EFFECT OF FALL FERTILIZATON, TAXONOMIC DIFFERENCES AND LIGHT INTENSITY ON FREEZE RESISTANCE OF AZALEAS

by

FRANK PORTER HENNING

(Under the direction of Timothy J. Smalley and Orville Lindstrom)

ABSTRACT

Environmental factors such as light, fertilization and temperature interact to influence the freeze resistance of woody plants, but the combined effects of these factors are not well understood. Freeze resistance, growth, flower production and chlorophyll fluorescence of container-grown *Rhododendron* species were analyzed to investigate interactions between timing of fall fertilization, fertilizer application rate, species, and light intensity treatments.

Influence of fertilization rate and timing on freeze resistance was examined in a study that exposed *Rhododendron* x*kurume* to five fertigation regimes. Fall fertigation increased leaf and stem dry weight (DW) without affecting freeze resistance compared to plants that received no additional fertilizer after Jul. 31. The high rate of N fertigation (125 $mg \cdot L^{-1}$ from Aug. 1 to Sept. 29 or Nov. 28) increased azalea leaf and stem DW, but reduced stem freeze resistance in Nov. and Mar. compared to plants that received N at 75 $mg \cdot L^{-1}$ from Aug. 1 to Sept. 29.

Rhododendron canescens (Michx.) Sweet and *R.* x*satsuki* ‘Wakaebisu’ were grown under three fall fertigation regimes to determine if extended fertilizer application in fall had divergent effects on freeze tolerance, nutrient uptake, growth and flower production of evergreen versus

deciduous plants. There were no interactions of fertilizer and taxa on growth, freeze tolerance, nor timing of cold acclimation/deacclimation. When fertigation was extended in fall 120 versus 60 days, stem N concentration increased, but growth was not affected.

To investigate the effects of fall fertilization and light intensity on photosynthesis and freeze resistance, an experiment with two fertilization treatments, and four light intensity treatments was initiated on *Rhododendron* x*kurume* 'Pink Pearl'. Fertigation and light intensity treatments did not interact in their effects on freeze resistance or chlorophyll fluorescence (*Fv/Fm)*. Plants grown in 50% photosynthetic photon flux (*PPF*) from May 1 through Sept.30 had greater DM compared to plants grown in 100% *PPF* during the same time period. The addition or removal of shade cloth beginning Oct.1 did not increase azalea stem freeze resistance compared to plants that were only exposed to 100%, or 50% *PPF* respectively. Fertigation and light intensity treatments affected *Fv/Fm*, but *Fv/Fm* was not correlated with stem freeze resistance.

INDEX WORDS: *Rhododendron*, *xkurume*, *canescens*, x*satsuki*, 'Wakaebisu'azalea, shade, photosynthesis, chlorophyll, cold hardiness, freeze tolerance, nitrogen, fertilization, fertigation, evergreen , deciduous, fluorescence

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DEDICATION

I dedicate this publication to my wife Megan for her unyielding support and encouragement throughout my entire graduate education process. Megan has made cheerful sacrifices that helped make this extended education effort possible. I will always be grateful for her unyielding encouragement, love and support.

I also dedicate this body of work to Caroline and Laura Scott Henning. Caroline and Laura Scott have grown up with the projects that are included in this study. It has been a pleasure to share student experiences with my daughters through many of their childhood years. I hope they continue to enjoy the pursuit of knowledge in their life-long learning experiences.

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CHAPTER 1

INTRODUCTION

Rhododendron species, which offer a variety of horticultural interests, are among the most widely produced ornamental plants (Chamberlain et al., 1996) and are often the largest proportion of broadleaf evergreen sales (Williams and Mussilo, 1984). Many varieties with good foliage, flower color, or growth habit may be damaged by freezing temperatures (Dirr, 1998) during production.

In temperate climates most woody nursery crops must survive the freezing temperature stresses of at least one winter before reaching marketable size (Bilderbeck and Bir, 1986). Stresses from freezing temperatures have a direct negative impact on nursery production because they decrease plant growth, flowering and reproduction below the genotype potential (Osmond et al., 1987). In addition to the stress itself, the threat of freezing temperature stresses may influence nursery production practices, and further limit production potential.

Cold acclimation is a complex process that is interdependent with other physiological functions (Mazur, 1963; Glerum, 1985). Fertilization, leaf retention and light intensity can influence the ability of plants to acclimate, attain, and maintain the ability to resist freeze-damage. Common selections of evergreen and deciduous azaleas found in nursery production are exposed to a wide variety of fertility regimes and a wide range of light conditions in the landscape (Anderson et al., 1991) and in nurseries (Anderson et al., 1991; Ingram and Midcap, 1979). Despite extensive research on the effects of fall fertilization on cold hardiness, few studies

investigated the timing of nutrient application or the influences of leaf retention or light intensity on cold hardiness.

Nursery production seeks to manage growing regimes to increase plant growth and promote salability in an efficient manner. The extent to which fertilization rate and application timing interact in their influence on freeze resistance is unclear. Late summer and fall fertilization may increase mineral nutrient reserves and promote spring growth (Meyer and Tukey, 1965; Bigras, 1989) and flowering (Kiplinger and Bresser,1951) of woody ornamentals . However, high rates of fertilization that promote growth may also play a role in the development and loss of cold acclimation and freeze resistance (Glerum, 1985).

Previous studies investigated the effects of fall fertilization on cold hardiness. However, few studies accurately controlled the timing of nutrient application. Additional research is needed to understand how fertilization application rate and timing interact in their effect on freeze resistance. Therefore, one objective of our studies was to investigate the influence of fertilization timing and rate treatments on growth, flower production and freeze resistance of 'Hinodegiri'azalea.

Plants that maintain their leaves throughout winter may respond differently to fall fertilization than deciduous plants. Leaf retention affects the carbohydrate supply that is required for active nutrient uptake and the transpiration stream that is responsible for the movement of inorganic ions within the xylem. For fertilizer ions from the substrate to reach the cytoplasm of a leaf or stem cell, they must be actively transported across plasma membranes at least three times (Pitman, 1977; De Boer and Wegner 1997), and carried upward in the xylem by the transpiration stream.

Previous fertilization rate and timing studies investigated treatment effects on evergreen and deciduous plants in separate studies. Little is known about whether extended fertilizer applications in fall influences growth and freeze resistance of evergreen and deciduous plants differently. A second objective of our studies was to determine if the effects of extended fall fertilization on nutrient uptake, cold hardiness, flowering and growth were different for a deciduous [*Rhododendron canescens* (Michx.) Sweet] and an evergreen azalea taxa (*R.* x*satsuki* 'Wakaebisu').

High photosynthetic photon flux (*PPF*) in fall and winter may affect the freeze resistance of plants when excessive excitation of the plant photosynthetic apparatus results in photoinhibition or photoxidation (Minkov et al., 1999). Application of shade in winter decreased photosytem II damage, reduced the incidence and degree of freeze damage, and improved *Pseudotsuga menziesii* seedling survival (Orlander, 1993). Azaleas that received some shade during the winter were observed to suffer less freeze damage than plants grown in high light intensities (Shumack et al., 1995; Sweet, 1960). Photoinhibition and photooxidation increased, and growth was reduced when *Rhododendron xkurume* 'Pink Ruffles' were grown in 100% versus 47% *PPF* (Andersen et al., 1991). Nursery growers may be able to increase control over freeze resistance, flowering, and growth of azaleas by managing fertilization and light intensity concomitantly during fall and winter. The third objective of our studies was to determine if fall fertilization and fall and winter light intensity treatments interact in their effects on *Rhododendron* x*kurume* 'Pink Pearl' stem freeze resistance, photoinhibition , flower production, and growth.

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CHAPTER 2

LITERATURE REVIEW

Rhododendrons

The genus *Rhododendron* contains over 900 species (Chamberlain et al., 1996; Dirr, 1998) with an indigenous distribution that ranges from tropical to polar climates (Sakai, 1987). Azaleas are among the most popular woody flowering landscape shrubs grown in nurseries, landscapes and public gardens (Williams and Mussilo, 1984; Chamberlain et al., 1996) and often account for the largest proportion of broadleaf evergreens sales (Williams and Mussilo, 1984).

Freezing Temperature Stress

Stress is defined as any factor that decreases plant growth and reproduction below the genotype's potential (Osmond et al., 1987). Abiotic stressors, such as cold temperature, limit crop productivity (Araus et al., 2002). One of the primary causes of crop loss in the nursery industry is freeze damage (van de Werken, 1987). Freezing temperatures impede production of many Rhododendron species with good foliage, flower color, or growth habit (Dirr, 1998). In temperate climates, most woody nursery crops must survive the freezing temperature stresses of at least one winter before reaching marketable size (Bilderbeck and Bir, 1986).

Freeze tolerant cells avoid protoplasmic freezing by allowing water to move from intracellular spaces and form ice in extracellular spaces (Levitt, 1980, Sakai and Larcher, 1987). Ice formation concentrates solutes in the intracellular spaces, decreases the water potential (ψ_w)

of extracellular space, and promotes the movement of water down the chemical potential gradient from inside the cells to intercellular spaces until equilibrium of ψ_w across the membrane is reestablished (Thomashow, 1998). As the intracellular spaces dehydrate, their volume decreases, solutes are concentrated, and the freezing point of the intracellular solution is lowered (Levit, 1980). In addition to the ability to resist intracellular freezing, plant cell survival depends on the ability to tolerate freeze-induced stresses such as mechanical, osmotic and toxic ion stresses that are associated with intercellular freezing (Guy, 2003).

Stress Interactions

A plant may experience multiple abiotic stresses either concurrently or at different times during the growing season (Tester and Bacic, 2005). Common abiotic stresses include temperature, water and light, availability of essential nutrients, and high concentrations of toxic-ions (Levitt, 1980; Verslues et al., 2005). Plants are often subjected to multiple stressors simultaneously, and they are generally better able to survive a given stress when other sources of stress are absent (van den Driessche, 1991).

Azaleas are often grown under intensive fertilization regimes and exposed to a wide range of light conditions in their natural habitat (Wang et al., 2008), in the landscape (Anderson et al., 1991) and in nurseries (Anderson et al., 1991; Ingram and Midcap, 1979).The decisions growers make about fertilization, irrigation, pruning, species selection, digging, and light can influence the ability of plants to acclimate, attain, and maintain the ability to resist freeze-damage (Bilderbeck and Bir, 1986).

Cold Hardiness, Acclimation and Deacclimation

Cold hardiness has been defined as the ability or capacity of a plant to survive an unfavorable low environmental temperature (Steponkus, 1984). Cold hardiness is a dynamic

process whereby plants interact and adapt to changes in their environment with continuous adjustments that are limited by genetic constraints (Levitt, 1972).

In temperate zones, plants display periodicity in their resistance to freezing temperature stresses, adjusting their development and metabolic activity to seasonal changes (Sakai and Larcher, 1987). During fall and winter, freezing-tolerant plants must acclimate to freezing temperature stress in order to become more resistant to freezing low temperatures (Guy, 2003). The process by which plants undergo transition from a lower to a higher level of freeze resistance is termed cold acclimation (Steponkus, 1984). The timing of this response is a critical component of freeze resistance because freeze injury often depends on the timing of cold acclimation in fall or deacclimation in spring (Williams et al., 1986). Cold acclimation is a complex process that is interdependent with other physiological functions (Mazur, 1963; Glerum, 1985). However, duration and intensity of low temperatures are generally considered the most significant factors affecting cold acclimation (Sakai and Larcher, 1987).

Deacclimation is the transition from a freeze tolerant to a non-tolerant state and is usually induced by exposure to temperatures above freezing (Bigras et al., 2001). Deacclimation of shoots before budbreak was documented for many woody species (Glerum, 1973). Cold hardiness of *Malus domestica* flower buds decreased by 10C or more as bud development proceeded from dormancy through anthesis (Ballard and Proebsting, 1978). Reducing temperatures below 4.5C by sprinkling to promote evaporative cooling delayed anthesis of apples by up to 17 days (Anderson et al., 1975), and peaches by up to 15 days (Chesness et al., 1977). Chilling requirements must be satisfied for many species, *Malus* (Rieger, 1989), *Fragaria* (Harris, 1973) and *Sedum* (Iles and Agnew, 1995), to deacclimate, break dormancy and resume growth. In a review of freeze protection practices, Rieger (1989) concluded that once the chilling

requirement has been met, phenological development of flower buds on fruit trees is related to the number of degree-hours above 4.5C that are accumulated.

Nutrition, Temperature and Growth

Nutrient uptake by woody plants occurs when growing medium temperatures are above freezing (Good and Tukey, 1969; Wright et al., 1983). At temperatures between 2C and 26C, Wright and Blazich (1983) found that plant N increased as both temperature and N rate increased and that N rate had a greater influence on N accumulation than temperature.

Spring growth of container grown *Ligustrum ibolium* Coe ex Rehder and *Euonymus alatus* (Thunb.) Siebold (Good and Tukey, 1969), *Juniperus chinensus* L. (Bigras et al., 1989a), *Forsythia* x*intermedia* Zabel (Meyer and Tukey, 1965), *Syringa vulgaris* L. (Meyer and Splittstoesser, 1969), and field grown *Malus domestica* Borkh. (Millard and Neilsen, 1989) increased after fertilizer applications during the previous fall. Nitrogen application increased tissue N and growth of *Syringa vulgaris* (Meyer and Splittstoesser, 1969). Kiplinger and Bresser (1951) found that the number of *Rhododendron* x*kurume* 'Coral Bells' flower buds increased from 127 to 171 as N applied through Sept. 20 was increased from 2.3 to 11.3 mg L^{-1}.

