Thermotolerance of Hemlock (*Tsuga spp.*) and Investigation of a Foliar Disorder in Crapemyrtle (*Lagerstroemia indica ×fauriei*)

by

Matthew Stephen Wilson

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 09, 2010

Keywords: Heat stress, direct injury, electrolyte leakage, rabbit tracks

Approved by

Jeff L. Sibley, Chair, Professor of Horticulture
D. Joseph Eakes, Professor of Horticulture
Gary J. Keever, Professor of Horticulture

Abstract

Foliar thermotolerance of six *Tsuga* species to direct heat injury was evaluated using electrolyte leakage. Species evaluated included: *T. canadensis, T. caroliniana, T. chinensis, T. diversifolia, T. heterophylla,* and *T. dumosa* (formerly *T. yunnanensis*). Species were exposed to 12 temperatures (25-60°C) for 30 minutes and electrolyte leakage from needle tissue was measured. *T. canadensis* and *T. dumosa* had similarly, higher predicted temperature midpoints (T_m) and *k*-values than *T. caroliniana* suggesting a higher resistance to direct heat injury when exposed to brief, supra-optimal temperatures. While differences were found in T_m and *k*-values between *T. canadensis* and *T. dumosa* when compared to *T. caroliniana*, no differences were found in T_m and *k*-values for *T. canadensis* or *T. dumosa* when were compared to *T. chinensis*, *T. diversifolia*, or *T. heterophylla*. Similarly, no differences were found for T_m and *k*-values of *T. caroliniana* when compared to *T. chinensis*, *T. diversifolia*, or *T. heterophylla*.

An online survey was conducted in cooperation with seven nursery and landscape associations in the southeastern U.S. to quantify the impact of a foliar disorder in *Lagerstroemia* spp. on the ornamental horticulture industry. Industry members were asked 16 questions to obtain demographic, disorder familiarity, and disorder significance information. No significant correlations between gross business income of participants and the ratings for the significance of “rabbit tracks” to the industry were found. Measures requiring a large amount of resources, expense, and costly solutions are not

recommended for diagnosis work or treatment, as industry members did not indicate significant losses or customer concern for effects of "rabbit tracks" on plant material quality.

A preliminary nutrition study was conducted on five *Lagerstroemia indica ×fauriei* cultivars using six modified nutrient solutions to evaluate foliar disorder occurrence in relation to treatment. Nutrient solutions were modified to exclude one macronutrient sulfur (S) or four micronutrients; copper (Cu), iron (Fe), manganese (Mn), or zinc (Zn), as prescribed by treatment. No occurrence of "rabbit tracks" for complete nutrient solution-treated plants was seen. There was no significant difference between any of the nutrient solutions for the occurrence of "rabbit tracks" foliar disorder on the five cultivars tested. Additionally, no significant relationship between modified nutrient solutions and percent chlorosis coverage, chlorosis stage, chlorosis progression, spot occurrence, spot pattern, or leaf distortion ratings were found. Chlorosis occurrence ratings due to treatment were found to be significant across all cultivars with iron-deficient nutrient solutions having higher incidence of chlorosis than zinc-deficient and complete nutrient solution treatments.

Acknowledgments

The author would like to express sincere gratitude and appreciation to Dr. Jeff Sibley for providing the opportunity to pursue the Master of Science degree in horticulture at Auburn. His guidance, support, and generosity will always serve as reminders of the wonderful mentorship and friendship provided to the author. The author would like to thank Drs. Gary Keever, Joe Eakes, John Ruter, and Brian Whipker for their guidance and patience in the pursuit of this degree and to Drs. Joe Eakes and Jeff Sibley for providing the opportunity and experience to instruct at the university level, providing real-world experience and training for the road ahead. The author would like to thank his parents Mark and Sandra Wilson, sister Kayla and brother-in-law Shane, and grandparents Mary Wilson and Henry and June Richards for their love, support and sacrifices to encourage the author to accomplish his goals and achievements. The author would also like to thank B.J. Brymer, Chris Marble, Jeremy Pickens, and Daniel Wells for the support and friendship provided through the partnership of Team MaterDirt, "Strangers we met, Friends we became, and Brothers we remain." In addition to aforementioned acknowledgments, the author would like to express gratitude to fellow Department of Horticulture faculty, staff, and graduate students for their friendship, support, and assistance. The author would like to thank the Auburn University Campus Club, Montgomery Federation of Garden Clubs, American Conifer Society, and Southern Nursery Association for the provision of scholarships for the author's academic pursuits.

Table of Contents

List of Tables

List of Figures

PART I THERMOTOLERANCE OF HEMLOCK (*TSUGA*)

Chapter I

Introduction and Literature Review

I. Hemlock cultivation history and species descriptions

The genus *Tsuga* currently consists of approximately 11 species (Table 1). Nine of the 11 species are commonly used within the landscape/nursery industries (Bitner, 2007; Dirr, 1998; Swartley, 1984). A member of the Pinaceae family, *Tsuga* may be differentiated from other family members such as *Pinus*, *Picea*, and *Abies* by its flat evergreen leaves that are joined to the stem via woody, short decurrent bases and pendulous female cones with imbricate scales (Radford et al., 1968). *Tsuga*, referred to by the common name hemlock, is unlike its family members in that it has a soft, fine texture in comparison to the coarse texture commonly associated with spruce (*Picea*) and fir (*Abies*) (Dirr, 1998). This soft texture gives hemlocks a special place within the landscape as specimen trees, unique hedges, in rock gardens, and other specialty uses (Forrest, 2005).

Typically found in mountainous regions, hemlocks thrive in areas with abundant rainfall and well-drained soil (Swartley, 1984). Hemlocks also may be found along streambeds in deeply shaded forests (Bitner, 2007). Four native species and one hybrid of *Tsuga*, as well as cultivated forms, can be found in North America. Along the eastern sector of the continent two species can be found within forests, *T. canadensis*

(L.) Carr. (Canadian or eastern hemlock) and *T. caroliniana* Engelm. (Carolina hemlock), and in the western sector the remaining three, *T. heterophylla* (Raf.) Sarg. (western hemlock), *T. mertensiana* (Bong.) Carr. (mountain hemlock), and *T.* ×*jeffreyi* (Henry) Henry [*heterophylla* x *mertensiana*]. In addition to native *Tsuga* species, four species of Asian origin are found to be in cultivation in North America and around the world. These species vary in origin, from *T. chinensis* (Franch.) Pritzel (Chinese hemlock), and *Tsuga dumosa* (D. Don) Eichler (Himalayan hemlock) in Central-Southern China to *T. diversifolia* (Maxim) Mast. (Northern Japanese hemlock) and *T. sieboldii* Carr. (Southern Japanese hemlock) in Japan (Swartley, 1984).

Of the North American *Tsuga*, the Canadian hemlock (*T. canadensis* (L.) Carr.) is the more prominently used specimen in the landscape. According to Dirr (1998), hemlock was introduced into cultivation around 1736 with the number of named cultivars and varieties currently exceeding 300. Canadian hemlock may be distinguished from other native hemlocks by its flatly arranged leaves with stalked cones and broad cone scales (Sargent, 1949). Noted for its conspicuous stomatal lines underneath leaves, relaxed branch tips, and small pendulous cones, Canadian hemlock often adorns mixed borders and plantings in the landscape as a prized specimen tree (Bitner, 2007). As a slow growing conifer with large variability within seedlings, many varieties and cultivars of *T. canadensis* have been developed such as 'Sargentii' with its pendulous branches and spreading growth habit reaching 10 m (30 ft) in spread and 'Bennett' growing only 0.6 x 1.2 m (2 x 4 ft) within ten years (Bitner, 2007; Dirr 1998). With such extensive use in cultivation, Canadian hemlock has encountered a major decline with the arrival of the hemlock woolly adelgid (*Adelges tsugae* Annan.). Brought to North America in the

1920's (Evans and Gregoire, 2007), from Asia and first recorded in eastern North America in 1951, hemlock woolly adelgid (HWA) has spread to cover 21% of Canadian hemlock's native range consisting of the Appalachian Mountain range from Nova Scotia to northern Alabama (Evans and Gregoire, 2007; Sargent, 1949). HWA is a member of the order Homoptera, a category of insects with two pairs of wings and piercing-sucking mouthparts that enable such insects to pierce plant tissues and extract sap, photoassimilates, and water from the plant (Evans and Gregoire, 2007; Pedigo, 2002). HWA's feeding method, efficient reproduction mechanisms, and lack of predators have allowed for mass infestations and continuous spread across North America, causing a decline in native hemlock stands along the eastern portion of the continent (Evans and Gregoire, 2007). Concern of HWA's potential to endanger existing habitats and landscapes of *Tsuga* is aiding research in finding and releasing predators with sufficient control and adaptability to surrounding environment to control HWA populations (McAvoy et al., 2007). In addition to developing predator controls for HWA, research evaluating several *Tsuga* species for potential host plant resistance with regard to plant nutrition and feeding preferences has been conducted to provide information useful for breeding programs to introduce species/ hybrids of hemlock resistant to HWA related death (Montgomery et al., 2009; Pontius et al., 2006; Tredici and Kitajima, 2004).

Another hemlock native along the eastern portion of North America is the Carolina hemlock (*T. caroliniana* Engelm.). Carolina hemlock is more isolated in its native range than Canadian hemlock as its range consists of southwestern Virginia to Northern Georgia (Dirr, 1998), typically at higher elevations than *T. canadensis*. Distinguishing characteristics from other native hemlocks are stalked cones with cone scales longer than wide and obtusely pointed bracts and bristle-like leaves not appearing to be flat, but two-ranked in arrangement off the stem (Bitner, 2007; Sargent, 1949; Swartley, 1984). Similar to Canadian hemlock, Carolina hemlock has two white stomatal lines underneath the leaf with a glabrous, dark green surface above. Reduced native range and reported lack of drought tolerance have not encouraged Carolina hemlock's use within the landscape (Bitner, 2007; Dirr, 1998). Like Canadian hemlock, Carolina hemlock is susceptible to HWA (Dirr, 1998; Montgomery et al., 2009).

North America also serves as the native range for two additional *Tsuga* species and one hybrid along its West coast. Western hemlock's (*T. heterophylla* (Raf.) Sarg.) native range extends from southern Alaska to Idaho and northern California (Dirr, 1998). Characterized by sessile cones with oval scales, *T. heterophylla* (Raf.) Sarg., also has blackish green leaves arranged in upright ranks along the stem with broad white stomatal lines underneath (Sargent, 1949; Swartley, 1984). Western hemlock is largely used by the timber and forestry industry, along with limited use in landscapes of western North America and western Europe (Bitner, 2007; Swartley, 1984). Western hemlock has shown less mortality in response to HWA infestations than eastern counterparts which was attributed to a greater presence of HWA-controlling predators in the West than host plant resistance (Pontius et al., 2006).

Mountain hemlock (*T. mertensiana* (Bong.) Carr.), western hemlock's companion in western North America, shares a similar native range from southeastern Alaska through British Columbia south to northern California (Sargent, 1949). More easily distinguished from other species of the genus, mountain hemlock is characterized by its convex leaves arranged along all sides of the stem with stomata on both lower and upper leaf surfaces and long, cylindrical cones with oblong scales (Sargent, 1949). Mountain hemlock also noticeably differs from other hemlocks by its blue-green leaves which have a somewhat prickly appearance due to the leaf arrangement along all sides of the shoots (Dirr, 1998). Mountain hemlock is found at high altitudes where it is tolerant of heavy snow loads. Preferring cool moist soils, mountain hemlock is reported to have difficulty adapting and growing in landscape and nursery situations because of excessive irrigation and susceptibility to *Phytophthora* root rots (Dirr, 1998; Swartley, 1984). Mountain hemlock serves the pulp industry mainly within the Pacific Northwest with a few cultivars released for use in landscapes (Bitner, 2007). As with the western hemlock, mountain hemlock has shown decreased mortality to HWA infestations in comparison to eastern North American species (Pontius et al., 2006).

Along with the two western species of *Tsuga*, a hybrid of the mountain and western hemlock (*T.×jeffreyi* (Henry) Henry) (USDA, 2007) exists. This hybrid is found in parts of British Columbia and Washington and closely resembles mountain hemlock in features and response to landscape situations but has been found to be highly susceptible to *Phytophthora* root rots (Dirr, 1998; Swartley, 1984).

Of the four Asian species of *Tsuga,* Himalayan hemlock (*T. dumosa* (D. Don) Eichler) and Chinese hemlock (*T. chinensis* (Franch.) Pritzel) are native to the mainland of the continent and the other two native to the neighboring islands of Japan. Himalayan hemlock's native range is from north-central India's Uttarakhand state along the Himalayan Mountains to Bhutan just east of Nepal and south China (Swartley, 1984). Himalayan hemlock may be distinguished among the hemlocks by its rigid leaves pointing upwards along the upper side of the shoot with prominent white bands underneath the leaves (Swartley, 1984). *T. dumosa* has the largest leaves of all the hemlocks and has pendulous branching. Introduced into North America in 1838, Himalayan hemlock is rarely found in landscapes. Recent observations (Wu and Raven, 1999) indicate that Yunnan hemlock (*T. yunnanensis* (Franch.) Pritzel), once considered a separate species, may be a subspecies of *T. dumosa* due to morphological and geographical distribution similarities (Table 1). Swartley (1984) describes Yunnan hemlock as being similar to the Himalayan hemlock with its pendulous foliage and creamy white bands underneath sparsely arranged large, shiny green leaves. Like Himalayan, Yunnan hemlock is rarely found in North American landscapes, with little available research concerning its HWA tolerance/resistance (Swartley, 1984).

Another mainland Asian hemlock includes *T. chinensis* (Franch.) Pritzel (Chinese hemlock) which is native to China's southwestern region of West Szechual in the Sichuan Province (Dirr, 1998; Swartley, 1984). Chinese hemlock may be characterized by a series of short leaves separating larger leaves evenly along the shoot and light green surface with narrow light green bands underneath leaf (Swartley, 1984). Introduced in 1901, Chinese hemlock is growing in availability in North America due to its resistance to the HWA (Dirr, 1998; Montgomery et al., 2009; Pontius et al., 2006; Tredici and Kitajima, 2004).

Non-mainland Asian species of *Tsuga* include *T. diversifolia* (Maxim.) Mast. (northern Japanese hemlock) and *T. sieboldii* Carr. (southern Japanese hemlock). The northern Japanese hemlock, being the more popular of the two Japanese hemlocks, is distinguished from other hemlocks by bristle-like foliage arranged tightly along the sides of the shoots, ovoid, shiny brown cones, and dark orange-brown bark (Swartley, 1984).

Introduced in 1861 from Central and Southern Honshu regions of Japan, northern Japanese hemlock has recently seen increased landscape use in Europe and North America due to its tight leaf arrangement and broad pyramidal structure lending to its use as specimen plants and bonsai plantings, as well as its noted resistance to the HWA (Bitner, 2007; Dirr, 1998; Pontius et al., 2006; Swartley, 1984). Unlike its northern counterpart, southern Japanese hemlock has not grown as quickly in demand for use in bonsai or in the landscape. Introduced in 1850 from Japan's South Honshu and southern islands, southern Japanese hemlock is a broad, shrubby tree with dark shining green foliage arranged along the bottom of shoots in irregular lengths with pendulous cones having vertically ribbed and incurved scales (Dirr, 1998; Swartley, 1984). Just as the northern Japanese hemlock, the southern Japanese hemlock has shown resistance to HWA, furthering interests in its use in North American landscapes (Montgomery et al, 2009; Pontius et al., 2006).

II. Temperature Effects

No literature quantifying the performance and heat tolerance of *Tsuga* has been published. Indigenous to numerous geographical locations, several species of hemlock may perform satisfactory and be well suited to other regions of the world. Evaluations of several species' resistance to HWA are prevalent and preventive measures to control HWA infestations using predatory insects dominate the literature (Evans and Gregoire, 2007; Pontius et al., 2007). In addition to evaluating species' tolerances of insect infestations for potential introduction into devastated areas, it is important to evaluate performance of potential species within these new environments. Some *Tsuga* species have been evaluated for their response to various environmental conditions, including light gradients, acid concentrations in soil, carbon dioxide levels, and low temperatures (Bond et al., 1999; Coleman et al., 1992; Khan et al., 2000; Murray et al., 1989; Ryan et al., 1986).

In looking at the native range of a species, one may observe that temperature can play a vital role in determining a species' ability to survive within a given environment and climate. An example of this can be seen in the distribution of broadleaf evergreens with broadleaves more commonly found in warm rather than cool climates. The disparate distribution of broadleaf evergreens and conifers may be partially explained from the standpoint of leaf area and its exposure to temperatures and conditions causing desiccation, death of foliage, and plant death. Adaptations and acclimation to survive these conditions is known as cold tolerance. As with cold tolerance, plants also have limits as to the heat stress they can survive. This is often referred to as heat tolerance or thermotolerance. Often used interchangeably with thermostability, thermotolerance and heat tolerance are characterized by the ability of plant tissues to survive heat stress whereas, thermostability is used to describe the ability of plant substances such as proteins, lipids, or nucleic acids to remain viable upon exposure to heat stress (Levitt, 1980).

Influences that may affect thermotolerance include genetics, nutrition, soil moisture, and maturity of the plant. Influences aside, temperature can affect the natural distribution of a given species given its degree, variations during seasonal and diurnal fluctuations, and the duration of exposure to high temperatures. Even though a species may be tolerant of higher temperatures, the time of day and the duration of heat stress with which it can withstand said temperatures without considerable damage also serve as factors in heat tolerance. This degree versus duration dilemma is termed the heat dose law as described by Belehradek (1957), which states that elevated temperatures held at lower levels over extended periods of time may cause as much damage as higher temperatures held over short periods of time. The relationship of high temperature and duration may be further explained by the type of injury each combination may cause. The two heat stress injury types are referred to as direct injury and indirect injury (Levitt, 1980).

A. Direct Injury

Direct injury may be described as the damage that occurs when tissues are exposed to extreme temperatures for short periods of time. Direct injury is the damage characterized under the degree portion of the heat dose law, where plants under supra-optimal temperatures may suffer heat stress and death when exposed to extreme temperatures for short periods of time (Levitt, 1980). Injury incurred under these brief, intense temperatures is a mechanical means of damage as compartmentalization provided by cell membranes fails and allows cell contents to flow out from the cell (Abass and Rajashekar, 1991). Direct injury occurs when biological systems are damaged through membrane damage via protein denaturation and lipid liquidfaction, nucleic acid damage, photosynthesis inhibition, and ion imbalance (Levitt, 1980; Sage and Reid, 1994; Schreiber and Berry, 1977; Sibley, 1997).

1. Membrane Damage

Direct injury is often associated and described via membrane damage where high temperatures cross critical temperature thresholds causing cellular structures and functions to be damaged, killing the protoplasm (Larcher, 1995; Levitt, 1980; Sibley;

1997). Membranes believed to experience stress are plasma, chloroplast, mitochondrial, and nuclear membranes (Levitt, 1980). It is believed exposure overwhelms proteins and lipids associated with membranes of cells and organelles within, causing leakage of contents of these bodies into the protoplasm and space outside the cell. Bernstam and Arndt (1937) observed the effects of 10 minute heat shocks on the slime mold *Physarum polycephalum* where increasing temperatures caused discontinuation of cytoplasmic streaming, leakage of protein metabolites, increased leakage of substances, and respiration stoppage. With the leakage of cellular contents, disruption of cellular processes accompanied. Aforementioned disruption of cellular processes of metabolic nature led to the argument and investigation as to the actual cause for death, whether direct injury to organ membranes or inhibition of metabolic pathways. Berry et al. (1975) and Krause and Santarius (1975) observed that leakage of ions from plasma membranes typically required as much as 11°C (19.8°F) higher temperatures than those necessary to inhibit photosynthetic function via thylakoid membrane damage, suggesting inhibition of metabolic pathways to be the contributing factor of death. Research conducted to further investigate the nature of the injury suggested protein denaturation, lipid liquidfaction, and nucleic acid damage to be potential contributors to direct heat injury and inhibition of metabolic pathways (Levitt, 1980).

2. Protein Denaturation

With proteins and lipids serving as the main constituents of cellular membranes, some previously and currently accepted theories of cellular death due to membrane damage suggest that membrane damage occurs due to coagulation of proteins at increased temperatures (Belehradek, 1935). However, early investigations concluded protein coagulation was the cause of cell damage as many organisms are killed by temperatures considered too low to denature proteins (Levitt, 1980). Contention and uncertainty between the two theories led to Aleksandrov's (1964) theory that although not all proteins making up cellular membranes are subject to coagulation at higher temperatures, one protein sensitive to stress under high temperatures may provide a weak point for membrane compromise. Cytoplasmic streaming is reduced at a lower temperature than other cellular processes, suggesting that proteins associated with cytoplasmic streaming are more susceptible to heat denaturation than others within the membrane (Aleksandrov, 1964). Fel'dman and Kamensteva (1967) further supported Aleksandrov by correlating stability of urease with respiration and protoplasmic movement rates at different temperatures. However, the correlation seemed to show a cause and effect relationship between temperature and protein denaturation indicating that some other factors must be considered such as reversible denaturation at higher temperatures (Levitt, 1980) and water content of cell prior to stress (Lorenz, 1939). With protein denaturation not fully accounting for cellular death, investigation into phospholipid and nucleic acid changes have been looked at to explain direct heat injury (Levitt, 1980; Van Uden and Vidal Leiria, 1976).

3. Lipid Liquidfaction

Theories supporting lipid liquefaction stated that membrane integrity loss occurred due to the phase change of lipids within the membrane from a liquid crystalline state to a solid gel form when exposed to high temperatures (Levitt, 1980). However, recent work shows that this phase change occurs at lower temperatures than normal growing temperatures and may not be the cause of cell death, but rather a result of death

(Lepeshlch, 1935; Levitt, 1980; Reinert and Steim, 1970). Although recent evidence suggest it is not the cause for cell death, rising temperatures increase the mobility of lipids encouraging the formation of lipid vesicles from the cell membrane (Levitt, 1980). The formation of vesicles from the membrane is believed to cause weakening of the membrane and increase susceptibility to cell death. It is this excessive fluidity paired with denaturation and aggregation of proteins within the membranes that may serve as factors for direct heat injury (Levitt, 1980).

4. Nucleic Acid Damage

In the same way proteins are subject to protein denaturation through temperature increases, nucleic acids are sensitive to temperature increases as well. Since nucleic acids do not serve as the primary constituents of cell membranes, they may serve as an indirect factor in heat injury as they are involved in lipid and protein synthesis (Levitt, 1980; Peacocke and Wallner, 1962). Investigations conducted on various bacteria and yeasts revealed that although DNA bases were not affected by increased temperatures, various types of RNA, mainly mRNA and tRNA, were found to be temperature-sensitive (Malcolm, 1968; Nash et al., 1969) and restrictive to protein and lipid synthesis. Inhibition of protein synthesis is important for membrane repair and shown to serve as a factor for thermotolerance (Levitt, 1980).

Although research suggests indirect injury to be a leading cause of species death and decline, direct injury to foliage and roots cannot be discounted as temperatures have been shown to reach and exceed 50 °C (122°F) in plant canopies (Pair and Still, 1982) and 62.8°C (145°F) in container media (Martin and Ruter, 1996). Exposure to supra-optimal temperatures for brief periods may contribute to cellular membrane and metabolic pathway damage as the result of direct heat injury.

B. Indirect Injury

As current research suggests, direct injury alone may not fully account for the death of various species exposed to elevated temperatures (Sibley et al., 1999). Quantification of temperatures necessary to denature proteins, lipids, and nucleic acids suggests that in addition to direct injury, indirect injury serves to limit and determine a species' thermal tolerance. Referring to Belehradek's heat dose law, indirect injury characterizes the duration portion of the model. Indirect injury may be described as the damage that occurs to an organism exposed to sub-lethal temperatures for extended durations of time (Levitt, 1980). Species decline and death have often been shown to occur at lower temperatures than those necessary to breakdown many biomolecules, justifying indirect injury's potential role in determining heat tolerance (Berry et al., 1975; Krause and Santarius, 1975; Levitt, 1980; Malcolm, 1968; Nash et al., 1969).

Not characterized by the breakdown of biomolecules such as proteins and lipids, indirect injury is commonly associated with the disruption of metabolic and cellular processes. Prolonged disruption of metabolic processes along with the accumulation of toxins, normally degraded during functioning metabolic pathways, are believed to cause slow cellular decline and death (Larcher, 1995; Sibley et al., 1999). Measures to quantify indirect injury include photosynthesis versus respiration rates, triphenyltetrazolium chloride (TTC) tests, C_{14} assimilation, toxin accumulation, chlorophyll fluorescence, and growth trials, while direct injury is measured through electrolyte leakage.

III. Electrolyte Leakage

Exposure to supra-optimal growth temperatures is one of the adverse conditions container-grown ornamentals face in the intensive production cycle of nursery production. Many ornamental plants are grown in colored containers set on pads often lined with black weed barriers that reflect heat collected throughout the day. This can be problematic for plants as the heat reflected off these surfaces collects in plant canopies and container media, exposing them to supra-optimal temperatures that can cause direct heat injury and reduced growth (Martin and Ruter, 1996; Pair and Still, 1982). Temperatures have been shown to reach and exceed 50°C (122°F) in plant canopies (Pair and Still, 1982) and 62.8°C (145°F) in container media (Martin and Ruter, 1996). Exposure to supra-optimal temperatures for brief periods may contribute to direct damage to cellular membranes and metabolic pathways as the result of direct heat injury.

Electrolyte leakage has been used as an accepted measure of cellular membrane damage in foliage, fruit, and root tissues (Dexter et al., 1932; Ingram and Buchanan, 1981; Stergios and Howell, 1973; Sullivan, 1972; Yadava et al., 1978). Electrolyte leakage measures thermal tolerance to direct injury (Levitt, 1980).

Direct injury occurs when tissues are exposed to extreme temperatures for short periods of time, causing compartmentalization provided by cell membranes to fail and allow cell contents to flow out from the cell (Abass and Rajashekar, 1991).

Electrolyte leakage determines heat tolerance via electrical conductivity measurements. As cells contain organelles, photoassimilates, nuclear bodies, and other components necessary for cellular growth, maintenance, and reproduction, these components can be exchanged between cells through protein channels within the phospholipid bi-layer of cell membranes. Since these components are made of organic and inorganic materials, they contain ions that contribute to electrical conductivity of the solution in which they are suspended and can be measured using an electrical conductivity meter. When plant tissue is placed into water containing no ions (de-ionized water), some of these cellular components may flow from the cell into the water as osmotic and solute potentials are equilibrated. When damage occurs to cellular membranes through stress or physical rupture, cellular contents flow freely into the surrounding solution. This “leakage” of cellular components into the surrounding solution can dramatically increase the electrical conductivity, depending on the severity of the damage. Electrolyte leakage serves as a measure of membrane damage in relation to stress (McKay, 1992; Sibley, 1997).

A model commonly used for electrolyte leakage procedures and data analyses for ornamental plants was developed by Ingram (1985) and Ingram and Buchanan (1984) and serves to provide the basis for direct heat stress determination of many ornamental plants. As outlined by Ingram and Buchanan (1984), electrolyte leakage is conducted by excising foliage, fruit, or roots from the plant and placing a known mass of each sample into test tubes containing a known volume of de-ionized water. Samples are treated within a thermostatically controlled water bath, cooler, or growth chamber at prescribed temperatures. Upon completing prescribed temperature treatment, test tubes are placed on ice and incubated in a cooler maintained at 4.4 °C (40°F) for 24 hours. Following incubation, samples are brought to room temperature and electrical conductivity measured using a conductivity meter. Electrical conductivities are measured again following autoclaving samples at 121°C and 1.4 kg·cm^{-2} (249.8°F and 20 lbs·in^{-2}) for 20 minutes and incubated 24 hours at 4.4 °C (40°F).

Once initial and final conductivity measurements are collected, electrolyte leakage may be expressed as a ratio of the initial electrical conductivity post-treatment to the conductivity post-autoclave. Electrolyte leakage response to temperature is expected to fit the sigmoidal equation:

$$L_e = [x - z) / (1 + \exp [- k (T - T_m])] + z$$

where L_e is the percent leakage, z = baseline level of electrolyte leakage, x = percent of electrolyte leakage observed across all temperatures, T_m = predicted temperature midpoint, also referred to as the “inflection point” for the response curve, k = the slope of the predicted response at T_m, and T = water bath temperature. The equation is used to determine critical midpoint temperatures (T_m) by fitting electrolyte leakage data across temperature treatments using Gauss-Newton method of non-linear regression approach. The critical temperature midpoint (T_m) serves as the estimate where 50 percent of the cells of a plant or one of its portions are damaged beyond repair and recovery/ survival is unlikely.

