ABSTRACT

FREY, HALEY HIBBERT. Factors Affecting Graft Success and Early Growth of Fraser fir (*Abies fraseri*). (Under the direction of Dr. John Frampton.)

Two studies were conducted to investigate factors influencing graft success and subsequent growth of Fraser fir (*Abies fraseri* (Pursh) Poir). The traditional time of grafting (late winter/early spring) was compared with eight summer/early fall grafting dates from mid-July through mid-October. Optimal grafting success (95%) was in the late winter/early spring (April) while the scions were dormant and the rootstocks were becoming active. Success of subsequent grafting dates decreased from 52% (14 July) to 0% (20 Oct.). Shade improved summer graft success (52% with, 38% without). Irrigation did not affect graft success or growth. Grafting of stored dormant scion material in summer/early fall was not successful (<1%). In a second study, success and subsequent growth of Fraser fir cleft grafts were studied in relation to season of grafting (late summer vs. spring), grafter, and origin of scion material (height in the tree and lateral branch order (first vs. second). Grafting in early September yielded only 3% success compared to 70% for mid-April. Grafters had significantly different graft success (86% for Grafter 1 with 5 years experience vs. 54% for Grafter 2 with 1 year experience). First-order laterals from the upper crown yielded the best graft success and growth.
(except plagiotropism). First-order laterals were better than second-order laterals for all growth measurements.
Factors Affecting Graft Success and Early Growth of Fraser fir (Abies fraseri)

by
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Chair of Advisory Committee

Dr. Frank A. Blazich

Dr. L. Eric Hinesley
Dedication

This is dedicated to the five-year old Haley. I hope she’s proud.
Biography

Haley Hibbert Frey grew up in Amherst, Virginia, on a 1 acre farm. From a young age, Haley knew she wanted to be a scientist. She left Amherst County High School in 1995 after her junior year to go to the Academy at St. Andrew’s Presbyterian College. Being away from the mountains with the trees completely surrounding her, she realized her passion for plant sciences. She transferred to Queens College in Charlotte, NC to complete her Bachelors of Science (2001). While enrolled, Haley worked as a gardener at Wing Haven, a botanical garden and bird sanctuary. In 2003, she went back to Queens, to teach microbiology labs for the Presbyterian School of Nursing.

While working as an instructor, she enrolled in graduate classes in Soil Science at the University of North Carolina at Charlotte. There she led a project observing the correlation of soil pH and texture on the growth of Leyland cypress along a hill slope (presented at the Eastern NC Christmas Tree Growers Association Annual Winter meeting in February 2007). She then applied and was accepted into the Master of Science program in the NCSU College of Natural Resources. During her final semester, Haley married Greg Frey on September 27, 2008 in her hometown of Amherst, VA.

Following graduation, she will return to Charlotte to pursue a career in natural resources.
Acknowledgements

I would first like thank the members of my graduate committee who have been great references and have guided me in the direction I needed to go throughout my research. Drs. John Frampton, Frank Blazich, and Eric Hinesley, have all had amazing patience with a novice scientist like myself. They have all been extremely helpful and enthusiastic throughout my MS studies and research. I would also like to acknowledge the staff of the North Carolina State University Christmas Tree Genetics Program (Anne Margaret Braham, Keith Reinhardt, and Brenda Flood) for additional help and support.

I would also like to thank Christmas tree growers, Herbie Johnson and Benny Vance of Crossnore, NC, who provided me with my field sites and plant material, Jim Rideout of the NC Division of Forest Resources Linville River Nursery, and Avery County Extension Director, Jerry Moody. Special thanks go to Avery County IPM Technician, Doug Hundley and his wife Meg. They were my Avery County family and provided me so much more than a place to stay. None of this could have happened without funding from the North Carolina Agricultural Research Service via the North Carolina State University Christmas Tree Genetics Program.
I would also like to thank my parents, Bill and Meg Hibbert, my sister and brother-in-law, Meredith and Frederick Gerber, my zoo, Jeter, Sam, and Tela, and most of all, my husband and my rock, Greg Frey.
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Introduction

In North Carolina, Christmas tree farming is over a $100 million industry. North Carolina has become the second largest Christmas tree producing state (behind Oregon) harvesting 5.5 to 6 million trees annually. There are over 12,000 hectares used by over 1500 growers for Christmas tree production in NC (NCDA & CS, 2004). The popular native species, Fraser fir \([\textit{Abies fraseri} (\text{Pursh}) \text{Poir}]\) accounts for 96% of all Christmas trees grown in NC (Rosier et al., 2006). It grows naturally at elevations above 1370 m on isolated mountain tops in the Southern Appalachians from southwest Virginia, through western North Carolina, and into eastern Tennessee (Liu, 1971). Fraser fir is grown for its fragrant aroma, dark green foliage, strong braches, and natural Christmas tree shape (Frampton, 2001).

One problem growers are currently facing is phytophthora root rot, a fatal disease caused by \textit{Phytophthora cinnamomi} Rands. When an area becomes infested, there is no known way to completely eliminate the pathogen from the soil, which can ravage entire fields. This can lead to abandonment of a field site which otherwise would be ideal for growing Fraser fir (Frampton, 2001; Hinesley and Frampton, 2002).

A possible solution is to graft Fraser fir scions onto rootstocks of compatible fir species that are resistant to Phytophthora. Momi fir (\textit{Abies
*firma* Sieb. & Zucc.) and Turkish fir (*Abies bornmuelleriana* Mattf.) are the two most resistant fir species to *P. cinnamomi*, respectively (Benson et al., 1998). The use of Phytophthora resistant *Abies* species as rootstocks for grafting Fraser fir scions may allow reclamation of previously infested fields that have been deemed unusable (Hinesley and Frampton, 2002).