The application of fertilizer at high rates late summer or fall may prolong growth and delay vegetative maturity (Fuchigami and Weiser, 1981). Cool, above freezing, temperatures that did not prevent nutrient uptake prevented *Ilex crenata* shoot elongation. Irrespective of the fertilization regime, Wright et al. (1983) concluded that day/night temperatures below 18C/14C prevented shoot elongation of *Ilex crenata*.

Nutrition, Temperature and Leaf Retention

Leaf retention affects the carbohydrate supply that is required for active nutrient uptake and the transpiration stream that is responsible for the movement of inorganic ions within the xylem.

For fertilizer ions from the substrate to reach the cytoplasm of a leaf or stem cell, they must be actively transported across plasma membranes at least three times (Pitman, 1977; De Boer and Wegner 1997) and carried upward in the xylem by the transpirational stream. Assimilation of $NH4^+$ increases demand for carbohydrates and a depletion of soluble carbohydrates may result if photosynthetic reserves are limited (Epstein, 1972).

In studies that investigated N uptake of deciduous species, defoliation of *Prunus domestica* L. subsp. *domestica* (Weinbaum et al., 1978) and shade applied to *Malus domestica* Borkh. (Firth and Nichols, 1975) and *Prunus domestica* L. subsp. *domestica* (Therios and Weinbaum, 1979) reduced carbon assimilation and inhibited N uptake. Compared to ungirdled *Prunus persica* L. Batsch, trees that were girdled after apical meristem growth ceased had a 5-fold decrease in N uptake compared to ungirdled trees (Jordan et al., 1998). These studies emphasize the importance of photosynthesizing leaves for the uptake of nitrogen.

Low temperatures reduced transpiration and photosynthetic activity but low temperatures above freezing did not prevent N uptake in species with persistent leaves [*Ilex crenata* Thunb. (Wright et al., 1983), *Ligustrum* x*ibolium* Coe ex Rehder (Good and Tukey 1969)]. Nitrogen application rate had more influence on total plant N than temperature when *Ilex crenata* plants were exposed to low temperatures above freezing (Wright et al., 1983).

Nutrition and Freeze Resistance

Over application of fertilizer in late summer or fall may extend the period of shoot elongation, delay vegetative maturity, postpone cold acclimation and increase the incidence of freeze damage (Fuchigami and Weiser, 1981). High rates of fertilization applied in fall reduced freeze resistance when growth cessation (Hellegren, 1981; Glerum, 1985) and bud development (Glerum, 1985) were delayed in fall, or when new vegetative growth was promoted either late

(Williams et al., 1986; Wright et al. 1978) or early (Benzian et al. 1974) in the growing season. A negative correlation was found between the application rate of granular fertilizers and freeze resistance for container grown *Pyracantha coccinea* M. Roem. and *Ilex crenata* Thunb. (Kelley, 1972), *Forsythia* x*intermedia* Zabel (Pellett, 1973) and *Rhododendron obtusum* (Lindl.) Planch. (Wright et al., 1978).

In their review of the relationship between nutrition and cold hardiness, Pellet and Carter (1981) concluded that supra optimum fertility levels can reduce cold hardiness, but plants fertilized at levels that promote optimum growth will cold acclimate at a similar rate and to the same degree as plants grown under lower fertility regimes. Within the range of near-deficient to supra optimal, tissue N content had little effect on freeze resistance of *Thuja plicata* or *Pseudotsuga menziesii* seedlings (Hawkins et al. 1995). Fertilization that prolonged the growth period of *Pinus strobus* needles did not impair hardening and seedlings with the highest nutrient concentrations had the least needle damage (Rikala and Repo, 1997). Nitrogen applied in fall to container grown *Ilex crenata* Thunb. (Havis et al., 1972), *Cornus alba* L. (Pellett, 1973), and field grown *Lagerstroemia indica* L. (Lindstrom et al., 2002) had no effect on freeze resistance. Compared with fertilization stopped in late summer, fall fertilization increased the level of N in *Juniperus chinensis* 'Pfizeriana' root and stem tissues. Spring growth was correlated with the N content of stems the previous fall, but cold hardiness of mature roots and stems was not reduced by prolonged fertilization in the fall (Bigras et al., 1987).

Liquid Fertilization and Freeze Resistance

Unlike other methods of nutrient application, liquid feed (LF) systems can be managed to supply nutrients at levels that match plant needs and LF can be managed to maintain nitrogen levels in the container medium late in the season (Wright and Niemiera, 1987). Greater growth

was reported with LF than controlled release fertilizer for *I. crenata* (Sharma et al., 1982), *Chamaecyparis, Pyracantha* (van der Boon, 1981), *Thuja occidentalis* and *Viburnum* x*burkwoodii* (Sanderson and Martin, 1974).

Several studies used liquid fertilization systems to control the timing of nutrient application and accurately analyze the effects of fall fertilizer application on freeze resistance. Field grown crape myrtle (*Lagerstroemia* L. 'Natchez') that received no fertilizer, or six bi-weekly applications either from mid-Jun. through Aug., or from late Aug. through Oct. had little or no effect on the development or degree of cold hardiness (Lindstrom et al., 2002). Hawkins et al. (1996) exposed container-grown seedlings of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] to three fertilization timing treatments and found differences in fertilization timing which did not affect mineral nutrient (N and P) concentrations of leaves influenced freeze resistance. Bigras et al., (1989b) found that fertilization through Sept. in Quebec, Canada increased spring growth of container grown *Juniperus chinensis* 'Pfizerana' shoots without reducing freeze resistance of shoots or roots compared to plants that were fertilized through Jul. 31.

Light, Temperature and Freeze Damage

At otherwise sub-lethal freezing temperatures, plants exposed to high light intensities may be damaged by rapid temperature changes, photoinjury, desiccation, or by a synergistic combination of these stresses (Christersson and von Fircks, 1988). Azaleas that received some shade during the winter were observed to suffer less freeze damage than plants grown in high light intensities (Shumack et al., 1994; Sweet, 1960). Shade application in winter decreased photosytem II damage, reduced the incidence and degree of freeze damage and improved *Pseudotsuga menziesii* seedling survival (Orlander, 1993).

Light intensity affects plant tissue temperature fluctuations. Cambial temperatures of trees were as much as 25^{o}C higher on south-facing than north-facing sides of the same trunk (Harvey 1923) and 15^{o}C higher than ambient air temperature on clear versus cloudy winter days (Jensen et al., 1970). Hamer (1975) applied 40% shade cloth on freeze nights to a 0.1 ha plot of dwarfed Malus domestica and found that bud temperatures in the covered plots increased by 0.5C near the ground.

Previous studies found that stresses related to fast cooling (6C $\cdot$ hr^{-1}) raised the lethal temperature of *Rhododendron* leaves and stems compared to slow cooling (2C $\cdot$ hr^{-1}) (Anisko, et al., 1994; Havis, 1964). Intracellular ice may form if cooling rates are too rapid and the speed at which water can diffuse from cells to form extracellular ice is insufficient (Bigras, 2001). Cooling rates of plants grown in high light exceeded 5C $\cdot$ hr^{-1} under field conditions (White and Weiser, 1964; Gross et al., 1991) and the leaves of *Thuja occidentalis* cooled at a rate of 9.5C $\cdot$ min^{-1} at sunset in winter (White and Weiser, 1964). On partly cloudy days, cambial temperatures of plants grown on exposed sites were reported to fluctuate as much as 3.3C $\cdot$ min^{-1}, fluctuations that resulted in alternating freeze-thaw cycles (Harvey, 1923). A positive relationship between light intensity and freeze damage was observed for *Rhododendron* x*kurume* and *R. simsii* azaleas based on leaf burn, stem damage and flower bud kill for plants grown in light intensities that varied from full sun to 72% shade (Sweet, 1960).

High light intensities promote desiccation, an indirect freeze injury, when plants are exposed to high light intensities and cold temperatures. Plants exposed to high light intensities may lose moisture through warm leaves faster than it can be replaced with water taken up from cold or frozen soil (Tranquillini, 1981, Bilderbeck and Bir, 1986). The affects of temperature on root and shoot hydraulic conductance have been attributed to either changes in membrane fluidity and

permability (Carvajal et al., 1996; Cochard et al., 2000) or changes in water viscosity (Hertel and Steudle, 1997; Cochard et al., 2000). The combination of high light intensities and low ambient temperatures may result in low root and high shoot hydraulic conductances that increase osmotic stress. High light intensities also promote osmotic stress when radiative heat increases transpiration while frozen soil, roots or stems restrict the transport and replacement of the water that is lost (Kramer and Boyer, 1995).

Photosynthesis and Freeze Resistance

Photosynthesis provides the energy that is needed for plants to develop maximum tolerance to freezing temperature stresses (Oquist et al., 2001). Photosynthesis is sensitive to growth factors including temperature, osmotic stress, nutrient availability and light (Larcher 1994). Light-induced photosynthetic stress and photoinhibition have been reported when plants that are unable to increase maximum rate of photosynthesis (A_{max}) are exposed to high light intensities (Olsen et al., 2002).

Low temperatures inhibit photosynthesis when the speed of enzymatic reactions of electron transport and carbon metabolism are reduced, chloroplast membrane function is impaired, or the rate of protein synthesis associated with repair of damaged PSII centers slows (Tiaz and Zeiger, 2006). Cold temperatures lower the light saturated rate of net photosynthesis and decrease the efficiency of electron transport and PSII (Oquist et al., 1983). Low temperatures reduce the range of light intensity to which a plant can acclimate (Strand and Lundmark, 1987; Sweet, 1960). Inability to maintain A_{max} as temperatures decrease may preclude sufficient acclimation and increase photosynthetic stress, even without an increase in light intensity. Stresses, such as low temperature, have been shown to reduce photosynthetic rates and increase a plants susceptibility to photoinhibition (Edwards, 1989; Hawkins et al., 1995). High light

intensities in fall and winter may affect the freeze resistance of plants when excessive excitation of the plant photosynthetic apparatus results in photoinhibition or photoxidation (Bertamini et al., 2005).

Genetic adaptations that allow plants to survive in low light intensities may prevent shade-adapted species from adequately acclimating when exposed to high light intensities (Olsen et al., 2002). In general, understory shrubs that are native to low-light environments, have low photosynthetic rates, or lack the ability to increase light saturated photosynthesis (Oquist et al., 1983). Excessive photosynthetic radiation absorbed by the leaves may result in photoinhibition or photoxidation when this absorption results in the inactivation or impairment of the photosynthetic reaction centers (Bertamini et al., 2005). In a light intensity study with the shade-adapted plants of *Illicium lanceolatum* and *I. parviflorum* 'Forest Green', Olsen et al. (2002) found that the maximum rate of net CO_2 assimilation and net leaf CO_2 assimilation at light saturation were decreased when plants were exposed to full light compared to 45% shade (2110 versus 1010 $\mu mol \cdot m^{-2} \cdot s^{-1}$ average daily maximum ambient *PPF*). In a similar investigation, Andersen et al. (1991) found that photoinhibition and photooxidation of *Rhododendron* x*kurume* 'Pink Ruffles' increased when exposed to full light (100% *PPF*) compared to 47% *PPF*.

In addition to genetic adaptations that limit A_{max}, sub-optimal nutrient supplies have been linked to reduced photosynthetic rates and increased photoinhibition (Hawkins et al., 1995). Sub-optimal nutrient supplies reduced wintertime photosynthetic efficiency of seedling *P. menziesii* (Bircher, 2001; Hawkins, 1995).

When plants are unable to dissipate light energy through photosynthetic quenching, absorbed light energy may be quenched through non-radiative dissipation (Adams et al., 1994), or this energy may degrade D1 reaction centers and cause inactivation of photosystem II reaction

centers (Ottander et al., 1995). Some plants respond to cool temperature with xanthophyll cycle conversions of violaxanthin to zeaxanthin and antheraxanthin. (Adams et al., 1994). De-epoxidation of violaxanthin is viewed as a protective, non-radiative means of dissipating (quenching) excitation energy (Adams et al., 1994). Without adequate photoprotection, plants may respond to light and temperature stresses by balancing between the photosystem II deactivation and reactivation. The deactivation of photosystem II that results when D1 proteins are degraded is countered by synthesis of D1 proteins and reactivation of photosystem II function (Aro et al., 1993).

Chlorophyll fluorescence is an indirect, non-destructive method for assessing photosynthetic performance. The ratio of variable to maximum fluorescence (F_v/F_m) indicates the proportion of the maximum possible fluorescence that was used for photosynthesis and is inversely proportional to photosynthetic stress. Fluorescence is linearly correlated with the quantum yield of net photosynthesis (Demig-Adams and Adams., 1992) and research conducted on *P. menziesii* demonstrated that F_v/F_m ratios were linearly related to stem freeze damage and short-term survival after exposure to sub-freezing temperatures (Birchler et al., 2001; Perks et al., 2004). Based on linear correlations between chlorophyll fluorescence (F_v/F_m) and LT_{50} of Douglas-fir seedling stems, Perks et al. (2004) demonstrated that F_v/F_m could be used to accurately predict stem LT_{50} and developed $F.LT_{50}$ (fluorescence-based empirical determination of LT_{50}), a non-destructive alternative to LT_{50} for estimating low temperature survival of seedling trees. Orlander (1993) found that following a frost, chlorophyll fluorescence (F_v/F_m) values of Norway spruce seedlings were reduced by high light intensities and that freeze damage could be reduced with the application of shade the day after frost.

