CHARACTERIZATION OF WATER STRESS DURING COLD
STORAGE AND ESTABLISHMENT FOR ACER PLATANOIDES
AND CRATAEGUS PHAENOPOYRUM

by

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(ABSTRACT)

This study examined the affects of desiccation during and after cold storage on the physiology, growth, and marketability of bare-root Acer platanoides (Norway maple), Crataegus phaenopyrum (Washington hawthorn) and Prunus x yedoensis (Yoshino cherry). Histological examination of Acer and Crataegus stems was also conducted. Maple and cherry trees were transplanted into pine bark-filled containers and subjected to mist or non-mist treatments. Xylem water potential increased (became less negative) for misted maple and cherry trees. Water potential increased for non-misted maple and decreased for non-misted cherry trees. Maple and hawthorn seedlings were subjected to cold storage durations of 2, 4, 6, 8, 10, and 12 weeks and storage treatments: whole plant covered, shoots exposed, roots exposed and whole plant exposed. Shoot (Ψₛ) and root (Ψᵣ) water potentials for all treatments and both species decreased during storage. For maple, (Ψₛ) and (Ψᵣ) of the exposed shoot treatment were the same as the whole plant covered treatment. In contrast, hawthorn (Ψₛ) and (Ψᵣ) of the exposed shoot treatment were lower (more negative) than for the whole plant covered treatment. Root hydraulic conductivity was the same for both species and decreased with increased storage duration and for treatments with
exposed roots. For the root covered treatments, maple root growth potential (RGP) increased while hawthorn RGP decreased with increased cold storage duration. RGP for both species remained low throughout storage for treatments exposing roots. Days to bud break for Acer and Crataegus seedlings decreased with increased storage time for the whole plant covered treatments but increased for both species when stored with exposed roots. Maple marketability, percent of trees with ≤ 10% shoot dieback, for root covered treatments was high for most storage durations. Hawthorn marketability was generally low except for the whole plant covered treatment during the first six weeks of storage. There was a high positive correlation between RGP and marketability for both maple and hawthorn. Histological examination revealed that Acer stems had a highly suberized periderm, and a uniform cuticle with few disruptions. Periderm suberization of Crataegus stems was variable and extensive peridermal cracking was evident. Cuticle wax decreased with increasing distance from the stem apex for both species. Collectively, results indicated that hawthorn stems had more pathways for water loss than maple shoots. While protection of roots of all bare-root stock is important, desiccation sensitive species such as Washington hawthorn require both root and shoot protection during storage and at transplanting to minimize water loss.
Acknowledgments

I would like to express my appreciation to my major professor, Dr. Alex Niemiera, for his advice, guidance, editorial aptitude and sense of humor throughout the course of this study. He allowed me the freedom to work and think independently while remaining closely involved with all aspects of this project. Heartfelt thanks go to Dr. Robert Wright, for his technical guidance and support, and for freely sharing his helpful insights concerning the industry, research and life in general. I am deeply indebted to my committee members, Dr. Gregory Welbaum, for his penchant to help a student no matter how large or small the problem, Dr. Roger Harris, for his timely advice and continual encouragement, and Dr. John Seiler, for his valuable suggestions concerning this research and for his quick wit.

Special thanks to Charlie Parkerson and Lancaster Farms Nursery, for their support, plant material and keen observations which provided the impetus to begin this, and many other nursery research projects. I appreciate the valuable assistance provided by the staff and students in the Dept. of Horticulture, particularly the help of Carol Leda, Joyce Shelton, Connie Wallace, Marilyn Echols, Elbert Perfator and Richard Harkess.

I would like to thank my wife, Jeanna, for her strength, perseverance and cheerful encouragement throughout these three years. I dedicate this work to her. Thanks also to my children, Jeremiah, Colin, and Jacqueline, for helping me keep my priorities straight (and not forgetting who I was when research kept me away!).

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INTRODUCTION

Most flowering and shade trees in the nursery industry are produced using bare-root trees as liners. Bare-root tree seedlings are commonly dug in late fall, placed in cold storage for up to six months, shipped and transplanted in the spring. Bare-root plants are particularly prone to desiccation damage due to the loss of roots at digging, storage duration and conditions, and transplanting conditions. Storage conditions for bare-root stock vary greatly among nurseries but generally consist of low temperature (0-5°C), high humidity (90-98%), and root protection such as moistened peat moss. Storage duration, conditions, and packaging methods can affect the degree of desiccation stress and the subsequent physiological quality of bare-root trees (Webb and von Althen, 1980). Studies have shown that tree species differ widely in their response to storage temperature (Tabbush, 1987) and duration (Ritchie et al., 1985). A complete understanding of the wide variation in species response to storage conditions remains unclear. Once bare-root trees are transplanted, desiccation stress is considered to be the main cause of death because stem and transpirational water loss exceeds water uptake by the transplanted root system (Kozlowski and Davies, 1975). Water stress research on bare-root nursery stock has primarily focused on lift date, length of storage (Hermann, 1964) and exposure to dry conditions during processing (Coutts, 1981). Minimizing postharvest desiccation stress by reducing water loss from bare-root seedlings has been shown to improve survivability of some species (Insely and Buckley, 1985). Species such as Yoshino cherry (Prunus x yedoensis Matsum.) and Washington hawthorn (Crataegus phaenopyrum Med.) often have low post-transplant survival rates due to a high
degree of desiccation sensitivity. Species that achieve a deeper degree of dormancy in
the field are more tolerant to desiccation stress after harvesting (Murakami et al.,
1990). The desiccation tolerance of certain tree species has also been linked to
morphological characteristics. Insley and Buckley (1985) reported that the finer root
system of Betula pubescens Ehrh. contributed to its desiccation sensitivity as
compared to the coarser rooted Fraxinus angustifolia Vahl. Sulaiman (1968) found
differences in the rate of water loss through the defoliated stems of Quercus alba L.
and Fraxinus pennsylvanica Marsh., and suggested that water loss may be correlated
to root volume. For most species of bare-root plants, current handling practices are
acceptable and post-transplant survival rates are high. Desiccation sensitive species,
however, may require modified cultural practices to ensure acceptable survival rates.
Survival rates of less than 80% are common for transplanted bare-root desiccation
sensitive species. Thus, knowledge of how storage and post-storage conditions
influence tree survival has a direct link to the economic viability of producing such
species.

Research objectives. Understanding how dormant bare-root tree seedlings lose
water during cold storage and re-establishment would be useful in developing
improved postharvest handling procedures for sensitive species. The desiccation
tolerant Norway maple (Acer platanoides) and desiccation sensitive Washington
hawthorn (Crataegus phaenopyrum) and Yoshino cherry (Prunus x yedoensis) were
used in this investigation to describe the qualities of water loss from bare-root trees.
With the above discussion in mind we established the following specific objectives:

1) investigate the effect of mist irrigation to reduce post-transplant water
stress in Prunus x yedoensis and Acer platanoides (Chapter One);
2) compare stem water loss rates and determine the relative impact of either shoot or root exposure during cold storage on water stress development in bare-root *Acer platanoides* and *Crataegus phaenopyrum* (Chapter Two);  

3) determine the influence of storage duration and root and shoot exposure treatments on the post-transplant shoot water potential and root hydraulic conductivity of *Crataegus phaenopyrum* and *Acer platanoides*, and determine if post-storage stem wax coating influenced shoot water potential and bud break (Chapter Three);  

4) determine the influence of root and shoot exposure during storage and prior to transplanting on root growth potential, timing of bud break and marketability of *Acer platanoides* and *Crataegus phaenopyrum* (Chapter Four);  

5) examine and compare the morphological features related to water loss in *Acer platanoides* and *Crataegus phaenopyrum* stems (Chapter Five).
Literature Cited


CHAPTER ONE

MIST IRRIGATION REDUCES POST-TRANSPLANT DESICCATION OF BARE-ROOT TREES

Abstract

Desiccation during storage and reestablishment is a major factor contributing to poor regrowth of transplanted bare-root trees. The effect of frequent overhead mist irrigation on reducing post transplant water stress in *Acer platanoides* L. 'Emerald Lustre' and *Prunus x yedoensis* was examined. Bare-root Norway maple (desiccation tolerant) and Yoshino cherry (desiccation sensitive) trees were transplanted into pine bark-filled containers and subjected to mist or non-mist treatments. Stem xylem water potential ($\Psi_s$), relative water content (RWC), and survivability were determined. Xylem water potential increased (became less negative) for misted maple and cherry trees. Water potential increased for non-misted maple and decreased for non-misted cherry trees. Twenty-seven percent of non-misted cherries were evaluated as nonmarketable due to stem dieback. Results of this study indicate that mist irrigation effectively reduces desiccation damage for desiccation sensitive species.
Introduction

Water stress research on bare-root nursery stock has primarily focused on lift date, length of storage (Hermann, 1964) and exposure to dry conditions during processing (Coutts, 1981). Water loss from stem tissue, however, can continue after transplanting (Schoneer and Ziegler, 1980). Stem desiccation after transplanting and prior to new root growth during periods of high vapor pressure deficit can contribute to stem dieback and poor regrowth in certain species (Insley, 1981). Desiccation sensitivity varies widely among tree species. For example, Crataegus phaenopyrum Med. is desiccation sensitive whereas Acer platanoides L. is desiccation tolerant (Murakami et al., 1990). Several species of Prunus have shown poor regrowth following transplanting (personal observation). Some nurseries suggest "sweating" (wrapping liners with plastic under warm, humid conditions) difficult-to-transplant species as a means of increasing survivability. There are, however, no reports on the use of irrigation to reduce post-transplant desiccation of bare-root stock. The objective of this study was to investigate the effect of mist irrigation to reduce post-transplant stress in a desiccation sensitive (Prunus x yedoensis) and desiccation tolerant (Acer platanoides) species.