Initial research conducted using electrolyte leakage determination were aimed at determining cold hardiness for many plant species, especially for conifers (Burr et al., 1993; Dexter et al., 1932; Murray et al., 1989; Stergios and Howell, 1973; Yadava et al., 1978). Modified electrolyte procedures for agronomic crops such as sorghum and soybeans followed (Martineau et al. 1979; Sullivan, 1972). With models for determining heat tolerance via modified electrolyte leakage procedures for *Pittosporum* (pittosporum) conducted by Ingram (1985) and Ingram and Buchanan (1984), evaluation of heat tolerance of other ornamentals increased leading to electrolyte leakage's acceptance for direct heat injury determinations. Ornamentals evaluated for direct heat tolerance include: *Ilex* (holly), *Illicium* (illicium), and *Juniperus* (juniper) (Ingram and Buchanan, 1981), *Pittosporum* (pittosporum) (Ingram, 1985), *Ilex* (holly) (Ingram 1986; Ruter, 1993), *Nyssa* (tupelo), *Cephalanthus* (button bush), and *Taxodium* (bald cypress) (Donovan, et al., 1990), *Magnolia* (magnolia) (Martin et al., 1991), *Acer* (maple) (Sibley et al., 1999), and *Cornus* (dogwood) (Hardin et al., 1999).

Based on the lack of information regarding thermotolerance of *Tsuga* species with regard to direct heat injury, the objective of this project was to evaluate eight of the recognized hemlock species (*T. canadensis, T. caroliniana, T. chinensis, T. diversifolia, T. dumosa / yunnanensis, T. heterophylla T. mertensiana*, and *T. sieboldii*). Thermotolerance of the leaf tissue cells was evaluated by assessing electrolyte leakage following imposed direct-heat stress.

Literature Cited

Abass, M. and C.B. Rajashekar. 1991. Characterization of heat injury in grapes using ^{1}Hnuclear magnetic resonance methods. Plant Physiol. 96:957-961.

Aleksandrov, V.Y. 1964. Cytophysiological and cytoecological investigations of heat resistance of plant cells toward the action of high and low temperature. Q. Rev. Biol. 39:35-77.

Belehradek, J. 1935. Temperature and living matter. Protoplama-Monogr. Berlin, 8: 277.

Belehradek, J. 1957. Physiological aspects of heat and cold. Annu. Rev. Physiol. 19:59-82.

Bernstam, V. A. and S. Arndt. 1937. Effects of supraoptimal temperatures on the *myxomyete Physarum polycephalum*. I. Protoplasmic streaming, respiration and leakage of protoplasmic substances. Arch. Mikrobiol. 92:251-261.

Berry, J.A., D.C. Fork, and S. Garrison. 1975. Mechanistic studies of thermal damage to leaves. Carnegie Inst. Washington, Yrbk. 74:751-759.

Bitner, R.L. 2007. Conifers for gardens: an illustrated encyclopedia. Timber Press, Portland, OR.

Bond, B.J., B.T. Farnsworth, R.A. Coulombe, and W.E. Winner. 1999. Foliage physiology and biochemistry in response to light gradients in conifers with varying shade tolerance. Oecologia 120:183-192.

Burr, K.E., S.J. Wallner, and R.W. Tinus. 1993. Heat tolerance, cold hardiness, and bud dormancy relationships in seedlings of selected conifers. J. Amer. Soc. Hort. Sci. 188:840-844.

Coleman, M.D., T.M. Hinckley, G. McNaughton, and B.A. Smith. 1992. Root cold hardiness and native distribution of subalpine conifers. Can. J. For. Res. 22:932-938.

Dexter, S.T., W.E. Tottinghan, and L.F. Graber. 1932. Investigation of the hardiness of plants by electrical conductivity. Plant Physiol. 7:63-78.

Dirr, M.A. 1998. Manual of woody landscape plants: their identification, ornamental characteristics, culture, propagation, and uses, 5th ed. Stipes, Champaign, IL.

Donovan, L.A., K.W. McLeod, and K.L. Grant. 1990. Direct heat injury of roots of woody swamp species. J. Therm. Biol. 15:245-249.

Evans, A.M., and T.G. Gregoire. 2007. A geographically variable model of hemlock woolly adelgid spread. Biol. Invasions 9:369-382.

Fel'dman, N.L. and I.E. Kamensteva. 1967. Urease thermostability in leaf extracts of two *Leucojum* species with different periods of vegetation. Tsitologiya 9:886-889.

Forrest, T. 2005. Cool conifers: the backbone of the four-season garden, conifers bring texture, form, and color. Garden Design 6:28-32.

Hardin, R.B., D.J. Eakes, J.L.Sibley, C.H. Gilliam, and G.J. Keever. 1999. Root membrane thermostability of *Cornus florida* L. provenances. J. Therm. Biol. 24:237-240.

Ingram, D.L. 1985. Modeling high temperature and exposure time interactions on *Pittosporum tobira* root cell membrane thermostability. J. Amer. Soc. Hort. Sci. 110:470-473.

Ingram, D.L. 1986. Root cell membrane heat tolerance of two dwarf hollies. J. Amer. Soc. Hort. Sci. 111:270-272.

Ingram, D.L. and D.W. Buchanan. 1981. Measurement of direct heat injury of roots of three woody plants. HortScience 16:769-771.

Ingram, D.L. and D.W. Buchanan. 1984. Lethal high temperatures for roots of three citrus rootstocks. J. Amer. Soc. Hort. Sci. 109:189-193.

Khan, S.R., R. Rose, D.L. Haase, and T.E. Sabin. 2000. Effects of shade on morphology, chlorophyll concentration, and chlorophyll fluorescence of four Pacific Northwest conifer species. New Forests 19:171-186.

Krause, G.H. and K.A. Santarius. 1975. Relative thermostability of the chloroplast envelope. Planta 127:285-299.

Larcher, W. 1995. Physiological plant ecology. Springer-Verlag, Berlin. 3rd ed.

Levitt, J. 1980. Responses of plants to environmental stresses. Vol. I. Chilling, freezing, and high temperature stresses. Academic Press, New York, N.Y.

Lepeshlch, W.W. 1935. Zur Kenntinis des Hitzetodes des Protoplasmas. Protoplasma 23:349-366.

Lorenz, R.W. 1939. High temperature tolerance of forest trees. Minn. Agr. Expt. Sta., Tech. Bul. 141:1-25.

Malcolm, N.L. 1968. A temperature-induced lesion in amino acid-transfer ribonucleic acid attachment in a psychrophile. Biochem. Biophys. Acta 157:493-503.

Martin, C.A., D.L. Ingram, and M.A. Jenks. 1991. Response of southern magnolia to supraoptimal root-zone temperatures. J. Therm. Biol. 16:281-285.

Martin, C.A. and J.M. Ruter. 1996. Growth and foliar nutrient concentrations of crape myrtle in response to disparate climate and fertilizer placement in large nursery containers. J. Environ. Hort. 14:9-12.

Martineau, J.R., J.E. Specht, J.H. Williams, and C.Y. Sullivan. 1979. Temperature tolerance in soybeans. I. Evaluation of techniques for assessing cellular membrane thermostability. Crop Sci. 19:76-78.

McAvoy, T.J., G.M.G. Zilahi-Balogh, S.M. Salom, L.T. Kok, and Z. Guoliang. 2007. Development and feeding of *Tetraphleps galchanoides*, a predator of the hemlock woolly adelgid. BioControl 52:339-350.

McKay, H.M. 1992. Electrolyte leakage from fine roots of conifer seedlings: a rapid index of plant viability following cold storage. Can. J. For. Res. 22:1371-1377.

Montgomery, M.E., S.E. Bentz, and R.T. Olsen. 2009. Evaluation of hemlock (*Tsuga*) species and hybrids for resistance to *Adelges tsugae* (Hemiptera: Adelgidae) using artificial infestation. J. Econ. Entomol. 102:1247-1254.

Murray, M.B., J.N. Cape, and D. Fowler. 1989. Quantification of frost damage in plant tissues by rates of electrolyte leakage. New Phytol. 113:307-311.

Nash, C.H., D.W. Grant, and N.A. Sinclair. 1969. Thermolability of protein synthesis in a cell-free system from the obligetely psychrophilic yeast *Candida gelida*. Can. J. Microbiol. 15:339-343.

Pair, J.C. and S.M. Still. 1982. Growth, hardiness, and leaf water potential of blue holly (*Ilex* ×*merserveae*) cultivars affected by exposure. HortScience 17:823-825.

Peacocke, A.R. and I.O. Wallner. 1962. The thermal denaturation of sodium deoxyribonuclease. II. Kenetics. J. Mol. Biol. 5:560-563.

Pedigo, L.P. 2002. Entomology and pest management, 4th ed. Prentice Hall, Upper Saddle River, N.J.

Pontius, J.A., R.A. Hallett, and J.C. Jenkins. 2006. Foliar chemistry linked to infestation and susceptibility to hemlock woolly adelgid (Homoptera: Adelgidae). Environ. Entomol. 35:112-120.

Radford, A.E., H.E. Ahles, and C.R. Bell. 1968. Manual of the vascular flora of the carolinas. University of North Carolina Press, Chapel Hill, NC.

Reinert, J.C. and J.M. Steim. 1970. Calorimetric detection of a membrane-lipid phase transition in living cells. Sci. 168:1580-1582.

Ruter, J.M. 1993. Foliar heat tolerance of two hybrid hollies. HortScience 28: 650-652.

Ryan, P.J., S.P. Gessel, and R.J. Zasoski. 1986. Acid tolerance of Pacific Northwest conifers in solution culture. I. Effect of high aluminum concentration and solution acidity. Plant and Soil 96:239-257.

Sage, R.F. and C.D. Reid. 1994. Photosynthetic response mechanisms to environmental change in C3 plants. p. 413-499. In: R.E. Wilkinson (editor). Plant-environment interactions. Marcel-Dekker, Inc., New York, N.Y.

Sargent, C.S. 1949. Manual of the Trees of North America. 2nd ed. Dover Publications, New York, N.Y.

Schreiber, U. and J.A. Berry. 1977. Heat-induced changes of chlorophyll fluorescence in intact leaves correlated with damage of the photosynthetic apparatus. Planta 136:233-238.

Sibley, J.L. 1997. Effects of elevated temperatures on container production of red maple (*Acer rubrum* L.) cultivars. Univ. of Ga., Athens, PhD Diss.

Sibley, J.L., J.M. Ruter, and D.J. Eakes. 1999. Root membrane thermostability of red maple cultivars. J. Therm. Biol. 24:79-89.

Stergios, B.G. and G.S. Howell, Jr. 1973. Evaluation of viability tests for cold stressed plants. J. Amer. Hort. Sci. 98:325-330.

Sullivan, C.Y. 1972. Mechanisms of heat and drought resistance in grain sorghum and methods of measurements. p.247-264. In: N.G.P. Rao and L.R. House (eds.) Sorghum in the seventies. Oxford & I.B.H. Publishing Co., New Delhi, India.

Swartley, J.C. 1984. The Cultivated Hemlocks. Timber Press, Portland, OR.

Tredici, P.D. and A. Kitajima. 2004. Introduction and cultivation of chinese hemlock (*Tsuga chinensis*) and it's resistance to hemlock woolly adelgid (*Adelges tsugae*). J. of Arbor. 30:282-287.

USDA, NRCS. 2007. The PLANTS Database. 23 October 2007. <http://plants.usda.gov/>.

Van Uden, N. and M.M. Vidal Leiria. 1976. Thermodynamic compensation in microbial thermal death: Studies with yeasts. Arch. Microbiol. 108:293-298.

Wu, Z. and P.H. Raven. 1999. Flora of China. Vol. 4. Science Press, Beijing.

Yadava, U.L., S.L. Doud, and D.J. Weaver. 1978 Evaluation of different methods to assess cold hardiness of peach trees. J. Amer. Soc. Hort. Sci. 103:318-321.

Table 1. Accepted species for genus *Tsuga*[Z]

Scientific Name	Author[Y]	Reference[X]	Date[W]	Source[V]
Tsuga canadensis	(L.) Carrière	Traité Gén. Conif. 189	1855	ACB
Tsuga americana	(Mill.) Farw.	Bull. Torrey Bot. Club 41: 629	1915	AC
Tsuga canadensis fo. pyramidalis	Jenkins	Hemlock Arboretum Bull. 6.	1934	B[U]
Tsuga canadensis fo. argentea	Nelson	Pinaceae: 32.	1866	B
Tsuga canadensis fo. aurea	(Nelson) Jäger u Beissn.	Ziergehölze: 495.	1884	B
Tsuga canadensis fo. canadensis				C[T]
Tsuga canadensis fo. densifolia	(Jenkins) Swartley	Heml. Arb. Bull. 6.	1934	B
Tsuga canadensis fo. globosa	Beissn.	Handbuch Coniferen-Benennung: 65.	1887	B
Tsuga canadensis fo. latifolia	(Sén.) Swartley	Conif. 19.	1868	B
Tsuga canadensis fo. microphylla	(Lindl.) Beissn.	Syst. Eintheil. Conif. 40.	1887	B
Tsuga canadensis fo. parvula	Vict. & J. Rousseau	Contr. Inst. Bot. Univ. Montreal 36: 13.	1940	AC
Tsuga canadensis fo. pendula	Jäger u Beissn	Ziergehölze ed. 2: 445.	1884	B
Tsuga canadensis fo. prostrata	Bean	Trees and Shrubs ed. 1, 3:482	1933	B
Tsuga canadensis fo. fastigiata	Beissn.	Handbuch Nadel: 402.	1891	B
Tsuga canadensis fo. macrophylla	(Beissn.) Swartley	Handb. Nadel. 404.	1891	B
Tsuga canadensis fo. minuta	(Teusch.) Rehder	Bibliogr. Cult. Trees: 648. (GCI)	1949	A
Tsuga canadensis fo. sparsifolia	(Beissn.) Rehder	Bibliogr. Cult. Trees: 647. (GCI)	1949	AB
Tsuga canadensis var. macrophylla	Hort			A[S]
Tsuga canadensis var. sargentii	Bean	Trees & Shrubs Hardy in the Br. Isles 2: 606. (GCI)	1914	A
Tsuga caroliniana	Engelm.	Bot. Gaz. 6(6): 223-224.	1881	AC
Tsuga chinensis	(Franch.) Pritz.	Bot. Jahrb. Syst. 29(2): 217	1901	ABC
Tsuga brunoniana var. chinensis	(Franch.) Mast.	Journal of the Linnean Society, Botany 26(179-180): 556.	1902	BC
Tsuga chinensis subsp. tchekiangensis	(Franch.) Pritzel in Diels subsp. tchekiangensis (Flous) Silba	J. Int. Conifer Preserv. Soc. 15(2): 64.	2008	A
Tsuga chinensis var. chinensis				C
Tsuga chinensis var. daibuensis	S.S. Ying	Bull. EBp. Forest Natl. Taiwan Univ. 114: 150	1974	ABC
Tsuga chinensis var. formosana	(Hayata) H.L. Li & H. Keng	Taiwania 5: 64, pl. 19	1954	BC
Tsuga chinensis var. forrestii	(Downie) Silba	Phytologia 68: 72	1990	AC
Tsuga chinensis var. patens	(Downie) L.K. Fu & Nan Li	Novon 7(3): 263	1997	AC
Tsuga chinensis var. robusta	C.Y. Cheng & L.K. Fu	Acta PhytotaB. Sin. 13(4): 83, pl. 18, f. 11-15	1975	ABC
Tsuga dumosa var. chinensis	(Franch.) E. Pritz.	Bot. Jahrb. Syst. 29(2): 217	1900	C
Tsuga formosana	Hayata	Gard. Chron., ser. 3, 43: 194	1908	AC
Tsuga forrestii	Downie	Notes Roy. Bot. Gard. Edinburgh 14(67): 18-19, pl. 194, f. 7	1923	AC
Tsuga patens	Downie	Notes Roy. Bot. Gard. Edinburgh 14(67): 16-17, pl. 194, f. 6	1923	AC
Tsuga tchekiangensis	Flous	Bull. Soc. Hist. Nat. Toulouse 69: 6, f. 1-12	1936	AC
Tsuga diversifolia	(MaBim.) Mast.	J. Linn. Soc., Bot. 18: 514	1881	ABC
Tsuga blaringhemii	Flous	Bull. Soc. Hist. Nat. Toulouse 69: 410	1936	AB
Tsuga diversifolia subsp. Blaringhemii	(Flous) A.E.Murray	Kalmia 12: 26.	1982	A
Tsuga dumosa	(D. Don) Eichler	Nat. Pflanzenfam. 2(1): 80	1887	ABC
Tsuga brunoniana	(Wall.) Carrière	Traité Gén. Conif. 188	1855	BC
Tsuga calcarea	Downie	Notes Roy. Bot. Gard. Edinburgh 14(67): 17-18, pl. 194, f. 3	1923	C
Tsuga chinensis subsp. wardii	(Downie) A.E. Murray	Kalmia 12: 26	1982	C
Tsuga dumosa subsp. leptophylla	(Hand.-Mazz.) A.E. Murray	Kalmia 12: 26	1982	AC
Tsuga dumosa var. dumosa				C
Tsuga dumosa var. yunnanensis	(Franch.) Silba	Phytologia 68: 73	1990	AC
Tsuga dura	Downie	Notes Roy. Bot. Gard. Edinburgh 14(67): 16, pl. 194, f. 2	1923	C
Tsuga intermedia	Hand.-Mazz.	Kaiserl. Akad. Wiss. Wien, Math.-Naturwiss. Kl., Anz. 61: 82	1924	C
Tsuga leptophylla	Hand.-Mazz.	Kaiserl. Akad. Wiss. Wien, Math.-Naturwiss. Kl., Anz. 61: 83	1924	ABC
Tsuga wardii	Downie	Notes Roy. Bot. Gard. Edinburgh 14(67): 17, pl. 17, f. 4	1923	C
Tsuga yunnanensis	(Franch.) E. Pritz.	Bot. Jahrb. Syst. 29(2): 217, in obs.	1901	C
Tsuga yunnanensis subsp. dura	(Downie) A.E. Murray	Kalmia 12: 26	1982	C
Tsuga heterophylla	(Raf.) Sarg.	Silva 12: 73	1898	ABC
Tsuga albertiana	(A. Murray bis) Senecl.	Conif. 18	1868	B
Tsuga longibracteata	W.C. Cheng	Contr. Biol. Lab. Chin. Assoc. Advancem. Sci. 7(1): 1, f. 1	1932	ABC
Tsuga mertensiana	(Bong.) Carrière	Traité Gén. Conif. (ed. 2) 250	1867	ABC
Tsuga balfouriana	(Rehder & E.H. Wilson) W.R. McNab	J. Linn. Soc., Bot. 19: 211	1882	C
Tsuga crassifolia	Flous	Bull. Soc. Hist. Nat. Toulouse 69: 412	1936	AC
Tsuga hookeriana	(A. Murray bis) Carrière	Traité Gén. Conif. (ed. 2) 252	1867	AB
Tsuga mertensiana subsp. crassifolia	(Bong.) Carrière subsp. crassifolia (Flous) Silba	J. Int. Conifer Preserv. Soc. 15(2): 65.	2008	A
Tsuga mertensiana subsp. grendicone	Farjon	Proc. Kon. Ned. Akad. Wetensch., C 91(1): 39	1988	AC
Tsuga mertensiana subsp. mertensiana				C
Tsuga mertensiana var. jeffreyi	(Henry) C.K. Schneid.	NEW 294	1913	AC
Tsuga mertensiana var. macrophylla	(Bong.) Carrière var. macrophylla Beissn. in Beissn.	Handb. Nadelholzk. 404.	1891	A
Tsuga mertensiana var. mertensiana				C
Tsuga pattoniana	(Jeffrey eB A. Murray bis) Engelm.	Gard. Chron., n.s., 756	1879	BC
Tsuga williamsonii	(Newb.) de Vos	Bered. Woordenb. Heest. Conif. 181	1867	BC
Tsuga oblongisquamata	(C.Y. Cheng & L.K. Fu) L.K. Fu & Nan Li	Novon 7(3): 263	1997	AC
Tsuga sieboldii	Carrière	Traité Gén. Conif. 186	1855	ABC
Tsuga araragi	(Siebold) Koehne	Deut. Dendrol. 10	1893	AB
Tsuga hanburyana				B
Tsuga sieboldii var. nana	(Endl.) Carrière			C
Tsuga sieboldii var. sieboldii				C
Tsuga tsuja	A. Murray bis	Proc. Roy. Hort. Soc. London 1862(2): 508, f. 151-153	1862	AB
Tsuga x jeffreyi	(Henry) A. Henry	Proc. Roy. Irish Acad. 34: 55	1919	BC

[Z]Data presented from taxonomic survey of published names and descriptions for currently accepted species of the genus *Tsuga* in 2010.

[Y]Author responsible for recorded specie existence, description, and / or agreement with previously recorded literature.

[X]Accepted literature citing current specie existence and description by referred author(s).

[W]Publishing date of referenced literature.

[V]Source of current taxonomic information.

[U]Swartley, J.C., 1984. The Cultivated Hemlocks. Timber Press, Portland, OR. 186.

[T]Tropicos. 10 May 2010. Tropicos Botanical Information System at the Missouri Botanical Garden, St. Louis. 10 May 2010. <http://www.tropicos.org/>.

[S]IPNI. 6 May 2010. The International Plant Names Index. 10 May 2010. <http://www.ipni.org/>.

Chapter II

Foliar Thermotolerance of *Tsuga* spp.

Abstract

Foliar thermotolerance of six *Tsuga* species to direct injury was evaluated using electrolyte leakage. Species evaluated included: *T. canadensis, T. caroliniana, T. chinensis, T. diversifolia, T. heterophylla,* and *T. dumosa* (formerly *T. yunnanensis*). Bare-root liners of the six species were potted into containers and grown for nine months prior to evaluation. Species were exposed to 25, 30, 35, 37.5, 40, 42.5, 45, 47.5, 50, 52.5, 55, and 60°C (77, 86, 95, 99.5, 104, 108.5, 113, 117.5, 122, 126.5, 131, and 140°F) for 30 minutes in thermostatically controlled water baths, and electrical conductivity determined subsequent each 24 hour incubation time following treatment and autoclaving. Electrolyte leakage, expressed as a ratio of the electrical conductivity taken 24 hours following treatment to the conductivity taken 24 hours following autoclaving, was determined. Electrolyte leakage response to temperature was sigmoidal. *T. canadensis* and *T. dumosa* had similarly, higher predicted temperature midpoints (T_m) and *k*-values than *T. caroliniana* suggesting higher resistance to direct injury when exposed to brief, supra-optimal temperatures. While differences were found for *T. canadensis* and *T. dumosa* when compared to *T. caroliniana*, no differences were found in T_m and *k*-values for *T. canadensis* or *T. dumosa* when each compared to *T. chinensis, T. diversifolia*, and *T. heterophylla*. Similarly, no differences were found for T_m and *k*-values of *T. caroliniana* when compared to *T. chinensis, T. diversifolia*, and *T. heterophylla*.

Introduction

Evaluation of plant response to environmental and biotic stress benefits industry professionals by providing practical information useful in selecting species for production and landscape use under various conditions. Research addressing heat tolerance of species and cultivars provides valuable information for plant production in areas pre-disposed to high temperatures. The genus *Tsuga* (hemlock) contains several species indigenous to numerous geographical locations. No literature quantifying *Tsuga*'s performance and heat tolerance has been published to date. With hemlock woolly adelgid (*Adelges tsugae* Annan.), causing decline of North American hemlock stands (Evans and Gregoire, 2007;), other species found to be more resistant to hemlock woolly adelgid (HWA) infestations may perform satisfactory and be well suited to other regions of the world (Montgomery et al., 2009; Tredici and Kitajima, 2004). Exposure to supra-optimal temperatures is one of the adverse conditions container-grown ornamentals face in the intensive production cycle of nursery production. Many ornamental plants are grown in black containers set on pads lined with black weed barriers that transfer heat collected throughout the day. This can be problematic for plants as the heat reflected off these surfaces collects in plant canopies and container media exposing them to supra-optimal temperatures that can cause direct heat injury and reduced growth (Martin and Ruter, 1996; Pair and Still, 1982). Temperatures have been shown to exceed 50°C (122°F) in plant canopies (Pair and Still, 1982) and 62.8°C (145°F) in container media (Martin and Ruter, 1996). Exposure to supra-optimal temperatures for brief periods may contribute to direct damage to cellular membranes and metabolic pathways as the result of direct heat injury.

Electrolyte leakage provides an indication of heat tolerance for cell membrane stability in response to extreme temperatures due to direct injury (Levitt, 1980). Electrolyte leakage can be used to predict heat tolerance via electrical conductivity measurements. Cells contain organelles, photoassimilates, nuclear bodies, and other components necessary for cellular growth, maintenance, and reproduction, with these components often exchanged between cells through protein channels within the phospholipid bi-layer of cell membranes. Since these components are made of organic and inorganic materials, they contain ions that contribute to electrical conductivity of the solution in which they are suspended, which can be measured using an electrical conductivity meter. When plant tissue is placed into water containing no ions (de-ionized water), these cellular components may flow from the cell into the water as osmotic and solute potentials are equilibrated. When damage occurs to cellular membranes through stress or physical rupture, cellular contents flow freely into the surrounding solution. This "leakage" of cellular components into the surrounding solution can dramatically increase the electrical conductivity, depending on the severity of the damage. Electrolyte leakage serves as a measure of membrane damage in relation to stress (McKay, 1992; Sibley, 1997).

Electrolyte leakage has been used as an accepted measure of cellular membrane damage in foliage, fruit, and root tissues (Dexter et al., 1932; Ingram and Buchanan, 1981; Stergios and Howell, 1973; Sullivan, 1972; Yadava et al., 1978). Electrolyte leakage measures thermal tolerance with respect to direct injury (Levitt, 1980). Direct injury occurs when tissues are exposed to extreme temperatures for short periods of time, causing compartmentalization provided by cell membranes to fail and allow cell contents to flow out from the cell (Abass and Rajashekar, 1991). Initial research conducted using electrolyte leakage determination were aimed at determining cold hardiness for many plant species, especially for conifers (Burr et al., 1993; Dexter et al., 1932; Murray et al., 1989; Stergios and Howell, 1973; Yadava et al., 1978).

Modified electrolyte procedures for agronomic crops such as sorghum and soybeans followed (Martineau et al., 1979; Sullivan, 1972). With models for determining heat tolerance via modified electrolyte leakage procedures for *Pittosporum* (pittosporum) conducted by Ingram (1985) and Ingram and Buchanan (1984), evaluation of heat tolerance of other ornamentals increased leading to electrolyte leakage's acceptance for direct heat injury determinations. Ornamentals evaluated for direct heat tolerance with predicted T_m values for foliage include: *Ilex cornuta* 'Burfordii' (46.5 ± 0.5°C), *Illicium anisatum* (50.5 ± 0.5°C), and *Juniperus chinensis* 'Parsonii' (48.5 ± 0.5°C) (Ingram and Buchanan, 1981), *Pittosporum tobira* (52.2 ± 0.2°C) (Ingram, 1985), *Nyssa aquatica* (50.9 ± 0.2°C), *Cephalanthus occidentalis* (51.0 ± 0.2°C), and *Taxodium distichum* (46.6 ± 0.6°C) (Donovan et al., 1990), *Magnolia* (52.5 ± 0.9-54.0 ± 0.4°C) (Martin et al., 1991), *Acer rubrum* (52.0 ± 0.8-53.3 ± 0.5°C) (Sibley et al., 1999), and *Cornus florida* (51.2 ± 0.5-52.4 ± 0.6°C) (Hardin et al., 1999).

However, electrolyte leakage may not serve as a complete indicator of a plant's performance under adverse conditions as photosynthetic and metabolic pathways are often more sensitive to high temperatures than membranes (Berry and Bjorkman, 1980; Larcher, 1995; Levitt, 1980; Sibley et al., 1999). Other means for evaluation of indirect injury include chlorophyll fluorescence, photosynthesis versus respiration rates, triphenyltetrazolium chloride (TTC) tests, and carbon (C_{14}) partitioning/ assimilation

(Berry and Bjorkman, 1980; Ruter, 1993; Sibley, 1997). The purpose of the following study was to examine the tolerance of six *Tsuga* species to direct foliar heat injury as determined by electrolyte leakage.

Materials and Methods

In January 2008, liners of six *Tsuga* species (*T. canadensis* (L.) Carr., *T. caroliniana* Engelm., *T. chinensis* (Franch.) Pritzel, *T. diversifolia* (Maxim) Mast., *T. heterophylla* (Raf.) Sarg., and *T. dumosa* (D. Don) Eichler (formerly *T. yunnanensis*) were potted into 6.0 L (#2 trade gal.) containers in a substrate of 6 pine bark:1 builders' sand (by volume) and amended with 6.6, 3.0, and 0.9 $kg{\cdot}m^{-3}$ (11.1, 5, and 1.5 $lbs{\cdot}yd^{-3}$) of 18N-2.6P-9.9K (18-6-12) 8-9 month Osmocote® (Scotts Co., Marysville, OH), dolomitic limestone, and Micromax® (Scotts Co.), respectively. Plants were grown on a container pad in Auburn, AL (32° 36'N×85° 29'W, USDA cold hardiness zone 8a) and irrigated with approximately 1.3 cm (0.5 in) water daily for nine months prior to foliar membrane thermostability determination in October 2008.