In the Christmas tree industry, Fraser fir is typically propagated by seed. Recently grafting has generated interest among Christmas tree growers to produce *Abies* planting stock that has resistance to Phytophthora (Hinesley and Frampton, 2002). Grafting of Fraser fir typically occurs in the spring, a busy time for the growers. Grafting in the late summer has been suggested as an alternative.

No research has been performed to date investigating the possible season effects on Fraser fir graft success nor possible benefits of shade and irrigation. The use of stored Fraser fir dormant scions also has not been investigated. In the first study, the objectives were to: 1) compare the success and growth of grafting freshly collected Fraser fir scions onto Turkish fir rootstocks during the traditional time (April) with 8 biweekly grafting times from mid-July through mid-October and 2) assess the effect of shade and irrigation treatments on graft success and growth for each grafting time. A supplemental study was also carried out to evaluate using dormant Fraser fir scions collected during April and stored in a freezer for grafting onto Turkish
fir rootstocks during the mid-July through mid-October times.

The use of scions from different crown positions (lower vs, upper) and from different lateral branch orders (first and second) has not been studied. Therefore, the objectives of the second study were to investigate effects of 1) season (late summer versus spring), 2) grafter experience, 3) effect of scion origin based on height (five heights), and 4) lateral branch order (first and second) on graft success and subsequent growth.
Literature Cited


Chapter 1

Grafting Fraser fir (*Abies fraseri*): Effect of Month of Grafting, Shade, and Irrigation

(In the format appropriate for submission to HortScience)
Grafting Fraser fir (*Abies fraseri*): Effect of Month of Grafting, Shade, and Irrigation

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Grafting Fraser fir \textit{(Abies fraseri)}: Effect of Month of Grafting, Shade, and Irrigation

\textit{Additional index words:} Abies fraseri, Abies bornmuelleriana, vegetative propagation, seasonal effects, \textit{Phytophthora cinnamomi}

\textbf{Abstract.} Grafting Fraser fir \textit{[Abies fraseri (Pursh) Poir.]} scions onto rootstocks of Turkish fir \textit{(Abies bornmuelleriana Mattf.)} is a strategy used by some Christmas tree growers in the southern Appalachian Mountains of North Carolina to reduce losses by phytophthora root rot caused by \textit{Phytophthora cinnamomi} Rands. This study compared the traditional time of grafting (late winter/early spring) with eight summer/early fall grafting dates from mid-July through mid-October. Shade and irrigation treatments were also superimposed on the grafting times. Results indicate that to ensure optimal grafting success of 95\%, grafting should be performed in the late winter/early spring (April) when scions are dormant and the rootstocks are becoming active. With summer/fall grafting of fresh scions, graft success decreased from 52\% in July to 0\% in October. Shade (40\%) improved summer graft success (52\% with, 38\% without). Irrigation did not significantly affect graft
success or growth. Grafting in summer/early fall of stored dormant scion material was not successful (< 1%).

North Carolina Christmas trees are worth over $100 million annually in wholesale value. Approximately 5.5 to 6 million Christmas trees are harvested yearly, ranking North Carolina second behind Oregon in the nation for the number of trees harvested. There are over 1500 Christmas tree growers in North Carolina utilizing over 12,000 ha for Christmas tree production. Fraser fir accounts for 95% of harvested Christmas trees produced from 24 western North Carolina counties (NCDA & CS, 2004). Fraser fir is grown for its fragrance, soft, dark green needles, strong branches, and natural Christmas tree shape (Frampton, 2001). Fraser fir is indigenous to isolated mountain tops at elevations between 1370 and 2037 m in southwestern Virginia, western North Carolina, and eastern Tennessee (Liu, 1971). Two problems facing North Carolina Christmas tree growers are the balsam wooly adelgid (*Adelges piceas* Ratz.) and phytophthora root rot.

Phytophthora causes a fatal root rot that has spread rapidly throughout western North Carolina soils and is nearly impossible to eradicate. It can take as few as 2 or 3 weeks from infection to death of Fraser fir (Benson, et al., 1998). Thus, there is a need in the region for planting stock of other fir species that are resistant or tolerant to this pathogen. To ameliorate the
impact of this disease, some Christmas tree growers in the region are grafting Fraser fir onto rootstocks of more resistant fir species. Grafting onto resistant rootstocks is a widely accepted method of managing phytophthora root rot (Hinesley and Frampton, 2002). Turkish fir and momi fir (*Abies firma* Sieb. & Zucc.) were resistant to *P. cinnamomi* in a controlled inoculation study (Benson et al., 1998). While momi fir was the most resistant, it has sharp, prickly, light green needles and breaks bud early in western North Carolina leaving it susceptible to late frosts. On the other hand, Turkish fir was somewhat less resistant than momi fir, and has desirable Christmas tree qualities more similar to Fraser fir. Using Phytophthora-resistant *Abies* species as rootstocks or transplants may allow use of previously infested fields (Hinesley and Frampton, 2002).

Typically, Fraser fir is grafted early spring (April), when the rootstock and scion are both dormant. This, however, is a busy time for the growers. The opportunity to graft at other times of the year (such as late summer or early fall) would allow Christmas tree growers more flexibility. Therefore, the objectives of the main study of this investigation were to: 1) compare success and growth of grafting freshly collected Fraser fir scions onto Turkish fir rootstocks during the traditional time (April) with 8 biweekly grafting times from mid-July through mid-October and 2) assess the effect of shade and irrigation treatments on graft success and growth for each grafting time.
A supplemental study was also conducted to evaluate using dormant Fraser fir scions collected during April and stored at -1 °C for grafting onto Turkish fir rootstocks during the mid-July through mid-October.

