. by 60 ft. Industrial Grade Electrical Tape, Harbor Freight Tools, Camarillo, CA). The control was no wrapping material. A shield-like incision was made with the assistance of a patch budding knife for uniformity of length (Fig. 2). The incision made on each tree was approximately 1 cm wide  $\times$  3 cm

long. After incisions were made and treatments were applied, two trees of each treatment were bundled together, with seven bundles total. The bundles were buried under moist cedar shavings in a wooden box for 4 weeks. The dimensions of the box were 0.61 m wide, 2.45 m long and 0.61 m deep. Heat was maintained at approximately 25-26 °C in the box by the use of a heating mat underneath the shavings (RHM 2110, 53.34 cm × 3.048 m, Redi-Heat Propagation Mat, Phytotronics Inc., Earth City, MO). Relative humidity was monitored with an AcuRite™ Digital Temperature and Humidity monitor (AcuRite™, Chaney Instrument Co., Lake Geneva, WI) and maintained around approximately 90% throughout the duration of the study, assisted with the use of a mist bottle to mist shavings and prevent desiccation.

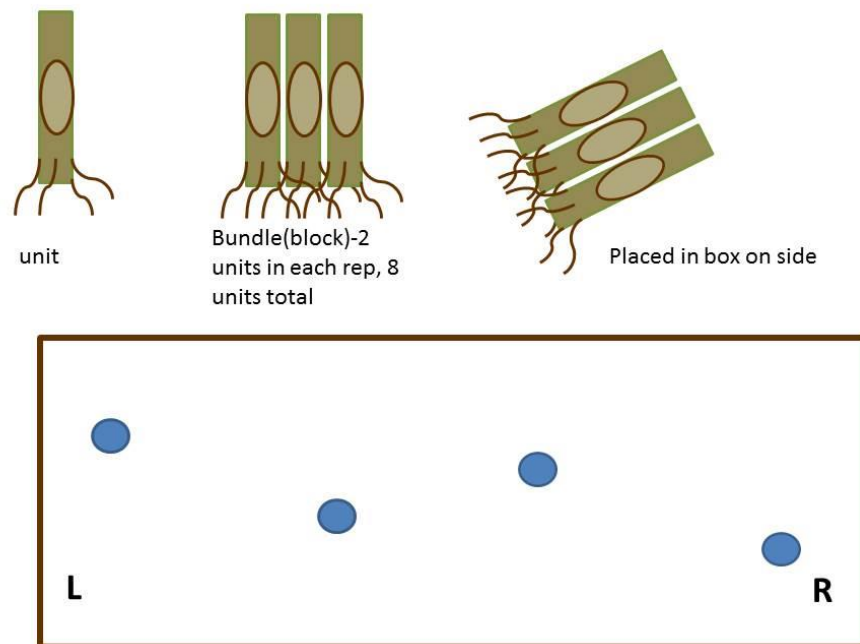


Fig. 2. Illustration of experimental setup for the February callus experiment. Brown rectangle represents the callus box viewed from above. Blue circles represent placement of HOBO sensors as they were for the experiment.

For the April experiment, actively growing ‘Elliott’ seedlings were used. These were from the same group as used in 2.2 and 2.4. Approximately 50 seedlings were taken from their location between the greenhouses and the tops were trimmed, leaving an approximate 20 cm stem with an average diameter of 8.7 mm. Trees were kept in their containers to minimize moisture stress on actively growing tissues. This time an incision was also made with the assistance of a patch budding knife, and was also cut as a patch bud would be (Fig. 3). Incision was 1 cm wide  $\times$  3 cm long. Trees were placed on the heating mat in the same wooden box from the February experiment, without cedar shavings.

