5-2014

INCREASING NURSERY CROP CANOPY DENSITY: IMPLICATIONS FOR SUSTAINABLE INSECT PEST MANAGEMENT

Whitney Michelle Yeary
University of Tennessee - Knoxville, wmcnutt1@utk.edu

To the Graduate Council:

I am submitting herewith a thesis written by Whitney Michelle Yeary entitled "INCREASING NURSERY CROP CANOPY DENSITY: IMPLICATIONS FOR SUSTAINABLE INSECT PEST MANAGEMENT." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

Amy Fulcher, Major Professor

We have read this thesis and recommend its acceptance:

William Klingemen, Jerome F. Grant

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

INCREASING NURSERY CROP CANOPY DENSITY: IMPLICATIONS FOR SUSTAINABLE INSECT PEST MANAGEMENT

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Whitney Michelle Yeary
May 2014

DEDICATION

I would like to dedicate this work to my husband, Robin, who put up with me when I was stressed, listened when I needed to vent, and held me when I needed it the most. I'm the luckiest girl in the world because you love me.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my major professor, Dr. Amy Fulcher for her guidance and encouragement during my graduate study here at the University of Tennessee.

I would like to thank my other committee members Dr. William E. Klingeman and Dr. Jerome F. Grant for their valuable suggestions and assistance on this project. I have learned more practical knowledge from them than from any other source.

Special thanks to Xiaocun Sun for her irreplaceable statistical analysis assistance.

Ed Kinsey of Kinsey Gardens and Dale Bryan of Freedom Tree Farm also deserve thanks for their contribution of plant material. Ed Kinsey was also a wealth of information on several topics.

Lastly, I would like to thank Deulin' and Banjo for slobbery kisses, shenanigans, and the reminder to take a break every now and then.

ABSTRACT

Consumers are attracted to woody ornamental plants that have symmetrical, dense canopies. In order to get the desired canopy density and symmetry, growers often manipulate growth by pruning or applying chemical plant growth regulators. Another method of acquiring a dense plant canopy is for growers to purchase in vitro-propagated liners instead of traditional cutting-propagated liners. This work analyzed the validity of all three methods on several woody ornamental species. Liners from Cutting-propagated (CP) and in vitro-propagated (IVP) sources were purchased and treatments of pruning and PGRs were applied. Pruning only increased the canopy density of rhododendron (*Rhododendron* L. 'Roseum Elegans') and was even more effective when IVP plants were pruned. PGRs were generally ineffective on all species with the exception of blueberry (*Vaccinum corymbosum* L. 'Duke'). IVP clethra (*Clethra alnifolia* L. 'Hummingbird') and rhododendron had greater canopy density than their CP counterparts.

A dense plant canopy attracts customers more easily than a sparse canopy. However, as canopy density increases, the grower's ability to achieve adequate spray penetration within the canopy decreases, causing insecticide application to be ineffective at controlling pests within the interior of the canopy. If insect pest populations within the plant canopy are not decreased by chemical application events, it is possible that natural enemy populations within the plant canopy will also be unaffected and therefore continue to aid in pest control. However, we cannot be certain that natural enemies will be within the plant canopy when an insecticide application occurs.

In order to achieve the most effective pest control strategy, growers should apply chemicals that control insect pests but do not harm natural enemies. Systemic insecticides are generally thought to be safer for insects that do not directly ingest the plant material. A worse-case scenario was conducted where natural enemies were trapped in arenas with residue of a systemic or contact insecticide. Reactions to both systemic and contact insecticides were inconsistent between three species implying that no insecticide is inherently "safe" for all natural enemies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The nursery industry depends on consumers' desires; ornamental plants are not considered a necessity like food and water. Because they are more aesthetic in nature, if a grower wants to keep his business in business, the plants he or she sells must be pleasing to the customer. Customers are attracted to plants that have full, dense canopies with healthy foliage and stems, striking foliage color, and symmetry (Christensen et al., 2008b; Glasgow, 1999; Hodges et al., 2008; Townsley-Brascamp and Marr, 1995). However, like humans, many ornamentals have an adolescent stage where they are awkward and leggy. This adolescent stage is generally the most economical time for growers to sell (least amount of inputs at this point and the greatest demand for plants of this size) (Christensen et al., 2008b). In order to get the desired canopy density and symmetry during this stage in plant development, it is often best to modify plant growth by pruning, applying chemical plant growth regulators, or perhaps by using a different propagation method. Pruning is highly labor intensive (Holland et al., 2007) and with one of the highest costs in most businesses (especially small, less automated businesses) being labor (Jones and Sluis, 1991), it can be quite expensive. Plant growth regulators generally use less labor and in some cases even produce more desirable results than pruning, but the cost of the product and application must be considered as well as phytotoxicity caused by the chemical treatment (Meijon et al., 2009; Systema and Ruesink, 1996; Woodson and Raiford, 1986). Some in vitro-propagated plants are anecdotally reported to have denser canopies than their cutting-propagated counterparts and often require no further modification before they are sold. In vitro plants, however, may be more expensive to purchase, especially new cultivars. It is important for growers to minimize inputs when possible for economic and environmental sustainability.

All of these methods can increase the number of branches, the density, and by extension the overall quality and attractiveness of plants. A denser plant profits the grower more than an open, less branchy plant, but there may be consequences to having a densely branched plant. Pests can

thrive in the dense canopy and as a plant's canopy density increases, the grower's ability to achieve adequate spray penetration into the canopy may decrease. Pesticide could be blocked by the foliage, effectively providing a pesticide-free refuge in the inner canopy and pesticide-laden foliage in the outer canopy. Pests within the canopy may be protected from pesticide as would the natural enemies, allowing the natural enemies to continue to aid in pest control.

CHAPTER 1. LITERATURE REVIEW

ECONOMIC IMPACTS OF THE GREEN INDUSTRY

The green industry consists of sod, ornamental and floriculture growers, landscape architects and designers, contractors, builders and maintainers of landscapes, any store with a lawn and garden center, wholesalers of plants, and garden centers (Hodges et al., 2011). In 2004, the United States green industry produced $148 billion in output and provided almost 2 million jobs (Hodges et al., 2011; Hall et al., 2006). In 2006, the sales of ornamental crops in the United States reached almost $17 billion (Jerardo, 2007). It is the fastest growing agricultural industry and has even experienced growth in times of economic decline (Hall et al., 2006).

According to the USDA (2007), the majority of ornamental plants are produced in the United States with major production areas in Florida and California. According to Chandler and Tanaka (2007), Europe and the United States consume and produce the most ornamental plants (including cut flowers) followed closely by Japan and China. The ornamental industry is also important to the economy of several developing countries such as Colombia, Costa Rica, Ecuador, Ethiopia and Kenya (Chandler and Brugliera, 2011). The United States nursery industry continues to increase its sales annually due to its ability to compete with a small import market. In 2004, the ornamental crop industry in the United States boasted over $15.3 billion in sales, making ornamentals the fourth most profitable agricultural crop after corn, soybeans, and vegetables (Hall et al., 2005). In 2007, sales were reported at $17 billion and in 2008 they were up to $27.1 billion (Hodges et al., 2011; USDA, 2007). The market is driven by an increasing demand for novelties and plants of high quality (Ascough et al., 2008).

PRUNING

High quality plants have dense canopies with evenly distributed branches. Plants are unattractive to buyers if they are sparse, asymmetrical, or leggy (Christensen et al., 2008b; Hodges et al., 2008); however, many ornamentals tend to have a naturally elongated growth (Christensen et al., 2008b). Efforts have been made, through plant breeding, to improve quality and beauty for

thousands of years, but conventional methods of breeding are limited by gene pool availability within crossable species (Auer, 2008). Therefore, growers often make plants more marketable not by species selection but by modifying the branch architecture and increasing flower production through cultural practices (Glasgow, 1999). Pruning is the main technique used in the nursery industry to manipulate plant growth and produce high quality plants. Pruning alters the future growth of a plant by changing the distribution of naturally occurring plant hormones. Apical dominance occurs in an actively growing stem when the concentration of auxin in the shoot apex suppresses the growth of the buds below it, causing the plant to grow vertically rather than laterally. When the shoot tip is removed, usually by pruning, the source of apical dominance is removed and the lower shoots are able to grow, causing the plant to be denser (Wade and Westerfield, 2012). Pruning is generally labor-intensive and therefore expensive (Holland et al., 2007) and does not always increase branching (Cochran and Fulcher, 2013; Cochran et al., 2013; Hester et al., 2013; Starman, 1991,) or plant quality, and it can remove flowers or floral primordial (Cochran and Fulcher, 2013; Cochran et al., 2013), potentially reducing the marketability of the plant. Pruning may also cause plants to be more susceptible to pests, pathogens, and environmental stresses (Clair-Maczulajtys et al., 1999).

PLANT GROWTH REGULATORS

Plant growth regulators (PGRs) are a promising alternative to hand pruning to increase branch number, canopy density, and overall plant quality. PGRs are synthetic chemicals that mimic the effects of plants' naturally occurring hormones (Halmann, 1990). Plant hormones control plant growth and development and are especially important in propagation because they induce responses such as dormancy in seeds and root initiation. Although the amount of each plant hormone is very minute, they stimulate and inhibit any physiological process controlling the rate, direction, and development of plant growth. Hormones are produced by the plant, but there are also several synthetically produced PGRs available on the market (Basra, 2000).

The naturally occurring plant hormones, also known as phytohormones, include auxin, cytokinin, gibberellins (GA), abscisic acid (ABA), and ethylene. The commonly associated function of each is as follows: auxin promotes and inhibits growth and is responsible for geotropism, phototropism and apical dominance. Cytokinin is responsible for cell division. GA regulates cell enlargement, stem elongation, seed germination, and flowering (Hooley, 1994; Ingles, 2001). ABA counters the effects of gibberellins and causes leaves and fruit to abscise. Ethylene influences geotropism, leaf abscission, and ripening of fruit (Ingles, 2001). More minor plant hormones include brassinosteroids, salicylic acid, polyamines, peptide hormones, and jasmonates. It is now recognized that many types of growth and development are not controlled by a single hormone, but instead the interaction of multiple hormones. Often, a main hormone induces development with one or more minor hormones modifying the growth (Kepinski, 2006).

PGRs were used even before scientists knew about plant hormones. For example, to stimulate synchronized flowering in pineapple (*Ananas comosus* L.) and mangos (*Mangifera indica* L.), fires were lit in nearby fields. Although this was not understood at the time, the fires generated ethylene which stimulated the mangos and pineapples to flower (Rodriguez, 1932). Lemons (*Citrus limon* L.) were heated to increase ripening, as well. Again, it was not the heat but the ethylene produced by inefficient heat sources that stimulated ripening (Denny, 1924). It was not until the 1940s that scientists began to recognize and understand what was happening, and since then, synthetic growth regulators have been used for many horticultural purposes (Basra, 2000).

PGRs have numerous applications in agriculture. Chlormequat chloride (CCC) [(2 - chloroethyl) trimethylammonium chloride] inhibits gibberellin, produces more compact plants, and is used commercially on wheat to shorten plants and prevent lodging. Glyphosine, a common pesticide and herbicide, is used to increase sugar cane yield and ripening time. DEF (S,S,S-tributyl phosphorotrithioate), a defoliant, is used to remove the leaves from cotton plants to make picking easier. Naphthalene acetic acid (NAA), an auxin plant hormone, is used to reduce extensive fruit set

in fruit trees, which causes the larger fruits to be produced; without NAA, the removal of fruit would have to be done manually, increasing labor cost to the grower and food cost to the consumer. Gibberellin extends the lemon harvest season by inhibiting the senescence of the rind, enabling the grower to harvest lemons when they are in the greatest demand. Gibberellin applied to artichokes promotes flower bud set to occur faster than it naturally would, making the artichokes marketable sooner (Basra, 2000; Gianfagna, 1990).

PGRs have also been used extensively to manipulate branch architecture. Lateral branching decreases tobacco (*Nicotiana*) leaf quality. Maleic hydrazide, an herbicide that inhibits cell division, applied to tobacco prevents lateral branching (Gianfagna, 1990). Applications of cytokinin have been shown to stimulate branching in apple (*Malus domestica* Borkh.) (Elfving, 1984, Forshey, 1982), peach (*Prunus persica* L.) (Elkner and Coston, 1986) and macadamia nut trees (*Macadamia integrifolia* Maiden & Betche) (Boswell et al., 1981). In apple, lateral branching increases when BA (6-benzylaminopurine), a synthetic cytokinin, alone or in combination with combination with GA_{4+7} is applied (Elfving, 1984; Forshey, 1982), but with both treatments the length of lateral branches decreases due to increased shoot competition (Greene and Miller, 1988). Branching is promoted at as little as 100 mgL^{-1} BA (Greene and Miller, 1988), but BA-induced lateral branches had fewer flower buds (Greene and Autio, 1990). Pecan (*Carya illinoinensis* Wangenh.) trees have a strong apical dominance that causes young lateral shoots to abscise before reaching maturity. Applying BA delays, but does not stop, abscission (Wood, 1988).

Although traditionally PGRs have been used in agriculture, PGRs are becoming more commonly applied during ornamental plant production as a replacement for, or in addition to, pruning to control excessive growth, encourage canopy density, and to promote flowering (Lutken et al., 2012). In some cases, PGRs in ornamentals have even been shown to promote healthier plants by increasing water efficiency (Navarro et al., 2007) and inhibiting pathogens (Jacobs and Berg, 2000).

PGRs in woody ornamental plant production

In woody ornamental production, it can be difficult to produce compact, well-branched plants. Nurseries producing several different species and cultivars have an even harder time due to each requiring a specific pruning schedule and protocol (Meijon et al., 2009). Applying PGRs to woody ornamentals can be a useful tool to lower production costs while increasing plant quality.

For ornamental plants, the focus of PGRs is often to control plant size, increase branching, promote flowering, and increase flower number while plants are growing in containers (Abdelgadir et al., 2010; Hammond et al., 2007; Holland et al., 2007; Lutken et al., 2012; Stuart, 1961). Shorter, densely-branched plants are more attractive to consumers (Glasgow, 1999) but are also advantageous to the grower because they use less water, require less space, and are easier to transport (Müller, 2011). Growers often provide excessive water and fertilizer to produce plants quickly, which can result in poor plant quality. Often plants that grow rapidly are tall with weak stems and sparse foliage. Other production practices can cause plants to need modification during production. For example, artificial lights used to maintain a longer photoperiod can cause leggy plants. Heat can also cause some species to elongate (Oerum and Christensen, 2001). PGRs can be used to combat these issues.

Azaleas (*Azalea japonica* L.) tend to have strong apical dominance in select branches, creating an irregular shaped plant canopy. To encourage lateral branching, growers manually pinch the shoot tips, a labor-intensive practice. Growth regulators (daminozide, paclobutrazol and CCC) and fatty acid chemical pinching agents applied to 'Johanna' and 'Blaauw's Pink' reduced shoot elongation. Daminiozide and paclobutrazol effectively controlled vegetative growth and promoted flowering. However, daminizide created abnormal flowers in 'Blaauw's Pink'. The effects of the PGRs lasted up to three years (Meijon et al., 2009). Daminozide has also been foliar applied to pieris (*Pieris japonica* Thunb. Debutante') to successfully improve flower budding on plants potted in the spring. The flower buds become dormant once formed and can be forced from October to November in time to sell for Christmas (Systema and Ruesink, 1996).

Foliar application of BA to Japanese holly (*Ilex crenata* Thunb.*)* increased lateral branching. BA application of 500 ppm produced the most lateral branches in holly, but decreased leaf size and shoot length (Wright, 1976). Container grown Foster's holly (*Ilex x attenuata* Ashe) were treated with five branch modifying treatments: pruned 6 inches back, pruned 12 inches (30.48 cm) back, painted with latex and Promalin [(active ingredients 6BA for cell division and GA_{4+7} for cell expansion) a PGR usually used for apple fruit production to increase fruit size and improve fruit shape], and a normally pruned control to identify a treatment that reduced pruning as hollies must be pruned one to three times each season. The control treatment produced the highest quality plants based on plant shape. The PGR treatment did not promote branch development. The buds did not swell, and in fact, the latex/Promalin combination had an adverse effect on the foliage (Midcap, 2000).

Dikegulac- sodium (2,3:4,6-bis-0-(1-methylethylidene) α-L-xylo-2-hexulofuranosonic acid) is a chemical pincher, a compound that affects the shoot apex by temporarily preventing apical dominance and enabling lateral branches to emerge (Arzee et al., 1977). Dikegulac has been mostly tested on azaleas (Desilva et al., 1976; Shu and Sanderson, 1980), zinnia (*Zinnia* L. 'Scarlet Flame'), chrysanthemum (*Chrysanthemum morifolium* Ram. 'Escapade') (Arzee et al., 1977), begonia (*Begonia semperflorens* L.) and New Guinea impatiens (*Impatiens* L. hybrids) (Starman, 1991). Following foliar applications of 0.05% to 0.4% dikegulac-sodium (Atrinal, pbi/Gordan corporation, Kansas City, Kansas) on wintercreeper (*Euonymus fortunei* Turcz. 'Colorata'), shoot elongation was reduced and lateral branching was increased. The most effective concentration was 0.1% dikegulac-sodium which produced plants with the most shoot growth, "optimum" branching, and only initial phytotoxicity (Johnson and Lumis, 1979). Crape myrtle (*Lagerstroemia indica* L.*)* is an important ornamental plant in many landscapes. It flowers in early summer, but once flowering starts, its growth stops for the rest of the season. Pruning is conventionally used to delay the bud set and promote growth until the end of the growing season. When mature flowers are sprayed

with dikegulac-sodium (Atrimmec, PBI/Gordon, Kansas City, MO), flowers abort and new branches develop thus eliminating the need to mechanically prune (Fain et al., 2001). Dikegulac-sodium has also been shown to increase branching and decrease shoot length in honeysuckle (*Lonicera x heckrottii* Rehd. 'Goldflame') (Bruner et al., 2002).

Uniconazole, an ABA inhibitor that is also effective as a fungicide, was used on 'Flame' and 'Sunglow' azalea, forsythia (*Forsythia suspense* Thunb.*)*, holly (*Ilex crenata* Tunb.'Compacta' and *Ilex X* 'Nellie R. Stevens'), and mountain pieris (*Pieris floribunda* Bth.*)*. Application of uniconazole at the labeled rate decreased growth as determined by dry weight of each species as the concentration applied increased when compared with controls (Warren et al., 1991).

Mefluidide, a highly active plant growth regulator, retards growth of turf grass (Parups and Cordukes, 1977) and woody plants, controls weeds in soybeans (*Glycine max* L.), and increases lateral branching in peach (Arnold et al., 1981). The mechanism of action for mefluidide seems to be its inhibition of growth and development of active meristems (Woodson and Raiford, 1986). Woodson and Raiford (1986) found that mefluidide increased lateral branching of Chinese hibiscus (*Hibiscus rosa-sinensis* L.), but the branches were shorter than untreated plants. Plants treated with mefluidide had delayed flowering but an increased number of flower buds. A double application of melfulidide inhibited height, lateral shoot number and shoot length when compared to the single application. At the lowest concentration (500mgL^{-1}) tip necrosis occurred. As dosage increased (up to 800mgL^{-1}), so did the severity of tip necrosis and plant defoliation. In this study pinching was more labor intensive than PGR application and stimulated longer lateral branches than those sprayed with mefluidide (Woodson and Raiford, 1986).

While PGRs can be effective at controlling growth, their effectiveness may not be consistent between years, species, and even among cultivars of the same species. A single application of daminozide inscreased the flower size of big leaf hydrangea 'Schenkenburg' (*Hydrangea macrophylla* Thunb. 'Schenkenburg'), but had no effect on 'Böttstein', 'Enziandom', 'Kasteln',

'Mathilde Cütges', 'Merritt's Supreme', and 'Red Star' (Bailey and Clark, 1992). Two applications of Dikegulac sodium increased flowering in bougainvillea 'Temple Fire' (*Bougainvillea glabra* Choisy 'Temple Fire'), but had no effect on 'Matina Kea White', 'Raspberry Ice', 'Royal Purple', and 'Summer snow' (Norcini et al., 1994). Dikegulac sodium also increased the number of branches of rhododendron 'Formosa' (*Rhododendron indica* L. 'Formosa'), but had no effect on 'Hexe' (Cohen, 1978).

Augeo® Label information

Augeo is a dikegulac sodium foliar spray PGR. It has been used effectively on bedding, herbaceous, and woody ornamental plants as well as plugs and liners. Augeo works by disrupting apical cell wall integrity to create a pinching effect. Augeo is also a mild GA synthesis inhibitor (not unlike the flurprimidol in Topflor). The label claims its advantages are lateral bud break, improved bud potential, and thicker, fuller plants. It can be used in greenhouses, field, and container nurseries and tree farms. Rates applied vary between 400 to 6400 ppm depending on the type of plant. Effects of Augeo application can be seen after 7 to 10 days. Phytotoxicity is commonly exhibited as yellow new growth, which cannot be prevented or reversed by adding nutrients. However, phytotoxic effects do not persist (OHP, 2012).

Configure® Label information

The active ingredient in Configure is a cytokinin, benzyladenine (6BA). It is used as a foliar spray and has been shown to increase lateral branching and promote flowering in certain ornamental crops. Configure can also reduce the overall height resulting in a more compact and marketable plant. Plants that Configure has been tested on with positive results include Christmas cactus (*Schlumbergera* Lem.*)*, hosta (*Hosta* L. spp.), and purple coneflower (*Echinacea purpurea* L.), none of which are woody plants (Fine Agrochemicals Limited, 2012).

Topflor® Label information

Topflor is a gibberellin biosynthesis inhibitor. Its active ingredient is flurprimidol. Topflor has been shown to decrease internode elongation resulting in a more compact and marketable plant. According to the Topflor label, even if internode elongation is not inhibited, the quality of the plant is increased due to darker leaf color, higher chlorophyll content, greater leaf thickness, stronger stems, and decreased water loss. It claims to have no phytotoxic effects. Application of Topflor can be done by foliar spray, chemigation, or drenching. After any use, there is a 12 hour reentry period. Topflor has successfully been used on poinsettia, acalypha, fuchsia, phlox, sage, butterfly bush, lantana, verbena, abelia, azalea, bougainvillea, holly, cotoneaster, rose, photinia, hydrangea, honeysuckle, gardenia, Manhattan euonymus, and crape myrtle (SePRO, 2011).

As with all PGRs, there are several factors that affect how well Topflor works. For instance, if the plant grows vigorously or is generally a taller plant, more Topflor may need to be applied to get the desired results. Environmental conditions are an important consideration when applying Topflor, as with many PGRs. Growers in warmer climates must apply more than those in cooler climates. More applications may be needed as temperatures increase.

An informal survey of attendees at the 2012 UT Turf and Ornamental Field Day showed that many landscape maintenance professionals in and around Tennessee use flurprimidol to maintain trees and woody shrubs in the landscape. They were not familiar with Topflor. Many used Shortstop® (paclobutrazol) (Greenleaf Chemical LLC, Henderson, NV).

Because PGRs are of a chemical nature, many people fear the environmental and worker safety repercussions of their use (de Castro et al., 2004; Sorensen and Danielsen, 2006). Many European countries have banned paclobutrazol and daminozide and it is likely that more will be added to the list (Lutken et al., 2012; Rademacher, 2000). Europe is focusing on genetic

modification instead of chemical control to achieve the desired aesthetic qualities (Lutken et al., 2012). However, GMOs can also be very controversial. Growers may have to find alternatives to PGRs to increase canopy density and plant quality in the very near future.

IN VITRO PROPAGATION

In vitro is a term that describes a collection of methods in which plants cells are grown and maintained in a controlled environment. When in vitro is used as a propagation method, it is called micropropagation or in vitro propagation (IVP). In IVP, very small pieces of plant tissue are regenerated into new plants in an artificial medium under sterile conditions (Macdonald, 1990).

The history of in vitro culture follows closely with major discoveries in plant science. Schleiden (1838) and Schwann (1839) theorized that all plant cells were totipotent, that is that each cell has the capability to grow into a complete plant. Haberlandt (1902) grew the first dividing callus cultures, but they did not survive longer than 6 months on an agar medium. Haberlandt (1902) concluded that this was because there was something missing from his medium. White and Braun (1941) established cell division and growth by adding auxin to the medium. Skoog (1948) isolated synthetic cytokinin from herring Sperm DNA, and it was discovered that growth of roots and shoots occurred based on the ratio of auxin and cytokinin, and in 1962, the universally used Murashige and Skoog medium was developed based on this concept (Hartmann et al., 1997; Murashige and Skoog, 1962).

The use of in vitro as a propagation technique is very advantageous because it can produce superior, more uniform, pathogen-free plants (Jones et al., 1996; Macdonald, 1990; Smulders and De Klerk, 2011) in a short time with little space requirements (Kozai and Kubota, 2001). In vitro also makes it easier to root some plants that would not otherwise root by traditional propagation methods. There are no seasonal limitations on in vitro production; it can be used year-round. As the plantlets are small, they take up little space, and so it is especially advantageous for breeding massive amounts of plants quickly, especially when new varieties and cultivars are released and

demand for the plant is expected to be great. The ability to produce multitudes of clones is also helpful in finding mutations, or conserving dwindling or rare populations (Christensen et al., 2008a). In some cases, plants may even flower and fruit earlier when propagated in vitro, compared to cuttings or seedlings (Jones et al., 1996, Nas et al., 2003; Stuart, 1961; Systema and Ruesink, 1996; Ticknor, 1968; Woodson and Raiford, 1986).

There are, however, some limitations of IVP. The IVP process is a complex one consisting of many steps. Media formulations differ between species and even between cultivars, and the formulae are often not in a common repository, but are considered proprietary to the individual company that, through trial and error, invented the protocols. Not all plant species can be propagated by micropropagation, or at least not economically (Kyte, 1983; Macdonald, 1990). Plants that can be propagated may not be "true to type", or visually different from the parent plants they were propagated from (Smulders and De Klerk, 2011; van Staden et al., 2006). As with all propagation methods, pests and media contamination can be a problem (Kyte, 1983). As of 1995, woody plant species were rarely cultured (Aitken-Christie et al., 1995) due to contamination, low growth rate, low rooting, poor survival during acclimation, and abnormalities that made plants unmarketable (Kurata, 1992). The biggest limitation, though, is the starting cost associated with producing plants by this method. Growers who want to produce their own IVP plants will find an upstart cost ranging from $15,000 to $250,000, depending on the size of the operation (Macdonald, 1990). Also, grower need to consider the type of staff they will be employing, knowing that labor in any business counts for as much as 80% of operating costs (Jones and Sluis, 1991) and that with micropropagation being a specialized skill, the salary of the labor force may be even higher (Macdonald, 1990). All of these reasons make IVP plants generally more costly to produce than cuttings or seedlings.

The development of commercial laboratories and nurseries for micropropagation in the United States began in the late 1970s and speedily grew until the early 1980s. In 1986, there were

250 laboratories (Jones, 1986). A survey done in 1992 concluded that there were only 125 IVP facilities left, due to mergers, buy-outs and competition leading to failures (Bridgen, 1992). Although IVP plants typically cost more to produce, Briggs Nursery (Olympia, Washington) is able to produce high quality plants due to their mass production techniques at prices competitive with or even cheaper than high quality cutting propagators (Lynne Caton, personal communication).

In vitro variation

When in vitro was first established as a viable way to propagate plants, researchers expected that clonally propagated plants would be exact copies of their parent plant, but soon found out that this is not always true (Karp, 1994). The in vitro protocols and environmental conditions are very unnatural and stressful for plants (Aitken-Christie et al., 1995; Desjardins et al., 2007; Gaspar et al., 2002; Molinier et al., 2006; Smulders and De Klerk, 2011; van Staden et al., 2006). Protocol stresses include wounding, multiple subcultures, and removal of beneficial microorganisms due to sterilization. Environmental stresses include heat from overhead lights, excessive levels of exogenous hormones, hyperhydration due to high humidity, little to no air exchange, and the forced development of heterotropism or photomixotrophism due to low CO_2 concentrations and sugar media (Aitken-Christie et al. 1995; Baranek et al., 2010; Gaspar et al., 2002; Kozai, 1991; Smulders and De Klerk, 2011). Variations can be broken down into 2 categories: Genetic or epigenetic (Hartmann et al., 1997; Smulders and De Klerk, 2011).

Genetic variation is relatively permanent because it is an actual change in the genetic code of the plant and is maintained unless a mutation takes place. In IVP, it's called somaclonal variation. Typically, this variation is not desired, but it can be useful in creating new cultivars. During callus formation, axillary shoots are considered more genetically stable then adventitious shoots, but both can be affected by the stresses of the in vitro environment, long exposure to hormones, and by-products in the media. Also, the longer a plant is in the in vitro environment, the more likely it is to

have a genetic variation from its parent plant. That is why it is important to constantly generate fresh cultured stock from parent plants (Hartmann et al., 1997).

Epigenetic variation is long-lasting change in the expression of genes that are not initiated by changes in the DNA sequence (Simmons, 2008). This change is often temporary, and plants usually grow out of it; however, some changes can be long-lasting and even transferred to sexually-produced progenies (Brettell and Dennis, 1991). Often, these changes are undesirable and unmarketable especially when an exact clone of the parent plant is desired, but there are also changes that lead to new and marketable variations and cultivars. For example, when Jain (1993) propagated *Begonia* x *elatior* Fotsch.and *Santpaulia ionantha* Wendel in vitro, he found several differences in flower morphology, flower number, and plant height and morphology. Some of these traits were selected and bred for introduction into the market (Jain, 1993).

Habituation is an epigenetic variation in which the plant reacts to plant hormones even after they are no longer available. In some cases, habituation occurs because the plant begins to produce its own hormone, but this is not true for all plant species (Hartmann et al., 1997). It was first thought that habituation was caused by large amounts of plant hormones in the culture environment, but now we know that habituation can occur in cultures where there is no increased hormone concentration. All that is required for habituation to occur is time in the in vitro environment (Pischke et al., 2006). Habituation is a phenomenon that occurs in the in vitro environment; it does not initiate once the plant is out of the in vitro environment (Kevers et al., 1996; Meins, 1989; van Staden et al., 2006).

Increased branching examples

Increased branching of IVP plants is often thought to be caused by exogenous hormones in the in vitro media which stimulates rapid, disorganized growth (Karp, 1994). More specifically, high cytokinin levels have been shown to induce adventitious shoot proliferation (Damasco et al.,

1997; Zakhlenyuk and Kunakh, 1987). However, bushiness is often found in plants that receive no hormones at all, indicating that exogenous hormones are not always the reason.

According to Smulders and De Klerk (2011) excessive bushiness is an "aberration" and in some cases, it does seem to be, especially in cut-flower production where stems are expected to be long. Calla lily (*Zantedeschia* Spreng.), a common cut-flower, is hard to propagate. In order for it to survive and grow in the in vitro environment, large concentrations of cytokinin are used. Certain calla lily cultivars have shown uncharacteristic bushiness after being transplanted to soil. This bushiness is maintained and passed on to new generations (D'Arth et al., 2002). Interestingly, even though the bushiness was induced by the high levels of cytokinin (D'Arth et al., 2002), when compared to nonbushy plants there was no difference in the cytokinin levels between the two phenotypes (D'Arth et al., 2007). Bushiness has also been studied in *Gerbera* L. where it was noted that various degrees of increased branching was dependent on the type of explant used (Topoonyanont and Debergh, 2001).

Another theory for in vitro "bushiness" is rejuvenation. Rejuvenation is another epigenetic change that occurs when plants are propagated in vitro. Rejuvenation is when an explant from mature plant material reverts to juvenile plant morphology, often leading to improved rooting, branching, and an increased growth rate (Brand and Lineberger, 1992; Webster and Jones, 1989). Cuttings taken from rejuvenated, in vitro-propagated stock plants also have an improved ability to root (Webster and Jones, 1989). Sometimes flowering still occurs on plant material that has reverted to its juvenile state, indicating that not all plant processes have become juvenile (Jones et al., 1996).