Materials and Methods

Two-year-old branched bare-root liners of 'Emerald Lustre' Norway maple and Yoshino cherry (30 per species) were shipped from Oregon to Virginia in Feb. 1992. Xylem water potential was measured prior to initiation of treatments (17 Feb.) using a portable pressure chamber (Model 3005, SoilMoisture Equipment Corp., Santa Barbara,
Calif.) on a 10.2 cm (4 in) stem section excised from each tree. A second piece of internodal shoot tissue, 4 cm (1.5 in), was collected from each tree and fresh weight (FW) was immediately measured for relative water content (RWC) determinations. Stem sections were then placed on water-saturated cotton in airtight beakers for 48 h and reweighed to determine turgid weight (TW). Dry weight (DW) was determined after oven drying stem sections to a constant weight in a 62°C oven for at least 72 h. Relative water content was calculated using the formula: $RWC = \frac{(FW-DW)}{(TW-DW)} \times 100$. Trees were transplanted into 100% pine bark-filled 18.9 liter plastic containers and dolomitic limestone was surface applied at 2.9 kg/cubic m. Containers were thoroughly irrigated by hand. Aluminum foil covers were fitted around tree stems and over container sides to prevent water (from mist) entering containers while allowing adequate oxygen exchange. The study was conducted in a greenhouse vented at 24°C during the day and heated to a night minimum of 18°C. Beginning on 17 Feb., half of the maple and cherry trees received overhead mist irrigation, 25 sec every 15 min, and the remaining trees received no mist irrigation. Xylem water potential and RWC measurements of stem sections were made between 1200 and 1400 HR for each tree 3, 6, and 9 days after initiation of irrigation treatments.

Plants were greenhouse-grown until May, then moved to an outdoor nursery. Sixty days after the termination of treatments plants were graded as marketable or unmarketable. Marketable plants exhibited less than 10% shoot dieback whereas unmarketable plants had > 50% shoot death. There were no intermediate categories of shoot dieback. Data were subjected to analysis of variance procedures. Treatments (species, irrigation) were in factorial combination with 15 single plant replications using a completely randomized design. Individual sample dates were analyzed separately.
Results and Discussion

For misted cherry and maple, $\Psi$'s increased (became less negative) from day 0 to day 3 (Fig. 1); after day 3, there were relatively small changes in water potential for either species. Water potential for all non-misted cherry trees decreased 1.2 MPa from day 0 until day 6 (Fig. 1). Thereafter, $\Psi$'s increased for trees that exhibited bud break but continued to decrease for those not breaking bud. Water potential of non-misted maples increased 0.6 MPa from day 0 to day 9 (Fig. 1), indicating that transplanting bare-root maples into a moist medium relieved a portion of pre-transplant water stress and prevented further desiccation. In support of this contention, Johnson et al. (1988) showed that one-year-old suberized Pinus roots were capable of absorbing water in the absence of young un-suberized roots. In contrast to maple, transplanting bare-root cherry trees into a moist medium without mist did not increase $\Psi$'s. An exception to this occurred when eight of the 15 non-irrigated cherry trees broke bud after day 6 and $\Psi$'s increased from -2.5 to -1.1 MPa (Fig. 1). This increase may have been related to new root initiation since regenerated roots generally exhibit greater hydraulic conductivity (Johnson et al., 1988) which may have been responsible for the change in $\Psi$'s. Although not monitored in this study, new root initiation for some species has been shown to occur concurrently with bud break (Wilcox, 1955).

The increase in RWC from day 0 to day 3 for misted cherry and maple was 12% and 7%, respectively (Fig. 2); after day 3, RWC remained relatively constant for both species. Relative water content of cherry was not measured beyond day 6 because bud break for non-misted cherries occurred about that time. Interestingly, the RWC for misted and non-misted maple (100% marketability) was generally less than non-misted cherry
(73% marketability) (Table 1) which reflects the desiccation tolerant nature of *Acer platanoides* (Murakami et al., 1990). In general, the magnitude of RWC differences between misted and non-misted trees of each species was similar to the magnitude in $\Psi$s differences for the respective treatments indicating that $\Psi$s and RWC are both reliable indicators of bare-root transplant water status.

Sixty days after treatment termination, 100% of misted cherries and maples and 100% of non-misted maples were evaluated as marketable (<10% stem dieback) (Table 1). In contrast, only 73% of non-misted cherries were deemed marketable. The shoot dieback and unmarketable status of non-misted cherries is most likely due to desiccation following transplanting. These results indicate that pre-bud break $\Psi$s and RWC values < -2.2 MPa and 70%, respectively, for *Prunus x yedoensis* signal significant plant water stress resulting in possible tissue damage. These values may be used to screen shipped bare-root trees of this species to determine post transplant establishment potential. More research is needed to determine critical $\Psi$s and RWC values for other desiccation intolerant species.

In summary, maintaining a film of water on transplanted bare-root trees prior to bud break increased the survival and plant quality of the desiccation intolerant *Prunus x yedoensis*. Other measures such as planting at a time when less stressful climatic conditions prevail, coating trees with film-forming compounds (Englert et al., 1993), and forcing plants out of dormancy may also increase survival.
Literature Cited


Table 1. Marketability assessment of Norway maple and Yoshino cherry 60 days after treatment termination.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Marketability(^y) (% of trees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mist</td>
<td>Cherry: 100(^z) Maple: 100</td>
</tr>
<tr>
<td>No mist</td>
<td>Cherry: 73  Maple: 100</td>
</tr>
</tbody>
</table>

\(^y\) Marketable plants = < 10% shoot dieback, unmarketable plants = > 50% shoot dieback.

\(^z\) \(n = 15\) trees per treatment.
Figure 1. Xylem water potential of Norway maple (A) and Yoshino cherry (B) as influenced by mist irrigation after transplanting. Each point is a mean of 15 measurements. Bars represent SE of means.
Figure 2. Relative water content of Norway maple (A) and Yoshino cherry (B) as influenced by mist irrigation after transplanting. Each point is a mean of 15 measurements. Bars represent SE of means.
CHAPTER TWO

COLD STORAGE METHOD AND DURATION AFFECTS SHOOT WATER POTENTIAL OF BARE-ROOT TREES

Abstract

Desiccation of bare-root tree seedlings during storage can result in reduced growth and poor quality after transplanting. For 12 weeks, shoot and root water potentials of bare-root Norway maple (Acer platanoides L.) and Washington hawthorn (Crataegus phaenopyrum Med.) seedlings were measured in response to four cold storage treatments: whole plant exposed, roots exposed, shoots exposed, whole plant covered. In another experiment, water loss was measured for stem sections of both species during four weeks of cold storage. Shoot and root water potentials for all treatments and both species decreased during storage. For maple, shoot and root water potentials of the exposed shoot treatment were the same as the whole plant covered treatment. In contrast, hawthorn shoot and root water potentials of the exposed shoot treatment were lower (more negative) than for the whole plant covered treatment. Most of the water stress experienced by roots and shoots of both species accumulated during the first six weeks of storage. Water loss was greater for hawthorn stem sections than for maple only during the first two weeks of storage. Results indicated that while protection of roots of all bare-root stock is important, sensitive species such as Washington hawthorn require both root and shoot protection to minimize water loss.
Introduction

Bare-root tree seedlings are commonly harvested during autumn and early winter, placed in cold storage and shipped in the spring. Storage conditions and packaging methods in storage can affect desiccation stress and the subsequent physiological quality of bare-root trees (Webb and von Althen, 1980). The desiccation tolerance of bare-root nursery stock is known to differ dramatically among species (Hermann, 1967; Tabbush, 1987). Englert et al. (1993) found differences in the dieback and survival of *Quercus rubra* L. and *Crataegus phaenopyrum* Med. when entire seedlings were subjected to a 48 hr drying period; however, overall water loss rates for these species were similar. Insley (1981) attributed the drying rate and survival variation between *Nothofagus obliqua* Mirb. and *Acer platanoides* L. to species root morphology differences. Of the various techniques used to reduce plant desiccation in storage, many growers protect only roots (Darby, 1961; Mullin et al., 1974; Racey et al., 1983) despite the fact that stem water loss is high for some species (Insley, 1981). The desiccation sensitive nature of *Crataegus phaenopyrum* (Washington hawthorn) and desiccation resistant nature of *Acer platanoides* (Norway maple) have been well documented (Englert et al., 1993; Murakami et al., 1990). There are, however, no reports on the relative contribution of shoots and roots to water stress development in storage for desiccation tolerant and resistant species. The objective of this study was to determine the relative impact of either shoot or root exposure during bare-root storage on water stress development in a sensitive and resistant species.
**Materials and Methods**

On January 14, 1993, 2 yr. old *Acer platanoides* and *Crataegus phaenopyrum* bare-root seedlings (approx. 24-36") were received in Blacksburg, VA. from Lawyer Nurseries, Plains, MT. Seedling bundles were wrapped in plastic sheeting and placed in cardboard boxes with the roots of each bundle packed in moistened, shredded newsprint. Transit time was approximately five days. Trees were sorted for uniformity and one-hundred forty-four of each species were placed on wooden racks in a walk-in cooler maintained at 70% ± 5% relative humidity and 2°C (35°F). At the time seedlings were placed into cold storage one of the following four treatments were randomly allocated to each tree: 1) whole plant covered in which the entire seedling was enclosed in a sealed 3-layer storage bag (Union Camp Corp., Tifton, GA), 2) shoot exposed in which seedling roots were enclosed in a storage bag sealed around the stem just above the root collar, 3) roots exposed in which shoots were enclosed in a storage bag sealed just below the root collar and 4) entire seedling exposed (no storage bag). Storage bags were compressed during plant insertion to minimize air space within the bag; all trees were placed horizontally on racks.

On January 28, February 11, February 28, March 11, March 28 and April 11 (2, 4, 6, 8, 10, and 12 weeks in storage, respectively) six hawthorn and six maple trees from each treatment were removed from cold storage. Shoot water potential (Ψs) and root water potential (Ψr) were measured on three seedlings from each species-treatment combination. The remaining three seedlings from each combination were placed on a lab bench and allowed to air-dry at 24°C (75°F) and 35% ± 5% relative humidity for 12 hr after which Ψs and Ψr were measured. Shoot and root water potential were measured using a portable pressure chamber (Model 3005, SoilMoisture Equipment Corp., Santa Barbara, CA) on a 10.2 cm (4 in.) stem section.
and a 7.6 cm (3 in.) root section excised from each tree. Data for all measured variables were subjected to analysis of variance. Species and cold storage treatments were replicated three times using a completely randomized design. Desiccation time (0 hr vs. 12 hr) and storage duration data were analyzed separately. Mean separation of treatment effects was performed by Duncan's multiple range test (P = 0.05). Slope of the least squares was determined for storage treatments over storage durations for each species.