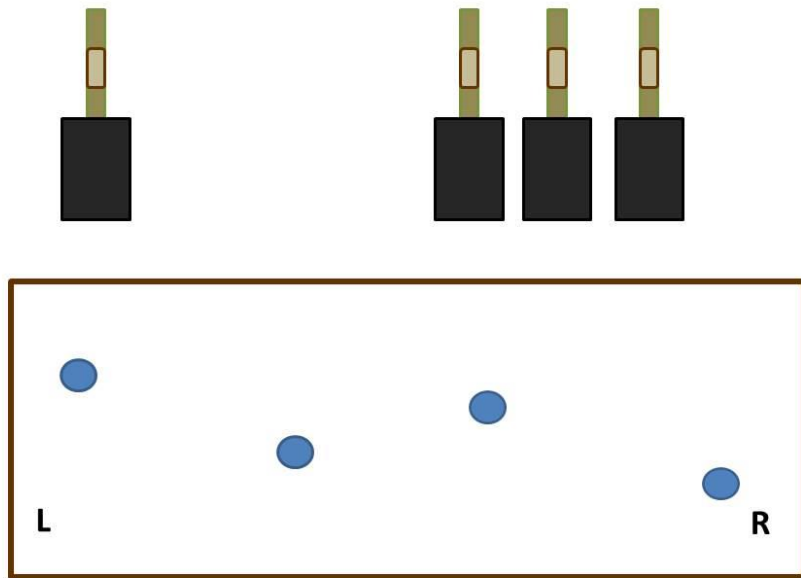


Fig. 3. Illustration of experimental setup for the April callus experiment. Brown rectangle represents the callus box viewed from above. Blue circles represent placement of HOBO sensors as they were for the experiment. This time, individual units were a treatment, and remained in containers (black boxes).

## 2.6 Statistical analysis

All statistical analyses were performed in JMP 11.0 (SAS Institute, Cary, NC, USA). All differences were considered significant at  $P < 0.05$ .

## CHAPTER III

### RESULTS

#### 3.1 T-budding

There were no significant differences between treatments in any of the two T-budding experiments, and the second experiment had no bud take success. The first experiment had 28 buds out of the 120 take, and treatment success among these was highly variable, as shown in Fig. 4.

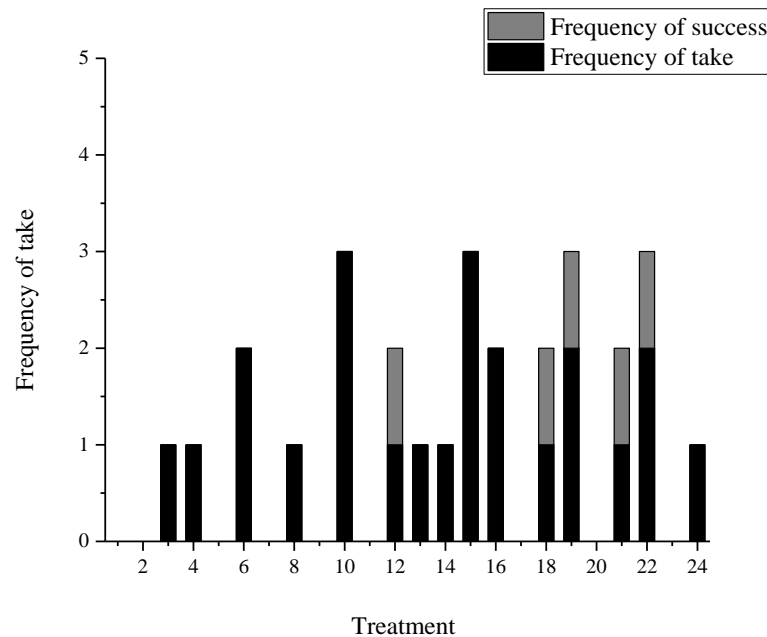


Fig. 4. Results from the April T-budding experiment conducted on 1-year-old potted ‘Elliott’ pecan seedlings. Frequency of take indicates the number of buds that still had green wood 3 weeks after budding occurred, and frequency of success refers to the number of buds which grew when forced after being checked at 3 weeks.

Of the 28 that successfully took, only five of these buds grew out once forced. Table 2 below shows which five treatments corresponded to the successful buds.

Table 2. List of the five treatments corresponding to those buds from the April T-budding experiment conducted on 1-year-old potted 'Elliott' pecan seedlings which grew out successfully.

Treatment number	Wrapping type	T incision	Bud size	Insulation
12	Buddy	Inverted	Small	No
18	Buddy and poly tape	Inverted	Large	No
19	Buddy and poly tape	Inverted	Small	Yes
21	Buddy and poly tape	Upright	Large	Yes
22	Buddy and poly tape	Upright	Large	No

The second T-budding experiment conducted in June had no successes at all. All buds were dead when checked at 3 weeks after budding. No statistical analysis was conducted for this portion of the project.

### 3.2 Cuttings

There was very little success with this experiment, with only 1 out of 24 forming adventitious roots. This cutting died approximately one month after the experiment was terminated. No statistical analysis was conducted.



### 3.3 V-grafting

In both years of V-grafting, some successes occurred. The second experiment conducted in 2015 had more success than the first in 2014.