Increased "bushiness" can also be a desirable trait, such as in fruit production. IVP has resulted in increased lateral branch growth in strawberry (*Fragaria ananassa* Weston), grape (*Vitis* L. 'Seyval') and thronless blackberry (*Rubus ulmifolius* Schott) (Damiano, 1980; Krul and Myerson, 1980; Swartz et al., 1981a, 1981b). Micropropagated strawberries grow more vigorously than

traditional, runner propagated strawberries (Damiano, 1980; Swartz et al., 1981a). Grapes that are micropropagated show rapid growth and greater branching than the stock plants they came from (Krul and Myerson, 1980). IVP, thornless blackberries have increased shoot growth, lateral branching, and flower production (Swartz et al., 1981b). When blueberries (*Vaccinum corymbosum* L. x *V. angustifolium* Ait. 'Northblue') are propagated in vitro, plants have significantly higher yields for the first three years. These yields are directly related to increased growth that leads to bushier plants (El-Shiekh et al., 1996). Grout et al. (1986) compared cutting- and in vitro-propagated plants and found that in vitro-propagated plants had 2-3x more lateral branches by the time they were 27 weeks old, but the length of branches on both in vitro- and cutting-propagated plants were not different. The growth rate was faster in in vitro-propagated plants, but evened off after week 34 (Grout et al. 1986). Fruit trees propagated in vitro also have more vegetative growth (Jones, 1994).

Some ornamentals may also benefit from increased branching caused by the in vitro environment. Growers of red maple have reported more branches among IVP plants when compared to those they would typically get from cuttings. Several other plants have responded similarly including rhododendron, red bud, and magnolia (personal communication, Phil Flanagan and Ed Kinsey). However, IVP plants tend to form more basal than apical branches (Personal communication, Phil Flanagan; personal experience), so IVP may not be recommended for urban trees or other plants where basal branches are not desired.

SPRAY PENETRATION

The application of pesticides and other liquid chemicals to plants involves a complex interaction of the equipment used, chemical/liquid composition (thickness, etc.), water quality, canopy structure, and environmental factors (temperature, precipitation, wind, etc.), all of which impact the effectiveness of the application (Yates et al., 1976). Droplet size and leaf coverage are also important factors to be considered. Coverage of the canopy with synthetic, contact chemical insecticide should be sparse (about 20-30 droplets per cm^2 with a droplet size of about 59 μm), but

when using more natural sprays such as soaps and oils it is important to completely cover the surface in order to effectively control pests (Syngenta, 2013; Zhu et al., 2011)

Newly hatched larvae of peach moth (*Grapholitha molesta* Busck*)* were exposed to carbaryl residues. As the number of droplets per cm^2 and dispersion of droplets increased, the time it took for the moth to be affected by the chemical decreased (Fisher and Menzies, 1976). Droplet size, density, and concentration of chemical pesticide (Dipel 8L, Valent BioSciences Corporation, Walnut Creek, CA) effects were studied on fourth instar spruce budworm (*Choristoneura fumiferana* Clem.*).* To mimic and increase in drop dispersion, one, two, or four droplets with diameters of 84, 66, and 52µm, respectively, were placed on needles of balsam fir (*Abies balsamea* L.*)*. When there were more droplets with smaller diameters (4 droplets, 52µm), mortality dropped considerably. It was suggested from this study that larger droplets were more effective. Another suggestion was that the active ingredient be increased so that droplets could be smaller and cover more crop area (Payne and Vanfrankenhuyzen, 1995).

Another study concluded that gypsy moth larvae feeding inability was more affected by Bt concentration rather than droplet density and dispersion (Falchieri et al., 1995). However, a similar study conducted by Maczuga and Mierzejewski (1995) using *Bt* and 2nd, 3rd, and 4th instar gypsy moth larvae concluded that as droplet size and dispersion increased, the time to mortality decreased.

Water sensitive paper (WSP) is a rigid paper with a coating that turns blue when water and other aqueous substances contact it. It was developed by Syngenta® (Greensboro, NC) to assess spray droplet size and dispersion. WSP can be used with either aerial or ground sprayers. When using WSP on trees and shrubs, cards should be place on the outside of the leaf on the periphery and inside the canopy at the top, the center, and the lower part of the tree to assess full coverage of the plant. There are some drawbacks to using WSP. Water droplets less than 50µm in diameter do not typically show up on WSP or do not leave a stain big enough to be noticed by computer

programs. Water sensitive paper should not be used in humid environments as the humidity can stain the paper. In this case the droplets may still be detected, but computer programs will not be able to distinguish between individual droplets. The droplet stain is not an accurate representation of the droplet size due to spreading (Hoffmann and Hewitt, 2005; Syngenta, 2012).

Once the paper is dried it can be analyzed by hand or by using a computer program. Analyzing by hand is a time-consuming process where the examiner must determine the average droplet size (in μm) and count the number of droplets per cm^2. Using a computer program can speed up the process considerably. DepositScan (USDA-ARS, Wooster, OH), a free and easy to use program for analyzing spray size and density, is run off of java-based ImageJ, an image analyzing program. WSP cards are scanned by common equipment such as a business card scanner, opened in the ImageJ program, and analyzed by DepositScan. Data from multiple cards can be compiled into one document that allows comparison of percent coverage, droplet density, and number of deposits. Like all other WSP analyzing programs, DepositScan cannot distinguish dots that overlap but it does account for drop spreading. DepositScan's accuracy also decreases as the droplet size decreases due to its use of pixels to determine droplet size (Zhu et al., 2011).

Spot densities that are too high (greater than 20% coverage) can be hard to read by these programs, and in fact, when using synthetic chemical pesticides, can be more coverage than is needed to be effective (Fox et al., 2003; Fox et al., 2008). However, getting sprays to penetrate into closed canopies (dense, heavily branched plants, and plants with large leaves) can be difficult especially in the nursery industry where production practices are designed to produce dense plants. Specialized equipment was developed to address this challenge including a canopy opener and a sprayer that can penetrate dense canopies (Zhu et al., 2006; Zhu et al., 2008).

BIOLOGICAL CONTROL

As of 1995, an estimated 2.5 million tons of pesticide are applied in the world each year (Pimentel, 1995). Chemical pest control has become the norm in our simplified monoculture type

of agriculture (Landis et al., 2005). Pesticides are used to control problematic insects, but reduction of beneficial insect populations and arthropod diversity is an undesirable side effect (Los and Allen, 1983). Restrictions of pesticide use (mostly in Europe and California), high cost of pesticides, complications of worker re-entry following applications, and interest in sustainable production by growers and the market have driven interest in new ways to control pests (Falconer, 1998).

Integrated pest management (IPM) is a method of managing a pest that includes the incorporation of multiple strategies. Strategies may include preventative measures such as scouting and sanitation, using pesticides that target your specific pest, crop rotation, choosing cultivars that are less susceptible, and biological control as well as several others (Brewer and Goodell, 2012; Ehler, 2006; Kogan, 1998; Stern et al., 1959). In the United States, IPM is used most prevalently in the southwestern region where, due to the warm climate, pest pressure is high (Hodges et al., 2008).

Biological control (BC) uses natural, living inhibitors of pests in order to control them. Conservation and augmentative BC are promising systems of utilizing natural enemies in plant production systems to reduce or eliminate dependence on conventional pesticides. Conservation includes maintaining a good environment for beneficial insects, fungi, nematodes, and pathogens to live, breed, feed, and overwinter. It is important to maintain and support a population of natural enemies to maximize their benefits. Augmentative biological control includes releasing natural enemies into the environment to increase the natural enemy population and decrease the pest population.

BC has been extensively utilized in greenhouse production; the enclosed space facilitates predator and parasite containment within the system. Augmentative BC is not widely used in nursery production, however successes have been documented. The black vine weevil (*Otiorhynchus sulcatus* Fabr.) is a major pest in herbaceous perennial as well as woody plants. Introducing the nematode *Heterohabditis*, reduced black vine weevils by 90-100% in all three trials

conducted (Gill et al., 2001). Azalea lace bug (*Stephanitis pyrioides* Scott), a prominent pest of azaleas can be controlled by a type of green lacewing (*Chrysoperla rufilabris* Burmeister*)* (Shrewsbury and Smith-Fiola, 2000). Spider mites (*Tetranychus urticae* Koch), one of the worst pests in commercial nurseries (Fulcher et al., 2012) can be controlled in the nursery by predacious mites called *Neoseiulus fallacis* (Garman) (Pratt and Croft, 2000).

Loss of natural enemies due to lack of enclosure is one challenge with BC in nursery production. It is important to release enough insects that they are able to find mates and maintain an effective population. Another potential concern of using natural enemies in any production system is the effect of pesticide residue on their ability to function properly. Other factors that affect natural enemies' ability to control pest is weather and their own natural enemies.

Natural enemies and complex canopies

Plant structure can affect natural enemy predator-prey interactions. Predators may prefer dense canopies to sparse canopies because denser canopies provide more shelter from heavy rain and other predators as well as provide abundant and diverse food sources (Halaj et al., 2000a, 2000b; Langellotto and Denno, 2004). The more complex the plant canopy, the more easily a predator can move to its prey. Canopy complexity also makes it more likely that the predator will continue to search for food on that particular plant (Skirvin, 2004).

Plant structure can also be detrimental to predators finding their prey. Andow and Prokyrm (1990) found that the parasitoid *Trichogramma nubilale* (Ertle and Davis) was more able to find its prey on a simple surface rather than a complex one and that it was more likely to give up the search on a complex surface. Experiments done on coleus (*Solenostemon scutellarioides* L.) and the parasitoid *Leptomastix dactylopii* (Howard) had similar results (Cloyd and Sadof, 2000). Several studies have been done on how grass architecture or other herbaceous plants affect predator-prey activity (Clark and Messina, 1998a, 1998b; Dobbs and Potter, 2014; Stiling and Moon, 2005), but no

studies were found at the time of this literature review preparation that dealt with woody ornamental canopy structure and natural enemies.

SYSTEMIC INSECTICIDES

Systemic insecticides are substances that are absorbed by and translocated throughout a plant (Bennett, 1949). They can be effective even in areas where spray does not penetrate and thus more effectively control pests within complex canopies (Ripper et al., 1949). Systemic insecticides were initially thought to be safer for biological control organisms (Ripper et al., 1949) because they are taken up by the plant and are ingested only by herbivorous insects. However, there is research that suggests that systemic insecticides can have negative consequences on insects used to biologically control pests when they come in direct contact with the insecticide or by ingesting prey that has ingested the pesticide (Szczepaniec et al., 2011). This can lead to an outbreak of secondary pests. For example, imidacloprid application caused an outbreaks of spider mites, *Tetranychus schoenei* (McG.) (Acari: Tetranychidae), where previously there were none (Szczepaniec et al., 2011).

PLANTS

Blueberry

In the 1990s, pruning was only practiced by 2/3 of Georgia's blueberry (*Vaccinum* spp. L.) growers, and even those that did prune did not do so annually (Florkowski et al., 1992). Highbush blueberries are upright, round, multi-stemmed bushes that grow 6-12' with a spread of 8-12'. They grow slowly and produce edible fruit. Although sometimes grown as an ornamental plant, they are most often grown in or around vegetable gardens or orchards. Dirr (1998) mentions that highbush blueberries are susceptible to several pests, but does not list any. Highbush blueberries are propagated by cuttings taken in mid-May and then placed without hormones in a milled pine bark media under mist and partial shade (Dirr and Heuser, 1987).

When highbush blueberries are propagated in vitro, plants have significantly higher yields for the first three years. These yields are directly related to increased growth that leads to bushier plants (El-Shiekh et al., 1996). Grout et al. (1986) compared cutting and in vitro-propagated plants. They found that in vitro-propagated plants had 2-3x more lateral branches than cutting-propagated plants by the time they were 27 weeks old, but the length of branches on both in vitro and cutting-propagated plants was not different. The growth rate was faster in in vitro-propagated plants, but evened off after week 34 (Grout et al., 1986).

Clethra

In the landscape, clethra (*Clethra alnifolia* L.*)* is a dense, leafy, oval shrub which often suckers and forms masses (Dirr, 2009). In nurseries, however, we do not always see a dense shrub but a leggy, unattractive plant that can easily go unnoticed (personal observation). The leaves are attractive, especially in the fall when they turn pale yellow to golden brown, but it's the panicle-shaped flowers that make this plant stand out as they are very fragrant and bloom over a long period of time in late spring (Dirr, 2009).

Like hydrangea (*Hydrangea* spp. Thunb.), clethra is a water loving plant and thrives in a wet spot. If it becomes too dry, clethra will drop its leaves. Clethra is generally pest-free though mite damage, typically from eriophyid mites (Eriophyidae), does occur when the air is dry and can be easily noted by clethra's terminal leaves crinkling.

Clethra cuttings root readily in the summer in sand and peat without the addition of synthetic hormones, but 1000 ppm KIBA may decrease the rooting time (Dirr 2009). Clethra typically roots in four weeks with 90-100% rooting. The harder the wood, the more difficult it is to root, but once rooted, clethra can outgrow a one gallon pot in a single growing season. Clethra can also be produced easily from seed (Dirr 2009), however another study suggests they do not self-pollinate well (Reed, 2006).

Hummingbird clethra (*Clethra alnifolia* L. 'Hummingbird') grows 30-40 inches (76.2-101.6 cm) tall and can spread by rhizomes to form large colonies. Hummingbird clethra differs from the species in that the leaves are darker green and narrower, and the flowers bloom earlier and more profusely. However, Hummingbird clethra is known to be weak and to flop, spread unabatedly, and become untidy (Dirr, 2009).

Magnolia

Sweetbay magnolia *(Magnolia virginiana* L.*)* was the first magnolia to be cultivated (Callaway, 1994). It is a native of the eastern coastal plain and the Mid-Atlantic States and is an important plant to the nursery industry as it is a tree commonly used in both commercial and residential landscapes (Zale et al., 2011). Sweetbay magnolia is a semi-evergreen tree that grows to about 20 feet (6.1 m) tall with a 20 feet (6.1 m) spread (Dirr, 2009). It often forms root suckers and can form small colonies. Although considered a tree, it is often planted in the landscape as a multi-stemmed shrub (Callaway, 1994). The large flowers have a distinctive lemony scent, are creamy white, and very attractive, but not as showy as some other magnolia species. The leaves are also quite attractive due to their soft, green adaxial surface and silver abaxial surface (Dirr, 1998). It can handle drought quite well (Nash and Graves, 1993), and unlike other magnolias, sweetbay magnolia grows very well in wet, even swampy soils and can also be placed in shade (Dirr, 1998). Sweetbay magnolia can survive in a wide range of soil pH levels (Zale et al., 2011) and does not have any serious insect problems or diseases, although Dirr (1998) mentions leaf minor and chlorosis.

Sweetbay magnolia can be propagated by cuttings. Softwood cuttings should be treated with 1000 ppm IBA quick dip then placed in sand under mist. This produces 100% rooting (Dirr, 2009). Hard wood cuttings should be treated with 10,000 ppm IBA, but even hormone treatment might not produce rooting (Dirr, 1998).

Propagation can also be done in vitro, though it is not easy for plants in the magnolia family. In general, magnolias contain high levels of phenolic acid which leach into the media and inhibit

growth, requiring magnolia explants to be constantly transferred to fresh media. However, this does not stop commercial labs from producing them. New, actively growing shoot tips are used as explants, but success has also been shown when using dormant shoot tips. Once shoot multiplication has occurred, shoots are stuck as cuttings in a greenhouse for roots to develop. Once rooted, plants are potted up and handled as a typical cutting plant (personal communication, Lynn Caton).

Rhododendron

Several books have been written exclusively on Rhododendron (*Rhododendron* spp. L.) (Chamberlain et al., 1996; Cullen, 2005; Greer, 1988), and there is an almost endless supply of papers have been published on their taxonomy (Cullen, 1980), phenology, diversity (Escaravage et al., 1998), and physiology (Crombie et al., 1985; Williams et al., 1982). More than 900 species occur within the genus *Rhododendron,* and the specie's ability to hybridize makes the cultivar list nearly endless (Dirr, 1998). Rhododendrons can be found at almost any nursery in the country and, despite the wide variety, are often the same cultivars.

'Roseum Elegans' is one such cultivar. It is an old standard with lavender-pink flowers. It can survive extreme heat and cold down to -25°F (-32°C) without suffering injury (Dirr, 1998; Lim et al., 1998). Many studies, including hardiness (Lim et al., 1998), plant-microbe interaction (Eccher and Martinelli, 2010), substrates (Matysiak and Nowak, 2008), genetic manipulation (Rowland et al., 2003) and PGR studies, have been conducted using 'Roseum Elegans'.

Applications of PGRs to rhododendrons have been extensively studied. Under field grown conditions, rhododendron often require a minimum of three years to flower when produced by cuttings and even longer than that if produced by seeds (Dirr, 1998). In 1961, it was found that growth retardants phosfon (tributyl-2,4-dichlorobenzylphosphonium chloride) and CCC induced flowering of rhododendron one year after propagation (Stuart, 1961). In 1975, ancymidol, phosfon, CCC, and daminozide were all found to promote compactness in field-grown rhododendron, with

ancymidol being the most effective (Cathey, 1975). Ancymidol also increased the number of flower buds on 'Roseum Elegans' by one to two times (Ticknor, 1968) and five times in 'Hummingbird' (Ryan, 1970). Paclobutrazol has been known to inhibit stem elongation and promote flowering in field grown 'Roseum Elegans' and 'Boursault' (Gent, 1995). Paclobutrazol applied to rooted Catawba creating a compact plant and increasing the number of flowers than applications applied after transplant (*Rhododendron catawbiense*) cuttings as a drench before transplant was found to be more effective in (Gent, 2004).

CHAPTER 2. ENHANCING ORNAMENTAL PLANT QUALITY CHARACTERISTICS WITH PRUNING, PLANT GROWTH REGULATORS, AND PROPAGATION TECHNIQUE OF *Clethra alnifolia* 'HUMMINGBIRD', *Magnolia virginiana* 'Henry Hicks', AND *Rhododendron* 'ROSEUM ELEGANS'

ABSTRACT

Woody ornamental plants are more marketable and considered higher quality when they have dense, symmetrical canopies. Pruning or plant growth regulators (PGRs) are often used to modify growth and increase branching and canopy density. Some evidence supports that in vitro propagation (IVP) produces plants with denser canopies than cutting-propagated (CP) plants. The purpose of this study was to determine whether pruning, PGRs or IVP would yield higher quality plants due to improved canopy characteristics. Augeo, Configure, Topflor, water or a pruning treatment were applied to clethra (*Clethra alnifolia* L. 'Hummingbird'), magnolia (*Magnolia virginiana* L. 'Henry Hicks'), and rhododendron (*Rhododendron* L. 'Roseum Elegans') IVP and CP plants. No treatment improved symmetry of any plant species. Pruning and PGRs were generally ineffective, but IVP clethra were denser, had more branches, and were higher quality than CP plants. No treatment increased the branch number, canopy density, or quality of magnolia. IVP plus one pruning to rhododendron produced more branches and greater density than CP rhododendron; a pruning treatment at the beginning of the study further increased the branch number and density of both propagation methods. PGRs were generally ineffective on rhododendron. Although the three species reacted differently to the treatments, the results suggest that IVP liners may be more advantageous to growers than CP liners for some plant species.

INTRODUCTION

Consumers of woody ornamental shrubs desire plants that are full and cover the surface of the container in which they are sold (Glasgow, 1999). Growers and retailers prefer plants that are well branched yet compact (Roh and Lawson, 1998) for easier transport, to reduce space required per crop, and to minimize toppling due to wind (Müller, 2011). Many ornamentals, however, have a naturally elongated habit (Christensen et al., 2008b; Lutken et al., 2012). Efforts have been made through plant breeding to improve ornamental quality, but conventional methods of breeding are

limited by gene pool availability within crossable species (Auer, 2008). In order to produce plants that satisfy the desires of both retail and wholesale customers, growers often modify growth with pruning. Pruning induces branching by removing apical buds, causing lateral buds to break (Wade and Westerfield, 2012). However, pruning is generally labor intensive, especially for species that require multiple pruning events within a single growing season (Meijon et al., 2009), and therefore, can be expensive for growers (Holland et al., 2007). Other drawbacks to pruning include a longer production time due to loss of biomass, the possibility that pruning will not increase branching, and flower reduction due to removal of wood with floral buds (Cochran and Fulcher, 2013; Hester et al., 2013; Holland et al., 2007; Starman, 1991).

Plant growth regulators (PGRs) are a potential alternative to pruning to improve plant quality while lowering labor costs. Foliar application of benzyladenine (BA) to Japanese holly (*Ilex crenata* Thumb.*)* increased branch number (Wright, 1976). Mefluidide increased lateral branching in peach (*Prunus persica* L.) (Arnold et al., 1981) and Chinese hibiscus (*Hibiscus rosa-sinensis* L.) (Woodson and Raiford, 1986). Augeo (dikegulac sodium), a chemical pincher, applied to Little Lime™ hydrangea (*Hydrangea paniculata* Siebold 'Jane') increased branching and branching symmetry without reducing panicle number (Cochran and Fulcher, 2013). Dikegulac sodium applied to euonymus (*Euonymus fortunei* Turcz.), crape myrtle (*Lagerstoemia indica* L.), and honeysuckle (*Lonicera x heckrottii* Rehd. 'Goldflame') increased branch number and decreased branch elongation, resulting in more compact plants (Bruner et al., 2002; Johnson and Lumis, 1979). Uniconazole, a GA_3 biosynthesis inhibitor, decreased growth when applied to azalea (*Rhododendron* L. 'Flame' and 'Sunglow'), forsythia (*Forsythia suspense* Thunb.*)*, holly (*Ilex crenata* Thunb. 'Compacta' and *Ilex* x 'Nellie R. Stevens), and mountain pieris (*Pieris floribunda* Bth.) resulting in more compact plants (Warren et al., 1991).

Several plant species have been observed developing more branches when propagated in vitro. In vitro propagation (IVP) resulted in increased branching of strawberry (*Fragaria ananassa*

Weston), grape (*Vitis* L. 'Seyval'), and thornless blackberry (*Rubus ulmifolius* Schott.) when compared with the cutting-propagated (CP) parent plants they came from (Damiano, 1980; Krul and Myersone, 1980; Swartz et al., 1981a, 1981b). IVP blueberries (*Vaccinum corymbosum* L. x *V. angustifolium* Ait. 'Northblue') had significantly higher yields for the first three years than their CP counterparts. These higher yields were directly related to the increased branching of the IVP plants (El-Shiekh et al., 1996). Fruit trees propagated in vitro also have increased vegetative growth (Jones, 1994).

Ornamental plant growers have reported more branches among IVP red maple liners when compared to CP liners, and other ornamental plants have been observed with similar results including rhododendron (*Rhododendron* spp.) and magnolia (*Magnolia grandiflora* L. and *M. virginiana* L.*)* (E. Kinsey, personal communication). However, there are no published reports on the effect of propagation technique on branch number, canopy density, or symmetry of woody ornamental shrubs or on differential efficacy of PGRs based on propagation technique. Therefore, the objectives of this study were to explore the effectiveness of branch-inducing treatments including pruning and PGRs [Configure® (BA), Augeo® (dikegulac-sodium), and Topflor® (flurprimidol)] and propagation technique (CP and IVP) on enhancing branch architecture and plant quality for clethra, magnolia, and rhododendron.

MATERIALS AND METHODS

Magnolia and clethra IVP plants (Briggs Nursery, Elma, WA) and rooted cuttings (Spring Meadow Nursery, Grand Haven, MI), both in 2 ¼ inch (5.72 cm) pots, arrived 25 April 2012 and 30 May 2013. They were kept in a greenhouse for two days and then were potted in sterilized 1-gal containers filled with 85% pine bark and 15% peat and placed outside under 50% shade to acclimate. After four days, the plants were moved to 25% shade in the nursery compound at the University of Tennessee in Knoxville, TN (35°56'46"N 83°56'18"W). Rhododendron IVP plants arrived 25 May 2012 and 30 May 2013 from Briggs Nursery and rooted cuttings were procured

from North Carolina in 2 3/8 inch (6.03 cm) sheet pots. All rhododendrons were potted in the same manner as above and kept in 25% shade for the remainder of the experiment. Two weeks after potting (WAP) clethra and magnolia were placed in full sun and all plants were top dressed with 19N–1.7P–6.6K, 5- to 6-month controlled release fertilizer with minors (Harrell's, Lakeland, FL) at 14 g (0.49 oz.) per container (medium-high label rate).

Initial growth measurements (plant height, widest width, and width 90° to widest width) and branch number [only included branches > 1.2 inches (3 cm)] were recorded 14 June 2012 and 10 June 2013. A subset of clethra, 10 CP plants and 10 IVP plants, were further analyzed for initial compactness. Each plant was destructively harvested, and the total leaf surface area and dry shoot mass were assessed. A ratio of leaf surface to height and dry shoot mass to height determined compactness (van Iersel and Nemali, 2004). Plants were then separated into treatment groups and branch-inducing treatments of a chemical pincher, Augeo (800 ppm), a cytokinin, Configure (600 ppm), or a GA_3 biosynthesis inhibitor, Topflor (150 ppm) were applied to foliage on 21 June 2012 and 13 June 2013 until the canopy was thoroughly wetted. Two control treatments, a hand pruning and a water spray, were also applied. The hand pruning treatment for clethra and magnolia consisted of pruning each stem to a lateral bud 6 inches (15.24 cm) from the substrate surface. For rhododendron, apical buds were removed with pruners.

In both years, CP and IVP clethra liners had been pruned several times during liner production, but neither was pruned just prior to shipping. Magnolias arrived unpruned in 2012, but in 2013 despite thorough instruction, the IVP magnolias were sheared just prior to shipping. Therefore, to match the IVP magnolia, CP plants were sheared upon arrival. In both years, the IVP rhododendron liners were mistakenly sheared just prior to shipping; CP liners were not pruned and were mostly apical cuttings. To account for this disparate treatment prior to the experiment, the objective with rhododendron shifted to comparing IVP plus one pruning with CP. For simplicity within the text and tables, IVP rhododendron refers to IVP rhododendron plus one pruning. As a

result of different plant growth stages between the two groups of rhododendron liners, branch-inducing treatments were applied based on phenological stage rather than WAP. Specifically, each group was treated when plants finished a flush and set apical buds. IVP plants flushed and set buds before the CP plants, such that treatments were applied IVP rhododendron on 5 July 2013 and to CP rhododendron on 25 July 2012.

Plants were hand-weeded as needed and watered by overhead automatic irrigation twice daily. Phytotoxicity symptoms were rated two weeks after treatment (WAT) on a 0 to 10 visual scale, where 0 represented no injury, and 10 represented complete kill. Branch number and growth measurements were recorded at 4, 8, and 12 WAT. Quality was determined at 12 WAT on a 1 to 5 scale. For clethra, a rating of 1 represented sparsely branched and asymmetrical plants with an open canopy, 2 represented sparsely branched and asymmetrical plants with a partially closed canopy, 3 represented densely branched and asymmetrical plants with a partially closed canopy, 4 represented densely branched, symmetrical plants, with a closed canopy but not necessarily completely covering the container surface, and 5 represented densely branched and symmetrical plants with a closed canopy that completely covered the container surface. Here, open and closed refer to the degree to which the canopy can be seen through when viewed from above. In magnolia, a rating of 1 represented a single branch or dominant central leader, 2 represented two branches or co-dominant leaders, 3 represented several branches emerging towards the apex of the plant, 4 represented a plant with a majority of basal branching, and 5 represented several branches emerging from the base, covering at least 90% of the container surface. In rhododendron, a rating of 1 represented plants with one strong leader with branch development on distal portion of stem only, causing minimal coverage of the pot surface, 2 represented two or more leaders with a narrow, columnar growth pattern and branch development occurring distally, 3 represented two or more stems with branch development occurring at the base, that covered at least 70% of the container surface when viewed from above, 4 represented multiple stems that covered

approximately 90% of the container surface, and 5 represented multiple stems from base and covered 100% of the container surface.

Experiments were conducted using a complete randomized design with repeated measures. There were 10 single plant replications for each treatment. Data were analyzed using the GLM procedure of SAS (version 9.3S; SAS Institute, Cary, NC). Analysis of variance (ANOVA) and mean comparisons were used to determine the effects of propagation method and PGRs on branch number, density, measured as branch number per unit height following the method of Randlkofer et al. (2009), symmetry (width 1 – width 2), quality, phytotoxicity, and cumulative branch number (week 12 branch number – week 0 branch number). Means were separated using Tukey's HSD, $\alpha = 0.05$. Each plant species was conducted and analyzed as a separate experiment.

RESULTS

Clethra

When water controls of both propagation methods were compared prior to PGR applications, IVP clethra were more compact (Table 1) and had more branches than CP clethra (Tables 2 and 3). IVP water controls had more branches than CP water controls for the duration of the 12-week experiment. Pruning did not increase branch number for either propagation method when compared with water controls and led to a temporary decrease in branch number in IVP in both years and CP in 2013 at 4 and 8 WAT. None of the PGR treatments increased branch number compared with the water controls. Topflor decreased the number of branches in CP clethra at 8 WAT only and in IVP clethra at 4 and 8 WAT in 2012 and at 8 and 12 WAT in 2013. In both years, the cumulative branch numbers for IVP and CP clethra were not different and no treatment increased the cumulative branch number. Topflor-treated clethra had a lower cumulative branch number for CP in 2012 and for IVP in 2013 than the respective water controls.

IVP water controls had a greater density than CP water controls for the duration of the experiment (Table 2 and 3). In both years, pruning neither increased nor decreased density of

clethra, regardless of propagation method when compared to their respective water controls. No PGR treatment was effective at increasing the density of CP plants in either year. Topflor increased the density of IVP plants at 12 WAT in 2012, but not in 2013. Symmetry was not different between IVP and CP water controls and no branch-inducing treatment increased symmetry (data not shown).

In both years, Augeo-treated clethra displayed phytotoxicity symptoms 2 WAT, regardless of propagation method (Tables 4 and 5). No other treatment caused phytotoxicity. Quality was higher in IVP clethra than in CP clethra in both years when water controls were compared. No treatment improved the quality of IVP plants, including pruning, but in 2013, Topflor-treated CP clethra had a higher quality than CP water and in both years, a higher quality than Augeo-treated clethra. Topflor-treated CP was not different from untreated IVP in both years.

Magnolia

In general, propagation technique did not influence initial branch number nor did either method influence branch number after branch-inducing treatments were applied (Tables 6 and 7). Neither pruning nor PGRs influenced branch number in either year. Cumulative branch number between IVP and CP water controls was not different. Neither pruning nor PGR treatments increased the total number of branches gained over the course of this study. In 2012, IVP magnolia was less dense than CP magnolia before branch-inducing treatments were applied. In 2013, there was no overall trend that one propagation method was denser than the other (Tables 6 and 7). Based on comparison of water controls, neither propagation method was denser after 0 WAT. Pruning did not increase CP magnolia density compared to the CP water controls in either year, however, pruning did increase IVP density compared to IVP water controls at 8 and 12 WAT in 2012. No PGRs were effective at increasing density during the course of this study, regardless of propagation method.

Propagation method and branch-inducing treatment had no effect on symmetry (data not shown). No treatment caused phytotoxicity symptoms in either year (Tables 8 and 9). In 2012, IVP water controls had a lower quality than CP water controls but pruned IVP plants had a higher quality than both IVP and CP water controls; pruning did not influence CP quality in either year. In 2012, Configure increased the quality of IVP plants, but no PGR treatment increased the quality of CP plants. In 2013, quality was not affected by propagation method or branch-inducing treatments.

Rhododendron

Before branch-inducing treatments were applied, IVP plants had more branches than CP plants in both years (Tables 10 and 11). In 2012, IVP water controls had more branches than CP water controls 4 WAT, but by 8 WAT, branch number was not different. In 2013, IVP water controls had more branches than CP water controls throughout the experiment. In 2012, pruning increased branching at 4 WAT for CP rhododendron, and at 8 WAT for IVP rhododendron, but was no longer significant by 8 WAT for CP and 12 WAT for IVP. In 2013, pruning did not increase branching in CP plants but consistently increased the number of branches in IVP plants. No PGR was effective at increasing branch number regardless of propagation method, and some decreased branching compared to pruning. This decrease in branch number was especially noticeable in IVP plants in 2013, where all PGRs had fewer branches than pruning. In 2012, IVP and CP water controls' cumulative branch numbers were not different and the branch-inducing treatments did not increase or decrease the cumulative branch number. In 2013, IVP water controls had a greater cumulative increase in branch number than CP water controls at each time point in the study. Pruning was the only branch-inducing treatment that increased the cumulative branch number in 2013, but only for IVP plants.

IVP water controls were denser than CP water controls at all time points, except 0 WAT in 2013 where IVP and CP density were not different. Pruning improved density of both propagation methods at 8 and 12 WAT in 2012, but improved only IVP rhododendron in 2013 at 4, 8, and 12

WAT. Topflor increased density of IVP plants in 2012 at 8 and 12 WAT, but no other PGR was effective at increasing density for either propagation method in either year. Symmetry was not different between IVP and CP water controls and no treatment increased symmetry (data not shown).