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CHAPTER 3

EFFECTS OF FALL FERTILIZATION ON FREEZE RESISTANCE, FLOWERING AND GROWTH OF 'HINODEGIRI' AZALEA[1]

[1]Henning, F.H., T. Smalley, O. Lindstrom, and J. Ruter. 2008. J. Environ. Hort. 26:135-138.

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Effects of Fall Fertilization on Freeze Resistance, Flowering, and Growth of 'Hinodegiri' Azalea[1]

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Abstract

Five fertigation treatments were initiated Aug. 1, 2002: 1) No additional fertilizer, 2) N at 75 mg · L^{-1} from Aug. 1 to Sept. 29, 3) N at 125 mg · L^{-1} from Aug. 1 to Sept. 29, 4) N at 75 mg · L^{-1} from Aug. 1 to Nov. 28, and 5) N at 125 mg · L^{-1} from Aug. 1 to Nov. 28. Freeze resistance of stems and leaves was analyzed monthly, Nov. to Feb.. Flower counts began prior to budbreak, and continued to flower expression. Fall fertigation increased azalea leaf and stem dry weight (DW) compared to azaleas that received no additional fertigation after Jul. 31. Fertigation N applied at 75 or 125 mg · L^{-1} from Aug. 1 to Nov. 28 did not increase leaf or stem DW compared to plants fertigated at the same N rate from Aug. 1 to Sept. 29. High N treatments (N at 125 mg · L^{-1} from Aug. 1 to Sept. 29 or Nov. 28) increased above ground DW, but reduced stem freeze resistance in Nov. and March compared to plants that received N at 75 mg · L^{-1} from Aug. 1 to Sept. 29. Early flower budbreak and decreased total flower production resulted when N was applied at 125 mg · L^{-1} from Aug. 1 to Nov. 28 compared to 75 mg · L^{-1} from Aug. 1 to Sept. 29.

Index words

Rhododendron x*kurume*, evergreen azalea, cold hardiness, nitrogen, liquid feed, fertigation.

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Significance to the Nursery Industry

Interactions between fertilizer application rate and timing can influence growth and freeze resistance of container-grown horticultural crops. Fall fertilization can be used to increase the growth of container-grown azaleas without increasing freeze damage. A moderate rate of fall fertilization (N at 75 mg $\cdot$ L^{-1}) (Bailey and Nelson, 2008) can be extended through late fall (Nov. 28) without reducing stem or leaf freeze resistance. A high rate of fertilization (N at 125 mg $\cdot$ L^{-1}) (Bailey and Nelson, 2008) may increase plant growth, compared to plants fertilized at a moderate rate. However, high rates of fertilization may reduce freeze hardiness, even when fertilization is terminated in Sept., well before the first freeze event. To maximize growth and stem freeze resistance, azalea producers in USDA Plant Hardiness 7 should maintain moderate fertility through Aug. and Sept. Sustaining moderate fertility levels past late Sept. achieves no additional growth benefit.

Introduction

Late summer and fall fertilization may increase mineral nutrient reserves and promote spring growth and flowering of woody ornamentals. Spring growth of container-grown ibolium privet (*Ligustrum ibolium* Coe ex Rehder) and winged burning bush (*Euonymus alatus* (Thunb.) Siebold) (Good and Tukey, 1969), Chinese juniper (*Juniperus chinensus* L.) (Bigras et al.,

1989a), border forsythia (*Forsythia* x*intermedia* Zabel) (Meyer and Tukey, 1965), common lilac (*Syringa vulgaris* L.) (Meyer and splittstoesser, 1969), and field-grown domestic apple (*Malus domestica* Borkh.) (Millard and Neilsen, 1989) increased after fertilizer applications during the previous fall. Kiplinger and Bresser (1951) reported that the number of flower buds per plant on azalea 'Coral Bells' (*Rhododendron* x*kurume* 'Coral Bells') increased from 127 to 171 as N fertigation applied through Sept. 20 was increased from 10 to 50 mg $\cdot$ L^{-1}.

High rates of fertilization applied in fall may reduce freeze resistance when growth cessation (Hellegren, 1981; Edwards, 1989) and bud development (Edwards, 1989) are delayed in fall, or when new vegetative growth is promoted either late (Williams et al., 1986; Wright et al., 1978) or early (Benzian et al., 1974) in the growing season. A negative correlation was found between the application rate of granular fertilizers and freeze resistance for container grown scarlet firethorn (*Pyracantha coccinea* M. Roem.) and Japanese holly (*Ilex crenata* Thunb.) (Kelley, 1972), *Forsythia* x*intermedia* (Pellet, 1973) and Kurume azalea (*Rhododendron obtusum* (Lindl.) Planch.) (Wright et al., 1978). However, N applied in fall to container-grown *Ilex crenata* (Havis, 1972), redosier dogwood (*Cornus alba* L.) (Pellet, 1973), and field grown crape myrtle (*Lagerstroemia indica* L.) (Lindstrom et al., 2002) had no effect on freeze resistance.

Several studies utilized fertigation systems to control the timing of mineral nutrient application and analyze accurately the effects of fall fertilizer application on freeze resistance. Hawkins et al. (1996) exposed container-grown seedlings of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] to three fertilization timing treatments and found differences in fertilization timing which did not affect mineral nutrient (N and P) concentrations of leaves influenced freeze resistance. Bigras et al. (1989b) reported that compared to container-grown *Juniperus chinensis*

L. fertilized through Jul. 31 in Quebec, Canada, fertilization through Sept. increased spring growth of shoots without reducing freeze resistance of shoots or roots.

Fall fertilization may increase plant growth and flower production, but additional research is needed to understand how application rate and timing affect freeze resistance. Therefore, the objective of this study was to investigate whether timing and rate of fertigation influence growth, flower production and freeze resistance of 'Hinodegiri'azalea.

Materials and Methods

Uniform rooted liners of *R.* x*kurume* 'Hinodegiri' were transplanted into 2.2 L (#1) containers May 1, 2002, and grown in an unsheltered, gravel container nursery bed located on the University of Georgia campus in Athens, GA. Plants were exposed to ambient air temperature that ranged from -12.4 to 37.3C (9.7 to 99.1F). The growing substrate was aged pine bark amended with 6.74 kg · m^{-3} (4 lb/yd^{-3}) dolomitic limestone and 1.67 kg · m^{-3} (1 lb/ yd^{-3}) Micromax® Micronutrients Granular (The Scotts Co., Marysville, OH). Prior to the initiation of fertility treatments on Aug. 1, all plants were fertigated daily with 0.5 L (0.13 gallon) solution that contained at N at 75 mg · L^{-1} (4% NH_4, 6% NO_3, and 10% urea), P at 33 mg · L^{-1} and K at 62 mg · L^{-1}. Beginning Aug. 1, 2002 plants were grown under five different fall fertility regimes, 1) no additional fertilizer beginning Aug. 1, 2) 60 days of extended fertigation (from Aug. 1 to Sept. 29) at a moderate rate (N at 75 mg · L^{-1}), 3) 60 days of extended fertigation (from Aug. 1 to Sept. 29) at a high rate (N at 125 mg · L^{-1}), 4) 120 days of extended fertilizer application (from Aug. 1 to Nov. 28) at a moderate rate (N at 75 mg · L^{-1}) and 5) 120 days of extended fertilizer application (from Aug. 1 to Nov. 28) at a high rate (N at 125 mg · L^{-1}). Fertilizer treatments were randomly assigned and applied using a constant liquid feed fertilizer application. Plants were fertigated daily with 0.5 L (0.13 gallon) solution of Harrell's (Sylacauga, AL) 16.0-3.5-6.6 (N-P-

K) liquid fertilizer that contained N as NO_3 (1.6%), and urea (14.4%). Electrical conductivity of leachate was monitored daily throughout the study to maintain electrical conductivity of the substrate below 1.0 $mS \cdot cm^{-1}$, using the Virginia Tech Extraction Method (Yeager et al., 1997). Floating row cover (Specialty Converting and Supply Inc., Nashville, GA) was applied for freeze protection when minimum temperatures fell below -6.7C (20F).

This experiment was a factorial arrangement of five fall fertigation treatments in a randomized complete block design with three replications. Leaves and stems were harvested from three azaleas per treatment-replication combination on Nov. 12, and December 11, 2002, and January 14, Feb. 18, and March 19, 2003. Samples from each treatment-replication combination were combined, wrapped in moist paper towels, put in plastic bags, and placed on ice for transport to the lab. Within four hours of collection leaves and stems were prepared for freezing. Leaf and stem freeze resistance was estimated using the technique outlined by Lindstrom et al. (Lindstrom et al., 2002). Thirty six leaf, and 5 cm (2 inch) long stem segments from each treatment–replication combination were analyzed under laboratory conditions. Four leaves and stem segments from each treatment-replication combination were removed from a freezing temperature bath at 3C (5F) temperature intervals. Samples were exposed to temperatures between -3 and -27C (26.6 and -16.6F) for freeze analysis in Nov., December, and March, and to temperatures between -6 and -30C (21.2 and -22F) in January, and Feb.. Controls were kept at 4C (39F) for the duration of the freezing test. The Spearman-Karber Method (Bittenbender and Howell, 1974) was used to estimate the temperature at which 50% of leaf or and stem samples were killed (T_{50}).

Flower counts began Feb. 24, 2003, prior to initial budbreak, and continued to full flower expression (F_{max}). Flowering was defined as budbreak showing petal color, and flowers were

counted on five plants per treatment-replication combination three times weekly. Linear regression analysis was used to characterize the relationship between day of year and flower count. Regression equations were then used to calculate F_{50} (date which 50% of flowers were in bloom, or $F_{max}/2$) for each treatment x replicate combination.

The same five azaleas from each treatment-replication combination used in F_{50} and F_{max} analyses were harvested May 5, 2003, separated into leaves and stems, and dried at 55C (131F) for 72 hr to determine leaf, stem and total above ground DW.

Dry weight, leaf freeze resistance, stem freeze resistance, flower production, and flower budbreak were analyzed using the GLM procedures (SAS Inst., Inc., Cary, NC.) Tukey's studentized range (HSD) means comparison test was used to detect differences in pairwise comparison of treatment means.

Results and Discussion

Fall fertilization increased plant growth. Fertilization extended through Sept. 29 (60 days) or Nov. 28 (120 days) increased leaf, stem and total plant DW of azaleas compared to plants that received no fertilization after 31 Jul. (Table 3.1). These results are similar to other studies reporting fall fertilization increased growth (Bigras et al., 1989a; Glerum, 1985; Millard and Neilsen, 1989). Results from this study demonstrated that compared to azaleas which received 60 days of extended fertilization at the moderate rate (N at 75 mg $\cdot L^{-1}$ from Aug. 1 to Sept. 29), the high rate of fertilization (N at 125 mg $\cdot L^{-1}$) applied during the same time period increased total plant DW. Extended fertilization for 120 days at the high rate (N at 125 mg $\cdot L^{-1}$ from Aug. 1 to Nov. 28) increased leaf and total plant DW relative to azaleas that received 60 days of extended fertilization at the moderate rate. However, compared to azaleas that received 60 days of extended fertilization at a moderate rate (N at 75 mg N $\cdot L^{-1}$, Aug. 1 to Sept. 29), dry weight

production was not increased when fertilization was extended at the same rate for 120 days (N at 75 mg · L^{-1}, Aug. 1 to Nov. 28).

Freeze resistance of azalea leaves in Nov., Dec., Jan, or Mar. was not affected by fall fertilization (Table 3.2). Higher levels of extended fall fertilization reduced leaf freeze resistance in Feb., as leaves of azaleas that received no fertilization from Sept. 29 to Nov. 28 had greater freeze resistance than azaleas that received the high rate of fertilization for 120 days (N at 125 mg · L^{-1}, Aug. 1 to Nov. 28).

Termination of fertigation in late summer (no fertilizer applied after Jul. 31) did not reduce freeze resistance of azalea stems compared to other fertigation treatments (Table 3.3). These results differ from those of Edwards (1989) who found that reduced fertilization in late summer increased conifer seedling freeze damage.

In Nov. and March, stem tissue from azaleas that received 60 days of extended fertilization at the high rate (N at 125 mg · L^{-1} from Aug. 1 to Sept. 29) was less freeze resistant than stem tissue of azaleas that received the moderate rate of fertilization (N at 75 mg · L^{-1}) during this same time period (Aug. 1 to Sept. 29). Stem tissue of azaleas that received the high rate of extended fertilization (N at 125 mg · L^{-1} from Aug. 1 to Nov. 28) was less freeze resistant in Nov., December, January and March than plants that received the moderate rate of fertilization (N at 75 mg · L^{-1}) through Sept. 29.

In the present investigation, fall fertilization treatments that increased total plant DW did not always reduce freeze resistance. Compared to azaleas that were not fertilized after Jul. 31, extended fertilization at a moderate rate (N at 75 mg · L^{-1}) through Sept. 29 or Nov. 28 increased leaf, stem and above ground DW without effect on stem freeze resistance. These results agree with the work of Bigras et al. (1989a) who found fall fertigation increased growth of *Juniperus*

chinensis L. without reducing stem freeze resistance. However, the high rate of fall fertigation (N at 125 mg · L^{-1} from Aug. 1 to Sept. 29 or Nov. 28) increased azalea growth, but reduced stem freeze resistance in Nov. and March compared to plants that either received no fall fertilization after Jul. 31, or received extended fertilization at a moderate rate (N at 75 mg · L^{-1}) through 29 Sept .