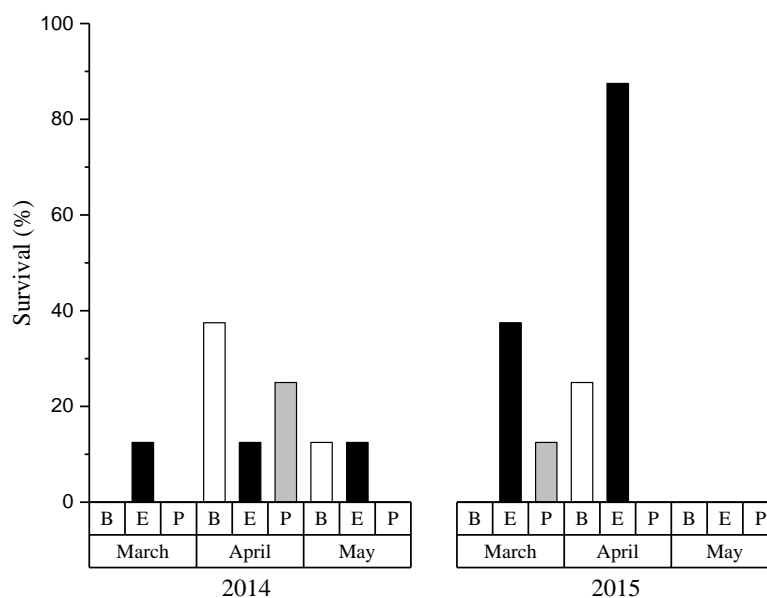


Fig. 5. Survival rates of V-grafts conducted on 'Choctaw' pecan seedlings in the orchard from both years. After grafts were performed, union was wrapped in three different tapes: Buddy tape (B), electrical tape (E) or Poly tape (P).

As shown in Fig. 5, success of the V-graft in the orchard was variable between the years. The most successful period was April 2015, with electrical tape having the highest success rate at 87.5%. While 2014 presented no real significant outcomes, April of that year also present some higher successes compared to the other months. Temperature and rainfall varied among the years, as shown below in Fig. 6.

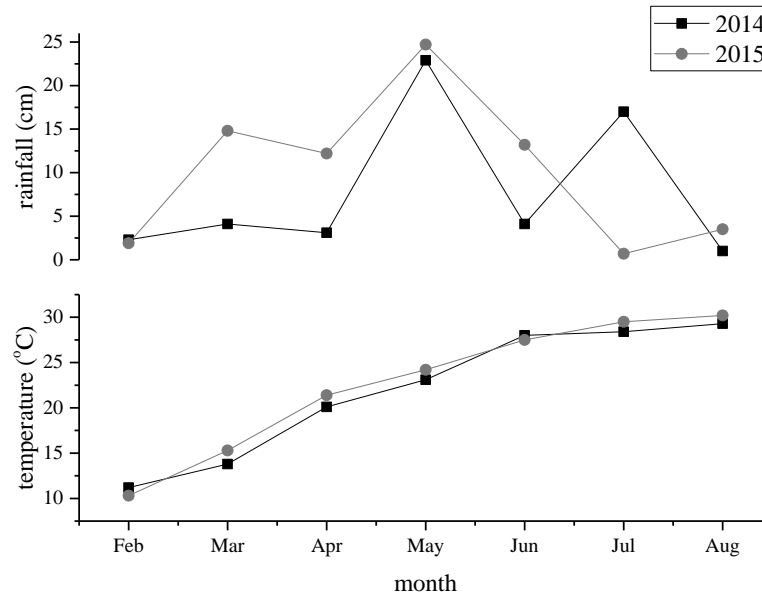


Fig. 6. Average monthly temperatures and monthly rainfall data collected during V-grafting of ‘Choctaw’ pecan seedlings at the TAMU orchard in both 2014 and 2015, from February to August.

Daily rainfall of each year also differed distinctly. Fig. 7 shows daily rainfall received for both years during months of budwood collection and grafting periods.