Very mild phytotoxicity occurred on Configure-treated CP plants in 2012. Leaves were thin and pale green; symptoms disappeared by 4 WAT (Table 12). Phytotoxicity symptoms did not occur in 2013 (Table 13). Quality of water controls was not affected by propagation method in either year (Tables 12 and 13). Topflor-treated CP had better quality than Augeo in 2012, but was not different from CP water controls (Table 12). No branch-inducing treatment increased IVP quality in 2012. IVP Configure and IVP Topflor had greater quality than CP water controls, CP Configure, and CP Topflor in 2013, but were not different from IVP water controls (Table 13).

DISCUSSION

Clethra

Initially, IVP plants were 126% and 62% more compact than CP plants according to the leaf surface area to height and shoot dry mass to height ratios, respectively (Table 1). Before PGR application, IVP water controls had more branches than CP water controls (Tables 2 and 3). IVP water controls had approximately double the average number of branches than CP water controls, 46 versus 24 branches, respectively, in 2012 and 16 versus 9 branches, respectively, in 2013. The number of initial branches between the two years was likely due to the time when the plants were potted into 1 gal containers. The 2012 plants had an extra month to grow and, thus, more time to develop branches. IVP water controls continued to have more branches than the CP water controls by 95%, 58%, and 51% in 2012 and 42%, 39%, and 39% in 2013 at 4, 8, and 12 WAT, respectively.

While pruning is the industry standard to create more well-branched plants, in this study pruning clethra often decreased the number of branches. More specifically, pruning decreased the number of IVP branches by 31 and 39 branches in 2012 and 10 and 19 branches in 2013 at 4 and 8

WAT, respectively and the number of CP branches by 10 and 20 branches at 4 and 8 WAT, respectively, in 2013. Pruning was also not effective at increasing the number of branches of 'Limelight' or Little Lime™ hydrangea (Cochran and Fulcher, 2013; Cochran et al., 2013) or density of Little Lime™ hydrangea (Cochran and Fulcher, 2013). Augeo and Configure are labeled to promote branching but did not increase the number of branches in clethra in this study compared to the water controls (OHP, 2012, Fine Agrochemicals Limited, 2012). Topflor, labeled for shorter internodes (SePRO, 2011), also did not increase the number of branches and, in fact, decreased the number of IVP branches by 29% and 22% at 4 and 8 WAT, respectively in 2012 and by 28% and 38% at 8 and 12 WAT, respectively in 2013 when compared to water controls.

Although IVP plants had more branches than CP plants at each time point, the cumulative branch number, i.e., the total increase in branch number from 0 to 12 WAT was not different between the two propagation methods indicating that the advantage that IVP plants had at the initiation of the experiment led to a better branched plant, but not because they produced more branches than CP plants during the experiment. Rather, IVP plants are more well branched due to beginning the experiment with more branches. Cumulative branch number was also not consistently different among the branch-inducing treatments. Light quality and quantity are known to affect branching (Franklin and Whitelam, 2005; Reddy et al., 2013). Reddy et al. (2013) observed that Arabidopsis (*Arabidopsis thaliana* L.) buds in high far red light did not form new branches. In this study, the well-branched IVP plants had more shade within the canopy, possibly causing buds within the canopy to remain dormant. Environmental conditions, water, and nutrient availability were no different for either propagation method, but they could have allocated these resources differently, such as one plant may have added canopy mass, while another plant may have used available resources to add root mass instead. In this study, we did not measure root mass. For whatever reason, even though IVP plants had more branches, they did not accumulate more branches than CP plants over the life of the study.

IVP water controls had a greater initial density than CP water controls by 100% and 81% in 2012 and 2013, respectively (Tables 2 and 3). However, in 2013, IVP plants had more branches but CP plants were shorter, creating less of a disparity between the two propagation methods in regard to density. IVP water controls remained denser than the CP water controls by 90%, 58% and 58% in 2012 and 194%, 56% and 41% in 2013 at 4, 8, and 12 WAT, respectively. Pruning, the industry standard, was ineffective at increasing clethra density in this study. PGRs were also ineffective at increasing CP clethra density. Topflor did increase density of IVP plants by 37% in 2012, but did not increase density in 2013.

For both propagation methods, Augeo was the only treatment to cause phytotoxicity (pale and stunted apical leaves) (Tables 4 and 5). Damage was not detectable by 4 WAT and therefore is not considered relevant to marketing the plants. IVP water controls had a higher quality than CP water controls by 74% and 18% in 2012 and 2013, respectively. No branch-inducing treatment increased quality over the water controls except for Topflor-treated CP plants, which had 65% higher quality than CP water controls and had as high a quality as IVP water controls. CP Topflor also had a higher quality than CP Augeo by 55% in 2012 and by 100% in 2013.

It is important to note that quality specifications were devised at the initiation of the experiment, prior to observation of the full effect of the treatments. While Topflor-treated CP clethra did have improved visual symmetry and density, as recognized by our quality measurements, in both years, Topflor-treated clethra (both propagation methods) had altered morphology including growth with shortened internodes, flattened apices, and leaves emerging from stems in whorls. Leaves were also darker and shinier than all other treatments, an effect listed on the Topflor label (SePRO, 2011). These anomalies continued throughout the study and persisted even after the scope of this study. Topflor-treated clethra were clearly visually different which may make them harder to sell as “true to type”. However, one year after treatment, Topflor-

treated clethra had a typical morphology, more branches and flowers, were denser and higher quality than water controls of both propagation methods (Appendix B).

In this study, IVP clethra were superior to CP plants due to their greater branch number, quality, and density. IVP clethra were $0.09 more expensive per plant than CP clethra (Briggs and Spring Meadow 2012 catalog prices); however the added cost may be worth the benefits to a grower. Pruning clethra did not increase branch number, density, or quality and sometimes decreased branch number. PGRs were also generally ineffective. For the highest quality clethra with the lowest labor investment, growers should purchase IVP plants rather than CP plants and not prune or apply PGRs.

Magnolia

In 2012, branch number for IVP and CP water controls was not different, but in 2013, IVP water controls had 80% more branches than CP water controls (Tables 6 and 7). In both years from 4 WAT to termination of experiment, IVP and CP controls were not different in branch number or cumulative branch number. Although they had the same number of branches before applying treatments, IVP water controls were 50% less dense than CP water controls in 2012 due to the greater height of the IVP plants. In 2013, density was not different between IVP and CP plants. There was no advantage to either propagation method following PGR treatment. Pruning did not increase density in CP plants in either year, but did increase IVP density by 80% and 90% at 8 and 12 WAT, respectively, in 2012.

IVP water controls had a 42% lower quality than CP water controls in 2012 but in 2013 were not different (Tables 8 and 9). This was due mainly to the IVP plants growing excessively tall in 2012 while CP plants remained compact by comparison. Pruning increased the quality of IVP water control by 68% in 2012, but had no effect on CP plants. In 2013, pruning did not improve quality of either propagation method, possibly because plants were pruned prior to the initiation of the experiment.

Unlike clethra, IVP magnolias did not have greater branch number, density, or quality. Pruning increased density and quality of IVP, but only in 2012 indicating that environmental conditions and/or cultural practices likely play a role in response. The PGRs used in this study were also ineffective at improving magnolia. In the case of magnolia, in vitro plants were $0.26 cheaper per plant to purchase. Since the water controls of the two propagation methods were not different, growers could purchase either for the same result.

Rhododendron

When initial measurements were taken, IVP water controls had 1.9 more branches than CP water controls in 2012 and 2.7 more branches in 2013 (Tables 10 and 11). In both years IVP water control had more branches than CP water control at 4 WAT, 3.9 versus 2.4, respectively, in 2012 and 6.6 versus 2.0, respectively, in 2013. This branch difference persisted throughout the experiment in 2013; IVP water controls had a higher branch number than CP water control by 250% and 271% at 8 and 12 WAT, respectively. In 2012, IVP and CP water controls' cumulative branch numbers were not different. In 2013, however, IVP water controls gained 4.1 (373%) more branches over the course of the experiment than CP water controls.

Pruning increased branch number, but not consistently. In 2012, pruning increased branch number of CP at 4 WAT by 1.6 branches, but the increase did not persist. Pruning was generally ineffective at increasing IVP branch number. In 2013, pruning increased the number of branches of IVP plants by 71%, 50%, and 49% at 4, 8, and 12 WAT, respectively. Pruning had no effect on CP plants in 2013. Pruning caused a greater cumulative branch number for IVP plants in 2013, but CP plants were unaffected. Pruned IVP plants gained 4 (77%) more branches than IVP water controls and 6.8 (283%) more branches than CP pruned plants over the course of the experiment.

PGRs were ineffective at increasing branch number as well as cumulative branch number and occasionally decreased branch number compared with the pruning treatment. Holland et al. (2007) found that cyclanilide applied to rhododendron 'Roseum Elegans' was not effective at

increasing branch number. The authors attributed this to the thick waxy surface of the leaves (Holland et al., 2007). However, multiple studies have shown PGRs affecting rhododendron growth and development. Dikegulac sodium, the active ingredient in Augeo increased the number of branches of rhododendron 'Formosa' (*Rhododendron indica* 'Formosa'*)*, but had no effect on 'Hexe' (Cohen, 1978). Ancymidol, phosfon, CCC, and daminozide promoted compactness in field-grown rhododendron, with ancymidol being the most effective (Cathey, 1975). Ancymidol also increased the number of flower buds on 'Roseum Elegans' by one to two times (Ticknor, 1968) and five times in *Rhododendron* 'Humming Bird' (Ryan, 1970). Paclobutrazol inhibited stem elongation and promoted flowering in field grown 'Roseum Elegans' and 'Boursault' (Gent, 1995).

In 2012, IVP water controls were initially 150% denser than CP water controls. In 2013, density was not different between the two propagation methods initially (Tables 10 and 11). IVP water controls were denser than CP water controls by 233%, 150%, and 113% in 2012 and 122%, 212%, and 214% in 2013 at 4, 8, and 12 WAT, respectively. Pruning increased density of IVP rhododendron in both years. In 2012, pruning increased the density of IVP plants by 85% and 76% at 4 and 8 WAT, respectively, such that IVP pruned rhododendron had a 362% and 275% greater density than CP water controls at 8 and 12 WAT, respectively. In 2013, pruning increased density of IVP by 85%, 52%, and 73% at 4, 8, and 12 WAT, respectively, such that IVP pruned rhododendron had a 311%, 375% and 443% greater density than CP water controls at 4, 8, and 12 WAT, respectively.

Topflor increased the density of IVP rhododendron by 75% and 65% at 8 and 12 WAT, respectively, in 2012, but did not increase density of IVP in 2013. No other PGR treatment was effective at increasing rhododendron density. Although applying Topflor to IVP plants created a denser plant than the water control, it was inconsistent from year to year and pruning, although more labor intensive, was more effective at consistently increasing density. However, 1 YAT, Topflor-treated plants of both propagation methods exhibited greater density than the water

controls and were the only treatment (including water control) that flowered (Appendix B). Under field-grown conditions, rhododendron do not bloom until three or more years of production (Gent, 1995). Other PGRs promoted flowering one year into production including the growth retardants phosfon and CCC for rhododendrons (Stuart, 1961) and daminozide and paclobutrazol for azaleas (Meijon et al., 2009). If Topflor decreases the time to flowering, it may allow producers to sell rhododendron earlier as consumers prefer plants in flower (Glasgow, 1999).

In 2012, Configure was the only PGR to cause phytotoxicity symptoms in the form of thin, light green apical leaves and only in CP plants (Table 12). In 2013, rhododendron was more affected by branch-inducing treatment than in 2012, but was not susceptible to phytotoxicity symptoms (Table 13). The increased branching yet lack of phytotoxicity symptoms may have been due to increased rainfall in 2013 or the fact that the plants were one month younger in 2013 than in 2012. Quality was not improved by either propagation method or branch-inducing treatment in either year (Tables 12 and 13).

As with the clethra, IVP rhododendron had a greater number of branches and density. However, it is important to point out that IVP rhododendron had been pruned just prior to the study, an additional pruning that CP were not subjected to, and may have given IVP plants an advantage. Pruning increased branch number and density of CP and IVP, but was more effective and consistent on IVP plants. Although they may be more expensive (cuttings were donated; price not available), purchasing IVP rhododendron may be the best option for growers. Pruning may be unnecessary, but can create an even more well-branched IVP plant.

Treatments were applied in June both years, but plants were acquired a month earlier in 2012 than in 2013 so plants were younger in 2013 when treatments were applied. Applying PGRs to plants when they are younger can be more effective than applying when they are older (Gent, 2004), however, treatments were more effective in 2012 than in 2013 (Topflor increased clethra

and rhododendron density in 2012 but not 2013). Regardless of year, and most noticeably in 2013, IVP plants were more sensitive to branch-inducing treatments than CP plants.

CONCLUSION

PGRs in this study were not effective at improving the overall quality and marketability of clethra, magnolia, or rhododendron. The supplier for the rooted cuttings used in this study prunes liners during propagation in order to provide the highest quality liner. Perhaps the PGRs did not increase branch number because resources had already been allocated to branching. Alternatively, perhaps PGRs were not effective because plants were not treated early enough in the growing season. Although, in general, PGRs did not decrease branch number, density, or quality compared with untreated plants, there was no advantage to investing in PGRs in this study.

Many alternatives to PGRs may improve plant quality such as creating a water deficit (Brown et al., 1992; Latimer, 1992; Latimer and Oetting, 1998; Latimer and Severson, 1997). Not only can drought stress control plant height, but it can also condition plants for shipping and after planting in the landscape (Herbert et al., 2010; Latimer and Oetting, 1998). Other alternatives include controlling temperature, light quality, and phosphorous levels. Lower day than night temperatures and an early morning decrease can retard plant growth (Oerum and Christensen, 2001). Reducing the transmission of far-red light controls growth in poinsettias (*Euphorbia pulcherrima* Willd.) (Clifford et al., 2004). In response to low phosphorus levels, root formation is favored over shoot growth, creating more compact plants (Lopez-Bucio et al., 2002; Ma et al., 2001). Mechanical stress and breeding are also often used to improve plant quality (Müller, 2011). However, conventional breeding approaches have not provided a timely solution (Heuvelink et al., 2009). Transgenic plants could also be an alternative to chemical control and/or pruning. However, there is a high cost to GMO breeding and research for ornamentals, and so far only one species, carnation (*Dianthus caryophyllus* L.*)* has been marketed; however, it was only modified for flower color, not compactness (Müller, 2011). Another restriction is negative perception to

molecular breeding and GMOs (GMO-Compass, 2013). Molecular breeding strategies, ones not requiring controversial recombinant DNA techniques (these are considered non GMOs), may be another way to produce compact plants that do not need any growth modification (Christensen et al., 2008b; Lutken et al., 2010). Another alternative can now be added to this list: IVP.

For clethra and rhododendron, IVP plants had more branches and a greater density and quality than the CP plants. The enhanced IVP branching to the juvenility gained through the IVP process (Brand and Lineberger, 1992; Webster and Jones, 1989). Brand and Lineberger, (1992) observed decreased internode length on IVP paper birch (*Betula papyrifera* Marshall) compared to cohorts that were CP. Decreased internode length would generate more branches per given unit of stem length, creating a mere dense plant canopy. In this study, however, we did not measure internode length. IVP plants were occasionally more responsive to PGRs than CP plants in this study. IVP plants are pruned several times while in culture; pruning produces physiological differences and may change the susceptibility of the plant to external factors (Clair-Maczulajtys et al., 1999), including PGRs.

Although the PGRs in this study were ineffective at increasing branch number, density, symmetry and quality of any of our species consistently, there may be other PGRs that are and/or other production environments in which these would be effective. Additionally, there may be other cultivars of these species for which these products would be effective as cultivar specificity among PGRs has been documented (Bailey and Clark, 1992; Cohen, 1978; Norcini et al, 1994). Another possibility is that our rate was not sufficient or multiple applications were required. In future studies, PGRs with other modes of action, application timing, and variable PGR rates should be evaluated for these plant species.

In this study, pruning was only effective at improving quality of the rhododendron, PGRs were ineffective on improving quality of all three species within our 12-week time frame, and IVP plants displayed innately higher branch numbers, canopy densities, and overall quality for both

clethra and rhododendron without any additional inputs (with the exception of the pre-experiment pruning to rhododendron) throughout the study. If cost effective (considering cost of liners and additional inputs needed for CP including pruning and/or PGRs), nursery produces may want to consider acquiring IVP liners for these species.

APPENDIX 1: TABLES

Table 1: Initial *Clethra alnifolia* 'Hummingbird' compactness in 2012 and 2013 (data pooled).

Propagation Method	Clethra compactness	
	Leaf area: height (cm^2/cm)	Shoot to leaf dry mass: height (g/cm)
CP	25:1 b[z]	0.34:1 b
IVP	56:1 a	0.55:1 a
DF	1	1
Significance	***[y]	***
P-value	<0.0001	<0.0001
F Statistic	81.07	41.05

[z]Means within a column followed by the same letter were not significantly different (Tukey's HSD α = 0.05).

[y]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significa

Table 2: Branch number and density of IVP and CP *Clethra alnifolia* 'Hummingbird' following branch-inducing treatments in 2012.

Treatment	Rate (ppm)[z]	Branches (number)					Density (Branches/cm)			
CP		0 WAT	4 WAT	8 WAT	12 WAT	Cumulative	0 WAT	4 WAT	8 WAT	12 WAT
Water	-	24 ± 3 b[y]	41 ± 4 bcd	69 ± 5 c	79 ± 7 bc	55 ± 6 abc	0.8 ± 0.1b	1.0 ± 0.1 e	1.2 ± 0.1 de	1.2 ± 0.1 c
Augeo	800	26 ± 3 b	46 ± 4 bc	65 ± 5 cd	73 ± 7 bcd	47 ± 6 bcd	0.8 ± 0.1 b	1.2 ± 0.1 cde	1.2 ± 0.1 de	1.2 ± 0.1 c
Configure	600	24 ± 3 b	44 ± 4 bc	64 ± 5 cd	71 ± 7 bcd	47 ± 6 bcd	0.8 ± 0.1 b	1.1 ± 0.1 de	1.1 ± 0.1 de	1.2 ± 0.1 c
Topflor	150	25 ± 3 b	26 ± 4 d	39 ± 5 e	44 ± 7 d	19 ± 6 d	0.9 ± 0.1 b	0.8 ± 0.1 e	1.1 ± 0.1 de	1.3 ± 0.1 c
Prune	-	27 ± 3 b	30 ± 4 cd	45 ± 5 de	55 ± 7 cd	28 ± 7 cd	0.9 ± 0.1 b	1.0 ± 0.1 e	0.9 ± 0.1 e	1.1 ± 0.1 c
IVP										
Water	-	46 ± 3 a	80 ± 4 a	109 ± 4.9 a	119 ± 7 a	74 ± 6 ab	1.6 ± 0.1 a	1.9 ± 0.1 ab	1.9 ± 0.1 abc	1.9 ± 0.1 b
Augeo	800	44 ± 3 a	81 ± 4 a	104 ± 5.1 ab	121 ± 7 a	73 ± 6 ab	1.6 ± 0.1 a	2.3 ± 0.1 a	1.8 ± 0.1 bc	2.1 ± 0.1 ab
Configure	600	42 ± 3 a	78 ± 4 a	113 ± 4.9 a	121 ± 7 a	79 ± 6 a	1.5 ± 0.1 a	1.9 ± 0.1 ab	2.0 ± 0.1 ab	2.1 ± 0.1 ab
Topflor	150	48 ± 3 a	57 ± 4 b	85 ± 5.1 bc	97 ± 7 ab	49 ± 6 bc	1.6 ± 0.1 a	1.5 ± 0.1 bcd	2.3 ± 0.1 a	2.6 ± 0.1 a
Prune	-	41 ± 3 a	49 ± 4 b	70 ± 4.9 c	99 ± 7 ab	58 ± 6 ab	1.5 ± 0.1 a	1.6 ± 0.1 bc	1.5 ± 0.1 cd	2.0 ± 0.1 b
DF		9	9	9	9	9	9	9	9	9
Significance		***[x]	***	***	***	***	***	***	***	***
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
F Statistic		15.68	31.39	26.53	17.14	9.70	16.97	22.29	20.87	13.33

[z]1 ppm = 1 mg·L^{-1}

[y]Means within a column followed by the same letter were not significantly different (Tukey's HSD $\alpha = 0.05$).

[y]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

Table 3: Branch number and density of IVP and CP clethra following branch-inducing treatments in 2013.

Treatment	Rate (ppm)[z]	Branches (number)					Density (Branches/cm)			
CP	Rate (ppm)[z]	0 WAT	4 WAT	8 WAT	12 WAT	Cumulative	0 WAT	4 WAT	8 WAT	12 WAT
Water	-	9 ± 1 b[y]	24 ± 2 cde	51 ± 3 bc	72 ± 6 bcde	63 ± 6 abcd	0.31 ± .047 bcd	0.31 ± 0.053 cd	0.98 ± 0.084 de	1.03 ± 0.089 bc
Augeo	800	7 ± 1 b	25 ± 2 bcd	48 ± 3 cd	75 ± 7 bcd	68 ± 7 abc	0.28 ± .050 d	0.66 ± 0.056 bcd	0.86 ± 0.088 de	1.01 ± 0.094 bc
Configure	600	8 ± 1 b	25 ± 2 bcd	54 ± 3 bc	70 ± 6 bcde	62 ± 6 abcd	0.31 ± .045 bcd	0.63 ± 0.051 cd	1.00 ± 0.081 cde	1.00 ± 0.085 bc
Topflor	150	7 ± 1 b	16 ± 2 ef	33 ± 3 de	44 ± 6 e	37 ± 6 d	0.28 ± .047 d	0.56 ± 0.053 d	1.10 ± 0.084 cde	1.00 ± 0.089 bc
Prune	-	7 ± 1 b	14± 2 F	31 ± 3 e	51 ± 6 de	44 ± 6 cd	0.27 ± .047 d	0.51 ± 0.053 d	0.73 ± 0.084 e	0.83 ± 0.089 c
IVP										
Water	-	16 ± 1 a	34 ± 2 ab	71± 3 a	100 ± 7 a	84 ± 7 a	0.56 ± .050 a	0.91 ± 0.056 ab	1.53 ± 0.088 ab	1.45 ± 0.094 A
Augeo	800	15 ± 1 a	42 ± 2 a	67 ± 4 ab	96 ± 7 ab	81 ± 8 a	0.53 ± .059 abc	1.14 ± 0.067 a	1.43 ± 0.11 abc	1.42 ± 0.11 Ab
Configure	600	16 ± 1 a	39 ± 2 a	73 ± 4 a	95 ± 7 abc	79 ± 7 ab	0.53 ± .052 ab	1.02 ± 0.059 a	1.60 ± 0.092 a	1.51 ± 0.099 A
Topflor	150	16 ± 1 a	28 ± 2 bc	51 ± 4 bc	62 ± 7 cde	13 ± 7 bcd	0.58 ± .052 a	0.86 ± 0.059 abc	1.82 ± 0.093 a	1.56 ± 0.099 a
Prune	-	16 ± 1 a	18 ± 2 def	52 ± 3 bc	86 ± 7 abc	70 ± 7 abc	0.54 ± .050 a	0.66 ± 0.056 bcd	1.13 ± 0.088 bcd	1.28 ± 0.094 ab
DF		9	9	9	9	9	9	9	9	9
Significance		***[x]	***	***	***	***	***	***	***	***
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
F Statistic		11.10	22.03	17.55	8.20	5.85	7.69	12.70	15.77	7.81

[z]1 ppm = 1 mg·L^{-1}

[y]Means within a column followed by the same letter were not significantly different (Tukey's HSD $\alpha = 0.05$).

[x]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

Table 4: Phytotoxicity and quality of IVP and CP *Clethra alnifolia* 'Hummingbird' following branch-inducing treatments in 2012.

Treatment	Rate (ppm)[z]	Phytotoxicity[y]	Quality[v]
CP		2 WAT	12 WAT
Water	-	0.0 ± 0.2 b[x]	2.3 ± 0.2 bc
Augeo	800	2.4 ± 0.2 a	2.2 ± 0.2 c
Configure	600	0.6 ± 0.2 b	2.4 ± 0.3 bc
Topflor	150	0.0 ± 0.2 b	3.4 ± 0.3 ab
Prune	-	0.1 ± 0.2 b	2.2 ± 0.3 c
IVP			
Water	-	0.0 ± 0.2 b	4.0 ± 0.3 a
Augeo	800	2.6 ± 0.2 a	4.0 ± 0.3 a
Configure	600	0.2 ± 0.2 b	3.6 ± 0.2 a
Topflor	150	0.0 ± 0.2 b	4.0 ± 0.2 a
Prune	-	0.0 ± 0.2 b	4.0 ± 0.2 a
DF		9	9
Significance		***[w]	***
P-value		<0.0001	<0.0001
F statistic		30.21	10.50

[z]1 ppm = 1 mg·L^{-1}

[y]Phytotoxicity symptoms rated on a 0 to 10 visual scale, where 0 represented no injury, and 10 represented complete kill.

[x]Means within a column followed by the same letter were not significantly different (Tukey's HSD α = 0.05)

[w]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

[v]Quality was determined on a 1 to 5 scale: 1=sparsely branched and asymmetrical plants with an open canopy, 2=sparsely branched and asymmetrical plants with a closed canopy, 3=more densely branched and asymmetrical plants with a closed canopy, 4=densely branched, symmetrical plants, and 5=densely branched and symmetrical plants that completely covered the container surface.

Table 5: Phytotoxicity and quality of IVP and CP *Clethra alnifolia* 'Hummingbird' following branch-inducing treatments in 2013.

Treatment	Rate (ppm)[z]	Phytotoxicity[y]	Quality[v]
CP		2 WAT	12 WAT
Water	-	0.2 ± 0.3 b[x]	1.7 ± 0.2 c
Augeo	800	1.7 ± 0.2 a	1.4 ± 0.2 c
Configure	600	0.0 ± 0.2 b	1.9 ± 0.2 bc
Topflor	150	0.0 ± 0.3 b	2.8 ± 0.2 ab
Prune	-	0.0 ± 0.3 b	1.9 ± 0.2 bc
IVP			
Water	-	0.0 ± 0.3 b	2.9 ± 0.2 ab
Augeo	800	1.9 ± 0.3 a	3.9 ± 0.3 a
Configure	600	0.0 ± 0.3 b	3.6 ± 0.3 a
Topflor	150	0.0 ± 0.3 b	3.8 ± 0.3 a
Prune	-	0.0 ± 0.3 b	3.0 ± 0.2 ab
DF		9	9
Significance		***[w]	***
P-value		<0.0001	<0.0001
F Statistic		8.18	13.06

[z]1 ppm = 1 mg·L^{-1}

[y]Phytotoxicity symptoms rated on a 0 to 10 visual scale, where 0 represented no injury, and 10 represented complete kill.

[x]Means within a column followed by the same letter were not significantly different (Tukey's HSD $\alpha = 0.05$).

[w]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

[v]Quality was determined on a 1 to 5 scale: 1=sparsely branched and asymmetrical plants with an open canopy, 2=sparsely branched and asymmetrical plants with a closed canopy, 3=more densely branched and asymmetrical plants with a closed canopy, 4=densely branched, symmetrical plants, and 5=densely branched and symmetrical plants that completely covered the container surface.

Table 6: Branch number and density of IVP and CP *Magnolia virginiana* 'Henry Hicks' following branch-inducing treatments in 2012.

Treatment	Rate (ppm)[z]	Branches (number)					Density (Branches/cm)			
CP		0 WAT	4 WAT	8 WAT	12 WAT	Cumulative	0 WAT	4 WAT	8 WAT	12 WAT
Water	-	2.9 ± 0.3	3.9 ± 0.4	5.6 ± 0.5 ab[y]	6.3 ± 0.6	3 ± 0.6	0.14 ± 0.014 a	0.14 ± 0.014 abc	0.16 ± 0.015 abc	0.16 ± 0.015 ab
Augeo	800	2.8 ± 0.3	3.4 ± 0.4	5.2 ± 0.5 ab	6.0 ± 0.6	3 ± 0.6	0.11 ± 0.013 a	0.10 ± 0.013 bc	0.13 ± 0.015 bc	0.13 ± 0.015 ab
Configure	600	2.7 ± 0.4	3.6 ± 0.4	5.5 ± 0.6 ab	6.5 ± 0.6	3 ± 0.6	0.10 ± 0.014 a	0.11 ± 0.014 abc	0.16 ± 0.015 abc	0.17 ± 0.015 ab
Topflor	150	2.8 ± 0.3	4.1 ± 0.4	5.6 ± 0.6 ab	6.0 ± 0.6	3 ± 0.6	0.12 ± 0.013 a	0.15 ± 0.013 ab	0.15 ± 0.015 abc	0.14 ± 0.015 ab
Prune	-	2.9 ± 0.3	3.9 ± 0.4	6.5 ± 0.5 a	7.2 ± 0.6	4 ± 0.6	0.12 ± 0.013 a	0.17 ± 0.013 a	0.21 ± 0.015 a	0.20 ± 0.015 a
IVP										
Water	-	2.1 ± 0.4	3.1 ± 0.4	5.3 ± 0.6 ab	5.6 ± 0.6	4 ± 0.6	0.07 ± 0.014 b	0.08 ± 0.014 c	0.10 ± 0.015 c	0.10 ± 0.015 b
Augeo	800	2.3 ± 0.4	2.8 ± 0.5	4.5 ± 0.6 ab	5.6 ± 0.7	3 ± 0.7	0.09 ± 0.015 b	0.08 ± 0.014 c	0.10 ± 0.016 c	0.11 ± 0.016 b
Configure	600	1.9 ± 0.4	2.9 ± 0.5	5.3 ± 0.6 ab	6.3 ± 0.7	6 ± 0.6	0.08 ± 0.015 b	0.09 ± 0.014 bc	0.15 ± 0.016 abc	0.16 ± 0.016 ab
Topflor	150	2.4 ± 0.4	3.0 ± 0.4	3.8 ± 0.6 b	5.3 ± 0.6	3 ± 0.6	0.08 ± 0.014 b	0.10 ± 0.014 bc	0.10 ± 0.015 c	0.11 ± 0.015 b
Prune	-	2.2 ± 0.4	2.9 ± 0.4	5.4 ± 0.6 ab	7.0 ± 0.6	5 ± 0.6	0.08 ± 0.014 b	0.13 ± 0.014 abc	0.18 ± 0.015 ab	0.19 ± 0.015 a
DF		9	9	9	9	9	9	9	9	9
Significance		NS[x]	NS	*	NS	NS	*	***	***	***
P-value		0.3699	0.2280	0.1522	0.4908	0.0743	0.0150	<0.0001	<0.0001	<0.0001
F Statistic		1.10	1.34	2.05	0.94	1.81	2.58	5.24	5.85	4.55

[z]1 ppm = 1 mg·L^{-1}

[y]Means within a column followed by the same letter were not significantly different (Tukey's HSD α = 0.05).

[y]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

Table 7: Branch number and density of IVP and CP *Magnolia virginiana* 'Henry Hicks' following branch-inducing treatments in 2013.