A second group of two-year old hawthorn and maple seedlings were used to determine stem water loss rates. Twelve randomly selected seedlings of each species were removed from cold storage and 13 cm (5 in) stem segments containing four buds were removed from each plant. Internodal stem diameter was measured at three points with a microcaliper to establish average stem diameter; we assumed the stems were approximately cylindrical. The average diameter and stem length (measured to the nearest 0.1 cm) were converted to approximate surface area. Cut stem surfaces were sealed with melted paraffin wax. Stem segments were then placed in cold storage maintained at 2°C (35°F) and 80% ± 5% RH. Water loss was determined gravimetrically after one, two, three, and four weeks, and expressed on a stem surface area basis (mg H_2O/cm^2). This experiment was repeated using the same experimental procedure except that buds were removed from hawthorn and maple stem segments and incisions sealed with paraffin.
Results and Discussion

Root Water Potential:

Species, storage duration, and storage treatment affected $\Psi_r$ and $\Psi_s$ (Table 1). Root water potential of hawthorn seedlings before placement into storage was -0.8 MPa which indicated that trees were not excessively stressed upon placement into storage (Table 2). Generally, hawthorn $\Psi_r$ decreased with increased time in storage for all treatments during the 12 week storage period with most of the decrease occurring in the first six weeks in storage (Table 2). At the end of 12 weeks, the rate of decrease in water potential values for the whole plant exposed and roots and shoots exposed treatments (slopes = -0.26, -0.26, -0.24, respectively) were higher than the whole plant covered treatment (slope = -0.12). Relative to the root exposed treatments, the shoot exposed (roots covered) treatment was effective in maintaining a higher water potential for the first six weeks. But by weeks 8 to 10, water potentials for the shoot exposed treatment were the same as for the root exposed treatments.

As with Washington hawthorn, Norway maple $\Psi_r$ decreased with storage time (Table 2). Root water potential values for storage treatments were clearly segregated into two groups: 1) treatments providing root covering (whole plant covered, shoots exposed), and 2) treatments with roots exposed (whole plant exposed, roots exposed) (Table 2). Water potentials for trees completely covered and with shoots exposed were the same for each storage duration, and with the exception of week four, roots exposed and whole plant exposed treatments were the same. At each storage duration, $\Psi_r$ for shoots exposed and whole plant covered treatments were less than values for roots exposed and whole plant exposed treatments.
Root water potential, for plants of both species and all storage treatments that were exposed to a 12 hr drying period at each storage duration, decreased over time (data not shown). For each species, the decrease in $\Psi_r$ during the 12 hr desiccation period was at least 1.5 MPa for all storage durations. At each sample date, maple $\Psi_r$ values were 0.4 to 0.7 MPa higher than hawthorn, however the difference between the 0 and 12 hr desiccation treatment (both species) decreased as the duration of storage increased. For example, after two weeks in storage $\Psi_r$ of hawthorn without a post-storage desiccation treatment averaged -1.7 MPa compared to -2.9 MPa for trees exposed to a 12 hr desiccation treatment; after 12 weeks of storage, values averaged -3.5 MPa and -3.9 MPa, respectively.

*Shoot Water Potential:*

Hawthorn $\Psi$s decreased with increased storage duration for each storage method (Table 3). Water potentials of completely covered trees were the same as trees of other storage treatments for the first four weeks in storage. However, $\Psi$s of completely covered trees remained relatively constant after four weeks in storage and were higher than potentials for the other treatments which decreased throughout the study. Relative to the pre-storage -1.1 MPa value, $\Psi$s at week twelve increased 109% for the whole plant covered treatment whereas the increase for other treatments was at least 209% (Table 3). Shoot water potentials for the shoots exposed treatment were the same as for the roots exposed and whole plant exposed treatments throughout the study.

Maple $\Psi$s for all storage treatments decreased with increasing storage duration (Table 3). Similar to $\Psi_r$, maple $\Psi$s were segregated according to storage method. With the exception of week four, potentials for the shoots exposed and whole plant
covered treatments were higher than the roots exposed and whole plant exposed treatments. Water potential values for trees in the shoots exposed treatment were the same as the whole plant covered treatment for all storage durations.

Trends in $\Psi_s$ over time and for species of stored trees receiving a 12 hr desiccation treatment were similar to trends for $\Psi_r$ data (data not shown). For each species, the decrease in $\Psi_s$ during the 12 hr desiccation period was at least 1.3 MPa for all storage durations. Maple $\Psi_s$ values were higher ($P = 0.05$) than hawthorn at each storage duration. All two-way and three-way interactions were significant for variables of the 0 hr desiccation treatment. Desiccation in storage and the various storage treatments contributed to water stress in the bare-root trees and provides a possible explanation for the two-way and three-way interactions. For hawthorn, there were large decreases in $\Psi_s$ and $\Psi_r$ for the root exposed treatments but a small decrease for the whole plant covered treatment. For maple, there were large decreases for the roots exposed treatments but a small decrease for the roots covered treatments.

Relative to storage treatment and duration high positive correlations existed between $\Psi_r$ and $\Psi_s$ for maple ($r = 0.93$) and hawthorn ($r = 0.92$). High positive correlations between root and shoot water potentials have also been demonstrated in other species indicating both root and shoot tissue can provide reliable $\Psi$ measurements (Sucoff et al., 1985). Generally, water stress increased with storage duration regardless of species or storage method. Maple seedlings that were completely covered or with their shoots exposed showed the lowest decrease in root or shoot water potential throughout the study (Tables 2 and 3). Root and shoot water potentials for shoots exposed and whole plant covered treatments were the same which indicated that water loss from maple stems was minimal and most likely related
to stem morphology. In contrast, hawthorn $\Psi_r$ and $\Psi_s$ of the shoots exposed to stem morphology. In contrast, hawthorn $\Psi_r$ and $\Psi_s$ of the shoots exposed treatment were usually much lower than trees completely covered (Tables 2, 3). In maple, $\Psi_r$ of trees with shoots exposed decreased 114% throughout storage while in hawthorn there was a 350% decrease for the same treatment. This finding indicated that Washington hawthorn stems were very susceptible to water stress while dormant which may be due to a morphological aspect that allows for a relatively high degree of moisture loss. Exposure of bare-root hawthorn trees to a 12 hr desiccation treatment resulted in a decrease in $\Psi_s$ and $\Psi_r$ of at least 1.3 MPa, regardless of storage treatment and generally caused extensive tissue damage. Thus, minimizing water stress by proper storage methods can be negated by exposure to desiccating conditions during planting. Sensitivity to exposure varies according to growth stage, species, and plant tissue. Hermann (1967) reported a decrease in sensitivity of Douglas-fir to root exposure from November to January. The critical exposure limit for Washington hawthorn appears to be well below 12 hr, even when seedlings are dormant as in this study.

**Stem Water Loss:**

Water loss from hawthorn and maple stem segments was highest during the first week of storage (Table 4). Hawthorn water loss was higher than maple during the first two weeks of storage; however, values for week three and four were the same for both species. Cumulative water loss for hawthorn exceeded maple. Similar results were obtained when the experiment was repeated using internodal stem segments containing no buds (data not shown). Stem water loss was standardized between species on a surface area basis, however, stem diameter differed which may have affected the volume:surface area ratio. Accordingly, stem capacitance may have differed between species. Water loss through lenticels has been shown to contribute
to overall water loss of stem tissue (Kozlowski, 1943). Analysis of hawthorn and maple stem lenticel number and distribution yielded no pattern or significant difference between species (data not shown). The stem water loss data presented in this study, however, does indicate that in early storage hawthorn stem tissue loses more water than maple (Table 4); this may be related to hawthorn stem dieback. In support of this contention, Englert et al. (1993) found that an application of film-forming antidesiccant compounds to Washington hawthorn stems reduced water loss and increased survival rates.

Results of this and other work (Hermann, 1967) indicate that roots of seedling nursery stock are extremely vulnerable to desiccation stress. Although protection of roots for all bare-root stock is imperative, desiccation sensitive species such as hawthorn require both shoot and root protection to minimize water stress.
Literature Cited


Table 1. Analysis of variance of the effect of cold storage duration, storage wrap method and desiccation treatment on shoot and root water potential of Norway maple and Washington hawthorn.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Shoot $\Psi$</th>
<th>Root $\Psi$</th>
<th>Shoot $\Psi$</th>
<th>Root $\Psi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species (Spec)</td>
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<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Storage Duration (SD)</td>
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<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Storage Trt (Trt)</td>
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<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Spec x SD</td>
<td>5</td>
<td>*</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Spec x Trt</td>
<td>3</td>
<td>**</td>
<td>**</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>SD x Trt</td>
<td>15</td>
<td>**</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Spec x SD x Trt</td>
<td>15</td>
<td>*</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Desiccation time

$^2$ Trees were air dried at 25°C (76°F) and 35% relative humidity.

$^\gamma$ NS, *, ** nonsignificant, or significant at $P=0.05$, or 0.01 level, respectively.
Table 2. Influence of cold storage duration and storage treatment on root water potential of 2-yr.-old Washington hawthorn and Norway maple.

<table>
<thead>
<tr>
<th>Storage treatment</th>
<th>Hawthorn</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Storage duration (weeks)</td>
<td>Root $\Psi$ (-MPa)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole plant exposed</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Roots exposed</td>
<td>2.4 a</td>
<td>3.2 a</td>
<td>3.8 a</td>
<td>4.0 a</td>
<td>4.0 a</td>
<td>4.0 a</td>
</tr>
<tr>
<td>Shoots exposed</td>
<td>1.2 c</td>
<td>1.8 c</td>
<td>2.8 b</td>
<td>3.2 b</td>
<td>3.5 a</td>
<td>3.6 a</td>
</tr>
<tr>
<td>Whole plant covered</td>
<td>1.4 bc</td>
<td>2.0 c</td>
<td>2.2 c</td>
<td>2.1 c</td>
<td>2.0 b</td>
<td>2.6 b</td>
</tr>
</tbody>
</table>

| Maple                    |          |             |          |       |       |       |
| Whole plant exposed      | 2.2 a\textsuperscript{2} | 2.4 b       | 2.8 a    | 3.6 a | 3.8 a | 3.8 a |
| Roots exposed            | 1.9 a    | 3.1 a       | 3.3 a    | 3.4 a | 3.4 a | 3.5 a |
| Shoots exposed           | 1.0 b    | 1.3 c       | 1.2 b    | 1.4 b | 1.8 b | 1.5 b |
| Whole plant covered      | 0.8 b    | 1.0 c       | 1.0 b    | 1.3 b | 1.6 b | 1.7 b |

\textsuperscript{2} Hawthorn pre-storage $\Psi = -0.8$ MPa, maple pre-storage $\Psi = -0.7$ MPa, n = 5.