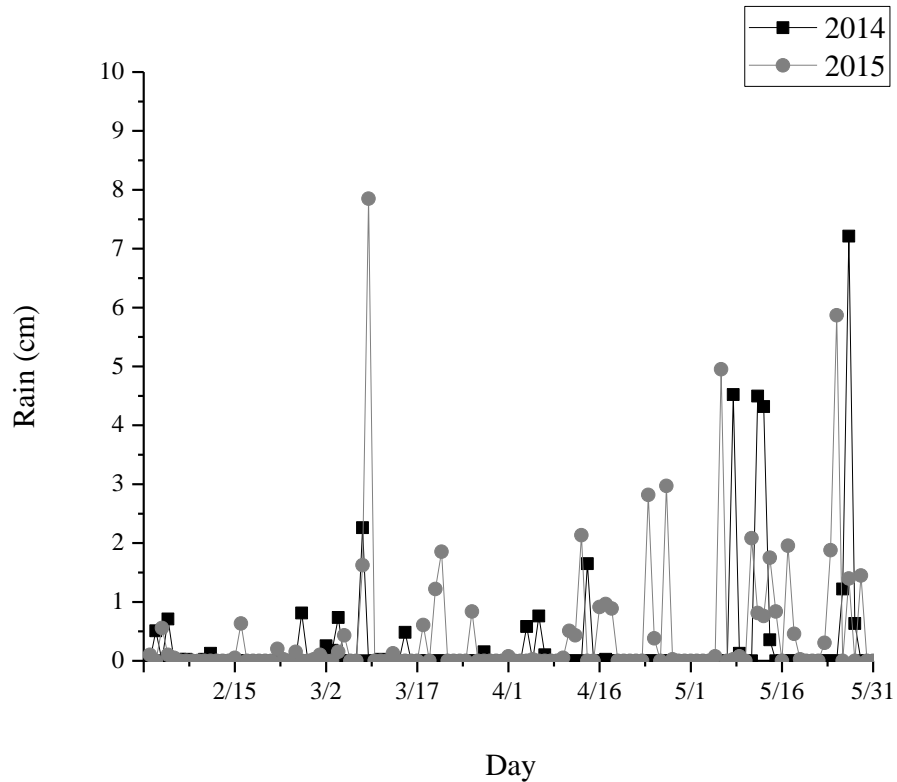


Fig. 7. Daily rainfall received from February to May in both 2014 and 2015 at Easterwood Field weather station, located at lat. 30.58917°, long. -96.36472° approximately 9 km east of the research site.

### 3.4 Callus development

The only significant difference occurred in the second callus experiment in April of 2015. This difference was between the control and treatments. There were no significant differences among treatments in both callus studies, as shown in Fig. 8 and Fig. 9.

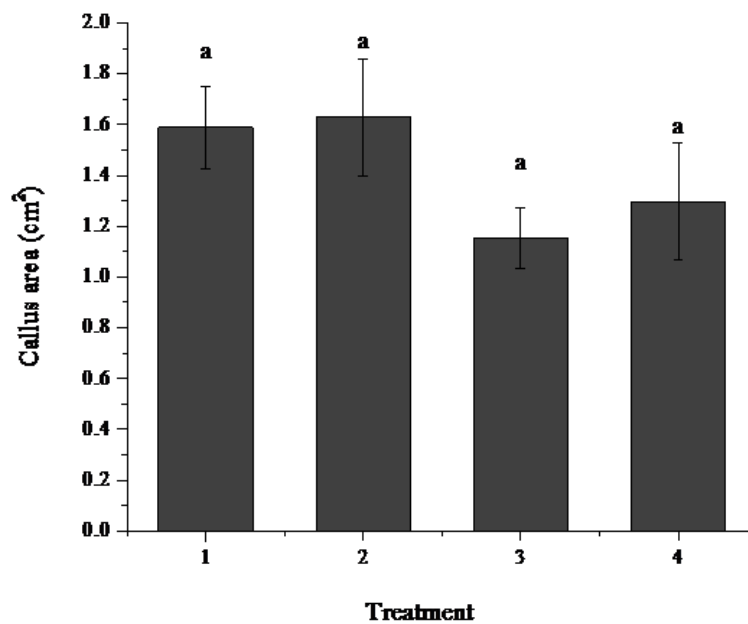


Fig. 8. Average areas of callus measured from wounded stem sections on ‘Elliot’ pecan seedlings from February 2015. Treatment numbers indicate the following treatments: 1- Control, 2- Buddy tape, 3- poly tape, 4- electrical tape.