Treatment	Rate (ppm)[z]	Branches (number)					Density (Branches/cm)			
CP		0 WAT	4 WAT	8 WAT	12 WAT	Cumulative	0 WAT	4 WAT	8 WAT	12 WAT
Water	-	1.0 ± 0.1 b[y]	3.2 ± 0.4	4.6 ± 0.5	9.0 ± 0.8 a	8.2 ± 0.7 ab[x]	0.06 ± 0.010 b	0.11 ± 0.015	0.11 ± 0.013	0.12 ± 0.012
Augeo	800	1.0 ± 0.2 b	4.1 ± 0.4	4.8 ± 0.6	8.8 ± 0.9 a	8.0 ± 0.8 abc	0.06 ± 0.011 ab	0.14 ± 0.018	0.11 ± 0.015	0.11 ± 0.014
Configure	600	1.0 ± 0.2 b	2.6 ± 0.4	5.4 ± 0.6	9.8 ± 0.9 a	8.8 ± 0.8 a	0.06 ± 0.010 ab	0.09 ± 0.017	0.12 ± 0.014	0.12 ± 0.013
Topflor	150	1.0 ± 0.2 b	2.4 ± 0.4	4.8 ± 0.6	8.1 ± 0.9 ab	6.9 ± 0.8 abcd	0.06 ± 0.011 ab	0.09 ± 0.018	0.11 ± 0.015	0.11 ± 0.014
Prune	-	1.0 ± 0.1 b	2.9 ± 0.4	4.0 ± 0.6	6.9 ± 0.8 ab	5.9 ± 0.7 abcd	0.05 ± 0.010 b	0.10 ± 0.016	0.11 ± 0.014	0.10 ± 0.013
IVP										
Water	-	1.8 ± 0.1 a	2.6 ± 0.4	4.5 ± 0.5	7.4 ± 0.8 ab	5.6 ± 0.7 abcd	0.09 ± 0.010 ab	0.09 ± 0.016	0.10 ± 0.014	0.12 ± 0.013
Augeo	800	1.3 ± 0.1 ab	3.1 ± 0.4	4.4 ± 0.6	5.9 ± 0.8 ab	4.6 ± 0.8 cd	0.08 ± 0.010 ab	0.12 ± 0.016	0.12 ± 0.014	0.10 ± 0.013
Configure	600	1.5 ± 0.2 ab	2.4 ± 0.4	3.5 ± 0.6	4.5 ± 0.9 b	3.3 ± 0.9 d	0.10 ± 0.010 a	0.13 ± 0.016	0.13 ± 0.014	0.11 ± 0.013
Topflor	150	1.4 ± 0.1 ab	2.8 ± 0.4	5.3 ± 0.6	6.4 ± 0.8 ab	5.0 ± 0.8 bcd	0.08 ± 0.010 ab	0.11 ± 0.017	0.14 ± 0.014	0.12 ± 0.013
Prune	-	1.5 ± 0.1 ab	2.5 ± 0.4	4.1 ± 0.5	6.6 ± 0.8 ab	5.2 ± 0.7 bcd	0.09 ± 0.010 ab	0.10 ± 0.016	0.10 ± 0.014	0.11 ± 0.013
DF		9	9	9	9	9	9	9	9	9
Significance		***[x]	NS	NS	**	***	**	NS	NS	NS
P-value		0.0005	0.1363	0.5483	0.0012	<0.0001	0.0010	0.3817	0.5425	0.8618
F Statistic		3.78	1.57	0.88	3.45	4.80	3.51	1.09	0.88	0.51

[z]1 ppm = 1 mg·L^{-1}

[y]Means within a column followed by the same letter were not significantly different (Tukey's HSD $\alpha = 0.05$).

[y]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

Table 8: Phytotoxicity and quality of IVP and CP *Magnolia virginiana* 'Henry Hicks' following branch-inducing treatments in 2012.

Treatment	Rate (ppm)[z]	Phytotoxicity[y]	Quality[v]
CP		2 WAT	12 WAT
Water	-	0.0 ± 0.7	3.8 ± 0.3 a[x]
Augeo	800	0.0 ± 0.7	3.4 ± 0.3 abc
Configure	600	0.1 ± 0.7	3.6 ± 0.3 ab
Topflor	150	0.2 ± 0.7	2.8 ± 0.3 abc
Prune	-	0.1 ± 0.7	3.3 ± 0.3 abc
IVP			
Water	-	0.0 ± 0.8	2.2 ± 0.3 c
Augeo	800	0.0 ± 0.8	2.6 ± 0.3 bc
Configure	600	0.0 ± 0.8	3.6 ± 0.3 ab
Topflor	150	0.2 ± 0.8	2.5 ± 0.3 bc
Prune	-	0.2 ± 0.8	3.7 ± 0.3 ab
DF		9	9
Significance		NS[w]	***
P-value		0.3019	<0.0001
F Statistic		1.20	4.34

[z]1 ppm = 1 mg·L^{-1}

[y]Phytotoxicity symptoms rated on a 0 to 10 visual scale, where 0 represented no injury, and 10 represented complete kill.

[x]Means within a column followed by the same letter were not significantly different (Tukey's HSD α = 0.05).

[w]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

[v]Quality was determined on a 1 to 5 scale: 1=a single branch or dominant central leader, 2=two branches or to dominant leaders, 3=several branches emerging towards the apex of the plant, 4=a plant with a majority of basal branching, and 5=several branches emerging from the base, covering at least 90% of the container surface.

Table 9: Phytotoxicity and quality of IVP and CP *Magnolia virginiana* 'Henry Hicks' following branch-inducing treatments in 2013.

Treatment	Rate (ppm)[z]	Phytotoxicity[y]	Quality[v]
CP		2 WAT	12 WAT
Water	-	0.0 ± 0.0	3.1 ± 0.4
Augeo	800	0.0 ± 0.0	3.4 ± 0.4
Configure	600	0.0 ± 0.0	3.9 ± 0.5
Topflor	150	0.0 ± 0.0	3.0 ± 0.4
Prune	-	0.0± 0.0	2.9 ± 0.4
IVP			
Water	-	0.0 ± 0.0	2.8 ± 0.4
Augeo	800	0.0 ± 0.0	2.8 ± 0.4
Configure	600	0.0 ± 0.0	3.0 ± 0.4
Topflor	150	0.0 ± 0.0	2.5 ± 0.4
Prune	-	0.0 ± 0.0	2.9 ± 0.4
DF		9	9
Significance		NS[w]	NS
P-value		.	0.6423
F Statistic		0.00	0.77

[z]1 ppm = 1 mg·L^{-1}

[y]Phytotoxicity symptoms rated on a 0 to 10 visual scale, where 0 represented no injury, and 10 represented complete kill.

[x]Means within a column followed by the same letter were not significantly different (Tukey's HSD $\alpha = 0.05$).

[w]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

[v]Quality was determined on a 1 to 5 scale: 1=a single branch or dominant central leader, 2=two branches or to dominant leaders, 3=several branches emerging towards the apex of the plant, 4=a plant with a majority of basal branching, and 5=several branches emerging from the base, covering at least 90% of the container surface.

Table 10: Branch number and density of IVP and CP *Rhododendron* 'Roseum Elegans' following branch-inducing treatments in 2012.

Treatment	Rate (ppm) [z]	Branches (number)					Density (Branches/cm)			
CP		0 WAT	4 WAT	8 WAT	12 WAT	Cumulative	0 WAT	4 WAT	8 WAT	12 WAT
Water	-	1.9 ± 0.3 b[y]	2.4 ± 0.3 b	2.9 ± 0.3 cde	3.0 ± 0.4 cd	1.1 ± 0.3 ab	0.14 ± 0.030 b	0.09 ± 0.026 c	0.08 ± 0.018 e	0.08 ± 0.016 d
Augeo	800	1.9 ± 0.3 b	2.5 ± 0.3 b	2.5 ± 0.3 de	2.6 ± 0.4 d	0.4 ± 0.3 b	0.15 ± 0.030 b	0.12 ± 0.026 c	0.09 ± 0.018 de	0.07 ± 0.016 d
Configure	600	1.9 ± 0.3 b	1.9 ± 0.3 b	2.1 ± 0.3 e	2.7 ± 0.4 cd	0.8 ± 0.3 ab	0.15 ± 0.032 b	0.09 ± 0.027 c	0.07 ± 0.019 e	0.06 ± 0.017 d
Topflor	150	2.0 ± 0.3 b	2.0 ± 0.3 b	2.0 ± 0.3 e	3.1 ± 0.4 bcd	1.1 ± 0.3 ab	0.15 ± 0.032 b	0.14 ± 0.027 c	0.12 ± 0.019 cde	0.14 ± 0.017 cd
Prune	-	1.9 ± 0.3 b	4.0 ± 0.3 a	4.0 ± 0.3 bcd	4.1 ± 0.4 bcd	2.0 ± 0.3 a	0.14 ± 0.030 b	0.21 ± 0.026 bc	0.17 ± 0.018 bcd	0.15 ± 0.016 c
IVP										
Water	-	3.8 ± 0.3 a	3.9 ± 0.3 a	3.9 ± 0.4 bcd	4.4 ± 0.4 abc	0.6 ± 0.3 ab	0.35 ± 0.033 a	0.30 ± 0.028 ab	0.20 ± 0.020 bc	0.17 ± 0.018 c
Augeo	800	3.9 ± 0.3 a	4.0 ± 0.3 a	4.4 ± 0.4 abc	4.9 ± 0.4 ab	1.1 ± 0.3 ab	0.41 ± 0.032 a	0.28 ± 0.027 ab	0.23 ± 0.019 b	0.20 ± 0.017 bc
Configure	600	3.8 ± 0.3 a	3.8 ± 0.3 a	3.8 ± 0.4 bcd	4.4 ± 0.4 abc	0.3 ± 0.3 b	0.36 ± 0.032 a	0.18 ± 0.027 ab	0.21 ± 0.019 b	0.17 ± 0.017 c
Topflor	150	4.0 ± 0.3 a	4.2 ± 0.3 a	4.8 ± 0.3 ab	5.9 ± 0.4 a	1.9 ± 0.3 a	0.40 ± 0.038 a	0.29 ± 0.032 ab	0.35 ± 0.023 a	0.28 ± 0.020 ab
Prune	-	4.2 ± 0.3 a	4.7 ± 0.3 a	5.6 ± 0.4 a	6.2 ± 0.4 a	1.9 ± 0.3 a	0.39 ± 0.038 a	0.36 ± 0.032 a	0.37 ± 0.023 a	0.30 ± 0.020 a
DF		9	9	9	9	9	9	9	9	9
Significance		***[x]	***	***	***	***	***	***	***	***
P-value		<0.0001	<0.0001	<0.0001	<0.0001	0.0003	<0.0001	<0.0001	<0.0001	<0.0001
F Statistic		12.88	12.78	12.27	11.98	3.96	15.45	12.13	25.14	19.95

[z]1 ppm = 1 mg·L^{-1}

[y]Means within a column followed by the same letter were not significantly different (Tukey's HSD $\alpha = 0.05$).

[x]Significance at P=0.01 (*), P=0.001 (**), P=0.0001(***), [NS] denotes non-significant

Table 11: Branch number and density of IVP and CP *Rhododendron* 'Roseum Elegans' following branch-inducing treatments in 2013.

Treatment	Rate (ppm) [z]	Branches (number)					Density (Branches/cm)			
CP		0 WAT	4 WAT	8 WAT	12 WAT	Cumulative	0 WAT	4 WAT	8 WAT	12 WAT
Water	-	1.0 ± 0.3 b[y]	2.0 ± 0.5 d	2.4 ± 0.7 c	2.4 ± 0.7 c	1.1 ± 0.6 d	0.09 ± 0.02 bc	0.09 ± 0.02 c	0.08 ± 0.03 d	0.07 ± 0.02 d
Augeo	800	1.0 ± 0.3 b	1.8 ± 0.5 d	2.8 ± 0.7 c	3.1 ± 0.7 c	2.1 ± 0.6 d	0.07 ± 0.02 c	0.08 ± 0.02 c	0.12 ± 0.03 cd	0.10 ± 0.02 d
Configure	600	1.1 ± 0.3 b	1.9 ± 0.5 d	2.1 ± 0.7 c	2.5 ± 0.7 c	1.4 ± 0.6 d	0.06 ± 0.02 c	0.09 ± 0.02 c	0.08 ± 0.03 d	0.09 ± 0.06 d
Topflor	150	1.0 ± 0.3 b	1.0 ± 0.6 d	1.4 ± 0.7 c	1.6 ± 0.7 c	0.5 ± 0.6 d	0.05 ± 0.02 bc	0.09 ± 0.02 c	0.15 ± 0.03 bcd	0.12 ± 0.02 cd
Prune	-	1.1 ± 0.3 b	3.1 ± 0.5 cd	3.1 ± 0.7 c	3.5 ± 0.7 c	2.4 ± 0.6 cd	0.07 ± 0.02 bc	0.16 ± 0.02 bc	0.14 ± 0.03 bcd	0.12 ± 0.03 cd
IVP										
Water	-	3.7 ± 0.3 a	6.6 ± 0.5 b	8.4 ± 0.7 b	8.9 ± 0.7 b	5.2 ± 0.6 bc	0.15 ± 0.02 ab	0.20 ± 0.02 b	0.25 ± 0.03 bc	0.22 ± 0.024 bc
Augeo	800	3.6 ± 0.3 a	6.7 ± 0.5 b	8.8 ± 0.7 b	9.9 ± 0.7 b	6.3 ± 0.6 b	0.14 ± 0.02 ab	0.21 ± 0.02 b	0.27 ± 0.03 ab	0.26 ± 0.024 b
Configure	600	3.3 ± 0.3 a	5.6 ± 0.6 bc	8.7 ± 0.7 b	9.1 ± 0.7 b	6.2 ± 0.6 b	0.12 ± 0.02 abc	0.16 ± 0.02 bc	0.23 ± 0.03 bc	0.22 ± 0.03 bc
Topflor	150	3.7 ± 0.3 a	6.0 ± 0.5 b	8.2 ± 0.7 b	9.8 ± 0.7 b	6.1 ± 0.6 b	0.15 ± 0.02 ab	021 ± 0.02 b	0.26 ± 0.03 ab	0.29 ± 0.02 ab
Prune	-	4.1 ± 0.3 a	11.3 ± 0.6 a	12.6 ± 0.7 a	13.3 ± 0.7 a	9.2 ± 0.6 a	0.17 ± 0.02 a	0.37 ± 0.02 a	0.38 ± 0.03 a	0.38 ± 0.03 a
DF		9	9	9	9	9	9	9	9	9
Significance		***[x]	***	***	***	***	***	***	***	***
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
F Statistic		20.15	35.34	29.33	34.81	22.02	6.14	15.11	11.14	16.53

[z]1 ppm = 1 mg·L^{-1}

[y]Means within a column followed by the same letter were not significantly different (Tukey's HSD $\alpha = 0.05$).

[x]Significance at P=0.01 (*), P=0.001 (**), P=0.0001(***), [NS] denotes non-significant

Table 12: Phytotoxicity and quality of IVP and CP *Rhododendron* 'Roseum Elegans' following branch-inducing treatments in 2012.

Treatment	Rate (ppm)[z]	Phytotoxicity[y]	Quality[v]
CP		2 WAT	12 WAT
Water	-	0.0 ± 0.3 b[x]	2.4 ± 0.3 ab
Augeo	800	0.1 ± 0.3 b	1.9 ± 0.3 b
Configure	600	2.2 ± 0.3 a	2.7 ± 0.3 ab
Topflor	150	0.1 ± 0.3 b	3.3 ± 0.3 a
Prune	-	0.0 ± 0.3 b	2.5 ± 0.3 ab
IVP			
Water	-	0.0 ± 0.3 b	2.9 ± 0.3 ab
Augeo	800	0.0 ± 0.3 b	2.8 ± 0.3 ab
Configure	600	0.0 ± 0.3 b	3.1 ± 0.3 ab
Topflor	150	0.0 ± 0.3 b	3.2 ± 0.3 ab
Prune	-	0.0 ± 0.3 b	2.5 ± 0.3 ab
DF		9	9
Significance		***[w]	*
P-value		<0.0001	0.0239
F Statistic		5.66	2.27

[z]1 ppm = 1 mg·L^{-1}

[y]Phytotoxicity symptoms rated on a 0 to 10 visual scale, where 0 represented no injury, and 10 represented complete kill.

[x]Means within a column followed by the same letter were not significantly different (Tukey's HSD α = 0.05).

[w]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

[v]Quality was determined on a 1 to 5 scale: 1=plants that had one strong leader with branch development on distal portion of stem only, causing minimal coverage of the pot surface, 2=two or more leaders with a narrow, columnar growth pattern and branch development occurring distally, 3=two or more stems with branch development occurring at the base, covering at least 70% of the container surface when viewed from above, 4=multiple stems that cover approximately 90% of the container surface, and 5=multiple stems from base and covered 100% of the container surface.

Table 13: Phytotoxicity and quality IVP and CP *Rhododendron* 'Roseum Elegans' following branch-inducing treatments in 2013.

Treatment	Rate (ppm)[z]	Phyto	Quality
CP		2 WAT	12 WAT
Water	-	0.0 ± 0.0	1.7 ± 0.3 bcd[y]
Augeo	800	0.0 ± 0.0	2.3 ± 0.3 abcd
Configure	600	0.0 ± 0.0	1.3 ± 0.3 d
Topflor	150	0.0 ± 0.0	1.4 ± 0.3 cd
Prune	-	0.0 ± 0.0	2.6 ± 0.3 abc
IVP			
Water	-	0.0 ± 0.0	2.9 ± 0.3 ab
Augeo	800	0.0 ± 0.0	2.8 ± 0.3 ab
Configure	600	0.0 ± 0.0	3.1 ± 0.3 a
Topflor	150	0.0 ± 0.0	3.2 ± 0.3 a
Prune	-	0.0 ± 0.0	2.4 ± 0.3 abcd
DF		9	9
Significance		NS[x]	***
P-value		.	<0.0001
F Statistic		0.00	6.07

[z]1 ppm = 1 mg·L^{-1}

[y]Phytotoxicity symptoms rated on a 0 to 10 visual scale, where 0 represented no injury, and 10 represented complete kill.

[x]Means within a column followed by the same letter were not significantly different (Tukey's HSD $\alpha = 0.05$).

[y]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant.

[v]Quality was determined on a 1 to 5 scale: 1=plants that had one strong leader with branch development on distal portion of stem only, causing minimal coverage of the pot surface, 2=two or more leaders with a narrow, columnar growth pattern and branch development occurring distally, 3=two or more stems with branch development occurring at the base, covering at least 70% of the container surface when viewed from above, 4=multiple stems that cover approximately 90% of the container surface, and 5=multiple stems from base and covered 100% of the container surface.

CHAPTER 3. SPRAY PENETRATION AND NATURAL ENEMY SURVIVAL IN DENSE AND SPARSE PLANT CANOPIES TREATED WITH CARBARYL: IMPLICATIONS FOR CONVENTIONAL AND BIOLOGICAL CONTROL

ABSTRACT

Ornamental plant producers often rely on chemical control to manage insect pests. However, cultural practices can influence plant architecture which may, in turn, affect pesticide penetration. The objectives of this study were to examine spray penetration in dense and sparse canopies, determine the effect of canopy density on beneficial insect survival following insecticide application, and provide understanding of the implication of canopy density on pest management for selected container-grown plants. To characterize spray penetration, water sensitive cards were placed on the exterior, middle and interior of China Girl® holly (*Ilex* ×*meserveae* S.Y. Hu 'Mesog') and oakleaf hydrangea 'Alice' (*Hydrangea quercifolia* Bartr. 'Alice') plants with dense or sparse canopies. Water was then applied with a CO_2 sprayer. To assess beneficial insect survival, hydrangeas were sprayed with an insecticide (carbaryl) in the same manner as water. Leaves were collected from each canopy position and placed in arenas with either a type of adult green lacewing (TAGL)(*Chrysoperla rufilabris* Burmeister) or adult convergent lady beetle (ACL) (*Hippodamia convergens* GM*)* to monitor survival over four days. Regardless of canopy density or plant species, the interior position of the canopy received less than 8% spray coverage. The middle position of sparse canopies received more coverage than the middle position of dense canopies. The middle and interior position of dense canopies protected greater than 50% of the ACL population while only the interior position of dense canopies protected greater than 50% of TAGL populations. Canopy density influenced spray penetration among both the small- and the large-leaved plant species. In this study, spray coverage within canopy interiors was low regardless of plant architecture, indicating that the interior of a plant could serve as a refugium for natural enemies. Additionally, the use of natural enemies and position of their release on crop plants may be critical to controlling pests, such as scales, that infest the trunk and other interior positions of the plant.

INTRODUCTION

Market forces, cultural practices, and pest management are inextricably linked during production of ornamental crops. Consumers of woody landscape plants prefer densely-branched plants over ones that are sparse (Glasgow, 1999). Therefore, growers endeavor to produce plants with dense canopies through the use of architecture-altering practices such as pruning and plant growth regulators (Cochran and Fulcher, 2013; Currey and Erwin, 2012; Gilman, 2012). However, increasing canopy density can affect pest management. A dense plant canopy can hinder foliar-applied insecticides from penetrating to the interior of the plant (Zhu et al., 2006; Zhu et al., 2008). Poor pesticide penetration can lead to problems controlling pests within the plant canopy or directly on the branches, such as scale insects (Hanks and Denno, 1993).

Ornamental plants are valued primarily for their aesthetic qualities (Bethke and Cloyd, 2009; Sadof and Raupp, 1996). Therefore, the economic threshold for an ornamental insect pest is often zero (Klingeman et al., 2000; van de Vrie, 1985). For example, a single female bagworm, *Thyridopteryx ephemeraeformis* (Haworth), can produce enough offspring to render a plant unmarketable (Horn and Sheppard, 1979; Raupp et al., 1989). Conventional chemicals are often the first and only control used in nursery crop production, in part, because they work quickly and can maintain pest populations at acceptable levels with minimal effort from the grower (Bethke and Cloyd, 2009; LeBude et al., 2012). Consumer's low tolerance for pest damage often motivates growers to apply pesticides as a preventative with the mindset that they are protecting their crops from pest damage (Briggs et al., 2002; Cho and Ki, 1999). If pest populations persist, growers may increase application frequency or the concentration of pesticide that they apply (Zhu et al., 2006). However, with adverse public perception of pesticide use (Falconer, 1998; Kher et al., 2013; Montella et al., 2012) and its effects on pollinators and other beneficial insects (Colin et al., 2004; Koppert Biological Systems, 2005; Mitsui Chemicals America, 2013; Szczepaniec et al., 2011), growers may need to reevaluate their practices.

It is well documented that natural enemies such as spiders and other predators prefer dense canopies to sparse canopies because dense canopies provide more shelter from heavy rain and other predators as well as provide abundant and diverse food sources (Halaj et al., 2000a; Halaj et al., 2000b; Langellotto and Denno, 2004). The more complex the plant canopy, the more connectors are available for a predator to move to its prey. Canopy complexity also increases the likelihood that the predator will continue to search for food on a particular plant (Skirvin, 2004). Using natural enemies with conventional pesticide application may increase the effectiveness of pest management in dense canopies.

Research on pesticide application to field-grown nursery crops has been conducted (Bache and Johnstone, 1992; Sánchez-Hermosilla et al., 2011; Zhu et al., 2006), but little information is available comparing spray penetration into dense and sparse plant canopies in a container nursery. There is little information on how to use natural enemies during woody ornamental plant production (LeBude et al., 2012) or how dense canopies may act as refugia for natural enemies. The objectives of this study were to 1) characterize spray penetration in dense and sparse canopies of select woody ornamental crops and 2) determine if denser canopies protect natural enemies from a foliar-applied insecticide, conserving their ability to effectively control arthropod pests.

MATERIALS AND METHODS

Spray penetration

Eighteen China Girl® hollies in 3-gal (11.4 L) containers were purchased (John Deere Landscaping, Knoxville, TN) on 23 January 2013 and placed in the North Greenhouse at the University of Tennessee in Knoxville, TN (35°56'46"N 83°56'18"W). On 18 February 2013, branches were counted. To create dense or sparse canopy densities, nine plants were pruned to 35 branches and the other nine were pruned to 75 branches (Figure 1), a 53% disparity. The appropriate disparity between dense and sparse plants was determined based on preliminary spray experiments and visually assessing the levels of canopy density to recreate the range found in

the marketplace. Pruning for both species consisted of thinning cuts to remove interior branches and create a more open canopy.

Twelve oakleaf hydrangeas were grown from 10.2 to 15.2 cm (4 to 6 inch) cuttings taken spring 2012. Plants were potted into 3 gal (11.4 L) containers filled with 85% pine bark and 15% peat. One week after transplanting, plants were top dressed with 19N-1.7P-6.6K, 5- to 6-month controlled release fertilizer with micronutrients (Polyon®, Harrell's Inc., Lakeland, FL) at 53 g per container (medium label rate). In October 2012, plants were placed in a plastic covered overwintering house until 4 February 2013 when they were placed in a walk-in cooler [~7°C (44°F), intermittent light]. They were watered periodically to prevent desiccation. On 5 March 2013 plants were moved to the North Greenhouse at which time they were pruned to 25.4 cm (10 inch) from substrate surface and again top dressed with 19N–1.7P–6.6K, 5- to 6-month controlled release fertilizer. By 27 March 2013, plants had leafed out and branches were counted. On 17 April 2013, six of the plants were pruned to 11 branches and six were pruned to 19 branches (Figure 2), a 42% disparity, creating dense and sparse canopy densities. To establish that the disparity in branch number created a disparity in density, hydrangea plant height was measured and then plants were destructively harvested at the conclusion of all four experiments in this study. All plant mass above the substrate surface was oven dried at 61°C (141 °F) for 72 hours. Once dry, weight was recorded. Density was calculated as shoot dry mass per unit of height according to van Iersel and Nemali (2004). For both the holly and the hydrangea, while the disparity between dense and sparse canopies was near 50%, both represented plant architecture commonly available in the marketplace.

For each species, the experiment was repeated four times. Spray penetration experiments for holly were conducted on 19 April and repeated on 30 April 2013 and for hydrangea were

conducted 17 May and repeated on 24 May 2013. On each date, the experiment was conducted two times. The same set of plants was used for each experiment.

Plants were placed on the ground in a row to simulate a nursery setting and spaced so that there was no contact between plants. Three 5.1- by 7.6-cm (2- by 3-inch) cards of water sensitive paper (WSP) (Syngenta Crop Protection AG, Basel, Switzerland) were placed on each plant, one per canopy position. The canopy positions were the exterior, middle and interior of the canopy (Figure 3). The exterior cards were attached in front of each plant with 5.1 cm (2 inch) alligator clips (Grand Rapids Industrial Products, Wayland, MI) on a wire attached to wooden poles to keep the cards at the same height of 46 cm (18.11 in) above the ground (Figure 4). Two alligator clips attached together with 15.2 cm (6 inches) of wire wrapped around the most central stem and held the interior card flush against the branch at 46 cm above the ground and the middle card half-way between the interior and exterior card, 46 cm above the ground. The exterior position served as the control as spray applied to cards in this position was unimpeded by leaves or branches.

Water was applied to the foliage simulating a pesticide application using a hand held CO_2 sprayer coupled with a Teejet® even flat spray tip TP8002E (Spraying Systems Company, Springfield, IL). The sprayer was operated at 30 PSI delivering 0.64 $L \cdot min^{-1}$ (0.17 GPM) flow rate. Many growers use handheld sprayers due to their ease of use in tight areas such as a greenhouse, and their ability to monitor where they have sprayed in real-time (Derksen et al., 2010). The nozzle was kept 46 cm above the ground and 0.61 m (2 feet) from the exterior cards and moved at a speed of 1.30 $m \cdot s^{-1}$ (4.7 KPH) [4.25 $feet \cdot s^{-1}$ (2.9 MPH)].

Cards dried on the plants and were immediately collected, labeled, and scanned with a business card scanner (WorldCard Office, Penpower Technology LTD., Fremont, CA). Spray penetration was analyzed using DepositScan scanning software (Zhu et al., 2011). Spray penetration was characterized by coverage (the percentage of WSP surface area that was covered by spray deposits) and droplet density (the number of droplets deposited on the cards per cm^2).

The percent of exterior coverage and the percent of exterior droplet density were calculated based on the spray captured in the middle and interior position compared with the exterior position (the control), which was not blocked by the plant canopy.

The experiment was arranged as a completely randomized design with nine replications for holly and six replications for hydrangea. Data were analyzed using the mixed procedure of SAS (Version 9.3S; SAS Institute, Cary, NC). Means were separated using Tukey's HSD at a significance level of 5% ($\alpha = 0.05$). Data for the two plant species were analyzed separately. Data were pooled between experiment repetitions within a species as data were not different.

Natural enemy survival

To determine how spray penetration affected natural enemy survival within dense and sparse plants, TAGL and ACL were confined to arenas with hydrangea leaves from dense and sparse plants that were sprayed with either water or carbaryl (1-naphthyl N-methylcarbamate, Sevin® SL, Bayer CropScience, Durham, NC) at 1 qt. per 100 gallons. Carbaryl was chosen because it was shown in previous research to be highly toxic to both TAGL and ACL populations (Yeary et al. unpublished data).

Arenas were built from 90 mm (3.54 inch) petri dishes (Fisher Scientific, Pittsburgh, PA) by removing a 7.6 cm (3 inch) diameter opening in the lid and replacing it with organdy fabric. A single 90 mm filter paper was placed in each arena to absorb excess moisture. For each arena, a hole was drilled in the lids of a 0.65 ml (3.04 oz.) microcentrifuge tube (Costar®, Corning, Corning, NY), plugged with cotton, and filled with a honey water solution (5% v/v) to serve as a food source. TAGL and ACL arrived 8 May and 9 May, respectively (Beneficial Insectaries, Redding, CA). Ten insects (TAGL or ACL) were added to each arena; prepared arenas remained in a walk-in cooler [~7 °C (44 °F), intermittent light] until spray applications were made, approximately 3 hours.

Oakleaf hydrangea was selected for this objective because the insects would not be able to avoid contact with the large leaf surface and any associated water or insecticide residue. To

determine if the hydrangea leaves or the arena environment affected natural enemy survival, twelve oakleaf hydrangeas from the spray penetration experiments (six with dense and six with sparse canopies) were sprayed with water using the same method as described in the spray penetration experiment. Leaves were allowed to dry on the plant and then were collected from the exterior, middle, and interior of each plant canopy and placed in plastic bags. Carbaryl was then applied and leaves were collected in exactly the same manner as the water. Each petiole was placed in a water pick and placed in its respective arena, one leaf per arena. While leaves were added, arenas remained in the cooler to prevent insects from warming and becoming active. Arenas were then moved to an insect rearing room with daytime temperatures maintained at 21 °C (69.8 °F). Survival was recorded 24, 48, 72, and 96 hours of exposure (HOE).

The experiment was a completely randomized design in a 3 (canopy positions) x 2 (canopy densities) factorial arrangement and analyzed with repeated measures using the GLM procedure of SAS. Means were separated using Tukey's LSD, $\alpha = 0.05$. ACL data were pooled, but TAGL data were separated by experiment due to survival differences between the two experiments.

RESULTS

Spray penetration

Hydrangea canopy density measurements comparing shoot dry mass to height established density between dense and sparse plants was different at 1.96:1 and 1.29:1, respectfully (*P*-value = 0.0001), supporting that branch differences and density are correlated (data not shown). Spray coverage was not different for the exterior positions of both dense and sparse plants, 28.4% versus 33.5% coverage and 38.2% versus 36.5% coverage, for holly and hydrangea, respectively (Tables 14 and 15). The middle and the interior positions had less coverage than the exterior position for each species. Coverage was not significantly affected by plant density in the interior position, but the middle position of dense plants received less coverage than the middle position of sparse plants, 4.5% versus 12.8% coverage and 0.8% versus 4.5 % coverage for holly and hydrangea,

respectively. For both species, within dense canopies, coverage was not different between the middle and interior positions, but in the sparse canopies of hollies, the interior position had less coverage than the middle position, 7.1% versus 12.8%.

Percentage of exterior coverage largely paralleled coverage for both holly and hydrangea (Tables 14 and 15). Percentage of exterior coverage was not different among exterior positions of sparse and dense plant architecture for either plant species. Percentage of exterior coverage was greater for the exterior position than either the middle or interior positions of both plant species. The interior position of sparse plants had lower percentage of exterior coverage than the middle position. However, percentage of exterior coverage at middle and interior positions among dense holly and hydrangea were not different, 12% versus 8% and 2% versus 1%, respectively.

Droplet density results were similar to coverage for both plant species (Tables 14 and 15). The exterior positions were not different among the dense and sparse plants for both holly (32 versus 33 deposits/cm^2 for dense and sparse, respectively) and hydrangea (56 versus 47 deposits/cm^2 for dense and sparse, respectively). In both species, deposits were lower in the middle and interior position than the exterior position regardless of plant architecture. Within the dense holly canopy, the exterior received 32 deposits/cm^2, while the middle and interior received only 7 deposits/cm^2 each, and within the dense hydrangea canopy, the exterior received 56 deposits/cm^2, while the middle and interior had only 2 deposits/cm^2. For sparse holly, the interior position had less droplet density than the middle position, 10 deposits/cm^2 versus 17 deposits/cm^2, but for sparse hydrangea, the droplet density in the middle and interior was not different.

Percentage of exterior droplet density paralleled droplet density for both holly and hydrangea (Tables 14 and 15). Percentage of exterior droplet density was not different among exterior positions of dense and sparse plant architecture for either plant species. Percentage of

exterior droplet density was also not different between middle and interior positions of dense hollies and hydrangeas, 22% versus 23%, and 3% versus 3%, respectively. For sparse plant canopies, the interior position was more protected from spray coverage than the middle position. For example, percentage of exterior droplet density was 49% versus 33% for middle and interior, respectively, for hollies and 26% versus 14%, respectively, for hydrangeas. However, percentage of exterior droplet density was greater for the exterior position than either the middle or interior positions of both plant species.

