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## CUTTING PROPAGATION AND CONTAINER PRODUCTION OF RUDY HAAG BURNING BUSH [Euonymus alatus Rudy Haag]

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## ABSTRACT OF THESIS

### CUTTING PROPAGATION AND CONTAINER PRODUCTION OF RUDY HAAG BURNING BUSH [*Euonymus alatus* 'Rudy Haag']

Softwood cuttings of *Euonymus alatus* and the cultivar 'Rudy Haag', a nearly seedless cultivar, were taken in Kentucky on two cutting dates. *E. alatus* 'Rudy Haag' cuttings taken in mid-May, untreated or treated with 1000 - 3000 ppm IBA, produced many roots on a high percentage of cuttings. Post-rooting shoot growth was reduced for the species with cuttings taken in June. 'Rudy Haag' produced new growth on cuttings taken in May with no IBA treatment. Transplanting resulted in a decrease in new growth.

Liner plants of *E. alatus* 'Compactus' and 'Rudy Haag' were planted into 1 and 3 gallon containers of two different types, conventional black plastic and root training. A second experiment exposed plants to two levels of supplemental fertilizer in addition to slow release. A subsample was sprayed with Fascination<sup>®</sup> (BA + GA<sub>4+7</sub>) at 1500 ppm in late July. No difference was seen in above-ground growth due to container type or supplemental fertilizer. A change in root morphology is seen with root trainers. Three gallon containers produced a larger plant than one gallon containers. 'Rudy Haag' sprayed with Fascination<sup>®</sup> were greater in size and branch number than those not sprayed.

**KEYWORDS:** *Euonymus alatus* 'Rudy Haag', cutting propagation, growth regulator, root trainer containers, root morphology

Amy Lynn Poston

May 2, 2007

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THESIS

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The Graduate School  
University of Kentucky

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THESIS

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A thesis submitted in partial fulfillment of the  
requirements for the degree of Master of Science in the  
College of Agriculture  
at the University of Kentucky

By

Amy Lynn Poston

Lexington, Kentucky

Director: Dr. Robert L. Geneve, Professor of Horticulture

Lexington, Kentucky

2007

## ACKNOWLEDGMENTS

I would like to thank Dr. Robert Geneve for providing me with the opportunity and funding to conduct this project, as well as, insight and direction in research, coursework advising, and professional guidance. I would also like to thank my committee: Win Dunwell and Mark Williams for their advice, constructive ideas, and support during conferences. Special thanks to Rhonda VanDyke for her support with statistical analyses. I would like to offer sincere gratitude to Sharon Kester for her daily help in the lab, greenhouse, and field. Also, thank you to Mary Evelyn Kester for her help in the field; Shari Dutton for her suggestions in the greenhouse; and fellow graduate students Amy Fulcher and Cindy Finneseth for their constructive ideas about the project.

I would like to extend appreciation to Darrell Slone, Bonka Vaneva, Dr. Robert McNiel, and the crew at the UK Horticulture farm for their help with the setup and maintenance of the container production portion of this project. In addition, thank you to Dena Garvue at Bernhiem Arboretum and Research Forest, John Swintosky at Louisville Metro Parks, and John Cramner at Valent Biosciences for allowing cutting and seed collection and for donation of materials for the project.

I am very grateful to have such remarkable friends in my life, especially Joseph C. Lentz, Sara Kinslow, Holly Dunn-Pendleton, Sherry Wright and Jennifer Kennedy, for their patience and friendship throughout this process. Finally, I would like to thank my family for their unconditional support and encouragement throughout my college career as they taught me to pursue my goals and give my best effort.



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# CHAPTER 1

## Introduction

*Euonymus alatus*, notably the ‘Compactus’ cultivar, is a popular shrub grown and sold by the landscape industry. *E. alatus* is known for its brilliant red fall color and abundant fruit set and is often utilized in the landscape as an accent plant, hedge, or foundation planting. While very popular for its aesthetics and wide range of adaptability, *E. alatus* has become invasive in many parts of the Eastern United States as its seed are spread by birds into forested areas. This raises both ecological and economical issues for the landscape industry in producing and selling *E. alatus* to meet the demand of the consumer.

‘Rudy Haag’ is another cultivar of *E. alatus* that is nearly seedless, however, it still retains most other desirable characteristics as ‘Compactus’. This cultivar was selected by a nurseryman from Louisville, Kentucky named Rudy Haag. Because of its sparse fruit set, ‘Rudy Haag’ makes a good alternative to other *E. alatus* cultivars without the threat of becoming invasive. This cultivar can create an economical advantage as it can be marketed as an environmentally friendly plant.

The most common propagation method for *E. alatus* is by cuttings, therefore, one objective of this research is to test various methods for propagating ‘Rudy Haag’ by cuttings to determine limiting factors. These methods include a quantitative analysis of rooting hormone concentration and two cutting dates where ‘Rudy Haag’ will be compared to the species. In addition, observations of post-rooting growth will be addressed as ‘Rudy Haag’ grows at a slower rate compared to other *E. alatus* cultivars.

Due to its slow growth rate, a second objective for this research is to provide a mechanism for growing 'Rudy Haag' to the same salable size in a time comparable to 'Compactus', a standard cultivar in the industry. For this objective, several container-production techniques were tested such as varying container sizes, use of a root-promoting container design, two supplemental-N fertilizer regimens, and use of a cytokinin-gibberellin growth regulator. The thesis is organized into the following sections: 1) Introduction 2) Literature review 3) Cutting propagation and 4) Production in containers.

## CHAPTER 2

### Literature Review

#### Introduction to *Euonymus alatus*

*Euonymus alatus* (Thunb.) Sieb., winged spindle tree, winged euonymus or burning bush, is a member of Celastraceae. *Euonymus* includes about 170 species of deciduous or evergreen, small trees and shrubs (Rehder, 1940). Members of this genus are native to Central America, Europe, Asia, Madagascar, and Australia. *E. alatus* is native to areas from Northeastern Asia to Central China, Manchuria, East Siberia, Korea and Japan and was introduced into the United States in 1860 (Dirr, 1998; Rudolf, 1974). The bark of some *Euonymus* species possesses medicinal properties (Bailey, 1917).

The flowers of *E. alatus* are small perfect axillary cymes that are yellowish in color, borne in clusters (Bailey, 1917), and bloom from May to June (Young and Young, 1992). The fruit is usually a 1- to 5-celled capsule and ripens in late summer through fall. Each fruit cell contains a single seed enclosed in a fleshy, orange aril (Dirr, 1998). Abundant fruit crops can be expected annually with natural dispersal of seeds soon after the fruit ripens (Young and Young, 1992).

*E. alatus* can be grown in hardiness zones 4 through 8 as a flat-topped shrub with a mounded spreading habit. It has opposite simple elliptic leaves that are 1 to 3 inches in length and green to brown stems with corky wings. Leaf color is a medium green in summer and brilliant red in the fall (Bailey, 1917). The species has a mature size of 15 to 20 feet in height and width, however, size varies depending on cultivar. *E. alatus* has a slow growth rate in which one major growth flush is produced in spring prior to a halt in

any additional growth (Dirr, 1998). It has been suggested that *E. alatus* has deep bud rest and requires a cold treatment to induce growth. For example, 90 – 120 days at 40° F can induce bud break of terminal and lateral buds (Dirr, 1987).

*E. alatus* withstands heavy pruning and is commonly used for specimen plantings, borders, screening, massing, and hedging. It is easily transplanted and very adaptable to a wide range of soil types due in part to its fibrous root system that has a mass of roots at the soil surface (Bailey, 1917; Dirr, 1998). It is purported to be relatively trouble-free (Grounds Maintenance, 1990) with few serious disease and insect problems and less susceptibility to *Euonymus* scale, which is more of a problem on evergreen types of *Euonymus* (Dirr, 1998; Grounds Maintenance, 1990).

Of the limited scientific research available for germination with this species, some suggest that seeds be planted in the fall or stratified for a 3-month period (Dirr and Heuser, 1987) while others suggest that members of this genus require both warm and prechilling treatments (Young and Young, 1992).

There are several available cultivars of *E. alatus* including ‘Compactus’ and ‘Rudy Haag’. ‘Compactus’ has less pronounced corky wings, branches more densely, and is more compact than the species. It has an overall rounded appearance with a height and width of 10 feet. ‘Compactus’ has very abundant seed set with thousands of seeds on a single plant. Other cultivars of importance in the nursery industry are ‘Angelica’, ‘Apterus’, ‘Chicago Fire’, ‘Fire Ball’, ‘Microphyllus’, ‘Monstrous’, ‘Nordine Strain’, and ‘October Glory’ (Dirr, 1998).

‘Rudy Haag’ is named for the Kentucky nurseryman who selected the plant from a seedling population (Geneve et al., 2006). It is more compact than ‘Compactus’ with a



mature size of 4 to 5 feet in height and width and is nearly seedless. This cultivar has pinkish-rose to red fall color (Dirr, 1998). ‘Rudy Haag’ has a slower growth rate than ‘Compactus’ requiring more time for growers to produce. However, the nearly-seedless quality of ‘Rudy Haag’ allows it to be marketed as a good replacement to other invasive cultivars (Geneve et al., 2006).

### **Invasive Characteristics of *Euonymus alatus***

The National Management Plan, Executive Order 13112, defines an invasive species as one that is non-native or alien to the ecosystem under consideration, as well as, whose introduction causes or is likely to cause economic or environmental harm or harm to human health (Executive Order 13112, Appendix 1). Approximately 5,000 introduced ornamental woody and herbaceous plant species have escaped from cultivation and now exist in natural ecosystems of the United States (Morse et al., 1995). It has been estimated that 50% of all invasive plants were introduced for ornamental use, indicating that the green industry has been a major contributor to the spread of invasive plants (Li et al., 2004).

Over the past 30 years, *E. alatus* has escaped from cultivation in the Eastern United States where it is disseminated by birds into open woods, mature second-growth forests, and into pastures located near ornamental plantings. It threatens these ecosystems by replacing native shrubs and competing for resources (Randall and Marinelli, 1996). Similar to other invasive plants, characteristics such as abundant seed production, aggressive root systems, colonization of disturbed areas, and being habitat

generalists allow these plants to thrive, dominate, and become invasive (Neal and Clyde, 2004).

*E. alatus* has been placed on the Federal List of Invasive Garden Plants and classified as invasive in many states (Table 2.1). In Iowa (Widrechner and Iles, 2002), *E. alatus* was documented as escaping into natural ecosystems. In Illinois, *E. alatus* was the dominant species documented as invasive in two natural hill prairie ecosystems formed by glacial drift (Behnke and Ebinger, 1989). In Kentucky, *E. alatus* has been listed as a severe threat defined as “an exotic plant species which possess characteristics of invasive species and spreads easily into native plant communities and displace native vegetation” (SEEPPC, 2000). While there is no current or pending legislation, the invasive plant council recommends voluntary non-use of *E. alatus*. One preferred method of reducing the sale of these plants is to label these species as potentially invasive in the nursery (Reichard and White, 2001).