**Materials and Methods**

*Study design.* This study was conducted at the North Carolina Division of Forest Resources Linville River Nursery near Crossnore. In April 2007, one thousand 3-1 (3 years grown in seed bed, 1 year grown in transplant bed) Turkish fir rootstocks were planted approximately 20 cm apart in raised, freshly tilled 1.2-m-wide raised nursery beds in the flood plain of the Linville River. The soil was in the Crossnore-Jeffery soil series (mixed mesic, Umbric Dystrochrepts). The rootstocks were planted in four blocks with each block consisting of three treatment plots of 50 rootstocks and one plot of 100 rootstocks. The smaller plots were 2 m long (10 rows x 5 columns of rootstocks). The larger plots were designed to accommodate the supplemental study and were 4 m long with 20 rows and five rootstocks in each column.

*Main study.* The main study was a randomized complete block design with a 2 x 2 factorial arrangement of treatments. There were two shade treatments (shade and no shade) and two irrigation treatments (irrigation and no irrigation).
The smaller plots were for shade + no irrigation, no shade + irrigation, and shade + irrigation treatments while the larger plots accommodated the control (no shade + no irrigation) treatment. Every 2 weeks starting 14 July 2007 through 20 Oct. 2007, single cleft grafts (Garner, 1979; Hinesley and Frampton, 2002) were performed with freshly collected scions. Terminal shoots were collected from 80 actively growing Fraser fir trees from a Christmas tree farm near Crossnore. Scions averaged 10 cm long and approximately 1 cm in diameter. Five grafts were randomly assigned to each of the 16 plots for each of the eight grafting times. The grafts were secured with 0.95 x 20.3 cm grafting rubber bands and then sealed with Doc Farwell’s Grafting Seal (Farwell Products, LLC, Wenatchee, Wash.).

*Supplemental study.* The supplemental study was a randomized complete block design. It was established only in the no shade + no irrigation (control) treatment plots which also accommodated grafts for the main study. The Fraser fir scions used for the supplemental study were collected on 6 Apr. 2007 from the same Christmas tree farm as mentioned in the main study. Two hundred scions were collected from 2-m-tall Christmas trees of an unknown seed source. Twenty dormant scions were then grafted randomly in the four control plots (five in each). The remaining 180 dormant scions were then placed in a plastic bag, the bag placed in a cooler, and the cooler was stored at -1 °C. For each grafting date in the main study, 20 stored dormant
Fraser fir scions were grafted onto randomly assigned Turkish fir rootstock in the four control plots.

*Shade and irrigation treatments.* On 10 July 2007, shade and irrigation treatments were installed. Irrigation was provided from a nearby building’s water supply through a filter and then to a 2-cm diameter garden hose 67 m to the study beds. The hose fed a 2.5-cm diameter polyvinyl chloride (PVC) pipe that ran the length of the two study beds (30 m). In each irrigation plot, the PVC pipe was joined to a 1-m section of 2.5 cm PVC pipe with 60 cm of 2.5-cm rubber garden hose attached to allow the tractors used at the nursery to pass over the irrigation system between the beds. The hose was then attached to a 1.2-m section of 2.5 cm PVC pipe. Four Blazing Saddle Tees (Blazing Products, Chesterfield, Mo.) were spaced every 30 cm on the PVC pipe. A 2-m long NETAFIM drip line hose (NETAFIM LTD., Tel Aviv, Israel) (emitters spaced 30 cm apart) was attached to each tee. The hoses were attached so the emitters were in a staggered pattern to create an overlap in irrigation. Each hose was closed at the ends with a figure eight clamp and held in place in the beds using metal U-shaped stakes. A pressure gauge was installed at the end of the main PVC pipe to measure water pressure. Irrigation flow rate for 1 emitter was tested and was found to be 465 mL·h⁻¹.

One HOBO Pro RH and Temperature Data Logger (Onset Computer Corp., Bourne, Mass.) was installed in each of the four plots in Block 1 to
record mean air temperature and mean relative humidity every hour. Shade treatments were installed using 2.5-cm rebar frames and 40% shade cloth.

New shade tents were installed 28 July 2007 to provide more room for the rootstocks to grow. Three PVC arches were constructed in each of the eight shaded plots. The anchors were 60-cm, 2.5-cm PVC sections with 2-cm PVC arches bent into the anchors. Two sections of 40% shade cloth were sewn together to form a 2.5-m wide, 4.2-m long tent to cover the arches to ground level on all sides. A 2.5-cm diameter plastic rain gauge was installed next to the main irrigation valve, and rainfall was checked twice weekly. A total of 25 mm of rain was required weekly or the irrigation was turned on to provide the weekly 25 mm.

Irrigation was discontinued 28 Oct. 2007 and shade removed 8 Nov. 2007. The grafts were left to over-winter in the beds. Beginning 8 May 2008, grafts were checked for budbreak every 2 days until all budbreak ceased (16 June 2008). Shade and irrigation treatments were again resumed 13 May 2008. At the end of the active growing season on 3 Aug. 2008, the following data were collected for each successful graft: leader elongation (millimeter), longest lateral bud elongation (millimeter), number of terminal buds broken, number of new lateral buds on the leader (elongated scion), and number of lateral buds per decimeter.

Statistical analysis. The main study was a randomized complete block
design with a 2 x 2 factorial arrangement of treatments. Except for graft success, data were analyzed for only the successful grafting dates (6 Apr., 14 July, 28 July, and 10 Aug.) for the main study using Proc GLM (SAS Inst., Inc., 2003), and all effects were considered fixed. An analysis of variance (ANOVA) was conducted to test for differences among main effects (block, date, shade, and irrigation) and all possible interactions for leader elongation, number of terminal buds broken, longest lateral length, number of lateral buds on leader, and number of lateral buds per length at $P < 0.05$. Least squared means were used for comparisons. Since there were no significant interactions for any growth characteristics, interaction terms were dropped from the model.