Based on their analysis of the effects of fertilization and temperature on the growth of *Ilex crenata*, Wright and Blazich (1983) recommended when plants have entered the cold acclimation phase, fertilizer applied at half the rate applied in the growing season may be beneficial to support growth and cold acclimation. Unlike the results of Wright and Blazich, in this study when fertilization was extended 60 days (through Sept. 29) versus 120 days (through Nov. 28) at the moderate rate (N at 75 mg · L^{-1}), sustaining fall fertilization for 120 days through Nov. 28 affected neither stem freeze hardiness nor DW accumulation. The high rate of fertilization (N at 125 mg · L^{-1}) reduced azalea freeze resistance in fall and early spring even when fertilization was terminated in Sept., well before the first freeze event.

The high rate of extended fertilization (N at 125 mg · L^{-1} from Aug. 1 to Nov. 28) promoted early flower budbreak and decreased the total number of flowers compared to azaleas that received N at 75 mg · L^{-1} from Aug. 1 to Sept. 29 (Table 3.4). These findings support those of Benzian et al. (1974), and Van Den Driessche (1991) who reported high tissue N content accelerated budbreak. However, using high N to promote early flowering and thus marketing of azaleas is not advisable because fertilization with high N rates reduces cold hardiness.

In this study, when fall fertilization was extended 60 days at a moderate rate (N at 75 mg · L^{-1} from Aug. 1 through Sept. 28), growth increased with no effect on freeze resistance. Sustaining this moderate rate of fertilization later in the season (through Nov. 28) did not further

increase growth or decrease freeze resistance. A high rate of fertilization (N at 125 mg $\cdot L^{-1}$) may increase azalea DW production. However, this high rate of fertilization may also reduce freeze hardiness in fall and early spring even when fertilization is terminated in Sept. well before the first freeze event. Therefore, results herein indicate azalea growers in Zone 7 should maintain moderate fertility levels through late Sept. Sustaining fertilization past late Sept. achieves no growth benefit, and high fertility rates may promote freeze damage.

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Table 3.1. Effect of fertilization treatments on leaf, stem and total above ground DW of *R.* x*kurume* 'Hinodegiri'.

N fertilization (mg N · L^{-1})		Dry weight (g)		
Aug. 1 to Sept. 29	Sept. 30 to Nov. 28	Leaf	Stem	Total
0	0	9.2 d[z,y]	8.3 b	17.4 d
75	0	17.2 bc	12.4 a	29.6 c
125	0	20.0 ab	14.2 a	34.3 ab
75	75	16.4 c	14.2 a	30.6 bc
125	125	22.0 a	15.0 a	37.1 a

[z] Each mean based on observations of five plants in each of three replicates.

[y] Mean separation within columns by Tukey's Studentized range test at $P \leq 0.05$.

Table 3.2. Effect of fertilization treatments on freeze resistance of leaf tissue of *R.* x*kurume* 'Hinodegiri'.

N fertilization ($mg \cdot L^{-1}$)		T_{50} (C, F)				
		Leaf harvest dates				
Aug. 1 to Sept. 29	Sept. 30 to Nov. 28	Nov.12	Dec. 11	Jan. 14	Feb. 18	Mar.19
0	0	-8.75 (16.3)a[z,y]	-20.0 (-4)a	-19.5 (-3.1)a	-21.0 (-5.8)b	-10.25 (13.6)a
75	0	-9.0 (15.8)a	-19.75 (-3.6)a	-22.5 (-8.5)a	-21.25 (-6.3)b	-10.25 (13.6)a
125	0	-9.5 (14.9)a	-18.0 (-0.4)a	-21.75 (-7.2)a	-20.0 (-4.0)b	-9.50 (14.9)a
75	75	-7.75 (18.1)a	-18.75 (-1.8)a	-19.0 (-2.2)a	-19.75 (-3.6)ab	-9.75 (14.5)a
125	125	-6.0 (21.2)a	-18.5 (-1.3)a	-21.0 (-5.8)a	-16.75 (1.9)a	-10.25 (13.6)a

[z]Each mean based on observations of four leaf samples at each of 9 different freezing temperature increments in each of 3 replicates.

[y]Mean separation within columns by Tukey's Studentized range test at $P \leq 0.05$.

Table 3.3. Effect of fertilization treatments on freeze resistance of stem tissue of *R.* x*kurume* 'Hinodegiri'.

N fertilization ($mg \cdot L^{-1}$)		T_{50} (C, F)				
Aug. 1 to Sept. 29	Sept. 30 to Nov. 28	Stem Harvest Dates				
		Nov. 12	Dec. 11	Jan. 14	Feb. 18	Mar.19
0	0	-13.5 (7.7)bc[z,y]	-22.5 (-8.5)b	-22.25 (-8.1)ab	-20.25 (-4.5)a	-6.42 (20.4)b
75	0	-15.5 (22.1)c	-21.25 (-6.3)b	-24 (-11.2)b	-20.75 (-5.4)a	-6.75 (19.9)b
125	0	-11.5 (11.3)ab	-20.75 (-5.4)b	-21 (-5.8)ab	-18.5 (-1.3)a	-4.5 (23.9)a
75	75	-13.75 (7.3)bc	-19.5 (-3.1)b	-22.25 (-8.1)ab	-18.75 (-1.8)a	-5.92 (21.3)ab
125	125	-9.25 (15.4)a	-9.5 (14.9)a	-18.75 (-1.8)a	-18.5 (-1.3)a	-4 (24.8)a

[z]Each mean based on observations of four stem samples at each of 9 different freezing temperature increments for each of 3 replicates.

[y]Mean separation within columns by Tukey's Studentized range test at $P \leq 0.05$.

Table 3.4. Effect of fertilization treatments on the day of year 50% of *R.* x*kurume* 'Hinodegiri' flowers were in bloom (F_{50}), and total number of flowers per plant.

Fertilization treatment (mg $N \cdot L^{-1}$)			
Aug. 1 to Sept. 29	Sept.30 to Nov. 28	F_{50} Day of the Year	Flower Production
0	0	85.4 (Mar. 26)a[z,y]	205.6ab
75	0	85.2 (Mar. 26)a	245.3a
125	0	83.4 (Mar. 24)ab	201.9ab
75	75	81.9 (Mar. 22)ab	206.2ab
125	125	80.1 (Mar. 21)b	151.3b

[z] Each mean based on observations of five plants in each of three replicates.

[y] Mean separation within columns by Tukey's Studentized range test at $P \leq 0.05$.

CHAPTER 4

EFFECT OF FALL FERTILIZATION ON FREEZE RESISTANCE OF DECIDUOUS VERSUS EVERGREEN AZALEAS[1]

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Effect of Fall Fertilization on Freeze Resistance of Deciduous Versus Evergreen Azaleas[1]

Authors

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Abstract

The effects of fall fertilization on cold hardiness, nutrient uptake, growth and flower production of evergreen versus deciduous azaleas were studied. *Rhododendron canescens* (Michx.) Sweet and *R.* x*satsuki* 'Wakaebisu' were grown in containers, outdoors in Athens, GA under three fall fertigation regimes applied daily as 0.5L solutions containing: 1) 75 mg $N \cdot L^{-1}$ from Aug. 1 through Sept. 29, 2) 75 mg $N \cdot L^{-1}$ from Aug. 1 through Nov. 28 and 3) 125 mg $N \cdot L^{-1}$ from Aug. 1 through Nov. 28. Stem freeze resistance was analyzed monthly Nov. through Mar. Growth of azaleas that received 120 days of extended fertigation (Aug. 1 through Nov. 28) was not increased compared to azaleas that received 60 days of extended fertigation (Aug. 1 through Sept. 29). Growth of the two taxa did not differ in their response to fertilization treatments. The high rate of extended fertilization (125 mg $N \cdot L^{-1}$ from Aug.1 through Nov. 28) reduced stem freeze resistance Nov. through Feb., while the moderate rate of extended fertilization (75 mg $N \cdot L^{-1}$ from Aug.1 through Nov. 28) reduced azalea freeze resistance in Dec. Fall fertilization regimes did not produce differences in the time or relative degree of freeze resistance development between *R. canescens* and *R.* x*satsuki.* The high rate of extended fertilization promoted early budbreak of *R.* x*satsuki* and postponed flower budbreak of *R. canescens*. Flower production of R. *canescens* was not affected by fall fertilization, but the high rate of extended fertilization increased flower production of R. *xsatsuki* compared to plants that received the moderate rate of fertilization (75 mg $N \cdot L^{-1}$ from Aug.1 through Sept. 29).

Index words

Rhododendron, *R. canescens*, *R.* x*satsuki,* 'Wakaebisu', fertigation, nitrogen, frost, cold, hardiness

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Significance to the Nursery Industry

The design of this study included fall fertilization and taxa treatments to determine if extending the application of fertilizer in fall affects the cold hardiness of evergreen and deciduous plants differently. Results indicated that nursery growers and landscape managers do not need different fall fertilization schedules for managing the freeze resistance of evergreen versus deciduous azaleas.

Extending fall fertilization through Nov.28, especially at the high rate (125 mg $N \cdot L^{-1}$), may reduce freeze hardiness of azaleas. The moderate rate of extended fertilization (75 mg $N \cdot L^{-1}$ from Aug.1 through Nov.28) decreased freeze hardiness of azaleas in Dec., while the high rate of extended fertilization (125 mg $N \cdot L^{-1}$) reduced freeze hardiness Nov. through February compared to azaleas that received 75 mg $N \cdot L^{-1}$ from Aug. 1 through Sept. 29. Nursery growers interested in building the nutrient reserves of their plants should avoid application of high rates of extended

fertilization, but may benefit from extended fertilization when it is applied at a moderate rate (75 mg $N \cdot L^{-1}$).

Introduction

Plants that maintain their leaves throughout winter may respond differently to fall fertilization than deciduous plants. Leaf retention affects the carbohydrate supply that is required for active nutrient uptake, and the transpiration pull that is responsible for the movement of inorganic ions within the xylem. For fertilizer ions from the substrate to reach the cytoplasm of a leaf or stem cell, they must be actively transported across plasma membranes at least three times (Pitman, 1977; De Boer and Wegner 1997), and carried upward in the xylem by the transpirational stream.

In studies that investigated N uptake of deciduous species, defoliation of *Prunus domestica* L. subsp. *domestica* (Weinbaum et al., 1978) and shade applied to *Malus domestica* Borkh. (Firth and Nichols, 1975) and *Prunus domestica* L. subsp. *domestica* (Therios and Weinbaum, 1979) reduced carbon assimilation and inhibited N uptake. Compared to ungirdled *Prunus persica* L. Batsch, trees that were girdled after apical meristem growth ceased had a 5-fold decrease in N uptake compared to ungirdled trees (Jordan et al., 1998). These studies emphasize the importance of photosynthates from photosynthesizing leaves for the uptake of nitrogen.

Low temperature may reduce transpiration and photosynthetic activity but low temperatures above freezing did not prevent N uptake of *Ilex crenata* Thunb. (Wright et al., 1983), or *Ligustrum* x*ibolium* Coe ex Rehder (Good and Tukey 1969), species with persistent leaves. Nitrogen application rate had more influence on total plant N than temperature when *Ilex crenata* plants were exposed to low temperatures above freezing (Wright et al., 1983).

Fall fertilization may increase spring growth (Good and Tukey, 1969; Bigras et al., 1989; Meyer and Tukey, 1965; Millard and Neilsen, 1989; 1969; Henning et al., 2008) and flower production (Kiplinger and Bresser, 1951). However, fall fertilization may also reduce the freeze resistance of woody ornamental plants (Kelley, 1972; Pellett, 1973; Wright et al., 1978; Henning et al., 2008).

In this research, a deciduous and an evergreen species of the genus *Rhododendron* were compared to determine if extended fertilizer applications in fall affect growth and freeze resistance of evergreen and deciduous plants differently. This information could help nurserymen determine if different fertilization schedules should be used for evergreen versus deciduous plants.

Materials and Methods

Uniform liners of *Rhododendron canescens* (a deciduous azalea) and *Rhododendron* x*satsuki* 'Wakaebisu' (an evergreen azalea) were planted in 2.2-L (#1) containers in a nursery production area at the University of Georgia's Riverbend Horticulture Research Facility in Athens, GA on May 1, 2003. The growing substrate was aged pine bark amended with 6.74 kg · m^{-3} (4 lb · yd^{-3}) dolomitic limestone and 1.67 kg · m^{-3} (1 lb · yd^{-3}) Micromax micronutrient mix (The Scotts Company, Marysville, OH).

Prior to the initiation of fertility treatments on Aug. 1 2003, all plants were fertigated daily with a 0.5 L (0.13 gallon) solution containing 75, 33 and 62.5 mg of N, P and K · L^{-1}. Beginning Aug.1, 2003, each *Rhododendron* taxa was grown under three different fall fertility regimes: 1) 60 days of extended fertilizer application from Aug.1 through Sept. 29 at a moderate rate (75 mg N · L^{-1}), 2) 120 days of extended fertilizer application from Aug.1 through Nov. 28 at a moderate rate (75 mg N · L^{-1}) and 3) 120 days of extended fertilizer application from Aug.1 through

Nov.28 at a high rate (125 mg $N \cdot L^{-1}$). The three fertility treatments were chosen based on a previous study (Henning et al., 2008), in which a high rate (125 mg $N \cdot L^{-1}$) of fertilization extended from Aug.1 through Nov.28 increased growth and decreased freeze resistance of *R.* x*kurume* 'Hinodegiri' compared to plants that received a moderate rate (75 mg $N \cdot L^{-1}$) of fertilization that was extended through either Sept. 29 or Nov. 28.