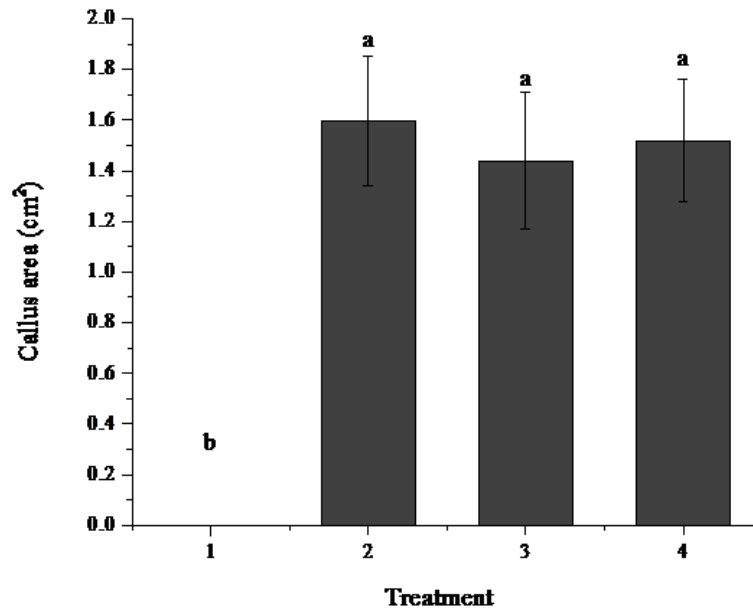


Fig. 9. Average areas of callus measured from wounded stem sections on ‘Elliot’ pecan seedlings from April 2015. Treatment numbers indicate the following treatments: 1- Control, 2- Buddy tape, 3- poly tape, 4- electrical tape.

Surface area measurements were also converted to percent of wounded area covered with callus tissue, as seen in Fig. 10.

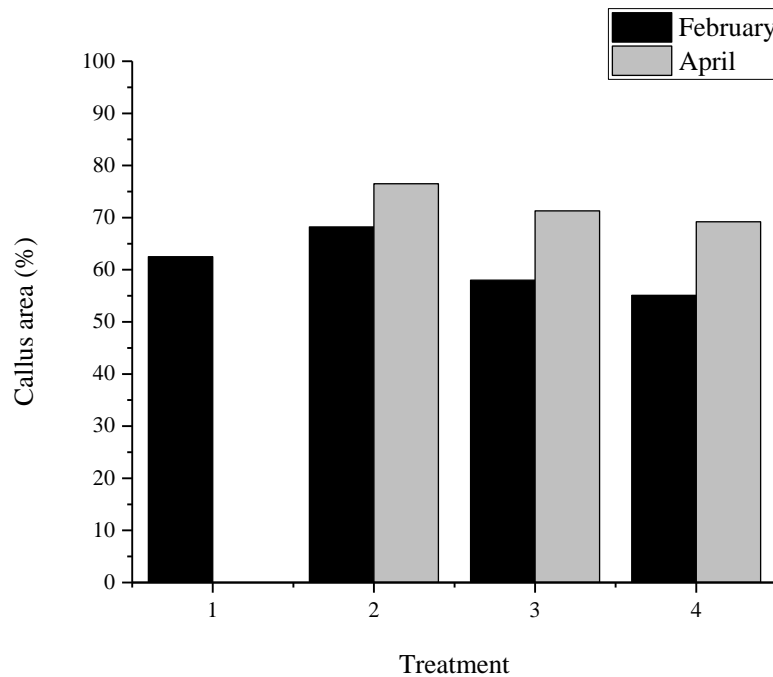


Fig. 10. Percent of wounded area covered with callus on ‘Elliot’ pecan seedlings. ‘Callus area’ refers to the percentage of callus tissue formed over a wounded area on the stem. Treatment numbers indicate the following treatments: 1- Control, 2- Buddy tape, 3- poly tape, 4- electrical tape.

No percent callus area was observed in April, as there was no surface area of callus tissue in the control treatment.

### 3.5 Image analysis and histology

The microscopy analysis showed a gap between rootstock and scion in both the unsuccessful and successful buds. However, gap size was drastically smaller in those

buds which had successful growth. Fig. 11 below shows a successful bud union with successful growth, while Fig. 12 shows a successful union with unsuccessful growth.