For the binomial response variable (graft success), a generalized linear model with a logistic link function was performed with Proc GENMOD (SAS Inst., Inc., 2003) using the same classification variables employed in the ANOVA for continuous variables. It was necessary to omit all of the possible interaction effects from the model to achieve convergence for the response variable. Type III likelihood ratio statistics were used to evaluate significant differences when detected. A Wald chi-square test was employed to assess differences among least squares means.

The supplemental study was a randomized complete block design. Data (except graft success) were analyzed for only the successful grafting
times using Proc GLM, and all variables were fixed. An ANOVA was conducted to test for differences among main effects (block and date) for leader elongation, number of terminal buds broken, longest lateral length, number of lateral buds on leader, and number of lateral buds per length at $P \leq 0.05$. Least squared means were used for comparisons. Since there were no significant interactions for any growth measurements, interaction terms were removed from the model.

The supplemental study binomial response variable (graft success) was analyzed with a generalized linear model with a logistic link function was performed with Proc GENMOD using the same classification variables employed in the ANOVA for continuous variables. It was necessary to omit all of the possible interactions (nonsignificant) from the model to achieve convergence for the response variable. Type III likelihood ratio statistics were used to evaluate effect differences. When detected, a Wald chi-square test was employed to assess differences among least squares means.

Results

**Main study.** Effect of grafting date was significant for graft success, mean leader elongation, longest lateral bud elongation, and number of lateral buds on the leader. Graft success was low (30% to 52%) for the first three
summer grafting dates and unsuccessful (0%) for 24 Aug. through 20 Oct. All unsuccessful summer grafting dates were not included in the ANOVAs for other traits.

Grafting date affected date of budbreak. The 28 July and 10 Aug. grafts first started breaking bud around 8 May 2008 (Fig. 1). Grafts made 6 Apr. and 28 July started breaking (10 May 2008), almost 2 weeks prior to budbreak (22 May 2008) of the seedlings from which the scion material originated. The remaining grafting dates (24 Aug. through 20 Oct.) did not start breaking bud until 26 May 2008. The pattern of budbreak for the four successful grafting times (6 Apr., 14 July, 28 July, and 10 Aug.) was similar. They all peaked around 1 June 2008, even though they all had different graft success values.

Shade was only significant ($P < 0.05$) for graft success (Table 1). Plots with the shade treatment had a success rate of 52% compared to 38% without shade (Table 2). Irrigation was not significant for success or any of the growth traits. There were no significant shade x irrigation interactions.

Supplemental study. Grafting date significantly affected ($P < 0.05$) graft success, mean leader elongation, number of terminal buds broken, mean lateral elongation, and number of newly formed lateral buds on the leader (Table 3). Graft success was 95% in April, 55% for the first summer grafting date (14 July 2007), 30% for 28 July 2007, and 50% for 10 Aug. 2007
Grafting in the summer using stored dormant scion material was not successful (0% for all dates). None of the grafts performed in summer/early fall using stored dormant scion material were successful (Table 4).

Similarly, grafting date also affected mean leader elongation and mean lateral elongation. The April grafting date had the shortest elongation with 117 mm for mean leader length and 99 mm for mean lateral elongation (Table 4). For both growth measurements, mean elongation increased for later grafting dates.

Mean number of terminal buds broken was highest (6.1) for the April grafting date, and decreased with later grafting dates. The mean number of lateral buds formed on the leader was lower for the first two July control plot grafting dates (6 Apr. 2007 and 14 July 2007, 5.9 and 5.8, respectively), and increased later (Table 4).

**Discussion**

Results indicate grafting date significantly affected graft success and subsequent growth. Graft success was 95% for April, 52% for 14 July, 30% for 28 July, 52% for 10 Aug., and < 1 % for 24 Aug. through 20 Oct. (Table 2). Grafting in early spring, when the scions were dormant and the rootstocks
were just becoming active was the best time to ensure graft success in Fraser fir (95%).

It is common practice to graft conifers, such as slash pine (Pinus elliottii Englem.), when the rootstock is active and the scion is dormant in winter or early spring (Mergen, 1955). The first week of April is the most successful time for grafting Douglas fir [Pseudotsuga menziesii (Mirb.) Franco] in the Pacific Northwest (vs. the first week of each month from May through September) (Copes, 1973). In pepper (Piper nigrum L.), grafting during late winter/early spring yields better results (> 90% success) than summer/late summer grafting (Vanaja et al., 2007). By July, shoot elongation of Fraser fir is ending, and ceases by August/September, but the cambium is still active (Lavender, 1981).

Frost hardening begins in September for some Abies species (Abies balsamea (L.) Mill.; Mellerowicz, 1992) which is also considered to be the end of the growing season due to the cessation of cambial growth (Mitchell, 1995). The first frost at the Linville River Nursery normally occurs in the last week of September.

Optimal day/night temperatures for growth of Fraser fir seedlings are 22 to 26/14 to 18 °C (Hinesley, 1981). Average mean day/night temperatures during the successful grafting dates (14 July through 10 Aug.) were 24/13 °C. The temperature at the graft union has a direct effect on Douglas fir graft
success (Copes, 1973). High day temperatures with warm nights are stressful for Fraser fir (Hinesley 1981). The increase in temperature directly affects development of a good graft union (Karadeniz, 2005), and graft failure during summer/late summer may be due to high temperatures (MacDonald, 1986). After 6 Aug. 2007, the day temperatures increased above the ideal range and then decreased to within the ideal range from 28 Aug. through 3 Sept. Temperatures increased above the range again from 4 Sept. to 11 Sept. After 13 Sept. 2007, both the day and night temperatures decreased below the ideal range (Fig. 2).