\textsuperscript{Y} Mean separation by Duncan's multiple range test, $P = 0.05$. Means in columns followed by the same letter are not significantly different.
Table 3. Influence of cold storage duration and storage treatment on shoot water potential of 2-yr.-old Washington hawthorn and Norway maple.

<table>
<thead>
<tr>
<th>Storage treatment</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Hawthorn</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Whole plant exposed</td>
<td>1.6 a\textsuperscript{2y}</td>
<td>2.3 a</td>
<td>3.0 a</td>
<td>3.1 a</td>
<td>3.1 a</td>
<td>4.0 a</td>
</tr>
<tr>
<td>Roots exposed</td>
<td>1.5 a</td>
<td>1.9 a</td>
<td>2.9 a</td>
<td>2.8 a</td>
<td>3.0 a</td>
<td>4.0 a</td>
</tr>
<tr>
<td>Shoots exposed</td>
<td>1.3 a</td>
<td>2.1 a</td>
<td>2.4 ab</td>
<td>2.7 a</td>
<td>2.8 a</td>
<td>3.4 a</td>
</tr>
<tr>
<td>Whole plant covered</td>
<td>1.5 a</td>
<td>2.2 a</td>
<td>2.0 b</td>
<td>1.8 b</td>
<td>2.0 b</td>
<td>2.6 b</td>
</tr>
<tr>
<td></td>
<td>Maple</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole plant exposed</td>
<td>1.5 a\textsuperscript{2y}</td>
<td>2.0 a</td>
<td>3.1 a</td>
<td>3.1 a</td>
<td>2.8 a</td>
<td>3.1 a</td>
</tr>
<tr>
<td>Roots exposed</td>
<td>1.4 a</td>
<td>2.1 a</td>
<td>2.3 b</td>
<td>2.9 a</td>
<td>3.2 a</td>
<td>3.2 a</td>
</tr>
<tr>
<td>Shoots exposed</td>
<td>0.7 b</td>
<td>1.5 ab</td>
<td>1.4 c</td>
<td>1.7 b</td>
<td>2.1 b</td>
<td>2.0 b</td>
</tr>
<tr>
<td>Whole plant covered</td>
<td>0.8 b</td>
<td>1.0 b</td>
<td>1.1 c</td>
<td>1.6 b</td>
<td>1.8 b</td>
<td>1.8 b</td>
</tr>
</tbody>
</table>

\textsuperscript{2} Hawthorn pre-storage $\Psi = -1.1$ MPa, maple pre-storage $\Psi = -0.9$ MPa, $n = 5$.

\textsuperscript{y} Mean separation by Duncan’s multiple range test, $P = 0.05$. Means in columns followed by the same letter are not significantly different.
Table 4. Water loss from Washington hawthorn and Norway maple stem segments during cold storage\(^x\)\(^y\).

<table>
<thead>
<tr>
<th>Species</th>
<th>Storage duration (weeks)</th>
<th>Cumulative loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Hawthorn</td>
<td>19.7 a</td>
<td>16.6 a</td>
</tr>
<tr>
<td>Maple</td>
<td>14.0 b</td>
<td>11.4 b</td>
</tr>
</tbody>
</table>

\(^x\) Water loss during cold storage at 2°C, 80% ± 5% RH.
\(^y\) Mean separation by t test at P = 0.05. Same letter within column indicates no significant difference.
CHAPTER THREE

EFFECT OF TRANSPLANTING ON SHOOT WATER POTENTIAL OF BARE-ROOT WASHINGTON HAWTHORN AND NORWAY MAPLE TREES

Abstract

Two-yr.-old Norway maple (Acer platanoides L.) and Washington hawthorn (Crataegus phaenopyrum Med.) trees were cold-stored for 2, 4, 6, 8, 10, and 12 weeks and stem water potentials were measured prior to and five days after transplanting. In a second experiment, a wax coating was applied to hawthorn trees at transplanting and shoot water potential was measured at two-day intervals for twelve days after transplanting; percent bud break was measured eight weeks after transplanting. In a third experiment, maple and hawthorn trees were stored for 2, 4, 6, 8, 10, and 12 weeks with the following tree covering treatments: whole plant covered, shoots exposed, roots exposed, whole plant exposed, and root hydraulic conductivity was measured for each storage duration. At each storage duration, maple stem water potentials after transplanting were the same as or higher than the pre-transplant potential value; hawthorn water potentials after transplanting were generally lower than pre-transplant values. Six to eight days after transplanting, hawthorn water potentials of wax covered stems were higher than unwaxed stems. Bud break percentages were higher for trees with waxed stems than for trees without wax. Root hydraulic conductivity was the same for both species and decreased with increased storage duration and for treatments exposing roots.
Introduction

Desiccation of bare-root nursery stock during storage and after transplanting can result in poor regrowth and is considered to be the main cause of post-transplant tree death (Coutts, 1981; Insley and Buckley, 1985; Kozlowski and Davies, 1975). Several studies have shown that tree species differ widely in their response to storage conditions (Hermann, 1967; Tabbush, 1987), temperature (Webb and von Althen, 1980), and duration (Ritchie et al., 1985). Bates and Niemiera (1994) found that, following storage and transplanting into a moist substrate, stem xylem water potentials ($\Psi_s$) increased (became less negative) for Norway maple (Acer platanoides), but decreased for Yoshino cherry (Prunus x yedoensis), which resulted in more stem dieback and reduced survival for cherry than maple. These results suggested species differences in stem water loss rates, root water absorption and conductivity or a combination of these factors. Sulaiman (1968) found differences in the rate of water loss through the defoliated stems of Quercus alba L. and Fraxinus pennsylvanica Marsh., however, there are no reports on the contribution of stem water loss and root hydraulic conductivity ($J_V$) to decreasing $\Psi$ after transplanting. Objectives of this study using bare-root trees were to 1) determine the influence of storage duration on the post-transplant $\Psi_s$ of desiccation sensitive Crataegus phaenopyrum and desiccation tolerant Acer platanoides, 2) determine how $J_V$ was influenced by cold storage duration and treatments, and 3) determine if post-storage stem wax coating influenced $\Psi_s$ and bud break.
Materials and Methods

Storage duration and $\Psi$. On January 14, 1993, 2-yr.-old bare-root *Acer platanoides* and *Crataegus phaeopryrum* seedlings 60-90 cm (24-36") tall were received in Blacksburg, VA. from Lawyer Nurseries, Plains, MT. During the five day shipping period seedlings were wrapped in plastic sheeting and placed in cardboard boxes with the roots of each bundle packed in moistened, shredded newsprint. Upon arrival, trees were sorted for uniformity, enclosed in storage bags (Union Camp Corp., Tifton, GA.) to reduce water loss and placed on wooden racks in a walk-in cooler maintained at 70% ± 5% relative humidity and 2°C (35°F). On January 28, February 11, February 28, March 11, March 28, and April 11, (2, 4, 6, 8, 10 and 12 weeks in storage, respectively) 16 hawthorn and 16 maple trees were randomly selected and removed from cold storage. Stem water potential was measured between 1200 and 1400 HR the same day using a portable pressure chamber (Model 3005, SoilMoisture Equipment Corp., Santa Barbara, CA) on a 10.2 cm (4 in) stem section excised from 8 trees of each species. The remaining trees were transferred to a greenhouse ventilated at 24°C (75°F) and heated at 18°C (64°F), transplanted into 100% pine bark-filled 3.8 l (1 gal) plastic containers and thoroughly irrigated. Stem water potential was then measured between 1200 and 1400 HR for these trees five days after transplanting. Storage duration and time of measurement factors were applied in a completely randomized design with eight single plant replications. Data were subjected to analysis of variance (ANOVA) and mean comparisons were made using a $t$ test. Species (maple and hawthorn) data were analyzed separately.

Storage treatment and root hydraulic conductivity. Plant material was identical to that used in the above experiment except that the time seedlings were
placed into cold storage, one of the following treatments was randomly allocated to each tree: 1) whole plant covered in which the entire seedling was enclosed in a sealed 3-layer storage bag (Union Camp Corp., Tifton, GA.), 2) shoot exposed in which seedling roots were enclosed in a storage bag sealed around the stem just above the root collar, 3) roots exposed in which shoots were enclosed in a storage bag sealed just below root collar and 4) entire seedling exposed (no storage bag). Storage bags were compressed during plant insertion to minimize air space within the bag; all trees were placed horizontally on racks. On January 28, February 11, February 28, March 11, March 28 and April 11, six hawthorn and six maple trees from each treatment were removed from cold storage and $J_v$ was measured on three seedlings from each species x treatment combination. The remaining three seedlings from each combination were placed on a lab bench and air-dried at 24°C (75°F) and 35\% ± 5\% relative humidity for 12 hr. For $J_v$ determinations, 15.3 cm (6 in.) excised primary lateral roots from each plant were submerged in distilled water; cortical tissue at the proximal end of the root was trimmed exposing the stele which was inserted into a section of Tygon tubing and fastened with a silicon washer. Root and tubing were mounted into the lid orifice of a pressure chamber with the proximal root end protruding through a gasket. The distal portion of the root system was immersed in a water-filled plastic container located in the pressure vessel. Water temperature was maintained at 20°C (68°F). Hydrostatic pressure was slowly increased to 0.5 MPa using compressed air. The volume of water that exited the cut root surface was measured with a pipette attached to the tubing. Water flow rates through tubing were recorded at 5-min intervals until the change in flow rate over time was the same for a minimum of three readings which indicated the system had reached equilibrium. The volume of water flow (flux) at equilibrium was expressed on a root dry weight basis.
Data were subjected to analysis of variance (ANOVA). Species and cold storage treatments were replicated three times using a completely randomized design. Desiccation time (0 hr vs. 12 hr) and storage duration data were analyzed separately. Slope of the least squares was determined for storage treatments over storage duration.