Natural enemy survival

There was no interaction between position and density on survival during the water control experiments for either TAGL or ACL. Water, hydrangea leaf, and arena environment had no effect on TAGL and ACL survivability (Tables 16 and 17). Therefore, the experimental conditions were considered acceptable and unlikely to influence the outcome of the experiments in which carbaryl was sprayed.

In experiments where carbaryl was applied, there was an interaction between position and density on survival of both insect species (Tables 18, 19, and 20). For both insects, plant architecture influenced survival at the middle and interior positions but not the exterior position. With the exception of ACL at 24 HOE, the middle of dense plants had greater survival than the middle of sparse plants (Table 20), and the interior of dense plants had a higher survival than the interior of sparse plants for both species by as much as 1100% and 446% for TAGL and ACL, respectively.

TAGL in the middle and interior positions of dense canopies consistently had a greater survivability than those in the exterior position as well as greater survivability than those in the sparse middle and interior positions (Table 18 and 19). In sparse canopies, TAGL survival in the exterior, middle, and interior positions was not different at any time point. In experiment one,

TAGL survival in the middle and interior positions of dense canopies was not different at 24 HOE, 76% and 85%, respectively, but beginning at 48 HOE, the TAGL in the middle position had lower survival than those in the interior position, 50%, 48%, and 35% survival in the middle position versus 72%, 63% and 60% survival in the interior position at 48, 72, and 96 HOE, respectively. In experiment two, TAGL survival was always greater in the interior position of dense canopies except for at 48 HOE when survival in the interior and middle positions were no different.

At 24 HOE, canopy position and plant density had no effect on ACL survival (Table 20). Survival in the exterior position was not affected by plant density. At 48, 72, and 96 HOE, ACL survival in the interior and middle positions of dense canopies was not different at 80%, 72%, and 71% and 73%, 65%, and 58%, respectively. However, both middle and interior positions were more protected positions than the exterior for dense plants. ACL survival in the interior and middle positions of sparse canopies was not different from the exterior position.

DISCUSSION

Spray penetration

Plant density measurements for hydrangea demonstrated that different levels of plant density were achieved. Densely-branched hydrangea plants were 52% denser than those pruned to create the sparsely dense plants (data not shown). Spray coverage was not different between the exterior positions for both dense and sparse hollies and hydrangea, indicating that spray applications were made consistently (Tables 14 and 15). As in the Derksen et al. (2001), Derksen et al. (2008) and Zhu et al. (1997) studies, there was less penetration in the interior than the exterior of the plant canopy. Within the dense holly canopy, as spray penetrated there was an 84% decrease in spray coverage at the middle position and 90% decrease in coverage at the interior position when compared with the coverage on the exterior of the plant (Table 14). Within the sparse holly canopy, there was a 62% and 79% coverage decrease at the middle and interior positions, respectively, when compared with the exterior position. Within the dense hydrangeas, a large-

leaved species, almost all spray penetrating the canopy was obstructed by foliage and branches; the middle and interior had less than 1% coverage (Table 15). Even within the sparse hydrangea canopy, coverage decreased 88% and 97% at the middle and interior positions, respectively, compared to the exterior position. Regardless of plant density, the interior of holly canopies received less than 8% coverage and hydrangea canopies received 1% or less coverage (Tables 14 and 15). The sparse holly plants received 184% and the hydrangea 463% more coverage in the middle of the canopy than the dense plants, indicating that some pest insects may be easier to control in sparse canopies due to higher insecticide coverage. Garcera et al. (2011) found that contact insecticides with 36-62% coverage were sufficient dependent upon insecticide used to manage all stages of California red scale (*Aonidiella aurantii* Mask.). Coverage documented in this study not be enough for a contact insecticide to effectively control scales, borers and other insects in the interior of the plant canopy (Garcera et al., 2011).

Within the dense canopy, droplet density is reduced from 56 deposits/cm^2 on the exterior position to 2 deposits/cm^2 on the middle and interior positions, a 96% reduction, in hydrangea and from 32 deposits/cm^2 on the exterior position to 7 deposits/cm^2 in the middle and interior position, a 78% reduction, in holly (Tables 14 and 15). Similar decreases in penetration have been documented in panicle hydrangea (*Hydrangea paniculata* 'DVPpinky' Siebold) where only 5% of the deposits found on the exterior of the canopy reached the interior (Derksen et al., 2012). Because of small droplet size, 2 deposits/cm^2 may not be enough to achieve adequate control of many insect species. The manufacturer of WSP recommends 20-30 deposits/cm^2 for contact insecticides (Syngenta Crop Protection AG, 2013). However, this recommendation may be pest and pesticide dependent. Within the sparse canopy, droplet density decreased by more than 70% from the exterior to the interior for both species. Regardless of density, the interior of holly and hydrangea canopies had 10 or fewer deposits/cm^2. The middle of sparse plants received a greater droplet density than the middle of dense plants. Spray penetration may be greater in sparse canopies, but

for pests that are in the interior, such as scale, pruning to create a sparse canopy may not be enough to ensure adequate coverage.

In hollies and hydrangea, percentage of exterior coverage and exterior droplet density largely supported coverage and droplet density data (Tables 1 and 2). For both species, coverage and droplet density in the dense middle and interior position were not different from the interior position of sparse plants. However, in hollies, the percentage of exterior coverage was greater in the interior of the sparse plants than the middle and interior of dense plants, 23% versus 12% and 8% of exterior, respectively (Table 1). Likewise, percentage of exterior droplet density was greater in the interior of sparse plants than the middle and interior of dense plants, 33% compared to 22% and 23%, respectively. For hydrangea, neither coverage nor droplet density was different between the middle and interior of sparse plants (Table 2). However, percentage of exterior coverage and percent of exterior droplet density were different. The middle position of sparse hydrangea had 17% of the exterior coverage whereas the interior position had 5% of the exterior coverage. For droplet density, the middle position had 26% of the exterior droplet density and the interior position had just half of that, 14%, of the exterior droplet density. Among hydrangea, droplet density was not different among dense middle and interior and sparse interior positions. However, the middle and interior positions of dense plants both had only 3% of the exterior droplet density, while droplet density at the sparse interior position had 14% of the exterior droplet density. The percentage of exterior coverage and percent of exterior droplet density show that even though the coverage and droplet density are similar between dense middle and interior and sparse interior positions, the most difficult place to achieve spray penetration is the middle and interior of dense plants.

The differences in canopy coverage loss between holly and hydrangea could be due to the size of the leaves or even due to the leaf morphology. China Girl® holly leaves are waxy, smooth, and convex (Dirr, 2009). The waxy surface repels droplets, allowing for more spray to deflect

further into the canopy (Kirkwood, 1999). The hydrangea leaves have trichomes, which are also water repellant, but spray droplets are more likely to fall off the leaf rather than deflect (Xu et al., 2011). The hydrangea leaves are also much larger than holly leaves, 2.5 × 3.2 cm (1 × 1.3 inch) versus 7.6 to 20.3 cm (3 to 8 inches) long and wide, allowing them to block the interior more effectively. The disparity in branch number between dense and sparse holly (53%) and hydrangea (42%) was due to leaf size as well as leaf spacing; fewer branches were removed in hydrangea because removing one branch removed significant canopy surface area, whereas with holly, several branches had to be removed to achieve a reduction in canopy surface area.

Foliage and branches inhibited the spray from penetrating into the canopy. To achieve better spray penetration, many landscape pesticide applicators place the wand within the canopy; however this is not feasible in a large nursery. Lee et al. (2000) and Tunstall et al. (1965) found that spraying plants from the bottom of the canopy at a 45° angle upwards towards the plant's crown increased spray penetration. In this study, the effects of this method were not tested. Other improvements to spray penetration have been made by changing the sprayer design. Derksen et al. (2012) found that using an air-assisted sprayer helped to increase the droplet density within hardy hydrangea (*Hydrangea paniculata* 'DVPpinky') canopies. They also found that increasing the spray volume (187 $L \cdot ha^{-1}$ to 374 $L \cdot ha^{-1}$) improved canopy penetration. Several studies have reported the abilities of air-assisted sprayers to increase deflection of leaf surfaces, allowing spray to penetrate better into the canopy when compared with other sprayers (Derksen et al., 2001; Derksen et al., 2012; Derksen and Sanderson, 1996; Ozkan et al., 2006; Piché et al., 2000; Womac et al, 1992). Zhu et al. (2006) developed an air-assisted sprayer with five-port nozzle to improve spray penetration and droplet density uniformity within yew (*Taxus* sp.) canopies. Other improvements on spray technology include intelligent sprayers that can sense plant presence and density in real time may allow growers to achieve greater spray penetration with reduced spray volume (Chen et al., 2012;

Jeon and Zhu, 2012). Applications that can be applied to deciduous trees in winter or before plants have leafed out in the spring would not have the same penetration issues.

Natural enemy survival

Canopy position and plant density had no effect on insect survival when water was applied to plants (Table 3 and 4). It can be concluded that, because survival remained greater than 90% and 70% for TAGL and ACL, respectively, that hydrangea leaf and arena environment were suitable conditions in which to conduct the insect survival experiments. For both insect species, when carbaryl was applied to plants, survival was not different in the exterior position for both dense and sparse plants indicating that the carbaryl was consistently applied (Tables 5, 6, and 7).

TAGL in the middle position of dense plants had a higher survival than those in the middle position of sparse plants by 230%, 900%, and 2300%, at 24, 48 and 72 HOE, respectively, in experiment one and 114%, 1380%, 2033%, and 1500% at 24, 48, 72, and 96 HOE, respectively, in experiment two and for ACL, 62%, 160%, and 205% at 48, 72, and 96 HOE (Tables 5, 6, and 7). None of the TAGL in the middle position of sparse plants survived to 96 HOE in experiment one (Table 5). The interior position of dense plants had higher TAGL survival than the interior position of sparse plants by 204%, 380%, 688%, and 1100% at 24, 48, 72, and 96 HOE, respectively, in experiment one and 196%, 547%, 569%, and 608% at 24, 48, 72, and 96 HOE, respectively, in experiment two despite spray coverage no being different between interior positions of dense and sparse plants (Tables 2, 5, and 6). Additionally, the interior position population never dropped below 60% for either species, which is consistent with survival in the water application experiments.

The interior and middle positions of both dense and sparse plants received less than 10% coverage of insecticide (Table 2), but only the interior and middle positions of dense plants protected greater than 50% of the ACL, and only the interior position of dense plants protected greater than 50% of TAGL over the course of the study. The low survivability even in areas with

limited penetration seems to indicate that even a small amount carbaryl can harm some natural enemies and may also be effective against pest insects. If a less toxic, more targeted insecticide were used instead of carbaryl, the results may have been very different, with survival within the interior of the canopy closer to 100%. This study was conducted in an unnatural environment where insects were confined to arenas with treated leaves; the data may have also been different if natural enemies were able to move around. In a natural setting, insects move around the canopy searching for prey, making them more likely to come into contact with residue from other canopy positions. It is also possible that in a natural setting, natural enemies may avoid insecticide residue. Tome et al. (2013) observed that tomato leafminers (*Tuta absoluta* Meyrick) avoided laying eggs where the insecticide azadirachtin was present. Silcox et al. (2012) found that the tawny mole cricket (*Scapteriscus vicinus* Scudder) avoided tunneling in areas where bifenthrin (Talstar EZ®), chlorantraniliprole (Acelepryn®), and fipronil (Chipco Choice) had been applied.

CONCLUSION

Growers are subject to market pressure to produce plants with dense canopies. However, plant producers and landscape managers need to understand the implications of plant architecture on pest control. Canopy density may affect both control of pest insects with contact insecticides and the ability to use natural enemies with chemical control. If a dense canopy is not necessary, chemical pest control may be more effective if plants have a sparser canopy. If growers must produce plants with a dense canopy, either improving spray penetration through better sprayer design or technique, or using systemic or contact insecticides that are compatible with natural enemies so that natural enemies may be incorporated economically may improve pest control. In future studies, other insecticides should be incorporated to see if the insecticide is a determining factor in natural enemy survival within dense canopies. Additional research conducted in a natural environment is needed to determine the distribution and movement of natural enemy species

within the plant canopy in order to further evaluate the significance of plant density on conventional and biological control.

APPENDIX 2: TABLES AND FIGURES

Table 14. Coverage and droplet density in the exterior, middle, and interior of China Girl® holly (*Ilex* ×*meserveae* 'Mesog') with dense or sparse branch architecture.

Canopy Density	Canopy Position	Coverage (%)	Percentage of exterior coverage (%)	Droplet Density (Deposits/cm^2)	Percentage of exterior droplet density (%)
Dense	Exterior	28.4 ± 1.9 a[z]	100 ± 3 a	32 ± 2 a	100 ± 3 a
	Middle	4.5 ± 1.8 c	12 ± 3 d	7 ± 2 c	22 ± 3 d
	Interior	2.7 ± 1.8 c	8 ± 3 d	7 ± 2 c	23 ± 3 d
Sparse	Exterior	33.5 ± 2.0 a	100 ± 3 a	33 ± 2 a	100 ± 3 a
	Middle	12.8 ± 1.9 b	38 ± 3 b	17 ± 2 b	49 ± 3 b
	Interior	7.1 ± 2.2 c	23 ± 3 c	10 ± 2 c	33 ± 3 c
	Num DF	5	5	5	5
	Den DF	85	85	85	85
	Significance	***[y]	***	***	***
	P-value	<.0001	<.0001	<.0001	<.0001
	F Statistic	46.19	225.81	38.49	164.11

[z]means followed by the same letter within a column were not significantly different (Tukey α = 0.05)

[y]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***)

Table 15. Coverage and droplet density in the exterior, middle, and interior of oakleaf hydrangea 'Alice' (*Hydrangea quercifolia* Bartr. 'Alice') with dense or sparse branch architecture.

Canopy Density	Canopy Position	Coverage (%)	Percentage of exterior coverage (%)	Droplet Density (Deposits/cm^2)	Percentage of exterior droplet density (%)
Dense	Exterior	38.2 ± 1.0 a[z]	100 ± 2 a	56 ± 3 a	100 ± 3 a
	Middle	0.8 ± 1.0 c	2 ± 2 c	2 ± 3 c	3 ± 3 d
	Interior	0.4 ± 1.0 c	1 ± 2 c	2 ± 3 c	3 ± 3 d
Sparse	Exterior	36.5 ± 1.0 a	100 ± 2 a	47 ± 3 a	100 ± 3 a
	Middle	4.5 ± 1.0 b	17 ± 2 b	15 ± 3 b	26 ± 3 b
	Interior	1.0 ± 1.0 bc	5 ± 2 c	8 ± 3 bc	14 ± 3 c
	Num DF	5	5	5	5
	Den DF	66	66	66	66
	Significance	***[y]	***	***	***
	P-value	<.0001	<.0001	<.0001	<.0001
	F Statistic	311.50	480.97	56.54	208.66

[z]means followed by the same letter within a column were not significantly different (Tukey α = 0.05)

[y]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***)

Table 16. A type of adult green lacewing (*Chrysoperla rufilabirs*) survival at exterior, middle and interior positions of dense and sparse oakleaf hydrangea 'Alice' (*Hydrangea quercifolia* Bartr. 'Alice') canopies sprayed with water.

Canopy position and density	Survival (% alive)			
	Hours of exposure			
	24	48	72	96
Exterior	99	98	96	91
Middle	98	96	93	91
Interior	99	98	93	90
Dense	98	97	93	90
Sparse	99	98	95	91
Position DF	2	2	2	2
Position Significance	NS[z]	NS	NS	NS
Position *P*-value	0.9125	0.3195	0.1880	0.8875
Position F Statistic	0.09	1.16	1.71	0.12
Density DF	1	1	1	1
Density Significance	NS	NS	NS	NS
Density *P*-value	0.2302	0.4831	0.4299	0.5513
Density F Statistic	1.47	0.50	0.63	0.36
Position x Density *P*-value	0.3102	0.4930	0.6591	0.7321

[z]means were not significantly different

Table 17. Adult convergent lady beetles (*Hippodamia convergens* GM*)* survival at exterior, middle and interior positions of dense and sparse and oakleaf hydrangea 'Alice' (*Hydrangea quercifolia* Bartr. 'Alice') canopies sprayed with water.

Canopy position and density	Survival (% alive)			
	Hours of exposure			
	24	48	72	96
Exterior	93	85	82	79
Middle	93	83	78	72
Interior	94	85	80	77
Dense	94	85	80	76
Sparse	92	83	81	76
Position DF	2	2	2	2
Position Significance	NS[z]	NS	NS	NS
Position *P*-value	0.9245	0.8000	0.7346	0.3980
Position F Statistic	0.08	0.22	0.31	0.93
Density DF	1	1	1	1
Density Significance	NS	NS	NS	NS
Density *P*-value	0.4269	0.5468	0.8600	0.9951
Density F Statistic	0.64	0.37	0.03	0.00
Position*Density *P*-value	0.8928	0.8842	0.8081	0.6478

[z]means were not significantly different

Table 18. Interaction of canopy position and plant density within oakleaf hydrangea 'Alice' (*Hydrangea quercifolia* Bartr. 'Alice') canopies sprayed with carbaryl on a type of adult green lacewing (*chrysoperla rufilabris* Burmeister) survival, experiment one.

Position	Density	Survival (% alive)			
		Hours of exposure			
		24	48	72	96
Exterior	Dense	35 ± 0.6 b[z]	3 ± 0.4 c	0 ± 0.2 c	0 ± 0.3 c
Exterior	Sparse	10 ± 0.7 b	2 ± 0.4 c	1 ± 0.2 c	1 ± 0.3 c
Middle	Dense	76 ± 0.6 a	50 ± 0.4 b	48 ± 0.3 b	35 ± 0.4 b
Middle	Sparse	23 ± 0.6 b	5 ± 0.4 c	2 ± 0.2 c	0 ± 0.3 c
Interior	Dense	85 ± 0.6 a	72 ± 0.4 a	63 ± 0.3 a	60 ± 0.4 a
Interior	Sparse	28 ± 0.6 b	15 ± 0.4 c	8 ± 0.2 c	5 ± 0.3 c
Position *P*-value		<.0001	<.0001	<.0001	<.0001
Density *P*-value		<.0001	<.0001	<.0001	<.0001
Position*Density DF		2	2	2	2
Position*Density Significance		*[y]	***	***	***
Position*Density *P*-value		0.0331	<.0001	<.0001	<.0001
Position*Density F Statistic		3.90	25.22	77.29	43.88

[z]means followed by the same letter within the same column and treatment group were not significantly different (Tukey α = 0.05)

[y]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***)

Table 19. Interaction of canopy position and plant density within oakleaf hydrangea 'Alice' (*Hydrangea quercifolia* Bartr. 'Alice') canopies sprayed with carbaryl on a type of adult green lacewing (*chrysoperla rufilabris* Burmeister), experiment two.

Position	Density	Survival (% alive)			
		Hours of exposure			
		24	48	72	96
Exterior	Dense	37 ± 1 c[z]	8 ± 1 b	5 ± 0 c	2 ± 0 c
Exterior	Sparse	27 ± 1 c	0 ± 1 b	0 ± 0 c	0 ± 0 c
Middle	Dense	75 ± 1 b	74 ± 1 a	64 ± 0 b	48 ± 0 b
Middle	Sparse	35 ± 1 c	5 ± 1 b	3 ± 0 c	3 ± 0 c
Interior	Dense	98 ± 1 a	97 ± 1 a	87 ± 0 a	85 ± 0 a
Interior	Sparse	33 ± 1 c	15 ± 1 b	13 ± 0 c	12 ± 0 c
Position *P*-value		0.0021	<.0001	<.0001	<.0001
Density *P*-value		<.0001	<.0001	<.0001	<.0001
Position*Density DF		2	2	2	2
Position*Density Significance		*[y]	***	***	***
Position*Density *P*-value		0.0162	<.0001	<.0001	<.0001
Position*Density F Statistic		4.75	29.89	50.28	44.71

[z]means followed by the same letter within the same column and treatment group were not significantly different Tukey α = 0.05)

[y]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***)

Table 20. Interaction of canopy position and plant density within oakleaf hydrangea 'Alice' (*Hydrangea quercifolia* Bartr. 'Alice') canopies sprayed with carbaryl on adult convergent lady beetles (*Hippodamia convergens* GM*)* survival *(*experiment one and two pooled).

Position	Density	Survival (% alive)			
		Hours of exposure			
		24	48	72	96
Exterior	Dense	73 ± 1	48 ± 1 b[z]	24 ± 1 b	10 ± 0 b
Exterior	Sparse	58 ± 1	39 ± 1 b	8 ± 1 b	5 ± 0 b
Middle	Dense	83 ± 1	73 ± 1 a	65 ± 1 a	58 ± 0 a
Middle	Sparse	68 ± 1	45 ± 1 b	28 ± 1 b	19 ± 0 b
Interior	Dense	85 ± 1	80 ± 1 a	72 ± 1 a	71 ± 0 a
Interior	Sparse	63 ± 1	38 ± 1 b	16 ± 1 b	13 ± 0 b
Position *P*-value		0.1542	0.0077	<.0001	<.0001
Density *P*-value		0.0614	<.0001	<.0001	<.0001
Position*Density DF		2	2	2	2
Position*Density Significance		NS[X]	*z	**	***
Position*Density *P*-value		0.7841	0.0190	0.0013	<.0001
Position*Density F Statistic		0.24	4.21	7.39	15.92

[z]means followed by the same letter within the same column were not significantly different (Tukey α = 0.05)

[x]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), NS signifies non-significance

Figure 1. Sparse (35 branches) and dense (75 branches) China Girl® holly (*Ilex* ×*meserveae* 'Mesog').

Figure 2. Dense (19 branches) and sparse (11 branches) oakleaf hydrangea 'Alice' (*Hydrangea quercifolia* Bartr. 'Alice').

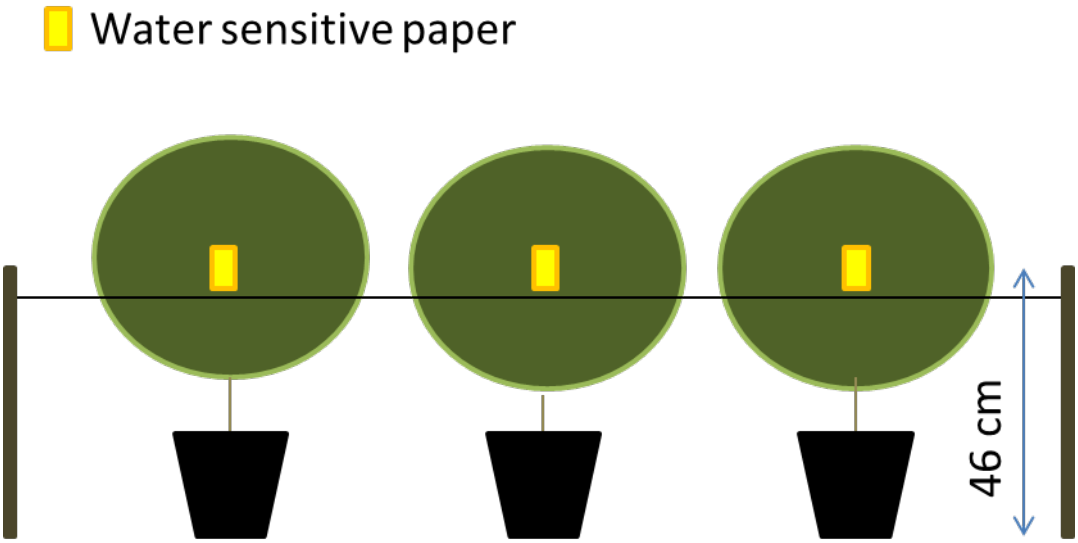

Figure 3. Water sensitive card placement at exterior canopy position.

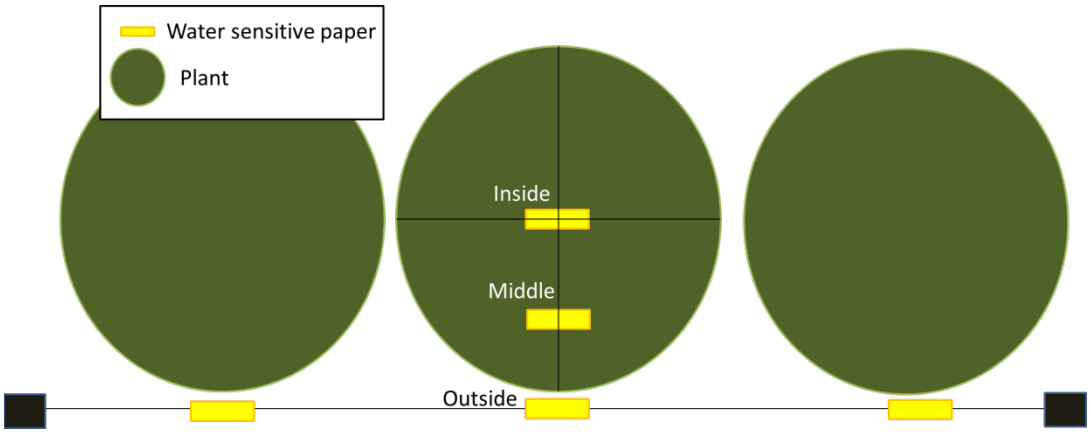

Figure 4. Water sensitive card placement positions from top view.

CHAPTER 4. EVALUATION OF THE EFFECT OF CONTACT AND SYSTEMIC INSECTICIDE EXPOSURE TO NATURAL ENEMY POPULATIONS IN A CONFINEMENT SCENERIO

ABSTRACT

Chemical pesticides can efficiently control insect pests and is often relied upon by nursery producers. With increased consumer concerns regarding insecticides, growers may choose to limit insecticide applications by incorporating natural enemies into their pest management program. This study assessed the effects of commonly used contact (bifenthrin and carbaryl) and systemic (imidacloprid and dinotefuran) insecticides on a type of adult green lacewing (TAGL) (*Chrysoperla rufilabris* Burmeister), adult convergent lady beetle (ACL) (*Hippodamia convergens* Guérin-Méneville), and insidious flower bug (IFB)(*Orius insidiosus* Say) to assess the safety of systemic insecticides on natural enemies. Insects were confined in arenas either with leaves sprayed to provide insecticide residues or treated with only water, and then allowed to air dry prior to use. Despite popular belief that systemic insecticides are safer to natural enemies than contact insecticides, both forms of insecticide were harmful to all three insect species. Bifenthrin, a contact insecticide, was the least harmful to TAGL and the systemic insecticide, dinotefuran, was not harmful to ACL. Carbaryl was the most harmful insecticide to both TAGL and ACL. All insecticides were harmful to IFB with bifenthrin being the most harmful. Not one of the insecticides chosen in this study was "safe" for all three natural enemy species.

INTRODUCTION

The pressure to produce new, unique, or easy-to-grow ornamental cultivars has led to breeding and selection largely focused on attractive flowers and foliage rather than plant health. When plants are bred for these specific traits, general pest resistance genes are inadvertently lost, leaving plants more susceptible to a wider range of pests (Tripp and van der Heide, 1996). For ornamental crops, which are valued solely for their aesthetics, the threshold for pest damage is often zero because a single pest can render a plant

unmarketable (Klingeman et al., 2000). For example, one female bagworm, *Thyridopteryx ephemeraeformis* (Haworth), can produce enough offspring to cause a major infestation on American arborvitae (*Thuja occidentalis* L.) (Horn and Sheppard, 1979; Raupp et al., 1989). However, achieving a pest level of zero is difficult, and thus, protecting nursery crops can be challenging. Chemical insect control has traditionally been an important part of nursery crop production because insecticides work quickly and can maintain pest populations at acceptable levels with minimal effort from the grower (Bethke and Cloyd, 2009). However, with negative consumer perceptions of pesticides due to concerns for environmental impacts (Falconer, 1998; Kher et al., 2013; Montella et al., 2012), worker safety (Kher et al., 2013), and the ability of insects to develop resistance to chemicals (Falconer, 1998; Montella et al., 2012), it is important for nurseries to consider more sustainable pest management options.

An alternative to relying soley on conventional pesticides is Integrated Pest Management (IPM). IPM combines several techniques, including biological control, to optimize pest management to meet the economic goal of the producer while minimizing ecological impacts (Ehler, 2006; Kogan, 1998). The purpose of IPM is not to eliminate all pests, but to limit the pest population to a manageable level, determined by economic factors. Biological control is one of the oldest forms of pest control with the earliest records dating to 304 BC when citrus growers used ants (*Oecophylla smaragdina* Fabr.) to protect trees from insect pests (Huang and Yang, 1987). Conservation biological control, is defined as enhancing survival of naturally occuring enemies to promote pest control (Barbosa, 1998; Landis et al., 2000). For growers, protecting natural enemies may mean using systemic insecticides instead of contact insecticides (Funderburk et al., 2013).

Systemic insecticides are substances that are absorbed by and translocated throughout a plant (Bennett, 1949). Systemics can be effective even in areas where spray does not penetrate and thus be more effective in controlling pests within complex canopies (Ripper et al., 1949). Systemics are long lasting in the plant; often only one application is needed per growing season, reducing the chemical and labor costs associated with multiple applications as may be needed with contact insecticides (Byrne et al., 2010; Reynolds, 1954). Systemics are also believed to be safer for biological control organisms because they are taken up by the plant and are injested by only phytophagous arthropods (Bellows Jr et al., 1988; Cloyd, 2010; Jeppson, 1953; Mizell and Sconyers, 1992; Rudinsky, 1959; Stapel et al., 2000). However, research suggests that systemic insecticides can limit functionality and even cause death to arthropods used to biologically control pests when they come in direct contact with the insecticide or by feeding on prey that has ingested the pesticide (Koppert Biological Systems, 2005; Szczepaniec et al., 2011).

Imidacloprid, a systemic neonicotinoid, is toxic to a wide range of economically important pest insects (Mullins, 1993). However, imidicloprid can also be harmful to non-target insects. Honey bees (*Apis* L.) gathering pollen from plants treated with imidacloprid at 70 times lower than 50% of the lethal concentration exhibited decreased activity (Colin et al., 2004). Imidacloprid is also harmful to insects used for biological control including TAGL larvae when used as a foliar spray but not as a drench and to IFB as both a foliar spray (Studebaker and Kring, 2003) and a drench (Koppert Biological Systems, 2005). Dinotefuran, another common systemic neonicotinoid insecticide, was established as an alternative to imidacloprid and is labeled as a reduced risk pesticide by the EPA. However, dinotefuran is also highly toxic to honeybees as well as silkworms (*Bombyx mori* L.), non-target beneficial insects (Mitsui, 2013), but has not been tested on natural enemies.

Several studies have been conducted on the effectiveness of insecticides at controlling target pests of ornamental crops (IR-4 Project, 2014; Mullins, 1993; Szczepaniec et al., 2013a) and limited independent research on the effects of insecticides on natural enemies has been conducted (Colin et al., 2004; Lucas et al., 2004 et al.; Szczepaniec et al., 2011; Szczepaniec et al., 2013a). However, little research has examined the compatibility of a range of insecticides on specific natural enemies common to the nursery industry. The objective of this study was to investigate the effects of commonly used contact and systemic insecticides on selected natural enemies, TAGL, ACL, and IFB, subjected to direct contact with insecticide residue in a confinement scenario to gain insight on which insecticides, if any, can be used cohesively with these natural enemies so growers can sustainably incorporate both forms of pest control into their IPM program.

MATERIALS AND METHODS

TAGL, ACL, and IFB were ordered from Rincon-Vintova (Ventura, CA) (2011) and from Beneficial Insectaries (Redding, CA) (2012). Insects, which arrived 4 May 2011 and 11 October 2012, were held in a cooler overnight and then used in assays once trees were treated the following morning. For TAGL and ACL, experimental arenas were built from 90 mm petri dishes (Fisher Scientific, Pittsburgh, PA) by removing a 7.6 cm (3 inch) diameter opening in the lid and covering the opening with organdy fabric to allow for gas exchange. A single 90 mm filter paper was placed in each arena to absorb excess moisture. Arenas for IFB were 76 mm (3 inch) (Gelman, Ann Arbor, MI) in diameter and were left intact as the IFB were small enough to climb through the organdy. For each arena, a hole was drilled in the lids of a 0.65 ml microcentrifuge tube (Costar®, Corning, Corning, NY), plugged with cotton, and filled with a honey water solution (5% v/v) to serve as a food source. On the morning of treatment, ten insects were placed in their respective arenas. Treated and air-

dried leaves had petioles inserted in a 5 mL centrifuge tube water source and then placed in arenas.