Fertilizer treatments were applied using a constant liquid feed fertilizer application of Harrell's (Sylacauga, AL) 16-3.5-6.6 (N-P-K) liquid fertilizer solution. Electrical conductivity of leachate was monitored daily using the Virginia Tech Extraction Method (Yeager et al., 1997) to maintain electrical conductivity of the media below 1.0 $dS \cdot m^{-1}$. Floating row cover (Specialty Converting and Supply Inc., Nahsville, GA) was applied for freeze protection when minimum temperatures fell below -6.7 C (20 F).

This experiment was arranged as a 2 x 3 factorial experiment with 2 taxa (*R. canescens and R.* x*satsuki)* and 3 fall fertilization regimes in a Randomized Complete Block Design with 3 replications. Uniform 4 cm stem sections were harvested from three azaleas per treatment-replication combination on Nov.15 and Dec.17, 2003, and Jan.16, Feb.18 and Mar.19, 2004. Four randomly selected stem sections from each replication were exposed to each of 10 progressively lower temperature intervals between -3 and -30 C (26.6 and -22 F) under laboratory conditions in order to estimate azalea freeze resistance (Lindstrom et al., 2002). The Spearman-Karber Method (Bittenbender and Howell, 1974) was used to estimate a LT_{50} value (temperature at which 50% of stems were killed).

On 17 Feb. 2004, stem sections were collected and pooled from five randomly selected azaleas from each Taxa x Fall fertilization replicate in order to determine stem tissue N concentration. Following harvest, stem sections were dried at 55 C (131 F) for 72 hours and

ground to pass a 0.2 mm ($7.9 \cdot 10^{-3}$ inch) mesh screen. Plant materials were analyzed by the UGA Soil, Plant and Water Laboratory for organic N using a LECO CHN-600 analyzer (LECO Corp., St. Joseph, MI).

Flower counts began 15 April 2004 prior to initial bud break and continued until all buds had flowered (F_{max}). Flowering was defined as bud break showing petal color; and flowers were counted on 5 plants per treatment-replication combination three times per week. Linear regression analysis was used to characterize the relationship between day of year and flower count. Regression equations were then used to calculate F_{50} (date which 50% of flowers were in bloom, or $F_{max}/2$) for each treatment-replicate combination.

The same 5 azaleas from each treatment-replication combination used in F_{50} and F_{max} were used to calculate plant growth index (PGI) on 21 May 2004. Maximum height (H), maximum width (W1) and width perpendicular to W1, W2 were used in the calculation GI = (H + (W1 + W2) / 2) / 2.

Stem N concentration, plant growth index, stem freeze resistance, flower production and flower budbreak were analyzed using analyzed using repeated measures analysis of variance (SAS Institute Inc., Cary, NC.). Tukey's HSD was used to test for differences between treatment means variance.

Results and Discussion

Fall fertilization treatments did not affect the growth of *R. canescens* and *R.* x*satsuki* differently. Because there was not a detectable interaction between fall fertilization and taxa treatments, growth data from this study do not support the use of different fertilization regimes for evergreen versus deciduous plants of this species. Previous studies investigated the effects of

fall fertilization on growth of evergreen and deciduous plants in separate studies which prevented direct comparison.

Due to the absence of interaction between fall fertilization and taxa treatments, growth data for the two azalea taxa were pooled within each fertilization treatment (Table 4.1). Extended fertigation through Nov. 28 with either 75 or 125 mg $N \cdot L^{-1}$ did not increase azalea growth compared to azaleas that received 75 mg $N \cdot L^{-1}$ from Aug. 1 through Sept. 29 (Table 4.1). Extended fertilization in fall did not increase azalea growth in this study, but previous horticulture research demonstrated that fall fertilization can increase growth of evergreen [*Rhododendron* x*kurume* 'Hinodegiri' (Henning et al., 2008), *Ligustrum ibolium* Coe ex Rehder and *Euonymus alatus* (Thunb.) Siebold (Good and Tukey, 1969), *Juniperus chinensus* L.(Bigras et al., 1989), *Forsythia* x*intermedia* Zabel (Meyer and Tukey, 1965)], and deciduous plants [*Syringa vulgaris* L. (Meyer and Splittstoesser, 1969)].

The effects of fall fertilization treatments on stem freeze resistance for the evergreen and deciduous taxa treatments were not different. Similar to growth data, cold hardiness data from this study do not support the use of different fall fertilization regimes for evergreen versus deciduous plants.

Fall fertilization and taxa treatments did not interact in their effect on freeze resistance, therefore stem freeze resistance data for the two taxa were pooled within each fall fertilization treatment (Table 4.2). Compared to azaleas that were fertilized with 75 mg $N \cdot L^{-1}$ for 60 days (Aug.1 through Nov. 28), stem tissue from azaleas that received the high rate of extended fertilization for 120 days (125 mg $N \cdot L^{-1}$, from Aug.1 through Nov. 28), had reduced freeze resistance in Nov., Dec., Jan., and Feb. (Table 4.2). However, compared to azaleas fertilized at a moderate rate (75 mg $N \cdot L^{-1}$) from Aug. 1 through Sept. 29, the moderate rate of extended

fertilization (Aug.1 through Nov. 28) only reduced the freeze resistance of azalea stem tissue in Dec. (Table 4.2). In other studies, fall fertilization reduced the freeze resistance of certain container-grown evergreen [*Rhododendron* x*kurume* (Henning et al., 2008), *R. obtusum* (Lindl.) Planch. (Wright et al., 1978), *Pyracantha coccinea* M. Roem. and *Ilex crenata* Thunb. (Kelley, 1972)], and deciduous [*Forsythia intermedia* Zabel (Pellett, 1973)] plants.

Taxa and fertilization treatments affected stem freeze resistance of the azaleas, but when freeze resistance was analyzed over time using repeated measures analysis, the interactive effects of taxa and fall fertilization treatments were not different over time (Table 4.3). The negative effects that high rates of fall fertilization have on the freeze resistance has been attributed to differences in growth cessation (Hellegren, 1981; Glerum, 1985), delayed bud development (Glerum, 1985) and early bud break (Benzian et al. 1974). In this study, fertilization and taxa treatments affected stem freeze resistance, but did not influence the time when freeze resistance increased in fall nor decreased in spring (Table 4.3).

In this study, fertilization with 125 mg $N \cdot L^{-1}$ from Aug.1 to Nov.28 promoted early anthesis of *R.* xsatsuki, but postponed flower budbreak of *R. canescens* compared to plants that received 75 mg $N \cdot L^{-1}$ from Aug.1 to Sept. 29 (Table 4.4). Similar to *R. canescens*, nitrogen applied in late fall to *Prunus persica* delayed the bloom up to 6 days on twig samples that were taken from the trees and placed in a heated greenhouse (Savage, 1970). A delay that was attributed to reduced carbohydrate reserves. However, *R.* xsatsuki, Ilex crenata 'Helleri' and Ilex cornuta 'Burfordi' (Gilliam and Wright, 1977), *Juniperus chinensis* 'pfizerana'(Bigras et al., 1989), and conifer seedlings (Benzian and Freeman 1974; Van den Driessche, 1991, Bigras, 1996) with high tissue nutrient content in fall began growth earlier the next spring.

Flower production of *R. canescens* was not affected by fertilization treatments, but the high rate of extended fertilization (125 mg N · L^{-1} from Aug.1 through Nov.28) more than doubled the flower production of *R.* x*satsuki* compared to plants that received 75 mg · L^{-1} from Aug.1 to Sept. 29 (Table 4.4). Kiplinger and Bresser (1951) found that the number of *Rhododendron* x*kurume* 'Coral Bells' flower buds increased from 126.5 to 170.9 as N applied through 20 Sept. was increased from 10 to 50 mg N · L^{-1}.

In this study, taxa and fertilization treatments interacted in their effect on azalea stem N concentration, which indicated that *R. canescens* and *R.* x*satsuki* responded differently to fall fertilization treatments. Stem N concentration of the deciduous *R. canescens* increased when fall fertilization was extended through Nov. 28 at either the moderate (75 mg N · L^{-1}) or the high rate (125 mg · L^{-1}) compared to plants that received 75 mg N · L^{-1} from 1 Aug. through Nov. 28, but the increase in stem N concentration was not greater in plants that received the high versus the moderate rate of extended fertilization (Table 4.5). One reason why extended fall fertilization through Nov. 28 increased the stem N concentration of *R. canescens* compared to plants that were only fertilized through Sept. 29 is leaf retention. The leaves of the deciduous *R. canescens* plants that received extended fall fertilization remained green and persisted for more than a month after fertilization was terminated Nov. 28, 2004. Active green leaves may have provided the photosynthates and transpirational pump needed for deciduous azaleas to maintain nutrient uptake throughout the period of fall fertilizer application.

Stem N concentration of *R.* x*satsuki* increased when fall fertilization was extended through Nov. 28 and the increase was greater when N was applied at 125 mg · L^{-1} versus 75 mg N · L^{-1}. Daily electrical conductivity measurements of media leachate indicated that fertilizer was removed from the growing media shortly after fertigation was terminated, well before leaves of

either taxa began to abscise. While stem N concentration of the evergreen taxa increased in response to the high rate of N fertilization, this response can not be attributed to the difference in leaf retention between the two taxa.

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Table 4.1. Effect of fertilization treatments on plant growth index (PGI[z]) measured May 21, 2004 and pooled for *R. cane*scens and *R.* xsatsuki 'Wakaebisu'.

N Fertilizer (mg N · L^{-1})		PGI
Aug. 1 to Sept. 29	Sept. 30 to Nov.28	
75[y]	0	46.5 a[x]
75	75	47.8 a
125	125	43.3 a

[z]Maximum height (H), maximum width (W1) and width perpendicular to W1, W2 were used in the calculation GI = (H + (W1 + W2) / 2) / 2.

[y]Data for the two azalea taxa were pooled within each fall fertilization treatment because fall fertilization and taxa treatments did not interact in their effect on growth.

[x]Mean separation within columns by Tukey's Studentized range test at $P \leq 0.05$.

Table 4.2. Effect of fertilization treatments on pooled freeze resistance (LT_{50}[z]) of *R. cane*scens and *R.* x*satsuki* 'Wakaebisu' stem tissue.

N fertilization (mg N · L^{-1})		---------- LT_{50} (C, F) ----------				
Aug. 1 to Sept. 29	Sept. 30 to Nov. 28	Stem harvest dates				
		Nov. 12	Dec. 11	Jan. 14	Feb. 18	Mar. 19
75	0[y]	-6.3 (20.7) b[x]	-20.4 (-4.7)c	-23.8 (-10.8) b	-24.8 (-12.6)b	-15.4 (4.3)a
75	75	-5.6 (21.9)b	-16.8 (1.8)b	-22.3 (-8.1) ab	-22.6 (-8.7)ab	-18.6 (-1.5)a
125	125	-3.4 (25.9)a	-13.6 (7.5)a	-19.9 (-3.8) a	-19.8 (-3.6)a	-19.6 (-3.3)a

[z] Temperature at which 50% of stems were killed

[y] Data for the two azalea taxa were pooled within each fall fertilization treatment because fall fertilization and taxa treatments did not interact in their effect on freeze resistance.

[x]Mean separation within columns by Tukey's Studentized range test at $P \leq 0.05$.

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Table 4.3. Repeated Measures Analysis of Variance for freeze resistance of azalea stems measured monthly Nov. through Mar.

Source of Variation	D.F.	P>F
Rep	2	0.57
Taxa	1	<0.01
Fertilization	2	<0.01
Taxa * Fertilization	2	0.17
Error	10	
Time	4	<0.01
Time * Rep	8	0.85
Time * Taxa	4	0.21
Time * Fertilization	8	0.70
Time * Taxa * Fertilization	8	0.90
Error	40	

Table 4.4. Effect of fertilization on the day of the year in which 50% of flowers were in bloom (F_{50}) and flower production (Fmax).

	N fertilization (mg · L^{-1})			
Taxa	Aug.1 to Sept. 29	Sept. 30 to Nov. 28	Flower Budbreak (F_{50}) Day of the Year	Flower Production
R. canescens	75	0	106.2 b	6.9 a[z]
	75	75	112.1 ab	4.5 a
	125	125	115.2 a	5.7 a
R. x*satsuki*	75	0	127.6 a	26.3 b
	75	75	127.6 a	40.0 ab
	125	125	126.7 b	54.8 a

[z]Mean separation within columns by Tukey's Studentized range test at P≤0.05.

Table 4.5. Effect of fertilization treatments on N concentration of azalea stem tissue harvested Feb.17, 2004.

Taxa	N fertilization (mg · L^{-1})		N Concentration (g · kg^{-1})
	Aug.1 to Sept. 29	Oct. 1 to Nov. 28	
R. canescens	75	0	7.7 b[z]
	75	75	13.5 a
	125	125	14.6 a
R. x*satsuki*	75	0	7.9 c
	75	75	10.7 b
	125	125	13.5 a

[z]Mean separation within columns by Tukey's Studentized range test at P≤0.05.

CHAPTER 5

INFLUENCES OF PHOTOSYNTHETIC PHOTON FLUX AND FALL FERTILIZATION ON FLUORESCENCE, FREEZE RESISTANCE AND GROWTH OF *RHODODENDRON* X*KURUME* 'PINK PEARL'[1]

[1]Henning, F., T. Smalley, O.Lindstrom, Jr., and J. Ruter. To be submitted to J. Environ. Hort.