Table 2.1: States on the Federal List of Invasive Garden Plants where *E. alatus* is considered invasive

---

Connecticut
Delaware
Indiana
Kentucky
Massachusetts
Missouri
New Hampshire
New Jersey
Ohio
Pennsylvania
Rhode Island
Tennessee
Virginia
Wisconsin
West Virginia

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(Burnell, 1996)

## **Economic Costs of Invasive Plant Species**

Each year, the total economic loss associated with the control of ecologically damaging exotic species totals 138 billion dollars (Pimentel et al., 2000). Approximately 35 billion dollars of this total is attributed to damage and control of invasive plants, ranking second to habitat loss. One major contributor to the threat of invasive species is the 'green' or landscape industry, which will be impacted economically as many of these plants are banned through legislation (Li et al., 2004). Some ornamental plants sold commercially by nurseries are those that have marketable aesthetic qualities and cultural characteristics including ease of propagation, rapid growth, disease tolerance, drought tolerance, salinity tolerance or hardiness; all of which could help some of these plants become established in natural habitats (Bell et al., 2003). In a recent report, of 235 identified woody plant species with invasive characteristics, approximately 82% have been used for landscaping and are introduced for horticultural purposes (Reichard, 1997). Other than nurseries, the introduction of invasive plants can be linked to landscape architects and designers, gardening media, botanical gardens, arboreta, garden clubs, plant enthusiasts, and the seed trade industry (Bell et al., 2003; Meyer, 1987; Reichard and White, 2001).

When assessing the damage and making recommendations for control of invasive plants, there can be controversy when the species is in an early stage of invasion or of high economic value (Fox et al., 2003), the latter of which is the case with *E. alatus*. Recent legislation pertaining to *E. alatus* has been passed in states such as New Hampshire, Connecticut, and Massachusetts. The Invasive Species Committee of New Hampshire listed several plant species to be prohibited from collection, transportation,

sale, distribution, propagation, and transplanting, including all cultivars. The state of New Hampshire has placed a ban on *E. alatus* along with Japanese Barberry (*Berberis thunbergii* DC.) and Norway maple (*Acer platanoides* L.), scheduled to take effect January 1, 2007 (Neal, 2004). As an example of the economic importance to the industry, *E. alatus* accounts for approximately 2.2 million dollars in wholesale value and 4.5 million dollars in retail value annually in the state of Connecticut, which would cause significant economic loss if *E. alatus* were banned in that state (Li et al., 2004).

‘Rudy Haag’, as a nearly seedless cultivar, would make an ecologically friendly alternative to ‘Compactus’ for use in the nursery trade. Additional alternatives include native red chokeberry (*Aronia arbutifolia* L.), non-invasive Koreanspice viburnum (*Viburnum carlesii* Hemsl.), highbush blueberry (*Vaccinium corymbosum* L.), spicebush (*Lindera benzoin* L. Blume), and winterberry holly (*Ilex verticillata* L. Gray) (Geneve et al., 2006; Neal, 2004). With progressive research, the potential to create sterile cultivars through genetic modification of economically important ornamental plants that are invasive (Li et al., 2004).

### **Overview of Cutting Propagation**

Cutting propagation is the primary means of propagating most woody ornamental landscape shrubs (Whitcomb, 1978). There are several factors that can affect the rooting potential of stem cuttings including species and specific cultivar needs; the source, position, and type of cutting taken; juvenility and condition of stock plant; wounding or leaf removal; stock plant etiolation and girdling; cutting date; or is influenced by growing

conditions such as media, mist, bottom heat, use of hormones, fertilizer, and supplemental lighting (Dirr, 1986; Hartmann et al., 2002).

Ensuring that the parent plant used to supply cuttings is in good condition will facilitate more vigorous and healthy cuttings. When taking cuttings, shaded portions of the parent plant should be avoided because the cutting will have lower levels of carbohydrates present making them less likely to root well (Whitcomb, 1978). There are many different types of cuttings including leaf cuttings, leaf-bud cuttings, root cuttings, and hardwood, semi-hardwood, and softwood stem cuttings (Hartmann et al., 2002). Softwood stem cuttings can be taken from new growth following a flush on the parent plant usually in the summer; however, this can occur throughout the growing season. Semi-hardwood cuttings are from partially matured wood also available throughout the season and dependent upon species (Blazich, 1987).

There is no one rooting medium that is ideal for all species, however, several combinations are possible using peat, perlite, ground pine bark, vermiculite, sand, and other media (Whitcomb, 1978). Six-pack containers or other propagation containers are recommended because they leave the cutting undisturbed, use less rooting medium, hasten establishment, and reduce crowding, stunting, disease problems, and damage to the root systems (Whitcomb, 1978).

Intermittent mist is often used on cuttings because it reduces the temperature of the leaves, lowers respiration, and increases relative humidity around the leaf surface (Langhans, 1955). The water left behind to accumulate on the leaf surface will contain any salts or debris from the water supply, which can have adverse effects on rooting (Chong and Daigneault, 1987). Because a cutting generally does not initiate roots for a

few weeks, the cutting is unable to take up and absorb nutrients. However, a controlled release fertilizer can be incorporated into the rooting medium to increase the growth after rooting. Osmocote 18-6-12 (6 to 9 month release) is recommended at a rate of 8 to 12 lbs/cu yd (Whitcomb, 1978).

Rooting hormones can improve rooting in some species but may have little effect on others. Generally, root quality in terms of number of roots and length are improved by treatment with hormones (Dirr, 1986). Indole-3-butyric acid (IBA) is the most widely used root promoting chemical in the nursery trade, along with 1-naphthaleneacetic acid (NAA), because it is nontoxic over a wide range of concentrations (Ruppert, 1974). These two auxins stimulate the formation of adventitious roots on cuttings of many species (Hartmann et al., 2002).

Rooting hormones can be applied as talc or as a liquid quick-dip solution where the hormone is dissolved in a solvent and water (Ruppert, 1974; Whitcomb, 1978). While different species require different amounts, the concentration of IBA should be considered when propagating by softwood cuttings because too little will have no effect, whereas, too much can cause injury or death to the cutting. Typically, a concentration of 2,000 to 4,000 ppm will result in good rooting for most shrubs and evergreens (Ruppert, 1974). Furthermore, some species will form roots over a wide range of IBA concentrations (Dirr, 1981; Ruppert, 1974). A study by Chong and Daigneault (1987) showed greater rooting with the use of IBA on cuttings from 20 different woody ornamentals, including *E. alatus*. Rooting of *E. alatus* was between 99 and 100 % for IBA concentrations of 2,500 to 10,000 ppm; compared to approximately 25% without

IBA. In addition, increasing levels of IBA caused adverse effects such as basal injury in all species, especially at concentrations above 20,000 ppm.

The time of year when cuttings are taken is an important factor influencing rooting of woody plants from stem cuttings (Blazich, 1987). When determining the seasonal timing of taking cuttings for a particular species, an attempt should be made to correlate physiological condition and maturity of the wood with stem rootability. Events such as flower induction in Rhododendron and bud dormancy in Douglas Fir can affect rootability more so than the age of the plant tissue (Roberts, 1969).

The importance of timeliness in cutting propagation is evident in a study with *Cotinus coggygia* 'Royal Purple' which showed that there was a seasonal variation in rooting response (Kelley and Foret, 1977). Each subsequent cutting date after June 11 showed a decrease in rooting response. The best rooting for this species was achieved with early cuttings. All cuttings responded to IBA with better root initiation, where those taken early showed the least response while mature cuttings showed the greatest response to auxin. Therefore, in some woody species, taking cuttings at the optimal time in combination with IBA can provide the greatest rooting potential.

### **Cutting Propagation of *Euonymus alatus***

There are many published reports on various cutting methods for *E. alatus* and, in general, it is considered to be an easy-to-root species (Chong and Daigneault, 1987) that requires no added hormone to achieve fast and abundant rooting (Hartmann et al., 2002). The use of mist on *E. alatus* resulted in delayed dormancy, delayed leaf abscission,

inhibited anthocyanin production, and increased production of root promoting substances (Tukey and Lee, 1971).

There is seasonal variation in rooting ability of *E. alatus* 'Compactus' where generally cuttings taken in spring and summer are easily rooted (Lee and Tukey, 1971). Cuttings taken from fully dormant plants in September also showed root initiation that was rapid and profuse, especially under mist (Tukey and Lee, 1971). One study suggests that *E. alatus* cuttings should be taken from June through the first half of August, will root in 5 to 7 weeks, and require 8000 ppm IBA (Whitcomb, 1978). Another reference suggests cuttings taken during the months of June, July, and August are preferred (Dirr and Heuser, 1987). Cuttings should be 4 to 6 inches in length, can be treated with 1000 to 3000 ppm IBA, and rooted under intermittent mist in a peat-perlite media. This recommendation produced over 90% rooting in 8 weeks. This reference suggests storing cuttings for approximately 100 days in a cooler to induce bud growth of *E. alatus*.

Lee and Tukey (1971) used *E. alatus* to test intermittent mist, exogenous hormones IBA and rutin (a phenolic compound or rooting cofactor), and seasonal variation in cutting time on rooting. This study concluded that mist improved rooting in all cases. IBA and rutin produced a larger and more compact root system in cuttings taken later in the season, however no significant increase was seen on early cuttings. This suggests that cuttings taken earlier in the summer may have sufficient naturally-occurring root-inducing substances and that the use of hormones will benefit cuttings taken later in the season (Lee and Tukey, 1971), whereas, hardwood cuttings of *E. alatus* are difficult to root (Dirr and Heuser, 1987; Lee and Tukey, 1971).



## **Overview of Container Production**

In the 1960's and 70's there was an increase in using containers to produce ornamental plants. Soilless mix soon became a complement to production in containers as it provided a greater amount of pore space (Whitcomb, 1982). Pine bark, either hardwood or softwood, is a common media used for container production. It has advantages such as being a renewable resource that can be processed to provide a standardized product, is currently available at lower cost to the grower than imported peat moss, and produces plant growth comparable to other organic soil amendments (Pokorny, 1979).

The growth of plants in containers is influenced by physical and chemical characteristics of the container environment including container volume, shape, and fertility (Keever and Cobb, 1987).

Root growth in containers is different from that in field grown plants due to the constrictive container wall, limited growth medium, and high water holding capacity of the media (Keever et al., 1985). In containers, roots generally take the path of moving out toward the sidewall of the container then downward following the contour of the container. Because of the restriction on space, the roots can make a half or full circle inside the container and circle around up to 5 times (Whitcomb, 1982). Container-grown plants can have kinked roots that have been deflected by the container wall, leading to long-term growth problems in the landscape (Nichols and Alm, 1983).

## **Container Size and Shape**

Selecting the correct container size and shape can improve both shoot and root growth when container shape is similar to the natural shape of root distribution; whereas, mismatching the container can cause growth retardation (Biran and Eliassaf, 1980).

Many woody ornamentals are grown in containers with a diameter to depth ratio of 1:1 (Keever et al., 1985). *E. alatus* 'Compactus' container production has been successful when close attention is paid to container size and fertilization. The use of 4-quart containers, slightly larger than one gallon, proved to be too small to maintain optimum growth for the two-year production period (Fuller, 1979).

A study of fertility and container size found an increase in top growth as the container depth, width, and volume increased for *Euonymus japonica* 'Microphylla', a member of the same genus as *E. alatus* and also with an extensive root system. The effect of container depth proved to be most influential on increasing top growth. In terms of root growth, the density of roots decreased as the container depth and width increased (Keever and Cobb, 1987). Selecting the optimal container size has helped in the reduction of costs in producing tree seedlings on a large scale (Rathore et al., 2004).

## **Root Pruning and Training Containers**

Special containers and other methods have been developed to prevent root circling including air-root-pruning containers, root training containers, and fabric bags, as well as, the use of copper applied to the inside container walls. For example, cupric hydroxide ( $\text{Cu}(\text{OH})_2$ ) (Ruter, 1994) and copper carbonate ( $\text{CuCO}_3$ ) (Arnold and Young,

1991) have been used as coatings on the insides of the container to control growth of root systems, and prevent circling.