Forty tulip poplar (*Liriodendron tulipifera* L.) trees grown in the field at the University of Tennessee Forest Resources Center, Cumberland Forest Unit in Oliver Springs, TN were placed into insecticide treatment groups, eight trees per treatment. Trees were two years old and 4-5.5 feet tall with a 1 inch caliper in 2011 and three years old, 6-9 feet tall with a 4 inch caliper in 2012. In 2011 the whole tree canopy was treated. In 2012, due to a much larger tree size, a single branch on each tree was treated. In both years foliage was covered with insecticide until runoff and then leaves were allowed to air dry on the tree before collection. Although foliage is not always thoroughly covered during a pesticide application, in this study we were interested in a scenario where the natural enemies were forced to come in immediate contact with pesticide residue. Trees were sprayed using a CO_2 sprayer at 30 PSI (Teejet® Even Flat Spray Tip, Springfield, IL, 0.17 GPM). Treatments chosen are widely used in commercial nursery operations and included: bifenthrin (Talstar® Select, FMC Corporation, Philadelphia, PA, MOA Group 3) at 40 fl. oz./per acre, carbaryl (Sevin® SL, Bayer CropScience, Durham, NC, MOA Group 1) at 1 qt. per 100 gallons, imidacloprid (Marathon® II, OHP, Inc., Mainland, PA, MOA Group 4a) at 6 ml per dbh (diameter at breast height), dinotefuran (Safari® 20 SG, Valent Professional Products, Walnut Creek, CA, MOA Group 4a) at 0.126 g per dbh, and were compared to a water spray control. Carbaryl, a carbamate, and bifenthrin, a pyrethroid, are both broad-spectrum contact insecticides. Imidacloprid and dinotefuran are systemic neonicotinoid insecticides. Dinotefuran is labeled as a drench only, but in a drench application, chemical may splash on the lower leaves of the treated plant or surrounding vegetation that natural enemies may inhabit.

Once air dried, three large, healthy leaves (one per insect species) were collected from each tree and placed in labeled re-sealable bags in a cooler for transport to campus. Leaf petioles were placed in water picks and placed in their respective arenas. Arenas were placed in a laboratory at the University of Tennessee in Knoxville, TN and maintained at 20°C with 8 hours of light. Survival was assessed every 24 hours over the course of four days. Insects that were not moving were recorded as dead and removed, and those that were moving were recorded as alive.

Each arena was an experimental unit with eight replicated units per insecticide treatment. Data were analyzed as a completely randomized design with repeated measures using the GLM procedure of SAS (9.3S; SAS Institute, Cary, NC). Means were separated using Tukey's LSD, $\alpha = 0.05$. Data were not pooled because results varied between years. Each insect species was analyzed separately.

RESULTS

TAGL

TAGL survival across the 96 hour experiment was generally higher in 2011 than 2012 (Tables 21 and 22). About the same numbers of TAGL exposed to bifenthrin survived compared to water controls. In 2012, following 96 hours of exposure (HOE) to bifenthrin, survival decreased below the untreated control. In 2011, carbaryl, dinotefuran, and imidacloprid each reduced TAGL survival, yet in 2012, survival after dinotefuran and imidacloprid exposure was not different than the water control. Carbaryl was not more toxic than the systemic insecticides in 2011. In 2012, however, carbaryl yielded the highest mortality at 24 and 48 HOE; at 72 and 96 HOE, carbaryl was still more toxic than the systemic insecticides while maintaining mortality not different from bifenthrin.

ACL

As with TAGL, ACL survival was generally greater during the 2011 trial than in 2012 (Tables 23 and 24). Compared with exposure to water-treated foliage alone, in 2011 survival on bifenthrin-treated foliage was consistently lower. In 2012, bifenthrin reduced ACL survival only at 48 HOE. In 2011, carbaryl consistently reduced ACL survival across all data collection points, yet in 2012, carbaryl had no effect on ACL survival until 96 HOE. Dinotefuran did not affect ACL survival in either year. Imidacloprid had no effect 24 and 48 HOE, but survival was reduced at 72 and 96 HOE in 2011 when compared with mortality across control treatments. In 2012, imidacloprid was no different than the water control at all data collection points except at 48 HOE when survival was decreased.

IFB

In both experiments, bifenthrin was one of the most highly toxic pesticides to adult *IFB* and substantially decreased survival at all time points to zero survival by 48 HOE (Tables 25 and 26). Compared to water control exposure, carbaryl reduced *IFB* survival at 48, 72, and 96 HOE in both years, yet was the least lethal insecticide in 2011. Both systemic insecticides were lethal to IFB, reducing survival at each time point for both years.

DISCUSSION

TAGL

TAGL survival among water-treated controls exceeded 80% throughout the experiment in 2011 but dropped below 60% in 2012 (Tables 21 and 22). This decrease in survival may have been due to the seasonal differences in the two experiments. Bifenthrin, when compared with control survival, caused no greater TAGL mortality at any time point (except 96 HOE in 2012), even in this confinement situation. Schuster and Stansly (2000) also found bifenthrin to be non-toxic to *Chrysoperla rulfilabris* (Burmeister) and *C. cubana*,

yet reported mortality in *C. carnea* (Stephens). Carbaryl was 51%, 72%, 69%, and 67% more lethal than bifenthrin in 2011 at 24, 48, 72, and 96 HOE, respectively. In 2012, carbaryl was more lethal than bifenthrin by 35% and 72% at 24 and 48 HOE. TAGL exposed to carbaryl had less than 50% survival compared to water, which is consistent with the Side Effects Database which shows that carbaryl is lethal to larval and adult stages of the same green lacewing species used in this study (Koppert Biological Systems, 2005). While survival was generally lower in 2012, neither systemic insecticide caused greater mortality than water exposure, but in 2011, the systemic insecticides reduced survival by 37%, 52%, 59%, and 58% for dinotefuran and 44%, 55%, 66%, and 70%, for imidacloprid, at 24, 48, 72 and 96 HOE, respectively. Imidacloprid has been reported as harmful to a type of green lacewing larvae when used as a foliar spray; however, foliar spray effects on TAGL have not been reported (Koppert Biological Systems, 2005).

ACL

Like the TAGL, ACL survival on water-treated foliage was higher in 2011 than in 2012 by 9%, 8%, 20%, and 24% at 24, 48, 72, and 96 HOE, respectively (Table 23 and 24). Greater survival could be explained, in part, to the time of year at which the ACL were collected (May in 2011 versus October in 2012). In the fall, ACL begin accumulating metabolic reserves needed for survival during overwintering (Hamedi et al., 2013). Hamedi et al. (2013) found that due to these reserves, storing *Hippodamia variegata* (Goeze) at 10°C between November and February resulted in a population decline.

In 2011, carbaryl exposure reduced ACL populations to below 50% at 24 HOE, 10% at 48 HOE, and 1% at 72 through 96 HOE (Table 23). By contrast, in 2012, carbaryl did not influence survival until 96 HOE when ACL survival decreased 49% compared to those exposed to water-treated foliage (Table 24). Despite lower ACL survival in 2012 than in

2011, by the end of the experiment, carbaryl-exposed ACL had 1% survival in 2011 and 31% in 2012. Carbaryl may have been less harmful in 2012 due to seasonal differences in ACL metabolic resources.

Like carbaryl, bifenthrin caused greater ACL mortality in 2011 than in 2012 (Table 23 and 24). In 2011, survival following bifenthrin exposure was reduced by 12%, 54%, 63% and 68% compared to water controls at 24, 48, 72 and 96 HOE, respectively. In 2012, however, survival was only negatively affected at 48 HOE (by 35%) when compared to the water controls. This decrease in survival is consistent with trials in corn (*Zea mays* L.) fields treated with bifenthrin, which decreased survival of larval multicolored Asian lady beetle *(Harmonia axyridis* Pallas) below that of control fields. In laboratory experiments within the same study, bifenthrin also decreased survival of multicolored Asian lady beetle adults (Galvan et al., 2005). Additionally, *Coccinella transversalis* (Fab.) and *Harmonia octomaculata* (Fab.) lady beetle populations were decreased following bifenthrin exposure on treated cotton (*Gossypium hirstum* L.) leaves compared to water controls (Ma et al., 2000).

ACL survival following exposure to dinotefuran in this study was not different from exposure to water in both years (Table 23 and 24). In field studies, Fulcher and Klingeman (2012) also found that dinotefuran exposure did not decrease ACL populations when compared with water exposure. Imidacloprid had no negative effect at 24 and 48 HOE in 2011, but caused a 42% decline in survival at 72 HOE and a 74% decline by 96 HOE. In 2012, imidacloprid caused a 32% decline in population by 48 HOE, yet was not different from water exposure at all other data collection points. In 2011, imidacloprid exposure caused survival rates to decline about 20% between each time point. In 2012, survival dropped quickly between 24 and 48 HOE and then decreased by less than 5% at subsequent

counts. Imidacloprid lady beetle toxicity has been demonstrated in laboratory tests exposing 12-spotted lady beetle larvae, (*Coleomegilla maculate* DeGeer), to imidacloprid which caused an 80% reduction in survival within 48 hours (Lucas et al., 2004). In another study, eggs and first- and second-instar multicolored Asian lady beetle all failed to survive when exposed to imidacloprid, yet imidacloprid did not kill adult beetles (Youn et al., 2003).

Although dinotefuran and imidacloprid are both neonicotinoid insecticides, their active ingredients differ in their physical chemistries (Toscano and Byrne, 2005; Wakita et al., 2005). Dinotefuran has greater water solubility and does not bind as easily with organic matter as imidacloprid (Wakita et al., 2005). These characteristics could help explain why ACL reacted differently to the two systemic insecticides. Several studies using dinotefuran and imidacloprid on various species of spider mites have also different reactions of spider mites to residues of the two insecticides (Gupta and Krischik, 2007; Sclar et al., 1998; Szczepaniec et al., 2011, 2013a, 2013b; Szczepaniec and Raupp, 2013)

IFB

When exposed to water-treated leaves, IFB populations decreased below 50% by 72 HOE in 2011 and 96 HOE in 2012, suggesting that IFB may not be suited to the experimental arena environment to which they were confined (Table 25 and 26). Fulcher and Klingeman (2012) conducted a similar study using modified Petri dishes attached to leaves of field-grown trees in which IFB survival did not decline as severely. The arena design was different for IFB than those used for TAGL and ACL in respect to size and potential for air exchange. High mortality may also be partly explained by the small body size that enabled a proportionately greater exposure than was received by the larger insects.

In both years, carbaryl was the least toxic insecticide yet was still highly lethal to IFB (Table 25 and 26). IFB survival, when exposed to carbaryl did not differ compared to water

control exposure at 24 HOE in 2011, yet was 46%, 58%, and 64% lower at 48, 72, and 96 HOE, respectively. In 2012, carbaryl consistently had a lower survival than the water control. More specifically, exposure to carbaryl decreased IFB survival by 40%, 72%, 63%, and 72% across time compared with water controls. This result is consistent with laboratory studies demonstrating carbaryl toxicity to IFB larvae and adults (Koppert Biological Systems, 2005).

In both years, bifenthrin was generally the most toxic pesticide tested, decreasing IFB survival by greater than 90% in 24 hours (Table 25 and 26). Bifenthrin has also been highly toxic to IFB larva and adults in laboratory studies (Koppert Biological Systems, 2005) and adults on corn (Al-Deeb et al., 2001).

Both systemic insecticides were consistantly toxic to IFB (Table 25 and 26). Dinotefuran decreased IFB populations by 84%, 96%, 98%, and 98% in 2011 and by 57%, 65%, 57%, and 78% in 2012 at 42, 48, 72, and 96 HOE, respectively. Imidacloprid decreased IFB populations by 63%, 87%, 100%, and 100% in 2011 and by 52%, 63%, 61%, and 78% in 2012 at 42, 48, 72, and 96 HOE, respectively. In laboratory tests, imidacloprid caused IFB mortality as both a foliar spray and a drench (Funderburk et al., 2013; Koppert Biological Systems, 2005). Imidacloprid applied to sorgum and corn seeds then grown into mature plants decreased IFB survival even on plants that did not contain prey (Al-Deeb et al, 2001). This decrease in survival may be due to IFB's omnivorous nature to feed not only on other insects, but also on plant material (Coll, 1996).

In this study, both contact and systemic insecticides were toxic to natural enemies. However, this study was conducted in an unnatural confinement scenario where insects were trapped with insecticide residue. In a nursery system, systemic insecticides have several potential advantages to conserving natural enemies and limiting pesticide exposure

to the environment when compared with contact insecticides. When systemic insecticides are applied as a drench, it is possible that little to no residue may reach the leaves for natural enemies to contact. Systemic insecticides are translocated throughout a plant, controlling pests even in areas where spray does not penetrate, such as complex or dense canopies (Ripper et al., 1949), potentially reducing the number of insecticide applications (Reynolds, 1954).

Further studies are needed that expose natural enemies several days after various pesticides are applied to determine when natural enemy populations may be safely introduced, or re-introduced, within a managed nursery or landscape as part of an augmentative biological control program. Although in this study bifenthrin was compatible with *C. rufilabris* and dinotefuran with *H. convergens*, effects of insecticides vary among species (Koppert Biological Systems, 2005; Szczepaniec et al., 2013a, 2013b). Bifenthrin should be tested with several species of lacewing, and dinotefuran with several species of lady beetle, to determine which natural enemies can be used concurrently with chemical control in an integrated pest management strategy.

CONCLUSION

Although exposure to systemic insecticide is perceived to be safer for natural enemies than contact insecticides, results of these research trials were mixed. Insecticides are essentially neurotoxins in which almost every chemical class can yield to decreases in birth rate and mobility among different insect species, even when not ingested (Haynes, 1988). In the studies reported here, systemic insecticides were toxic to all three natural enemy species tested, yet varied by year and with hours of exposure. If using natural enemy-based biological control, the contact insecticide bifenthrin may be the best option to help control insect pests while conserving adult TAGL populations. The systemic insecticide

dinotefuran was safe for adult ACL. Therefore, if using ACL as a biological control, the best chemical option may be dinotefuran. IFB survival was negatively affected by all tested insecticides; however, if chemical controls must be used, choosing carbaryl may help conserve some portion of the IFB population.

APPENDIX 3: TABLES

Table 21. Survival of a type of adult green lacewing (*Chrysoperla rufilabris*) when exposed to contact and systemic insecticides in May 2011.

Treatment	Survival (%)			
	24 HOE	48 HOE	72 HOE	96 HOE
Water	100 a[z]	100 a	96 a	83 a
Bifenthrin	99 a	88 a	65 ab	64 ab
Carbaryl	49 b	25 b	20 c	21 c
Dinotefuran	63 b	48 b	39 bc	35 bc
Imidacloprid	56 b	45 b	33 bc	25 bc
DF	4	4	4	4
Significance	***[y]	***	***	***
p-value	0.0002	<.0001	<.0001	0.0002
F Statistic	7.67	13.20	11.51	7.73

[z] Means followed by the same letter within a column were not significantly different (Tukey α = 0.05)
[y] Significance at P=0.01 (*), P=0.001 (**), P=0.0001(***)

Table 22. Survival of a type of adult green lacewing (*Chrysoperla rufilabris*) when exposed to contact and systemic insecticides in October 2012.

Treatment	Survival			
	24 HOE	48 HOE	72 HOE	96 HOE
Water	93 a[z]	83 a	66 a	59 a
Bifenthrin	91 a	54 a	40 ab	24 bc
Carbaryl	59 b	15 b	9 b	6 c
Dinotefuran	83 a	74 a	65 a	61 a
Imidacloprid	84 a	64 a	48 a	43 ab
DF	4	4	4	4
Significance	***[y]	***	***	***
p-value	0.0007	<.0001	0.0001	<.0001
F Statistic	6.26	12.09	7.72	9.01

[z] Means followed by the same letter within a column were not significantly different (Tukey α = 0.05)
[y] Significance at P=0.01 (*), P=0.001 (**), P=0.0001(***)

Table 23. Survival of adult convergent lady beetles (*Hippodamia convergens*) exposed to contact and systemic insecticides in May 2011.

Treatment	Survival (%)			
	24 HOE	48 HOE	72 HOE	96 HOE
Water	95 a[z]	85 a	83 a	80 a
Bifenthrin	84 b	39 b	31 c	26 b
Carbaryl	43 b	10 c	1 c	1 b
Dinotefuran	93 a	80 a	73 ab	62 a
Imidacloprid	84 a	69 a	48 bc	21 b
DF	4	4	4	4
Significance	***[y]	***	***	***
p-value	<.0001	<.0001	<.0001	<.0001
F Statistic	19.36	30.73	26.17	22.95

[z] Means followed by the same letter within a column were not significantly different (Tukey α = 0.05)
[y] Significance at P=0.01 (*), P=0.001 (**), P=0.0001(***)

Table 24. Survival of adult convergent lady beetles (*Hippodamia convergens*) exposed to contact and systemic insecticides in October 2012.

Treatment	Survival (%)			
	24 HOE	48 HOE	72 HOE	96 HOE
Water	86 ab[z]	78 a	66 ab	61 ab
Bifenthrin	75 b	51 b	46 b	40 bc
Carbaryl	79 ab	61 ab	49 b	31 c
Dinotefuran	93 a	84 a	78 a	65 a
Imidacloprid	70 b	53 b	49 b	44 abc
DF	4	4	4	4
Significance	**[y]	***	**	***
p-value	0.0039	0.0003	0.0012	0.0009
F Statistic	4.68	7.01	5.69	5.93

[z] Means followed by the same letter within a column were not significantly different (Tukey α = 0.05)
[y] Significance at P=0.01 (*), P=0.001 (**), P=0.0001(***)

Table 25. Survival of adult insidious flower bugs (*Orius insidiosus*) exposed to contact and systemic insecticide in May 2011.

Treatment	Survival (%)			
	24 HOE	48 HOE	72 HOE	96 HOE
Water	94 a[z]	71 a	45 a	39 a
Bifenthrin	5 c	0 c	0 c	0 b
Carbaryl	66 a	38 b	19 b	14 b
Dinotefuran	15 bc	3 c	1 c	1 b
Imidacloprid	35 b	9 c	0 c	0 b
DF	4	4	4	4
Significance	***[y]	***	***	***
p-value	<.0001	<.0001	<.0001	<.0001
F Statistic	27.56	31.18	31.80	19.69

[z] Means followed by the same letter within a column were not significantly different (Tukey α = 0.05)
[y] Significance at P=0.01 (*), P=0.001 (**), P=0.0001(***)

Table 26. Survival of adult insidious flower bug (*Orius insidiosus*) exposed to contact and systemic insecticides in October 2012

Treatment	Survival (%)			
	24 HOE	48 HOE	72 HOE	96 HOE
Water	100 a[z]	71 a	54 a	36 a
Bifenthrin	9 c	0 c	0 c	0 b
Carbaryl	60 b	20 bc	20 b	10 b
Dinotefuran	43 b	25 b	23 b	8 b
Imidacloprid	48 b	26 b	21 b	6 b
DF	4	4	4	4
Significance	***[y]	***	***	***
p-value	<.0001	<.0001	<.0001	<.0001
F Statistic	17.51	18.96	11.78	12.21

[z] Means followed by the same letter within a column were not significantly different (Tukey α = 0.05)
[y] Significance at P=0.01 (*), P=0.001 (**), P=0.0001(***)

CONCLUSION

Growers of woody ornamental plants will continue to produce plants with dense canopies as long as the market demands them. Regardless of effectiveness, growers prune or apply PGRs as a standard practice for manipulating canopy density, wasting both time and, more importantly, money. Although in vitro propagation is not a new concept, many growers are unaware of the potential benefits, namely increased canopy density without further inputs such as pruning or PGRs, as has been shown in this study. However, plants among differing species or even among cultivars within the same species do not always react in the same ways given the same treatment, and purchasing in vitro plants may not be beneficial for all.

Although plants with dense canopies are more attractive and easier to sell, the grower runs the risk of not being able to control pest infestations as easily as he would among plants with sparse canopies. As shown in this study, chemical control may not penetrate into the center of dense canopies, leaving pests within largely unaffected. In theory, natural enemies incorporated into this system would be able to consume pests that the chemical control did not reach. However, natural enemies do not stay stationary within the canopy. They are constantly moving, thereby increasing their chance of contacting chemical residue. It may be hard to find a grower who does not use chemical control. Therefore, in order to incorporated natural enemies into a successful pest management program, a grower should be selective on the type of chemical control used. In this study, dinotefuran did not affect adult convergent lady beetle populations. A grower should be able to release convergent lady beetles and apply dinotefuran without reducing the population released, but further studies need to be done on mating, egg laying, and larval survival to prove that any chemical control is truly “safe”.

LIST OF REFERENCES

Abdelgadir, H.A, A.K. Jager, S.D. Johnson, and J. van Staden. 2010. Influence of plant growth regulators on flowering, fruiting, seed oil content, and oil quality of *Jatropha curcas*. South African J. Bot. 76:440-446.

Aitken-Christie, J., T. Kozai, and M.A.L. Smith. 1995. Automation and environmental control in plant tissue culture, Springer, N. Y.

Al-Deeb, M.A., G.E. Wilde, and K.Y. Zhu. 2001. Effect of insecticides used in corn, sorghum, and alfalfa on the predator *Orius insidiosus* (Hemiptera : Anthocoridae). J. Econ. Entomol. 94: 1353-1360.

Andow, D.A. and D.R. Prokrym. 1990. Plant structural complexity and host-finding by a parasitoid. Oecologia 82:162-165.

Arnold, C.E., J.H. Aldrich, and J.A. Cornell. 1981. Physiological dwarfing of peach with mefluidide. HortScience 16:170-171.

Arzee, T., H. Langenauer, and J. Gressel. 1977. Effects of dikegulac, a new growth-regulator, on apical growth and development of three compositae. Botanical Gaz. 138:18-28.

Ascough, G.D., J.E. Erwin, J. van Staden. 2008. Biotechnology and ornamental horticulture. South African J. Bot. 74:357-357.

Auer, C. 2008. Ecological risk assessment and regulation for genetically-modified ornamental plants. Critical Rev. Plant Sci. 27:255-271.

Bache, D.H. and D. Johnstone. 1992. Microclimate and spray dispersion. Ellis Horwood, New York, NY.

Bailey, D.A. and B. Clark. 1992. Summer applications of plant growth retardants affect spring forcing of hydrangeas. HortTechnology 2:213-216.

Baranek, M., B. Krizan, E. Ondrusikova, and M. Pidra. 2010. DNA-methylation changes in grapevine somaclones following in vitro culture and thermotherapy. Plant Cell Tissue and Organ Culture, 101, 11-22.

Barbosa, P. 1998. Conservation Biological Control. Academic Press, New York, NY.

Basra, A. 2000. Plant growth regulators in agriculture and horticulture. Their role and ocmmercial uses. Haworth Press, Birmingham, NY.

Bellows Jr., T., J. Morse, L. Gaston, and J. Bailey. 1988. The fate of two systemic insecticides and their impact on two phytophagous and a beneficial arthropod in a citrus agroecosystem. J. Econ. Entomol. 81: 899-904.

Bennett, S.H. 1949. Preliminary experiments with systemic insecticides. Ann. Appl. Biol. 36:160-163.

Bethke, J.A. and R.A. Cloyd. 2009. Pesticide use in ornamental production: What are the benefits? Pest Mgt. Sci. 65:345-350.

Boswell, S.B., E.M. Nauer, and W.B. Storey. 1981. Axillary buds sprouting in macadamia induced by two cytokinins and a growth inhibitor. HortScience 16:46-46.

Brand, M.H. and R.D. Lineberger. 1992. In vitro rejuvenation of *Betula* (Betulaceae): Morphological evaluation. Amer. J. Bot. 79:618-625.

Brettell, R.I.S. and E.S. Dennis. 1991. Reactivation of a silent AC following tissue-culture is associated with heritable alterations in its methylation pattern. Mol. Gen. Genet. 229:365-372.

Brewer, M.J. and P.B. Goodell. 2012. Approaches and incentives to implement integrated pest management that addresses regional and environmental issues. In: M.R. Berenbaum (ed.). Annu. Rev. Entomol. 57:41-59.

Bridgen, L.H. 1992. A survey of plant tissue culture laboratories in North America. Proc. Intl. Plant Prop. Soc. 42:427-430.

Briggs, J., T. Whitwell, R.T. Fernandez, and M.B. Riley. 2002. Effect of integrated pest management strategies on chlorothalonil, metalaxyl, and thiophanate-methyl runoff at a container nursery. J. Amer. Soc. Hort. Sci. 127:1018-1024.

Brown, D.R., D.J. Eakes, B.K Behe, and C.H. Gilliam. 1992. Moisture stress: an alternative method of height control to B-nine (daminozide). J. Environ. Hort. 10:232-232.

Bruner, L.L., G.J. Keever, J.R. Kessler, and C.H. Gilliam. 2002. Atrimmec suppresses shoot length and promotes branching of *Lonicera x heckrottii* 'Goldflame' (Goldflame honeysuckle). J. Environ. Hort. 20:73-76.

Byrne, F.J., R.D Oetting, J.A. Bethke, C. Green, and J. Chamberlin. 2010. Understanding the dynamics of neonicotinoid activity in the management of *Bemisia tabaci* whiteflies on poinsettias. Crop Protection 29:260-266.

Callaway, D.J. 1994. The world of magnolias. Timber Press, Portland, O.R.

Cathey, H.M. 1975. Comparative plant growth retarding activities of ancymidol with ACPC, phosphon, chlormequat, and SADH on ornamental plant species. HortScience 10:204-216.

Chamberlain, D., R. Hyam, G. Argent, G. Fairweather, and K.S. Walter. 1996. The genus *Rhododendron*: its classification and synonymy. Royal Botanic Garden, Edinburgh.

Chandler, S. and Y. Tanaka. 2007. Genetic modification in floriculture. Critical Rev. Plant Sci. 26:169-197.

Chandler, S.F. and F. Brugliera. 2011. Genetic modification in floriculture. Biotechnol. Lett. 33:207-214.

Chen, Y., H. Zhu, and H.E. Ozkan. 2012. Development of a variable-rate sprayer with laser scanning sensor to synchronize spray outputs to tree structures. Trans. ASAE 55(3):773-781.

Cho, S. and N. Ki. 1999. Autonomous speed sprayer guidance using machine vision and fuzzy logic. Trans. ASAE 42:1137-1143.

Christensen, B., S. Sriskandarajah, M. Serek, and R. Muller. 2008a. In vitro culture of *Hibiscus rosa-sinensis* L.: Influence of iron, calcium and BAP on establishment and multiplication. Plant Cell Tissue and Organ Cult. 93:151-161.

Christensen, B., S. Sriskandarajah, M. Serek, and R. Muller. 2008b. Transformation of *Kalanchoe blossfeldiana* with *rol*-genes is useful in molecular breeding towards compact growth. Plant Cell Rpt. 27:1485-1495.

Clair-Maczulajtys, D., I. Le Disquet, and G. Bory. 1999. Pruning stress: Changes in the tree physiology and their effects on the tree health. Acta. Hort. 496:317-324.

Clark, T.L. and F.J. Messina. 1998a. Foraging behavior of lacewing larvae (Neuroptera : Chrysopidae) on plants with divergent architectures. J. Insect Behavior 11:303-317.

Clark, T.L. and F.J. Messina. 1998b. Plant architecture and the foraging success of ladybird beetles attacking the Russian wheat aphid. Entomologia Experimentalis Et Applicata 86:153-161.

Clifford, S.C., E.S. Runnkle, F.A. Langton, A. Mead, S.A. Foster, S. Pearson, and R.D. Heins. 2004. Height control of poinsettia using photoselective filters. HortScience 39:383-387.

Cloyd, R. 2010. Systemic insecticides 101. Amer. Nurseryman 210: 18-21.

Cloyd, R.A. and C.S. Sadof. 2000. Effects of plant architecture on the attack rate of *Leptomastix dactylopii* (Hymenoptera : Encyrtidae), a parasitoid of the citrus mealybug (Homoptera : Pseudococcidae). Environ. Entomol. 29:535-541.

Cochran, D.R. and A. Fulcher. 2013. Type and rate of plant growth regulator Influence vegetative, floral growth, and quality of Little Lime™ Hydrangea. HortTechnology 23:306-311.

Cochran, D.R., A. Fulcher, and G. Bi. 2013. Efficacy of dikegulac sodium applied to pruned and unpruned 'Limelight' hydrangea grown at two locations in the Southeastern United States. HortTechnology 23:836-842.

Cohen, M.A. 1978. Influence of dikegulac-sodium, Off-Shoot-O and manual pinching on Rhododendrons. Scientia Hort. 8:163-167.

Colin, M.E., J.M. Bonmatin, I. Moineau, C. Gaimon, S. Brun, and J.P. Vermandere. 2004. A method to quantify and analyze the foraging activity of honey bees: Relevance to the sublethal effects induced by systemic insecticides. Arch. Environ. Contamination and Toxicology 47:387-395.

Coll, M. 1996. Feeding and ovipositing on plants by an omnivorous insect predator. Oecologia 105:214-220.

Crombie, D., M. Hipkins, and J. Milburn. 1985. Gas penetration of pit membranes in the xylem of *Rhododendron* as the cause of acoustically detectable sap cavitation. Functional Plant Biol. 12:445-453.

Cullen, J. 1980. Revision of Rhododendron. 1. Subgenus Rhododendron sections Rhododendron and Pogonanthum. Notes from the Royal Botanic Garden, Edinburgh 39.

Cullen, J. 2005. Hardy rhododendron species: A guide to identification. Timber Press, Portland.

Currey, C.J. and J.E. Erwin. 2012. Foliar applications of plant growth regulators affect stem elongation and branching of 11 kalanchoe species. HortTechnology 22:338-344.

D'Arth, S.M., S.I. Simpson, J.F. Seelye, and P.E. Jameson. 2002. Bushiness and cytokinin sensitivity in micropropagated Zantedeschia. Plant Cell Tissue and Organ Culture 70:113-118.

D'Arth, S., S. Simpson, J. Seelye, and P. Jameson. 2007. Bushiness and cytokinin profile in dormant and sprouting tubers of Zantedeschia. Plant Cell Tissue and Organ Culture 89:185-191.

Damasco, O., S. Adkins, I. Godwin, and M. Smith. 1997. Use of a SCAR-based marker for the early detection of dwarf off-types in micropropagated Cavendish bananas. Intl. Symp. Biotechnol. Tropical and Subtropical Species Part 2 461:157-164.

Damiano, C. 1980. Strawberry micropropagation. Sci. and Edu. Administration Publ. ARR: 11-22.

Debnath, S.C. 2007. Influence of indole-3-butyric acid and propagation method on growth and development of in vitro- and ex vitro-derived lowbush blueberry plants. Plant Growth Regulat. 51:245-253.

de Castro, V.L., K.P. Goes, and S.H. Chiorato. 2004. Developmental toxicity potential of paclobutrazol in the rat. Intl. J. Environ. Health Res. 14:371-380.

Denny, F.E. 1924. Effect of ethylene upon respiration of lemons. Botanical Gaz. 77:322-329.

Desilva, W.H., P.F. Bocion, and H.R. Walther. 1976. Chemical pinching of azalea with dikegulac. HortScience 11:569-570.

Derksen, R., J. Altland, and J. Rennecker. 2012. Fate of preemergence herbicide applications sprayed through containerized hydrangea canopies. J. Environ. Hort. 30:76.

Derksen, R., S. Miller, H. Ozkan, and R. Fox. 2001. Spray deposition characteristics on tomatoes and disease management as influenced by droplet size, spray volume, and air-assistance. ASAE Annual International Meeting, ASAE paper No. 01-1120.

Derksen, R., C. Ranger, L. Canas, J. Locke, H. Zhu, and C. Krause. 2010. Evaluation of handgun and broadcast systems for spray deposition in greenhouse poinsettia canopies. Trans. ASAE 53:5-12.

Derksen, R. and J. Sanderson. 1996. Volume, speed, and poinsettia foliar deposits. Trans. ASAE 39:5-9.

Derksen, R.C., J. Frantz, C.M. Ranger, J.C. Locke, H. Zhu, and C.R. Krause. 2008. Comparing greenhouse handgun delivery to poinsettias by spray volume and quality. Trans. ASAE 51:27-33.

Desjardins, Y., J.F. Dubuc, and A. Badr. 2007. In vitro culture of plants: a stressful activity! In: A. Romano (ed.). III Intl. Symp. Acclimatization and Establishment of Micropropagated Plants 812:29-50.

Dirr, M.A. 1998. Manual of Woody Landscape Plants. Stipes Publishing, Champagne, IL.

Dirr, M.A. 2009. Manual of Woody Landscape Plants: Their identification, ornamental characteristics, culture, propagation and uses. Stipes Publishing, Champagne, IL.