Shoot growth of the scions was also affected by grafting date. The earlier the material was grafted, the higher the success, but the shorter the elongation of the leader and the laterals (Table 2).

Mean shoot growth of the successful later summer grafts were approximately twice as long with 201 mm for 28 July and 279 mm for 10 Aug. (24 Aug. was 353 mm, but was not included in the analyses due to low success rate of < 1%) as both the 2007 and 2008 mean shoot growth of the grafts performed in April (128 mm for 2007 and 117 mm for 2008). This may have been due to a selection process of the most vigorous scion/rootstock combinations in the summer grafting periods, therefore they elongate more. However, shoot growth of the 10 Aug. grafts were longer than the maximum lengths of the growth in 2007 and 2008 for the April grafts and the 14 July
grafts. We cannot explain this phenomenon. Shade improved the success of late summer grafts (52% shade vs. 38% no-shade) (Table 2). Shade may have lowered ambient air temperatures, and thus improved graft success.

Water stress can affect graft success in conifers, and transpiration increases as temperature increases (Beeson and Proebsting, 1988). Failure of grafts can be due to the inability of the scions to receive water from the rootstocks before the graft union has formed (Barnett and Weatherhead, 1989; Mergen, 1955). In the present investigation, water stress was likely not an issue. Weekly rainfall was sufficient (> 25 mm) for all but 4 weeks of the 14 week study. When weekly rainfall was not sufficient (weeks of 11 Aug., 10 Sept., 24 Sept., and 9 Oct.), supplemental irrigation made up the difference for irrigation treatments only. Irrigation, however, was not significant for graft success or any growth measurements suggesting that water stress was not a primary factor contributing to unsuccessful graft union formation in this study.

In the supplemental study, use of stored dormant scion material for grafting Fraser fir in the summer was not successful (0%). The dormant scion material used for this study was stored for a range of 14 weeks to 28 weeks in a plastic bag placed inside of a cooler and the cooler maintained at -1 °C without moisture, so they might have desiccated. Douglas fir scions had a water deficit after 7 d of cold storage (Copes, 1969) and only had a success rate of < 40% after 14 d. Grafting Sitka spruce (Picea sitchensis Bong.)
scions immediately after collection may ensure success of > 90% (Barnett and Weatherhead, 1989). Use of stored dormant Fraser fir scions was not successful for this study; however, dormant Fraser fir scions can be held for several weeks, especially at -2.2 to -1.1 °C, with no ill effects. Unless more successful storage techniques can be developed, it is recommended to graft Fraser fir in the early spring with freshly collected dormant scion
Literature Cited


Table 1. ANOVA for successful Fraser fir grafts performed 14 July 2007, 28 July 2007, and 10 Aug. 2007 on Turkish fir rootstocks near Crossnore, North Carolina. There were no significant interactions.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Success</th>
<th>Leader length</th>
<th>No. buds broken</th>
<th>Lateral length</th>
<th>No. lateral buds</th>
<th>No. lateral buds/length</th>
</tr>
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<tbody>
<tr>
<td>Block</td>
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<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Grafting date</td>
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<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Shade</td>
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<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Irrigation</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, * Nonsignificant or significant at $P \leq 0.05$, respectively.
Table 2. Least square means of successful Fraser fir grafts performed on 14 July 2007, 28 July 2007, and 10 Aug. 2007 onto Turkish fir rootstocks near Crossnore, North Carolina.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Grafting date</th>
<th>Shade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14-Jul-07</td>
<td>28-Jul-07</td>
</tr>
<tr>
<td>Success (%)</td>
<td>52 b</td>
<td>30 a</td>
</tr>
<tr>
<td>Leader length (mm)</td>
<td>142 a</td>
<td>201 b</td>
</tr>
<tr>
<td>Lateral length (mm)</td>
<td>89 a</td>
<td>102 a</td>
</tr>
<tr>
<td>Lateral buds (no.)</td>
<td>6.2 a</td>
<td>7.7 a</td>
</tr>
<tr>
<td>Lateral buds/length (no./dm)</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Any two means within a row followed by the same letter are not significantly different at $P \leq 0.05$ according to a Wald Chi-square test.

NS Nonsignificant at $P \leq 0.05$. 
Table 3. ANOVA for successful Fraser fir scions grafted onto Turkish fir rootstocks in control plots performed in 2007 near Crossnore, North Carolina. There were no significant interactions.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Success</th>
<th>Leader length</th>
<th>No. buds broken</th>
<th>Lateral length</th>
<th>No. lateral buds</th>
<th>No. lateral buds/length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>3</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Date</td>
<td>3</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, * Nonsignificant or significant at P ≤ 0.05, respectively.
Table 4. Least square mean comparisons for successful 2007 Fraser fir scions grafted onto Turkish fir rootstocks in control plots near Crossnore, North Carolina.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6-Apr-07</td>
</tr>
<tr>
<td>Success (%) (overall)</td>
<td>95 a</td>
</tr>
<tr>
<td>Success (%) (dormant grafts)</td>
<td>95</td>
</tr>
<tr>
<td>Success (%) (non-dormant grafts)</td>
<td>NA</td>
</tr>
<tr>
<td>Leader length (mm)</td>
<td>117 a</td>
</tr>
<tr>
<td>Lateral length (mm)</td>
<td>99 a</td>
</tr>
<tr>
<td>Lateral buds (no.)</td>
<td>5.9 a</td>
</tr>
<tr>
<td>Terminal buds broken (no.)</td>
<td>6.1 a</td>
</tr>
</tbody>
</table>

Any two means within a row followed by the same letter are not significantly different at $P \leq 0.05$ according to a Wald Chi-square test.

NS Nonsignificant at $P < 0.05$. 