Air-root-pruning containers were developed to address the many problems associated with root development in conventional black plastic containers. The first account of air-root pruning occurred with tree seedlings grown in square, bottomless containers placed on top of a raised wire bench. The air-root-pruning, which occurs because of necrosis and death of the root tip as it makes contact with the air, caused a decrease in root elongation and wrapping inside the container and an increase in lateral branch root development (Davis and Whitcomb, 1975).

There are many container designs for air-root-pruning containers, also referred to as root trainers and porous-walled containers (Appleton, 1993). While the response may vary among different tree and shrub species, generally root development and growth inside these specialized containers is such that roots are more evenly distributed throughout the container, have increased branch-root development where newly formed roots will elongate rapidly, and show an increase in white root tips. The increase in root tips at the time of transplanting allows for better and faster establishment. There is an increase in absorption of nutrients and subsequently an increase in plant growth due to the greater number of roots and root tips (Whitcomb, 1982). Khedkar and Subramanian (1997) found that the development of *Tectona grandis L* (Teak) roots increased in root-training containers, especially lateral roots. The plants from root-training containers were sturdier, healthier and had an increase in collar girth. Similar studies with porous containers have shown that roots stop growing when reaching the container wall (Privett and Hummel, 1992) and increase in new roots following transplanting (Marler and Willis,

1996). The production of many other species has been improved with the use of air-root-pruning containers, including *Casaurina equisetifolia* (Ironwood), by increasing in height and diameter of seedlings and reducing root circling (Rathore et al., 2004; Srivastava et al., 1998). Overall, these specialized containers can potentially lead to better survival during establishment and an increase in productivity (Khedkar and Subramanian, 1997).

In contrast, some studies show imprecise effects on root mass, trunk diameter, or height with air-root-pruning containers. For example, there was a decrease in the length of *Acer rubrum* L. (Red Maple) roots deflected by the container wall and a reduction in interior root mass with less roots on the inside of the root ball (Marshall and Gilman, 1998). *Swietenra mahagoni* L. (Mahogany) grown in these containers had lower root mass and a higher shoot to root ratio than plants grown in conventional plastic containers (Fitzpatrick et al., 1994).

Rootmaker containers (Rootmaker<sup>®</sup> Products Co., Huntsville, AL), a brand of root training containers, were first designed by C.E. Whitcomb in 1981 by cutting offset vertical slits into a conventional plastic container in an attempt to prevent root circling and stimulate root branching. A study using these modified containers showed an increase in top and root weight of 63% and 38% respectively, a 158% increase in the number of branches per plant, and a 187% increase in the presence of 2-inch long roots following transplanting (Whitcomb, 1982). The current version of the Rootmaker container works because of raised ridges or insertion of sharp angles, causing a disruption of the smooth inner surface of the container wall with which the root interacts (Whitcomb and Williams, 1985). These containers can be easily used in the nursery as they can be

filled using existing commercial pot fillers, can be easily stacked when not in use (Whitcomb, 1982).

### **Plant Nutrition in Containers**

The absorption of nutrients by plants, and subsequent plant growth, is related to an adequate supply of nutrients in the soil solution. There are many factors to consider when determining the use of fertilizers on container grown plants such as type of fertilizer, concentration of components, rate and method of application, and timing of application (Wright, 1986).

In a recent survey of best management practices, 100% of nurseries surveyed use controlled-release fertilizers as their primary source of plant nutrients (Fain et al., 2000). Controlled release, or slow-release, fertilizers are popular because of the availability of nutrients over the entire growing season, a reduction in costs including capital and labor, and a reduction in the amount of nutrients lost through leaching and runoff (Sharma, 1979). There are many different controlled-release fertilizers available on the market made for a range of plants and with varying release rates. It is important that controlled-release fertilizer provide sufficient nutrients initially, followed by a uniform supply that is synchronous with the nutrient requirements for a particular plant species (Sharma, 1979). The fertilizer within Osmocote (Sierra Chemical Co., Milpitas, CA) is encapsulated to retard the solubility of the fertilizer so it is not immediately available (Sharma, 1979). Following release of the nutrients, the capsule is left behind even though the fertilizer within has been completely exhausted (Matkin, 1970).

Traditional methods of fertilizing container plants such as the use of uncoated organic materials, or ureaforms, has proven to be insufficient in the second year of container production of *E. alatus* (Kelley, 1962). Ureaforms caused an excess of nitrates to be accumulated in the container shortly after application and a deficient supply of nutrients for the remainder of the growing season. The use of a controlled-release fertilizer would not incur these negative effects. Two types of controlled-release fertilizers, Nutricote (16:4.4:8.3) and Osmocote 8-9 month (18:4.8:4.3), resulted in an increase in shoot growth with the latter, Osmocote, on more than half of the 16 species tested for this study; primarily due to the higher nitrogen concentration, as well as, the difference in release rate (Worrall et al., 1987).

Controlled release fertilizer in combination with a supplement of liquid fertilizer has resulted in some success in producing additional growth flushes in ornamental crops that may produce only a single flush of growth per season (Cobb and Keever, 1984; Musselwhite et al., 2004; Yeager and Wright, 1981). While this was unsuccessful for *Buxus spp.* L. (Boxwood) (Cobb and Keever, 1984), additional flushes were produced successfully with *Ilex crenata* Thunb. 'Helleri' (Japanese Holly) by increasing shoot weight. This is induced by the higher rate of nitrate applied, whereas a low level of nitrate will favor root growth (Yeager and Wright, 1981). When producing *E. alatus* 'Compactus' in containers, a single application of Osmocote (18-6-12) in the spring was not enough to sustain the growth of the plant through the entire growing season, therefore a second application at half the rate or the use of a liquid supplement has been suggested (Fuller, 1979).

Nutrient availability in the substrate can be determined using a pour-through method procedure (Wright, 1986). This procedure is a quick way to determine the nutrient concentration in the container and does not require specialized material or handling of substrate and does not pose a threat to rupturing slow-release fertilizer capsules that can lead to an error in nutrient readings. A pour-through nutrient extraction is performed by adding a sufficient amount of distilled water to the container in order to obtain 50 mL of leachate in a collection vessel. This leachate can then be tested for pH, soluble salts or electrical conductivity (EC), or sent to a soil lab for nutrient analysis (Yeager et al., 1983). With this method, soluble salts can be monitored on a regular basis throughout the growing season (Wright, 1986).

### **Use of Plant Growth Regulators**

Growth regulators, particularly cytokinins and gibberellins, have many applications for both foliage and ornamental plants to regulate or stimulate growth. Growth regulators have been primarily used to reduce plant height, increase branching and to produce a second growth flush. They have been successfully used to increase the number of axillary buds, induce lateral budbreak, promote axillary shoot formation, and delay leaf chlorosis of cut flowers (Grzesik, 1989; Henry, 1985; Leonard and Nell, 2004; Meane and Debergh, 1982; Mulgrew and Williams, 1985; Wilson and Nell, 1983). Growth regulators can be applied using many different methods such as foliar sprays, paste applications, soil drenches, or absorption into cut stem through floral foams or dips (Carpenter and Rodriguez, 1971; Grzesik, 1989; Leonard and Nell, 2004).

The success of using growth regulators to accomplish the aforementioned applications varies by species or cultivar, type of growth hormone used, hormone concentration, application method, seasonal timing, environmental conditions, treatment duration, plant age, and bud condition (Grzesik, 1989; Meane and Debergh, 1982; Ohkawa, 1979). Thus, it is difficult to predict whether the use of growth regulators will be necessary or beneficial because there can be differences in response by different cultivars of the same species (Knavel, 1971), which has been seen in *Rosa* L. (Ohkawa, 1979).

In order to reduce plant height, Paclobutrazol is a growth regulator commonly used on bedding plants and woody ornamentals. This growth regulator, which acts as a gibberellin inhibitor, resulted in decreased growth, earlier and increased flower production, and darker foliage when applied to *Hibiscus rosa-sinensis* L. and *Pelargonium hortorum* Bailey (pro sp.) (Andrasek, 1989). Paclobutrazol has also been used to successfully reduce vegetative growth and increase flower-bud production in *Eucalyptus* L'Hér. (Griffin et al., 1993).

To increase branching on plants, pinching is an effective method used to induce lateral shoot growth as it physically removes the apical meristem thus reducing apical dominance exerted by the apical buds (Berghage et al., 1989). Growth hormones can be used as an alternative to manual pinching to increase branch number. Cytokinins promote bud development and reduce apical dominance (Sachs and Thimann, 1967). Cytokinins applied exogenously have proven to promote lateral bud growth and branching in a number of woody plants (Carpenter and Rodriguez, 1971; Carpenter and Carlson, 1971; Mulgrew and Williams, 1985; Parups, 1971; Williams and Billingsley,



1970) and foliage plants (Carpenter and Carlson, 1971; Henry, 1985; Jackson and Lingle, 1971; Wilson and Nell, 1983).

As a means of producing a second flush of growth, growth regulators applied as foliar sprays have proven beneficial for increasing development of shoots or renewal canes in rose plants (Carpenter and Rodriguez, 1971) and increasing lateral shoot elongation in *Verbena* L. (Svenson, 1991). However, the waxy coating on the leaves of some woody ornamentals such as *Camellia* L. and *Rhododendron* L. prevent the absorption of growth regulators applied as sprays and show no significant response (Richards and Wilkinson, 1983).

The optimal time during the season to apply growth hormones to induce bud break varies among species. Application to fully dormant buds possibly stimulates cell activity and overcomes the effects of natural inhibitors (Williams and Billingsley, 1970). However, success in increasing bud development has also been accomplished when growth regulators were sprayed at the time of bud break (Mulgrew and Williams, 1985).

Cytokinins can be used in combination with gibberellins to promote branching, as well as, produce a second growth flush. Williams and Billingsley (1970) tested gibberellin ( $GA_{4+7}$ ) in combination with cytokinin, benzyladenine (BA), and the synthetic cytokinin benzylaminopurine (BAP) as mechanisms for increasing the number of primary branches in *Malus* P. Mill. The two growth hormones in combination caused an increase in bud break and a two-fold increase in the total growth of primary branches. Application of BA or BAP alone or in combination with GA caused an increase in the number of buds breaking dormancy, however, GA used alone had no effect. Therefore, increase in bud-break is due to cytokinin and shoot elongation is attributed to the gibberellins (1970). GA

has enhanced stem elongation in many other nursery crops when used in combination with pinching agents, such as Atrinal (Grzesik, 1989). Both cytokinin and gibberellin have been shown to increase flowering following application or in the subsequent growing season (Carpenter and Rodriguez, 1971; Jackson and Lingle, 1971; Knavel, 1971; Richards and Wilkinson, 1983).

## CHAPTER 3

### Cutting Date and Hormone Use in Cutting Propagation of *Euonymus alatus* ‘Rudy Haag’

#### Introduction

*Euonymus alatus* (Thunb.) Sieb. is a popular woody landscape shrub utilized by the landscape industry as an accent plant, hedge, or foundation planting. While very popular, this shrub has escaped cultivation throughout much of the Eastern United States due to its abundant seed production that is dispersed by birds (Randall and Marinelli, 1996). As a habitat generalist, *E. alatus* competes in natural ecosystems due to its aggressive root systems and colonization of disturbed areas (Neal and Clyde, 2004). In Kentucky, *E. alatus* has been listed as a severe threat, which is defined as “an exotic plant species, which possess characteristics of invasive species, spreads easily into native plant communities, and displaces native vegetation”. Voluntary non-use of this plant is recommended in Kentucky (SEEPPC, 2000). ‘Rudy Haag’ is an *E. alatus* cultivar that is nearly seedless and can be utilized as an ecologically friendly alternative to other cultivars such as ‘Compactus’ (Geneve et al., 2006). However, while there is little published research on nursery production or propagation of ‘Rudy Haag’, it is purported to be slower-growing than other *E. alatus* cultivars, requiring additional production time.