Influences of photosynthetic photon flux and fall fertilization on fluorescence, freeze resistance and growth of *Rhododendron* x*kurume* 'Pink Pearl'[1]

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Abstract

We investigated the influences of fall fertilization and light intensity on photosynthesis and freeze resistance of *Rhododendron* x*kurume* 'Pink Pearl' grown outdoors in containers under nursery conditions. The study included two main-plot fall fertilization treatments: 1) 0.5 L solution containing 75 mg $N \cdot L^{-1}$ applied for 60 days from Aug.1 through Sept. 29 and 2) 0.5 L solution containing 125 mg $N \cdot L^{-1}$ applied for 120 days from Aug. 1 through Nov. 28, and four subplot light intensity treatments 1) 100% ambient photon flux density (*PPF*) from May 1, 2004 through May 1, 2005, 2) shade fabric rated to reduce *PPF* by 50% from May 1 through Sept. 30, 2004, followed by 100% *PPF* from Oct. 1, 2004 through May 1 2005, 3) 100% *PPF* from May 1 through Sept. 30, 2004 followed by 50% *PPF* from Oct.1, 2004 through May 1, 2005, and 4) 50% *PPF* from May 1, 2004 through May 1 2005. Fertilizer application and light intensity treatments did not interact in their effects on the freezing temperature that killed 50% of stem samples (LT_{50}), or the date when anthesis of 50% of flowers began (F_{50}). The high rate of extended fertigation (125 mg $N \cdot L^{-1}$ applied Aug. 1 through Sept. 28) reduced freeze resistance of azalea stems and advanced anthesis (F_{50}) by 4.9 days compared to plants that received moderate fertigation (75 mg $N \cdot L^{-1}$ from Aug.1 through Sept. 29). The high rate of extended fall fertigation did not increase leaf or stem dry weight compared to plants that received the moderate

rate of fertigation, but plants grown in 50% *PPF* from May 1 through Sept. 30 had greater dry weight compared to plants grown in 100% light during the same time period. The addition or removal of shade cloth beginning Oct. 1 did not enhance azalea stem freeze resistance compared to plants that were only exposed to 100%, or 50% *PPF* respectively. The ratio of variable to maximum fluorescence (F_v/F_m), a non-destructive measure that is inversely proportional to photosynthetic stress, was used to assess Photosystem II function. Light intensity treatments affected leaf F_v/F_m, but fluorescence was not correlated with stem LT_{50}.

Index Words

Rhododendron, x*kurume*, azalea, shade, light, photosynthesis, chlorophyll, cold hardiness, nitrogen, SPAD, fertigation

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Significance to the Nursery Industry

The high rate of extended fertilization used in this study reduced stem freeze resistance, and hastened flowering by nearly 5 days, but did not increase leaf or stem dry weight compared to plants fertilized with the moderate fertilization regime. Azalea growth increased with the application of shade. However, based on the results of this study, shade fabric (rated to reduce

PPF by 50%) has limited value for freeze protection. Neither the application of shade fabric immediately after liners were transplanted in spring (May 1), nor shade fabric applied in fall (Oct. 1) had a biologically significant effect on stem freeze resistance (LT_{50}) compared to plants grown in 100% *PPF*. The ratio of variable to maximum fluorescence (F_v/F_m) is a rapid non-destructive measure that is inversely proportional to photosynthetic stress, and has been used to estimate stem cold hardiness. In this study fluorescence was not a useful indicator of freeze resistance, as azalea stem LT_{50} and F_v/F_m were not related.

Introduction

Rhododendron species are among the most popular woody flowering landscape shrubs grown in nurseries, landscapes, and ornamental gardens (Williams and Mussilo, 1984; Chamberlain et al., 1996). They are often grown under intensive fertilization regimes and are commonly exposed to a wide range of light conditions in their natural habitat (Wang et al., 2008), in the landscape (Anderson et al., 1991) and in nurseries (Anderson et al., 1991; Ingram and Midcap, 1979). These divergent fertility and light regimes could influence cold hardiness.

Previous research found that high rates of extended fall fertilization increased azalea growth, but also reduced cold hardiness (Wright et al., 1978; Henning et al., 2008). High rates of fertilization applied in fall were reported to reduce freeze resistance when growth cessation (Hellegren, 1981; Glerum, 1985) and bud development (Glerum, 1985) are delayed in fall, or when new vegetative growth is promoted either late (Williams et al., 1986; Wright et al. 1978) or early (Benzian et al. 1974) in the growing season.

Azaleas that received some shade during the winter were observed to suffer less freeze damage than plants grown in high light intensities (Bilderbeck and Bir, 1986; Shumack et al., 1995; Sweet, 1960). Low temperatures inhibit photosynthesis and decrease a plant's

photosynthetic quenching capacity when the speed of enzymatic reactions of electron transport and carbon metabolism are reduced, chloroplast membrane function is impaired, or the rate of protein synthesis associated with repair of damaged PSII centers slows (Tiaz and Zeiger, 2006). Shade application in winter decreased photosystem II damage, reduced the incidence and degree of freeze damage and improved *Pseudotsuga menziesii* seedling survival (Orlander, 1993). Shade may be useful for reducing freeze damage in nursery production.

Photosynthesis is a useful measure of plant performance because photosynthesis is sensitive to growth factors including temperature, nutrient availability and light (Larcher 1994). Chlorophyll fluorescence is linearly correlated with the quantum yield of net photosynthesis (Adams et al., 1990), making fluorescence an indirect, non-destructive method for assessing photosynthetic performance. The ratio of variable to maximum fluorescence (F_v/F_m) is a measure of PSII reaction center efficiency that is inversely proportional to photosynthetic stress. Research conducted on *P. menziesii* demonstrated that F_v/F_m ratios were linearly related to stem freeze damage and short-term survival after exposure to sub-freezing temperatures (Birchler et al., 2001; Perks et al., 2004).

In this publication, the term freeze resistance is used to define the ability or capacity of a plant, or plant tissue to survive unfavorable low environmental temperatures (Steponkus, 1984). The development of freeze resistance is a process that is interdependent with other physiological processes (Glerum, 1985). Light intensity (Oleander, 1993; Birchler et al., 2001; Perks et al., 2004) and mineral nutrition (Henning et al., 2008) are know to affect freeze resistance, but little is known about the interactive effects of these variables. Because light intensity and fertility affect freeze resistance, nursery growers may be able to reduce freeze damage and increase flowering, and growth of azaleas by managing fertilization and light intensity concomitantly

during fall and winter. This study was designed to determine if fall fertilization and fall and winter light intensity treatments interact in their effects on *Rhododendron* x*kurume* 'Pink Pearl' stem freeze resistance, photoinhibition , flower production, and growth.

Materials and Methods

On May 1, 2004, 480 uniform *Rhododendron* x*kurume* 'Pink Pearl' liners were transplanted into 2.2 L (#1) containers and grown outdoors in a container nursery production area at the University of Georgia's Horticulture Research facility in Athens, GA. Plants were exposed to ambient air temperature that ranged from a summertime high of 35.3C on Aug. 4, 2004 to a wintertime low of -10.9C on Jan. 24, 2005 (95.5 to 12.4F). The growing substrate was aged pine bark amended with 6.74 kg · m^{-3} (4 lb · yd^{-3}) dolomitic limestone, and 1.67 kg · m^{-3} (1 lb · yd^{-3}) Micromax micronutrient mix (The Scotts Company, Marysville, OH).

This experiment was a split-plot design with two main-plot fall fertilization treatments, four subplot light intensity treatments, and three replications. High and low *PPF* subplot light intensity treatments consisted of uncovered hoop house plots, and plots covered with black woven polypropylene fabric rated to reduce *PPF* by 50% (Progress Grower Supply, Canton, GA) respectively. Immediately after liners were transplanted on May 1, 2004, plants were exposed to one of four subplot light intensity treatments 1) Low light (50% *PPF*) from May 1, 2004 through May 1, 2005, 2) Low light (50% *PPF*) May 1, through Sept. 30, 2004, followed by High Light (100% *PPF*) from Oct. 1, 2004 through May 1, 2005, 3) High light (100% *PPF*) from May 1, through Sept. 30, 2004 followed by Low light (50% *PPF*) from Oct.1 through May 1, 2005, or 4) High Light (100% *PPF*) from May 1, 2004 through May 1, 2005. Daily *PPF* was measured throughout the study in high and low light plots using LI-190 sensors (Li-Cor, Lincon, NE) located in covered and uncovered plots. During the course of the study, daily *PPF* ranged from

1.2 to 47.5 mol $\cdot$ $m^{-2}\cdot$ d^{-1} in high light plots and from 0.7 to 21.7 mol $\cdot$ $m^{-2}\cdot$ d^{-1} in low light plots. Shade fabric reduced daily *PPF* 43 – 67% compared to the light level of uncovered plots.

The main-plot fertility treatments used in this experiment were chosen based on a previous study conducted by Henning et al. (2008), in which a high rate of fertigation (125 mg N $\cdot$ L^{-1}) extended for 120 days (from Aug. 1 through Nov. 28) increased growth and decreased freeze resistance of *Rhododendron* x*kurume* compared to plants that received a moderate rate of fertigation (75 mg N $\cdot$ L^{-1}) that was extended either 60 days (though Sept.29), or 120 days (through Nov. 28). Prior to the initiation of fertigation treatments on Aug. 1, all plants were fertigated daily with a 0.5 L (0.13 gallon) solution containing 75 mg N $\cdot$ L^{-1} applied using a constant liquid feed fertilizer application of Harrell's (Sylacauga, AL) 16-3.5-6.6 (N-P-K), N as NO_3 (1.6%), and urea (14.4%). The two main-plot fertigation treatments were initiated Aug.1, 2004. Plants received either 1) 60 days of extended fertigation from Aug.1 through Sept. 29 at a moderate rate (75 mg N $\cdot$ L^{-1}), or 2) 120 days of extended fertigation from Aug. 1 through Nov. 28 at a high rate (125 mg N $\cdot$ L^{-1}).

To analyze azalea stem freeze resistance, uniform stem sections were harvested from 72 plants, three azaleas per treatment-replication combination, on Nov. 22 and Dec.21, 2004 and Jan.19, Feb. 21 and Mar. 22, 2005. Stem sections were only collected one time from each azalea plant that was sampled. Samples from each treatment-replication combination were combined, wrapped in moist paper towels, sealed in plastic bags, and placed on ice for transport to the lab. Within four hours of collection, stem sections were prepared for freezing. Stem freeze resistance was estimated using the technique outlined by Lindstrom et al. (2002). Forty, 5 cm (2 inch) long stem segments from each treatment–replication combination were analyzed under laboratory conditions. Four stem segments from each treatment-replication combination were removed from

a freezing temperature bath at 3C (5F) temperature intervals. Samples were exposed to temperatures between -3 and -27C (26.6 and -16.6F) for freeze analysis in Nov., Dec. and Mar., and to temperatures between -6 and -30C (21.2 and -22F) in Jan. and Feb. Four stem segments were kept at 4C (39F) for the duration of the freezing test and used as controls for comparison with frozen samples. The Spearman-Karber Method (Bittenbender and Howell, 1974) was used to estimate the freezing temperature at which 50% of stem samples were killed (LT_{50}).

Modulated chlorophyll fluorescence was measured outdoors in experimental plots with a portable fluorometer (pulse amplitude modulated fluorimeter, miniPAM Photosynthesis Yield Analyzer, Heinz Walz GmbH, Effeltrich, Germany). Five leaves were analyzed on five plants from each treatment-replication combination on Dec. 23, 2004 and Jan. 21, Feb.23 and Mar. 24, 2005. Readings were taken on the upper most fully expanded, mature leaves located below the terminal bud of hardened new growth. Leaves were analyzed prior to sunrise to ensure that they were dark-adapted and that energy quenching was relaxed. Leaf portions were exposed to a saturating pulse (>8000 $\mu mol.m^{-2}.s^{-1}$) to obtain a dark adapted measure of quantum efficiency of photosystem II (F_v/F_m). MINI-PAM fluorescence measurements were averaged within each treatment-replication combination.

A SPAD-502 chlorophyll meter (Minolta, Ramsey, N.J.) was used to estimate total leaf chlorophyll content (Markwell et al., 1995) on the same 5 plants that were used for fluorescence analysis. SPAD readings were taken on 5 leaves per plant, midway between the leaf mid vein and margin and these measurements were averaged within each treatment-replication combination.

Flower counts began March 1, 2005, prior to anthesis and continued to full flower expression (F_{max}). Flowering was defined as budbreak showing petal color. Flowers were counted three

times weekly on the same five plants per treatment-replication combination that were used for fluorescence and chlorophyll analyses. Flower counts were averaged within each treatment-replication combination. Linear regression analysis was then used to characterize the relationship between the day of year and flower count. An equation for the relationship between flower count and day number was developed for each of the 24 treatment-replicate combinations, then the corresponding $F_{max}/2$ was used to calculate F_{50} (date which 50% of flowers were in bloom).

The five azaleas from each treatment-replication combination that were used for chlorophyll and flower analysis were harvested May 1, 2005, separated into leaves and stems, and dried at 55C (131F) for 72 h. Leaf, stem and total above ground dry weight were determined and averaged within each treatment-replication combination.

In order to monitor changes that occur over the time period between fall and spring, the interactive effects of fertilization and light intensity treatments on stem freeze resistance, chlorophyll fluorescence and SPAD chlorophyll readings were analyzed using repeated measures GLM procedure (SAS Institute Inc., Cary, NC). Dry weight, flower budbreak and flower production were analyzed using the GLM procedure (SAS Institute Inc., Cary, NC). Tukey's studentized range (HSD) means comparison test was used to detect differences in pairwise comparisons of treatment means.