*Stem wax treatment.* Dormant 2-yr.-old *Crataegus phaenopyrum* bare-root seedlings were received from Lawyer Nurseries, Plains, MT on February 16, 1994 and placed in cold storage maintained at 90% relative humidity and 2°C (35°F). Within storage, 78 trees were enclosed in storage bags (approx. 10 trees/bag) and 78 trees were unbagged. On February 24, 78 seedlings from each group (bagged and unbagged) were removed from storage and $\Psi_s$ was measured between 1200 and 1400 HR on six trees of each group. The entire shoot system of 36 trees per storage treatment group were submerged in melted TissuePrep (Fisher Scientific Co., Fair Lawn, NJ) paraffin and then dipped into cold water to solidify the wax. The remaining 36 uncoated trees from each group served as the control. All seedlings were then immediately transplanted into 100% pine bark-filled 3.8 l (1 gal) containers and placed on raised benches in a greenhouse which was ventilated at 24°C (75°F) and heated at 18°C (64°F). Shoot water potentials were measured between 1200 and 1400 HR on six trees from the wax treated and untreated controls 2, 4, 6, 8, 10, and 12 days after transplanting. Percent bud break (number of growing buds/total bud number) was measured for each tree 8 weeks after transplanting. Plants in the stem wax treatment and time after transplanting treatments were arranged using a completely randomized design replicated six times. Data were subjected to analysis of variance (ANOVA) and mean comparisons were made using the *t* test. Storage treatment (bagged and unbagged) data were analyzed separately.
Results and Discussion

Storage duration and $\Psi$. Pre-transplant maple $\Psi_s$ decreased with increasing cold storage duration (Table 1). This affect of storage duration on $\Psi_s$ was the same as found by Bates (1994) in a previous study. For the first six weeks in storage, pre-transplant maple $\Psi_s$ were $\geq$ -1.2 MPa. Post-transplant $\Psi_s$ values were the same as the respective pre-transplant values for the first six weeks in storage. After eight weeks in storage, pre-transplant values were $\leq$ -1.5 and post-transplant $\Psi_s$ were higher than the respective pre-transplant values. For hawthorn, $\Psi_s$ decreased more rapidly during storage than maple, reaching a low of -2.25 MPa after twelve weeks (Table 1). Also in contrast to maple, post-transplant $\Psi_s$ were lower than respective pre-transplant values for four of the six durations. In a similar study, Bates and Niemiera (1994) reported post-transplant recovery from water stress for Norway maple and lack of recovery for the desiccation sensitive Yoshino cherry. Post-transplant recovery or the lack of recovery from pre-transplant induced water stress may be related to water absorption by roots, conductivity of the pre-bud break root system, stem water loss characteristics, or a combination of these factors.

Storage treatment and root hydraulic conductivity. Root hydraulic conductivity values were the same for both species within each post-storage desiccation treatment ($P = 0.05$, data not shown). Root hydraulic conductivity (averaged over species) for trees (0 hr desiccation treatment) decreased rapidly with increased storage duration for roots exposed (slope = -5.03) and whole plant exposed treatments (slope = -4.06) (Fig. 1). Compared to the roots exposed and whole plant exposed treatments, the decrease in $J_v$ was low for the shoots exposed (slope = -1.32) and entire seedling covered (slope = -1.43) treatments. At each storage duration, root
hydraulic conductivity for roots exposed and entire seedling exposed treatments were less than for shoots exposed and entire seedling covered treatments. Relative to the 0 hr desiccation treatment, air drying trees for 12 hr greatly reduced water conductivity rates resulting in no differences between storage treatments (Fig. 1).

Root hydraulic conductivity data showed that water flow in roots of both species was very sensitive to the storage conditions of this study and a 12 hr exposure to ambient conditions (Fig. 1). Water stress in loblolly pine has been shown to reduce water absorption because of an apparent reduction in root cell permeability (Brix, 1960). The relatively large decrease in \( J_v \) after a 12 hr desiccation period indicated the necessity for growers to protect root systems of bare-root plants during planting. Results of this and other work (Insley and Buckley, 1985) support the contention that roots of seedling nursery stock are extremely vulnerable to desiccation stress. The lack of differences in \( J_v \) between species, implied that the movement of water through roots was the same for desiccation sensitive and desiccation tolerant species. Hence, the difference in post-transplant \( \Psi_s \) responses between maple and hawthorn (Table 1) was apparently related to species specific stem water loss characteristics. In support of this contention, Bates (1994) reported that hawthorn \( \Psi_s \) decreased more rapidly and to a greater extent during cold storage than Norway maple \( \Psi_s \).

**Stem wax treatment.** Stem water potential for unwaxed hawthorn seedlings that were unstressed (covered) during storage decreased 69% during the twelve days after transplanting compared to only 28% for the wax covered seedlings (Table 2). Thus, the wax covering greatly ameliorated post-transplant water stress. Bud break (percent of total buds emerging) for unstressed trees with wax-coated stems was 26% higher than for trees without the wax coating (Table 2). Stressed hawthorn seedlings (uncovered in storage) exhibited the same \( \Psi_s \) trends as unstressed seedlings (Table 2)
although values were lower and differences between wax and no wax treatments occurred four days after transplanting compared to eight days for unstressed trees. This finding is in agreement with Murakami et al. (1990) who reported that hawthorn bud break decreased from 78% to 27% when $\Psi_s$ decreased from -0.8 to -2.5 MPa. The wax coating data of the current work indicated that water exiting through hawthorn stem tissue was responsible for increasing water stress. The low bud break percentages for the unwaxed trees of both stressed and unstressed experiments demonstrated the lack of desiccation tolerance of hawthorn. From a commercial standpoint, bud break percentages for the unwaxed treatments are unacceptable.

In summary, we found that bare root maple and hawthorn water stress increased with increasing cold storage duration. Maple $\Psi_s$ decreased to -1.3 MPa after six weeks of storage, whereas hawthorn $\Psi_s$ decreased to -1.4 MPa after only two weeks of storage. (Table 1). Compared to $\Psi_s$ during storage, $\Psi_s$ of transplanted maples either increased or remained the same. In contrast, $\Psi_s$ of transplanted hawthorn generally decreased. The desiccation sensitive nature of hawthorn is most likely related to water loss from stems since coating hawthorn stems with wax at transplanting minimized water stress and maximized bud break (Table 2). We concluded that water flow in roots was not responsible for the sensitivity because there was no difference in $J_v$ between hawthorn and maple (Fig. 1). Survival rates for transplanted bare-root hawthorn trees are relatively low in the nursery industry. Thus, understanding the nature of desiccation sensitivity can be used to improve water relations during cold storage and after transplanting thereby increasing post-transplant survivability. From this and other work (Bates, 1994; Englert et al., 1993), we recommend that the shoot and roots of desiccation sensitive species be enclosed in a
bag during cold storage and that stems be treated with an antidesiccant at transplanting.
Literature Cited


Fig. 1. Hydraulic conductivity (flux) of cold-stored seedling roots measured after 0 hr and 12 hr desiccation treatments. Storage treatments: whole plant exposed (◯), roots exposed (▲), shoots exposed (▼), whole plant covered (■). Means averaged over species, n=6. Vertical bars represent ± 1 SE.
Table 1. Influence of cold storage duration on shoot water potential of pre- and post-transplant 2-yr.-old Washington hawthorn and Norway maple.

<table>
<thead>
<tr>
<th>Measurement time</th>
<th>Initial$^z$</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Maple</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-transplant</td>
<td>0.90</td>
<td>0.92 a$^y$</td>
<td>1.14 a</td>
<td>1.25 a</td>
<td>1.55 a</td>
<td>1.80 a</td>
<td>1.84 a</td>
</tr>
<tr>
<td>5 days post-transplant</td>
<td>0.75 a</td>
<td>0.95 a</td>
<td>1.15 a</td>
<td>1.20 b</td>
<td>1.40 b</td>
<td>1.45 b</td>
<td></td>
</tr>
<tr>
<td><strong>Hawthorn</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-transplant</td>
<td>1.10</td>
<td>1.45 a</td>
<td>1.85 a</td>
<td>1.92 a</td>
<td>1.84 a</td>
<td>1.93 a</td>
<td>2.25 a</td>
</tr>
<tr>
<td>5 days post-transplant</td>
<td>1.82 b</td>
<td>2.10 a</td>
<td>2.27 b</td>
<td>2.23 b</td>
<td>2.30 b</td>
<td>2.41 a</td>
<td></td>
</tr>
</tbody>
</table>

$^z$ Pre-storage $\Psi_s$.

$^y$ Mean separation by t test at $P=0.05$. Same letter within column (by species) indicates no significant difference, $n = 8$. 

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Table 2. Influence of wax coating on shoot water potential and bud break of unstressed (bagged in storage) and stressed (unbagged in storage) 2-yr.-old Washington hawthorn after transplanting.

<table>
<thead>
<tr>
<th>Stem treatment</th>
<th>Initial $\Psi^Z$</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>Bud break$^Y$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wax coated</td>
<td>0.76 a$^x$</td>
<td>0.72 a</td>
<td>0.69 a</td>
<td>0.70 a</td>
<td>0.60 a</td>
<td>0.57 a</td>
<td>0.55 a</td>
<td>85 a</td>
</tr>
<tr>
<td>No wax coating</td>
<td>0.74 a</td>
<td>0.77 a</td>
<td>0.90 a</td>
<td>0.94 a</td>
<td>1.00 b</td>
<td>1.20 b</td>
<td>1.25 b</td>
<td>59 b</td>
</tr>
<tr>
<td>Wax coated</td>
<td>1.82 a</td>
<td>1.53 a</td>
<td>1.15 a</td>
<td>1.03 a</td>
<td>0.98 a</td>
<td>1.02 a</td>
<td>1.13 a</td>
<td>71 a</td>
</tr>
<tr>
<td>No wax coating</td>
<td>1.77 a</td>
<td>1.79 a</td>
<td>1.88 b</td>
<td>1.95 b</td>
<td>2.02 b</td>
<td>2.26 b</td>
<td>2.25 b</td>
<td>26 b</td>
</tr>
</tbody>
</table>

$^Z$ Stem water potential prior to transplanting.
$^Y$ % bud break 8 weeks after transplanting.
$^x$ Mean separation within columns by t test, P = 0.05. Means followed by the same letter are not significantly different, n = 6.
CHAPTER FOUR

EFFECT OF COLD STORAGE AND PRE-TRANSPLANT DESICCATION ON ROOT GROWTH POTENTIAL AND BUD BREAK OF BARE-ROOT WASHINGTON HAWTHORN AND NORWAY MAPLE

Abstract

Two-year-old Washington hawthorn (Crataegus phaenopyrum Med.) and Norway maple (Acer platanoides L.) seedlings were subjected to varying cold storage durations and the storage treatments: whole plant covered, shoots exposed, roots exposed and whole plant exposed. After storage, half the seedlings were immediately planted and half received a 12 hr desiccation treatment prior to transplanting. Root growth potential (RGP), time to bud break, and marketability were measured. For the root covered treatments, Norway maple RGP increased while Washington hawthorn RGP decreased with increased cold storage duration. RGP for both species remained low throughout storage for treatments exposing roots. The 12 hr desiccation treatment reduced RGP for both species with hawthorn being more affected than maple. Days to bud break for both species decreased with increased storage time for whole plant covered treatments, but increased for both species when stored with exposed roots. Maple marketability for root covered treatments was high for most storage durations. Hawthorn marketability was generally low except for the whole plant covered treatment during the first six weeks of storage. For the respective storage durations, hawthorn RGP, time to bud break and marketability values for the
shoots exposed treatment were similar to the roots exposed treatments for hawthorn. In contrast, values for the shoots exposed treatment were similar to the whole plant covered treatment for maple. There was a high positive correlation between RGP and marketability for both species.