*E. alatus* is commercially propagated by cuttings using standard methods employed for most landscape shrubs (Whitcomb, 1978). There are several factors that can affect the rooting potential of stem cuttings; however, the timing, or date, of cutting collection can be the most important factor in rooting response of many plants due to seasonal variation (Blazich, 1987; Kelley and Foret, 1977). *E. alatus* can be successfully

propagated from cuttings any time the plants are in leaf (Dirr and Heuser, 1987) and are easy to root as softwood cuttings in spring and summer (Lee and Tukey, 1971).

Hardwood cuttings of *E. alatus* are difficult to root or root with no success (Dirr and Heuser 1987; Lee and Tukey, 1971).

Auxins, such as indole-3-butyric acid (IBA), are commonly used to enhance rooting in stem cuttings. While *E. alatus* does not require the use of added hormones to achieve quick and abundant rooting (Hartmann, et al., 2002), their use may enhance rooting of cuttings taken later in the season, as is seen with other woody landscape shrubs (Kelley and Foret, 1977). However, use of auxin at a high concentration on cuttings can have a residual effect by suppressing bud-break or growth flushes post-rooting (Hartmann et al., 2002). The mode of action is thought to involve auxin-stimulated ethylene production (Sun and Bassuk, 1993).

The objectives of this study were to determine the impact of collection date and auxin concentration on 'Rudy Haag' and compare it with the species to confirm that cutting propagation will not be a limiting factor for propagation and post-rooting shoot growth.

## **Materials and Methods**

Softwood stem cuttings of *E. alatus* and *E. alatus* 'Rudy Haag' were taken from the University of Kentucky campus and Bernheim Arboretum and Research Forest, respectively, on two cutting dates of May 15 and June 28, 2005. Four to six inch stem tips were cut and kept in a cooler until planting. Cuttings were re-cut, treated with IBA at 0, 1000, 3000, and 6000 ppm as a quick dip solution, and stuck into 6-cell packs (3 ½" X

5 ¼” X 2 5/16 ”; Hummert International, Earth City, MO) with a 1:3 (v:v) ratio of perlite to 280-MetroMix (Sun Gro, Bellevue, WA). Flats of cuttings were placed in an intermittent mist-bed misting at 10 seconds every 16 minutes with bottom-heat (24°C). Greenhouse conditions for the first set of cuttings were a 24/20°C day-night temperature and ambient light, whereas, the second set of cuttings experienced a 24/21°C day-night temperature and ambient light. After 30 days, the flats were removed from the mist bed and roots were evaluated. Following evaluation, half of the cuttings were returned to the six-pack containers and half were potted-up into a 4” standard plastic container. Plants were given a weekly application of a 200 ppm N liquid fertilizer solution (Peter’s 20-10-20 Peat-lite Special, Scotts Company, Marysville, OH). All cuttings were moved into over-wintering structures in early November and percentage of cuttings with new shoot growth was recorded.

The experiment was designed as a nested (2 cultivars x 2 cutting dates x 4 levels of IBA) factorial with 72 cuttings per treatment and six cuttings per sub-sample. For each cutting date, 6-packs of the cuttings were placed in a completely randomized manner. Percentage rooting, number of roots, and roots per rooted cutting were evaluated 30 days after sticking using SAS (The SAS Institute, Cary, NC). An arcsin transformation was necessary to do statistical analysis on the percentage rooting and mean separations were obtained using Tukey’s HSD.

## **Results**

Cultivar, cutting date, and IBA treatment showed significant main effects for rooting percentage, roots per cutting, and roots per rooted cutting (Table 3.1). Averaged

over cutting date and IBA treatments, 'Rudy Haag' rooted at a higher percentage (79.9%), had a higher number of roots per cutting (13.0) and roots per rooted cutting (14.6) compared to the species at 61.6% rooting, 6.4 roots per cutting, and 8.0 roots per rooted cutting.

Cutting date had the most impact on all rooting responses (Table 3.1). Both the species and 'Rudy Haag' had reduced rooting in June compared to May, but the species showed a greater reduction. Averaged over IBA treatments, the species showed a 60.8% reduction in rooting percentage compared to only 30.7% for 'Rudy Haag'. There was a corresponding loss in the magnitude of rooting as evidenced by an overall reduction in roots per cutting and roots per rooted cutting. Adjusted for rooting percentage (roots per rooted cutting), the species showed a greater reduction in rooting capacity (72.8%) compared to a 57.1% reduction in 'Rudy Haag'.

Overall, there was a linear effect of IBA on rooting percentage and a quadratic effect on roots per cutting and roots per rooted cutting (Table 3.1). There was very little effect of IBA concentration on rooting percentage in 'Rudy Haag' regardless of the time cuttings were taken. However, there was a significant increase in rooting percentage in the species as concentration increased for cuttings taken in June. Treating cuttings with IBA showed increased roots per cutting and roots per rooted cutting compared to untreated cuttings in the species and 'Rudy Haag'.

'Rudy Haag' cuttings showed a reduced capacity to flush new shoot growth following rooting compared to the species (Table 3.2). For the species, cuttings had greater than a 95% capacity for new shoot growth when taken in May and an average capacity of 72.5% in cuttings taken in June. There was no apparent impact of IBA

treatment on the capacity to flush new growth in the species regardless of the time cuttings were taken. However, compared to cuttings left in the original 6-pack containers, cuttings moved to 4-inch containers following rooting showed a reduced capacity (3.7% for May and 41.0% for June) for new shoot growth. Overall, 'Rudy Haag' flushed new growth in only 6 percent of cuttings. The only significant new growth occurred in May cuttings that were not treated with IBA and left in the original 6-pack rooting containers, where 42% of cuttings showed new growth.

## **Discussion**

In general, *E. alatus* 'Rudy Haag' was easier to root from cuttings compared to the species (Table 3.1). The time cuttings were taken was the most significant factor in obtaining a high percentage of cuttings producing a high number of adventitious roots. Another cultivar, *E. alatus* 'Compactus', has been reported to have seasonal variation in rooting ability (Lee and Tukey, 1971). Cuttings of *E. alatus* 'Compactus' taken in June rooted at approximately 90 to 100%, with and without auxin at 2000 ppm, whereas cuttings taken in August rooted at approximately 45 and 70%, respectively. This seasonal variation was also seen in this study with cuttings of 'Rudy Haag' where those taken in mid-May rooted at greater than 92%, with or without the addition of IBA, and those taken in late June had less than 73% rooting (Table 3.1). This suggests that application of IBA was not necessary when taking cuttings of *E. alatus* 'Rudy Haag' early in the growing season, similar to reports for *E. alatus* 'Compactus' (Lee and Tukey, 1971). This response to cutting date was more exaggerated with the species. 'Rudy Haag' had more roots per cutting when taken early than did the species.

Seasonality, or the time that cuttings are taken, can have a significant effect on rooting of many woody ornamentals due to the physiological condition of the stock plants (Roberts, 1969). For example, cuttings of *Cotinus coggygia* Scop. ‘Royal Purple’ rooted best when taken in early to mid-summer during periods of active shoot growth (Cameron et al., 2005; Kelley and Foret, 1977). The decrease in rooting between cutting dates in *E. alatus* could be due to seasonal effects on the stock plants, but the additional stress on the cuttings taken in June compared to May because of increased in vapor-pressure deficit can not be ruled out.

The use of IBA when propagating *E. alatus* ‘Rudy Haag’ responded in a similar manner to the species where the effect of IBA was different for each of the two cutting dates. Cuttings taken in late June did not root well without auxins and responded more to IBA than did May cuttings. There is likely a sufficient amount of endogenous auxins in cuttings taken from stock plants in May as they rooted at high percentages without addition of IBA. Similarly, *Cotinus coggygia* Scop. ‘Royal Purple’ responded more to IBA in July compared with those taken in June, of which IBA was not necessary (Kelley and Foret, 1977).

Results for production of new shoots following rooting (Table 3.2) suggest that the cutting date and auxin concentration have effects on *E. alatus* ‘Rudy Haag’ as is indicated by a low percentage of plants producing new growth. Only those cuttings of ‘Rudy Haag’ taken in May that were not treated with auxin produced any significant shoot growth following rooting. This is similar to *Ligustrum ovalifolium* Hassk. with reduced rooting and decreased subsequent shoot growth in cuttings taken in September compared to those taken in June (Pridham, 1942). This may be due to increased bud



dormancy as daylength shortens. Shoot growth, following rooting, in late summer can be induced by extending the day length or addition of fertilizer (Hartmann et al., 2002). It is important for cuttings to produce a flush of growth prior to over-wintering in order to increase carbohydrate reserves and increase survivability. Extending daylength has increased shoot growth in other woody plants such as *Cornus florida* L. var. *rubra* and *Acer palmatum* Thunb. 'Bloodgood' (Goodman and Stimart, 1987).

There was an IBA effect on shoot growth for 'Rudy Haag' taken in May. The absence of shoot growth with the use of IBA suggests that there could be a carry-over effect of auxin (Hartmann et al., 2002). Auxin applied to cuttings may have a residual effect by suppressing bud-break or growth flushes post-rooting, as is seen in cuttings of *Rosa* L. species. This phenomenon has been documented with rose stem cuttings due to increased synthesis of ethylene in the upper part of the cuttings (Sun and Bassuk, 1993).

The container effect on shoot growth could be due to source-sink relationships. Transplanting rooted cuttings into 4 inch containers caused a decrease in new shoot growth post-rooting, especially for the cuttings taken in June. Similar results have been documented with *Prunus nersica* 'Spring Crest' (Ran et al., 1992) where an increase in container volume caused an increase in root weight and subsequently increasing N-uptake. The allocation of N to the top portions of the plant decreased with increasing container volume resulting in increased root systems. Therefore, the decrease in shoot growth for *E. alatus* cuttings transplanted into 4 inch pots may be due to this increase in container volume. Another possibility for the decrease in shoot growth after transplanting may be due to shock, which can result in reduced growth (Pridham, 1942).

'Rudy Haag' cuttings may have reduced capacity for new growth compared to the species because it is genetically a more dwarf cultivar. Overall, this is a disadvantage for nursery production because the first-year liner will be smaller than other cultivars that would have the capacity to flush new growth.

In general, cuttings of *E. alatus* 'Rudy Haag' taken in May and grown in six pack containers resulted in a high percentage of rooting and the greatest capacity to flush new growth, indicating that there are no substantial problems with propagation of this cultivar for nursery production. Cost in labor is also reduced for cuttings taken in May because the use of IBA is not necessary to induce rooting. However, if cutting date is in question, 1000 to 3000 ppm IBA can be used to increase root development without the threat of injury to the cutting due to toxicity, which is similar in concentration as is recommended for other woody ornamentals (Ruppert, 1974).

Table 3.1: Use of IBA during cutting propagation for cuttings taken in mid-May and late-June of *E. alatus* and *E. alatus* ‘Rudy Haag’ and the effects on rooting and root development.