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Fig. 1. Timing of budbreak in 2008 of Fraser fir scions grafted onto Turkish fir rootstocks in 2007 (by date grafted) near Crossnore, North Carolina.
Fig. 2. Daily day and night temperatures in Crossnore, North Carolina for July through October 2007 (provided by http://averyweather.com/Archive/Crossnore).
Chapter 2

Grafting Fraser fir (*Abies fraseri*): Effect of Scion Origin (Crown Position and Branch Order) and Season

(In the format appropriate for submission to HortScience)
Grafting Fraser fir (*Abies fraseri*): Effect of Scion Origin
(Crown Position and Branch Order) and Season

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___________________________________

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Grafting Fraser fir (*Abies fraseri*): Effect of Scion Origin (Crown Position and Branch Order) and Season

Additional index words: *Abies fraseri*, vegetative propagation, grafting, plagiotropism, topophysis, maturation, crown position

**Abstract.** Success and subsequent growth of Fraser fir (*Abies fraseri* (Pursh) Poir.) cleft grafts were studied in relation to season of grafting (late summer vs. spring), grafter, and origin of scion material [height in the tree and lateral branch order (first vs. second)]. Grafting in early September yielded only 3% success compared to 70% in mid-April. Grafters had significantly different graft success (86% for Grafter 1 with 5 years experience vs. 54% for Grafter 2 with 1 year experience). Grafting success and subsequent growth were best for scions taken from the upper crown. First-order branch tips yielded significantly better results than second-order branch tips for all growth measurements.

North Carolina is the second largest Christmas tree producing state, behind Oregon, annually harvesting 5.5 to 6 million trees worth a
reported $100 million. There are over 1500 Christmas tree growers using 12,000 ha for Christmas trees production in North Carolina (NCDA & CS, 2004). The native species, Fraser fir (Abies fraseri) accounts for 96% of all Christmas trees grown in North Carolina (Rosier et al., 2006). In its natural habitat, it occurs at elevations above 1370 m in the southern Appalachian Mountains from southwest Virginia, through western North Carolina, and into eastern Tennessee (Liu, 1971). Fraser fir is grown for its fragrant aroma, dark green foliage, and natural Christmas tree shape, and strong branches (Frampton, 2001).

Fraser fir is typically propagated by seed, but there is also interest in grafting Fraser fir onto rootstocks of other Abies species with more resistance to root rot caused by Phytophthora cinnamomi Rands (Hinesley and Frampton, 2002). Phytophthora resistant Abies species can be used in previously infested fields that have been abandoned. Additionally, grafting provides an opportunity to clonally propagate select trees with desirable Christmas tree phenotypes.

Grafting of conifers is performed in the early spring when stock plants are dormant, but spring is a busy time for Christmas tree growers due to the intensity of insect and disease scouting and field applications of pesticides and herbicides. Although grafting in late
summer could be an alternative grafting time for growers, there is no research concerning possible seasonal effects on Fraser fir grafting success and subsequent growth. Another issue is availability of desirable grafting material. The normal procedure is to use dormant scion material from the upper crown of the tree. Comparisons of Fraser fir scions from upper vs. lower crown branches and from different branch positions (first-order and second-order) have not been investigated.

Plagiotropism is radial asymmetry in growth. It can reduce uniformity of rooted stem cuttings and grafted material and decrease overall shoot growth (Timmis et al., 1992). The most obvious problem with plagiotropism is that there is not a vertical leader; it continues growing like a branch. If plagiotropism is negligible, using different portions of the tree for scions could increase the availability of material for grafting Fraser fir. Therefore, the objectives of this study were to investigate effects of 1) season (late summer vs. spring), 2) grafter experience, 3) effect of scion origin based on height (five heights), and 4) lateral branch order (first and second) on graft success and subsequent growth.
Methods and Materials

On 1 Sept. 2007, two hundred 3-4 (3 years grown in seedbeds, 4 years grown in transplant beds = 7 years old from seed) Fraser fir transplants growing under uniform fertility were selected in a nursery bed at a Christmas tree farm near Crossnore, North Carolina. At the same time, 10 Fraser fir trees growing in an adjacent field for 8 years (13 years from seed) were randomly selected for scion material. Scions were selected from five heights starting from the top progressing down the tree (1 – upper crown, 2 – upper mid-crown, 3 – mid-crown (approximately at 1.3 m), 4 – lower crown, and 5 – lowest portion of the crown) (Fig. 1).

Scions were collected at each height for two lateral branch orders (first and second). The scion average size was 10 cm long and varied from 2 to 10 mm depending on the height from which it was collected. The scion diameter decreased progressing down the tree. Since the terminal whorl had already been removed from the trees, the shoots collected from the first height differed from those of the other heights. For the first height, the first-order lateral scions were the uppermost lateral shoots. For the same height, the second-order scions were the next highest lateral shoots and had slightly more
horizontal-like growth. Scions were placed into pre-labeled bags with aluminum tags indicting tree number, height number, and branch order. All scions were grafted the same day as collected.

Scions were grafted onto the rootstocks using a single cleft graft (Garner, 1979; Hinesley and Frampton, 2002). If a scion was smaller in diameter than the rootstock, at least one side of the cambia of the rootstock and the scion were aligned. The grafts were wrapped with 0.95 x 20.3 cm grafting rubber bands and sealed with Doc Farwell's Grafting Seal (Farwell Products, LLC, Wenatchee, Wash.). Two grafters completed the grafts (50 grafts each) on 1 Sept. 2007 and then again on 18 Apr. 2008 for a total of 200 grafts in this study.