Electrolyte leakage procedures were similar to those described by Sullivan (1972) and modified by Ahrens (1988), Ingram and Buchanan (1981), and Ruter (1993). Recently matured needles from current-season growth were excised from petioles with a scalpel, weighed to 0.75 g (0.03 oz) samples, placed in test tubes containing 3 ml (0.1 fl oz) of de-ionized water and treated within a thermostatically controlled water bath at 12 temperatures [25, 30, 35, 37.5, 40, 42.5, 45, 47.5, 50, 52.5, 55, and 60 °C (77, 86, 95, 99.5, 104, 108.5, 113, 117.5, 122, 126.5, 131, and 140 °F)] for 30 minutes. Each temperature treatment contained replications of 5 test tubes for each species (temp N=30, total N=360). Upon completing a prescribed temperature treatment, test tubes were filled with 20 ml of de-ionized water, placed on ice, and incubated in a cooler maintained at 4.4 °C (40°F) for 24 hours. Following incubation, samples were brought to room temperature and electrical conductivity measured (Accumet Excel XL50, Fisher Scientific, Pittsburgh, PA). Samples were autoclaved at 121°C and 1.4 $kg \cdot cm^{-2}$ (249.8°F and 20 $lbs \cdot in^{-2}$) for 20 minutes and incubated for 24 hours at 4.4 °C (40°F), after which electrical conductivities were again determined. Electrolyte leakage was expressed as a ratio of the initial electrical conductivity post-treatment to the conductivity post-autoclave. Electrolyte leakage response to temperature has been reported to be sigmoidal with different species (Ahrens and Ingram, 1988; Ingram and Buchanan, 1981; Martineau et al., 1979; Ruter, 1993; Sibley et al., 1999). The sigmoidal equation:

$$L_e = [x - z) / (1 + \exp [- k (T - T_m])] + z$$

where L_e was the percent leakage, z = baseline level of electrolyte leakage, x = maximum percent of electrolyte leakage observed across all temperatures, T_m = predicted temperature midpoint, also referred to as the "inflection point" for the response curve, k = the slope of the predicted response at T_m, and T = water bath temperature used to determine critical midpoint temperatures (T_m) by fitting electrolyte leakage data across temperature treatments (Sibley et al., 1999). Gauss-Newton method of non-linear regression was used to analyze electrolyte leakage data with correlations performed by PROC CORR procedure using SAS Version 9.1.3 (SAS Institute, Cary, NC). Critical midpoint temperatures and *k*-values of fitted response curves were determined for each species (Table 1). Differences were determined by failures of species' T_m values, plus and minus T_m variance, to overlap. *K*-values were used to distinguish slope differences between T_m values and describe the range of temperatures around the T_m for which the species is predicted to withstand before experiencing greater than 50% damage.

Results and Discussion

Gauss-Newton method of non-linear regression met convergence criteria for all species and temperatures except for *T. dumosa*, where the 60°C (140°F) treatment failed to converge due to variability. Analysis of *T. dumosa* was conducted using temperatures 25-55°C (77-131°F) in order to meet convergence criteria. Hereafter all further references to *T. dumosa* are for 25-55°C (77-131°F). Electrolyte leakage and temperature were correlated across all species (r= 0.77, p= <0.0001, N=355). *T. canadensis* and *T. dumosa* had similarly, higher predicted temperature midpoints (T_m) and *k*-values than *T. caroliniana* suggesting higher resistance to direct injury when exposed to brief, supra-optimal temperatures. Predicted T_m for needles of *T. dumosa* 53.2 ± 0.2°C (127.7 ± 0.4°F) and *T. canadensis* 52.3 ± 0.2°C (126.2 ± 0.4°F) were ≈ 3.3 °C (5.7°F) and ≈ 2.4°C (4.2°F) greater than for needles of *T. caroliniana* 49.9 ± 0.7°C (122.0 ± 1.2°F) (Table 1) (Figures 1-6). While differences were found for *T. canadensis* and *T. dumosa* when compared to *T. caroliniana*, no differences were found in T_m and *k*-values for *T. canadensis* or *T. dumosa* when each compared to *T. chinensis*, *T. diversifolia*, and *T. heterophylla*. Similarly, no differences were found for T_m and *k*-values of *T. caroliniana* when compared to *T. chinensis*, *T. diversifolia*, and *T. heterophylla*.

K-values indicated narrower response curves for needles of *T. dumosa* and *T. canadensis* (0.97 ± 0.14 and 0.90 ± 0.16) compared to those of *T. caroliniana* (0.41 ± 0.10) (Table 1), indicating higher temperatures would be necessary to cause direct heat injury to foliage of *T. dumosa* and *T. canadensis.* Narrow *k*-values and low variability also indicated a narrower tolerance range for supra-optimal canopy temperatures around the critical temperature midpoint, suggesting the T_m value predicted is an accurate estimate of the beyond recovery temperature (Donovan et al., 1990; Martin et al., 1991; Ruter, 1996). This narrow tolerance range is the result of the steep slope of the predicted response curve and less variability found within the data for *T. canadensis* and *T. dumosa*, indicating that the higher temperatures of the critical temperature midpoint range for *T. canadensis* and *T. dumosa* are more accurate representations of the temperatures necessary to induce direct injury to the foliage than those predicted for *T. caroliniana*.

In addition to data collected and analyzed in 2008, data were analyzed from a similar study performed in 2003 using four of the same species, *T. canadensis*, *T. caroliniana*, *T. diversifolia*, and *T. heterophylla* with two additional species (*T. mertensiana* and *T.sieboldii*) (Appendix A). Predicted critical temperature midpoints (T_m) in 2008 and 2003 were similar for *T. canadensis* and *T. diversifolia*, respectively (Tables 1 and A1). Predicted T_m values were lower for *T. caroliniana* in 2008 than 2003, where *T. caroliniana* was shown to have one of the highest T_m values of the six species evaluated (Table 1A). Data analyzed from 2003 also indicated that the *k*-value for *T. caroliniana* was found to be highly variable in relation to *T. caroliniana* in 2008. Comparison of the two data sets indicated that most T_m values in 2003 had more variability than those in 2008. Variability between the two studies may have been due to differences in tissue age, acclimation, collection procedures, and sample preparation.

While differences were observed for foliar thermotolerance as direct injury, the occurrence of temperatures necessary to induce direct injury at the critical temperature midpoint range is unlikely. While temperatures have been shown to reach and exceed

50°C (122°F) in plant canopies (Pair and Still, 1982), it is unlikely that said temperatures are maintained long enough to significantly damage plants. Various factors such as automated irrigation regimes and plant-to-plant shading may serve to alleviate high temperatures within plant canopies, reducing the opportunity for foliage to be damaged directly. The temperatures predicted from direct injury, while accurate indicators of membrane thermotolerance, have little practical implication for species performance at elevated temperatures for extended periods of time. Because photosynthetic and metabolic pathways are often more sensitive to high temperatures than membranes, plants may suffer damage and decline at lower temperatures occurring over longer periods of time (Berry and Bjorkman, 1980; Larcher, 1995; Levitt, 1980; Sibley et al., 1999). This damage and decline occurs as the result of indirect injury and may be a better indicator of species performance and longevity in production and the landscape.

Further investigations into indirect injury and electrolyte leakage from hemlock foliage and roots are necessary to verify aforementioned results as exposure duration, tissue age, season, and stage of acclimation may contribute to varied responses for thermotolerance (Donovan et al, 1990; Levitt, 1980; Martineau et al, 1979). With further investigations into indirect heat injury, certain species of hemlock may prove more suitable for production and use in heat-disposed areas than others.

Literature Cited

Abass, M. and C.B. Rajashekar. 1991. Characterization of heat injury in grapes using ^{1}Hnuclear magnetic resonance methods. Plant Physiol. 96:957-961.

Ahrens, M.J. and D.L. Ingram. 1988. Heat tolerance of citrus leaves. HortScience 24:747-748.

Berry, J.A. and O. Bjorkman. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annu. Rev. Plant Physiol. 31:491-543.

Burr, K.E., S.J. Wallner, and R.W. Tinus. 1993. Heat tolerance, cold hardiness, and bud dormancy relationships in seedlings of selected conifers. J. Amer. Soc. Hort. Sci. 188:840-844.

Dexter, S.T., W.E. Tottinghan, and L.F. Graber. 1932. Investigation of the hardiness of plants by electrical conductivity. Plant Physiol. 7:63-78.

Donovan, L.A., K.W. McLeod, and K.L. Grant. 1990. Direct heat injury of roots of woody swamp species. J. Therm. Biol. 15:245-249.

Evans, A.M., and T.G. Gregoire. 2007. A geographically variable model of hemlock woolly adelgid spread. Biol. Invasions 9:369-382.

Hardin, R.B., D.J. Eakes, J.L.Sibley, C.H. Gilliam, and G.J. Keever. 1999. Root membrane thermostability of *Cornus florida* L. provenances. J. Therm. Biol. 24:237-240.

Ingram, D.L. 1985. Modeling high temperature and exposure time interactions on *Pittosporum tobira* root cell membrane thermostability. J. Amer. Soc. Hort. Sci. 110:470-473.

Ingram, D.L. 1986. Root cell membrane heat tolerance of two dwarf hollies. J. Amer. Soc. Hort. Sci. 111:270-272.

Ingram, D.L. and D.W. Buchanan. 1981. Measurement of direct heat injury of roots of three woody plants. HortScience 16:769-771.

Ingram, D.L. and D.W. Buchanan. 1984. Lethal high temperatures for roots of three citrus rootstocks. J. Amer. Soc. Hort. Sci. 109:189-193.

Larcher, W. 1995. Physiological plant ecology. Springer-Verlag, Berlin. 3rd ed.

Levitt, J. 1980. Responses of plants to environmental stresses. Vol. I. Chilling, freezing, and high temperature stresses. Academic Press, New York, N.Y.

Martin, C.A., D.L. Ingram, and M.A. Jenks. 1991. Response of southern magnolia to supraoptimal root-zone temperatures. J. Therm. Biol. 16:281-285.

Martin, C.A. and J.M. Ruter. 1996. Growth and foliar nutrient concentrations of crape myrtle in response to disparate climate and fertilizer placement in large nursery containers. J. Environ. Hort. 14:9-12.

Martineau, J.R., J.E. Specht, J.H. Williams, and C.Y. Sullivan. 1979. Temperature tolerance in soybeans. I. Evaluation of techniques for assessing cellular membrane thermostability. Crop Sci. 19:76-78.

McKay, H.M. 1992. Electrolyte leakage from fine roots of conifer seedlings: a rapid index of plant viability following cold storage. Can. J. For. Res. 22:1371-1377.

Montgomery, M.E., S.E. Bentz, and R.T. Olsen. 2009. Evaluation of hemlock (*Tsuga*) species and hybrids for resistance to *Adelges tsugae* (Hemiptera: Adelgidae) using artificial infestation. J. Econ. Entomol. 102:1247-1254.

Murray, M.B., J.N. Cape, and D. Fowler. 1989. Quantification of frost damage in plant tissues by rates of electrolyte leakage. New Phytol. 113:307-311.

Pair, J.C. and S.M. Still. 1982. Growth, hardiness, and leaf water potential of blue holly (*Ilex*×*merserveae*) cultivars affected by exposure. HortScience 17:823-825.

Ruter, J.M. 1993. Foliar heat tolerance of two hybrid hollies. HortScience 28:650-652.

Ruter, J.M. 1996. High-temperature tolerance of heritage river birch roots decreased by pot-in-pot production systems. HortScience 31:813-814.

SAS Institute Inc. 2004. SAS OnlineDoc® 9.1.3. Cary, NC: SAS Institute Inc.

Sibley, J.L. 1997. Effects of elevated temperatures on container production of red maple (*Acer rubrum* L.) cultivars. Univ. of Ga., Athens, PhD Diss.

Sibley, J.L., J.M. Ruter, and D.J. Eakes. 1999. Root membrane thermostability of red maple cultivars. J. Therm. Biol. 24:79-89.

Stergios, B.G. and G.S. Howell, Jr. 1973. Evaluation of viability tests for cold stressed plants. J. Amer. Hort. Sci. 98:325-330.

Sullivan, C.Y. 1972. Mechanisms of heat and drought resistance in grain sorghum and methods of measurements. p.247-264. In: N.G.P. Rao and L.R. House (eds.) Sorghum in the seventies. Oxford & I.B.H. Publishing Co., New Delhi, India.

Tredici, P.D. and A. Kitajima. 2004. Introduction and cultivation of chinese hemlock (*Tsuga chinensis*) and it's resistance to hemlock woolly adelgid (*Adelges tsugae*). J. of Arbor. 30:282-287.

Yadava, U.L., S.L. Doud, and D.J. Weaver. 1978 Evaluation of different methods to assess cold hardiness of peach trees. J. Amer. Soc. Hort. Sci. 103:318-321.

Table 1. Response of *Tsuga* (hemlock) species to elevated foliar temperature[z].

Species	Zone[y]	T_m[x]	T_m[w]	*k* -values[v]
T. canadensis	5	52.3 ± 0.2	126.2 ± 0.4	0.90 ± 0.16
T. caroliniana	5	49.9 ± 0.7	122.0 ± 1.2	0.41 ± 0.10
T. chinensis	8	51.4 ± 0.9	124.6 ± 1.6	0.34 ± 0.08
T. diversifolia	8	52.1 ± 0.5	125.7 ± 0.9	0.84 ± 0.31
T. dumosa [u]	8	53.2 ± 0.2	127.7 ± 0.4	0.97 ± 0.14
T. heterophylla	5	51.2 ± 0.4	124.1 ± 0.8	0.66 ± 0.15

[z]Data presented from electrolyte leakage study in 2008.

[y]USDA Hardiness Zones for location of nursery responsible for the supply of stocks or seedlings

[x]Means and standard errors for predicted critical temperature (Celsius) parameters determined by least squares approach Gauss-Newton method of non-linear regression.

[w]Means and standard errors for predicted critical temperature (Fahrenheit) parameters determined by least squares approach Gauss-Newton method of non-linear regression.

[v]K-values for Tm[x] (Celsius).

[u]*Tsuga dumosa* had 60°C (131°F) treatment removed during analysis to meet convergence criteria for non-linear regression analysis.

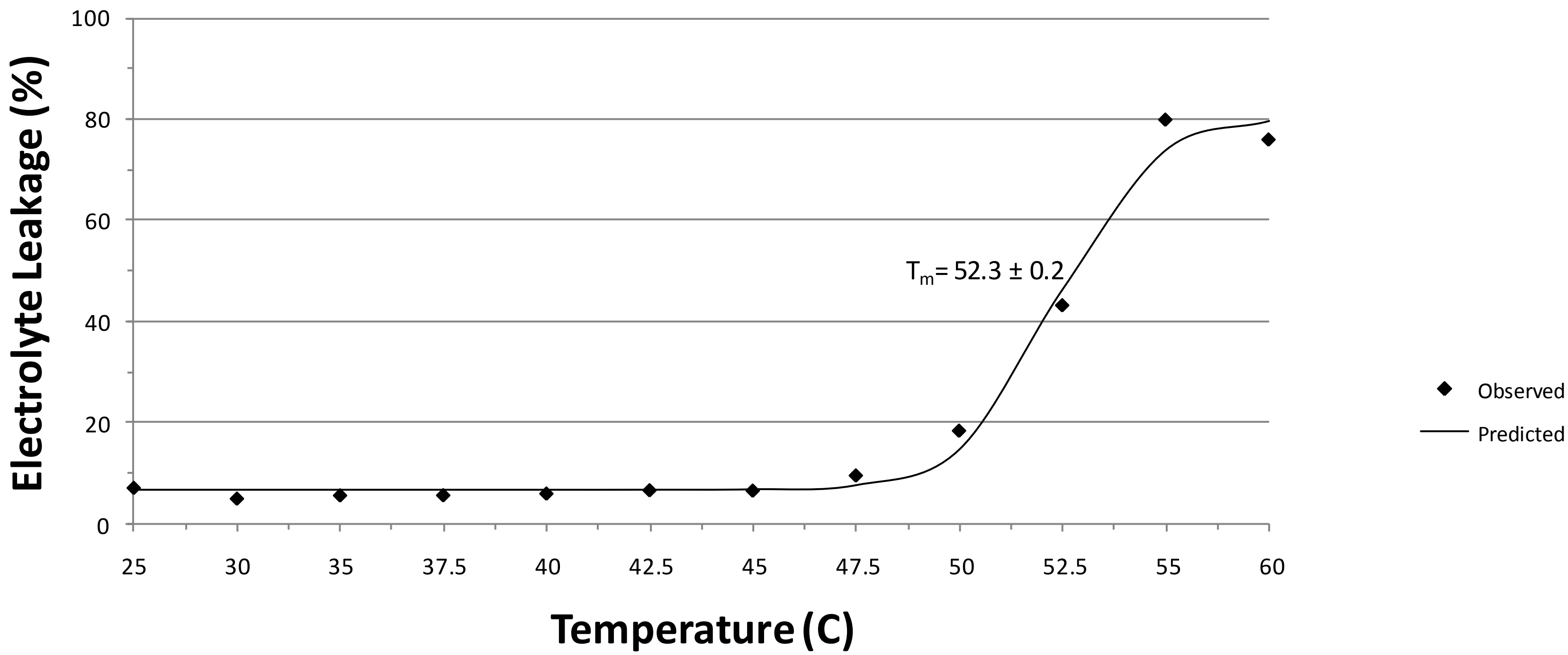


Figure 1. Foliar electrolyte leakage of *Tsuga canadensis* exposed to 25 to 60° C for 30 min. in 2008.

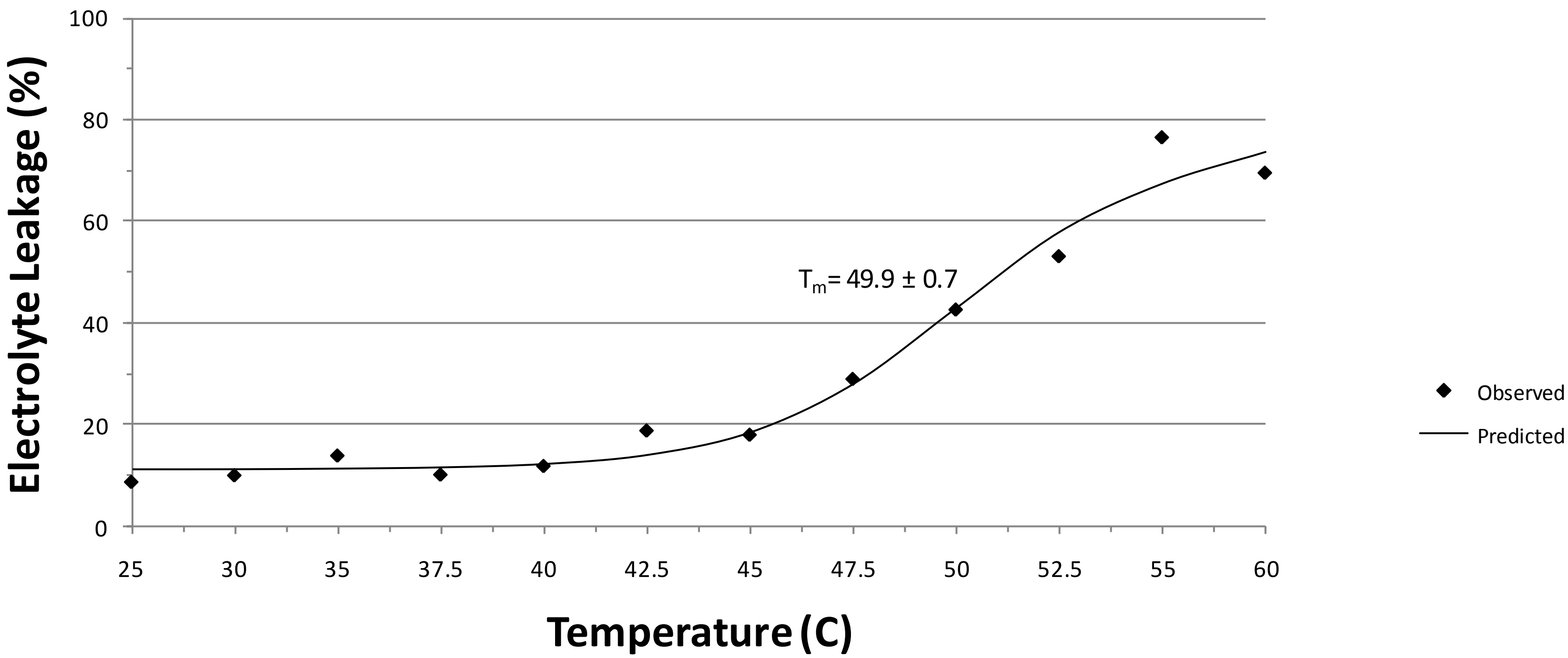


Figure 2. Foliar electrolyte leakage of *Tsuga caroliniana* exposed to 25 to 60° C for 30 min. in 2008.

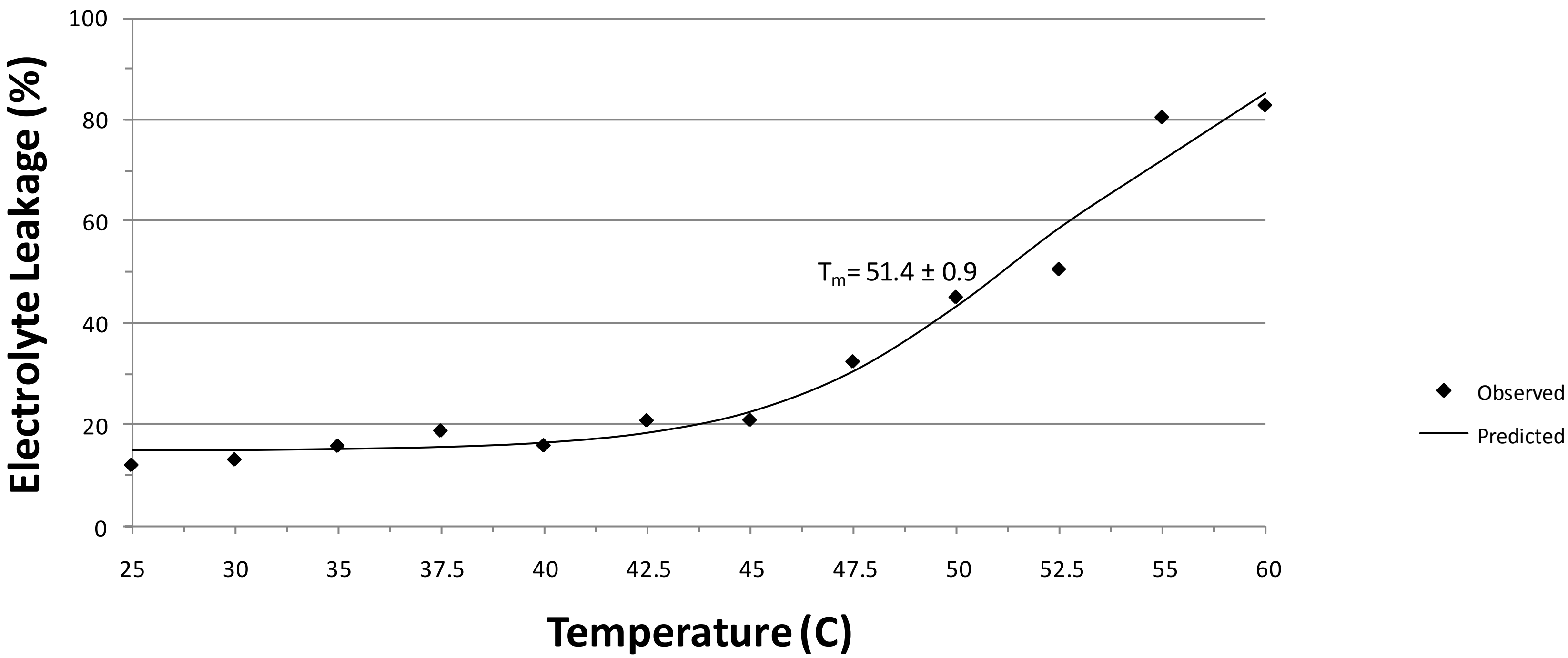


Figure 3. Foliar electrolyte leakage of *Tsuga chinensis* exposed to 25 to 60° C for 30 min. in 2008.

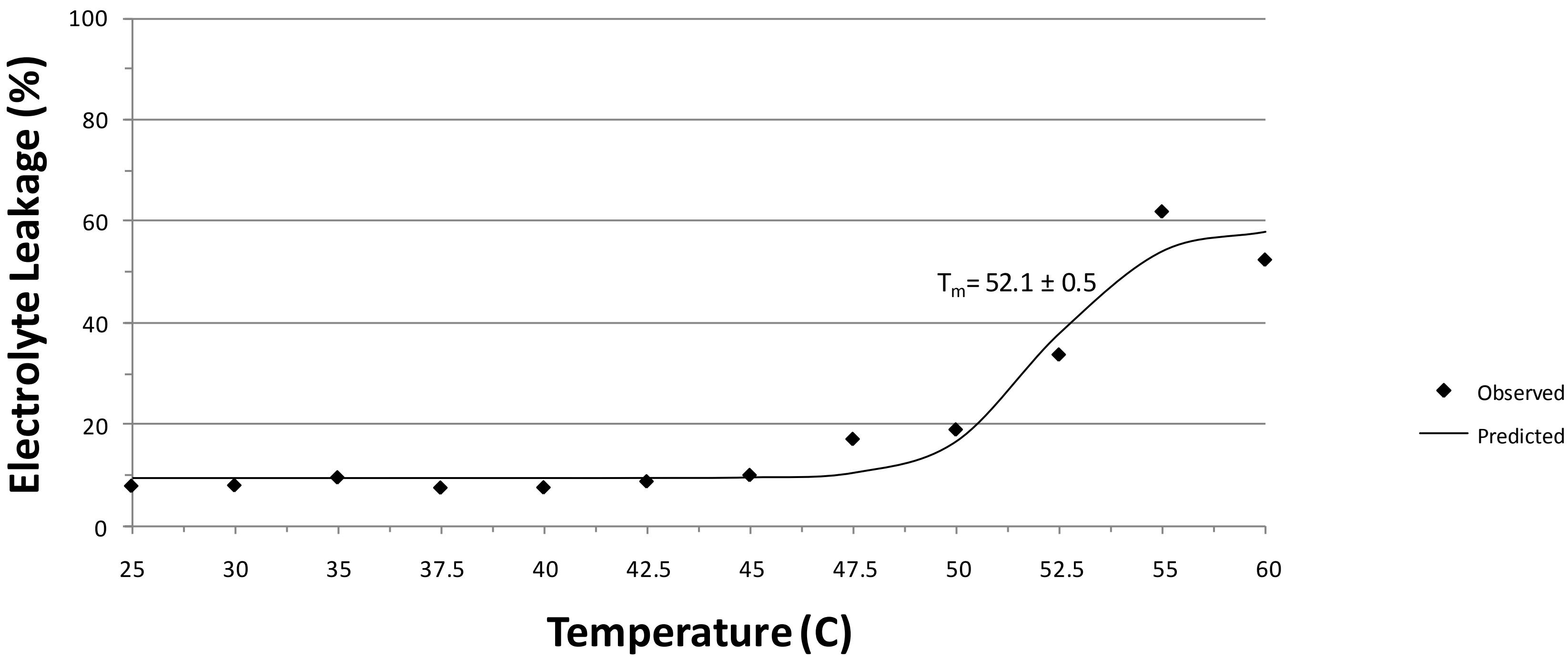


Figure 4. Foliar electrolyte leakage of *Tsuga diversifolia* exposed to 25 to 60° C for 30 min. in 2008.