Cultivar	Timing	[IBA] (ppm)	Rooted cuttings (%)	Roots per cutting	Roots per rooted cutting
<i>E. alatus</i>	May 15	0	85.2	5.7	6.7
		1000	87.3	9.1	10.3
		3000	92.5	17.2	18.2
		6000	88.8	13.2	14.8
	June 27	0	10.0	0.2	1.7
		1000	15.6	0.4	2.4
		3000	41.8	1.5	3.5
		6000	71.3	4.2	6.0
<i>E. alatus</i> ‘Rudy Haag’	May 15	0	92.5	12.7	13.4
		1000	95.8	19.3	19.3
		3000	95.8	23.7	23.7
		6000	93.5	24.6	25.7
	June 27	0	71.0	3.9	5.6
		1000	72.6	7.3	10.3
		3000	47.3	2.8	5.6
		6000	70.6	9.4	13.5

ANOVA	Rooted cuttings	Roots per cutting	Roots per rooted cutting
Cultivar	35.71 ** Z	48.84 **	45.67 **
Timing	183.49 **	543.51 **	26.06 **
IBA			
linear	15.11 **	95.80 **	35.17 **
quadratic	0.09 NS	14.06 **	13.73 **
Cultivar x timing	5.91 *	19.44 **	0.84 NS
Cultivar x IBA			
Linear	17.21 **	1.03 NS	1.37 NS
quadratic	1.33 NS	2.73 NS	2.46 NS
Timing x IBA			
linear	8.08 **	14.91 **	28.45 **
quadratic	3.38 NS	44.23 **	23.45 **
Cultivar x timing x IBA	11.04 **	1.31 NS	0.40 NS

Z NS, \*, \*\*, indicates non significant, significant at  $p \leq 0.05$ , and  $p \leq 0.01$ , respectively.

Table 3.2: New growth post-rooting of *E. alatus* and *E. alatus* 'Rudy Haag'.

		Cuttings producing new growth (%)							
		<i>E. alatus</i>				<i>E. alatus</i> 'Rudy Haag'			
		Cutting date							
		May 15		June 28		May 15		June 28	
IBA (ppm)	Container size								
	6-pack	4" pot	6-pack	4" pot	6-pack	4" pot	6-pack	4" pot	
0	100	100	82	40	42	0	0	0	5
1000	100	95	96	55	0	0	4	0	0
3000	100	95	91	55	0	0	0	0	0
6000	100	95	96	65	9	0	0	0	0

## CHAPTER 4

### Use of a Cytokinin-gibberellin Growth Regulator, Supplemental-N Fertilizer, and Varying Container Sizes and Types to Increase First-season Growth of *Euonymus alatus* ‘Rudy Haag’

#### Introduction

*Euonymus alatus* (Thunb.) Sieb. and its cultivars, especially ‘Compactus’, are popular shrubs grown and sold by the landscape industry. Despite their popularity, these shrubs have been cited as escaping cultivation throughout much of the Eastern United States due to their abundant seed production, which is dispersed by birds (Randall and Marinelli, 1996), and aggressive root systems allowing these plants to invade natural ecosystems and colonize disturbed areas (Neal and Clyde, 2004). ‘Rudy Haag’ is a nearly seedless cultivar that could be utilized as an ecologically friendly alternative to other cultivars such as ‘Compactus’.

*E. alatus* and its cultivars are considered slow growing during nursery production (Sandrock et al., 2005). They typically produce one major growth flush in spring prior to a halt in any additional growth (Dirr, 1998). This is possibly due to deep bud rest that may require a cold treatment to induce breaking of terminal and lateral buds (Dirr, 1987). ‘Rudy Haag’ has an even slower growth rate compared to other *E. alatus* cultivars. It has been purported that ‘Rudy Haag’ has fewer branches and less extensive growth because it is more dwarf, therefore, requiring more time in the nursery to produce a plant of salable size. The additional time required to produce ‘Rudy Haag’ has slowed its use by the nursery industry as an alternative to other *E. alatus* cultivars.

Several production practices have been shown to accelerate plant production in containers including the use of optimal container size, use of root training containers, fertility, and use of growth regulators (Whitcomb, 1986). An increase in pot size can increase plant growth due to the additional volume of growth medium (Keever et al., 1985). In addition to optimizing container size, the use of air-root pruning containers can help to induce additional growth by developing a more fibrous root system and increasing lateral roots (Khedkar and Subramanian, 1997). Deflected roots, which are kinked or grow along the sides of the container wall, are common among plants grown in standard plastic containers (Nichols and Alm, 1983). Rootmaker containers (Rootmaker<sup>®</sup> Products Co., Huntsville, AL), a brand of root training containers, first designed by C.E. Whitcomb in 1981, have raised ridges or insertion of sharp angles, causing a disruption of the smooth inner surface of the container wall with which the root interacts (Whitcomb and Williams, 1985). These and other air-root pruning and root training containers were designed to prevent root circling and stimulate root branching causing an increase in shoot and root weights and in number of branches per plant. This occurs as the root is pruned when exposed to an opening or slit in the container sidewall (Whitcomb, 1982). Root training containers have proven beneficial for species such as *Casaurina equisetifolia*, by reducing root circling and causing an increase in height and diameter of seedlings (Rathore et al., 2004; Srivastava et al., 1998), and with *Tectona grandis L.* by increasing lateral root development (Khedkar and Subramanian, 1997).

Nutrient absorption and plant growth is related to adequate nutrient supply of the substrate solution (Wright, 1986). The majority of growers rely on controlled-release fertilizer for container production (Fain et al., 2000). Controlled release fertilizers,

however, may not provide adequate nutrients in a timely fashion to support optimal shoot growth. For Example, the use of supplemental nitrogen by means of liquid fertilizer pulses in addition to a control-release fertilizer has been successful in inducing an additional flush of growth for *Euonymus japonica* Thunb. 'Microphylla' (Cobb and Keever, 1984). The use of supplemental fertilizer has also produced additional growth flushes of *Ilex crenata* Thunb. 'Helleri' with an increase in shoot weight. This increase in shoot weight is induced by the higher rate of nitrate applied, whereas, a low level of nitrate favors root growth (Yeager and Wright, 1981).

Growth regulators have been used to increase branching and promote elongation of axillary shoots in many foliage, bedding, and woody plants in order to produce more desirable plants and shorten the time of production. The use of cytokinin was successfully increased bud development and in some cases bud break in woody plants such as some cultivars of *Rosa* L. (Carpenter and Rodriguez, 1971; Ohkawa, 1979; Parups, 1971; Richards and Wilkinson, 1984), *Malus* P. Mill. (Brome and Zimmerman, 1976; Kender and Carpenter, 1972; Williams and Billingsley, 1970), *Azalea* L. (Jackson and Lingle, 1971), and *Picea* A. Dietr. (Mulgrew and Williams, 1985), by releasing buds from apical dominance (Sachs and Thimann, 1967). Gibberellins have been used either alone or in conjunction with cytokinins to increase lateral branch elongation (Knavel, 1971; Williams and Billingsley, 1970; Zieslin and Pines, 1987). The combination of cytokinin and gibberellin produced a second flush of growth on another slow-growing woody ornamental with summer dormancy, *Buxus spp.*, applied to plants as a foliar spray in mid-June (Musselwhite et al., 2004b).

The objectives of the current study were to evaluate container size, use of air-root pruning containers, supplemental fertilizer, and use of a growth regulator on the growth of *E. alatus* ‘Compactus’ and *E. alatus* ‘Rudy Haag’ during nursery container production.

## **Materials and Methods**

### *Plant species and general growth conditions*

Rooted one-year old liners of *E. alatus* ‘Rudy Haag’ and *E. alatus* ‘Compactus’ (Spring Meadow, Grand Rapids, MI) were used for this study. Container size and type varied but all plants were potted, on April 12, 2006, in southern pine bark (Barky Beaver, Professional Grow Mix, Moss, TN), top-coated with 3-4 month Osmocote Plus 15-9-12 (Scotts Company, Marysville, OH) slow release fertilizer at a rate of 9 grams per gallon of container size, and trickle irrigated using one pressure-compensating line per gallon of container size. Prior to potting, an initial sample of 15 plants for each cultivar were measured for growth index and destructively harvested to obtain root and shoot fresh and dry weights.

### *Container size and type*

Each cultivar was grown in either a traditional Classic<sup>®</sup> (Nursery Supplies, Inc., Columbus, OH) or RootMaker (RootMaker<sup>®</sup> Products Company, LLC, Huntsville, AL) design of approximately one and three gallon-size containers (Classic – 225 cu in., 7.75” top diameter x 7” depth; 699 cu in., 11” x 9.5”; Rootmaker – 190 cu in., 6.25” x 6”, 673 cu in., 10.5” x 9.5”). All containers were filled with a volume of media equal to the volume of the Rootmaker containers for both one and three gallon treatments. After one



season, height, mean width, growth index  $[(Ht + (Wdt_1 + Wdt_2)/2)/2]$ , and branch number were evaluated on all plants. Additional data was collected on three plants for shoot and root dry weight for each cultivar, container type, and container size combination. Of the plants from this subsample, those in one gallon containers were measured for total root length, length per root diameter class, and surface area by using digital images from the washed root systems (WinRhizo Pro v.2007c, Regent, Canada). In addition, three roots in contact with the container wall were selected from one plant per cultivar and container type of 1 gallon size by cutting the primary root at the base of the stem. These roots were further evaluated for root topology, including topological index, average link length, and number of root tips per cm using a link analysis with a threshold value of 170 to create the analysis overlay.

#### *Supplemental fertilizer*

Thirty plants within the two Classic container sizes received supplemental liquid fertilizer using Peters Professional 20-10-20 (Scotts Company, Marysville, OH) at 200 ppm-N and intervals of none (control), once, or twice per month. Growth index and branching were evaluated after one year's growth. Throughout the growing season, monthly pour-through substrate analysis was conducted for pH and electrical conductivity (EC) to monitor available nutrients.

#### *Fascination treatment*

One third of the plants from the container size and type experiment and one third of the plants from the supplemental fertilizer study were treated with 1500 ppm of

Fascination<sup>TM</sup> (Valent, Walnut Creek, CA) using a backpack sprayer to foliar runoff on July 26, 2006. Fascination cytokinin and gibberellin growth hormone with 1.8% 6-Benzyladenine : 1.8% Gibberellin <sub>A4+7</sub>. Height, width, growth index and branching were evaluated at the end of the growing season.

#### *Statistical design and evaluation*

For the container type study, a total of 240 plants were allocated at random in a factorial design with a total of 8 treatment combinations (2 cultivars x 2 container types x 2 container sizes) with 30 plants per treatment. For the supplemental fertilizer study, a total of 360 plants were allocated at random in a factorial design with a total of 12 treatment combinations (2 cultivars x 2 container sizes x 3 fertilizer rates) with 30 plants per treatment. For treatment with Fascination, a sub-sample of 10 plants from both the container type and supplemental fertilizer experiments was selected at random from each treatment combination, for a total of 240 plants. Response variables, including height, mean width, growth index and branching were evaluated with the Proc GLM command using SAS software to obtain analysis of variance and mean separations.

Initial measurements of growth index, as well as, shoot and root fresh and dry weights were evaluated with SAS using a two group comparison t-test on the cultivars. Statistical evaluation on the root architecture study included response variables of total root length, total root surface area, diameter classification and total root length per diameter class. A total of 3 root systems per cultivar and container type of 1 gallon size were evaluated.

## Results

At the start of the canning operation, each cultivar was measured for growth index (cm), and fresh and dry weights for the root and shoot portions of the plant (Table 4.1). A strong cultivar effect is seen with *E. alatus* 'Compactus' having a significantly higher growth index (19.24 cm) than *E. alatus* 'Rudy Haag' (13.60 cm), thus starting out the season as larger plants. In addition, the root, shoot and total fresh and dry weights of 'Compactus' were significantly greater than 'Rudy Haag'. Both fresh and dry root:shoot ratios were not significantly different between the two cultivars.