Results and Discussion

Analysis of variance over time revealed that fall fertilization and light intensity treatments did not interact in their influence on azalea freeze resistance, chlorophyll fluorescence, F_{50}, or dry weight. In the absence of interaction, data for the four light intensity treatments were pooled within each fall fertilization treatment and data for the two fertilization treatments were pooled with each light intensity treatment.

The high rate of extended fertilization (125 mg $N \cdot L^{-1}$, applied Aug. 1 through Nov. 30) reduced freeze resistance (LT_{50}) of azalea stems from every harvest and reduced LT_{50} of stems harvested Nov. 22 by more than 6C compared to a moderate rate of fertilization (75 mg $N \cdot L^{-1}$, applied Aug.1 through Sept. 30) (Table 5.1). In a previous study with *Rhododendron* x*kurume* 'Hinodegiri', high rates of extended fertigation reduced cold hardiness of azaleas compared to treatments that received moderate rates of fertilization (Henning et al., 2008).

The photosynthetic activity and efficiency of photosystem II (F_v/F_m) of evergreen species are often reduced by exposure to cold temperatures, and cold acclimation (Demmig-Adams and Adams, 1992; Adams et al., 1994). Maximal quantum yield of dark-adapted R. x*kurume* leaves in this study were below the expected maximum yield (0.83), but were comparable to values observed by Wang et al. (2008) who reported F_v/F_m values for *R. ponticum* and *R. catawbiense* of 0.76 and 0.74 for nonacclimated leaves in Aug., and 0.66 and 0.59 for cold acclimated leaves in Nov. (Table 5.2). Nitrogen is a major component of the photosynthetic apparatus, including chlorophyll *a* and *b*, and RuBisCO. The same fall fertilization treatments that produced differences in azalea stem LT_{50} had no detectable effect on F_v/F_m (Table 5.2). Consistent with the results for the moderate (75 mg $N \cdot L^{-1}$, applied Aug.1 through Sept. 29) and high rate of extended fertigation (125 mg $N \cdot L^{-1}$, applied Aug. 1 through 28) used in this study, Birchler (2001) detected no differences in chlorophyll fluorescence (F_v/F_m) of fertilized Douglas Fir seedlings that received 80, 160 or 320 kg $N \cdot ha^{-1}$. The effects of fertilization on fluorescence in this study extend the findings made on conifer species in previous studies to a woody angiosperm ornamental, that within the range where N was neither deficient nor toxic, additional N had little effect on light harvesting (Birchler et al., 2001; Hawkins et al., 1995).

Shade removal on Oct 1 and the associated increase in light intensity did not enhance azalea freeze resistance compared to plants grown in low light (50% *PPF*) throughout the study (Table 5.3). Exposure to low light (50% *PPF*) from May 1 through Sept. 30, 2004, followed by shade cloth removal and exposure to high light (100% *PPF*) beginning Oct. 1., produced azaleas that were less freeze resistant on Nov. 22 than plants grown in high light throughout the study (Table 5.3). These findings contrast with observations made on nursery plants grown in North Carolina (Bilderbeck and Bir, 1986), that shade cloth removal in fall induced more rapid cold acclimation and decreased stem splitting.

In Jan., azalea stems harvested from plants that only received low light (50% *PPF*) were more freeze resistant than plants with 100% *PPF* (Table 5.3). A small difference in stem freeze resistance in Jan. has limited biological significance for most regions of the temperate zone because freeze injury most frequently occurs during the fall or spring when the species is not at its maximum hardiness (Williams et al., 1986). In addition, fall application of shade (50% *PPF* on Oct. 1) to plants that were previously grown in high light (100% *PPF*) did not affect stem freeze resistance compared to plants that were grown in high light throughout the study. Results from this study contrast with the previous reports by Sweet (1960) who found a positive relationship between light intensity and freeze damage (stem splitting) in *Rhododendron* x*kurume* and *R. simsii* grown in Georgia that were exposed to light intensities that varied from 28 to 100% *PPF*, by Shumack et al. (1994), who observed that azaleas in Alabama that received shade during winter suffered less cold damage, and by Bilderbeck and Bir (1986) who noted that nursery plants in North Carolina acclimate to freezing temperatures more slowly in shade than in the sun. The apparent contrast in results is because we were measuring freeze tolerance and the

other authors were measuring freeze damage. We found that the effects of shade application on azalea freeze tolerance were minimal, but shade can influence radiative heating and cooling, reduce freeze desiccation and photosynthetic excitation, which can reduce freeze damage without affecting freeze tolerance.

Treatments that exposed plants to 100% *PPF* after Oct. 1 increased azalea photoinhibition (F_v/F_m was lower) in Dec. compared to plants exposed to 50% *PPF* after Oct. 1 (Table 5.4). These data support research reporting that cold acclimation and low temperatures reduce the range of light intensity to which a plant can acclimate (Strand and Lundmark, 1987; Oquist, 2001).

Photoinhibition was greater (F_v/F_m was lower) from Dec. through Mar. for azaleas that were grown in 50% *PPF* between May 1 and Sept. 30 followed by 100% *PPF* from Oct. 1 though harvest, compared to azaleas that received the converse treatment (100% *PPF* between May 1 and Sept. 30 followed by 50% *PPF* from Oct. 1 through harvest). One explanation for these results is acclimation of the xanthophyll cycle, which plays a major role in protective energy dissipation when photosynthetic excitation is excessive. Demmig-Adams et al. (1995) found that leaves that developed in high light contained greater concentrations of xanthophyll cycle components than leaves developed in low light conditions on the same plant.

High light intensities in fall and winter may affect the freeze resistance of plants when excessive excitation of the plant photosynthetic apparatus results in photoinhibition or photoxidation (Minkov et al., 1999). Based on linear correlations between chlorophyll fluorescence (F_v/F_m) of needles and LT_{50} of Douglas-fir seedling stems that were tested monthly under laboratory conditions, Perks et al. (2004) demonstrated that F_v/F_m could be used to accurately predict stem LT_{50}, and developed $F.LT_{50}$ (fluorescence-based empirical determination

of LT_{50}). Using Pearson's correlation, leaf fluorescence (F_v/F_m) and LT_{50} were not correlated (r = 0.02) in our study. Fertilization and light treatments affected azalea stem freeze resistance (Tables 5.1 and 5.3) and light treatments affected leaf chlorophyll fluorescence (Table 5.4), but azalea stem freeze resistance and leaf fluorescence were not related.

SPAD-502 chlorophyll meter (Minolta, Ramsey, NJ), a non-destructive measure of leaf chlorophyll concentrations (Olsen et al., 2002, Markwell et al., 1995), and fluorescence (F_v/F_m), a non destructive measure of PSII reaction center efficiency (Demig-Adams and Adams., 1992) were not correlated (r = 0.20). These results support those of Olsen et al. (2002) who found that chlorophyll concentration were poorly fitted to SPAD readings.

In this study, SPAD did not indicate that high light increased photobleaching, as SPAD readings from azaleas that were grown in 100% *PPF* throughout the study were not reduced compared to plants grown in 50% *PPF* (Table 5.5).

The manufacturer of the SPAD-502 does not recommend the use of this instrument on leaves with measured values greater than 50. Values that exceeded 50 were reported in this study, but SPAD data had low standard errors and were tightly clustered.

Photosynthetic adaptations to light intensity include biochemical, physiological and morphological changes (Larcher, 1994). Based on visual observations made in this study, morphologic adaptations to high irradiance were present. Leaves from plants that were grown in 100% *PPF* from May 1 through Sept. 30 were not chlorotic, but they appeared smaller and thicker than plants that those of plants that were exposed to 50% *PPF* during the same time period. These observations are similar to those of Anderson et al. (1991), who reported reduced average, and total leaf area, and an increase in specific leaf mass among azaleas that were exposed to 100% compared to 47% *PPF* and irrigated so that soil moisture was not limiting.

Nitrogen is an integral component of the photosynthetic apparatus and is linked to photosynthesis and the ability of plants to orderly dissipate light. Birchler et al. (2001) found that chlorophyll fluorescence of fertilized *Pseudotsuga menziesii* seedlings was constantly higher than that of unfertilized seedlings. Fertilization and light intensity interacted in their effect on SPAD readings. Low rates of photosynthesis and low capacity for energy dissipation predispose the photosynthetic apparatuses of shade-adapted plants to photoinhibition. Among plants fertigated 60 days at a moderate rate (75 mg $N \cdot L^{-1}$ applied from Aug. 1 through Sept. 29), exposure to 100% *PPF* from May 1 through Sept.30 followed by exposure to 50% *PPF* increased SPAD readings in Dec., Jan., and Feb. compared to other treatments grown under the same fertigation regime (Table 5.5). SPAD results suggest that plants acclimated to 100% *PPF* conditions and experienced less chlorophyll damage in winter when moved to 50% *PPF*.

Contrasting reports appear in the horticultural literature for effects of light intensity on the timing of azalea anthesis. *Rhododendron* x*kurume* 'Coral Bells' that were grown outdoors and shaded by a lath bloomed before plants that were exposed to 100% *PPF* (Kiplinger and Bresser, 1951). When 15 varieties of azaleas were grown under greenhouse conditions, the average bloom date was 1 day later for plants grown in 50% *PPF* compared to 100% *PPF* (Stuart, 1964).

In a review of freeze protection practices, Rieger (1989) concluded that once the chilling requirement has been met, phenological development of flower buds on fruit trees is related to the number of degree-hours that are accumulated. Reducing temperatures by sprinkling to promote evaporative cooling delayed anthesis of apples by up to 17 days (Anderson et al., 1975), and peaches by up to 15 days (Chesness et al., 1977). Radiative heating in high light increased plant tissue temperatures up to 15 C above ambient air temperature on bright, clear winter days (Jensen et al., 1970). In this study, we hypothesized that the heat load reductions associated with

shade would delay anthesis. The average daily maximum temperature between Jan. 1 and Mar. 31, 2005 measured 18.2 C in low light (50% *PPF*) versus 21.4 C in high light (100% *PPF*) plots. Unlike temperature reductions associated with irrigation during the period of degree-hour accumulation, the lower temperatures associated in 50% *PPF* plots did not postpone azalea $F_{50,}$ the date when 50% of flowers were in bloom (data not shown). The absence of an anthesis response to different light intensity treatments may be partially explained by Anisko et al. (1994) who reported that azalea tissues deharden rapidly when exposed to warm air temperatures once chilling requirements have been satisfied.

In contrast with the absence of a response to light intensity treatments, azalea anthesis was affected by fall fertilization. Azaleas that received the high rate of extended fertilization (125 mg $N \cdot L^{-1}$ from Aug. 1 through Nov. 28) bloomed nearly 5 days earlier than plants that received moderate fertilization (75 mg $N \cdot L^{-1}$ from Aug.1 through Sept.29) (Table 5.6). Previous studies reported that Ilex crenata 'Helleri' and Ilex cornuta 'Burfordi' (Gilliam and Wright, 1977), *Juniperus chinensis* 'pfizerana'(Bigras et al., 1989), and conifer seedlings (Benzian and Freeman 1974; Van den Driessche, 1991, Bigras ,1996) with high tissue nutrient content in fall begin growth earlier the next spring.

Fertigation and light treatments interacted in their effect on flower production (Table 5.7). However, regardless of the fertilizer treatment, more flowers were produced by plants that were grown in 50% *PPF* throughout the study compared to other light treatments. These results are similar to those of Sweet (1960), who found that flower production of 'Hinodegiri', 'Snow', and 'Formosa' azaleas increased when light intensity was reduced.

The dry weights of plants that received 60 days of extended fall fertigation at a moderate rate, and those that received 120 days of extended fertigation at the high rate did not differ (Table

5.8). Regardless of the fertilization treatment, leaf, stem and total above ground dry weight were greater among azaleas that were grown in 50% *PPF* from May 1 through September 30, than among plants grown in 100% *PPF* during the same time period (Table 5.8). Anderson et al. (1991) grew *Rhododendron* x 'Pink Ruffles' under three light intensities and found that plants grown in 29% PPF had less photoinhibition, less photooxidation, and greater growth than plants grown in 100% *PPF*.

This study provides additional data to support the conclusion that high rates of extended fall fertilization should be discouraged. High rates of extended fall fertilization reduced stem freeze resistance and promoted early anthesis without increasing DW. Shade treatments did not have a biologically significant impact on azalea cold hardiness, or the timing of flower anthesis. The application of shade fabric moderated air temperatures, reduced photosynthetic damage and promoted increases in azalea dry weight, and flower production. Reports of reduced freeze damage on plants grown in shaded versus exposed sites were likely caused by indirect effects of the shade canopy on freeze desiccation, air temperature, or photosynthetic damage rather than a direct effect on freeze resistance. Chlorophyll fluorescence was not related to stem freeze resistance, and was not a useful predictor of stem LT_{50}.

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Table 5.1. Effects of fall fertigation treatments on pooled[z] freeze resistance (LT_{50}[y]) of stem tissue of *R.* x*kurume* 'Pink Pearl' grown in Athens, GA.

N Fertilizer (mg $N \cdot L^{-1}$)		LT_{50} (^oC)				
		Stem Harvest Dates				
Aug. 1 to Sept. 29	Sept. 30 to Nov. 28	Nov. 22	Dec. 21	Jan. 19	Feb. 21	Mar. 22
75	0	-10.6 b[x]	-21.8 b	-24.8 b	-19.5 b	-9.9 b
125	125	-4.4 a	-10.6 a	-20.9 a	-14.9 a	-4.0 a

[z] Data for the four light intensity treatments were pooled within each fall fertilization treatment because fall fertilization and light treatments did not interact in their effect on freeze resistance.