Introduction

Rapid root regeneration is a critical factor in the successful establishment and survival of transplanted tree seedlings (Stone et al., 1962; Smith, 1986; Watson, 1986). Root growth potential (RGP), defined as the ability of a bare-root seedling to grow roots when placed in a favorable environment (Ritchie, 1985), has been positively correlated with field performance for numerous conifers (Feret and Kreh, 1985; Ritchie and Dunlap, 1980; Sutton, 1987) and deciduous trees (von Althen and Webb, 1978; Webb, 1977). Such trees with relatively high RGP's are able to quickly exploit water and nutrients beyond the original planting hole, thus hastening establishment.

Bare-root trees are commonly dug in late fall, placed in cold storage, and shipped and replanted in the spring. Seedlings handled in this manner are subject to desiccation during storage and handling prior to transplanting. Desiccation during these periods can lead to xylem cavitation and loss of hydraulic conductivity (Sperry et al., 1988), stem dieback (Englert et al., 1993) and reduced RGP (Webb and von Althen, 1980). Desiccating conditions affect species differently with regard to tissue (Coutts, 1981) and growth stage vulnerability (Mullin, 1963); survival, therefore is often species specific (Insley and Buckley, 1985). Bates (1994) found that shoots of
Washington hawthorn lost water more rapidly in cold storage than shoots of Norway maple and that water loss through hawthorn stems negatively impacted root water potential. These results indicated that while root protection for all bare-root stock is imperative, desiccation sensitive species such as hawthorn require both shoot and root protection to minimize water stress during cold storage. There is little information available comparing the impact of water loss during cold storage on the RGP and bud break of desiccation sensitive and tolerant species. Therefore, the objective of this study was to determine the influence of root and shoot exposure during storage and handling prior to transplanting, on RGP, timing of bud break and marketability of a desiccation tolerant (Norway maple) and desiccation sensitive (Washington hawthorn) species.

Materials and Methods

On January 14, 1993, 2-yr.-old Norway maple (Acer platanoides L.) and Washington hawthorn (Crataegus phaenopyrum Med.) bare-root seedlings (approx. 24-36") were received in Blacksburg, VA. from Lawyer Nurseries, Plains, MT. Prior to shipping, trees were held in 2°C (35°F) and 98% relative humidity cold storage for approximately 30 days at Lawyer Nurseries. Seedling bundles were wrapped in plastic sheeting and placed in cardboard boxes with the roots of each bundle packed in moistened, shredded newsprint. Transit time was approximately five days. Trees were sorted for uniformity and two-hundred forty of each species were placed on wooden racks in a walk-in cooler maintained at 70% ± 5% relative humidity and 2°C (35°F). At the time seedlings were placed into cold storage one of the following four
treatments were randomly allocated to each tree: 1) whole plant covered in which the entire seedling was enclosed in a sealed 3-layer storage bag (Union Camp Corp., Tifton, GA), 2) shoot exposed in which seedling roots were enclosed in a storage bag sealed around the stem just above the root collar, 3) roots exposed in which shoots were enclosed in a storage bag sealed just below the root collar and 4) entire seedling exposed (no storage bag). Storage bags were compressed during plant insertion to minimize air space within the bag; all trees were placed horizontally on racks. Two-yr.-old Washington hawthorn and Norway maple were also grown outdoors in raised beds at the VA TECH nursery, Blacksburg, VA.

On January 28, February 11, February 28, March 11, March 28 and April 11 (2, 4, 6, 8, 10, and 12 weeks in storage, respectively) 10 Washington hawthorn and 10 Norway maple trees from each treatment were randomly selected and removed from cold storage. Locally grown seedlings of each species were also dug from nursery beds on the above dates. All trees from each species x treatment combination (and locally grown trees) were planted into 46 x 10 x 41 cm (length x width x height) trays containing 100% pine bark (DeWald and Feret, 1988). The RGP test was conducted in a greenhouse where air temperatures were maintained at 24°C (75°F) day, 16°C (60°F) night and day length was extended to 16 hours with sodium vapor lamps. After 28 days, half the seedlings were carefully removed from the trays, the pine bark washed from the roots and the number of new white roots counted for each seedling. Days to first bud break and marketability were determined for the remaining five trees from each combination. Marketable plants exhibited less than 10% shoot dieback. Trees of each species and storage treatment were removed from cold storage at each cold storage duration and exposed to desiccating conditions prior to the RGP test. All seedlings receiving the desiccation treatment were placed on a lab bench and
allowed to air-dry at 24°C (75°F) and 35% ± 5% relative humidity for 12 hours. Data were subjected to analysis of variance. A factorial set of treatments: 2 species, 4 cold storage treatments, 6 storage durations was replicated five times using a completely randomized design. Desiccation time (0 hr vs. 12 hr) data were analyzed separately.

Results and Discussion

Norway maple RGP (number of new roots per transplanted seedling) generally increased over time and was highest for treatments covering roots (whole plant covered, shoots exposed) compared to treatments without root covering (0 hr desiccation; Fig. 1). RGP for plants that had roots covered and received a 12 hr desiccation treatment increased until week eight and decreased thereafter. RGP was near zero at each storage duration for treatments exposing roots (whole plant exposed, roots exposed). Hawthorn RGP (0 hr desiccation) was greatest for the whole plant covered treatment and increased from week two to week four and decreased thereafter. RGP for the shoot exposed treatment was intermediate between the whole plant covered treatment and the two treatments exposing roots for weeks four through ten. As with Norway maple, the 12 hr desiccation treatment reduced Washington hawthorn RGP for all treatments, with the whole plant covered treatment being higher for week four through eight than the other covering treatments. There was an increase in RGP on week four for the root covered treatments, with RGP decreasing thereafter.

The increase in RGP over time for maple is in agreement with Webb (1976) who reported increasing RGP with increased storage duration for *Fraxinus americana*
L. seedlings. Also, entirely covered hawthorn plants had higher RGP than plants that only had roots covered which indicated that stem water loss influenced root regeneration. This finding coincides with water potential data of Chapter 2 where, at each storage duration, hawthorn plants which were completely covered had higher root water potentials than plants with only roots covered. This suggested that water lost through the stems negatively impacted root water relations. Apparently, the negative effect of storage duration on hawthorn root emergence is at least in part due to water stress acquired in storage. Commercial nurseries typically cover only the roots of bare-root trees during storage (Lawyer Nurseries, personal communication). The results of the present study indicate that completely covering desiccation sensitive species such as Washington hawthorn during storage will improve new root initiation after transplanting. Reduced RGP of both species after a 12 hr desiccation treatment (current work) demonstrated the importance of protecting roots during the transplanting process.

RGP of many deciduous hardwood species follows a predictable hormone-mediated pattern, whereby new root initiation is low in autumn and winter, increases rapidly through spring, and returns to low levels by summer (P. Feret, personal communication). Washington hawthorn and Norway maple seedlings grown outdoors and harvested from VA TECH nursery beds exhibited such a pattern of RGP development (data not shown). RGP for locally grown hawthorn and maple also was very similar to RGP trends for cold-stored hawthorn (whole plant covered treatment) and maple (whole plant covered and shoots exposed treatments), (Fig. 1 and 2).

It is interesting to note that root hydraulic conductivity (Rhc) decreased for both hawthorn and maple with increased storage duration (Chapter 3, Fig. 1), while maple RGP generally increased with increased storage duration (Fig. 1 and 2). These
differences in response to storage duration may be related, in part, to seedling root type and root water relations. Dormant cold-stored seedlings have highly suberized older roots which usually exhibit low water conductivity. The RGP tests of this study measured initiation of new roots after transplanting, which typically possess high conductivity. Also, during cold storage root water potential ($\Psi_r$) may decline due to increased moisture stress (Chapter 2, Table 2). With the initiation of new roots after transplanting, however, $\Psi_r$ increases (unpublished data).

Differences among storage covering treatments in the number of days required for maple seedlings to break bud were not evident until six weeks of cold storage (Table 1). At eight weeks of storage and thereafter, the time to bud break for treatments exposing roots was greater than both treatments covering roots. After four weeks storage, the time to hawthorn bud break was less when the whole plant was covered compared to other treatments and the days to bud break decreased with storage time (Table 1). Also, stored for twelve weeks, plants that were not totally covered did not show signs of bud break after 90 days. The stems of these plants were visibly desiccated. In a study using green ash (Fraxinus pennsylvanica Marsh.) and paper birch (Betula papyrifera Marsh.), Harris et al. (1993) reported that days to bud break decreased as cold storage duration increased. The trees of that study were heeled in with moist vermiculite and experienced no stem dieback. The bud break data for the whole plant covered storage treatment (both species) of this study are in agreement with Harris et al. however, as storage desiccation increased (roots exposed, whole plant exposed storage treatments) days to first bud break and stem dieback also increased.