From the evaluation of all plants at the end of the season, a significant main cultivar effect remains for height and growth index, however not for mean width and branch number (Table 4.2). Averaging over Fascination treatment, container type, and container size, 'Compactus' was significantly greater in height (26.8 cm) and growth index (23.8) compared to 'Rudy Haag' at 19.3 cm in height and 20.3 cm for growth index.

The use of Fascination on plants had the most impact on all growth responses (Table 4.2). Both 'Compactus' and 'Rudy Haag' showed an increase in growth and branch number, however, 'Rudy Haag' showed a greater percentage increase in height (38%), mean width (80%), growth index (59%), and branch number (83%) compared to 'Compactus' with an increase in height (12%), mean width (24%), and growth index (16%), and an increase in branch number of 16%.

The type of container used, either rootmaker or classic, did not have a significant main effect on any of the responses measured (Table 4.2). However, the size of the container did show a significant main effect on height, mean width, and growth index,

but not for branch number (Table 4.3). Averaging over cultivar, Fascination, and container type, plants grown in one gallon containers had a lower height (21.6 cm), mean width (19.9), and growth index (20.8), compared to those grown in three gallon containers, which were 24.5 cm in height, 22.1 cm in width, and 23.3 cm in growth index, which is an increase in size of 13%, 11%, and 14% respectively.

Because growth indices and branching measurements do not take into account growth below the soil line, fresh and dry weights were incorporated into the results. Disregarding Fascination treatment, following the first season of container production, all treatments, compared to the start of the season, showed an increase in root, shoot, and total fresh and dry weights and an increase in both fresh and dry root:shoot ratios (Tables 4.5 and 4.6). For fresh weight, the cultivar showed a significant main effect on all responses measured. Averaging over container type and size, 'Compactus' was greater in root fresh weight (57.1g), shoot fresh weight (26.2), total fresh weight (83.3), and root:shoot ratio (2.4) than 'Rudy Haag' with root, shoot, and total fresh weights of 11.8, 7.9, and 19.7 g respectively, and a root:shoot ratio of 1.54 (Table 4.5). Relative to the initial total fresh weight of the liner plants, 'Compactus' increased in size by 475.4% and 'Rudy Haag' increased by only 169.9%. The use of standard (Classic<sup>®</sup>) containers showed a positive impact on root (40.2 g) and shoot (20.0 g) weight compared to use of Rootmaker containers, which resulted in an average of 28.7 g root fresh weight and 14.2 g shoot fresh weight. The use of different container types had no effect on the root:shoot ratio. The container size had a significant main effect on root and shoot fresh weight with an increase as container size increased. Averaged over cultivar and container type, the root (40.2 g), shoot (21.3 g), and total (61.5 g) fresh weight of plants grown in three

gallon containers were greater compared to plants grown in one gallon containers, which had a weights of 28.7, 12.8, and 41.6 g, respectively. Dry weights indicated similar significant effects of cultivar, container type, and container size on fresh weights in terms of comparing 'Compactus' to 'Rudy Haag' (Table 4.6).

Root analysis using WinRhizo software measured total root length, root length per diameter classes, and surface area of a sub-sample of roots from one gallon containers in a split plot design (3 roots X 3 root systems per cultivar X 2 container types) (Table 4.7). The cultivar had the most significant main effect on the total root length, length of roots in each diameter class, and on total surface area. Averaging over the container type, 'Compactus' was greater in total root length (283.7 m) and surface area (3384.9 cm<sup>2</sup>) than 'Rudy Haag', which had a total root length of 52.86 m and surface area of 1408.6 cm<sup>2</sup> (Table 4.8). In comparing the length of roots in each diameter class, 'Compactus' had a greater percentage of roots in the '< 0.3 mm' diameter class (43%) than 'Rudy Haag' (26%), whereas, 'Rudy Haag' had more roots in the '0.3 to 0.6 mm' diameter range (67%) than did 'Compactus' (52%). Container type had a significant main effect on the length and surface area (Table 4.7). Averaging over cultivar, Classic containers had a total root length of 205.7 m and a surface area of 2576.55 cm<sup>2</sup> compared to Rootmaker containers which resulted in less total root length (130.9 m) and surface area (1512.7 cm<sup>2</sup>). Rootmaker containers caused a decrease in the length of roots in the '0.3 to 0.6 mm' range with only 56% of the total length falling into this diameter class, compared to Classic containers which have 63% of their roots in this range (Table 4.9). Although not significant, there was an increase in the number of roots in the lower diameter class when using Rootmaker containers.

Although the total root length was significantly greater for ‘Compactus’, ‘Rudy Haag’ had significantly more roots tips per root unit length (0.65) than did ‘Compactus’ (0.45) (Table 4.10). The type of container used caused the average link length (inter-branch distances and root tip lengths) to be shorter for plants grown in Rootmaker containers (0.51) than Classic containers (0.62) suggesting a more tightly packed root system.

The topological index for altitude shows no apparent trend, however, the external path length topological index was higher for plants grown in Classic containers (1.59) than for Rootmaker containers (1.20), suggesting a more dichotomous or branched root system achieved with the Rootmaker container. Higher values for both indices represent a more herringbone root system (Table 4.10).

The use of supplemental fertilizer did not show any significance on height, mean width, growth index, or branch number (Table 4.11). The use of growth regulator had a strong significant effect on all responses in a manner similar to the results of the container type experiment. There was a strong interaction of cultivar with the addition of growth regulator, again, showing increases in height, mean width, growth index, and branch number for *E. alatus* ‘Rudy Haag’.

## Discussion

*E. alatus* 'Compactus' is commonly referred to as a dwarf cultivar, but 'Rudy Haag' is smaller in comparison. This was evident at the start of container production with *E. alatus* 'Compactus' having a size 1.5 times greater than *E. alatus* 'Rudy Haag' and almost twice the mass in fresh and dry weight (Table 4.1). Results for cutting propagation indicated that 'Rudy Haag' is much slower growing than the species due to the lack of new shoot growth following rooting (Chapter 3). In this study, the total fresh weight gained relative to the initial total fresh weight of the liner plants showed that 'Compactus' increased in size by over 400% during the growing season, whereas, 'Rudy Haag' had a much smaller percentage increase of 170%. The difference in size of these two plants places limitations on using *E. alatus* 'Rudy Haag' as an adequate replacement for 'Compactus' due to a longer production time to obtain plants at the same salable size.

Plants grown in larger containers showed an approximately 12% increase in height, width, and growth index, with no increase in branch number (Table 4.2). Similar to results for other woody ornamentals, container size was shown to have a large effect on growth for *Prunus nersica* 'Spring Crest' with dry weight increasing as a function of volume, which followed a curvilinear relationship (Ran et al., 1992). *Euonymus japonica* Thunb. 'Microphylla' has increased top growth and decreased root growth with an increase in container depth and width (Keever et al., 1985). A container size effect was indicated in the production of *E. alatus* 'Compactus' as one gallon containers were not adequate for the sustaining the second-season's growth due to the restricting volume (Fuller, 1979).

Root training containers are designed to prevent root circling by the use of ridges and openings in the container wall (Whitcomb and Williams, 1985). Root training containers were tested in two different sizes to evaluate growth of *E. alatus*. There was no significant increase in above-ground growth or branching for either cultivar or container size with the use of these containers (Table 4.3). When averaged over cultivar and container size, there was a 39.7 and 40.6% increase in root fresh and dry weight, respectively, using Classic containers compared to RootMaker containers (Tables 4.5 and 4.6). This is accounted for by an overall reduction in root length (Table 4.7) and an increase in the percentage of smaller diameter roots in Rootmaker container. Therefore, the results of this experiment suggest using standard nursery containers proved to be more beneficial in producing more top and root growth. Because *E. alatus* has an inherent fibrous root system without the use of root training containers, their use is of no benefit the first year and will increase production costs. This is especially true for the 3 gallon size containers where few roots reached the container wall of the root training containers. Standard nursery containers greater than one gallon and up to three gallons in size could be recommended for container production of *E. alatus* through the second year of production.

Overall, the root system of *E. alatus* ‘Rudy Haag’ was smaller than *E. alatus* ‘Compactus’ (Table 4.8). A more in-depth analysis of the root systems of these cultivars helped in understanding the influence of container size and type on the structure of the root system. The total root length and surface area is reduced with the use of root training containers. Similar results were obtained for *Azalea* Smith (Mahogany) where the use of root pruning containers resulted in a lower root mass and no additional above-



ground growth (Fitzpatrick et al., 1994). There is an increase, although not significant, in the number of fine roots with a diameter less than 0.3 mm for both cultivars when using RootMaker containers, with approximately a 10% increase for 'Compactus' and 7% increase in 'Rudy Haag' (Table 4.7). The average link length, or inter-branch distances, was significantly reduced with the use of one-gallon root training containers and the number of root tips per root length increased, especially for 'Rudy Haag' which increased by 20.3% (Table 4.10). This suggests that the standard containers produce a coarser root system whereas the root-training containers produce a finer, more compact root system. While a finer root system is more desirable, the large reduction in surface area (Table 4.7) due to the use of root training containers for this species reduces the capability for water absorption. The difference of root weight may be due to lower moisture levels in RootMaker containers compared to Classic containers, which is documented with air-root pruning containers due to increased gas exchange occurring at the openings in the container wall, thus increasing drainage (Arnold and McDonald, 1999). More frequent irrigation may be required for these and other root training containers, such as RootMaker, compared to conventional black plastic containers.

Topological indices are used to compare treatment effects on root architecture, described by the number of primary and secondary roots, degree of branching and plasticity of branching (Fitter, 1987). The slope of the regression for the altitude (the number of links in the longest unique path from the base of the root to the exterior link) plotted against the magnitude (number of tips for that root) on a log scale is an indicator of root architecture. This topological index has a possible range of 0 to 1, where higher values indicate a more herringbone root structure, which is the most expensive for the

plant to produce in terms of resources. A lower value suggests a more dichotomous (branched) root system. A second topological index represents the slope of the regression for the external pathlength (the total number of links in all possible unique paths from the base of the root to all exterior links) against magnitude, on a log scale. This can be used in the same manner as the altitude slope with a range of 0 to 1.92 as it measures the same underlying properties of the root system (Fitter and Strickland, 1991). While the altitude topological index did not show a definite trend, the index for external pathlength indicated that the root system produced in standard containers had a more herringbone architecture than root systems subjected to root training containers. Overall, the increase of root tips per length and the lower value for topological index suggest a more branched root system with the use of RootMaker containers than of Classic containers.

In addition to using different sized containers and root training containers, supplemental N fertilizer was used to possibly increase the size of *E. alatus* 'Rudy Haag' to that of 'Compactus'. While the use of supplemental liquid fertilizer resulted in no additional height of *E. alatus* 'Rudy Haag', an increase in width was indicated for this cultivar (Table 4.11). However, the use of supplemental N fertilizer in addition to controlled release fertilizer showed no positive effect on overall growth index or branching for either cultivar. This is contrary to another species, *Euonymus japonica* Thunb. 'Microphylla', where the use of a supplemental N fertilizer increased plant growth and other responses with increasing concentration (Cobb and Keever, 1984). *E. alatus* is said to reach optimum growth with an N concentration of 200 ppm which can be provided by a control release fertilizer application alone (Sandrock et al., 2005). The use of supplemental-N fertilizer during container production did not increase the growth or

produce a second growth flush in *Buxus spp.*, another slow-growing woody shrub (Musselwhite et al., 2004a).