Dirr, M.A. and C.W. Heuser. 1987. The reference manual of woody plant propagation: From seed to tissue culture. Varsity Press, Athens, GA.

Dobbs, E.K. and D.A. Potter. 2014. Conservation biological control and pest performance in lawn turf: Does mowing height matter? Environ. Mgt. 1-12.

Eccher, T. and M. Martinelli. 2010. Inoculation of rhododendron cultivars in vitro with different strains of ericoid endomycorrhizae. IV Intl. Symp. Acclimatization and Establishment of Micropropagated Plants 865:327-332.

Ehler, L.E. 2006. Integrated pest management (IPM): Definition, historical development and implementation, and the other IPM. Pest Mgt. Sci. 62:787-789.

Elfving, D.C. 1984. Factors affecting apple-tree response to chemical branch-induction treatments. J. Amer. Soc. Hort. Sci. 109:476-481.

Elkner, T.E. and D.C. Coston. 1986. Effect of BA + GA_{4+7}, BA, and daminozide on growth and lateral shoot development in peach. J. Amer. Soc. Hort. Sci. 111:520-524.

El-shiekh, A., D.K. Wildung, J.J. Luby, K.L. Sargent, and P.E. Read. 1996. Long-term effects of propagation by tissue culture or softwood single-node cuttings on growth habit, yield, and berry weight of 'Northblue' blueberry. J. Amer. Soc. for Hort. Sci. 121:339-342.

Escaravage, N., S. Questiau, A. Pornon, B. Doche, and P. Taberlet. 1998. Clonal diversity in a Rhododendron ferrugineum L. (Ericaceae) population inferred from AFLP markers. Mol. Ecol. 7:975-982.

Fain, G B., C.H. Gilliam, and G.J. Keever. 2001. Response of *Lagerstroemia* X 'Tuscarora' to Pistill and Atrimmec. J. Environ. Hort. 19:149-152.

Falchieri, D., K. Mierzejewski, and S. Maczuga. 1995. Effects of droplet density and concentration on the efficacy of *Bacillus thuringiensis* and carbary against gypsy moth larvae (*Lymantria dispar* L.). J. Environ. Sci. Health Part B-Pesticides Food Contaminants and Agri. Wastes 30:535-548.

Fall Creek Farm and Nursery, Inc. 2013. Northern Highbush: High Chill. 13 August 2013. <http://fallcreeknursery.com>.

Falconer, K.E. 1998. Managing diffuse environmental contamination from agricultural pesticides: An economic perspective on issues and policy options, with particular reference to Europe. Agri. Ecosystem Environ. 69:37-54.

Fine Agrochemicals Limited. 2012. Configure (label). 15 May 2012. <http://www.fine-americas.com/DocFrame/DocView.asp?id=1075&sec=-1>.

Fisher, R.W. and D.R. Menzies. 1976. Effect of spray droplet density and exposure time on the immobilization of newly-hatched oriental fruit moth larvae. J. Econ. Entomol. 69:438-440.

Florkowski, W.J., E.E. Hubbard, and G.W. Krewer. 1992. Cultural practices used by Georgia's expanding blueberry industry. J. Environ. Hort. 10:224-227.

Forshey, C.G. 1982. Branching responses of young apple-trees to applications of 6-benzylamino purine and gibberellin A_{4+7}. J. Amer. Soc. Hort. Sci. 107:538-541.

Fox, R.D., R.C. Derksen, R.C., J.A. Cooper, C.R. Krause, and H.E. Ozkan. 2003. Visual and image system measurement of spray deposits using water-sensitive paper. Appl. Eng. Agri. 19:549-552.

Fox, R.D., R.C. Derksen, H. Zhu, R.D. Brazee, and S.A. Svensson. 2008. A history of air-blast sprayer development and future prospects. Trans. Asabe 51:405-410.

Franklin K.A. and Whitelam, G.C. 2005. Phytochromes and shade-avoidance responses in plants. Annu. Bot. 96:169–175.

Fulcher, A., W.E. Klingeman, J.-H. Chong, A.V. LeBude, G.R. Armel, M.R. Chappell, S. Frank, F. Hale, J. Neal, S. White, J. Williams-Woodward, K. Ivors, C. Adkins, A. Senesac, and A. Windham. 2012. Stakeholder vision of future direction and strategies for Southeastern U.S. nursery pest research and extension programming. J. Integ. Pest Mgt. 3(2):D1-D8.

Funderburk, J., M. Srivastava, C. Funderburk, and S. McManus. 2013. Evaluation of imidacloprid and cyantraniliprole for suitability in conservation biological control program for *Orius insidius* (Hemiptera:Anthocoridae) in field pepper. Florida Entomol. 96:229-231.

Galvan, T.L., R.L. Koch, and W.D. Hutchison. 2005. Toxicity of commonly used insecticides in sweet corn and soybean to multicolored Asian lady beetle (Coleoptera : Coccinellidae). J. Econ. Entomol. 98:780-789.

Garcera, C., E. Molto, and P. Chueca. 2011. Effect of spray volume of two organophosphate pesticides on coverage and on mortality of California red scale *Aonidiella aurantii* (Maskell). Crop Protection 30:693-697.

Gaspar, T., T. Franck, B. Bisbis, C. Kevers, L. Jouve, J.F. Hausman, and J. Dommes. 2002. Concepts in plant stress physiology. Application to plant tissue cultures. Plant Growth Regulat. 37:263-285.

Gent, M.P.N. 1995. Paclobutrazol or uniconazol applied early in the previous season promotes flowering of field-grown *Rhododendron* and *Kalmia*. J. Plant Growth Regulat. 14:205-210.

Gent, M.P.N. 2004. Efficacy and persistence of paclobutrazol applied to rooted cuttings of rhododendron before transplant. HortScience 39:105-109.

Gianfagna, T.J. 1990. Natural and synthetic growth regulators and their use in horticultural and argronomic crops, p. 751-773. In: P.J. Davies (ed.). Plant hormones and their role in plant growth and development. Kluwer Academic Publishers, Dordrecht, Netherlands.

Gill, S., J. Lutz, P. Shrewsbury, and M. Raupp. 2001. Evaluation of biological and chemical control methods for black vine weevil, *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae), in container grown perennials. J. Environ. Hort. 19:166-170.

Gilman, E.F. 2012. An illustrated guide to pruning. Cengage Learning, Clifton Park, NY.

Glasgow, T. 1999. Customer perceptions of plant quality. PhD Diss., N. Carolina State Univ.

GMO-Compass. 2013. 19 July 13 < http://www.gmo-compass.org/eng/gmo/db/>.

Greene, D.W. and W.R. Autio. 1990. Vegetative responses of apple-trees following benzyladenine and growth-regulator sprays. J. Amer. Soc. Hort. Sci. 115:400-404.

Greene, D.W. and P. Miller. 1988. Effects of growth-regulator sprays and notching on growth and branching of Starkimson Delicious apple-trees. J. Amer. Soc. Hort. Sci. 113:18-23.

Greer, H.E. 1988. Greer's guidebook to available rhododendrons: species and hybrids.

Grout, J.M., P.E. Read, and D.K. Wildung. 1986. Influence of tissue-cultured and leaf-bud propagation on the growth habit of Northblue blueberry. J. Amer. Soc. Hort. Sci. 111:372-375.

Gupta, G. and V.A. Krischik. 2007. Professional and consumer insecticides for management of adult Japanese beetle on hybrid tea rose. J. Econ. Entomol. 100:830-837.

Haberlandt, G. 1902. Kulturversuche mit isolierten Pflanzenzellen. Sitzungsber. Akad. der Wiss. Wien, Math. Naturwiss K1 111:69-92.

Halaj, J., A.B. Cady, and G.W. Uetz. 2000a. Modular habitat refugia enhance generalist predators and lower plant damage in soybeans. Environ. Entomol. 29:383-393.

Halaj, J., D.W. Ross, and A.R. Moldenke. 2000b. Importance of habitat structure to the arthropod food-web in Douglas-fir canopies. Oikos 90:139-152.

Hall, C.R., A.W. Hodges, and J.J. Haydu. 2005. Economic impacts of the green industry in the United states: final report to the national urban and community forestry advisory committee. UT Extension. 12 April 2013. <http://www.ufei.org/files/pubs/EconomicImpactsoftheUSGreen%20Industr(NUCFACfinal report).pdf >.

Hall, C.R., A.W. Hodges, and J.J. Haydu . 2006. The economic impact of the green industry in the United States. HortTechnology 16:345-353.

Halmann, M. 1990. Synthetic plant growth regulators. Adv.Agron. 43:47-105.

Hamedi, N., S. Moharramipour, and M. Barzegar. 2013. Temperature-dependent chemical components accumulation in *Hippodamia variegata* (Coleoptera: Coccinellidae) during overwintering. Environ. Entomol. 42:375-380.

Hammond, H.E., R.K. Schoellhorn, S.B. Wilson, and J.G. Norcini. 2007. Differing blanketflower cultivar and ecotype responses to plant growth regulators. HortTechnology 17:552-556.

Hanks, L.M. and R.F. Denno. 1993. Natural enemies and plant water relations influence the distribution of an armored scale insect. Ecol. 1081-1091.

Hartmann, H.T., D.E. Kester, F.T. Davies, and R.L. Geneve. 1997. Hartmann and Kester's plant propagation: Principles and practices. Prentice Hall, Upper Saddle River, N.J.

Haynes, K.F. 1988. Sublethal effects of neurotoxic insecticides of insect behavior. Annu. Rev. Entomol. 33:149-168.

Hester, K.A., G. BI, M.A. Czarnota, A. Fulcher,G.J. Keever, J.H. Lieth, J.D Orsi, B.E. Whipker, K. Sullivan, and C.L. Palmer. 2013. Impact of Augeo, Configure, and Florel on hydrangea branching. J. Environ. Hort. 31(1):27-29.

Heuvelink, E., F. Van Noort, and J. Sleegers. 2009. Recept voor een compacte plant. Vakblad voor de bloemisterij 52:52-53.

Hodges, A.W., C.R. Hall, B.K. Behe, and J.H. Dennis. 2008. Regional analysis of production practices and technology use in the US nursery industry. HortScience 43:1807-1812.

Hodges, A.W., C.R. Hall, and M.A. Palma. 2011. Economic contributions of the green industry in the United States in 2007-08. HortTechnology 21:628-638.

Hoffmann, W.C. and A.J. Hewitt. 2005. Comparison of three imaging systems for water-sensitive papers. Appl. Eng. Agri. 21:961-964.

Holland, A.S., G.J. Keever, J.R. Kessler, and F. Dane. 2007. Single cyclanilide applications promote branching of woody ornamentals. J. Environ. Hort. 25:139-144.

Hooley, R. 1994. Gibberellins - perception, transduction and responses. Plant Mol.Biol. 26:1529-1555.

Horn, D.J. and R.F. Sheppard. 1979. Sex-ratio, pupal parasitism, and predation in two declining populations of the bagworm, *Thyridopteryx ephemeraeformis* (Haworth) (Lepidoptera:Psychidae). Ecol. Entomol. 4:259-265.

Huang, H.T., and P. Yang. 1987. The ancient cultured citrus ant. Bioscience 37, 665-671.

Ingles, J.E. 2001. Ornamental horticulture: Science, operations, & management. Delmar, United States.

IR-4 Project. 2014. The IR-4 Ornamental Horticulture Program Database. 29 March 2014. <http://ir4.rutgers.edu/Ornamental/Ornamentals.cfm>.

Jacobs, K.A. and L.C. Berg. 2000. Inhibition of fungal pathogens of woody plants by the plant growth regulator paclobutrazol. Pest Mgt. Sci. 56:407-412.

Jain, S.M. 1993. Somaclonal variation in *Begonia X Elatior* and *Saintpaulia-Ionantha* L. Scientia Hort. 54:221-231.

Jeon, H.Y. and H. Zhu. 2012. Development of a cariable-rate sprayer for nursery liner applications. Trans. ASAE 55:303-312.

Jeppson, L.R., 1953. Systemic insecticides- entomological aspects of systemic insecticides. J. Agr. Food Chem. 1:830-832.

Jerardo, A. 2007. Floriculture and nursery crops yearbook. Economic Research Service, United States Department of Agriculture, Washington, DC, USA.

Jimenez, M., K.M. Klonsky, and R.L. De Moura. 2009. Sample costs to establish and produce fresh market blueberries. University of California Cooperative Extension. 12 June 2012. <coststudies.ucdavis.edu/files/blueberryvs2009.pdf>.

Johnson, A.G. and G.P. Lumis. 1979. Chemical pruning of *Euonymus fortunei* 'Colorata' with dikegulac-sodium. HortScience 14:626-627.

Jones, J.B. 1986. Determining markets and market potential of horticultural corps, p. 175-182. In: R.H. Zimmerman, R.J. Griesbsach, F. Hamerschlag, and R.H. Lawson (eds.). Tissue culture as a plant production system for horticultural crops. Martinus-Nijhoff, Dordrecht, Netherlands.

Jones, J.B. and C.J. Sluis. 1991. Marketing of micropropagated plants. p. 141-154. In: R.H. Zimmerman, and P.C. DeBergh (eds.). Micropropagation: Technology and application. Kluwer Academic, Dordrecht, Netherlands.

Jones, O.P. 1994. Physiological change and apparent rejuvenation of temperate fruit trees from micropropagation, p. 323-331. In: P.J. Lumsden, J.R. Nicholas, and W.J. Davies (eds.). Physiology, Growth and Development of Plants in Culture. Springer, Netherlands.

Jones, O.P., M. Welander, B.J. Waller, and M.S. Ridout. 1996. Micropropagation of adult birch trees: Production and field performance. Tree Physiol. 16:521-525.

Karp, A. 1994. Origins, causes and uses of variation in plant tissue cultures, p. 139-151. In: I.K. Vasil and T.A. Thorpe (eds.). Plant cell and tissue culture. Springer, Netherlands.

Kepinski, S. 2006. Integrating hormone signaling and patterning mechanisms in plant development. Current Opinion in Plant Biol. 9:28-34.

Kevers, C., M. Filali, G. Petitpaly, D. Hagege, M. Rideau, and T. Gaspar. 1996. Habituation of plant cells does not mean insensitivity to plant growth regulators. In Vitro Cellular Dev. Biol.-Plant 32:204-209.

Kher, S.V., J. De Jonge, M.T.A. Wentholt, R. Deliza, J.C. De Andrade, H.J Cnossen, N.B.L. Luijckx, and L.J. Frewer. 2013. Consumer perceptions of risks of chemical and microbiological contaminants associated with food chains: a cross-national study. Intl. J. Consumer Studies 37:73-83.

Kirkwood, R.C. 1999. Recent developments in our understanding of the plant cuticle as a barrier to the foliar uptake of pesticides. Pesticide Sci. 55:69-77.

Fulcher, A. and W.E. Klingeman. 2012. Effect of contact and systemic insecticides used for scale pests on beneficial insects. SNA Res. Conf. 57:108-115.

Klingeman, W.E., S.K. Braman, and G.D. Buntin. 2000. Evaluating grower, landscape manager, and consumer perceptions of azalea lace bug (Heteroptera : Tingidae) feeding injury. J. Econ. Entomol. 93:141-148.

Kogan, M. 1998. Integrated pest management: Historical perspectives and contemporary developments. Annu. Rev. Entomol. 43:243-270.

Koppert Biological Systems. 2005. Side effects database. Netherlands: Koppert biological systems.

Kozai, T. 1991. Photoautotrophic micropropagation. In Vitro Cellular Dev. Biol.-Plant 27:47-51.

Kozai, T. and C. Kubota. 2001. Developing a photoautotrophic micropropagation system for woody plants. J. Plant Res. 114:525-537.

Krul, W.R. and J. Myerson. 1980. In vitro propagation of grape. Sci. Educa. Administration Publ. ARR:35-43.

Kurata, K. 1992. Transplant production systems. Kluwer Academic Publishers, Dordrecht, Netherlands.

Kyte, L. 1983. Plants from Test Tubes: and introduction to micropropagation. Timber Press, Portland, OR.

Landis, D.A., S.D. Wratten, and G.M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annu. Rev. Entomol. 45:175-201.

Landis, D.A., F.D. Menalled, A.C. Costamagna, and T.K. Wilkinson. 2005. Manipulating plant resources to enhance beneficial arthropods in agricultural landscapes. Weed Sci. 53:902-908.

Langellotto, G.A. and R.F. Denno. 2004. Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. Oecologia 139:1-10.

Latimer, J.G. 1992. Drought, paclobutrazol, abscisic acid, and gibberellic acid as alternatives to daminozide in tomato transplant production. J. Amer. Soc. Hort. Sci. 117:243-247.

Latimer, J.G. and R.D Oetting. 1998. Greenhouse conditioning affects landscape performance of bedding plants1. HortScience 21:227-229.

Latimer, J.G, and R.F. Severson. 1997. Effect of mechanical and moisture-stress conditioning on growth and cuticle composition of broccoli transplants. J. Amer. Soc. Hort. Sci. 122:788-791.

LeBude, A.V., S.A. White, A.F. Fulcher, S. Frank, W.E. Klingeman, J-H. Chong, M.R. Chappell, A. Windham, K. Braman, F. Hale, W. Dunwell, J. Williams-Woodward, K. Ivors, C. Adkins, and J. Neal. 2012. Assessing the integrated pest management practices of southeastern US ornamental nursery operations. Pest Mgt. Sci. 68:1278-1288.

Lee, A., P. Miller, J. Power, J. Cross, A. Gilbert, C. Glass, W. Taylor, P. Walklate, and N. Western. 2000. The application of pesticide sprays to tomato crops, p. 383-390. In: Pesticide application. Univ. of Surrey, Guildford, UK.

Lim, C.C., R. Arora, and E.C. Townsend. 1998. Comparing Gompertz and Richards functions to estimate freezing injury in *Rhododendron* using electrolyte leakage. J. Amer. Soc. Hort. Sci. 123:246-252.

Lopez-Bucio, J., E. Hernandez-Abreu, L. Sanchez-Calderon, M.F. Nieto-Jacobo, J. Simpson, and L. Herrera-Estrella. 2002. Phosphate availability alters architecture and causes changes in hormone sensitivity in the Arabidopsis root system. Plant Physiol. 129:244-256.

Los, L.M. and W.A. Allen. 1983. Abundance and diversity of adult Carabidae in insecticide-treated and untreated alfalfa fields. Environ.Entomol. 12:1068-1072.

Lucas, E., S. Giroux, S. Demougeot, R.M. Duchesne, and D. Coderre. 2004. Compatibility of a natural enemy, *Coleomegilla maculata* lengi (Col., Coccinellidae) and four insecticides used against the Colorado potato beetle (Col., Chrysomelidae). J. Appl. Entomol. 128:233-239.

Lutken, H., J.L. Clarke, and R. Muller. 2012. Genetic engineering and sustainable production of ornamentals: current status and future directions. Plant Cell Rpt. 31:1141-1157.

Lutken, H., J.S. Jensen, S.H. Topp, H. Mimbus, R. Muller, and S.K. Rasmussen. 2010. Production of compact plants by overexpression of AtSHI in the ornamental Kalanchoë. Plant Biotechnol. J. 8:211-222.

Ma, D.L., G. Gordh, and M.P. Zalucki. 2000. Toxicity of biorational insecticides to *Helicoverpa* spp. (Lepidoptera : Noctuidae) and predators in cotton field. Intl J. Pest Mgt. 46:237-240.

Ma, Z., D.G. Bielenberg, K.M. Brown, and J.P. Lynch. 2001. Regulation of root hair density by phosphorus availability in Arabidopsis thaliana. Plant Cell and Environ. 24:459-467.

Macdonald, B. 1990. Practical woody plant propagation for nursery growers. Timber Press, Portland, OR.

Maczuga, S.A. and K.J. Mierzejewski. 1995. Droplet size and density effects of *Bacillus thuringiensis* kurstaki on gypsy moth (Lepidoptera, Lymantriidae) larvae. J. Econ. Entomol. 88:1376-1379.

Matysiak, B. and J. Nowak. 2008. Coir substrates for rooting of ornamental ericaceous plants. Prop. Ornamental Plants 8:76-80.

Meijon, M., R. Rodroguez, M.J. Canal, and I. Feito. 2009. Improvement of compactness and floral quality in azalea by means of application of plant growth regulators. Scientia Hort. 119:169-176.

Meins, F. 1989. Habituation - heritable variation in the requirement of cultured plant cells for hormones. Ann. Rev. Genet. 23:395-408.

Midcap, J. 2000. Pruning Foster's holly. The University of Georgia. 23 October 2011. <http://www.canr.org/pastprojects/1999013.pdf>.

Mitsui Chemicals America, Inc. 2013. Dinotefuran [Online]. Mitsui Chemicals America, inc. Rye Brook, NY. 16 April 2013. <www.mitsuichemicals.com> .

Mizell, R.F. and M.C. Sconyers. 1992. Toxicity of imidacloprid to selected arthropod predators in the laboratory. Florida Entomol. 75:277-280.

Molinier, J., G. Ries, C. Zippfel, and B. Hohn. 2006. Transgeneration memory of stress in plants. Nature 442:1046-1049.

Montella, I.R., R. Schama, and D. Valle. 2012. The classification of esterases: an important gene family involved in insecticide resistance - A review. Memorias Do Instituto Oswaldo Cruz 107:437-449.

Müller, R. 2011. Physiology and genetics of plant quality improvement. Univ. Copenhagen, PhD diss.

Mullins, J.W. 1993. Imidacloprid - A new nitroguanidine insecticide. Acs Symposium Series 524:183-198.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, 15:473-497.

Nas, M.N., P.E. Read, V. Miller, and P.A. Rutter. 2003. In vitro "rejuvenation" of woody species is temporary, p. 211-215. In: Hammerschlag, F.A., and P. Saxena (eds.). Biotechnology in horticultural crop improvement: achievements, opportunities and limitations. In: Acta Hort. Proc. 625.

Nash, L.J. and W.R. Graves. 1993. Drought and flood stress effects on plant development and leaf water relations of five taxa of trees native to bottomland habitats. J. Amer. Soc. Hort. Sci. 118:845-850.

Navarro, A., M.J. Sanchez-Blanco, and S. Banon. 2007. Influence of paclobutrazol on water consumption and plant performance of *Arbutus unedo* seedlings. Scientia Hort. 111:133-139.

Norcini, J.G., J.H. Aldrich, and J.M. McDowell. 1994. Flowering response of Bougainvillea cultivars to dikegulac. HortScience 29:282-284.

Oerum, J. and J. Christensen. 2001. Produktionsokonomiske analyser af mulhederne for en reduceret pesticidanvendelse I dansk gartneri. Statens Jordbrugs- og Fiskeriokonomiske Institut 128:1-81.

OHP. 2012. Augeo plant growth regulator (label). 15 May 2012. < http://www.fine-americas.com/_Attachments/Resources/578_S4.pdf>.

Ozkan, H., H. Zhu, R. Derksen, H. Guler, and C. Krause. 2006. Evaluation of various spraying equipment for effective application of fungicides to control Asian soybean rust. Aspects Appl. Biol. 77:423.

Parups, E.V. and W.E. Cordukes. 1977. Growth of turfgrasses as affected by atrinal and embark. HortScience 12:258-259.

Payne, N.J. and K. Vanfrankenhuyzen. 1995. Effect of spray droplet size and density on efficacy of *Bacillus thuringiensis* Berliner against the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera, Tortricidae). Canadian Entomol. 127:15-23.

Piché, M., B. Panneton, and R. Thériault. 2000. Field evaluation of air-assisted boom spraying on broccoli and potato. Trans. ASAE 43:793-799.

Pimentel, D. 1995. Amounts of pesticides reaching target pests - environmental impacts and ethics. J. Agri. Environ. Ethics 8:17-29.

Pischke, M.S., E.L. Huttlin, A.D. Hegeman, and M.R. Sussman. 2006. A transcriptome-based characterization of habituation in plant tissue culture. Plant Physiol. 140:1255-1278.

Pratt, P.D. and B.A. Croft. 2000. Screening of predatory mites as potential control agents of pest mites in landscape plant nurseries of the Pacific Northwest. J. Environ. Hort. 18:218-223.

Rademacher, W. 2000. Growth retardants: Effects on gibberellin biosynthesis and other metabolic pathways. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51:501-531.

Randlkofer, B., F. Jordan, O. Mitesser, T. Meiners, and E. Obermaier. 2009. Effect of vegetation density, height, and connectivity on the oviposition pattern of the leaf beetle *Galeruca tanaceti*. Entomologia Experimentalis Et Applicata 132:134-146.

Raupp, M.J., J.A. Davidson, C.S. Koehler, C.S. Sadof, and K. Reichelderfer. 1989. Economic and aesthetic injury levels and thresholds for insect pests of ornamental plants. Florida Entomol. 72:403-407.

Reddy, S.K., H.V. Holalu, J.J. Casal, and S.A. Finlayson. 2013. Abscisic acid regulates axillary bud outgrowth responses to the ratio of red to far-red light. Plant Physiol. 163:1047-1058.

Reed, S.M. 2006. Evaluation of self-compatibility in *Clethra alnifolia*. HortScience 41:1040-1040.

Reynolds, H. 1954. Entomological aspects of systemic pesticides. Agr. Chem. 113.

Ripper, W.E., R.M. Greenslade, and L.A. Lickerish. 1949. Combined chemical and biological control of insects by means of a systemic insecticide. Nature 163:787-789.

Rodriguez, A.G. 1932. Influence of smoke and ethylene on fruiting of pineapple. J. Agri. 15.

Roh, M.S. and R.H. Lawson. 1998. Requirements for new floral crops - Perspectives for the United States of America, p. 29-38. In: Considine, J. A., and J. Gibbs (eds.). Proc. III Intl. Symp. New Floricultural Crops 454.

Rowland, L.J., Dhanaraj, A.L., Polashock, J.J., and R. Arora. 2003. Utility of blueberry-derived EST-PCR primers in related *Ericaceae* species. HortScience 38:1428-1432.

Rudinsky, J. 1959. Systemics in the control of forest insects. Journal of Forestry 57, 284-286.

Ryan, G.F. 1970. Effects of succinic acid 2,2-dimethyl hydrazide and phosphorus treatments on rhododendron flowering and growth. J. Amer. Soc. Hort. Sci. 95:624-626.

Sadof, C.S. and M.J. Raupp. 1996. Aesthetic thresholds and their development, p. 203-226. In: L.G. Higley and L.P. Pedigo (eds.). Economic thresholds for integrated pest management. Lincoln, NE: University of Nebraska Press.

Sánchez-Hermosilla, J., V.J. Rincón, F. Páez, F. Agüera, and F. Carvajal. 2011. Field evaluation of a self-propelled sprayer and effects of the application rate on spray deposition and losses to the ground in greenhouse tomato crops. Pest Mgt. Sci. 67:942-947.

Schleiden, M.J. 1838. Beitrage zur Phylogenesis. Translated by H. Smith. Sydenham Society, London.

Schuster, D.J., and P.A. Stansly. 2000. Response of two lacewing species to biorational and broad-spectrum insecticides. Phytoparasitica 28:297-304

Schwann, T. 1839. Mikroskopische untersuchungen uber die uebereinstimmung in der structure und dem wachsthum der thiere und pflanzen, Berlin, G.E. Reimer.

Sclar, D.C., D. Gerace, and W.S. Cranshaw. 1998. Observations of population increases and injury by spider mites (Acari: Tetranychidae) on ornamental plants treated with imidacloprid. J. Econ. Entomol. 91:250-255.

SePRO, C. 2011. Topflor ornamental plant growth regulator (label). 15 May 2012. <http://www.sepro.com/documents/Topflor_Label.pdf>.

Shrewsbury, P.M. and D.C. Smith-Fiola. 2000. Evaluation of green lacewings for suppressing azalea lace bug populations in nurseries. J. Environ. Hort. 18:207-211.

Simmons, D. 2008. Epigenetic influence and disease. Nature Educ. 1(1):6.

Shu, L.J. and K.C. Sanderson. 1980. Dikegulac sodium influences shoot growth of greenhouse azaleas. HortScience, 15:813-814.

Skirvin, D.J. 2004. Virtual plant models of predatory mite movement in complex plant canopies. Ecol. Modelling 171:301-313.

Smulders, M.J.M., and G.J. De Klerk. 2011. Epigenetics in plant tissue culture. Plant Growth Regulat. 63:137-146.

Sorensen, M.T. and Danielsen, V. 2006. Effects of the plant growth regulator, chlormequat, on mammalian fertility. Intl. J. Andrology 29:129-132.

Starman, T.W. 1991. Response of Kientzler New-Guinea impatiens to manual and chemical pinching. HortScience 26:856-857.

Stapel, J., A. Cortesero, and W. Lewis. 2000. Disruptive sublethal effects of insecticides on biological control: altered foraging ability and life span of a parasitoid after feeding on extrafloral nectar of cotton treated with systemic insecticides. Biol. Control 17:243-249.

Stern, V.M., R.F. Smith, R. Van Den, R. Bosch, and K.S Hagen. 1959. The Integrated control concept. Hilgardia 28:81-101.

Stiling, P. and D.C. Moon. 2005. Quality or quantity: the direct and indirect effects of host plants on herbivores and their natural enemies. Oecologia 142:413-420.

Stuart, N.W. 1961. Initiation of flower buds in rhododendron after application of growth retardants. Science 134:50-52.

Studebaker, G.E. and T.J. Kring. 2003. Effects of insecticides on Orius insidiosus (Hemiptera: Anthocoridae), measured by field, greenhouse and petri dish bioassays. Florida Entomol. 86(2):178-185.

Swartz, H.J., G.J. Galletta, and R.H. Zimmerman. 1981a. Field performance and phenotypic stability of tissue culture-propagated strawberries. J. Amer. Soc. Hort. Sci. 106:667-673.

Swartz, H.J., G.J. Galletta, and R.H. Zimmerman. 1981b. Phenotypic stability and field performance of tissue-culture propagated thornless blackberry. HortScience 16: 418-418.

Syngenta Crop Protection AG. 2013. Water-sensitive paper for monitoring spray distribution. 12 Dec 2013. <http://www.qinstruments.com/uploads/media/wsp_use-it-in-agriculture_01.pdf>.

Systema, W. and J.B. Ruesink. 1996. Forcing *Pieris japonica* 'Debutante'. Scientia Hort. 65:171-180.

Szczepaniec, A., S.F. Creary, K.L. Laskowski, J.P. Nyrop, and M.J. Raupp. 2011. Neonicotinoid insecticide imidacloprid causes outbreaks of spider mites on elm trees in urban landscapes. PLoS one 6(5), e20018.

Szczepaniec, A. and M.J. Raupp. 2013. Direct and indirect effects of imidacloprid on fecundity and abundance of *Eurytetranychus buxi* (Acari: Tetranychidae) on boxwoods. Exp. Appl. Acarol. 59:307-318.

Szczepaniec, A., B.B. Raupp, and M.J. Raupp. 2013a. Effects of dinotefuran and imidacloprid on target and non-target arthropods on American elm. Arboriculture Urban For. 39:231-235.

Szczepaniec, A., M.J. Raupp, R.D. Parker, D. Kerns, and M.D. Eubanks. 2013b. Neonicotinoid insecticides alter induced defenses and increase susceptibility to spider mites in distantly related crop plants. PLoS One 8, e62620.

Ticknor, R.L. 1968. Growth regulator tests on *Rhododendron*. Amer. Nursery 128:47-48.

Topoonyanont, N. and P.C. DeBergh, P.C. 2001. Reducing bushiness in micropropagated Gerbera. Plant Cell Tissue Organ Culture 67:133-144.

Toscano, N.C. and F.J. Byrne. 2005. Laboratory and field evaluations of neonicotinoid insecticides against the glassywinged sharpshooter. Pierce's Disease Research Symposium, San Diego, California, pp. 380-383.

Townsley-Brascamp, W. and N.E. Marr. 1995. Evaluation and analysis of consumer preferences for outdoor ornamental plants, p. 199-208. In: E. Matsuo and P.D. Relf (eds.). Horticulture in Human Life, Culture, and Environment. Acta Hort., Koyoto, Japan.

Tripp, R. and W. van der Heide. 1996. The erosion of crop genetic diversity: challenges, strategies and uncertainties. Natural Resource Perspectives. odi.

Tunstall, J., G. Matthews, and A. Rhodes. 1965. Development of cotton spraying equipment in Central Africa. Emp. Cott. Gr. Rev. 42:131-145.