On 2 Aug. 2008, the grafts were observed for success (0 = graft failure, 1 = graft success). If successful, scion leader elongation, number of buds broken on the scion, lateral elongation, number of lateral buds on the shoot (leader), number of lateral buds per decimeter, plagiotropism of scion, and plagiotropism of the graft union were all measured. Plagiotropism was measured with the aid of a template with a 90° angle divided into five equal sections (Fig. 2). Shoots or graft unions were placed into one of five categories: 0 to 4 (0 = 90° (orthotropic; no plagiotropism), 1 = < 90° to 67.5°, 2 = < 67.5° to 45°, 3 = < 45° to 22.5°, and 4 = < 22.5°). There were few shoots or
graft unions with plagiotropic values greater than 1, so plagiotropism was subsequently classified as either a “1” (plagiotropic) or “0” (no plagiotropism).

**Experimental design and statistical analysis.** The experiment was a completely randomized design. Statistical analyses were only performed on data from the spring grafts as the success of late summer grafts was extremely poor (3%). Data were analyzed using Proc GLM and Proc GENMOD (SAS Inst., Inc., 2003) and all variables were considered fixed effects. An analysis of variance (ANOVA) was conducted to test for differences among main effects (grafter, tree(grafter), height, branch order) and interactions (height x branch order) for leader elongation, number of terminal buds broken, longest lateral length, number of lateral buds on leader, and number of lateral buds per decimeter at $P \leq 0.05$. Least squared means were used for comparisons.

For binomial response variables (graft success, scion plagiotropism and graft union plagiotropism), a generalized linear model with a logistic link function was performed with Proc GenMod using the same classification variables employed in the ANOVA for continuous variables. It was necessary to omit the ‘height x order’ interaction from the model to achieve convergence for the latter two
response variables. Type III likelihood ratio statistics were used to evaluate an effect difference. When detected, a Wald chi-square test was employed to assess differences among least squares means.

**Results**

*Seasonal effects.* Grafts made on 1 Sept. 2007 only had a success rate of 3% compared to 70% success for grafts made on 18 Apr. 2008. Consequently, data for late summer grafting were not included in the analyses.

*Grafter effects.* The two grafters had different levels of experience. Grafter 1 had 5 years of experience, whereas Grafter 2 only had 1 year. There was a significant difference between grafters for success rate (Table 1); Grafter 1 had a success rate of 86% compared to 54% for Grafter 2.

*Height effects.* The height at which the scions were collected had a significant effect \((P < 0.05)\) on all variables (Table 1). Scions from the upper crown (Height 1) were significantly better than all other heights for graft success (90%), mean leader elongation (125 mm), mean number of terminal buds broken (5.3), mean longest lateral length (2.5 mm), mean number of lateral buds on leader (3.8), and
number of lateral buds per decimeter (24) at \( P \leq 0.05 \) (Table 2).

Scions collected from the upper mid-crown (Height 2) had the shortest mean leader length (40 mm) and lateral length (1.0 mm) (Table 2). Scions taken from Height 3 (1.3 m) had the lowest graft success (50%) and number of terminal buds broken (3.1), but had the second longest mean leader elongation (64 mm), the second longest lateral length (1.8 mm), and ranked second for number of lateral buds on the leader (0.6). Scions from Height 4 had the second highest graft success (70%), but had the second lowest number of terminal buds broken (3.4), number of lateral buds formed on the leader (0.4), and lateral buds per decimeter (0.6). Scions from the lowest portion of the crown (Height 5) had the fewest newly forming lateral buds (0.3) and the fewest lateral buds per decimeter (0.5). Height 5, however, ranked second for terminal broken buds (3.6).

**Branch order effects.** There was a significant difference \( (P \leq 0.05) \) between the first-order laterals and the second-order laterals for all variables, except for graft success and plagiotropism (Table 1). The first-order grafts had longer leaders (83 mm) and laterals (2.0 mm), more terminal buds broken (4.3), more lateral buds forming on the leader (1.9), and more buds per meter (1.5) than the second-order (45 mm, 1.1 mm, 3.2, 0.4, and 0.4, respectively) (Table 2).
There was a significant height x order interaction ($P < 0.05$) for mean number of terminal buds broken (Fig. 3). While there was no significant difference between branch orders for terminal buds broken from scions collected at the uppermost height (Height 1), second-order shoots broke significantly fewer buds than first-order shoots collected from the other heights.

**Discussion**

Grafting during the traditional grafting period of early spring was dramatically more successful than late summer grafting (Table 2). In addition, the grafter with more experience had a much greater success rate.

Perhaps the summer grafts were not successful because the rootstock and the scion were not at the proper physiological stages. Traditionally, grafting of conifers, such as slash pine (*Pinus elliottii*, Englem.) is done in early spring when the scion is dormant and the rootstock is capable of producing the callus needed for proper healing of the graft union (Mergen, 1955). Callus formation is less likely after the end of August for Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco]) (Copes, 1973) when the rootstock is preparing for frost
hardening and the cambium is becoming less active as in balsam fir
[Abies balsamea (L.) Mill.] (Mellerowicz et al., 1992). There is a direct
effect of cambial activity on the amount of callus formation across the
graft union as cambial activity slows for winter in Douglas fir in the

For the grafts made in the spring, the origin of the scion (height
and branch order) was important to graft success (Table 1). Scions
from the upper crown had the highest graft success, longest leader
and lateral elongation, most terminal buds broken, most new laterals
formed on the leader, most laterals per length of the leader, and the
lowest incidence of plagiotropism. This indicates the top of the tree is
the best place to collect scion material.

Results suggest the possibility of using shoots from other
portions of the tree than just the upper crown since grafting success
was relatively good for all scion material. However, using the upper
crown and first-order laterals will ensure greater success of both
forming graft unions and exhibiting normal (orthotropic) growth
patterns.

First-order scions yielded leader and lateral elongation almost
twice as long as second-order scions (Table 2). The first order shoots
also had five times the number of lateral buds forming on the leader.
These results indicate the most terminal apical shoot on a branch (regardless of height origin) has a greater chance of producing a successful graft that exhibits preferred growth and structure than a secondary shoot on the same branch.