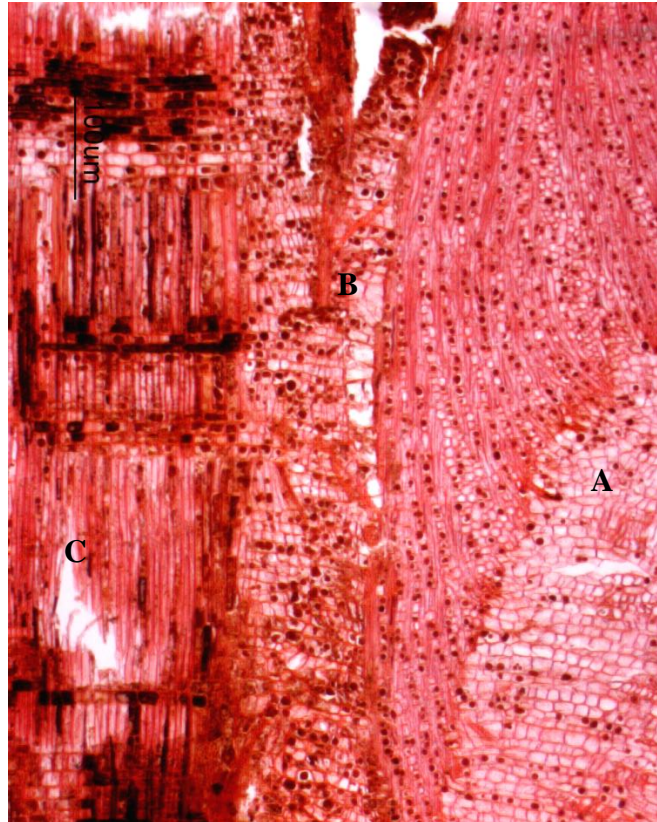


Fig. 11. Image of a successful bud union and growth (taken at 20x magnification) of pecan budded seedlings. Letters A, B and C indicate the scion, callus bridge formation, and rootstock tissues, respectively.

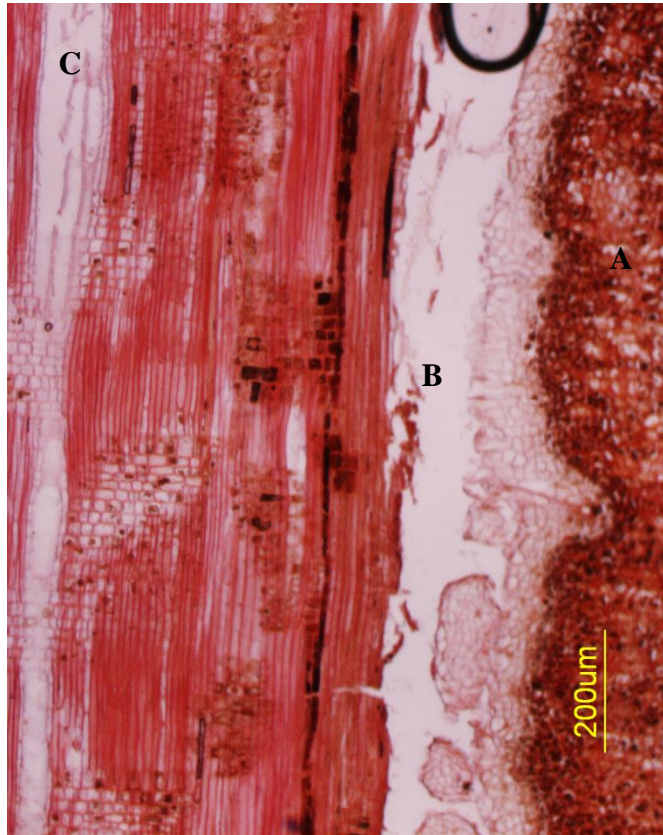


Fig. 12. Image of a successful bud union with unsuccessful growth (taken at 10x magnification) of pecan budded seedlings. Letters A, B and C indicate the scion, callus bridge location, and rootstock tissues, respectively.



## CHAPTER IV

### DISCUSSION AND CONCLUSION

Overall, the V-grafting experiment produced the most promising results. Variable success between both years of the V-grafting experiments could be a result of several factors such as environmental conditions, grafter's experience, cambial contact and wrapping materials. Most propagators consider a success rate of 75% or higher to be acceptable for pecan trees (Nesbitt et al., 2002). The electrical tape, used in both years of V-grafting, had the most success in the second year during April with 7 out of 8 (87.5% success) grafts successfully growing. Electrical tape is sticky and thick, and perhaps pulls the stock and scion closer together for better cambial contact. Singha (1990) stated that electric tape is easy to use and readily available, especially for amateur horticulturists, and more importantly reported no deleterious effects on the tree from the use of the tape. One reason for the partial success could be that the V-grafting also offers a larger contact area between stock and scion, giving them a better chance to align for callus bridge formation (Mng'omba and du Toit, 2013), as opposed to the T-bud method, which has a very small area of cambium contact between the stock and scion. Mng'omba and du Toit (2013) also stated that longer cut surface lengths of cambial contact offer a chance for stem sizes to better match up between unevenly sized stock and scion. During the grafting period in 2015, temperatures were warmer and more days of rain were received than the previous year. This could account for some differences observed between the years, as temperature and moisture are critical in callus and graft union

formation (Brison, 1986; Hartmann et al., 2011). Grafter experience could also be a contributing factor. The same person performed the grafts during both years, but 2014 was the first year for them to use the V-graft method. Technique may have improved, contributing to higher success rates in 2015. Vigor of the seedlings in the orchard may be another contributing factor to success compared to greenhouse experiments.