Marketability for maples with roots covered (whole plant covered and shoots exposed storage treatments) was 100% beyond six weeks of cold storage (Table 2).
For treatments exposing roots only, marketability was 40% or less at all storage durations. In contrast, hawthorn marketability for the whole plant covered treatment was 100% after four weeks of storage but declined to 20% by week ten and was 0% for the roots exposed treatments for all storage durations (Table 4). Compared to the root exposed treatments, only covering the roots (shoots exposed) increased marketability for the first six weeks in storage. Exposing Norway maple seedlings to a 12 hr desiccation treatment prior to transplanting resulted in 0% marketability for all storage treatment x duration combinations except the whole plant covered treatment at weeks eight and ten (80% and 80%, respectively) and the shoots exposed treatment at weeks six, eight and twelve (60%, 40% and 20%, respectively). A 12 hr desiccation treatment rendered hawthorn seedlings unmarketable for all storage treatments and durations (data not shown).

A major objective in assessing forest seedling RGP is to predict field survival and performance. Similarly, nurserymen are interested in post-transplant survival and subsequent marketability. In this study, a high positive correlation existed between RGP and marketability for Norway maple \( r = 0.86 \) and Washington hawthorn \( r = 0.91 \). Webb (1977) reported a strong correlation between RGP and field survival for *Acer saccharum* Marsh., *Acer saccharinum* L., and *Fraxinus americana* L. This work demonstrated that the impact of storage duration and plant part exposure during storage on seedling physiology was species specific. Maple root exposure and hawthorn root and shoot exposure during storage had a negative effect on RGP, time to bud break and marketability.
Literature Cited


Fig. 1. Root growth potential (number of new roots per seedling ≥ 0.5 cm) of cold-stored Norway maple seedlings measured after 0 hr and 12 hr pre-transplant desiccation treatments. Storage treatments: whole plant covered (◇), shoots exposed (△), roots exposed (□), whole plant exposed (○). Vertical bars represent ± S.E. of means, n = 5.
Fig. 2. Root growth potential (number of new roots per seedling ≥ 0.5 cm) of cold-stored Washington hawthorn seedlings measured after 0 hr and 12 hr pre-transplant desiccation treatments. Storage treatments: whole plant covered (▼), shoots exposed (▲), roots exposed (■), whole plant exposed (●). Vertical bars represent ± S.E. of means, n = 5.
Table 1. Influence of cold storage duration and storage treatment on the number of days between transplanting and first bud break for 2-yr.-old Norway maple and Washington hawthorn.

<table>
<thead>
<tr>
<th>Storage treatment</th>
<th>Storage duration (weeks)</th>
<th>Days to first bud break</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Whole plant covered</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>37 a²</td>
<td>35 a</td>
</tr>
<tr>
<td>Shoots exposed</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>39 a</td>
<td>36 a</td>
</tr>
<tr>
<td>Roots exposed</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>36 a</td>
<td>33 a</td>
</tr>
<tr>
<td>Whole plant exposed</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32 a</td>
<td>36 a</td>
</tr>
</tbody>
</table>

² Mean separation by Duncan’s multiple range test, P = 0.05.

Y Means in columns followed by the same letter are not significantly different.

Asterisk (*) indicates that plants had not broken bud 90 days after transplanting.
Table 2. Influence of cold storage duration and storage treatment on the marketability of 2-yr.-old Norway maple and Washington hawthorn.

<table>
<thead>
<tr>
<th>Storage treatment</th>
<th>Storage duration (weeks)</th>
<th>Marketability (%) (^ \text{*} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Whole plant covered</td>
<td>40</td>
<td>40</td>
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<tr>
<td>Shoots exposed</td>
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<td>100</td>
</tr>
<tr>
<td>Roots exposed</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Whole plant exposed</td>
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<td>20</td>
</tr>
<tr>
<td>Whole plant covered</td>
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<td>100</td>
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<td>Shoots exposed</td>
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<td>60</td>
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<tr>
<td>Roots exposed</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Whole plant exposed</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^*\) Percent marketability: number of plants with \( \leq 10\% \) shoot dieback ÷ total number of plants \((n = 5)\), evaluated after 90 days.
CHAPTER FIVE

A COMPARISON OF MORPHOLOGICAL FEATURES AFFECTING WATER LOSS IN NORWAY MAPLE AND WASHINGTON HAWTHORN STEMS

Abstract

_Crataegus phaenopyrum_ (Washington hawthorn) stems are known to become water stressed during cold storage at a faster rate than those of _Acer platanoides_ (Norway maple). Histological examination revealed that maple stems had a highly suberized periderm, and a uniform cuticle with few disruptions. Periderm suberization of hawthorn stems was variable and extensive peridermal cracking was evident. Cuticle wax decreased with increasing distance from the stem apex for both species. No differences in lenticellar characteristics were found between species. Collectively, results indicated that hawthorn stems had more pathways for water loss than maple stems, which may provide a possible explanation for differences in maple and hawthorn stem water loss rates.
Introduction

Shoot surfaces of woody plants have specialized protective layers that minimize water loss to the atmosphere. Primary organs such as leaves and young stems are covered by a cuticle whereas older secondary organs develop a periderm (Fahn, 1990) which consists of layered phellogen, phellem and phelloderm. Cells within these layers vary in the degree of suberization (Schonherr and Ziegler, 1980). Suberin is a lipid polymer and water permeability of suberized cells is low (Holloway et al., 1972). Cuticles provide an effective barrier to water movement and enable plants to withstand conditions of water stress (Schonerr and Schmidt, 1979). Cuticles are layered lipid membranes composed of soluble waxes and an insoluble polymer matrix, primarily containing pectin, cellulose and lipids (Juniper and Jeffree, 1983). Schonerr (1976a) found that the cuticular resistance to water transport was attributable to the soluble lipids embedded in the polymer matrix. Cuticle thickness and composition vary between plant species and are influenced by environmental conditions (Baker, 1974). Seiler (1985) reported increased epicuticular wax content on Alnus glutinosa leaves exposed to twelve weeks of sublethal water stress.

Plant lenticels, loose arrangements of cells in the periderm, are assumed to function in gas exchange (Fahn, 1990). The stems of deciduous trees can lose significant amounts of water through lenticels and other disruptions in the periderm. Kozlowski (1943) reported that Liriodendron tulipifera L. lost approximately 2 g water/100 cm² stem surface area per week during the winter. In temperate zones, lenticels typically become occluded by a layer of suberized cells at the end of the growing season. This closing layer minimizes water loss during dormancy and is ruptured with the renewal of growth in the spring (Fahn, 1990). The lack of, or
incomplete development of a closing layer may play a role in the loss of water vapor while seedlings are in cold storage (S.E. Sheckler, personal communication).

We have shown that the water relations differ between the desiccation tolerant Acer platanoides and the desiccation sensitive Crataegus phaenopyrum during and after cold storage (Ch.2, Ch.3). However, there are no reports on the cuticular, peridermal and lenticular characteristics of these species and on the possibility that these anatomical aspects are responsible for the species specific water loss traits. The objective of this study was to compare the degree of stem suberization, lenticular characteristics and cuticular wax content of Norway maple (desiccation tolerant) and Washington hawthorn (desiccation sensitive) stems.

Materials and Methods

Plant materials. On January 25, 1994, two-yr.-old Norway maple (Acer platanoides L.) and Washington hawthorn (Crataegus phaenopyrum Med.) bare-root seedlings (approx. 24-36") were received in Blacksburg, VA from Lawyer Nurseries, Plains, MT. Prior to shipping, trees were held in 2°C (35°F) and 98% relative humidity cold storage for approximately 60 days at Lawyer Nurseries. During transit, seedling bundles were wrapped in plastic sheeting and placed in cardboard boxes with the roots of each bundle packed in moistened, shredded newsprint. Trees were sorted for uniformity upon arrival and one-hundred fifty of each species were placed in a 3-layer storage bag (Union Camp Corp., Tifton, GA) on wooden racks in a walk-in cooler maintained at 90% ± 5% relative humidity and 2°C (35°F). Before seedlings were removed from cold storage for morphological studies, the terminal 30 cm (12 in)
of a randomly selected branch of each species was excised. Each 30 cm branch was then divided into three 10 cm (4 in) segments (distal, 0-10 cm; middle, 11-20 cm; proximal, 21-30 cm). Cut ends of segments were sealed with melted paraffin wax and segments placed in plastic trays containing moistened paper towels.

**Lenticel studies.** Twelve distal, middle and proximal stem segments of each species were removed from cold storage. Internodal stem diameter of each segment was measured at three points with a microcaliper to establish average stem diameter. Stems were assumed to be approximately cylindrical and the average diameter and stem length (measured to the nearest 0.1 cm) were converted to approximate surface area. Lenticels from each segment were counted using a stereomicroscope and colored marker to identify lenticels appearing on the segment surface as the segment was moved across the field. Five distal, middle and proximal stem segments were selected from the original twelve segments per species and a single lenticel was excised from each stem segment and fixed in formalin:acetic acid:alcohol (FAA). Fixed lenticels were dehydrated in an ethanol series (25, 40, 50, 70, 80, 95, 100% and absolute), embedded in paraffin, and sectioned at 10 μm (Berlyn and Miksche, 1976). Sections were mounted and stained using saffranin which stains lignin and suberin red (Pearse, 1985). Sections were examined with a Zeiss light microscope to determine the presence of closing cell layers. Lenticel counts were subjected to ANOVA.

**Cuticle studies.** Five distal, middle and proximal stem segments of each species were removed from cold storage. A 0.75 cm cross-sectional portion of each stem segment was excised at a point near the middle of the stem segment. Tissue was fixed, sectioned and stained as described previously. Wax content was determined on five 27 cm (9 in) stem segments per species. Wax was removed by dipping stem sections for 30 seconds in 7 ml of chloroform contained in a preweighed glass test
tube. The wax was filtered through Whatman No. 1 filter paper, evaporated to dryness under nitrogen and the test tube reweighed to determine net wax content (Jeffree et al., 1971). Each 27 cm stem segment was divided into nine 3 cm (1.2 in) long sections to calculate wax content as a function of distance from the stem apex.

Periderm suberization and analysis of hawthorn stem cracking. Three distal, middle and proximal stem segments of each species were removed from cold storage. A 0.75 cm cross-sectional portion of each stem segment was excised at a point near the middle of the stem segment. Tissue was fixed and sectioned as described previously. Sections were stained using Sudan IV in ethylene glycol (Jensen, 1962) to detect relative amounts of suberin in periderm layers. Additional middle and proximal hawthorn stem segments containing longitudinal cracks were fixed, stained with Sudan IV and sectioned. Sections were examined with a light microscope to determine the depth and approximate dimensions of stem cracks. An environmental scanning electron microscope (ESEM) was used for examination of stem surface characteristics. Use of this instrument eliminated the need for tissue fixation, dehydration and coating.