Rooted stem cuttings of Fraser fir are affected by branch order and crown position (Rosier et al., 2005). Some believe there is a branch “memory” from the original orientation of Douglas fir scion (Timmis et al., 1992). In the present investigation, scions taken from lateral branches retained their plagiotropic growth tendencies after grafting, possibly due to the formation of more xylem and compression wood on the adaxial (top) side of the bending stems than on the abaxial (bottom) side. Compression wood is formed as a response to gravitational disorientation to reposition the branch to an equalized position in Fraser fir (Wise et al., 1986) (Fig. 2). Plagiotropism of the scions is most likely due to topophysis, which is the effect of the position of the propagules in the source plant on the phenotype of the cloned progeny plants (Hartmann et al., 2002).

Scions from the mid-crown (Height 3) had variable results for most of the growth measurements, possibly as a result of shearing. During shearing, this height possibly had more growth removed than the lower portion of the tree to retain the desired conical shape.


Table 1. ANOVA of binomial and continuous variables for Fraser fir grafts performed in April 2008 near Crossnore, North Carolina.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Success</th>
<th>Binomial variables</th>
<th>Continuous variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grafter</td>
<td>1</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Tree(grafter)</td>
<td>8</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Height</td>
<td>4</td>
<td>*</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Order</td>
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<td>NS</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Height x order</td>
<td>4</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<tr>
<td>Grafter</td>
<td>1</td>
<td>*</td>
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<td>NS</td>
</tr>
<tr>
<td>Tree(grafter)</td>
<td>8</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Height</td>
<td>4</td>
<td>*</td>
<td>NS</td>
<td>*</td>
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<tr>
<td>Order</td>
<td>1</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Height x order</td>
<td>4</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
</tr>
</tbody>
</table>

There was a total of 100 spring grafts performed.

Longest lateral bud elongation.

Number of newly formed lateral buds along the leader by decimeter.

Lateral branch order (first or second).

NS: * Nonsignificant or significant at $P < 0.05$, respectively.
Table 2. Significant mean values of Fraser fir graft success and growth traits (successful April 2008 grafts only) performed near Crossnore, North Carolina.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Grafter</th>
<th>Tree (range)</th>
<th>Height</th>
<th>Order</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Success (%)</td>
<td>86⁺, 54⁻</td>
<td>30-100</td>
<td>90 a</td>
<td>70 b</td>
</tr>
<tr>
<td>Leader length (mm)</td>
<td>NS</td>
<td>NS 125 a</td>
<td>40 b</td>
<td>64 b</td>
</tr>
<tr>
<td>Terminal buds broken (no.)</td>
<td>NS</td>
<td>2.6-5.2</td>
<td>5.3 a</td>
<td>3.4 b</td>
</tr>
<tr>
<td>Lateral length (mm)</td>
<td>NS</td>
<td>0.4-2.1</td>
<td>2.5 a</td>
<td>1.0 b</td>
</tr>
<tr>
<td>Lateral buds (no.)</td>
<td>NS</td>
<td>-0.03-5.6</td>
<td>3.8 a</td>
<td>0.5 b</td>
</tr>
<tr>
<td>Lateral buds/length (no./dm)</td>
<td>NS</td>
<td>-1-2</td>
<td>24 a</td>
<td>0.8 b</td>
</tr>
</tbody>
</table>

⁺Any two means within a row followed by the same letter are not significantly different at \( P \leq 0.05 \) according to a Wald Chi-square test.

⁻Tree to tree variation was not a main area of study.

⁺⁺Grafter 1 percent success.

⁻⁻Grafter 2 percent success.

NSNonsignificant at \( P \leq 0.05 \).
Fig. 1. Height and lateral branch order origin of Fraser fir scions (thinner lines represent whorls of branches not sampled).
Fig. 2. Evaluation of the extent of plagiotropism in April 2008 Fraser fir grafts: (A) orthotropic growth of scion, (B) scion plagiotropism, (C) graft union plagiotropism, and (D) orthotropic growth of scion from a plagiotropic graft union (recovery).
Fig. 3. Height x lateral branch order interaction effect on mean number of terminal buds broken on the leader for successful Fraser fir grafts performed in April 2008 in Crossnore, North Carolina.
Conclusions

Grafting in early spring, when scions are dormant and rootstocks are just starting activity, is the best time to ensure graft success of Fraser fir. Summer grafting of Fraser fir scions onto Turkish fir rootstocks was marginally successful, but further research is needed to determine if grafting can be more successful other times of the year (i.e., April though June, or bench grafting during the winter months). Turkish fir was shown to be a viable rootstock for overall Fraser fir grafting. Other *Abies* species should be observed for successful grafting of Fraser fir scions during different times of the year.

Further research is needed to observe the seasonal timing of graft unions between Fraser fir and Turkish fir to note how temperature affects the formation of the graft union. Since weather varies from year to year, grafting in the late summer/early fall should be observed in the greenhouse or in a controlled Phytotron study with different day/night temperatures.

Grafting of stored dormant Fraser fir scions onto Turkish fir rootstocks was not successful for summer/early fall grafting. Different storage methods should be investigated for extending the life of stored dormant material. Collection of Fraser fir scions from different heights and lateral branch order showed that there was the possibility of using shoots from parts of the tree than just the upper crown. However, using scions from the upper crown and first-order lateral
branches can ensure a greater success of forming a graft union and exhibiting normal (orthotropic) growth patterns. More observation time is needed to see if plagiotropic grafts will recover to grow more orthotropically or revert back to plagiotropic growth. The physiological processes controlling plagiotropic growth of Fraser fir scions also need to be studied.
Appendix
Fig. 1. Linville River Nursery irrigation layout.