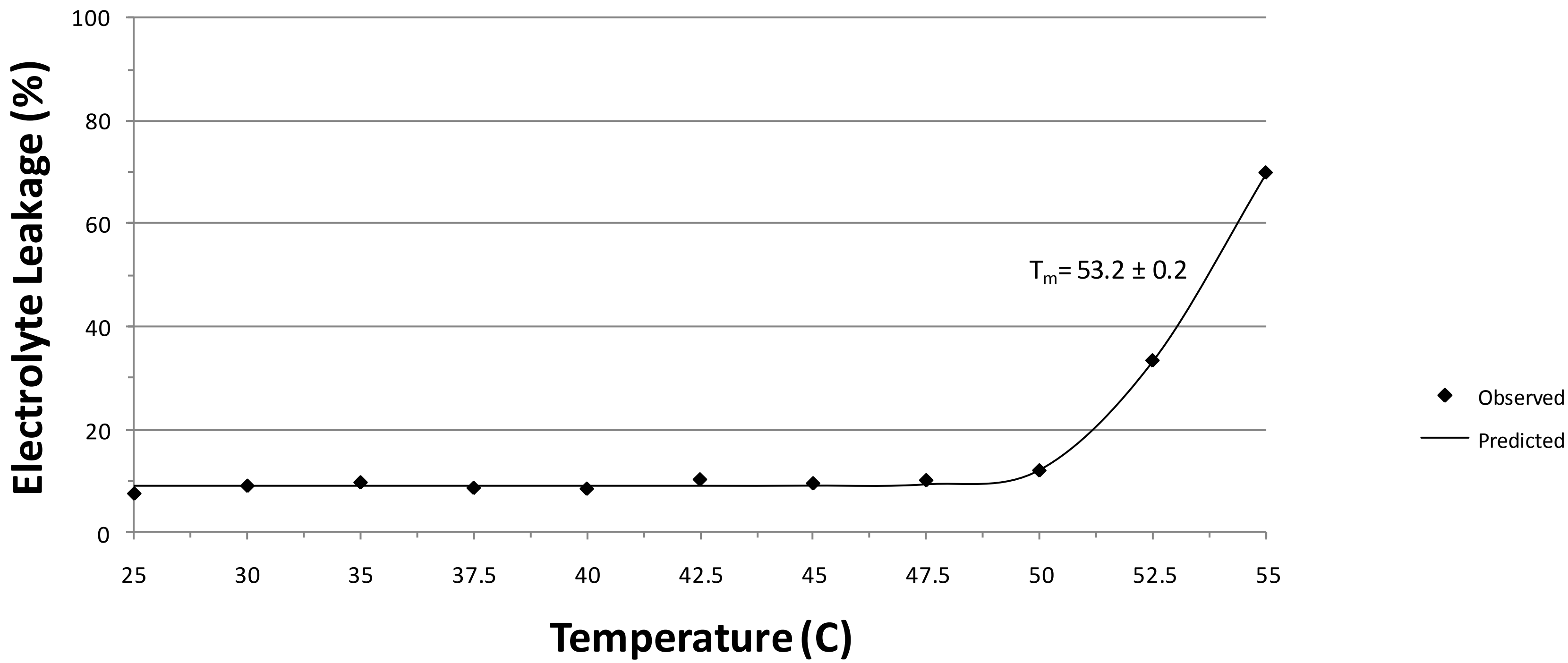


Figure 5. Foliar electrolyte leakage of *Tsuga dumosa* (*Tsuga* yunnanensis) exposed to 25 to 55° C for 30 min. in 2008.

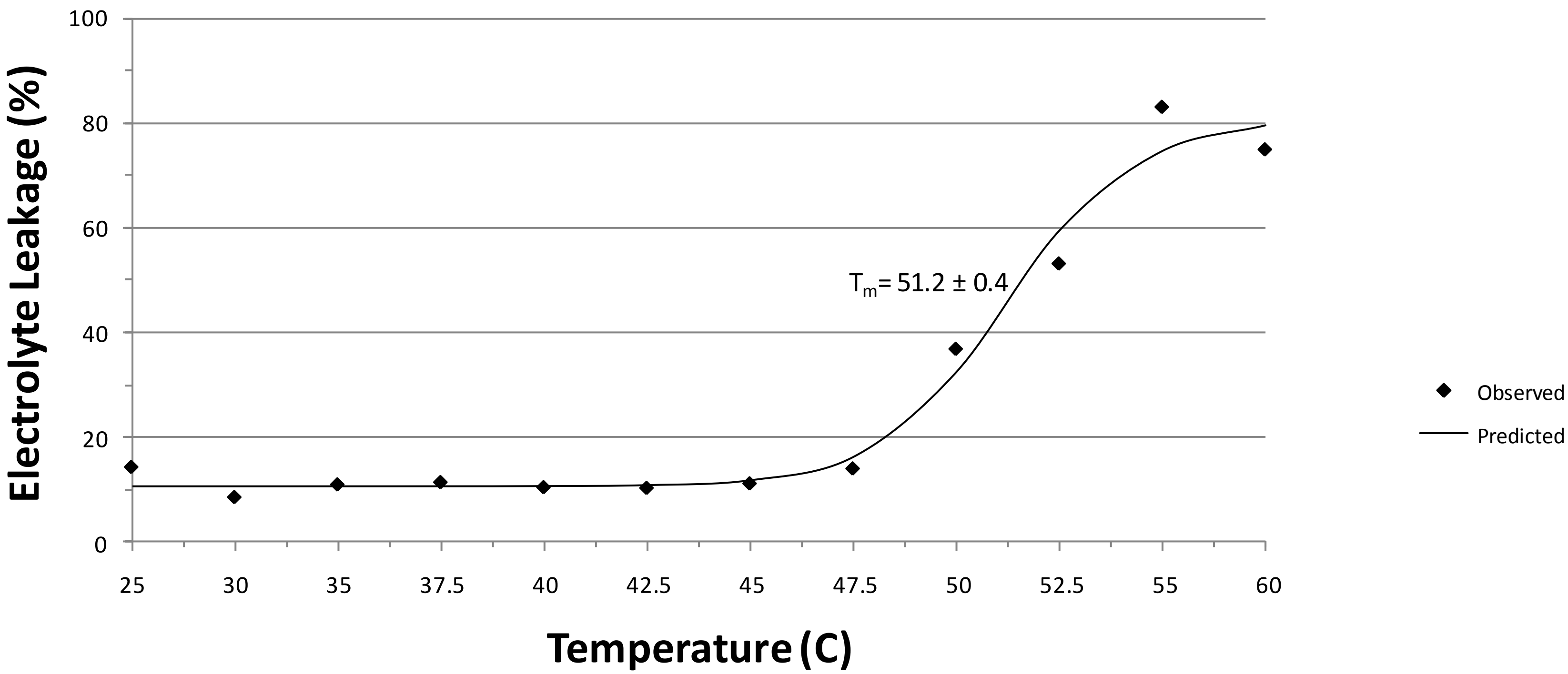


Figure 6. Foliar electrolyte leakage of *Tsuga heterophylla* exposed to 25 to 60° C for 30 min. in 2008.

PART II INVESTIGATION OF A FOLIAR DISORDER IN CRAPEMYRTLE (*LAGERSTROEMIA INDICA ×FAURIEI*)

Chapter I

Introduction and Literature Review

I. The Genus *Lagerstroemia*

The genus *Lagerstroemia* is comprised of approximately 50 species consisting mainly of woody shrubs and small trees (Graham et al., 1987). *Lagerstroemia* is native to Southeast Asia with origins in India, China, Korea, Japan, the Philippines, and Australia (Egolf and Andrick, 1978; Furtado and Montien, 1969). As one of 31 genera currently assigned to the *Lythraceae* family (Graham et al., 1993), *Lagerstroemia* is characterized within the family by alternate to sub-opposite leaves sometimes whorled with three leaves at each node (Dirr, 1998) and inflorescences of simple racemes, compound cymes, and lateral/terminal panicles (Graham et al., 1987). Flowers are conspicuous ranging from white to rose-purple in color. Flowers are perfect, with corolla segments 5-8-merous and stamens ranging in count from 15 (*L. subcostata* Koehne) to 200. Stamens are often dimorphic as characterized by *L. indica* L. (Graham et al., 1987).

Lagerstroemia speciosa (L.) Pers. and *L. piriformis* Koehne have been cultivated in native habitats of Southeast Asia for centuries and prized for furniture making as timber and wood quality from the two species share similar characteristics to teak (Cabrera, 2004; Egolf and Andrick, 1978; Everett, 1969; Everett, 1981). *L. speciosa* is also valued for medicinal use to treat diabetes, as chemical compounds within the leaves used in a tea have had similar effects insulin (Cabrera, 2004; Kakuda et al., 1996). Taxonomist Carl Linnaeus described and named *Lagerstroemia* after his friend Magnus von Lagerstrom of the Swedish East Indies Company in 1759 (Byers, 1997). Byers (1997), Dirr (1998) and Egolf and Andrick (1978) cite introduction of *Lagerstroemia* in Europe and North America in the 1750s. Early use of *Lagerstroemia* or crapemyrtle, "crape myrtle", "crepemyrtle, or "crepe myrtle", as commonly known and spelled, consisted of a limited number species within the North American landscape. *L. indica,* and *L. reginae* Roxb. are reported to be among the first seedlings planted in the gardens of George Washington's Mt. Vernon estate in 1799 (Byers, 1997; Egolf and Andrick, 1978). Crapemyrtle's recognition within the American landscape developed slowly as early introductions lacked cold hardiness, resistance to powdery mildew (*Erysiphe lagerstroemiae* E. West), and variety of flower color (Egolf, 1981). Introductions of new cultivars for the American landscape by Donald R. Egolf beginning in 1967, opened up a greater use of the species with selections from the cross of *Lagerstroemia indica* and *Lagerstroemia fauriei* Koehne (*L. indica* ×*fauriei*).

Early use of *L. indica* in the North American landscape can likely be attributed to its long bloom period, variety of flower colors (red, pink, white, lavender, and purple), and diversity of plant sizes and habits (Byers, 1997; Cabrera, 2004). However, increased susceptibility to powdery mildew limited use in humid, southern landscapes where powdery mildew could thrive and make plants unattractive by distorting growth (Knox, 1995). Until the 1960s, cultivars of *L. indica* were the predominant crapemyrtles in North America. In 1956, John Creech of the USDA's New Crops Research Branch, brought seeds of *L. fauriei* back from Japan to evaluate. Exfoliating, cinnamon-colored bark, greater cold hardiness, and reported resistance to powdery mildew of *L. fauriei*, led to inter-specific breeding of *L. indica* and *L. fauriei* by Donald Egolf of the U.S. National Arboretum (Byers, 1997; Cabrera, 2004; Knox, 1995). Egolf and Andrick (1978) reported offspring of the inter-specific cross to exhibit powdery mildew resistance previously known in *L. fauriei* parentage (Egolf, 1981). Further breeding from this single ascension of *L. fauriei* served as the parent to subsequent cultivars (Cabrera, 2004; Pooler, 2006). Resulting cultivars of the *L. indica ×fauriei* cross began to diversify the flower color, bark color, size, and disease resistance for crapemyrtle selections for the landscape, adding 22 hybrid cultivars to the over 200 registered cultivars of *Lagerstroemia* (Cabrera, 2004; Pooler, 2006).

II. Foliar Disorder in Crapemyrtle

Growers began to notice a perplexing leaf disorder occurring on cultivars of the *Lagerstroemia indica ×fauriei* cross in the 1980s. Most prevalent on cultivars of ‘Muskogee’, ‘Tuskegee’, ‘Miami’, and ‘Natchez’ hybrid crapemyrtles (Cabrera and Lopez, 2004), the foliar disorder has been routinely identified in container production and landscapes, with a few reports in field production. The disorder is casually referred to as “rabbit tracks” or “rabbit tracking” and typically occurs on the second flush of foliage in the spring. Symptoms of the disorder include bronze elliptical spots on both sides of the mid-vein leaving a series of translucent spots visible from upper and lower leaf surfaces and distortion of new growth when severe (Figure 1). Disorder progression suggests the effect of the disorder is aesthetic and not detrimental to affected plant health and survival as the disorder’s symptoms appear to diminish with continued shoot growth (Figure 2).

III. Preliminary Work on Rabbit Tracks Foliar Disorder

Preliminary experiments conducted by Sibley and Ruter (Wilson et al., 2007) during the 1990s and early 2000s sought to determine the cause of “rabbit tracks” foliar disorder through a nutritional approach. Initial work began in 1993 and 1994 with tissue samples collected from affected and unaffected ‘Natchez’ crapemyrtles in the landscape. Eight plants were included in this analysis with four affected and four unaffected plants. Fifty leaves and one soil sample from each specimen were analyzed for interactions between specific nutrient concentrations and occurrence of disorder. Data suggested investigation of several nutrients as potential causes for the disorder for which the following experiments were conducted.

In 1995, Sibley evaluated the effects of three manganese and three zinc concentrations (0, 1x, and 2x) within modified Hoagland’s nutrient solutions on *Lagerstroemia indica ×fauriei* ‘Tuskegee’ and ‘Natchez’. Plants were grown in 100% coarse sand in # 1 trade-gallon containers. Plants were grown in a controlled environment greenhouse and examined for presence of “rabbit tracks” disorder. Conclusions of both the manganese and zinc studies suggest further investigation of other nutrients is warranted because both cultivars exemplified the disorder in all treatments (Wilson et al., 2007).

In 1997, an experiment was conducted using 54 ‘Natchez’ crapemyrtle 10 cm (4 in) potted liners, rooted in unamended 6 pine bark: 1 sand substrate. Liners were potted up to #3 trade-gallon containers with four different rates of dolomitic limestone [0, 3, 6, and 9 $kg{\cdot}m^{-3}$ (0, 5, 10, and 15 $lbs{\cdot}yd^{-3}$)]. Differing lime rates were used to evaluate the effect of limited nutrient availability caused by increased substrate pH on the presence of "rabbit tracks". Results of the experiment did not indicate correlation between substrate pH and the presence of the "rabbit tracks" foliar disorder (Wilson et al., 2007).

Sibley continued nutritionally based experiments in 1999 evaluating four concentrations of phosphorus (0x, 1x, 2x, and 3x) based on recommended phosphorus rates using phosphoric acid, H_3PO_4 in a modified Hoagland's solution. Bare root liners of 'Natchez' crapemyrtles were grown using 100% sand in #1 trade-gallon containers inside a controlled environment greenhouse. All of the plants in the phosphorus experiment displayed the disorder in all treatments suggesting further investigation (Wilson et al., 2007).

In 2003 and 2004, Sibley and Ruter (Wilson et al., 2007) conducted a study to examined the role of nickel in the occurrence of "rabbit tracks" using a foliar spray and substrate drench. This experiment was conducted using the hybrid crapemyrtle cultivars 'Miami', 'Muskogee', and 'Natchez'. One year old plants in #3 trade-gallon containers were potted up to #7 trade-gallon containers in 6 pine bark : 1 sand substrate. Treatments consisted of a foliar spray of urea at 2 $lbs \cdot 100\ gal^{-1}$ with a surfactant at 0.14 $fl\ oz \cdot gal^{-1}$, along with 0.30 $oz \cdot gal^{-1}$ nickel sulfate foliar spray applied at 100 $gal \cdot acre^{-1}$ and a drench treatment with 0.16 $oz \cdot gal^{-1}$ nickel sulfate drench applied at 16.9 fl oz per container. Treatments were applied once in the fall and again in the spring during leaf expansion. Plants were grown outdoors on a container pad under overhead irrigation. Results of the nickel study did not explain the cause of the leaf disorder in the three hybrid crapemyrtle cultivars using nickel foliar spray and drench as all the plants displayed the disorder (Wilson et al., 2007). Additional preliminary experiments evaluating various rates of substrate and foliar-applied nutrients on the occurrence of “rabbit tracks” were conducted in 2007 and 2008 (Appendix C).

In addition to nutrition based experiments, an enzyme-linked immunosorbent assay (ELISA test) was conducted in 1997 and 2008 to see if any viruses or bacteria were present in samples of affected leaf tissue that could be transferred to young tobacco plants or evaluated in a plate reader. In reviewing the ELISA test for the presence of plant viruses, no transferable virus was found. Not all plant viruses are easily transferable to other plants and not all crops are susceptible to other plant species’ viruses. Tobacco was used due to its susceptibility to many known plant viruses. ELISA tests conducted in 2008 for the bacterium *Xylella fastidiosa* for which crapemyrtle is a host (Huang, 2007) exhibited positive readings for all samples. The peroxidase reagent kit used for most ELISA test of *Xylella fastidiosa* was believed to react with phytochemicals associated with crapemyrtle causing all samples to exhibit bacteria. According to personal communication with John Murphy, an Auburn University plant pathology professor, further work using polymerase chain reaction (PCR) method to determine *Xylella* presence and “rabbit tracks” foliar disorder interaction and development of ELISA reagent kits compatible with *Lagerstroemia* are needed (Murphy, personal communication).

Cabrera and Lopez of Texas A&M University conducted a cultivar and foliar nutrient survey of 13 crapemyrtle cultivars of three species and one hybrid (5 *L. indica*, 3 *L. fauriei*, 4 *L. indica* ×*fauriei*, and 1 *L. speciosa*) as a part of a salinity tolerance evaluation in 2003. Cabrera and Lopez (2004) reported zero percent of *L. speciosa* cultivars exhibited symptoms of “rabbit tracks” disorder whereas, 94.4 and 97.5% of the cultivars for *L. fauriei* and *L. indica ×fauriei* exhibited symptoms of the disorder (N=390, at 30 plants per cultivar). Increased incidence for disorder occurrence in cultivars of *L. fauriei* and *L. indica ×fauriei* suggests that abnormalities associated with gene expression for cultivars with *L. fauriei* parentage may be a factor for “rabbit tracks” presence as seen in flea beetle and powdery mildew resistance between *L. indica* and *L. fauriei* (Cabrera et al., 2003; Hagan et al., 1998). In addition to potential genetic influences, Cabrera and Lopez (2004) observed that some of the nutrient content of foliage samples were strongly associated with “rabbit tracks” occurrence. Manganese (Mn) and zinc (Zn) were observed to have a potential relationship with disorder presence as the percentage of plants exhibiting “rabbit tracks” was highest for plants with lower Mn levels (*L. fauriei* and *L. indica ×fauriei*). In addition to Mn, Zn was found strongly associated with the foliar disorder. Observations of the raw data indicated foliar Zn concentrations less than 90 to 100 ppm were found to be associated with increased percentage of affected plants (Cabrera and Lopez, 2004). Consistent with visual symptoms, the foliar nutrient survey suggests micronutrient (Fe, Mn, Cu, and Zn) deficiency or toxicity may be involved as the disorder appears on growth subsequent to the first foliage flush. The occurrence of symptoms on secondary growth indicates a potential immobile nutrient deficiency/toxicity as most micronutrient elements are unable to translocate from older foliage to newer foliage (Mengel and Kirkby, 2001). In addition to genetic and nutrient content, studies examining interactions between genetic and nutritional influences are warranted. Conversation with Fenny Dane, an Auburn University horticulture professor, nutrient status and other environmental factors may initiate or terminate gene expression for protein biosynthesis leading to disorders of photosynthetic and metabolic enzyme production, carbon partitioning, and plant growth and development (Dane, personal communication; Galangau et al., 1988; Migge et al., 2000), potentially inducing the “rabbit tracks” foliar disorder.

Cabrera and Lopez (2004) and Wilson et al. (2007) are the only literature describing work to diagnose “rabbit tracks” foliar disorder in *Lagerstroemia*. The purpose of the following work was to explore the significance of “rabbit tracks” disorder to the nursery and landscape industry through an industry sponsored survey and to examine the disorder’s occurrence through a hydroponic nutrition study.

Literature Cited

Byers, D. 1997. Crapemyrtle: a grower's thoughts. Owl Bay, Auburn, AL.

Cabrera, R.I. 2004. Evaluating and promoting the cosmopolitan and multipurpose *Lagerstroemia*. Acta Hort. 630:177-184.

Cabrera, R.I., J.A. Reinert, and C. McKenney. 2003. Resistance among crape myrtle (*Lagerstroemia* spp.) cultivars to the flea beetle (*Altica litigate*). South. Nurs. Assoc. Res. Conf. 48:164-167.

Cabrera, R I. and R.E. Lopez. 2004. A leaf interveinal chlorosis-necrosis disorder in crape myrtle. South. Nurs. Assoc. Res. Conf. 49:90-93.

Dane, F. 2010. Personal communication. 18 June 2010.

Dirr, M.A. 1998. Manual of woody landscape plants: their identification, ornamental characteristics, culture, propagation, and Uses. 5th ed. Stipes, Champaign, IL.

Egolf, D.R. 1981. 'Muskogee' and 'Natchez' *Lagerstroemia*. HortScience 16:576.

Egolf, D.R. and A.O. Andrick. 1978. The *Lagerstroemia* handbook/checklist. Amer. Soc. Botanical Gardens and Arboreta, Inc. Los Angeles.

Everett, T.H. 1969. Living trees of the world. Doubleday, N.Y.

Everett, T.H. 1981. The New York botanical garden illustrated encyclopedia of horticulture. Vol. 6. Garland, N.Y.

Furtado, C.X. and S. Montien. 1969. A revision of *Lagerstroemia* L. (Lythraceae). Gard. Bul. Straits Settlem. 24:185-335.

Galangau, F., F. Daniel-Vedele, T. Moureaux, M.F. Dorbe, M.T. Leydecker, and M. Caboche. 1998. Expression of leaf nitrate reductase genes from tomato and tobacco in relation to light-dark regimes and nitrate supply. Plant Physiol. 88:383-388.

Graham, A., J.W. Nowicke, J.J. Skvarla, Graham, S.A., V. Patel, and S. Lee. 1987. Palynology and systematics of the Lythraceae. II. Genera *Haitia* through *Peplis*. Amer. J. Bot. 74:829-850.

Graham, S.A., J.V. Crisci, and P.C. Hoch. 1993. Cladistic analysis of the Lythraceae *sensu lato* based on morphological characters. Bot. J. Linn. Soc. 113:1-33.

Hagan, A.K., G.J. Keever, C.H. Gilliam, J.D. Williams, and G. Creech. 1998. Susceptibility of crapemyrtle cultivars to powdery mildew and Cercospora leaf spot in Alabama. J. Environ. Hort. 16:143-147.

Huang, Q. 2007. Natural occurrence of *Xylella fastidiosa* in a commercial nursery in Maryland. Can. J. Plant Path. 29:299-303.

Kakuda, T., I. Sakane, T. Takihara, Y. Ozaki, H. Takeuchi, and M. Kuroyanagi. 1996. Hypoglycemic effect of extracts from *Lagerstroemia speciosa* L. leaves in genetically diabetic KK-A(y) mice. Biosci. Biotechnol. Biochem. 60:204-208.

Knox, G.W. 1995. Crape myrtles for the deep south. Amer. Nurseryman 6 (11):70-77.

Mengel, K. and E.A. Kirkby. 2001. Principles of plant nutrition. 5th ed. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Migge, A., C. Bork, R. Hell, and T.W. Becker. 2000. Negative regulation of nitrate reductase gene expression by glutamine or asparagines accumulating in leaves of sulfur-deprived tobacco. Planta. 211:587-595.

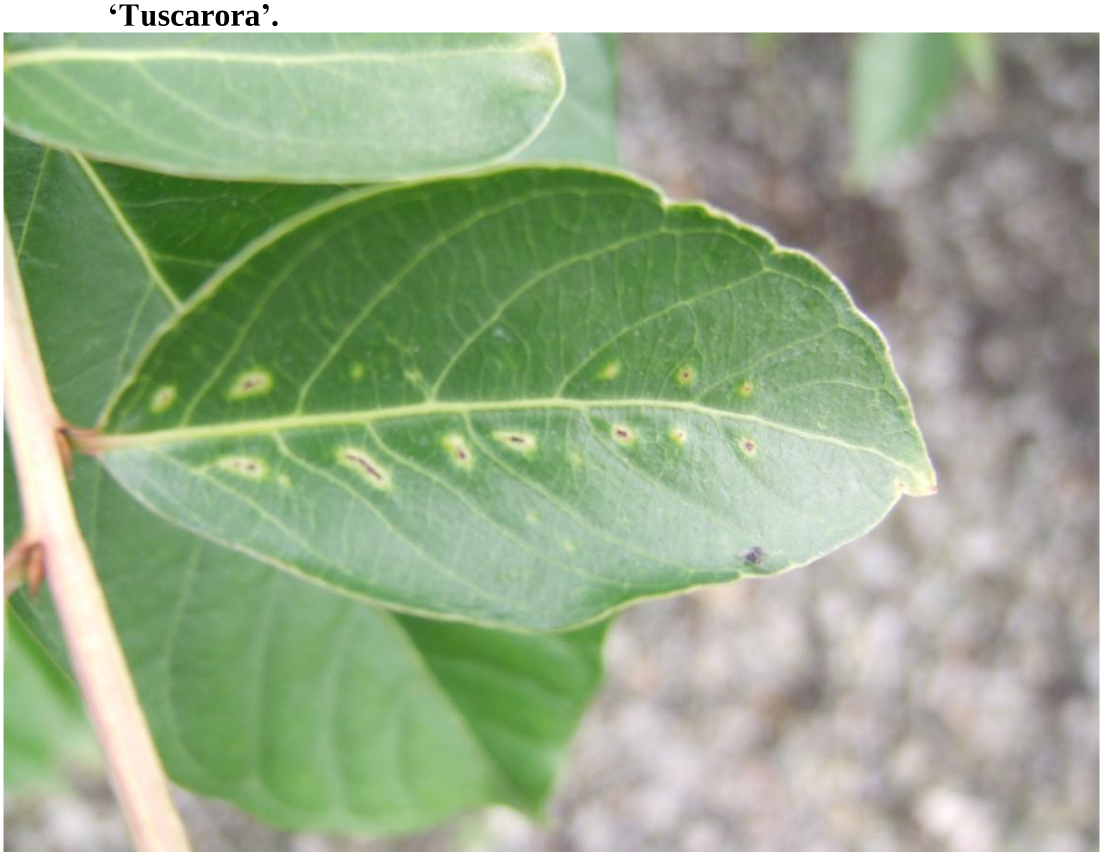
Murphy, J.F. 2008. Personal communication. 22 July 2008.

Pooler, M.R. 2006. Crapemyrtle – *Lagerstroemia indica*. P. 428-449. In: N.O. Anderson (editor). Flower breeding and genetics: Issues, challenges, and opportunities for the 21st century. Vol. 2. Springer, N.Y.

Wilson, M.W., J.L. Sibley, D.J. Eakes, and J.M. Ruter. 2007. Investigation of rabbit tracks: a foliar disorder of crapemyrtle. South. Nurs. Assoc. Res. Conf. 52:590-593.

Figure 1. "Rabbit tracks" foliar disorder in *Lagerstroemia indica ×fauriei* 'Tuskegee'.

Figure 2. Progressed "rabbit tracks" foliar disorder in *Lagerstroemia indica ×fauriei* 'Tuscarora'.



Chapter II

Survey of “Rabbit Tracks” Foliar Disorder’s Extent, Effect, and Significance to Ornamental Horticulture Industries

Abstract

With rapid growth and use of hybrid crapemyrtles, growers began to notice a perplexing leaf disorder occurring on cultivars of the *Lagerstroemia indica* L. × *Lagerstroemia fauriei* Koehne crosses in the 1980s. The disorder is casually referred to as “rabbit tracks” or “rabbit tracking” and typically occurs on the second flush of foliage in the spring. No work quantifying the impact of the foliar disorder to ornamental horticulture industries has been published to date. In August 2008, an online survey was conducted in cooperation with seven nursery and landscape associations in Alabama, Florida, Georgia, Louisiana, South Carolina, Tennessee, and Texas. Industry members within the seven associations were asked 16 questions to obtain demographic, disorder familiarity, and disorder significance information. No significant correlations between gross income of participants and the ratings for significance to the industry for “rabbit tracks” foliar disorder were found. Percentages of participants’ responses were reported. Thirty percent of industry members surveyed reported never hearing the term “rabbit tracks” and 40% reported experience with the disorder. Data collected from the survey served to estimate the need for significant measures to diagnose and treat the disorder.

Measures requiring large amount of resources, expense, and costly solutions are not recommended for diagnosis work or treatment, as industry members did not indicate significant losses or customer concern for “rabbit tracks” effect to plant material quality.

Introduction

With rapid growth and use of hybrid crapemyrtles, growers began to notice a perplexing leaf disorder occurring on cultivars of the *Lagerstroemia indica* L., *Lagerstroemia fauriei* Koehne crosses in the 1980s. Most prevalent on cultivars of ‘Muskogee’, ‘Tuskegee’, ‘Miami’, and ‘Natchez’ hybrid crapemyrtles (Cabrera and Lopez, 2004), the foliar disorder has been routinely identified in container production and landscapes, with a few reports in field production. The disorder is casually referred to as “rabbit tracks” or “rabbit tracking” and typically occurs on the second flush of foliage in the spring. Symptoms of the disorder include bronze elliptical spots on both sides of the mid-vein leaving a series of translucent spots visible from upper and lower leaf surfaces and distortion of new growth when severe. Disorder progression suggests the effect of the disorder is aesthetic and not detrimental to affected plants’ health and survival as the disorder’s symptoms appear to diminish with continued growth of subsequent foliage. Preliminary work including a cultivar survey and nutrition experiments have attempted to diagnose the cause of “rabbit tracks” with no work quantifying the impact of the foliar disorder to ornamental horticulture industries.