One means of inducing a second flush of growth is the use of a cytokinin-gibberellin growth regulator on slow-growing woody ornamentals with summer dormancy. Application of Fascination (BA + GA<sub>4+7</sub>) was successful at inducing a second growth flush in ‘Rudy Haag’ when sprayed in late July. *Buxus* is similar in growth pattern to *E. alatus* as they are both known to be slow growing due to summer dormancy. Similar results for inducing growth were achieved with Promalin (BA + GA<sub>4+7</sub>) applied to *Buxus sempervirens* L. ‘Subfruticosa’, *B. sempervirens* L. ‘Vardar Valley’ and *B. sinica* L. var. *insularis* Nakai ‘Justing Brouwers’ as a foliar spray in mid-June increased new shoot number (Musselwhite et al., 2004b).

Single application of Promalin or BA alone increased branching in other woody ornamentals such as *Ilex crenata* Thunb. ‘Helleri’, *Ilex vomitoria* Ait. ‘Stoke’s Dwarf’, *Photinia x Fraseri* Dress, *Nandina domestica* Thunb. ‘Harbour Dwarf’, and *Rhododendron x ‘Formosa’* azalea (Keever and Foster, 1990). Increased branching as a result of Promalin treatment occurred with concentrations of 2000 to 5000 ppm. For *E. alatus*, Fascination was applied at a concentration of 1500 ppm, which is included in this range.

The effect of using Fascination on *E. alatus* ‘Rudy Haag’ was larger than its effect on ‘Compactus’. At the end of the growing season ‘Rudy Haag’ reached the same size with equal branching as *E. alatus* ‘Compactus’. The ultimate reason for bud-break and shoot growth is due to either a pinching effect of the growth regulator or due to a change in the hormone concentrations in the plant. The results here suggest that ‘Rudy

Haag' was under greater hormonal control for apical dominance which is overcome by the addition of cytokinin and gibberellin. The use of Fascination resulted in a change in hormone balance in terminal and lateral buds causing both bud types to break simultaneously, producing a uniform increase in size.

To determine whether *E. alatus* 'Rudy Haag' is a GA mutant, the use of the cytokinin-gibberellin growth regulator should be compared with those that contain only cytokinin. GA is generally attributed to increased elongation, whereas BA is more efficient in causing budbreak. This effect of using the cytokinin-gibberellin growth regulator may be a matter of application timing. Future experiments may test differences in timing of application.

In conclusion, the use of root training containers or additional supplemental N fertilizer did not significantly increase growth of *E. alatus* 'Rudy Haag' to a comparable size of 'Compactus' for the first year of growth. The use of larger containers slightly increased growth. Using a growth hormone with a combination of cytokinin and gibberellin caused *E. alatus* 'Rudy Haag' to increase in growth and branch number, thus successfully reaching a similar size and branch number as 'Compactus'. The use of the growth regulator to produce a second flush of growth in *E. alatus* 'Rudy Haag' resulted in a similar sized plant to untreated *E. alatus* 'Compactus', allowing *E. alatus* 'Rudy Haag' to be marketed as ecologically friendly alternative to 'Compactus'.

Table 4.1: Growth index, root fresh and dry weights, shoot fresh and dry weights, and corresponding root:shoot ratios for a sample of 15 plants for each cultivar prior to container production of *E. alatus* 'Compactus' and 'Rudy Haag'.

Cultivar	Growth index (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Total fresh weight (g)	Fresh root:shoot ratio	Root dry weight (g)	Shoot dry weight (g)	Total dry weight (g)	Dry root:shoot ratio
<i>E. alatus</i> 'Compactus'	19.24	6.12	8.36	14.48	0.69	1.25	2.50	3.75	0.47
<i>E. alatus</i> 'Rudy Haag'	13.60	2.75	4.55	7.30	0.61	0.60	1.46	2.05	0.42
ANOVA									
Cultivar	34.13 <sup>**Z</sup>	9.26 <sup>**</sup>	12.42 <sup>**</sup>	11.60 <sup>**</sup>	0.85 <sup>NS</sup>	8.25 <sup>**</sup>	10.05 <sup>**</sup>	9.93 <sup>**</sup>	0.59 <sup>NS</sup>

<sup>Z</sup> <sup>NS</sup> and <sup>\*\*</sup>, indicate non significant and significant at  $p \leq 0.01$ , respectively.

Table 4.2: Height, mean width, growth index, and branch number of *E. alatus* ‘Compactus’ and ‘Rudy Haag’ following the first growing season in standard (Classic) and root training (RootMaker) containers in 1 and 3 gallon sizes with and without the addition of Fascination, a cytokinin-gibberellin growth regulator.

Cultivar	Fascination treatment	Container type	Container size (gallon)	Height (cm)	Mean width (cm)	Growth index (cm)	Branch number
<i>E. alatus</i> ‘Compactus’	No	Classic	1	25.7	18.0	21.8	23
			3	29.3	19.0	24.1	24
	Yes	RootMaker	1	22.5	18.3	20.4	21
			3	24.0	19.0	21.5	21
		Classic	1	26.3	22.8	24.6	23
			3	30.7	23.0	26.8	25
RootMaker	1	26.1	20.8	23.5	23		
	3	29.7	25.2	27.4	24		
<i>E. alatus</i> ‘Rudy Haag’	No	Classic	1	15.3	14.0	14.6	15
			3	16.9	16.2	16.5	19
	Yes	RootMaker	1	14.2	14.8	14.5	15
			3	18.4	15.8	17.1	14
		Classic	1	21.8	25.3	23.6	23
			3	24.1	28.4	26.3	26
RootMaker	1	21.0	25.5	23.3	27		
	3	23.0	29.9	26.4	27		

Table 4.2 (cont.)

ANOVA	Height	Mean width	Growth index	Branch number
Cultivar	66.98 ** Z	0.91 NS	30.72 **	3.09 NS
Growth regulator	25.31 **	259.88 **	104.17 **	92.31 **
Container type	2.37 NS	0.39 NS	0.74 NS	1.18 NS
Container size	10.03 **	17.35 **	15.92 **	1.02 NS
Cultivar x growth regulator	3.66 NS	56.97 **	19.88 **	30.25 **
Cultivar x container type	1.27 NS	0.15 NS	0.95 NS	0.09 NS
Cultivar x container size	0.17 NS	1.17 NS	0.02 NS	1.28 NS
Growth regulator x container type	0.44 NS	0.08 NS	0.36 NS	0.08 NS
Growth regulator x container size	0.03 NS	3.20 NS	0.72 NS	0.23 NS
Container type x container size	0.01 NS	0.80 NS	0.09 NS	1.25 NS
Cultivar x growth regulator x container type	1.79 NS	0.14 NS	0.67 NS	0.11 NS
Cultivar x growth regulator x container size	0.33 NS	0.11 NS	0.08 NS	0.04 NS
Cultivar x container type x container size	0.49 NS	0.86 NS	0.02 NS	3.53 NS
Growth regulator x container type x container size	0.05 NS	2.96 NS	0.29 NS	0.06 NS
Cultivar x growth regulator x container type x container size	0.33 NS	0.26 NS	0.39 NS	0.01 NS

Z NS, \*, \*\*, indicates non significant, significant at  $p \leq 0.05$ , and  $p \leq 0.01$ , respectively.

Table 4.3. Impact of container size on growth as averaged over cultivar, container type, and use of growth regulator.

Container Size	Height	Width	Growth index
1 gallon	21.6 b <sup>Z</sup>	19.9 b	20.8 b
3 gallon	24.5 a	22.1 a	23.3 a

<sup>Z</sup> Means in each column followed by the same letter are not significantly different as determined by Tukey's HSD test. Alpha = 0.05.



Table 4.4: Interaction effect between cultivar and growth regulator on width, growth index and branch number for *E. alatus* ‘Compactus’ and ‘Rudy Haag’.

Cultivar	Fascination treatment	Height (cm)	Width (cm)	Growth index (cm)	Branch number
<i>E. alatus</i> ‘Compactus’	No	25.3	18.6 b <sup>Z</sup>	22.0 b	21.0 b
	Yes	28.2	22.9 a	25.6 a	24.4 a
<i>E. alatus</i> ‘Rudy Haag’	No	16.2	15.2 b	15.7 b	15.0 b
	Yes	22.5	27.3 a	4.9 a	27.5 a

<sup>Z</sup>Means in each column for each cultivar followed by the same letter are not significantly different as determined by Tukey’s HSD test. Alpha = 0.05.

Table 4.5: Growth index, root, shoot, and total fresh weight with corresponding root : shoot ratios, and growth during the first season (relative growth rate) for *E. alatus* ‘Compactus’ and ‘Rudy Haag’ with standard (Classic) and root training (RootMaker) containers in 1 and 3 gallon sizes.

Cultivar	Container type	Container size	Growth index (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Total fresh weight (g)	Fresh root:shoot ratio	Relative Growth (%)
<i>E. alatus</i> ‘Compactus’	Classic	1 gallon	22.33	62.44	24.86	87.30	2.66	502.9
		3 gallon	22.37	67.50	34.48	101.98	1.94	604.3
	RootMaker	1 gallon	17.20	34.20	16.07	50.27	2.76	247.2
		3 gallon	20.40	64.31	29.42	93.73	2.21	547.3
<i>E. alatus</i> ‘Rudy Haag’	Classic	1 gallon	14.67	12.17	6.72	18.89	1.77	158.8
		3 gallon	20.63	18.51	13.74	32.25	1.35	341.8
	RootMaker	1 gallon	12.10	6.11	3.65	9.76	1.67	33.7
		3 gallon	13.73	10.34	7.56	17.90	1.38	145.3
ANOVA								
Cultivar			11.39 <sup>**Z</sup>	157.93 <sup>**</sup>	58.21 <sup>**</sup>	133.68 <sup>**</sup>	10.35 <sup>**</sup>	50.68 <sup>**</sup>
Container type			6.97 <sup>*</sup>	10.01 <sup>**</sup>	5.81 <sup>*</sup>	9.76 <sup>**</sup>	0.08 <sup>NS</sup>	13.65 <sup>**</sup>
Container size			2.98 <sup>NS</sup>	10.05 <sup>**</sup>	12.50 <sup>**</sup>	13.09 <sup>**</sup>	3.52 <sup>NS</sup>	16.44 <sup>**</sup>
Cultivar x container type			0.14 <sup>NS</sup>	1.42 <sup>NS</sup>	0.23 <sup>NS</sup>	0.98 <sup>NS</sup>	0.18 <sup>NS</sup>	0.00 <sup>NS</sup>
Cultivar x container size			0.48 <sup>NS</sup>	2.91 <sup>NS</sup>	1.57 <sup>NS</sup>	2.77 <sup>NS</sup>	0.28 <sup>NS</sup>	0.39 <sup>NS</sup>
Container type x container size			0.03 <sup>NS</sup>	2.53 <sup>NS</sup>	0.00 <sup>NS</sup>	1.15 <sup>NS</sup>	0.08 <sup>NS</sup>	0.55 <sup>NS</sup>
Cultivar x container type x container size			1.43 <sup>NS</sup>	3.54 <sup>NS</sup>	0.51 <sup>NS</sup>	2.39 <sup>NS</sup>	0.00 <sup>NS</sup>	2.48 <sup>NS</sup>

<sup>Z</sup> NS, \*, \*\*, indicates non significant, significant at  $p \leq 0.05$ , and  $p \leq 0.01$ , respectively.