Container trees usually have small root systems and are frequently irrigated and fertilized, while field-grown trees develop a larger root system to find water and nutrients (McEachern, 2007). This could explain the very low success rates of both T-budding experiments on potted trees. In fact, Smith and Goff (2014) reported in their study on patch bud that vigorously growing rootstocks of varying size all had similar success, and that those buds placed on stock of lower vigor were not successful. Genetic diversity of the seedlings used for rootstocks could also be a contributing factor. The seedling nature of pecan accounts for much of its vigor and diversity in growth rate (Grauke, 2007; Sparks, 2005). One issue that could have affected the success rate of the upright T-budding is heavy sap flow, which could have entered the T-bud and soaked it to the point of preventing gas exchange for the healing process (Hartmann et al., 2011). The inverted T is often recommended for species that have higher sap flow, and pecan seedlings growing in greenhouse in constantly well-watered medium could potentially have had more active sap flow with constant growing conditions. This is supported by the microscopy images taken that showed that even among the few successful buds, the graft union was weak. The weak graft union could be a result of one or more of these factors.

Rooting on softwood pecan cuttings had a very low success (4%). Based on previous studies, this result is not surprising. While there was one successful cutting, this died soon after transplant. McEachern (1973) reported that transplant stage of cuttings was critical and requires much care not to damage fragile, new root growth. Early studies by Gossard (1944) stated that the young tissue of softwood cuttings may not have enough internal food reserves to produce both roots and shoots, therefore much assistance with hormones may be needed. While our study used a high concentration of IBA/NAA, perhaps additional studies with higher concentration ratio may be more successful.

Differences in callus production underneath the different wrapping materials were not significant among the treatments. We expected that the different wrapping materials would produce different results; however, it seemed that either the conditions or experiments did not allow this to happen. Studies by Singha (1990) found that apple trees whip grafted and covered with readily available wrapping materials such as parafilm, nursery tape, electrical tape and duct tape were all successful and shoots were of similar lengths at the end of the growing season. Smaller nurseries and growers may choose to take advantage of cheaper, more readily available materials such as electrical tape since studies show that there are no differences between growth when trees are grafted using these materials.

In conclusion, grafting still remains to be one of the better, if not the only commercially feasible, methods for propagation of pecan. While V-grafting does seem to offer some potential as a method of propagation, more studies should be conducted to

observe the long-term effects and outcome of the method. Propagation of pecan by cuttings would be ideal to provide more uniform rootstock; however, pecan trees still do not respond well to this method. Perhaps additional studies, along with future technology could offer success for pecan cuttings.

## REFERENCES

- Alexander, D.M. and W.J. Lewis. 1998. Budding techniques, p. 14-19. Grafting and budding fruit and nut trees: a practical guide. CSIRO.
- Allan, P., M.O. Brutsch, I.E. Smith, and B.N. Wolstenholme. 1980. Improved techniques for pecan propagation by hardwood cuttings. *Pecan Qrtly.* 14:7-14.
- Arpaci, S., O. Aksu, and H. Tekin. 1997. Determination of the best suitable grafting method on different pistachio rootstocks. *Proc. II Intl. Symp. Pistachios and Almonds.* Davis, CA p. 443-446.
- Brison, F.R. 1986. Pecan culture. Texas Pecan Growers Assoc. , College Station, TX.
- Ebrahimi, A., K. Vahdati, and E. Fallahi. 2006. Improved success of persian walnut grafting under environmentally controlled conditions. *Intl. J. Fruit Sci.* 6:3-12.
- Geneve, R.L. 2001. Plant propagation techniques: a historical perspective. *Comb. Proc. Intl. Plant Prop. Soc.* p. 370-375.
- Gerardi, L. 1954. Grafting methods adapted to nut trees. *Proc. Northern Nut Growers Assn.* 45: 41-43.
- Gossard, A.C. 1944. The rooting of pecan softwood cuttings under continous mist. *Proc. Amer. Soc. Hort. Sci.* 44:251-254.
- Graham, E.T. and W.R. Trentham. 1998. Staining paraffin extracted, alcohol rinsed and air dired plant tissue with an aqueous mixture of three dyes. *Biotech. Histochem.* 73:178-185.