Cabrera and Lopez (2004) and Wilson et al. (2007) are the only literature describing work to diagnose “rabbit tracks” foliar disorder in *Lagerstroemia*. From casual observation and correspondence with growers, Cabrera and Lopez (2004) stated “rabbit tracks” did not seem to have apparent effects on the popularity or sales of crapemyrtle. In

Alabama, the nursery, landscape, and horticulture retail industries contributed $2.7 billion dollars and 41,800 jobs to Alabama's economy in 2007 (ALNLA, 2009). Previous work performed by Adrian et al. (1998) evaluated the fixed and variable costs associated with producing crapemyrtle over three years using three production systems. Average production cost for producers using the three production systems ranged from $21.52 to $23.73 per plant with total costs ranging from approximately $450,000 to $508,000 for a 6 ha (15A) nursery with a 4 ha (10A) production area (Adrian et al., 1998) plus 2.52 % annual inflation rate for years 1996 to 2007 (USBLS, 2010). While most growers produce additional plant material besides crapemyrtle, work conducted by Adrian et al. (1998) showed the production price associated with growing each plant. Crop failure due to disease or improper management can cause growers to incur larger costs as the production costs can account for most of the wholesale/retail value of the crop, leaving small profit margins. The purpose of the following work was to explore the "rabbit tracks" disorder's significance to the nursery and landscape industry through an industry sponsored survey in eight southeastern states.

Materials and Methods

In July 2008, the internal review board (IRB) of Auburn University approved an online survey to nursery and landscape grower associations to evaluate the significance of "rabbit tracks" to their industry (Appendix B). Nursery and landscape grower associations were identified to participate in an online survey through the American Nursery and Landscape Association website (ANLA, 2009). Association directors of ten southeastern U.S. states (Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, and Texas) were contacted and asked to participate in the on-line survey. Association directors were given an opportunity to preview the online survey and suggest changes. All associations with the exception of Arkansas, Mississippi, and North Carolina agreed to participate. In August 2008, approval from association directors was obtained and a letter to industry professionals was given to associations to include on association websites, emails, newsletters, and magazines (Appendix B). Associations were responsible for disseminating information to its members to avoid collection of personal information as requested by association directors for protection of member information and for IRB approval.

To increase association and participant response, pre-contact emails, short web address, and easy-to-answer questions were incorporated into the survey's design and implementation (Dillman, 2007). The survey was designed and hosted on SurveyMonkey.com™ (SurveyMonkey, 2008). The survey used a short web uniform resource locator (URL) to redirect participants to the online survey hosted on SurveyMonkey.com™ server (www.auburn.edu/rtsurvey). Incorporating a short web address ensured that the survey could be easily passed along to industry members through word-of-mouth communication and email (Dillman, 2007). Upon following the link to the survey, respondents were directed to a survey introduction page (Appendix B), which explained the purpose of the survey and assured participants that no personally identifying information was collected from the survey. Survey respondents were asked to click on a button to agree to participate in the survey. Upon agreement to the terms and conditions of the survey, participants were directed to the first page of the survey for a series of 14 multiple-choice questions with two optional short answer questions (16 total) (Appendix B). Questions related to the nature, size, location (state), and income of their business, whether they had previous "rabbit tracks" foliar disorder knowledge, what cultivars experienced the symptoms, and experience/opinion of the disorder's significance to the industry (Appendix B). Response collection for the survey began on August 20, 2008 with an initial deadline for October 31, 2008 (72 days). Due to late dissemination of association media and to collect more responses, the deadline was extended to December 31, 2008 (133 days).

Upon closure of the survey, survey data were collected and entered into an Excel© spreadsheet (Microsoft, 2007). Data were imported into statistical software (SPSS, 2007) where variable and response data were given values to perform correlations using the Pearson correlation procedure (SPSS, 2007).

Results and Discussion

Data revealed that 61 horticulture industry members participated in the survey (N=61). Since no collection of email addresses or personal information was conducted through the survey, there was no definite number as to how many were ultimately contacted about the survey. Correspondence with association directors estimates that a total of approximately 5,500 potential contacts were made through association emails, websites, newsletters, and magazines. States with the greatest participation in the survey with 41% and 31% of the total surveyed were Florida and Texas (Figure 1), which are among the largest crapemyrtle producers in the U.S. Association emails (68.8%) and website postings (15.6%) were reported to be the most effective in gaining participation in comparison to association newsletters (7.8%) and magazine (1.5%) articles, personal communication (6.2%), or general internet searches (0.0%) (Figure 2). Problems associated with online surveys are that online surveys are not favored among all groups of respondents as elderly or access-challenged participants may find electronic surveys challenging (Dillman, 2007). Mail-in surveys with included return-postage may increase response, but require additional labor and expense to enter data and pay for postage and survey labor. The survey was conducted electronically to reduce data entry time, expense, and error.

In order to determine the significance of “rabbit tracks” to the horticulture industry, correlations were performed using the Pearson correlation procedure (SPSS, 2007) for the gross income of participants versus the ratings for “rabbit tracks” significance to the industry. Correlations did not indicate a significant relationship between significance ratings for “rabbit tracks” and gross business income ($p=0.265$, $N=55$). No correlations between gross income and other ratings were significant, therefore only frequencies in relation to total responses (percentages) were further reported.

The survey was designed to gather demographic information, industry experience with “rabbit tracks”, and industry ratings for significance of “rabbit tracks” to the ornamental horticulture industry. Of the participants that responded to the survey, 62.3% were growers, 11.5% landscapers, 3.3% retailers, 3.3% re-wholesalers (landscape distribution center), 1.6% grower/retailer, 4.9% grower/landscaper, 3.3% grower/re-wholesaler, 3.3% grower/retailer/landscaper, and 6.6% of others were industry professionals such as consultants and extension specialists (Figure 3). Participants were allowed to select all applicable categories to describe their business. Gross sales of industry professionals’ business averaged over $500,000 with 20% earning less than $200k, 20% between $200-$500k, 18.3% between $500k-$1million, and 41.7% earning over 1 million dollars (Figure 4). Participants described their plant selection produced/offered as consisting largely of landscape staples (i.e. common shrubs, trees, etc.) (43.9%), 28.1% specialty crops (i.e. only trees, azaleas, etc.), 15.8% niche market crops (herbs, natives, etc.), and 12.2% indoor/foliage plants (Figure 5). Of the respondents, 91.4% attributed 0 to 25% of their total sales to crapemyrtle, 6.9%: 26-50%, 0.0%: 51-75%, and 1.7%: 76-100% (Figure 6).

Of those who responded, 31.0, 67.2 and 1.7% of those surveyed had, had not, or possibly had heard the term “rabbit tracks”, referring to crapemyrtle foliar disorder (Figure 7). However, 41.4, 48.3, and 10.3% reported had, had not, or possibly had seen the “rabbit tracks” foliar disorder in crapemyrtle when presented with a photograph (Appendix B) of the disorder (Figure 8). Participants acknowledging yes to noticing the disorder, revealed location of experience with “rabbit tracks” disorder. Twenty-three percent saw the disorder in container production, 4.9% in the landscape, 1.6% in field production, and 8.2% in all three locations (Figure 9). Along with location of disorder occurrence, respondents were asked to name the cultivars of crapemyrtle on which they observed “rabbit tracks”. ‘Natchez’ was observed to have the highest occurrence (38.9%), followed by ‘Muskogee’ (25.9%), ‘Tuscarora’ (11.1%), ‘Tuskegee’ (3.7%), and ‘Firebird’ (3.7%) (Figure 10). With ‘Natchez’ as the most commonly commercially grown cultivar, it is possible that cultivars with greater frequency of “rabbit tracks” occurrence were not grown by all growers surveyed. Thus, other cultivars may prove to show greater propensity to exhibit the disorder than ‘Natchez’. Respondents with disorder experience reported 83.3% had not tried to remedy the disorder, while 16.7% had tried to remedy the disorder (Figure 11). Participants reported attempting nothing

(33.3%), fungicide (22.2%), micronutrient application (22.2%), or others had discontinued crapemyrtle production as a means to cure the disorder and believed the solutions to be beneficial in alleviating or remedying the disorder (Figure 12).

To determine the significance to the industry of the “rabbit tracks” disorder, sales estimates and ratings were collected. It was found that 77.4% of industry members never had customers comment on the presence of “rabbit tracks” on affected products, while 22.6% experienced customer comments (Figure 13). In addition, only 6.7% of participants ever had buyers/customers refuse to purchase disorder-affected plants (Figure 14). When asked to rate the likelihood (1=lowest, 4=highest) that a grower, retailer, or customer would refuse to purchase affected plant material, respondents estimated that retailers are the most likely to reject plants affected by the disorder (2.6) followed by growers (2.5 and customers (2.2) (Figures 15 and 16). When asked to rate the significance of “rabbit tracks” foliar disorder to the ornamental horticulture industry, industry members reported medium (38.1%) to low (36.4%) significance ratings compared to high (18.2%) and no significance (7.2) (Figure 17).

Conclusions from the “rabbit tracks” survey reveal that industry members do not view the “rabbit tracks” foliar disorder of crapemyrtle as a significant detriment to the ornamental horticulture industry. Thirty percent of industry members surveyed reported never hearing the term “rabbit tracks” and 40% reported experience with the disorder. Data collected from the survey served to estimate the need for measures to diagnose and treat the disorder. Measures requiring large amount of resources, expense, and costly solutions are not recommended for diagnosis work or treatment, as industry members did not indicate significant losses or customer concern for “rabbit tracks” effect to plant material quality.

Literature Cited

Adrian, J.L., C.C. Montgomery, B.K. Behe, P.A. Duffy, and K.M. Tilt. 1998. Cost comparisons for infield, above ground container and pot-in-pot production systems. J. Environ. Hort. 16:65-68.

ALNLA. 2009. Economic impact of Alabama’s green industry: green industry growing. Alabama Nursery and Landscape Assn. Spec. Rpt. 7.

Cabrera, R I. and R.E. Lopez. 2004. A leaf interveinal chlorosis-necrosis disorder in crape myrtle. South. Nurs. Assoc. Res. Conf. 49:90-93.

Dillman, D.A. 2007. Mail and internet surveys: the tailed design method. 2nd ed. John Wiley and Sons, Inc. N.J.

Microsoft. 2007. Microsoft excel© 2007 for windows™. Microsoft Corp., Inc. Redmond, Wash.

SPSS. 2007. IBM SPSS statistics 15. SPSS Inc. Chicago.

SurveyMonkey. 2008. SurveyMonkey.com™. SurveyMonkey.com, LLC. Palo Alto, Calif. <http://www.surveymonkey.com/>.

USBLS. 2010. Inflation calculator. U.S. Bureau of Labor Statistics, Washington, D.C. 20 Jun 2010. < http://www.bls.gov/data/inflation_calculator.htm/>.

Wilson, M.W., J.L. Sibley, D.J. Eakes, and J.M. Ruter. 2007. Investigation of rabbit tracks: a foliar disorder of crapemyrtle. South. Nurs. Assoc. Res. Conf. 52:590-593.

Figure 1. Participant response to "rabbit tracks" crapemyrtle survey question: Which state is your business located?

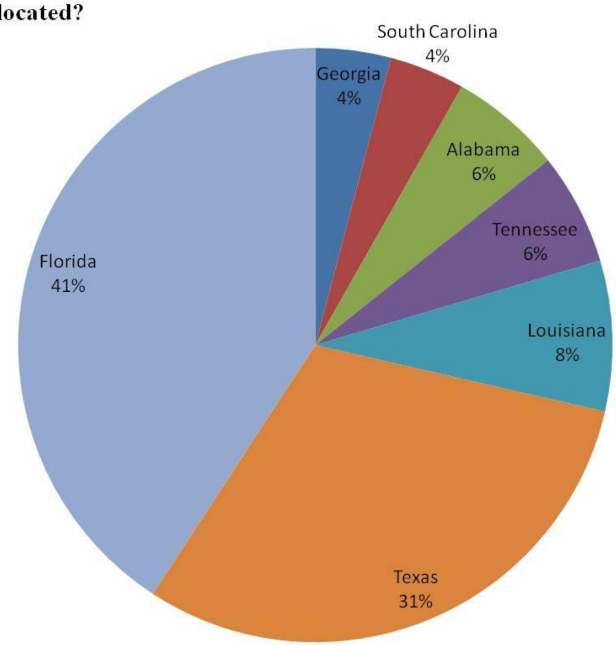


Figure 2. Participant response to "rabbit tracks" crapemyrtle survey question: What form of communication did you receive knowledge of this survey? Select all that apply.

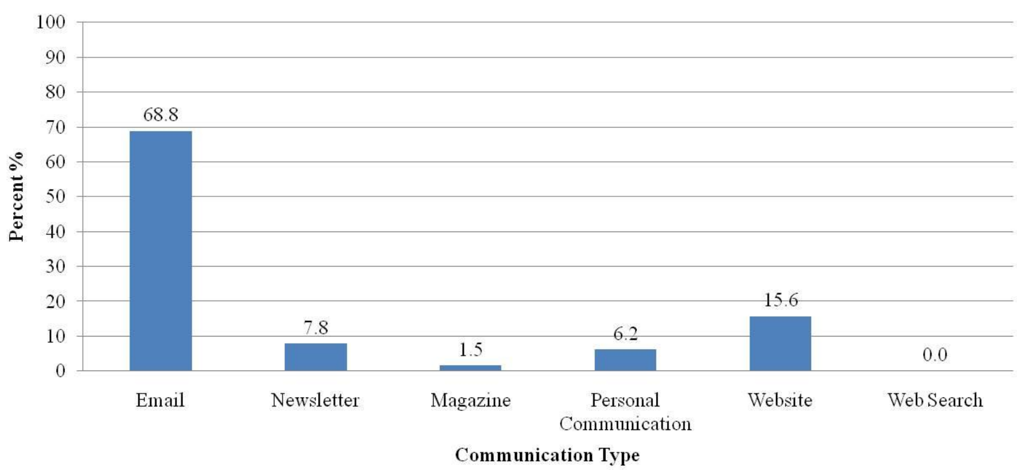


Figure 3. Participant response to "rabbit tracks" crapemyrtle survey question: What type of business do you manage/own? Select all that apply.

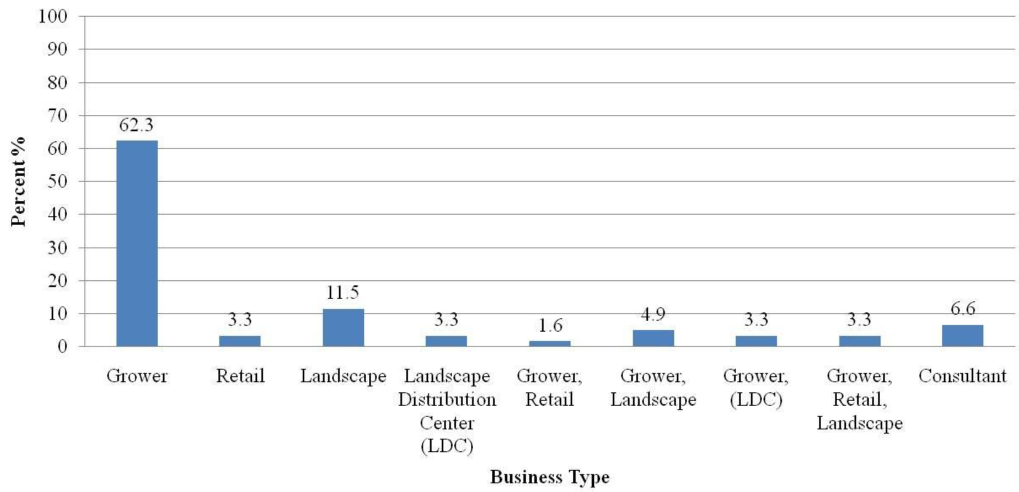


Figure 4. Participant response to "rabbit tracks" crapemyrtle survey question: What bracket best describes your gross sales?

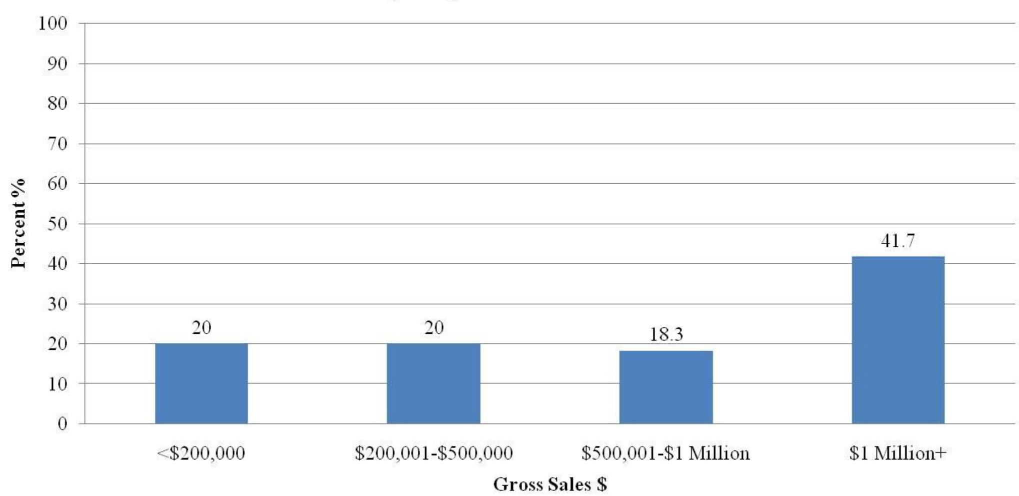


Figure 5. Participant response to "rabbit tracks" crapemyrtle survey question: If grower/wholesaler/retailer, what term best describes your plant selection? Select all that apply.

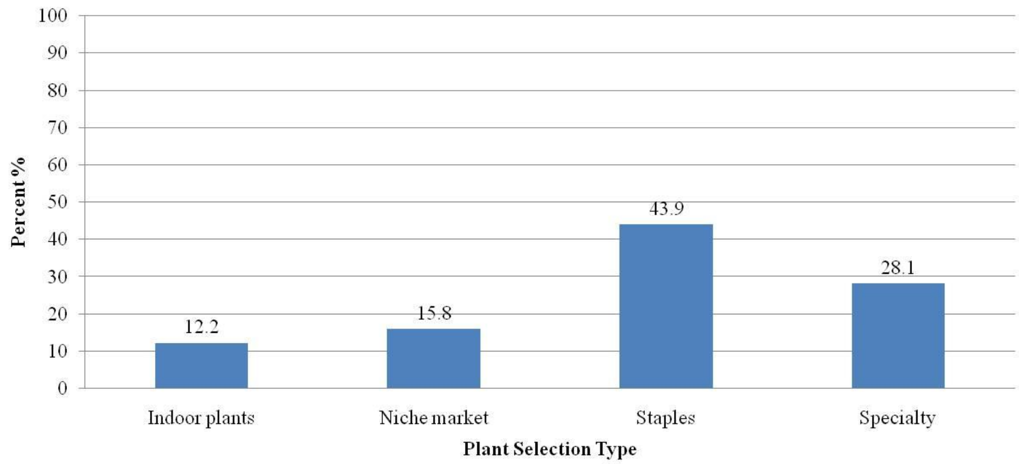


Figure 6. Participant response to "rabbit tracks" crapemyrtle survey question: What percentage of your crops/sales may be attributed to crapemyrtle?

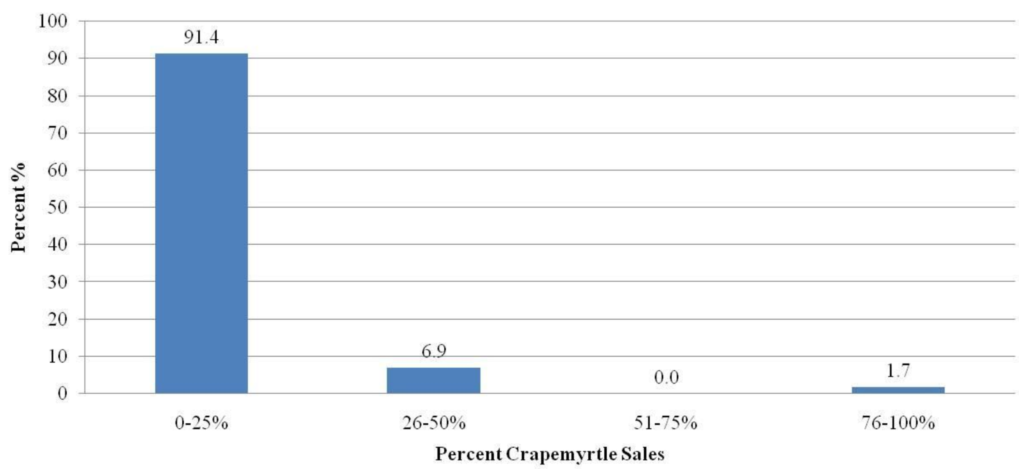


Figure 7. Participant response to "rabbit tracks" crapemyrtle survey question: Have you ever heard of a foliar disorder termed "rabbit tracking" or "rabbit tracks" believed to affect crapemyrtle (*Lagerstroemia*)?

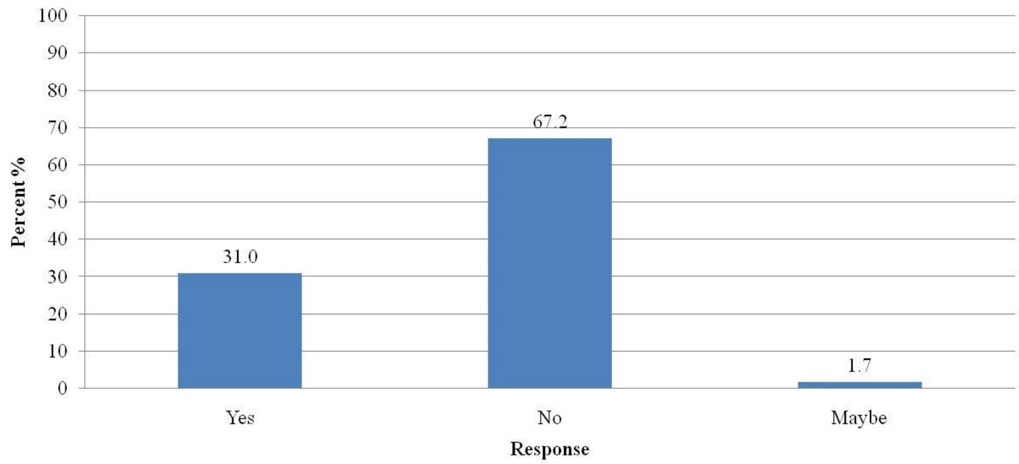


Figure 8. Participant response to "rabbit tracks" crapemyrtle survey question: Have you ever seen the symptoms as shown in the image above on crapemyrtles grown by you or another grower in late spring-early summer?

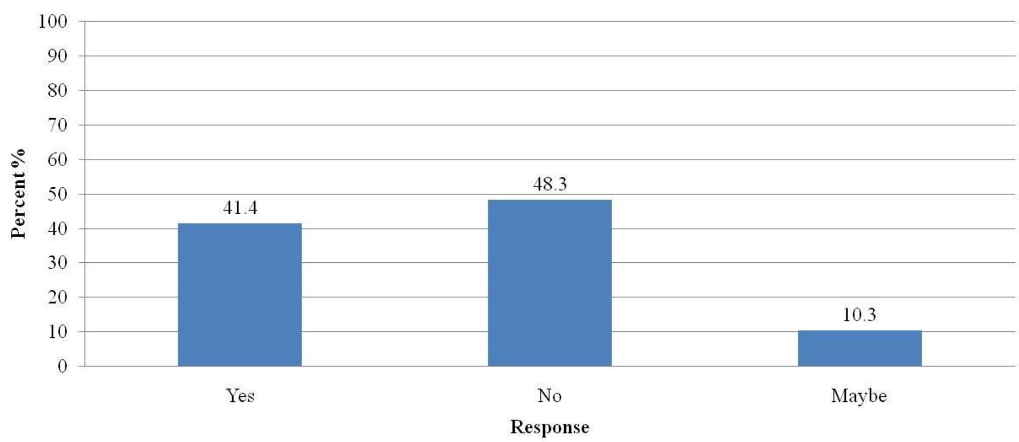


Figure 9. Participant response to "rabbit tracks" crapemyrtle survey question: Did you observe this disorder in field production, container production, or the landscape? Select all that apply.

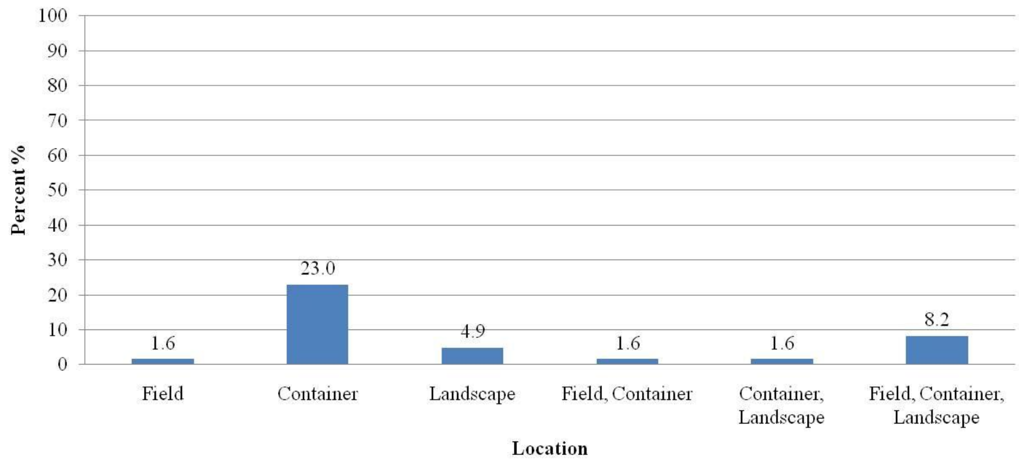


Figure 10. Participant response to "rabbit tracks" crapemyrtle survey question: What cultivars have you noticed to be affected greatest?

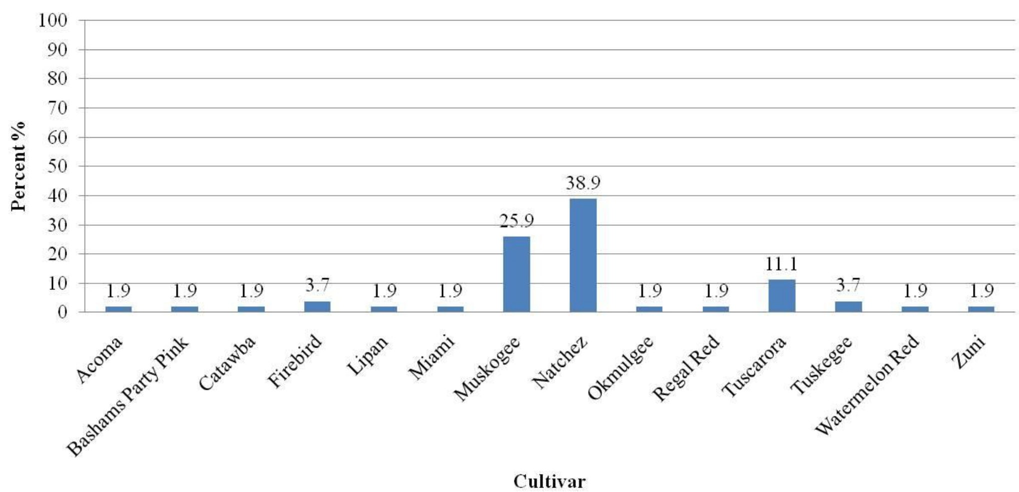


Figure 11. Participant response to "rabbit tracks" crapemyrtle survey question: Have you or another grower attempted to remedy this disorder via chemical sprays, substrate amendments, or environmental controls?

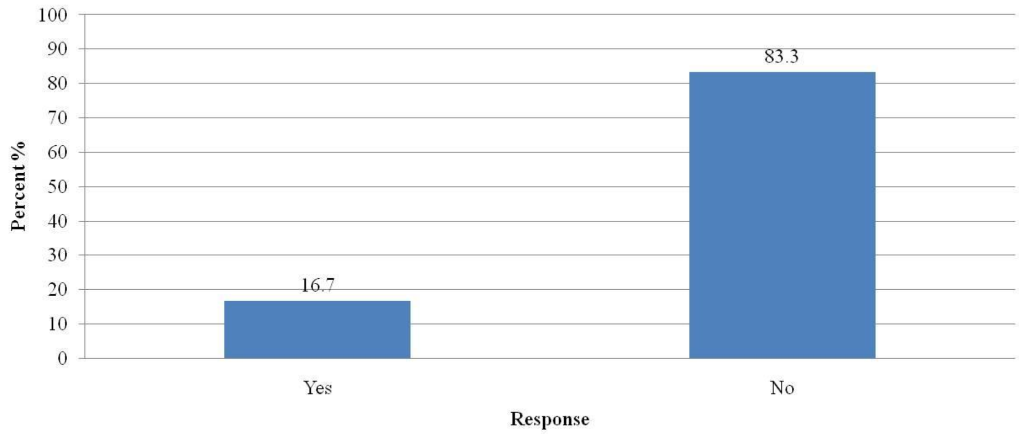


Figure 12. Participant response to "rabbit tracks" crapemyrtle survey question: If so, what remedies, if any, have you found to be beneficial in remedying "rabbit tracks"?