Table 4.6: Root, shoot, total dry weight, and root:shoot ratio following the first growing season for *E. alatus* ‘Compactus’ and ‘Rudy Haag’ with standard (Classic) and root training containers in 1 and 3 gallon sizes.

Cultivar	Container type	Container size	Root dry weight (g)	Shoot dry weight (g)	Total dry weight (g)	Dry root:shoot ratio
<i>E. alatus</i> ‘Compactus’	Classic	1 gallon	12.28	13.61	25.90	0.90
		3 gallon	14.96	15.71	30.67	0.95
	RootMaker	1 gallon	6.39	8.35	14.74	0.88
		3 gallon	13.7	12.73	26.43	1.09
<i>E. alatus</i> ‘Rudy Haag’	Classic	1 gallon	2.49	3.06	5.54	0.82
		3 gallon	3.63	5.52	9.16	0.66
	RootMaker	1 gallon	1.35	1.84	3.19	0.71
		3 gallon	2.27	2.91	5.18	0.79
ANOVA						
Cultivar			157.21 <sup>***Z</sup>	84.36 <sup>***</sup>	123.46 <sup>***</sup>	11.66 <sup>***</sup>
Container type			10.34 <sup>**</sup>	8.95 <sup>**</sup>	10.45 <sup>**</sup>	0.31 <sup>NS</sup>
Container size			16.18 <sup>**</sup>	6.14 <sup>*</sup>	10.78 <sup>**</sup>	0.46 <sup>NS</sup>
Cultivar x container type			2.40 <sup>NS</sup>	1.20 <sup>NS</sup>	1.82 <sup>NS</sup>	0.19 <sup>NS</sup>
Cultivar x container size			6.97 <sup>*</sup>	0.53 <sup>NS</sup>	2.61 <sup>NS</sup>	1.81 <sup>NS</sup>
Container type x container size			2.16 <sup>NS</sup>	0.05 <sup>NS</sup>	0.62 <sup>NS</sup>	2.77 <sup>NS</sup>
Cultivar x container type x container size			2.63 <sup>NS</sup>	0.83 <sup>NS</sup>	1.62 <sup>NS</sup>	0.10 <sup>NS</sup>

<sup>Z</sup> NS, \*, \*\* indicates non significant, significant at  $p \leq 0.05$ , and  $p \leq 0.01$ , respectively.

Table 4.7: Total root length, surface area, volume, and average root diameter for *E. alatus* ‘Compactus’ and ‘Rudy Haag’ following the first season of container production using two container types.

Cultivar	Container type	Total root length (m)	Length of roots in diameter classes			Surface area (cm <sup>2</sup> )
			< 0.3 mm (m)	0.3 to 0.6 mm (m)	> 0.6 mm (m)	
<i>E. alatus</i> ‘Compactus’	Classic	343.56	132.58 (38.6 %)	191.14 (55.7)	19.72 (5.7)	4241.34
	RootMaker	223.88	107.80 (48.2)	107.74 (48.1)	8.26 (3.7)	2528.47
<i>E. alatus</i> ‘Rudy Haag’	Classic	67.85	15.44 (22.8)	47.67 (70.3)	4.70 (6.9)	911.75
	RootMaker	37.87	11.16 (29.5)	24.20 (63.9)	2.50 (6.6)	496.87
ANOVA		Total root length	< 0.3	0.3 to 0.6	> 0.6	Surface area
Cultivar		55.33 **Z	50.25 **	42.58 **	36.56 **	46.32 **
Container type		5.81 *	0.93 NS	9.44 *	15.80 **	11.76 **
Cultivar x container type		2.09 NS	0.46 NS	2.97 NS	7.25 **	5.00 NS

Z NS, \*, \*\*, indicates non significant, significant at  $p \leq 0.05$ , and  $p \leq 0.01$ , respectively.

Table 4.8: Cultivar effect represented by LS means of total root length, surface area, and root volume averaged over container type and size for *E. alatus* ‘Compactus’ and ‘Rudy Haag’.

Cultivar	Total root length (m)	Surface area (cm <sup>2</sup> )
<i>E. alatus</i> ‘Compactus’	283.72 a <sup>Z</sup>	3384.9 a
<i>E. alatus</i> ‘Rudy Haag’	52.86 b	704.3 b

<sup>Z</sup> Means in each column followed by the same letter are not significantly different as determined by Tukey’s HSD test. Alpha = 0.05.

Table 4.9: Container type effect represented by LS means of total root length, surface area, and root volume averaged over cultivar and container size for *E. alatus* ‘Compactus’ and ‘Rudy Haag’.

Container type	Length of roots in the 0.3 to 0.6 mm diameter class (m)	Total root length (m)	Surface area (cm <sup>2</sup> )
Classic	119.41 a <sup>Z</sup>	205.7 a	2576.5 a
RootMaker	65.97 b	130.9 b	1512.7 b

<sup>Z</sup> Means in each column followed by the same letter are not significantly different as determined by Tukey’s HSD test. Alpha = 0.05.

Table 4.10: Topological analysis of single roots including total root length, number of tips, tips per length, average link length and topological indices of *E. alatus* ‘Compactus’ and ‘Rudy Haag’ following the first growing season in standard (Classic) and root training (RootMaker) containers.

Cultivar	Container type	Total root length (cm)	Number of tips (magnitude)	$\frac{\text{Number of tips}}{\text{root length}}$	Average link length (cm)	Altitude topological index	External path length topological index
<i>E. alatus</i> Compactus	Classic	320.80	142.00	0.44	0.56	0.32	1.40
	RootMaker	309.33	137.22	0.46	0.47	0.42	1.27
<i>E. alatus</i> ‘Rudy Haag’	Classic	186.26	109.22	0.59	0.67	0.47	1.78
	RootMaker	174.12	120.78	0.71	0.54	0.14	1.13
<b>ANOVA</b>							
Cultivar		12.25 <sup>**Z</sup>	1.77 <sup>NS</sup>	12.86 <sup>**</sup>	4.27 <sup>NS</sup>		
Container type		0.10 <sup>NS</sup>	0.03 <sup>NS</sup>	1.67 <sup>NS</sup>	6.46 <sup>*</sup>		
Cultivar x container type		0.00 <sup>NS</sup>	0.19 <sup>NS</sup>	0.86 <sup>NS</sup>	0.17 <sup>NS</sup>		

<sup>Z</sup> NS, \*, \*\*, indicates non significant, significant at  $p \leq 0.05$ , and  $p \leq 0.01$ , respectively.

Table 4.11: Height, mean width, growth index, and branch number of *E. alatus* ‘Compactus’ and ‘Rudy Haag’ following the first growing season in standard (Classic) containers in 1 and 3 gallon sizes, with and without the addition of a cytokinin-gibberellin growth regulator, and use of supplemental N fertilizer.

Cultivar	Fascination treatment	Container size (gallons)	Fertilizer supplement per month	Height (cm)	Mean width (cm)	Growth index (cm)	Branch number
<i>E. alatus</i> ‘Compactus’	No	1 gallon	None	25.7	17.9	21.8	23
			Once	28.1	20.2	24.2	20
			Twice	27.2	20.0	23.6	22
		3 gallon	None	29.3	19.0	24.1	24
			Once	27.1	20.8	23.9	22
			Twice	25.7	18.7	22.2	20
	Yes	1 gallon	None	26.3	22.8	24.6	23
			Once	27.8	23.2	25.5	21
			Twice	30.9	22.1	26.5	25
		3 gallon	None	30.7	23.0	26.8	25
			Once	32.7	25.9	29.3	20
			Twice	27.0	20.4	23.7	25
<i>E. alatus</i> ‘Rudy Haag’	No	1 gallon	None	15.3	14.0	14.6	15
			Once	17.2	14.8	16.0	17
			Twice	15.2	16.0	15.6	14
		3 gallon	None	16.9	16.2	16.5	19
			Once	20.0	16.5	18.2	15
			Twice	20.6	18.7	19.7	18



Yes	1 gallon	None	21.8	25.3	23.6	23
		Once	23.4	26.2	24.8	34
		Twice	21.9	26.0	24.0	26
	3 gallon	None	24.1	28.4	26.3	26
		Once	23.2	25.8	24.5	27
		Twice	20.1	23.7	21.9	30

ANOVA	Height	Mean width	Growth index	Branch number
Cultivar	103.20 ** Z	0.21 NS	59.03 **	0.73 NS
Growth regulator	18.43 **	254.42 **	85.78 **	76.72 **
Container size	2.90 NS	3.05 NS	3.70 NS	1.12 NS
Fertilizer	1.12 NS	2.09 NS	1.78 NS	0.13 NS
Cultivar x growth regulator	3.09 NS	58.07 **	17.71 **	49.57 **
Cultivar x container size	0.14 NS	1.18 NS	0.48 NS	0.28 NS
Cultivar x fertilizer	0.03 NS	3.18 *	0.34 NS	4.77 **
Growth regulator x container size	0.28 NS	1.05 NS	0.61 NS	0.08 NS
Growth regulator x fertilizer	0.19 NS	5.74 **	1.54 NS	2.49 NS
Container size x fertilizer	1.46 NS	2.79 NS	2.32 NS	3.88 *
Cultivar x growth regulator x container size	1.98 NS	1.96 NS	2.48 NS	0.28 NS
Cultivar x growth regulator x fertilizer	0.96 NS	0.37 NS	0.91 NS	3.05 *
Cultivar x container size x fertilizer	1.47 NS	1.40 NS	1.19 NS	4.69 **
Growth regulator x container size x fertilizer	1.54 NS	1.21 NS	1.78 NS	0.75 NS
Cultivar x growth regulator x container size x fertilizer	0.62 NS	1.48 NS	1.03 NS	0.01 NS

Z NS, \*, \*\*, indicates non significant, significant at  $p \leq 0.05$ , and  $p \leq 0.01$ , respectively.

## CHAPTER 5

### Conclusions

The purpose of this research was to test *Euonymus alatus* ‘Rudy Haag’, a nearly seedless cultivar of burning bush, in both propagation and production systems in order to assess its suitability as an environmentally friendly alternative to other invasive cultivars. The objectives were to define limitations that may occur during propagation and to test various production methods that may help to produce ‘Rudy Haag’ to a similar size as ‘Compactus’ in the same amount of time. The results of the research presented in this thesis suggest that it is possible to propagate and grow ‘Rudy Haag’ to a marketable size without additional production time.

In order to achieve a high percentage of rooted cuttings with adequate root growth, cuttings of ‘Rudy Haag’ should be taken in mid-May, for Kentucky. ‘Rudy Haag’ only produced new growth on cuttings taken in May with no IBA treatment, therefore, IBA is not recommended unless the ideal cutting date has passed. Transplanting following rooting is not recommended as cuttings moved into 4-inch containers resulted in a decrease in new growth compared to plants in 6-pack containers. During container production, no differences were seen in above-ground growth due to container type or supplemental liquid fertilizer treatment. Root morphology studies indicated a change in root morphology with the use of root training containers, however additional size was not gained either above or below ground. The size of the container can affect growth as the use of three gallon containers in this research produced a larger plant than did one gallon containers. Overall, the use of Fascination (BA + GA<sub>4+7</sub>) resulted in *E. alatus* ‘Rudy Haag’ increasing in size branch number due to the induction

of a second flush of growth. At the end of the season, 'Rudy Haag' plants that were sprayed had reached the same size, statistically, as 'Compactus', the nursery standard which is considered invasive in many parts of the United States.

Plants examined during the subsequent growing season will be evaluated for any carry-over effects of the treatments posed during the first season.

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