[y] Temperature at which 50% of stems were killed

[x] Mean separation within columns by Tukey's Studentized range test at $P \leq 0.05$.

Table 5.2. Effects of fall fertigation treatments on pooled[z] chlorophyll fluorescence (F_v/F_m) of *R.* x*kurume* 'Pink Pearl' leaves.

N Fertilization (mg $N \cdot L^{-1}$)		Chlorophyll fluorescence (F_v/F_m)			
Aug. 1 to Sept. 29	Sept. 30 to Nov. 28	Measurement Dates			
		Dec. 23	Jan. 21	Feb. 23	Mar. 24
75	0	0.66 a[y]	0.66 a	0.66 a	0.66 a
125	125	0.70 a	0.65 a	0.66 a	0.66 a

[z] In the absence of interaction, data from 4 light intensity treatments were pooled within each fall fertilization treatment, so that each mean is based on observations of 5 measurements from 20 plants in each of three replicates.

[y]Mean separation within columns by Tukey's Studentized range test at $P \leq 0.05$.

Table 5.3. Effects of light intensity (*PPF*) on stem tissue freeze hardiness (LT_{50}) of *R.* x*kurume* 'Pink Pearl'.

Light intensity (% *PPF*)		---------- LT_{50} (^{o}C) ----------				
May 1 to Sept. 30	Oct.1 to May 1	Stem Harvest Dates				
		22 Nov.	21 Dec.	19 Jan.	21 Feb.	22 Mar.
50	50	-6.6 ab[zy]	-14.1 a	-24.9 b	-15.8 a	-7.9 a
50	100	-5.1 a	-15.9 a	-23.0 ab	-17.1 a	-6.5 a
100	50	-8.8 ab	-17.0 a	-21.9 ab	-17.1 a	-6.9 a
100	100	-9.5 b	-17.9 a	-21.6 a	-18.8 a	-6.6 a

[z]In the absence of interaction, data from two fertilization treatments were pooled within each light intensity treatment, so that each mean is based on observations of 8 stem samples at each of 9 different freezing temperature increments for each of 3 replicates.

[y]Mean separation within columns by Tukey's Studentized range test at $P \leq 0.05$.

Table 5.4. Effects of light intensity on chlorophyll fluorescence (F_v/F_m) of *R.* x*kurume* 'Pink Pearl' leaves.

Light intensity (% *PPF*)		Chlorophyll fluorescence (F_v/F_m)			
May 1 to Sept. 30	Oct.1 to May 1	Measurement Dates			
		23 Dec.	21 Jan.	23 Feb.	24 Mar.
50	50	0.70 a[zy]	0.67 ab	0.67 ab	0.68 ab
50	100	0.64 b	0.59 b	0.60 b	0.61 b
100	50	0.74 a	0.72 a	0.72 a	0.72 a
100	100	0.64 b	0.63 ab	0.65 ab	0.65 ab

[z] In the absence of interaction, data from two fertilization treatments were pooled within each light intensity treatment, so that each mean is based on observations of 5 measurements from 10 plants in each of three replicates.

[y]Mean separation within columns by Tukey's Studentized range test at $P \leq 0.05$.

Table 5.5. Effects of daily 0.5L fall fertigation and light intensity treatments on *R.* x*kurume* 'Pink Pearl' SPAD-502 chloropyhll meter reading (SPAD).

N Fertilization (mg $N \cdot L^{-1}$)		Light Intensity (% *PPF*)		SPAD			
				Measurement Dates			
Aug.1 to Sept. 29	Sept.30 to Nov. 28	May 1 to Sept. 30	Oct. 1 to May 1	Dec. 23	Jan. 21	Feb. 23	Mar. 24
75	0	50	50	43.4 b[zy]	42.6 b	40.1 b	44.3 ab
75	0	50	100	38.5 b	37.5 b	34.7 b	40.6 b
75	0	100	50	54.0 a	51.2 a	47.3 a	47.8 a
75	0	100	100	43.2 b	40.9 b	38.6 b	42.3 ab
125	125	50	50	53.2 a	52.0 a	50.2 a	44.2 ab
125	125	50	100	55.2 a	52.8 a	50.6 a	40.0 b
125	125	100	50	53.8 a	52.5 a	53.3 a	48.9 a
125	125	100	100	55.6 a	54.7 a	54.0 a	44.1 ab

[z] Each mean based on observations of 5 measurements from 5 plants in each of three replicates.

[y]Mean separation within columns by Tukey's Studentized range test at $P \leq 0.05$.

Table 5.6. Effects of daily 0.5L fall fertigation treatments on the day of the year when 50% of *R.* x*kurume* 'Pink Pearl" flowers were in bloom (F_{50}).

N Fertilization (mg $N \cdot L^{-1}$)			
Aug. 1 to Sept. 29	Sept. 30 to Nov. 28	F_{50} Day of the Year	
75	0	101.2	a[zy]
125	125	96.3	b

[z] In the absence of interaction, data from 4 light intensity treatments were pooled within each fall fertilization treatment, so that each mean is based on observations of 20 plants in each of three replicates.

[y]Mean separation within columns by Tukey's Studentized range test at $P \leq 0.05$.

Table 5.7. Effects of daily 0.5L fall fertigation and light intensity treatments on total flower production of *R.* x*kurume* 'Pink Pearl'.

N Fertilizer (mg $N \cdot L^{-1}$)		Light intensity (% *PPF*)			
Aug. 1 to Sept 29	Sept. 30 to Nov. 28	May 1 to Sept. 30	Oct. 1 to May 1	Flower Production	
75	0	50	50	185	a[zy]
75	0	50	100	142	b
75	0	100	50	155	b
75	0	100	100	129	b
125	125	50	50	235	a
125	125	50	100	177	b
125	125	100	50	97	c
125	125	100	100	140	cb

[z] Each mean based on observations of 5 plants in each of three replicates.

[y]Mean separation within columns by Tukey's Studentized range test at $P \leq 0.05$.

Table 5.8. Effect of light intensity treatments on leaf, stem and total above ground dry weight of R. x*kurume* 'Pink Pearl' plants.

Light Intensity (%*PPF*)		Dry weight (g)					
May 1 to Sept. 30	Oct. 1 to May 1	Leaf		Stem		Total	
50	50	21.3	a[z,y]	18.4	a	39.7	a
50	100	19.1	a	17.1	a	36.3	a
100	50	10.0	b	4.8	b	14.5	b
100	100	9.7	b	4.3	b	14.4	b

[z] In the absence of interaction, two fertilization treatments were pooled within each light intensity treatment, so that each mean is based on observations of 10 plants in each of three replicates.

[y] Mean separation within columns by Tukey's Studentized range test at $P \leq 0.05$.

CHAPTER 6

CONCLUSIONS

Previous research has demonstrated that the survival mechanisms plants use to adapt and respond to freezing temperature stresses can be affected by temperature, light intensity, and nutrients (Glerum, 1985). In addition to the influences that single factors or stressors have, multiple cultural practices may produce stresses that combine in their effect on freeze tolerance of plants (van den Driessche, 1991). Studies that simultaneously apply multiple cultural practices known to affect plant freeze tolerance in the same experiment in order to investigate the interactive effects are rare, and interactive effects are not well understood. The objectives of this research were to investigate the influences of fall fertilization rate, fall fertilization timing, leaf retention, and light intensity on the freeze resistance, growth, photosynthesis, and flower production of azaleas.

This research demonstrated that interactions between fertilizer application rate and timing influenced growth and freeze resistance of container-grown *R.* x*kurume* 'Hinodegiri'. When fall fertilization was extended 60 days at a moderate rate (75 mg $N \cdot L^{-1}$ from Aug.1 through Sept. 29), growth increased with no effect on azalea freeze resistance compared to plants that received no additional fertilization after Jul. 31. When fall fertilization was extended 120 days (75 mg $N \cdot L^{-1}$ from Aug. 1 through Nov. 28) there was neither increased growth, nor decreased freeze resistance compared to azaleas that were fertilized at a moderate rate (75 mg $N \cdot L^{-1}$) from Aug.1 through Sept. 29. A high rate of fertilization (N at 125 mg $\cdot L^{-1}$) increased azalea DW production. However, the high rate of fertilization reduced freeze hardiness in fall and early spring even

when fertilization is terminated Sept. 29, well before the first freeze event. The results indicated that azalea growers in Zone 7 should maintain moderate fertility levels through late Sept. This study demonstrates that sustaining fertilization past late Sept. has few growth benefits, and high rates of fertilization promote freeze damage.

Plants that maintain their leaves throughout winter may respond differently to fall fertilization than deciduous plants. Previous studies did not investigate interactions between leaf retention (evergreen versus deciduous) and fall fertilization.

Taxa and fertilization treatments interacted in their effect on azalea stem N concentration, an indication that *R. canescens* and *R.* x*satsuki* responded differently to fall fertilization treatments. Stem N concentration of the deciduous *R. canescens* increased when fall fertilization was extended through Nov. 28 at either the moderate (75 mg $N \cdot L^{-1}$) or the high rate (125 mg $\cdot L^{-1}$) compared to plants that received a moderate fertilization (75 mg $N \cdot L^{-1}$) from Aug. 1 through Nov. 28. However, the increase in stem N concentration was not greater in plants that received the high (125 mg $\cdot L^{-1}$) versus the moderate rate (75 mg $N \cdot L^{-1}$) of extended fertilization. Leaves of the deciduous *R. canescens* that received extended fall fertilization remained green and persisted for more than a month after fertilization was terminated Nov. 28, and continued to provide the photosynthates and transpirational pump needed for these deciduous azaleas to maintain nutrient uptake throughout the period of fall fertilizer application. Stem N of the evergreen *R.* x*satsuki* increased when fall fertilization was extended through Nov.28, and the increase was greater when N was applied at 125 mg $\cdot L^{-1}$ versus 75 mg $N \cdot L^{-1}$. Differences in N uptake between *R.* x*satsuki* and *R. canescens* were attributed to *R.* x*satsuki*'s capacity to sustain uptake and storage of nutrients, perhaps because its evergreen leaves were metabolically active for a longer period of time in the fall.

Despite differences in stem N concentrations that were produced by fall fertilization treatments, fertilization treatments did not interact in their effects on growth or freeze resistance of evergreen versus deciduous azaleas. The moderate rate of extended fertilization (75 mg $N \cdot L^{-1}$ from Aug.1 through Nov. 28) decreased freeze hardiness of both azaleas in Dec., while the high rate of extended fertilization (125 mg $N \cdot L^{-1}$) reduced freeze hardiness Nov. through Feb., compared to azaleas that received moderate fertilization (75 mg $N \cdot L^{-1}$) from Aug. 1 through Sept. 29.

The results of the two aforementioned studies indicate that nursery growers interested in building the nutrient reserves of their plants should avoid the application of high rates of extended fertilization, but may benefit from extended fertilization when it is applied at a moderate rate. Notwithstanding effects on nutrient reserves, the absence of interaction between fall fertilization and taxa treatments indicate that nursery growers and landscape managers do not need different fall fertilization schedules to manage growth and freeze resistance of evergreen versus deciduous azaleas.

Analysis of variance over time revealed that fall fertilization and light intensity treatments did not interact in their influence on *Rhododendron, xkurume* freeze resistance, chlorophyll fluorescence, F_{50} (the date which 50% of flowers were in bloom), or DW. The high rate of extended fertigation (125 mg $N \cdot L^{-1}$, applied Aug. 1 through Nov. 30) reduced LT_{50} of stems harvested Nov. 22 by more than 6C , and reduced freeze resistance (LT_{50}) of azalea stems that were harvested monthly from Nov. through Apr. compared to plants that received a moderate rate of fertilization (75 mg $N \cdot L^{-1}$) applied Aug.1 through Sept. 29). These data support the conclusion that high rates of extended fertilization should be discouraged.

Previous reports indicated that nursery growers could reduce freeze damage by managing light to reduce (Oleander, 1993; Shumack, 1997; Sweet, 1960), or increase (Bilderbeck 1986) light intensity during fall and winter. This study provides no support for altering light intensity in fall, as neither an increase in light intensity from 50% *PPF* to 100% *PPF*, nor a decrease from 100% *PPF* to 50% *PPF* on Oct. 1 had a biologically significant influence on azalea freeze resistance compared to plants that were grown throughout the study in 100% or 50% *PPF* respectively. While shade had minimal affects on freeze resistance, it increased azalea growth, as DW of azaleas was greater among grown in 50% *PPF* from May 1 through Sept. 29 versus plants grown in 100% *PPF* during the same time period. So, growers should consider shade for its influence on growth and protective affects rather than direct affects on cold hardiness. Shade moderates radiative heating and cooling, and reduces freeze desiccation, and photosynthetic excitation, but does not have a biologically significant affect on freeze resistance.

Fluorescence-based empirical determination of LT_{50} ($F.LT_{50}$) was recommended as a non-destructive alternative to LT_{50} for estimating low temperature survival of seedling trees (Perks et al., 2004; Orlander, 1993). Fluorescence and freeze resistance data from this study do not support the link between *Fv/Fm* and LT_{50} for azaleas, and suggest that fluorescence may have limited usefulness for predicting LT_{50} of azaleas.

We hypothesized that the heat load reductions associated with the application of shade fabric would delay anthesis, but reduced heat loads associated with shade application during the period of degree-hour accumulation did not postpone azalea $F_{50.}$ However, azalea anthesis was affected by fall fertilization. Fertigation treatments that hastened azalea anthesis also reduced stem freeze resistance, and support the correlation between anthesis and loss of stem tissue freeze resistance.

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