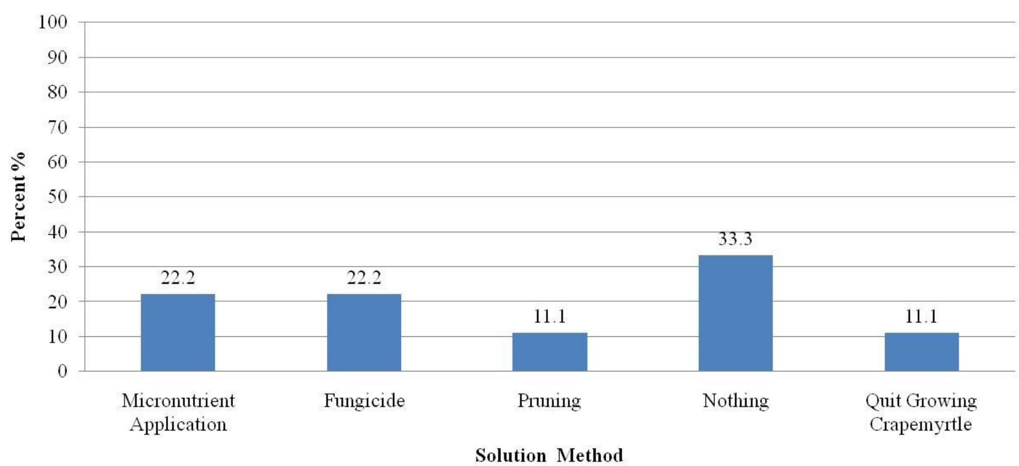


Figure 13. Participant response to "rabbit tracks" crapemyrtle survey question: Have you ever had customers/buyers comment on the "rabbit tracks" symptoms?

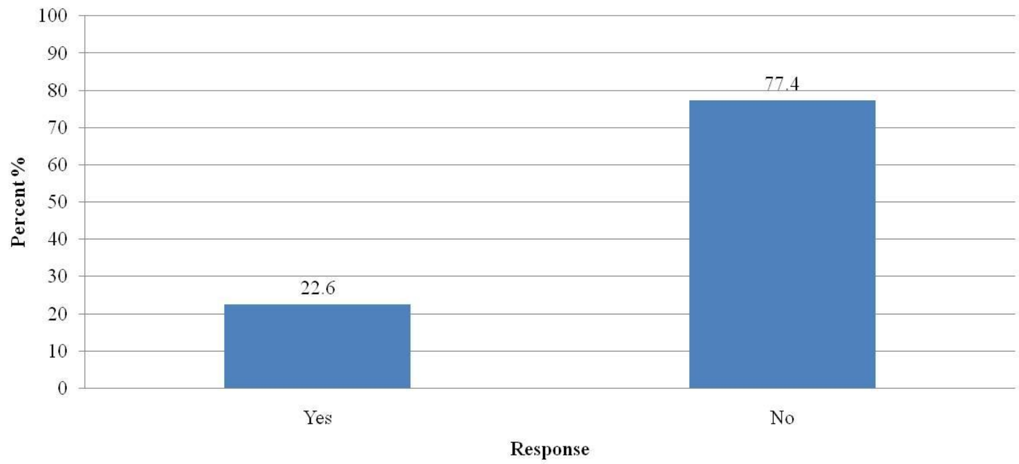


Figure 14. Participant response to "rabbit tracks" crapemyrtle survey question: Have customers/buyers refused to purchase affected plant material?

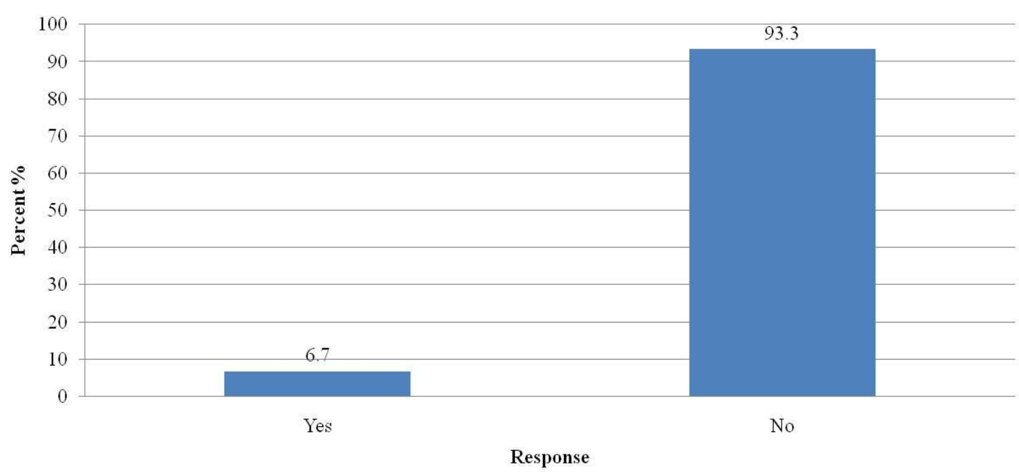


Figure 15. Participant response to "rabbit tracks" crapemyrtle survey question: Rate the likelihood you, buyers, or customers would reject purchasing affected material (0%- least likely, 100% most likely to reject).

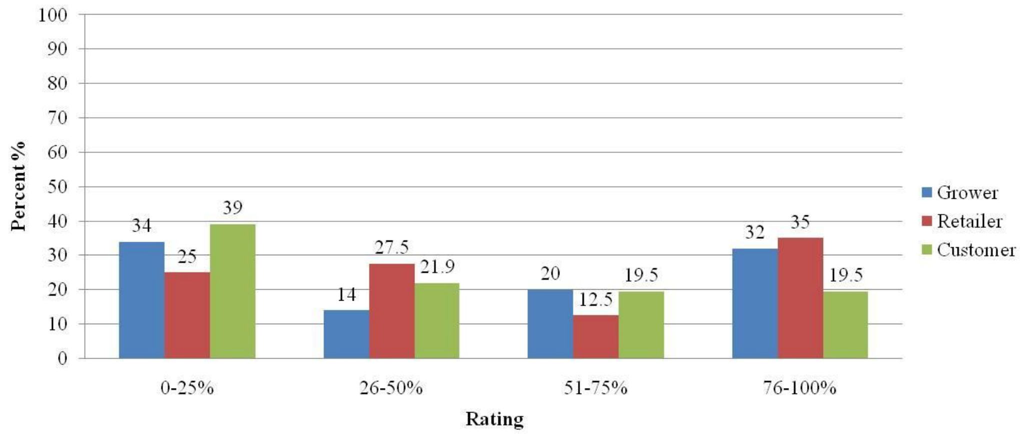


Figure 16. Average rating for rejection of "rabbit tracks"-affected plant by grower, retailer, and customer groups as rated by "rabbit tracks" crapemyrtle survey participants.

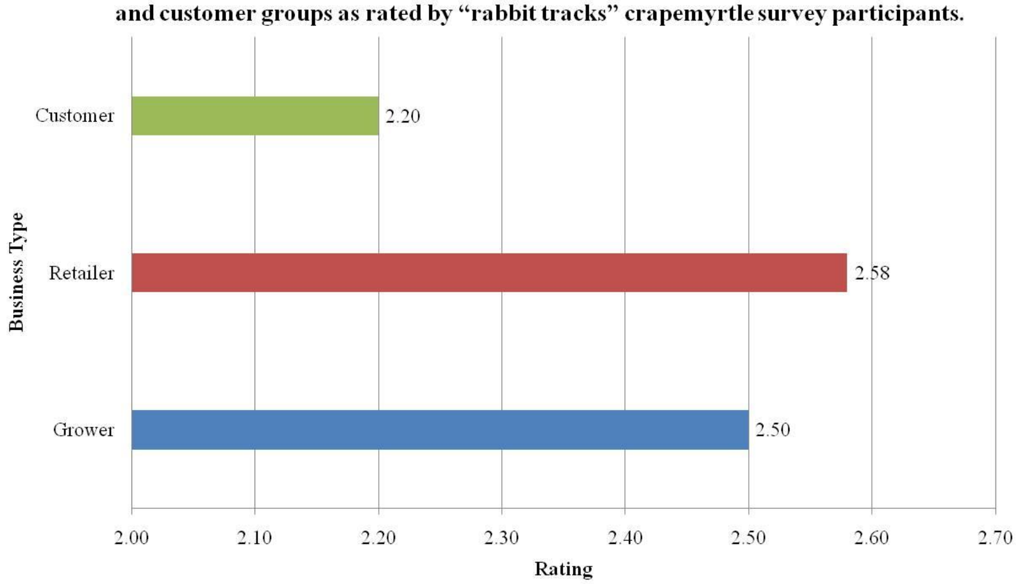
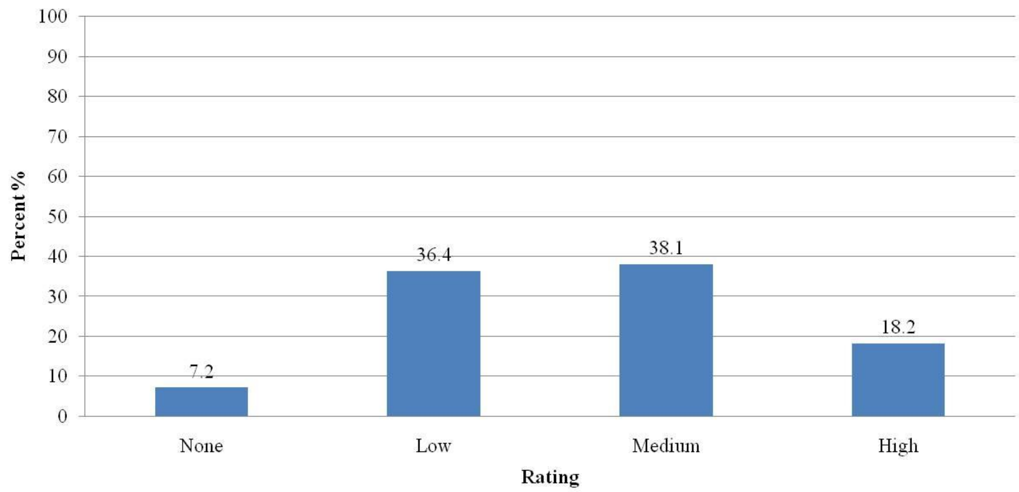


**Figure 17. Participant response to "rabbit tracks" crapemyrtle survey:
Rate your opinion of "rabbit tracks" significance to the industry.**



Chapter III

A Nutritional Approach to Diagnosing "Rabbit Tracks" Foliar Disorder in Five Cultivars of *Lagerstroemia indica ×fauriei*

Abstract

In September 2008, five *Lagerstroemia indica ×fauriei* cultivars 'Miami', 'Muskogee', 'Natchez', 'Tuscarora', and 'Yuma' were positioned with roots suspended by polystyrene disc wedges into gutters of six nutrient solutions. Treatments were used to test for "rabbit tracks" foliar crapemyrtle disorder and included one complete nutrient solution and five modified nutrient solutions to exclude sulfur (S), copper (Cu), iron (Fe), manganese (Mn), or zinc (Zn). Disorder and chlorosis occurrence, chlorosis coverage, chlorosis stage, chlorosis progression, spot occurrence, spot pattern, and leaf distortion ratings were taken 81 days later in December 2008. Data analyses revealed no evidence to suggest any of the modified nutrient solution treatments had an effect on the occurrence of "rabbit tracks" foliar disorder for the five cultivars tested. No relationship occurred between modified nutrient solutions and chlorosis coverage, chlorosis stage, chlorosis progression, spot occurrence, spot pattern, or leaf distortion ratings. However, chlorosis occurrence ratings due to treatment were found to be significant across all cultivars. Death of nearly 11% of the plants studied resulted in less ability of the generalized mixed linear models procedure (Proc Glimmix) to determine any significant relationship between disorder or symptom occurrences.

Introduction

With rapid growth and use of hybrid crapemyrtles, growers began to notice a perplexing leaf disorder occurring on cultivars of *Lagerstroemia indica ×fauriei* crosses in the 1980s. Most prevalent on cultivars of ‘Muskogee’, ‘Tuskegee’, ‘Miami’, and ‘Natchez’ hybrid crapemyrtles (Cabrera and Lopez, 2004), the foliar disorder has been routinely identified in container production and landscapes, with a few reports in field production. The disorder is casually referred to as “rabbit tracks” or “rabbit tracking” and typically occurs on the second flush of foliage in the spring. Symptoms of the disorder include bronze elliptical spots on both sides of the mid-vein leaving a series of translucent spots visible from upper and lower leaf surfaces and distortion of new growth when severe. Disorder progression suggests the effect of the disorder is aesthetic and not detrimental to affected plants’ health or survival as the disorder’s symptoms appear to minimize with continued growth of subsequent foliage.

Consistent with visual symptoms, foliar nutrient surveys conducted by Sibley (1993 and 1994), as well as Cabrera and Lopez (2003) suggest micronutrient (Fe, Mn, Cu, and Zn) deficiency or toxicity may be involved as disorder appears on subsequent growth to first foliage flush (Cabrera and Lopez, 2004; Wilson et al., 2007). Symptoms occurring on secondary growth indicate immobile nutrient deficiency/toxicity as most micronutrient elements are unable to translocate from older foliage to newer foliage (Mengel and Kirkby, 2001).

Cabrera and Lopez (2004) and Wilson et al. (2007) are the only literature describing work to diagnose “rabbit tracks” foliar disorder in *Lagerstroemia*. The purpose of the following work was to diagnose a causal agent for “rabbit tracks” foliar disorder by inducing disorder in a hydroponic nutrition study using six nutrient solutions.

Materials and Methods

In September 2008, 24, 6.4×10.2×154.9 cm (2.5×4×61 in) polyvinyl chloride (PVC) residential drainage gutters with PVC end-caps [10.0 L (2.6 gal)] spaced 10.2 cm (4 in) apart were constructed to contain six hydroponic nutrient solutions. Polystyrene foam sheets were cut to fit on top of the gutters and were supported with PVC gutter guards used to keep residential gutters clear of debris (Figure 1). Holes were cut 10.2 cm (4 in) apart in the polystyrene sheets and gutter guards for plant spacing and root clearance. Polystyrene sheets were labeled according to nutrient solution for gutter and labeled for cultivar of *Lagerstroemia indica ×fauriei* used in the study (Figure 2). Main effects nutrient solution and cultivar subplot designs were randomly assigned in SAS 9.1 (SAS, 2008). Gutters were aerated with two 80 watt air pumps (EcoPlus® Commercial Air 5) using 0.64 cm (0.25 in) Raindrip® black, polyethylene tubing (NDS, Inc., Woodland Hills, Calif.) (Figure 3). Air pumps provided approximately $1.2 \cdot 10^{-5} m^3 \cdot s^{-1}$ ($0.03\ gal \cdot s^{-1}$) to obtain a 70% air to solution exchange rate for each gutter. Air tubing was submersed within each gutter using a poly-coated shelving bracket to maintain complete aeration of solution (Figure 4). A bench top warming coil was placed under gutters to maintain water temperature of 23.9°C (75°F). A datalogger (Spectrum Technologies, Inc.) with ambient and water temperature sensors was placed on the bench with gutters readings were recorded every 30 minutes. Supplemental lighting initiated on October 15 to maintain and extend day length to represent photoperiod from May 18 to July 16, 2008 (NOAA, 2008) was provided using high-pressure sodium lamps at approximately 96.2

μmols·m^{-2}. Lamps were set to turn on prior to sunrise and extend after sunset for corresponding sunrise and sunset times (i.e. Sunrise May 18, 2008: 4:44; Sunset: 18:43 CST) (NOAA, 2008).

On September 18, 2008, five *Lagerstroemia indica ×fauriei* cultivars 'Miami', 'Muskogee', 'Natchez', 'Tuscarora', and 'Yuma' were positioned with roots suspended by polystyrene disc wedges into gutters of six nutrient solution treatments (Figure 5). Three 15.2 cm (6 in) tall bare-root liners for each cultivar were planted in each gutter at 10.2 cm (4 in) spacing (N= 15 plants/gutter, N=360). Plants were rooted in fall of previous year and had one season of growth. Liners were trimmed to 15.2 cm (6 in) in height and all foliage removed to stimulate new growth. Plants were arranged using a completely randomized split-plot design with four treatment replications containing three subsamples for each cultivar. Gutters were filled with 10 L of one of six #1 modified Hoagland nutrient solutions (Table 1), one containing a complete formulation of all prescribed elements and five modified to exclude one macronutrient sulfur (S) or four micronutrients; copper (Cu), iron (Fe), manganese (Mn), or zinc (Zn) (Whipker, 2008). All nutrient solutions were mixed with municipal water inside the greenhouses. Nutrient content of irrigation water was analyzed at the conclusion of the study (Table 3). Nutrient solutions were completely exchanged on a weekly basis with additional nutrient solutions added as necessary to maintain volume.

The experiment was terminated on December 8, 2008 (81 days after planting), as solution temperatures began to drop to 16°C (60.8°F) and cause dormancy of plants to initiate. Plants did not exhibit the amount of growth typical for a growing season for crapemyrtle. Growth in nutrient solutions induced more root development and growth than foliage. Plant foliage increased approximately 20.4 cm (8 in). Foliar nutrient analyses of two representative plants from each treatment were conducted using *L. indica* ×*fauriei* 'Yuma'. All leaves of plants analyzed were dried for 48 h at 68.3°C (155°F) and sent to the Auburn University Soil Testing Laboratory for complete nutrient analyses (Table 2). Data for "rabbit tracks" occurrence and nutrient disorder symptoms were collected using a binomial rating scale (0=no disorder, 1=disorder present) and multinomial rating scale (i.e. chlorosis coverage ratings; 0=no chlorosis, 1=entire chlorosis, 2=interveinal chlorosis, and 3=intraveinal chlorosis). Other disorder symptoms collected included chlorosis occurrence, stage, and coverage ratings, chlorosis/necrosis spot occurrence and spot pattern ratings, and leaf distortion occurrence. Occurrence and rating data were analyzed using generalized linear mixed models with Proc Glimmix procedure in SAS (Version 9.2; SAS Institute, Cary, NC). Treatment was included in the model as the fixed factor with cultivar and cultivar/treatment interactions included as random factors.

Results and Discussion

Data analyses revealed no evidence to suggest any of the modified nutrient solution treatments had an effect on the occurrence of "rabbit tracks" foliar disorder for the five cultivars tested (p=1.000). No significant relationship between modified nutrient solutions and percent chlorosis coverage, chlorosis stage, chlorosis progression, spot occurrence, spot pattern, or leaf distortion ratings was observed. However, chlorosis occurrence ratings due to treatment were found across all cultivars with iron-deficient nutrient solutions having higher incidence of chlorosis than zinc-deficient and complete nutrient solution treatments (p=0.473). Additionally, the lack of significant control or induction of the rabbit tracks disorder in all the treatments and the presence of the disorder in the study may serve to refute the hypothesis that drought could be a causal agent for the disorder as all plants were immersed in the nutrient solution without drought occurrence.

Death of nearly 11% of the plants studied, resulted in less ability of the generalized mixed linear models procedure (Proc Glimmix) to determine any significant relationship between disorder or symptom occurrences. Death in combination with the onset of dormancy and irrigation water nutrient content (Table 3), were likely contributors to analyses weaknesses. Replication of the experiment during the optimal growing season for crapemyrtle (May-July) may provide more conclusive results as to the role of nutrients in the occurrence of “rabbit tracks”. Results from such work may confirm or refute prior hypotheses proposed by investigations from Cabrera, Lopez and Sibley (Cabrera and Lopez, 2004; Wilson et al., 2007). Further studies investigating “rabbit tracks” occurrence in relation to modified nutrient solutions are needed to diagnose the causal agent for rabbit track foliar disorder in crapemyrtle.

Literature Cited

Cabrera, R I. and R.E. Lopez. 2004. A leaf interveinal chlorosis-necrosis disorder in crape myrtle. South. Nurs. Assoc. Res. Conf. 49:90-93.

Mengel, K. and E.A. Kirkby. 2001. Principles of plant nutrition. 5^{th} ed. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Mills, H.A. and J.B. Jones. 1996. Plant analysis handbook ii. MicroMacro Publishing, Inc. Athens, Ga.

NOAA, 2008. National oceanic & atmospheric administration earth system research laboratory sunrise/sunset calculator. Washington, D.C. 20 August 2008. < http://www.srrb.noaa.gov/highlights/sunrise/sunrise.html>.

SAS Institute Inc. 2009. SAS OnlineDoc® 9.2 Cary, NC: SAS Institute Inc.

Whipker, B.E. 2008. Personal communication. 15 August 2008.

Wilson, M.W., J.L. Sibley, D.J. Eakes, and J.M. Ruter. 2007. Investigation of rabbit tracks: a foliar disorder of crapemyrtle. South. Nurs. Assoc. Res. Conf. 52:590-593.

Table 1. Modified nutrient solution formulations with reagent stock solution ml·L^{-1} of treatment solution for rabbit tracks crapemyrtle nutrition study in 2008[Z].

Stock Solution	Molarity	Complete	-S	-Fe	-Mn	-Zn	-Cu
KNO_3	1M	0.005	0.005	0.005	0.005	0.005	0.005
$Ca(NO_3)_2 \cdot H_2O$	1M	0.005	0.005	0.005	0.005	0.005	0.005
KH_2PO_4	1M	0.001	0.001	0.001	0.001	0.001	0.001
$MgSO_4 \cdot 7H_2O$	1M	0.002	nil	0.002	0.002	0.002	0.002
$MgCl_2 \cdot 6H_2O$	1M	nil	0.002	nil	nil	nil	nil
FeDTPA	1M	0.00175	0.00175	nil	0.00175	0.00175	0.00175
$MnCl_2 \cdot 4H_2O$	10 mM	0.00090	0.00090	0.00090	nil	0.0009	0.00090
$ZnCl_2 \cdot 7H_2O$	10 mM	0.00015	0.00015	0.00015	0.00015	nil	0.00015
$CuCl \cdot 2H_2O$	10 mM	0.00015	0.00015	0.00015	0.00015	0.00015	nil
H_3BO_3	100 mM	0.00045	0.00045	0.00045	0.00045	0.00045	0.00045
$Na_2MoO_4 \cdot 2H_2O$	1 mM	0.00010	0.00010	0.00010	0.00010	0.00010	0.00010

[Z]Formulation amounts were prescribed using work and protocol from Whipker, B.E. 2008. Personal correspondence. 15 August 2008.

Table 2. *Lagerstroemia indica ×fauriei* 'Yuma' leaf tissue nutrient concentrations for six modified nutrient solutions.[Z]

	N	P	K	Ca	Mg	Fe	B	Mn	Zn	Cu
Solution[Y]			**% DW[X]**					**ppm**		
S	4.37	0.54	2.04	1.48	1.24	65.45	48.66	212.64	10.90	7.38
Cu	3.70	0.48	2.09	1.12	1.21	59.75	41.99	166.11	14.11	9.85
Fe	3.25	0.25	1.80	0.92	1.15	47.45	45.47	157.01	19.07	7.09
Mn	4.63	0.48	2.04	1.27	1.17	54.75	44.18	139.61	14.18	6.72
Zn	4.23	0.52	2.18	1.40	1.19	112.68	48.31	187.70	18.45	17.37
Complete	3.94	0.50	1.88	1.32	1.17	62.83	47.02	209.26	16.66	10.33
Sufficiency Range[W]	1.69-2.54	0.15-0.29	0.65-1.28	1.13-3.10	0.28-0.65	47-134	22-126	180-828	14-151	2-18

[Z]Mean nutrient concentration values are averages of 2 plants per treatment.

[Y]Represents nutrient excluded from modified nutrient solution.

[X]Percent based on leaf tissue dry weight.

[W]Sufficiency range reported for summer leaves of 9 hybrid crapemyrtles in botanical garden/arboretum.
Mills, H.A. and J.B. Jones. 1996. Plant analysis handbook ii. MicroMacro Publishing, Inc. Athens, Ga.

Table 3. Nutrient concentrations of irrigation water used in modified nutrient solutions for "rabbit tracks" crapemyrtle hydroponic study in 2008.[Z]

	NO_3-N	P	K	Ca	Mg	Fe	B	Mn	Zn	Cu
Sample			**ppm**					**ppm**		
Irrigation Water	0.28	0.34	3.07	28.81	10.54	<0.1	<0.1	<0.1	0.24	<0.1

[Z]Mean nutrient concentration values are the averages of 4 samples.

Figure 1. Top-view of hydroponic gutter system for "rabbit tracks" crapemyrtle nutrition study in 2008.

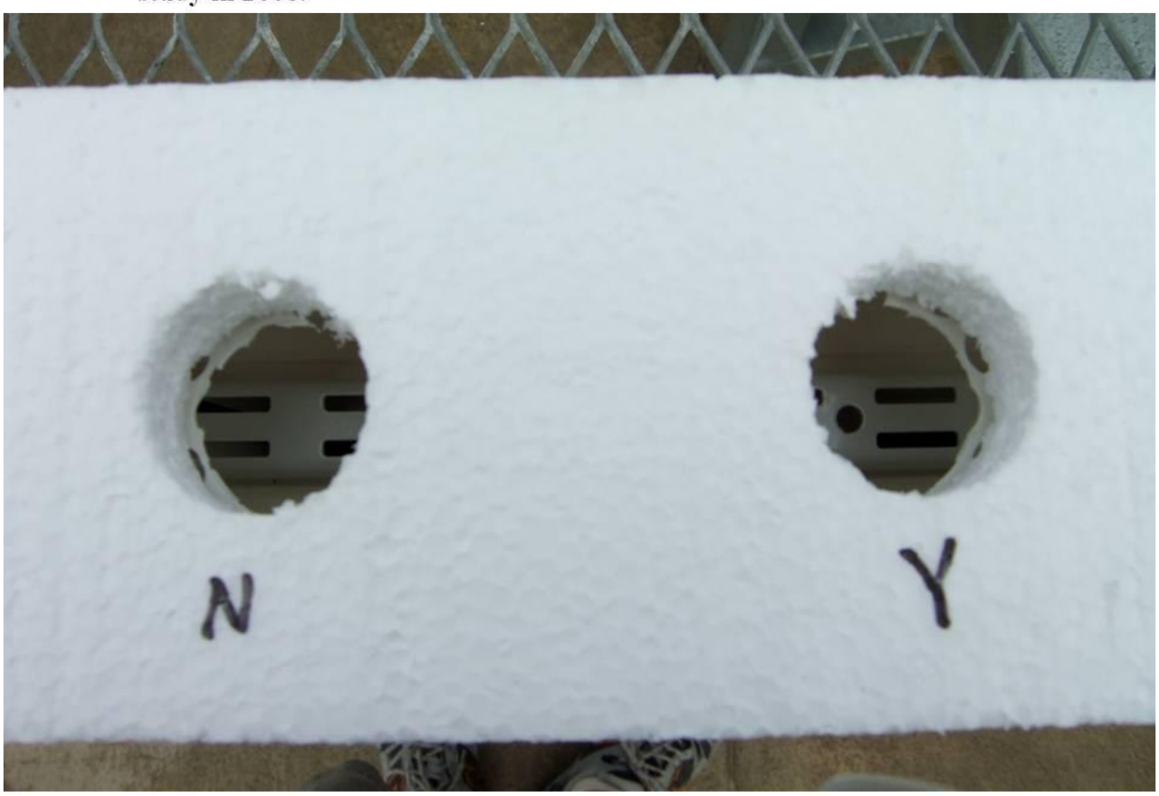


Figure 2. Top-view of polystyrene cover with gutter guard support and subsample plot spacing and labels.

Figure 3. Aeration system of hydroponic gutter system for "rabbits tracks" crapemyrtle nutrition study in 2008.

Figure 4. Air tube submersion bracket for hydroponic gutter system in "rabbit tracks" crapemyrtle nutrition study in 2008.

Figure 5. "Rabbit tracks" crapemyrtle nutrition study 81 days after planting in 2008.

Chapter IV

Final Discussion

Thermotolerance of Hemlock (Tsuga spp.)

Results from work conducted in 2003 and 2008 indicate that *T. canadensis* and *T. dumosa* had similarly, higher predicted temperature midpoints (T_m) and *k*-values than *T. caroliniana* suggesting higher resistance to direct injury when exposed to brief, supra-optimal temperatures with no differences found in T_m and *k*-values for *T. canadensis* or *T. dumosa* when each was compared to *T. chinensis*, *T. diversifolia*, or *T. heterophylla*. Similarly, no differences were found for T_m and *k*-values of *T. caroliniana* when compared to *T. chinensis*, *T. diversifolia*, or *T. heterophylla*.

Nature of work conducted and results from 2003 and 2008 indicate a need for further investigations into the thermotolerance or heat tolerance of *Tsuga* species. Though direct foliar injury may reveal a facet of hemlock tissues' ability to withstand intense temperatures for brief periods of time, direct injury lacks the ability to quantify the capacity of a species to sustain necessary growth to survive in adverse conditions. While conclusions derived from direct injury evaluations may serve as an indicator to predict the ability/inability of a species to survive these conditions, investigations into species performance, growth, and adaptation are necessary to validate claims from direct injury observations. Evaluations into response of *Tsuga* species to elevated temperatures with other diagnostic means such as net photosynthesis, night respiration rates, triphenyltetrazolium chloride (TTC) tests, C14 assimilation, toxin accumulation, chlorophyll fluorescence, and growth trials are warranted to fully understand both direct and indirect injury.