Results and Discussion

Lenticellar characteristics: At each stem location, maple lenticel number was the same for hawthorn (Table 1). However lenticel number (for both species) was higher (P = 0.05) at the distal location than at the proximal location. Decreasing lenticel numbers (with increasing distance from the stem apex) may be due to normal developmental patterns common in tree seedlings. Lenticel number (per unit stem
surface area) would tend to decrease as stem diameter increases, given a relatively constant rate of lenticel production. Microscopic analysis of lenticel anatomy showed the presence of suberized complementary cells surrounding the lenticellar opening (Fig. 1). Cellular disruptions within all observed lenticels did not extend through the periderm or into cortical tissue. Lack of differences between species appears to discount lenticels as a factor contributing to differences in stem water loss rates observed between maple and hawthorn as shown by Bates (1994).

**Cuticular wax:** Examination of hawthorn stem cross-sections using light microscopy revealed that a relatively thick (10-20 μ), uniform cuticle was present on the distal portion (0-10 cm) (Fig. 2). Maple cuticle at this stem location appeared as thick as hawthorn (photo not shown). Cuticle wax content decreased with increasing distance from the stem apex for hawthorn and maple (Fig. 3). However, hawthorn wax content at 27 cm from the apex was 0.10 mg/cm² whereas the corresponding value for maple was > 0.2 mg/cm². Cuticle wax content can be an important factor affecting cuticular transpiration (DeLucia and Berlyn, 1984). Low wax content may contribute to the dieback of seedling tips which often occurs after transplanting. Removal of wax from the cuticles of *Citrus* leaves increased their permeability to water by a factor of 300-500 (Schonerr, 1976b). The species wax content differences do not appear to be large enough to account for the differing stem water loss rates observed between hawthorn and maple in previous studies (Bates, 1994). Decreasing wax content with increasing distance from the stem apex may be due to decreasing production in the older portions of the stem as well as wax erosion by environmental factors.

**Tissue suberization:** Microscopic analysis of distal segment cross-sections showed a higher degree of suberization in maple periderm cells (Fig. 4A) than in
hawthorn periderm (Fig. 4B). The relatively high degree of maple periderm suberization found 10 cm from the stem apex continues into the middle (10-20 cm) and proximal (20-30 cm) stem locations (pictures not shown). In contrast, the relatively low amount of hawthorn periderm suberization at the distal location is also evident at the middle stem location (Fig. 5A). Only at the proximal stem location (20-30 cm) is an increase in relative amounts of suberization apparent (Fig. 5B). Thus, as with the cuticular wax distribution (low wax content at proximal location), the low degree of suberization in the desiccation sensitive hawthorn may contribute to increased water loss and typically low post-transplant survival rates. *Betula papyifera* Marsh. and *Crataegus phaenopyrum* Med. have been characterized as desiccation sensitive species (Engler, et al., 1993a). The periderm of *Betula pendula* Roth. was shown to be very permeable to water transport as a result of little or no suberization of cell layers within the periderm (Schonerr and Ziegler, 1980).

*Stem cracking:* Examination of hawthorn stem surfaces using scanning electron and light microscopy revealed the presence of small longitudinal cracks or fissures (Fig. 6A and 6B). The cracks were noticeable to appear between 10-20 cm from the stem apex and continued toward the proximal end of the stem. Few cracks were observed within 7 cm of the stem tip. Dimensions of the cracks were highly variable, depending upon distance from stem apex. Near the apex cracks generally were less than 75 microns wide and less than 400 microns long (Fig. 6A), while 20-30 cm from the stem apex cracks were usually 0.5-1.0 mm wide by 4-10 mm long (Fig. 6B). Cross sectioning stems through these cracked regions revealed that the depth of the openings usually extended at least through the cuticle and epidermis if present, and often extended through the periderm into cortical tissue (Fig. 5A and 5B). Cross sections made above or below the stem cracks disclosed the presence of open cavities.
enclosed by a layer of periderm several cells thick (Fig. 5B). This suggested that the amount of cortical tissue exposed by the crack exceeds the actual dimensions of the crack. Cracking in the middle (10-20 cm) section of the stem was estimated to account for 3 to 7% of the stem surface area and cracks in the proximal (20-30 cm) portion of the stem accounted for 5 to 12% of the stem surface area (data not shown). The location at which stem cracks usually appear on hawthorn stems corresponds with stem locations with low epicuticular wax content (Fig. 2). In contrast to hawthorn, examination of maple stems using scanning electron and light microscopy revealed a generally uniform stem surface with few disruptions in the cuticle or epidermis (Fig. 7).

In summary, periderm suberization and cuticular characteristics in the terminal 30 cm of maple stems are rather uniform and would tend to mitigate stem water loss. In contrast, hawthorn had relatively low wax content at the proximal stem sections, lower levels of suberization, and more cracks in the stem surface than maple which could collectively contribute to stem water loss. These findings, at least in part, explain the relative desiccation tolerance and high survival rates of Norway maple and the desiccation sensitivity and low survival rates of Washington hawthorn (Bates, 1994; Englert et al., 1993b). An additional factor not analyzed in this study but may impact the desiccation characteristic of these species is stem diameter. Norway maple typically tends to produce a single rather stout central leader while Washington hawthorn produces several relatively thin branches. If hawthorn does loss water rapidly through periderm cracking, its capacitance would be lower than that of maple because of the larger surface area:volume ratio. Insley and Buckley (1985) reported that the finer root system of Betula pubescens Ehrh. contributed to its desiccation sensitivity in bare-root storage as compared to the coarser rooted Fraxinus
*angustifolia* Vahl. The capacitance of stem tissue could become important in bare-root storage because the seedling has no means to replace water lost through roots or shoots.
Literature Cited


Table 1. Average number of lenticels (per square centimeter of stem surface area) at three stem locations for 2-yr.-old Norway maple and Washington hawthorn.

<table>
<thead>
<tr>
<th>Species</th>
<th>Stem location (cm)</th>
<th>Number of lenticels (no./cm²)</th>
<th>mean</th>
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<tbody>
<tr>
<td></td>
<td>Distal (0-10)</td>
<td>Middle (10-20)</td>
<td>Proximal (20-30)</td>
</tr>
<tr>
<td>Maple</td>
<td>13&lt;sup&gt;z&lt;/sup&gt;</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Hawthorn</td>
<td>9</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Significance&lt;sup&gt;y&lt;/sup&gt;</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

<sup>z</sup> Mean of 12 replicates.

<sup>y</sup> ns = not significant at p = 0.05.
Figure 1. Cross-sections of lenticels 7-10 cm from stem apex of (A) Washington hawthorn, and (B) Norway maple stained with saffranin. Arrows indicate dark red regions of suberized complementary cells. Bar = 50 μm.
Figure 3. Relationships between cuticular stem wax content and distance from stem apex for Washington hawthorn Norway maple. Data points are means of eight measurements.
Figure 4. Cross-sections of (A) Norway maple and (B) Washington hawthorn stems, 7-8 cm from stem apex, illustrating differences in degree of periderm suberization, (arrows indicate stem periderm). Bar = 50 μm.
Figure 5. Light micrograph of cross-section through stem crack (A) located 10-20 cm from stem apex and (B) 20-30 cm from stem apex. Arrows indicate regions of darkly stained suberized cell layers. Bar = 50 μm.
Figure 6. Analysis of Washington hawthorn stem cracking. (A) Scanning electron micrograph of stem cracks located 10-20 cm from stem apex. Bar = 50 μm. (B) Photograph illustrating stem cracks located 20-30 cm from stem apex. Bar = 1 mm.
Figure 7. Norway maple stem surface, illustrating lack of cracks and cellular disruptions. (A) Scanning electron micrograph 10-20 cm from stem apex. Bar = 50 μm. (B) Photograph 20-30 cm from stem apex. Bar = 1 mm.
Significance to the Nursery Industry

Desiccation during postharvest processing, storage and re-establishment can reduce the quality and regrowth of bare-root trees. Cold storage of bare-root nursery stock after fall lifting is a common ornamental tree production practice that allows for greater flexibility in spring shipping and availability of planting stock. Cold storage desiccation of bare root trees is reduced through the use of water vapor barriers such as polyethylene. However, water loss from bare-root trees can continue during cold storage when only the roots are covered and the shoots remain exposed. The impact of storage conditions on trees are species specific. Norway maple is considered to be desiccation tolerant and Washington hawthorn is relatively desiccation sensitive. Our work showed that hawthorn stems are more susceptible to water loss during cold storage than Norway maple stems. Compared to the desiccation tolerant maple, bare-root hawthorn trees developed more water stress in storage and transplanted bare-root hawthorn trees had less ability to recover from this water stress. Roots of both species were susceptible to desiccation during cold storage. When roots of Norway maple were covered during storage, transplanted trees broke bud quickly and were 100% marketable. However, hawthorn required root and shoot covering during storage for quick bud break and relatively high marketability. Following storage, trees of both species received a 12 hour unprotected exposure to ambient lab conditions; after transplanting these trees had very low root growth potential and marketability percentages. Thus, during the transplanting process, trees need to be protected from desiccating conditions. Coating stems of the desiccation sensitive hawthorn trees with an antidesiccant compound before transplanting greatly reduced water stress and improved growth. While growers should take precautions to protect
the roots of all bare-root stock from desiccating conditions during and after storage, desiccation sensitive species such as Washington hawthorn require both root and shoot protection to minimize water loss and increase survival.
Vita

Ricky Martin Bates was born on November 4, 1958 in Cincinnati, Ohio. He attended and graduated from Immaculate Heart of Mary Elementary School and Anderson High School. He received his Bachelor of Science degree in Agriculture from West Virginia University in 1981 and his Master of Science degree in Horticulture from West Virginia University in 1986. In the Fall of 1986 he returned to Cincinnati, Ohio to work for the Ohio Cooperative Extension Service as the County Extension Agent in Horticulture. From 1987 until 1991 the author worked for the Virginia Cooperative Extension Service in Botetourt County, VA as the County Extension Agent, Agriculture. In the Fall of 1991 he continued his education in the Department of Horticulture, Virginia Polytechnic Institute and State University and successfully completed requirements for his Doctor of Philosophy degree in Horticulture in August 1994. The author is married and has three children.