USDA 2007. Floriculture and nursery crops yearbook. USDA Economic Research Service. US Department of Agriculture.

van de Vrie, M. 1985. Spider mites- their biology, natural enemies and control. In: W. Helle and M.W. Sabelis (eds.). Spider mites: Their biology, natural enemies and control. Vol. 1. Elsevier, Amsterdam.

van Iersel, M.W. and K.S. Nemali. 2004. Drought stress can produce small but not compact marigolds. HortScience 39:1298-1301.

van Staden, J., C.W. Fennell, and N.J. Taylor. 2006. Plant stress in vitro: the role of phytohormones, p. 56-62. In: M.G. Fari, I. Holb, and G.D. Bisztray (eds.). Proc. Vth Intl. Symp. in Vitro Culture Hort. Breeding, Vols 1 and 2.

Wade, G.L. and R.R. Westerfield. 2012. Basic Principles of Pruning Woody Plants. 15 May 2012. <http://www.caes.uga.edu/applications/publications/files/pdf/B%20949_3.PDF>.

Wakita, T., N. Yasui, E. Yamada, and D. Kishi. 2005. Development of a novel insecticide, dinotefuran. J. Pesticide Sci. 30:122-123.

Warren, S.L., F.A. Blazich, and M. Thetford. 1991. Whole-plant response of selected woody landscape species to uniconazole1. J. Environ. Hort. 9:163-167.

Webster, C.A. and O. Jones. 1989. Micropropagation of the apple rootstock. M.9: effect of sustained subculture on apparent rejuvenation in vitro. J. hort. Sci. 64:421-428.

White, P.R. and A. Braun. 1941. Crown gall production by bacteria-free tumor tissues. Science 94:239-241.

Williams, E.G., B.R. Knox, and J.L. Rouse. 1982. Pollination sub-systems distinguished by pollen tube arrest after incompatible interspecific crosses in *Rhododendron* (Ericaceae). J. Cell Sci. 53:255-277.

Womac, A., J. Mulrooney, and W. Scott. 1992. Characteristics of air-assisted and drop-nozzle sprays in cotton. Trans. ASAE 35.

Wood, B.W. 1988. Axillary shoot abscission in pecan and its relationship to growth regulators. J. Amer. Soc. Hort. Sci. 113:713-717.

Woodson, W. R. and T.J. Raiford. 1986. Induction of lateral branching in Chinese hibiscus with mefluidide. HortScience 21:71-73.

Wright, R.D. 1976. 6-Benzylaminopurine promotes axillary shoots in *Ilex crenata* Thunb. HortScience 11:43-44.

Xu, L.Y., H.P Zhu, H.E Ozkan, W.E. Bagley, and C.R. Krause. 2011. Droplet evaporation and spread on waxy and hairy leaves associated with type and concentration of adjuvants. Pest Mgt. Sci. 67:842-851.

Yates, W.E., N.B. Akesson, and R.W. Cowden. 1976. Criteria for minimizing drift residues on crops downwind from aerial applications. Trans. Asabe 17:627-632.

Youn, Y.N., M.J Seo, J.G Shin, C. Jang, and Y.M Yu. 2003. Toxicity of greenhouse pesticides to multicolored Asian lady beetles, *Harmonia axyridis* (Coleoptera : Coccinellidae). Biol. Control 28:164-170.

Zakhlenyuk, O. and V. Kunakh. 1987. Cytophysiological and cytogenetic effects of adenine derivatives in *Haplopappus gracilis* tissue culture. Soviet Plant Physiol. 34(3);475-483..

Zale, P.J., D.K. Struve, D.K., P. Jourdan, and D.M. Francis. 2011. Rapid assessment of genetic variation for morphological traits in sweetbay magnolia using a container production system. J. Amer. Soc. Hort. Sci. 136:135-144.

Zhu, H., R.D. Brazee, R.C. Derksen, R.D. Fox, C.R. Krause, H.E. Ozkan, and K. Losely. 2006. A specially designed air-assisted sprayer to improve spray penetration and air jet velocity distribution inside dense nursery crops. Trans. ASAE 49:1285-1294.

Zhu, H., R.D. Brazee, R.D. Fox, R.C. Derksen, and H.E. Ozkan. 2008. Development of a canopy opener to improve spray deposition and coverage inside soybean canopies: part 1. mathematical models to assist opener development. Trans. ASAE 51:1905-1912.

Zhu, H., C. Krause, R. Fox, R. Brazee, and R. Derksen. 1997. Techniques for assessing spray drift and canopy penetration in nurseries. ASAE Paper, no. 975006.

Zhu, H.P., M. Salyani, and R.D. Fox. 2011. A portable scanning system for evaluation of spray deposit distribution. Computers and Electronics in Agr. 76:38-43.

APPENDIX

APPEDIX A: EFFECTS OF PGRS AND PRUNING ON IN VITRO-PROPAGATED BLUEBERRY

ABSTRACT

Blueberries (*Vaccinum* spp.) are quickly becoming a popular ornamental plant. In order to produce high quality ornamental blueberries, growers typically prune them to encourage new growth. In vitro propagated (IVP) blueberries are innately more vigorous growers than cutting- propagated (CP) blueberries negating the necessity to prune as often before selling them. The objective of this study was to explore whether various Plant growth regulators (PGRs) and pruning could increase vegetative growth of IVP blueberry (*Vaccinum* 'Duke') even further. Pruning did not affect quality. Augeo increased the branch number and canopy density consistently. Although IVP blueberry has an advantage over CP blueberry, IVP plants can still be further improved by the use of PGRs.

INTRODUCTION

Highbush blueberries are quickly becoming a popular ornamental. Although they are often grown for their fruit, their bell-shaped flowers, habit, and fall color can be assets to a landscape. Propagation by cuttings has been the traditional method; they can be propagated by single-node cuttings without the need of any hormones, making them easy and inexpensive to propagate by cuttings (Dirr, 1998, Dirr, 2009). In the first four years of production, highbush blueberries are manually pruned once a year at an estimated time of 1 minute/plant or 22 hours/acre in order to discourage fruit production and encourage growth. After the first four years, they are manually pruned twice a year especially if used in fruit production (Jimenez et al., 2009). All of this labor-intensive pruning is very expensive to the grower.

Blueberries propagated in vitro have increased branching and more vigorous vegetative growth when compared to cutting-propagated (CP) plants (Debnath, 2007). In a study done in 1986 by Grout et al., CP and in vitro-propagated (IVP) plants were compared. They found that IVP plants had 2-3x more basal branches by the time they were 27 weeks old without decreasing branch length. The growth rate was faster in tissue cultured plants but evened off after week 34 (Grout et al., 1986). As a direct result of increased branching, IVP plants may have significantly higher yields for the first three years of fruit production compared with CP plants (El-Shiekh et al., 1996).

PGRs are a potential alternative to pruning to improve plant quality while lowering the labor costs of manual pruning. Foliar application of benzyladenine (BA) to Japanese holly (*Ilex crenata)* increased branch number (Wright, 1976). Mefluidide increased lateral branching in peach (Prunus persica) (Arnold et al., 1981) and Chinese hibiscus (*Hibiscus rosa-sinensis*) (Woodson and Raiford, 1986). Augeo (dikegulac sodium), a chemical pincher, applied to Little Lime™ hydrangea (*Hydrangea paniculata* 'Jane') increased branching and branching symmetry without reducing panicle number (Cochran and Fulcher, 2013). Dikegulac sodium applied to euonymus (*Euonymus fortunei*), crape myrtle (*Lagerstoemia indica*), and honeysuckle (*Lonicera x heckrottii* 'Goldflame') increased branch number and decreased branch elongation, resulting in more compact plants (Bruner et al., 2002; Johnson and Lumis, 1979). Uniconazole, a GA_3 biosynthesis inhibitor, decreased growth when applied to azalea (*Rhododendron* 'Flame' and 'Sunglow'), forsythia (*Forsythia suspense)*, holly (*Ilex crenata* 'Compacta' and *Ilex x*'Nellie R. Stevens), and mountain pieris (*Pieris floribunda*) resulting in more compact plants (Warren et al., 1991).

'Duke' blueberry is a northern highbush, widely planted cultivar. It consistently produces large, firm, high quality berries. 'Duke' blooms later than other cultivars, but

ripens early which protects the blossoms from spring frosts (Fall Creek Farm and Nursery, 2013). There is much research about the in vitro effects on blueberry and so our objective with this experiment was to explore whether various PGRs and pruning could increase vegetative growth of IVP blueberry even further.

MATERIALS AND METHODS

Vaccinum 'Duke' 2 1/4-inch tissue cultured plants (Briggs Nursery, Elma, WA) arrived 25 April 2012. They were kept in a greenhouse for two days and then were potted in sterilized 1-gal containers filled with 85% pine bark and 15% peat on 27 April 2012. Plants were placed outside under 50% shade to acclimate. After four days, the plants were moved to 25% shade in the nursery compound at the University of Tennessee in Knoxville, TN (35°56'46"N 83°56'18"W). Two weeks after potting plants were placed in full sun and top dressed with 19N–1.7P–6.6K, 5- to 6-month controlled release fertilizer with minors (Harrell's, Lakeland, FL) at 14 g per container (medium-high label rate).

Initial plant height, widest width, width 90° to widest width, and branch number (>3 cm) were recorded 16 July 2012. Plants were then separated into treatment groups and either 800 PPM Augeo, 600 PPM Configure, or 150 PPM of Topflor were foliar applied until the foliage was thoroughly covered. Two controls, a hand pruned and a water spray, were also used. The hand pruning treatment consisted of pruning each stem to a lateral bud 6 inches from the substrate surface. Plants were then randomized.

Plants were hand-weeded as needed and watered by overhead automatic irrigation twice daily. A Phytotoxicity symptom rating was recorded 2 weeks after treatment (WAT) on a 0 to 10 visual scale; 0 representing no injury and 10 representing complete kill. Branch number and growth index measurements were recorded every four, eight, and twelve WAT and 1 year after treatment (YAT). Quality was determined 12 WAT and 1 YAT on a 1-5

scale. A rating of 1 = sparsely branched and asymmetrical plants with an open canopy, 2 = sparsely branched and asymmetrical plants with a closed canopy, 3 = more densely branched and asymmetrical plants with a closed canopy, 4 = densely branched symmetrical plants, 5 = densely branched and symmetrical plants that completely covered the pot surface.

The experiment was a completely randomized design with repeated measures. There were 10 single plant replications for each treatment. Branch number, Symmetry (width 1 – width 2) and density (branch number / height) were used to assess the treatments. Data were analyzed using the GLM procedure of SAS (version 9.3S; SAS Institute, Cary, NC), α=0.05.

RESULTS

Pruning did not increase branch number or canopy density (Table 27 and 28). Augeo-treated plants had more branches and were denser than water control at 4, 8 and 12 WAT. Topflor treatment increased density compared with water controls 8 and 12 WAT. Canopy densities were not different 8 WAT, but 12 WAT, Topflor-treated plants were not as dense as those treated with Augeo. Neither branch number nor density was different among the treatments by 1 YAT.

At 4 WAT only, pruned plants were more symmetrical than water controls. PGRs were no different from the water controls at any time point (Table 29). At 12 WAT, Augeo was more symmetrical than Configure, but by 1 YAT, symmetry was not different among treatments. Augeo had the highest phytotoxicity affecting almost 40% of each plant with symptoms of small, white tips (Table 30). Configure also showed signs of phytotoxicity in the form of lighter leaves with red spots that covered about 10% of each plant. Phytotoxicity symptoms were no longer visible by 4 WAT.

No treatment had a higher quality than the water or pruned control at 12 WAT (Table 30). However, at 1 YAT, Topflor had a higher quality than water control, a rating of 3.9 vs. a rating of 2.4, respectively. All other branch-inducing treatments were not different from the water controls.

DISCUSSION

Pruning did nether increased or decreased branch number. Augeo had 41%, 45%, and 39%, more branches than water controls at 4, 8, and 12 WAT, but was no different than any other treatment by 1 YAT (Table 27). Pruning was ineffective at improving density (Table 28). Augeo-treated plants were 48%, 100%, and 118% dense than water controls at 4, 8, and 12 WAT and Topflor was 72% and 60% denser than water controls at 8 and 12 WAT. By 1 YAT, no treatment was denser than water control.

Symmetry was inconsistent over the life of the experiment (Table 29). Pruned plants were 57% more symmetrical than water controls at 4 WAT only, and no other branch-inducing treatments were more symmetrical than water controls at any other time point, including 1 YAT. Quality was not different among treatments 12 WAT, but 1 YAT, Topflor plants were visually more dense, compact, and symmetrical than the water controls (Table 30).

Pruning was ineffective at increasing branch number or density and was inconsistent at improving symmetry of IVP 'Duke' blueberry. Augeo-treated IVP blueberries were more well-branched and denser than water controls or any other branch-inducing treatment, but improvements did not last 1 YAT. No treatment was effective at improving the quality of 'Duke' blueberry at 12 WAT, but by 1 YAT Topflor-treated plants were visually higher quality than untreated plants. If selling within a single growing season, applying Augeo to IVP 'Duke' blueberry may increase branch number as well as density. However, if

selling IVP blueberry the following season, Augeo may have to be reapplied to keep its advantage.

CONCLUSION

Pruning did not increase the quality of IVP blueberry. However, we may conclude that the ability of IVP plants to grow vigorously does not negate the effects of PGRs. PGRs can be used to further improve IVP blueberries and possibly other plant species as well.

APPENDIX 4: TABLES

Table 27: IVP Blueberry (*Vaccinum* 'Duke') branch number following application of branch-inducing treatments in 2012.

Treatment	Rate (PPM)[z]	Branch number			
		4 WAT	8 WAT	12 WAT	1 YAT
Water	-	29 b[y]	31 b	33 b	43
Augeo	800	41 a	45 a	46 a	53
Configure	600	25 b	30 b	31 b	52
Topflor	150	29 b	35 b	35 b	47
Pruned	-	31 b	33 b	33 b	40
DF		4	4	4	4
Significance		***[x]	***	***	NS
P-value		<.0001	0.0001	<.0001	0.2418
F Statistic		7.33	7.12	7.71	1.46

[z]1 PPM = 1 $mg \cdot L^{-1}$

[y]Means within a column followed by the same letter were not significantly different (Tukey's HSD $\alpha = 0.05$).

[x]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

Table 28: IVP Blueberry (*Vaccinum* 'Duke') density after following application of branch-inducing treatments in 2012.

Treatment	Rate (PPM)[z]	Density			
		4 WAT	8 WAT	12 WAT	1 YAT
Water	-	0.79 bc[y]	0.60 b	0.45 c	0.77
Augeo	800	1.17 a	1.20 a	0.95 a	1.02
Configure	600	0.58 c	0.65 b	0.54 c	0.97
Topflor	150	0.93 ab	1.03 a	0.72 b	1.01
Pruned	-	0.88 abc	0.68 b	0.55 bc	0.82
DF		4	4	4	4
Significance		***[x]	***	***	NS
P-value		.0002	<.0001	<.0001	0.3536
F Statistic		6.76	14.93	17.37	1.15

[z]1 PPM = 1 mg·L^{-1}

[y]Means within a column followed by the same letter were not significantly different (Tukey's HSD α = 0.05).

[x]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

Table 29: IVP 'Blueberry (*Vaccinum* 'Duke') symmetry following application of branch-inducing treatments in 2012.

Treatment	Rate (PPM)[z]	Symmetry			
		4 WAT	8 WAT	12 WAT	1 YAT
Water	-	14.3 a[y]	11.5	9.5 ab	9.6
Augeo	800	9.5 ab	7.3	4.7 b	9.3
Configure	600	11.9 ab	10.8	12.2 a	16.0
Topflor	150	10.9 ab	9.1	6.6 ab	11.0
Pruned	-	5.9 b	7.5	6.3 ab	6.0
DF		4	4	4	4
Significance		*[x]	NS	*	NS
P-value		0.0196	0.6080	0.0220	0.2145
F Statistic		3.23	0.68	3.15	1.56

[z]1 PPM = 1 mg·L^{-1}

[y]Means within a column followed by the same letter were not significantly different (Tukey's HSD α = 0.05).

[x]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

Table 30: IVP Blueberry (*Vaccinum* 'Duke') phytotoxicity and quality following application of branch-inducing treatments in 2012.

Treatment	Rate (PPM)[z]	Phytotoxicity	Quality	
		2 WAT	12 WAT	1 YAT
Water	-	0.0 c[y]	2.2	2.4 b
Augeo	800	3.4 a	2.4	3.0 ab
Configure	600	0.9 b	2.1	3.0 ab
Topflor	150	0.0 c	2.9	3.9 a
Pruned	-	0.0 c	2.5	3.0 ab
DF		4	4	4
Significance		***[x]	NS	*
P-value		<.0001	0.3909	0.0421
F Statistic		69.45	1.05	3.23

[z]1 PPM = 1 mg·L^{-1}

[y]Means within a column followed by the same letter were not significantly different (Tukey's HSD α = 0.05).

[x]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

APPENDIX B: LINGERING EFFECTS OF PRUNING, PLANT GROWTH REGULATORS, AND PROPAGATION TECHNIQUE ON WOODY ORNNAMENTAL SPECIES ONE YEAR AFTER TREATMENT

ABSTRACT

Growers often prune or apply PGRs to create the canopy density and symmetry that consumers desire. In vitro propagation (IVP) liners can also be used for the same effect. However, the advantages of these methods typically wear off, and pruning and PGRs are often reapplied multiple times within the same growing season. The objective of this study was to observe the benefits of pruning, PGRs, and IVP one year after a single application. Water, PGRs or pruning were applied to cutting-propagation (CP) and IVP liners of clethra (*Clethra alnifolia* 'Hummingbird'), magnolia (*Magnolia virginiana* ' Henry Hicks'), and rhododendron (*Rhododendron* 'Roseum Elegans') in 2012. One year after treatment (YAT), growth and quality measurements were taken. At 1 YAT, Topflor-treated IVP clethra had the highest branch number, flower number, density and quality. Magnolia was unaffected by all treatments. Topflor application increased the density of both IVP and CP and the quality of CP rhododendron and even promoted flowering. Although Topflor was ineffective when first applied to clethra and Rhododendron, 1 YAT when all other treatments had worn off, the Topflor-treated plants were superior, making them easier for growers to sell with the least input one year after purchase and treatment.

INTRODUCTION

Consumers of woody ornamental shrubs desire plants that are full and cover the surface of the container they are sold in (Glasgow, 1999). Growers and retailers prefer plants that are well branched and compact (Roh and Lawson, 1998) for easier transport, to reduce space required per crop, and to minimize toppling due to wind (Müller, 2011). Many ornamentals, however, have a naturally elongated habit (Christensen et al., 2008, Lutken et

al., 2012). In order to produce plants that satisfy the desires of both retail and wholesale customers, growers often modify growth with pruning and PGRs.

Several plant species have been observed developing more branches without the need to prune when propagated in vitro (Damiano, 1980, Krul and Myerson, 1980, Swartz et al., 1981b, Swartz et al., 1981a, Jones, 1994, El-Shiekh et al., 1996). There is even research to suggest that IVP propagation effects are persist across time. For example, in a study conducted in 1996, IVP blueberries continued to have significantly more branches and higher yields for the first three years post-treatment than their cutting-propagated (CP) counterparts (El-Shiekh et al., 1996). *Zantedeschia*, an ornamental plant, has excessive bushiness when produced in vitro that persists and is even passed on to new generations (D'Arth et al., 2002).

However, there have also been studies that have shown that the benefits of IVP wear off over time (Grout et al., 1986). Chemical PGR effects also do not persist and so chemicals are often reapplied during the growing season Therefore, the objective of this study was to explore the lingering effectiveness of PGRs [Configure® (BA), Augeo® (dikugulac-sodium), and Topflor® (flurprimidol)] and propagation technique (CP and IVP) on enhancing branch architecture and plant quality for *Clethra alnifolia* 'Hummingbird', *Magnolia virginiana* 'Henry Hicks', and Rhododendron 'Roseum elegans' one year after treatment (YAT) to determine which treatments are most cost-effective in the long-run.

MATERIALS AND METHODS

Magnolia virginiana 'Henry Hicks' and *Clethra alnifolia* 'Hummingbird' tissue cultured plants (Briggs Nursery, Elma, WA) and rooted cuttings (Spring Meadow Nursery, Grand Haven, MI) in 2 ¼ inch pots arrived 25 April 2012. They were kept in a greenhouse for two days and then were potted in sterilized 1-gal containers filled with 85% pine bark

and 15% peat and placed outside under 50% shade to acclimate. After four days, the plants were moved to 25% shade in the nursery compound at the University of Tennessee in Knoxville, TN (35o56'46"N 83o56'18"W). *Rhododendron* 'Roseum elegans' tissue culture plants arrived 25 May 2012 from Briggs Nursery and rooted cuttings were procured from North Carolina. All rhododendrons were potted up in the same manner as above and kept in 25% shade for the remainder of the experiment. Two weeks after potting (WAP) Clethra and magnolia were placed in full sun and all plants were top dressed with 19N–1.7P–6.6K, 5- to 6-month controlled release fertilizer with minors (Harrell's, Lakeland, FL) at 14 g per container (medium-high label rate).

Initial plant height, widest width, width 90° to widest width, and branch number (>3cm) were recorded 14 June 2012. Plants were then separated into treatment groups and branch-inducing treatments of 800 PPM Augeo, 600 PPM Configure, or 150 PPM of Topflor were foliar applied on 21 June 2012 until the foliage was thoroughly wetted. Two control treatments, a hand pruned and a water spray, were also applied. The hand pruning treatment for clethra and magnolia consisted of pruning each stem to a lateral bud 6 inches from the substrate surface. For rhododendron, apical buds were manually removed with pruners.

CP and IVP clethra liners were pruned several times during liner production, but neither were pruned just prior to shipping to us. Magnolias arrived unpruned. The in vitro-propagated rhododendron liners were mistakenly sheared just prior to shipping; liners from cuttings were not pruned and were mostly apical cuttings. In order to account for this disparate treatment prior to the experiment, our objective with rhododendron shifted to comparing in vitro propagation plus one pruning with cutting propagation. For simplicity within the text and tables, in vitro-propagated rhododendron refers to in vitro-propagated

rhododendron plus one pruning. As a result of different plant growth stages between the two groups of rhododendron liners, they were treated with PGRs based on phenological stage rather than WAP. Specifically, each group was treated when plants finished a flush and set apical buds. In vitro-propagated plants flushed and set buds before the cutting propagated plants, and as a result, rhododendron produced by cuttings were sprayed after the in vitro-propagated plants (5 July 2012).

Plants were hand-weeded as needed and watered by overhead automatic irrigation twice daily. Branch number and growth measurements were recorded 1 year after treatment (YAT) on 18 June 2013. Quality was determined on a 1 to 5 scale. For clethra, a rating of 1 represented sparsely branched and asymmetrical plants with an open canopy, 2 represented sparsely branched and asymmetrical plants with a closed canopy, 3 represented more densely branched and asymmetrical plants with a closed canopy, 4 represented densely branched, symmetrical plants, and 5 represented densely branched and symmetrical plants that completely covered the container surface. In magnolia, a rating of 1 represented a single branch or dominant central leader, 2 represented two branches or to dominant leaders, 3 represented several branches emerging towards the apex of the plant, 4 represented a plant with a majority of basal branching, and 5 represented several branches emerging from the base, covering at least 90% of the container surface. In rhododendron, a rating of 1 represented plants that had one strong leader with branch development on distal portion of stem only, causing minimal coverage of the pot surface, 2 represented two or more leaders with a narrow, columnar growth pattern and branch development occurring distally, 3 represented two or more stems with branch development occurring at the base, covering at least 70% of the container surface when viewed from

above, 4 represented multiple stems that cover approximately 90% of the container surface, and 5 represented multiple stems from base and covered 100% of the container surface.

Experiments were conducted using a complete randomized design in with repeated measures. There were 10 single plant replications for each treatment. Data were analyzed using the GLM procedure of SAS (version 9.3S; SAS Institute, Cary, NC). Analysis of variance (ANOVA) and mean comparisons were used to determine the effects of propagation method and PGRs on branch number, density (branch number / height) (Randlkofer et al., 2009), symmetry (width 1 – width 2), quality, and phytotoxicity. Means were separated using Tukey's HSD, $\alpha = 0.05$. Each plant species was analyzed as a separate experiment.

RESULTS

Clethra

By 1 YAT, IVP and CP water controls had the same number of branches, flowers, density, symmetry and quality (Table 31). Pruning treatment did not increase branch number, flower number, symmetry or quality of the water controls of either IVP or CP. However, pruning did increase the density of IVP water controls.

No branch-inducing treatment had a greater branch or flower number or density than the CP water controls, but Configure-treated CP had a greater symmetry and Topflor treatment led to a higher quality. Topflor treated IVP plants had more branches, flowers, and higher density and quality than IVP and CP water controls. Symmetry was unaffected by branch-inducing treatments.

Magnolia

Branch number, density, symmetry and quality were all unaffected by propagation method or branch-inducing treatment (Table 32).

Rhododendron

Branch number, density, symmetry, and quality were all unaffected by propagation method or pruning by 1 YAT (Table 33). Topflor application in 2012 increased the density of both IVP and CP plants and the quality of CP plants in 2013, but no other PGR was effective at increasing branch number, density, symmetry, or quality.

Discussion

Clethra

Before branch-inducing treatments were applied in 2012, IVP plants had an advantage in regards to branch number and density (Objective 1 data), but by 1 YAT, IVP and CP water controls branch number, flower number, density, symmetry, and quality were not different (Table 31). Pruning often caused a decrease in the number of branches in 2012 (objective 1 data), but by 1 YAT, clethra that were pruned were not different than plants that were not pruned. Pruning was also ineffective at increasing flower number, symmetry or quality. Pruning increased IVP density 1 YAT by 63%, but CP clethra was unaffected.

In 2012, Topflor had fewer branches than IVP water controls (Objective 1 data), but 1 YAT, Topflor-treated IVP had 110 more branches and 113 more flowers than IVP water controls, a 72% and 88% increase, respectively. Topflor increased density of IVP by 168%. Topflor treatment also increased the quality of both CP and IVP clethra, receiving a quality rating of 4 versus the water control rating of 2 for both propagation methods.

For clethra, propagation method alone no longer matters 1 YAT. When Topflor was applied to IVP clethra liners in 2012, the results did not justify the cost of the PGR. However, 1 YAT, Topflor-treated IVP clethra had a higher branch number, flower number, density and quality.

Magnolia

Just as in 2012, magnolia remained nonplussed by pruning, PGRs or propagation method (Table 32).

Rhododendron

In 2012, before branch-inducing treatments were applied, IVP plants had a greater branch number and density than CP, and pruning produced denser, more well-branched rhododendrons than the water controls of both propagation methods. However, 1 YAT, IVP plants has no advantage over CP plants and pruning had lost its affect (Table 33). Topflor increased the density of CP rhododendron by 114% and IVP rhododendron by 66% when compared to their respective water controls. Topflor-treated IVP rhododendron had a 118% greater density than CP water control. Topflor treatment also increased the quality of CP plants (quality rating of 1.4 versus 4.5 for water and Topflor, respectively. Topflor treated CP rhododendron also had another advantage 1 YAT. Under field grown conditions, Rhododendron often takes three years to flower (Gent, 1995). However, rhododendrons treated with Topflor, both IVP and CP, flowered 1 YAT (Figure 6). Other PGRs have been shown to affect flowering in rhododendron. Application of growth retardants Phosfon and CCC promoted flowering one year into production (Stuart, 1961). Ancymidol increased the number of flower buds in *Rhododendron* 'Roseum elegans' (Ticknor, 1968). Daminiozide and paclobutrazol promoted flowering in azaleas (Meijon et al., 2009).

CONCLUSION

If a grower plans on selling plants at the end of one growing season, IVP clethra and rhododendron are more advantageous than CP plants (Objective 1 data). However, holding on to Clethra and Rhododendron for a year and selling them in the early summer may be easier as customers prefer plants that are flowering (Figure 5 and 6) (Glasgow, 1999).

APPENDIX 5: TABLES AND FIGURES

Table 31: Branch number, flower number, density, symmetry and quality of clethra (*Clethra alnifolia* 'Hummingbird') one year after application of branch-inducing treatments.

Clethra		Branch number	Flower number	Density	Symmetry	Quality
Cut	Water	169 bc[z]	140 b	2.1 bc	12.0 a	2 b
	Augeo	158 bc	132 b	2.1 bc	8.0 ab	2 b
	Configure	177 bc	151 b	2.3 bc	2.6 b	2 b
	Topflor	154 bc	130 b	2.9 bc	7.7 ab	4 a
	Prune	151 c	132 b	2.4 bc	5.6 ab	2 b
TC	Water	153 bc	128 b	1.9 c	11.8 ab	2 b
	Augeo	199 bc	153 b	2.7 bc	10.5 ab	3 ab
	Configure	173 bc	147 b	2.2 bc	3.8 ab	2 b
	Topflor	263 a	241 a	5.1 a	10.0 ab	4 a
	Prune	211 ab	178 ab	3.1 b	5.6 ab	3 ab
DF		9	9	9	9	9
Significance		***[y]	***	***	**	***
F Statistic		7.48	5.52	14.97	2.86	7.44
P-value		<0.0001	<0.0001	<0.0001	0.0093	<0.0001

[z]Means within a column followed by the same letter were not significantly different (Tukey's HSD α = 0.05).
[y]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

Table 32: Branch number, density, symmetry, and quality of magnolia (*Magnolia virginniana* 'Henry Hicks') one year after application of branch-inducing treatments.

Magnolia		Branch number	Density	Symmetry	Quality
Cut	Water	18.60	0.27	9.22	4.00
	Augeo	18.29	0.25	6.14	3.57
	Configure	18.60	0.26	8.67	4.00
	Topflor	18.00	0.28	4.57	3.86
	Prune	14.17	0.20	5.83	4.50
TC	Water	14.86	0.20	4.57	3.17
	Augeo	15.40	0.22	11.00	4.00
	Configure	16.17	0.25	7.00	4.00
	Topflor	17.40	0.24	7.67	3.83
	Prune	12.50	0.18	5.80	4.17
DF		9	9	9	9
Significance		NS	NS	NS	NS
F Statistic		1.97[y]	1.95	1.34	1.15
P-value		0.0622	0.0654	0.2368	0.3457

[z]Means within a column followed by the same letter were not significantly different (Tukey's HSD α = 0.05).
[y]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

Table 33: Branch number, density, symmetry and quality of rhododendron (*Rhododendron* 'Roseun Elegans') one year after application of branch-inducing treatments.

		Branch number	Density	Symmetry	Quality
Cut	Water	14.20 bc[z]	0.22 c	6.40	1.40 c
Cut	Augeo	12.50 c	0.22 c	10.17	1.50 c
Cut	Configure	13.83 bc	0.21 c	11.50	2.00 bc
Cut	Topflor	17.40 abc	0.47 a	5.14	4.50 a
Cut	Prune	12.50 c	0.27 bc	4.83	1.60 bc
TC	Water	16.40 abc	0.29 bc	5.25	2.50 bc
TC	Augeo	20.80 a	0.39 ab	13.00	2.33 bc
TC	Configure	18.33 ab	0.35 abc	10.00	2.60 bc
TC	Topflor	19.29 a	0.48 a	5.50	3.38 ab
TC	Prune	15.75 abc	0.35 abc	11.00	3.00 abc
DF		9	9	9	9
Significance		***	***	NS	***
F Statistic		7.25	9.47	1.97	8.29
P-value		<0.0001	<0.0001	0.0595	<0.0001

[z]Means within a column followed by the same letter were not significantly different (Tukey's HSD α = 0.05).
[y]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***), [NS] denotes non-significant

Figure 5: Clethra (*clethra alnifolia* 'Hummingbird') one year after application of branch-inducing treatments

Figure 6: Topflor-treated rhododendron (*Rhododendron* 'Roseum Elegans') in flower one year after treatment application.

Vita

Whitney M. Yeary was born in Tulsa, Oklahoma. She graduated from Frisco High School in Frisco, TX in 2006 and then went on to the University of Tennessee for higher education. She fancied herself a chemist, an anthropologist, and an accountant, but none of these career paths seemed to fit. Eventually, she wondered over the bridge to an unfamiliar part of campus where Dr. Robert Augé introduced her to the agriculture campus and the wonderful possibilities of plants. In 2009, while still working toward her degree, she took a job working at Oak Ridge National Laboratory where she became fascinated with plant-microbe interactions and in vitro propagation. In 2010, she received her Bachelor of Science in Plant Sciences with a concentration of plant production and a minor in business administration. In the fall of 2011, she married Robin Yeary (also acquired from the University of Tennessee). She is currently at the University of Tennessee where she is pursuing her master's degree in plant science. Upon finishing her degree, Whitney will join her husband at Sevier Blumen, their cut-flower business.

.