- Grauke, L.J. 2007. Pecan rootstocks, p. 49-51. In: L.A. Stein and G.R. McEachern (eds.). Texas pecan handbook. Texas AgriLife Extension Service, College Station, TX.
- Griffin, J. and N. Bassuk. 1996. Preliminary progress on the asexual propagation of oaks. Combined Proc. Intl. Plant Prop. Soc. p. 487-494.
- Hare, R.C. 1977. Rooting of cuttings from mature water oak. South. J. Appl. For. 1:24-25.
- Hartmann, H.T., D.E. Kester, F.T. Davies, and R.L. Geneve. 2011. Plant propagation: principles and practices. 8th ed. Prentice Hall, Upper Saddle River, NJ.
- Ichikawa, H. 1958. A few findings in pecan propagation. J. Hort. Assn. Japan 27:101-107.
- Maguire, T. and R. Harun. 2007. Air root pruning to accelerate the growth of *Elaeagnus x ebbingei* from vegetative cuttings. <<http://www.docstoc.com/search/root-stock>>.
- McDowell, E.M. and B.F. Trump. 1976. Histologic fixatives suitable for diagnostic light and electron microscopy. Arch. Lab. Med. 100:405-414.
- McEachern, G.R. 1973. The influence of propagation techniques, the rest phenomenon, and juvenility on the propagation of pecan, *Carya illinoensis* (Wang) K. Koch, stem cuttings, Texas A&M Univ.
- McEachern, G.R. 2007. Methods of establishing a pecan orchard, p. 71-73. In: L.A. Stein and G.R. McEachern (eds.). Texas pecan handbook. Texas AgriLife Extension Service, College Station, TX.

- McFadden, S.E. 1962. Comparison of wrapping and scion coating materials in grafting pecans. *Proc. Fla. State Hort. Soc.* 75:421-426.
- Mng'omba, S.A. and E.S. du Toit. 2013. Effect of diagonal cut surface length on graft success and growth of *Mangifera indica*, *Persia americana*, and *Prunus persica*. *HortScience* 48:481-484.
- Nesbitt, M.L., W.D. Goff, and L.A. Stein. 2002. Effect of scionwood packing moisture and cut-end sealing on pecan graft success. *HortTechnology* 12:257-260.
- Nishihira, T. 2013. Buddy tape description, Shigyo Company, Truckee, CA.
- NRCS. 2014. Soil survey for Burleson County, Texas. Web soil survey, U.S. Dept. Agr. 11 November 2014. <<http://websoilsurvey.nrcs.usda.gov/>>.
- Preece, J.E. 2003. A century of progress with vegetative plant propagation. *HortScience* 38:1015-1025.
- Preece, J.E., J.W. Van Sambeek, P.H. Henry, and J. Zaczek. 2002. Forcing the tissue, *American Nurseryman* Vol. 196, pp. 26-29.
- Reed, C.A. 1926. Nut-tree propagation. U.S. Dept. Agr. Bul. 1501.
- Ruzin, S.E. 1999. Plant microtechnique and microscopy. Oxford University Press, Inc. , New York, NY.
- Singha, S. 1990. Effectiveness of readily available adhesive tapes as grafting wraps. *HortScience* 25:579.
- Smith, M.W. and H. Chiu. 1980. Seasonal changes in the rooting of juvenile and adult pecan cuttings. *HortScience* 15:594-595.

- Smith, M.W. and W.D. Goff. 2014. Patch budding pecan: girdling, tipping, age, and size of budwood and rootstock for budding; girdling, 2,3,5-triodobenzoic acid and 6-benzylaminopurine for bud forcing. HortTechnology 24:512-518.
- Sparks, D. 2005. Adaptability of pecan as a species. HortScience 40:1175-1189.
- Taylor, G. and R.E. Odom. 1969. Relationship of carbohydrate and nitrogen content to rooting in pecan, *Carya illinoensis*, stem cuttings as influenced by preconditioning treatments prior to propagation. The Plant Propagator 15:5-10.
- Taylor, G.G. and R.E. Odom. 1970. Some biochemical compounds associated with rooting of *Carya illinoensis* stem cuttings J. Amer. Soc. Hort. Sci. 95:146-151.
- Thompson, T.E. and L.J. Grauke. 1991. Pecans and other hickories (*Carya*), p. 839-906. In: J.N. Moore and J.R. Ballington Jr. (eds.). Acta Horticulturae 290: Genetic resources of temperate fruit and nut crops. Acta Hortic.
- Wally, Y.A., M.M. El-Hamady, S.T. Boulos, and M.A. Salama. 1980. Physiological and anatomical studies on pecan hardwood cuttings. Egypt. J. Hort. 8:89-100.
- Whatley, B.T., S.O. Thompson, and J.H. Jefferson. 1966. Propagation of *Carya illinoensis* (pecan) from cuttings. Comb. Proc. Intl. Prop. Soc 16:205-209.
- Youssef, N., B.W. Wood, C.C. Reilly, and I. Yates. 1993. Use of ethephon for rooting of pecan leafy stem cuttings. Proc. Northern Nut Growers Assn. 84:148-155.