Plants with tissues observed to survive intense temperatures have also displayed poor suitability to elevated supra-optimal temperatures because of reduced photosynthetic capacity and increased respiration rates, damage to photosystem pathways, and decreased carbon assimilation, all areas accepted as necessary for plant growth and survival. More work investigating these factors is warranted in validating direct injury prediction claims for the ability of various species of hemlock to grow and survive suitably in conditions and locations predisposed to elevated temperatures. Investigations into indirect injury are better suited to predict the survival of a species in heat-disposed areas as the temperatures used in indirect injury work are more representative of the natural environmental conditions a species would be exposed to in heat-disposed climates.

Survey of "Rabbit Tracks" Foliar Disorder's Extent, Effect, and Significance to Ornamental Horticulture Industries

Resulting analysis of survey data revealed important information for approaching "rabbit tracks" foliar disorder of crapemyrtle diagnosis. While the survey was conducted after initial experiments to determine the disorder's causal agent, information gathered served to quantify the disorder's significance and effect to various ornamental horticulture industries. Less than half of the industry members surveyed had heard or seen "rabbit tracks" foliar disorder in crapemyrtle, revealing limited awareness of the disorder among industry members. There was no significant correlation between business gross income and disorder significance to industry ratings, indicating low economic significance for larger industry members. Overall significance to industry ratings ascribed low to medium industry significance ratings for the disorder.

Data collected from the survey served to estimate the need for significant measures to diagnose and treat the disorder. Measures requiring large amount of resources, expense, and costly solutions are not recommended for diagnosis work or treatment, as industry members did not indicate significant losses or customer concern for “rabbit tracks” effect to plant material quality.

A Nutritional Approach to Diagnosing “Rabbit Tracks” Foliar Disorder in Five Cultivars of *Lagerstroemia indica ×fauriei*

Data analysis revealed no evidence to suggest any of the modified nutrient solution treatments had a significant effect on the occurrence of “rabbit tracks” foliar disorder for the five *Lagerstroemia indica ×fauriei* cultivars tested ‘Miami’, ‘Muskogee’, ‘Natchez’, ‘Tuscarora’, and ‘Yuma’. No significant relationship between modified nutrient solutions and chlorosis coverage, chlorosis stage, chlorosis progression, spot occurrence, spot pattern, or leaf distortion ratings. However, chlorosis occurrence ratings due to treatment were found to be significant across all cultivars. Additionally, the lack of significant control or induction of the rabbit tracks disorder in all the treatments and the presence of the disorder in the study may serve to refute the hypothesis that drought could be a causal agent for the disorder as all plants were immersed in the nutrient solution without drought occurrence.

Death of nearly 11% of the plants studied, resulted in less ability of the generalized mixed linear models procedure to determine any significant relationship between disorder or symptom occurrences. Death in combination with the onset of dormancy and irrigation water nutrient content, were likely contributors to analyses weaknesses. Replication of the experiment during the optimal growing season for crapemyrtle (May-July) may provide more conclusive results as to the role of nutrients in the occurrence of “rabbit tracks”. Results from such work may confirm or refute prior hypotheses proposed by investigations from Cabrera, Lopez and Sibley. Further studies investigating “rabbit tracks” occurrence in relation to modified nutrient solutions are needed to diagnose the causal agent for rabbit track foliar disorder in crapemyrtle.

Appendix A

Foliar Thermotolerance of *Tsuga* spp. in 2003.

Abstract

Initial measures for foliar thermotolerance of six *Tsuga* species were evaluated using electrolyte leakage in 2003. Species evaluated included: *T. canadensis, T. caroliniana*, *T. diversifolia*, *T. heterophylla*, *T. mertensiana*, and *T.sieboldii*. Liners of the six *Tsuga* species were potted into containers and grown for twelve months prior to evaluation. Species were exposed to 25, 30, 35, 37.5, 40, 42.5, 45, 47.5, 50, 52.5, 55, and 60°C (77, 86, 95, 99.5, 104, 108.5, 113, 117.5, 122, 126.5, 131, and 140°F) for 30 minutes in thermostatically controlled water baths, and electrical conductivity determined subsequent each 24 hour incubation time following treatment and autoclaving. Electrolyte leakage, expressed as a ratio of the electrical conductivity taken 24 hours following treatment to the conductivity taken 24 hours following autoclaving, was determined. Electrolyte leakage response to temperature was sigmoidal. Predicted temperature midpoints (T_m) of *T. canadensis*, *T. caroliniana*, *T. heterophylla*, *T. mertensiana*, and *T.sieboldii* were ≈ 1.6°C (2.9°F) higher than that of *T. diversifolia.* Highly variable T_m and *k*-values void significant extrapolations and conclusions concerning heat tolerance.

Materials and Methods

In September 2002, liners of six *Tsuga* species (*T. canadensis* (L.) Carr., *T. caroliniana* Engelm., *T. diversifolia* (Maxim) Mast., *T. heterophylla* (Raf.) Sarg, *T. mertensiana* (Bong.) Carr., and *T. sieboldii* Carr.) were potted into 6.0 L (#2 trade gal.) containers in a substrate of 6 pine bark:1 builders' sand (by volume) and amended with 6.6, 3.0, and 0.9 $kg \cdot m^{-3}$ (11.1, 5, and 1.5 $lbs \cdot yd^{-3}$) of 18N-2.6P-9.9K (18-6-12) 8-9 month Osmocote® (Scotts Co., Marysville, OH), dolomitic limestone, and Micromax® (Scotts Co.), respectively. Plants were grown on a container pad in Auburn, AL (32° 36'N×85° 29'W, USDA cold hardiness zone 8a) and irrigated with approximately 1.3 cm (0.5 in) water daily for twelve months prior to foliar membrane thermostability determination in September 2003.

Recently matured needles from current-season growth were excised from petioles with a scalpel, weighed to 0.5 g (0.02 oz) samples, placed in test tubes containing 1 ml (0.03 fl oz) of de-ionized water and treated within a thermostatically controlled water bath at 12 temperatures [25, 30, 35, 37.5, 40, 42.5, 45, 47.5, 50, 52.5, 55, and 60 °C (77, 86, 95, 99.5, 104, 108.5, 113, 117.5, 122, 126.5, 131, and 140 °F)] for 30 minutes. Each temperature treatment contained replications of 6 test tubes for each species (temp N=36, total N=432). Upon completing a prescribed temperature treatment, test tubes were filled with 20 ml of de-ionized water, placed on ice, and incubated in a cooler maintained at 4.4 °C (40°F) for 24 hours. Following incubation, samples were brought to room temperature and electrical conductivity measured (Accumet Excel XL50, Fisher Scientific, Pittsburgh, PA). Samples were autoclaved at 121°C and 1.4 $kg \cdot cm^{-2}$ (249.8°F and 20 $lbs \cdot in^{-2}$) for 20 minutes and incubated for 24 hours at 4.4 °C (40°F), after which electrical conductivities were again determined. Electrolyte leakage was expressed as a ratio of the initial electrical conductivity post-treatment to the conductivity post-autoclave.

Electrolyte leakage response to temperature was sigmoidal as reported with other species. Therefore, a sigmoidal equation was used to understand the data:

$$L_e = [x - z) / (1 + \exp [- k (T - T_m])] + z$$

where L_e was the percent leakage, z = baseline level of electrolyte leakage, x = percent of electrolyte leakage observed across all temperatures, T_m = predicted temperature midpoint, also referred to as the “inflection point” for the response curve, k = the slope of the predicted response at T_m, and T = water bath temperature used to determine critical midpoint temperatures (T_m) by fitting electrolyte leakage data across temperature treatments. Gauss-Newton method of non-linear regression was used to analyze electrolyte leakage data with correlations performed by PROC CORR procedure using SAS Version 9.1.3. Critical midpoint temperatures and *k*-values of fitted response curves were determined for each species (Table A1). Differences were determined by failures of species’ T_m values, plus and minus T_m variance, to overlap. *K*-values were used to distinguish slope differences between T_m values and describe the range of temperatures around the T_m for which the species is predicted to withstand before experiencing greater than 50% damage.

Results and Discussion

Gauss-Newton method of non-linear regression met convergence criteria for all species and temperatures. Electrolyte leakage and temperature were correlated across all species (r= 0.74, p= <0.0001, N=432). Predicted temperature midpoints (T_m) revealed species *T. canadensis* 53.7 ± 0.4°C (128.7 ± 0.7°F), *T. caroliniana* 54.2 ± 0.6°C (129.6 ±

1.1°F), *T. heterophylla* 53.7 ± 0.4°C (128.7 ± 0.6°F), *T. mertensiana* 55.6 ± 0.6°C (132.2 ± 1.1°F), and *T.sieboldii* 56.3 ± 2.1°C (133.3 ± 3.8°F) had higher T_m values ≥ 1.6°C (2.9°F) than *T. diversifolia* 52.1 ± 0.5°C (125.8 ± 0.8°F) (Table A1) (Figures A1-A6). Highly variable T_m and *k*-values void significant extrapolations and conclusions concerning heat tolerance.

T. canadensis and *T. heterophylla* had similar T_m values to *T. caroliniana* and *T.sieboldii* (≈ 1.6°C / 2.8°F) and narrower *k*-values (0.075 ± 0.05) than *T. caroliniana* and *T.sieboldii*, indicating higher temperatures would be necessary to cause direct heat injury to foliage of *T. canadensis* and *T. heterophylla*. Narrow *k*-values and low variability also indicated a narrower tolerance range for supra-optimal canopy temperatures around the critical temperature midpoint, suggesting the T_m value predicted is an accurate estimate of the beyond recovery temperature. High variability for T_m and *k*-values for *T. caroliniana, T. mertensiana,* and *T. sieboldii,* warrants further replications in determining their foliar thermotolerance with other *Tsuga* species.

Following the work in 2003, it was determined that further investigations of electrolyte leakage from hemlock foliage were necessary to verify aforementioned results as tissue age and stage of acclimation may contribute to varied responses for thermotolerance. Replications of the aforementioned experiment and procedures along with experiments examining different tissue age and acclimation may better predict the heat tolerance of hemlock species populations. With further investigations into direct heat injury, certain species of hemlock may prove more suitable for production and use in heat-disposed areas than others. These investigations began in 2008.

Table A1. Response of *Tsuga* (hemlock) species to elevated foliar temperature.[z]

Species	T_m[y]	T_m[x]	*k* -values[w]
T. canadensis	53.7 ± 0.4	128.7 ± 0.7	0.50 ± 0.09
T. caroliniana	54.2 ± 0.6	129.6 ± 1.1	0.58 ± 0.18
T. diversifolia	52.1 ± 0.5	125.8 ± 0.8	0.50 ± 0.09
T. heterophylla	53.7 ± 0.4	128.7 ± 0.6	0.54 ± 0.09
T. mertensiana	55.6 ± 0.6	132.2 ± 1.1	0.47 ± 0.09
T. sieboldii	56.3 ± 2.1	133.3 ± 3.8	0.31 ± 0.10

[z]Data presented from electrolyte leakage study in 2003.

[y]Means and standard errors for predicted critical temperature (Celsius) parameters determined by least squares approach Gauss-Newton method of non-linear regression.

[x]Means and standard errors for predicted critical temperature (Fahrenheit) parameters determined by least squares approach Gauss-Newton method of non-linear regression.

[w]K-values for Tm[x] (Celsius).

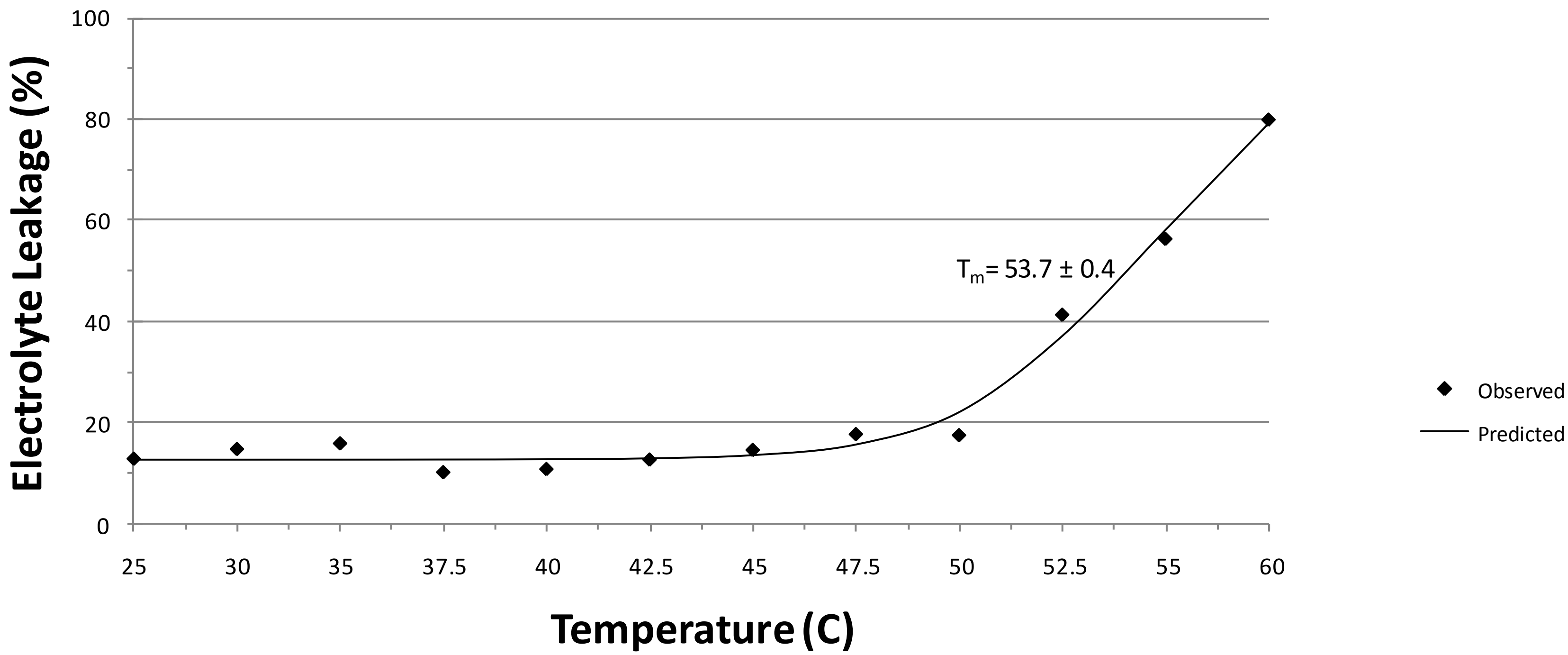


Figure A1. Foliar electrolyte leakage of *Tsuga canadensis* exposed to 25 to 60° C for 30 min. in 2003.

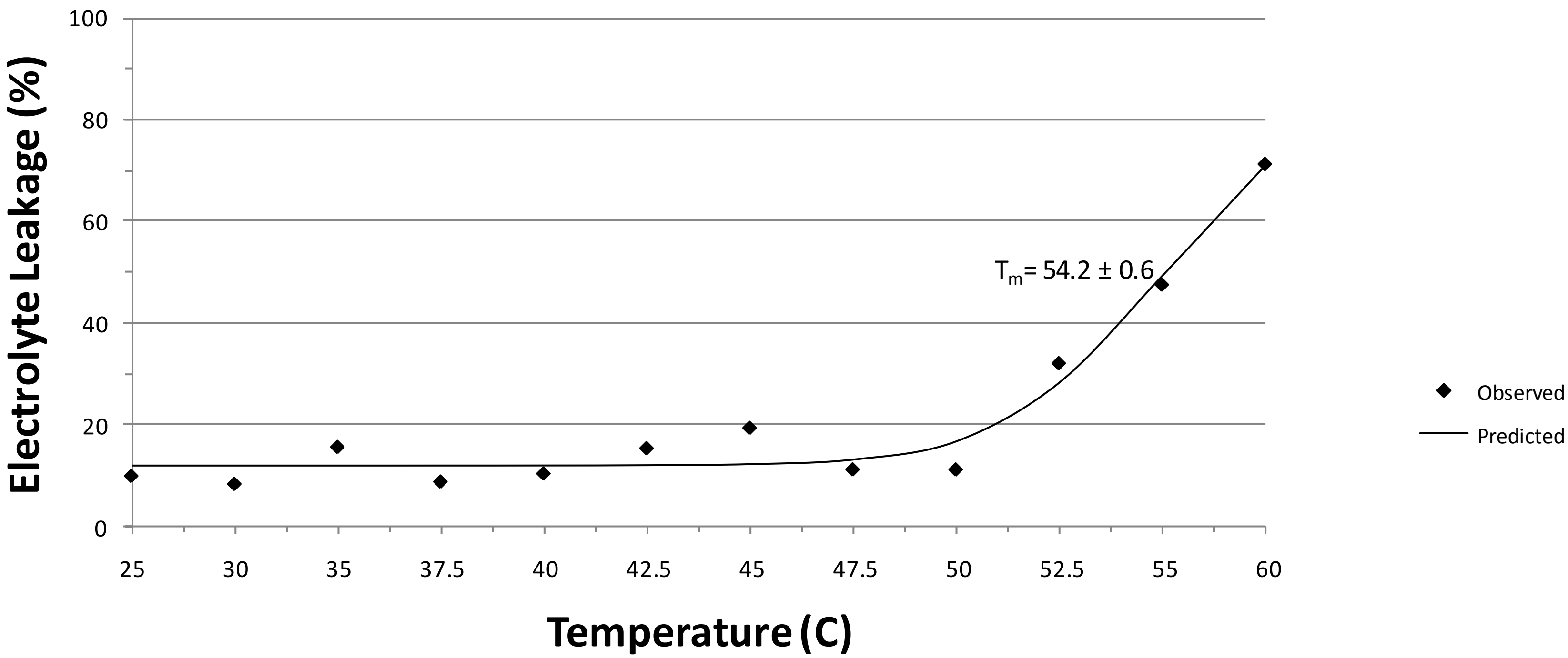


Figure A2. Foliar electrolyte leakage of *Tsuga caroliniana* exposed to 25 to 60° C for 30 min. in 2003.

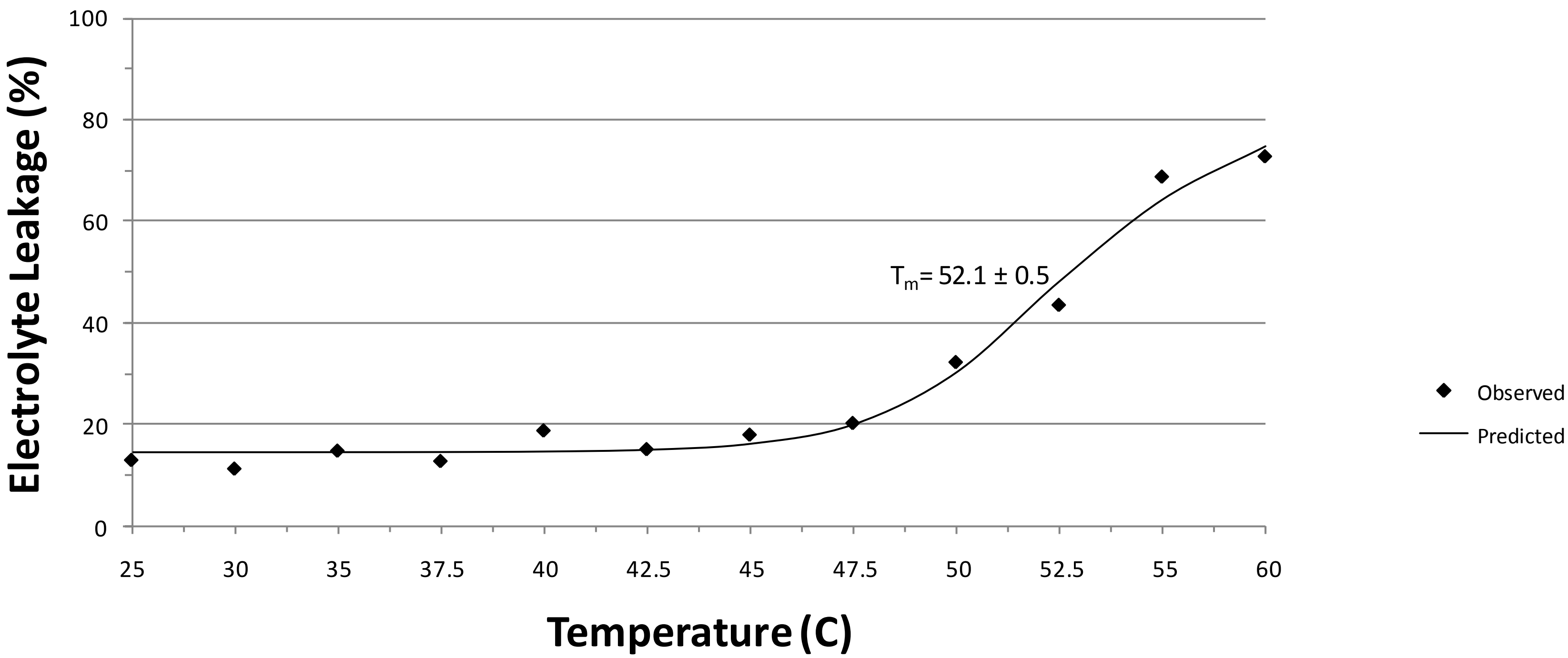


Figure A3. Foliar electrolyte leakage of *Tsuga diversifolia* exposed to 25 to 60° C for 30 min. in 2003.

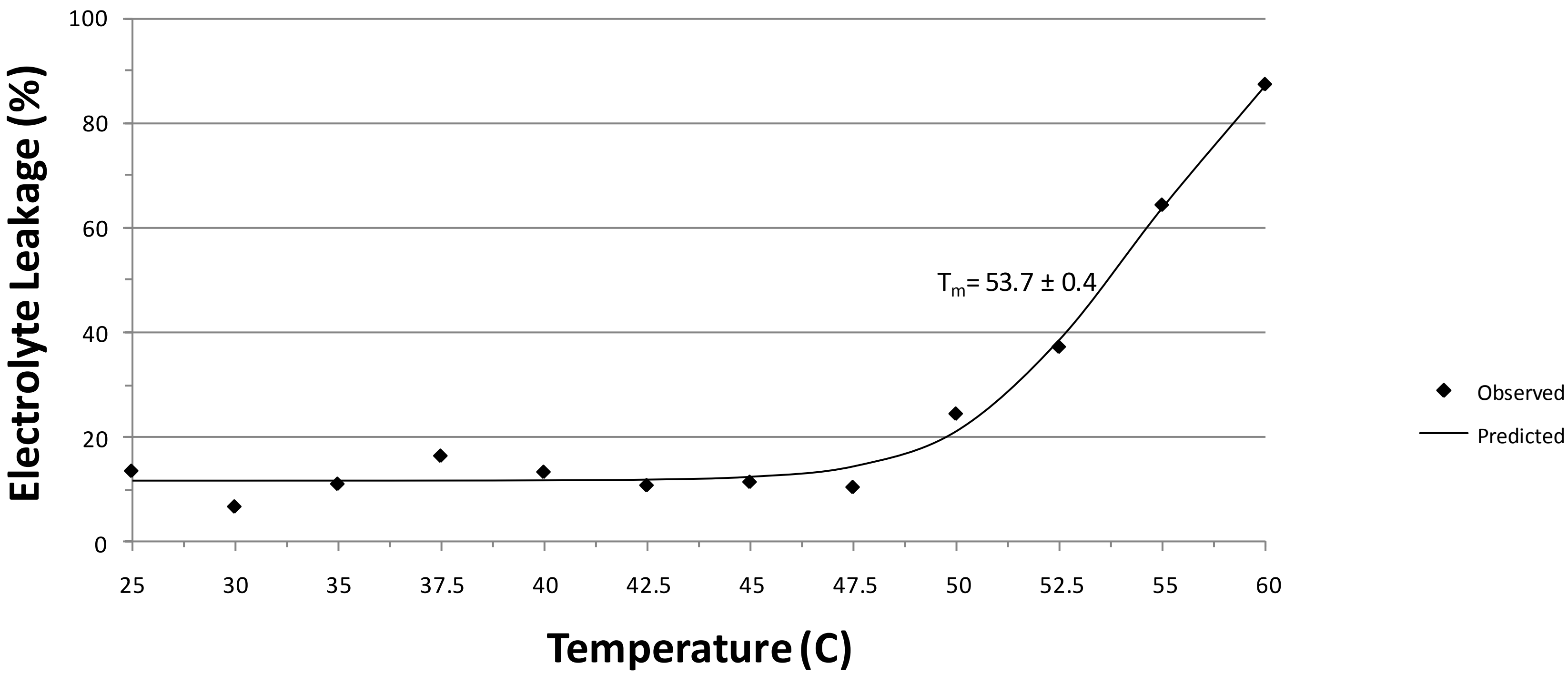


Figure A4. Foliar electrolyte leakage of *Tsuga heterophylla* exposed to 25 to 60° C for 30 min. in 2003.

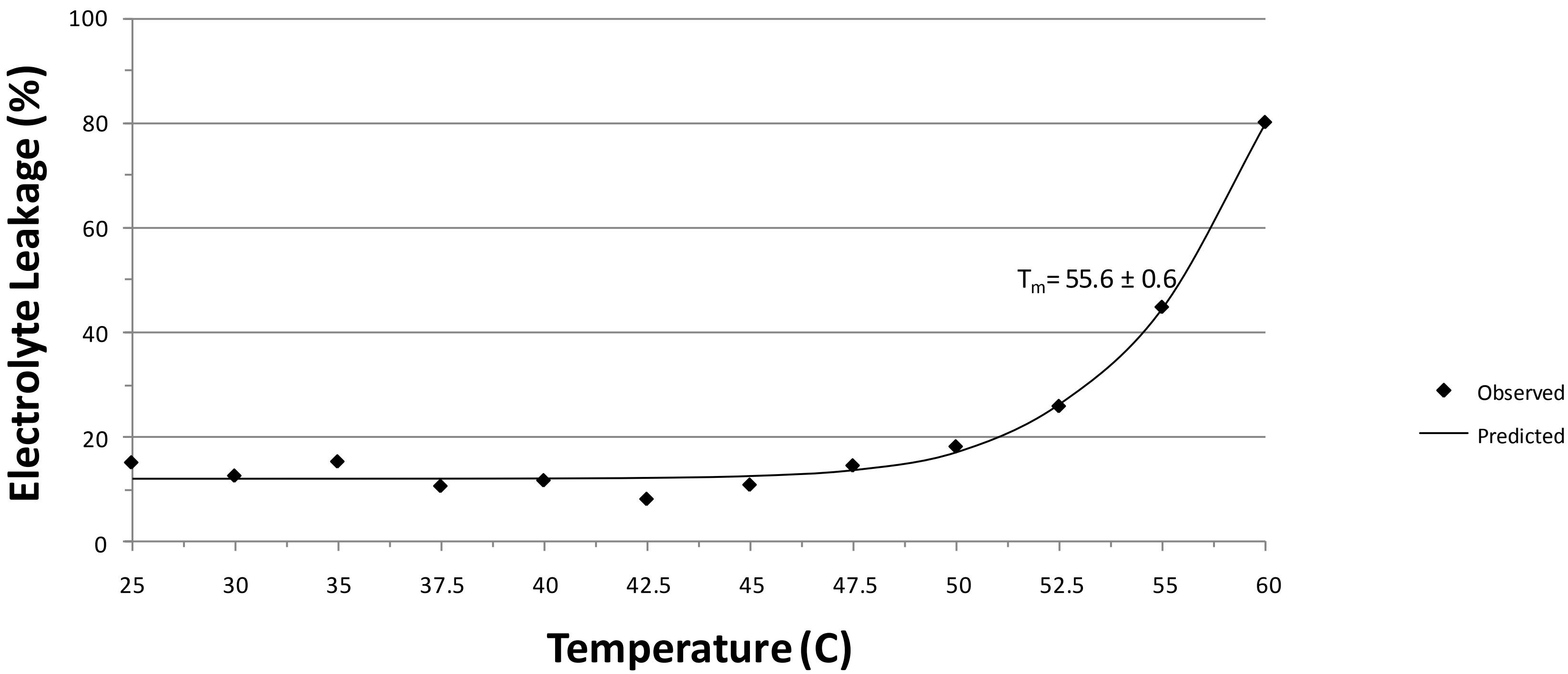


Figure A5. Foliar electrolyte leakage of *Tsuga mertensiana* exposed to 25 to 60° C for 30 min. in 2003.

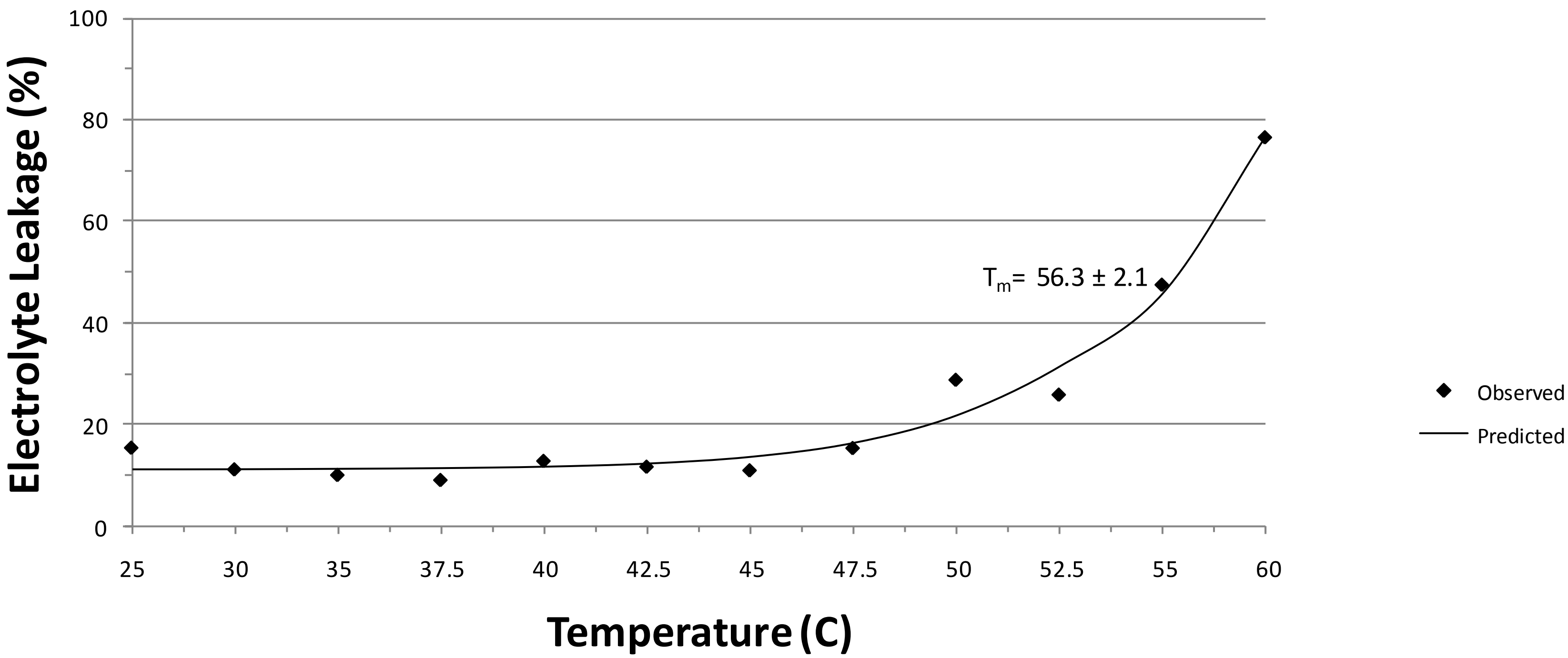


Figure A6. Foliar electrolyte leakage of *Tsuga sieboldii* exposed to 25 to 60° C for 30 min. in 2003.

Appendix B

Survey of "Rabbit Tracks" Foliar Disorder's Extent, Effect, and Significance to Ornamental Horticulture Industries

Figure B1. Institutional review board letter of approval for survey.

1856 2006
AUBURN
UNIVERSITY
Sesquicentennial

Office of Human Subject Research
307 Samford Hall
Auburn University, AL 36849

Telephone: 334-844-5966
Fax: 334-844-4391
hsubjec@auburn.edu

July 25, 2008

MEMORANDUM TO: Matthew Wilson
Horticulture

PROTOCOL TITLE: "Survey of 'Rabbit Tracks' Foliar Disorder's Extent, Effect, and Significance to Ornamental Horticulture Industries"

IRB FILE NUMBER: 08-0711

Thank you for submitting your determination request to the Institutional Review Board for review. According to your description of this project and the intended use, the IRB has determined that your activities as described **do not constitute "human subjects research"** according to the existing guidelines and statutes.

> *Research* means a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge. Activities which meet this definition constitute research for purposes of this policy, whether or not they are conducted or supported under a program, which is considered research for other purposes. For example, some demonstration and service programs may include research activities.
>
> *Human subject* means a living individual about whom an investigator (whether professional or student) conducting research obtains:
>
> (1) data through intervention or interaction with the individual, or
> (2) identifiable private information.

If there are any changes made which would constitute human subjects research, or if there are any events adverse or otherwise which concern the investigator(s), we encourage you to contact this office for further consultation.

We wish you success in your endeavors and look forward to working with you in your future research activities.

Sincerely,

[redacted]

Kathy Jo Ellison, RN, DSN, CIP
Chair of the Institutional Review Board
for the Use of Human Subjects in Research

cc: Dr. Jeffrey Sibley

Figure B2. Flyer emailed to nursery and landscape association directors.

Have You Seen This?

The picture above depicts symptoms of a foliar disorder on crapemyrtle termed “Rabbit Tracks”. Since the mid-1980’s, growers have noticed this disorder on cultivars of the *L. indica* ×*L. fauriei* cross such as ‘Natchez’, ‘Miami’, ‘Muskogee’, ‘Tuskegee’, and ‘Tuscarora’. Symptoms of this disorder typically are characterized by bronze, elliptical spots surrounded by chlorosis along both sides of the mid-vein. As symptoms become more severe, distortion of the leaf margins (margin curl) may occur. This foliar disorder occurs on the second flush of growth in the spring (May-June) with subsequent growth of second flush often minimizing symptoms during growing season causing the affected plants to appear to “grow out” of the disorder.

Currently, Dr. Jeff Sibley, a horticulture professor at Auburn University, is researching potential causes of the disorder. Along with finding its cause, part of the research includes gathering information from growers, landscapers, and retailers/wholesalers about their professional experience with the disorder and their opinion of the disorder’s significance to the industry. The (insert state grower/landscape association name) would like to encourage members to participate in an online survey to aid in the research of this disorder. The survey consists of 14 simple, multiple choice and two optional, short answer questions. The survey is open to growers, landscapers, retail/wholesale businesses, and researchers, irregardless of disorder knowledge and may be accessed at the following web address:

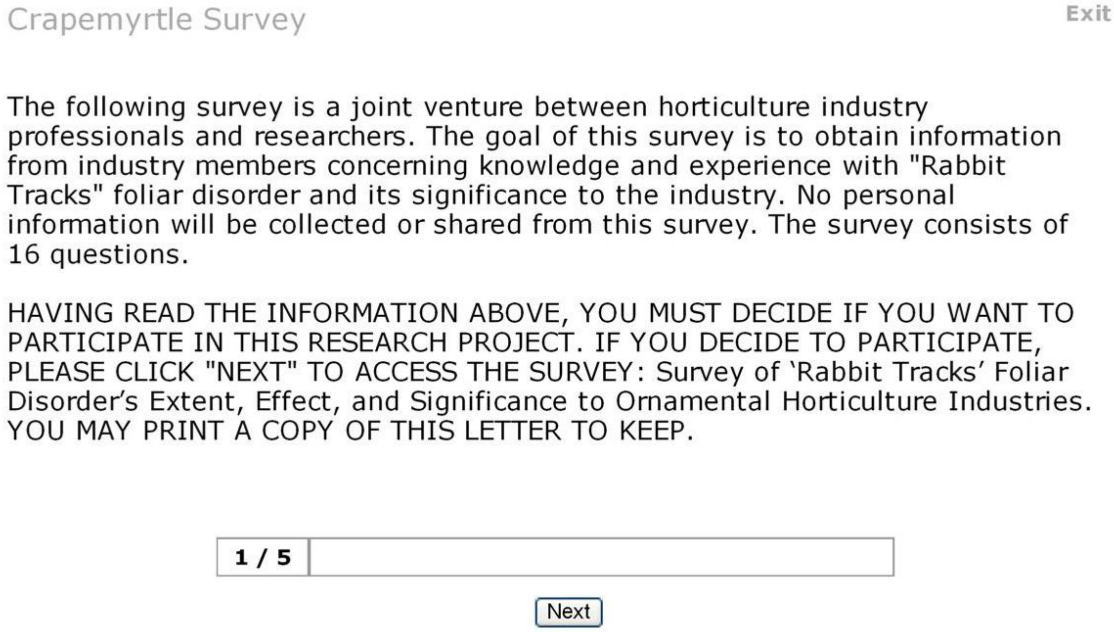
www.auburn.edu/rtsurvey

Your participation is essential and invaluable in providing researchers with information concerning this disorder and improvement of the horticulture industry.

Surveys are to be completed and submitted by XX/XX/XX. Questions or concerns may be addressed to:

Matt Wilson
Graduate Research Assistant
Department of Horticulture
101 Funchess Hall
Auburn University, AL 36849-5408
wilsoms@auburn.edu

Figure B3. Preliminary on-line survey information and participant approval page.



Crapemyrtle Survey Exit

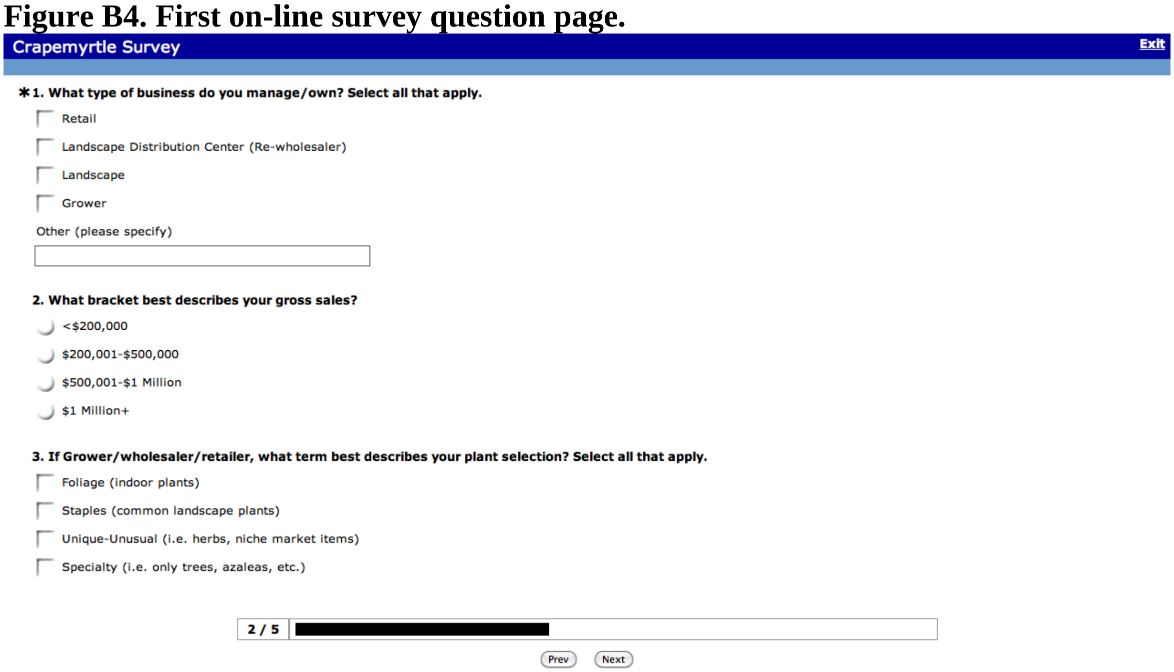
The following survey is a joint venture between horticulture industry professionals and researchers. The goal of this survey is to obtain information from industry members concerning knowledge and experience with "Rabbit Tracks" foliar disorder and its significance to the industry. No personal information will be collected or shared from this survey. The survey consists of 16 questions.

HAVING READ THE INFORMATION ABOVE, YOU MUST DECIDE IF YOU WANT TO PARTICIPATE IN THIS RESEARCH PROJECT. IF YOU DECIDE TO PARTICIPATE, PLEASE CLICK "NEXT" TO ACCESS THE SURVEY: Survey of 'Rabbit Tracks' Foliar Disorder's Extent, Effect, and Significance to Ornamental Horticulture Industries. YOU MAY PRINT A COPY OF THIS LETTER TO KEEP.

1 / 5

Next

Figure B4. First on-line survey question page.



Crapemyrtle Survey Exit

*1. What type of business do you manage/own? Select all that apply.

- Retail
- Landscape Distribution Center (Re-wholesaler)
- Landscape
- Grower

Other (please specify)

2. What bracket best describes your gross sales?

- <$200,000
- $200,001-$500,000
- $500,001-$1 Million
- $1 Million+

3. If Grower/wholesaler/retailer, what term best describes your plant selection? Select all that apply.

- Foliage (indoor plants)
- Staples (common landscape plants)
- Unique-Unusual (i.e. herbs, niche market items)
- Specialty (i.e. only trees, azaleas, etc.)

2 / 5

Prev Next

Figure B5. Survey of “rabbit tracks” foliar disorder’s extent, effect, and significance to ornamental horticulture industries.

---

1. What type of business do you manage/own? Select all that apply.

   ___Grower
   ___Landscape Distribution Center (Re-wholesaler)
   ___Retail
   ___Landscape
   ___Other (please specify)______________________________

2. What bracket best describes your gross sales?

   ___<$200,000
   ___$200,001-$500,000
   ___$500,001-$1 Million
   ___$1 Million+

3. If Grower/wholesaler/retailer, what term best describes your plant selection? Select all that apply.

   ___Foliage (indoor plants)
   ___Unique-Unusual (i.e. herbs, niche market items)
   ___Staples (common landscape plants)
   ___Specialty (i.e. only trees, azaleas, etc.)

4. What percentage of your crops/sales may be attributed to crapemyrtle?

   ___0-25%
   ___26-50%
   ___51-75%
   ___76-100%

5. Have you ever heard of a foliar disorder termed "Rabbit Tracking or Rabbit Tracks" believed to affect crapemyrtle (Lagerstroemia)?

   ___Yes
   ___No
   ___Maybe

6. Have you ever seen the symptoms as shown in the image below on crapemyrtles grown by you or another grower in late spring-early summer?

   ___Yes
   ___No
   ___Maybe

7. Answers No to Question 6, go to Question 13. Answers Yes or Maybe. Did you observe this disorder in field production, container production, or the landscape? Select all that apply.

___Field
___Container
___Landscape

8. What cultivars have you noticed to be affected greatest?

____
____
____

9. Have you or another grower attempted to remedy this disorder via chemical sprays, substrate amendments, or environmental controls?

___Yes
___No

10. If so, what remedies, if any, have you found to be beneficial?

____
____
____

11. Have you ever had customers/buyers comment on the "Rabbit Tracks" symptoms?

___Yes
___No
___Maybe

12. Have customers/buyers refused to purchase affected plant material?

___Yes
___No

13. Rate the likelihood you, buyers, or customers would reject purchasing affected material (0%- least likely, 100% most likely to reject).

Buyer	0-25%	26-50%	51-75%	76-100%
Grower	______	______	______	______
Retailer	______	______	______	______
Customer	______	______	______	______

14. Rate your opinion of "Rabbit Tracks" significance to the industry.

	None	Low	Medium	High
Significance to industry	______	______	______	______

15. What form of communication did you receive knowledge of this survey? Select all that apply.

___Email
___Newsletter
___Magazine
___Personal Communication
___Website
___Web Search
___Other (please specify)

16. Which state is your business located?

___Alabama
___Arkansas
___Florida
___Georgia
___Louisiana
___Mississippi
___North Carolina
___South Carolina
___Tennessee
___Texas

Appendix C

Preliminary Experiments Evaluating ‘Rabbit Tracks’ Occurrence to Various Rates of Substrate and Foliar-Applied Nutrients

Experiment 1 Occurrence of “Rabbit Tracks” Foliar Disorder in ‘Natchez’ Crapemyrtle using Various Substrate-Incorporated Manganese Rates

In May 2007, 2.7 L (2.9 qt) containers of *Lagerstroemia indica ×fauriei* ‘Natchez’ were potted into 10.20 L (10.78 qt) containers in a substrate of 6 pine bark:1 builders’ sand (by volume) and amended with 9.9, 3.0, and 0.9 kg·m^{-3} (16.6, 5, and 1.5 lbs·yd^{-3}) of 18N-2.6P-9.9K (18-6-12) 8-9 month Polyon® (Agrium Inc., Loveland, CO), dolomitic limestone, and Micromax® (Scotts Co., Marysville, OH), respectively. Treatments were based on the standard rate of Mn supplied by Micromax® (Scotts Co.) at 0.9 kg·m^{-3} (1.5 lbs·yd^{-3}) (0.02 kg·m^{-3}/0.04 lbs·yd^{-3} Mn) serving as the 1x rate. Additional amounts of manganese sulfate ($MnSO_4$) were withheld or incorporated in each substrate as treatments to obtain 0x, 1x, 2x, 3x, and 4x Mn rates. At planting, each plant was cut to 45 cm (18 in) in height to stimulate new growth. Plants were grown on a container pad in Auburn, AL (32° 36’N×85° 29’W, USDA cold hardiness zone 8a) with overhead irrigation at approximately 1.3 cm (0.5 in) water daily for two months. Plants were arranged using a complete randomized design with 5 treatments and 10 repetitions (N=50). In July 2007, disorder occurrence ratings were conducted from 0 to 5 (0=no disorder symptoms and 5=showing most). Analysis of the data revealed significant treatment effects from the addition of $MnSO_4$ ($p=0.0333$, $\alpha=0.05$) with a linear decrease in disorder symptoms as $MnSO_4$ was increased ($p=0.0023$, $\alpha=0.05$) (Table C1). Further investigation of substrate-applied manganese effects on “rabbit tracks” occurrence for crapemyrtle is warranted to confirm results.

Experiment 2- Occurrence of “Rabbit Tracks” Foliar Disorder in ‘Natchez’ Crapemyrtle Using Various Manganese Concentrations Applied as a Foliar Spray.

In June 2007, 2.7 L (2.9 qt) containers of *Lagerstroemia indica ×fauriei* ‘Natchez’ were potted into 10.2 L (10.8 qt) containers in a substrate of 6 pine bark:1 builders’ sand (by volume) and amended with 9.9, 3.0, and 0.9 $kg{\cdot}m^{-3}$ (16.6, 5, and 1.5 $lbs{\cdot}yd^{-3}$) of 18N-2.6P-9.9K (18-6-12) 8-9 month Polyon® (Agrium Inc., Loveland, CO), dolomitic limestone, and Micromax® (Scotts Co., Marysville, OH), respectively. At potting, each plant was cut to 63 cm (25 in) in height to stimulate new growth. Treatments consisted of seven rates of manganese (Mn) based on the standard rate supplied by Micromax® (Scotts Co.) when incorporated at 0.9 $kg{\cdot}m^{-3}$ (1.5 $lbs{\cdot}yd^{-3}$) (0.02 $kg{\cdot}m^{-3}$/0.04 $lbs{\cdot}yd^{-3}$ Mn) which served as the 1x rate. Additional amounts of manganese sulfate ($MnSO_4$) were withheld or added to 700 ml (23.7 fl oz) water and sprayed to the foliage of prescribed treatment plants to obtain 2x, 3x, 4x, 5x, and 6x Mn rates. Plants under 0x and 1x treatments contained 0.0 and 0.9 $kg{\cdot}m^{-3}$ (0.0 and 1.5 $lbs{\cdot}yd^{-3}$) Micromax® applied to substrate only (no foliar application). Plants were grown on a container pad in Auburn, AL (32° 36’N×85° 29’W, USDA cold hardiness zone 8a) with overhead irrigation at approximately 1.3 cm (0.5 in) water daily for two months. Plants were arranged using a complete randomized design with 7 treatments and 5 repetitions of each treatment (N=35). In July 2007, disorder occurrence ratings were conducted from 0 to 5 (0=no disorder symptoms and 5=showing most). Analysis of the data revealed significant treatment effects with the addition of $MnSO_4$ at 1x, 2x, 3x, 4x, and 5x rates ($p=0.0195$, $\alpha=0.05$) with a linear decrease in disorder symptoms as $MnSO_4$ was increased ($p=0.0019$, $\alpha=0.05$) (Table C2). Analysis of data also revealed a slight, but insignificant quadratic relationship between treatment and disorder ratings due the limited control of the disorder provided by the highest manganese rate (6x). This could be due to antagonistic effects that high concentrations of manganese could have on the availability of other nutrients with potential roles in the occurrence of the foliar disorder, inducing foliar disorder symptoms due to competition with manganese to be absorbed and utilized by the plants.

Experiment 3- Occurrence of "Rabbit Tracks" Foliar Disorder in 'Natchez' Crapemyrtle Using Copper Hydroxide Foliar Spray Applications at Various Stages of Development.

In August 2007, 2.7 L (2.9 qt) containers of *Lagerstroemia indica ×fauriei* 'Natchez' were potted into 10.2 L (10.8 qt) containers in a substrate of 6 pine bark:1 builders' sand (by volume) and amended with 9.9, 3.0, and 0.9 $kg \cdot m^{-3}$ (16.6, 5, and 1.5 $lbs \cdot yd^{-3}$) of 18N-2.6P-9.9K (18-6-12) 8-9 month Polyon® (Agrium Inc., Loveland, CO), dolomitic limestone, and Micromax® (Scotts Co., Marysville, OH), respectively. At potting, plants were cut to 63 cm (25 in) in height, as prescribed by treatment, to stimulate new growth. Treatments consisted of no Kocide™ or one foliar spray application of copper hydroxide (Kocide™ 2000, DuPont, Wilmington, DE) at the rate of

3.4 kg·ha^{-1} (3 lbs·a^{-1}) during three stages of growth (before pruning, first stage of growth-after pruning, and second stage of growth-after pruning). Stages of growth were determined in relation of time and pruning application. All plants were pruned on same day with Kocide™ applied immediately prior to pruning, 1 month after pruning (first growth stage), or 2 months after pruning (second growth stage). Plants were grown on a container pad in Auburn, AL (32° 36'N×85° 29'W, USDA cold hardiness zone 8a) with overhead irrigation at approximately 1.3 cm (0.5 in) water daily for two months. Plants were arranged using a complete randomized design with 4 treatments and 3 repetitions of each treatment (N=12). Visual observations conducted on September 29 and October 6 reveal that no foliar disorder appeared on any treatments. Plants were moved into the greenhouse in October and plants were grown for two additional months for observations. On December 7, no disorder symptoms were observed for any treatments. The experiment was terminated with no data collected. Further studies evaluating the impact of copper on "rabbit tracks" foliar disorder are warranted (Chapter 3).

Experiment 4- Occurrence of "Rabbit Tracks" Foliar Disorder in 'Muskogee' and 'Tuskegee' Crapemyrtles Using Various Rates of Substrate-Incorporated Sulfur and Gypsum.

In September 2007, 6.4 cm (2.5 in) rooted cuttings of *Lagerstroemia indica ×fauriei* 'Muskogee' and 'Tuskegee' were potted into 2.7 L (2.9 qt) containers in a substrate of 6 pine bark:1 builders' sand (by volume) and amended with 9.9 and 0.9 kg·m^{-3} (16.6 and 1.5 lbs·yd^{-3}) of 18N-2.6P-9.9K (18-6-12) 8-9 month Polyon® (Agrium Inc., Loveland, CO) and Micromax® (Scotts Co., Marysville, OH), respectively.

Each plant was cut to 5 cm and 10 cm (5 cm for ‘Tuskegee’ and 10 cm for ‘Muskogee’) in height 24 days after planting to stimulate new growth. Treatments consisted of various rates and combinations of elemental sulfur (Hi-Yield® Chemical Co., Bonham, TX) and pelletized gypsum ($CaSO_4$) as follows:

1. 0.0 sulfur, 0.0 gypsum, 5 dolomitic lime $kg \cdot m^{-3}$ (Control)
2. 0.0 sulfur, 0.0 gypsum, 0 dolomitic lime $kg \cdot m^{-3}$
3. 0.6 sulfur, 0.0 gypsum, 0 dolomitic lime $kg \cdot m^{-3}$
4. 1.2 sulfur, 0.0 gypsum, 0 dolomitic lime $kg \cdot m^{-3}$
5. 0.0 sulfur, 1.2 gypsum, 0 dolomitic lime $kg \cdot m^{-3}$
6. 0.0 sulfur, 2.4 gypsum, 0 dolomitic lime $kg \cdot m^{-3}$
7. 0.0 sulfur, 3.6 gypsum, 0 dolomitic lime $kg \cdot m^{-3}$
8. 0.6 sulfur, 1.2 gypsum, 0 dolomitic lime $kg \cdot m^{-3}$
9. 0.6 sulfur, 2.4 gypsum, 0 dolomitic lime $kg \cdot m^{-3}$
10. 0.6 sulfur, 3.6 gypsum, 0 dolomitic lime $kg \cdot m^{-3}$
11. 1.2 sulfur, 1.2 gypsum, 0 dolomitic lime $kg \cdot m^{-3}$
12. 1.2 sulfur, 2.4 gypsum, 0 dolomitic lime $kg \cdot m^{-3}$
13. 1.2 sulfur, 3.6 gypsum, 0 dolomitic lime $kg \cdot m^{-3}$

Plants were grown on a container pad in Auburn, AL (32° 36’N×85° 29’W, USDA cold hardiness zone 8a) with overhead irrigation at approximately 1.3 cm (0.5 in) water daily for one month before being moved to a greenhouse to prevent dormancy after which, they were hand watered daily with approximately 0.6 cm (0.3 in) of water. Plants were arranged using a complete randomized design with 13 treatments and 5 repetitions of each treatment (N=65). On December 7, no disorder symptoms were observed for any treatment, therefore the experiment was terminated with no data collected. Further studies evaluating the potential impact of sulfur and calcium on “rabbit tracks” foliar disorder are warranted (Chapter 3).

Experiment 5- Occurrence of "Rabbit Tracks" Foliar Disorder in 'Natchez' Crapemyrtle Using Various Rates of Substrate-Incorporated Copper Sulfate.

In June 2008, 2.7 L (2.9 qt) containers of *Lagerstroemia indica ×fauriei* 'Natchez' were potted up into 10.2 L (10.8 qt) containers in a substrate of 6 pine bark:1 builders' sand (by volume) and amended with 10.5, 3.0, and 0.9 $kg·m^{-3}$ (17.6, 5, and 1.5 $lbs·yd^{-3}$) of 17N-2.1P-9.1K (17-5-11) 8-9 month Polyon® (Agrium Inc., Loveland, CO), dolomitic limestone, and Micromax® (Scotts Co., Marysville, OH), respectively. Treatments consisted of various rates of substrate-incorporated copper sulfate ($CuSO_4$) [0, 3.0, 4.5, 5.9, 7.4, 8.9, 10.1, and 11.9 $kg·m^{-3}$ (0, 5, 7.5, 10, 12.5, 15, 17, and 20 $lbs·yd^{-3}$)]. At potting, each plant was cut to 25.4 cm (10 inches) in height and were grown on a container pad in Auburn, AL (32° 36'N×85° 29'W, USDA cold hardiness zone 8a) and overhead irrigated with approximately 1.3 cm (0.5 in) water daily for two months. Plants were arranged using a randomized complete block design with 8 treatments and 10 repetitions of each treatment (N=80). In July of 2008, no disorder symptoms were observed for any treatment, therefore the experiment was terminated with no data collected. Further studies evaluating the impact of copper on "rabbit tracks" foliar disorder are warranted (Chapter 3).

Table C1. Effects of substrate-incorporated manganese rates on occurrence of rabbit tracks foliar disorder in *Lagerstroemia indica ×fauriei* 'Natchez'.

	Treatment		Median Rating[z]
Mn Rate	kg·m-3 Micromax®	$kg{\cdot}m^{-3}$ $MnSO_4$	Scale 0-5
0x	0.0	0.0	3.0
1x	0.9	0.0	2.0
2x	0.9	0.0695	3.0
3x	0.9	0.1389	2.0
4x	0.9	0.2084	1.0

[z] Median incidence ratings for rabbit tracks calculated using PROC GLIMMIX in SAS 9.2 with α= 0.05. Rating was from 0 (no occurrence) to 5 (disorder prevalent).

Table C2. Effects of foliar-applied manganese rates on occurrence of rabbit tracks foliar disorder in *Lagerstroemia indica ×fauriei* 'Natchez'.

	Treatment		Median Rating[z]
Mn Rate	$kg{\cdot}m^{-3}$ Micromax®	$mg{\cdot}l^{-1}$ $MnSO_4$	Scale 0-5
0x	0.0	0.0	3.0
1x	0.9	0.0	2.0
2x	0.9	69.5	1.0
3x	0.9	138.9	1.0
4x	0.9	208.4	1.0
5x	0.9	277.9	1.0
6x	0.9	347.4	2.0

[z]Median incidence ratings for rabbit tracks calculated using PROC GLIMMIX in SAS 9.2 with α= 0.05. Rating was from 0 (no occurrence) to 5 (disorder prevalent).