

Chapter One

Literature Review

The history of boxwood is a rich one. The earliest record in taxonomic history of boxwood dates back to the Pliocene era (Larson, 1996). Fossils, identified as members of the genus *Buxus* L., have been found throughout Europe, and the American Boxwood Society claims the boxwood to be our oldest ornamental garden plant due to its use in ancient Egyptian hedges (Larson, 1996). The plant was used extensively in Italian gardens during the Renaissance and named by Linnæus in 1753 A.D. using binomial nomenclature (Larson, 1996).

The history of boxwood in America is a rich one as well. Despite the Americas having no native boxwood species of their own, they remain a favorite landscape plant throughout the country. The plant can be found in gardens from Ontario to Florida, from Long Island to California (Larson, 1996; Dirr, 1998). New interest is being shown in boxwood gardening due to their resistance to deer predation. However, commercial producers of boxwood have encountered a myriad of problems while trying to supply the public with boxwood for their gardens.

Nematodes, winter injury, *Phytophthora*, *Volutella*, leaf miners, psyllids, mites, scale, and even dogs plague the health of the boxwood (Cohen et al., 1978; Moody et al., 1978; Neumann, 1977). Problematic issues with nutrition, pH, solar exposure, soil type, and drainage also exist. With so many potential difficulties, commercial growers can become exasperated in their attempts to produce these plants, a misfortune which is further compounded by the plant's inherent slow growth rate. Boxwood typically produces a single flush of growth in the spring, the shoots elongate and budbreak ceases. Plants remain seemingly dormant for the rest of the year, producing only slight, erratic growth at best. For commercial growers, this summer "dormancy" makes production of boxwood expensive and time consuming. To encourage growers to produce these plants, the dormancy problem deserves examination. If a solution is found, production time can be reduced, and plant quality could be improved.

Dormancy has been defined as "a temporary suspension of visible growth of any plant structure containing a meristem" (Lang et al., 1987). Terminology has been

developed that defines three types of dormancy: endodormancy, paradormancy, and ecodormancy. Ecodormancy results from unfavorable environmental conditions; that is, an environmental (eco-) stimulus that does not originate within the plant inhibits growth. Drought (hydrational ecodormancy), high or low temperatures (thermal ecodormancy), or nutrient deficiency (nutritional ecodormancy) could potentially inhibit growth, thereby inducing ecodormancy. Endodormancy is regulated by physiological factors within (endo-) the dormant plant structure. For example, seeds of some plant species remain dormant until they have met the necessary chilling requirement (cryogenic endodormancy). Paradormancy is regulated by physiological factors outside (para-) of the dormant structure, as with apical dominance (apical paradormancy). Accumulation of auxin in the apical bud prevents the lateral bud from breaking. In this situation, the lateral bud is dormant, and is under the external control of the apical bud (Lang et al., 1987).

Using this definition, boxwood exhibits “a temporary suspension of visible growth” in the apical and lateral buds following the maturation of the spring shoot growth, and, therefore, boxwood can be said to be dormant. This phenomenon may be referred to as summer “dormancy”, and the potential causes of boxwood dormancy will be discussed further.

I. Nutrition.

To obtain optimal growth, nitrogen availability is critical. Nitrogen is a major component of amino acids, nucleic acids, and chlorophyll. Required for plant carbohydrate use, it is also necessary for nutrient uptake and root growth and development (Brady and Weil, 1999; Foth and Ellis, 1997; Havlin et al., 1999). For *B. sempervirens* L. ‘Suffruticosa’, the nitrogen range in uppermost mature leaf tissue is from 3.0% to 3.26% (Mills and Jones, 1996). Although the research on boxwood nutrition is not exhaustive, a considerable number of nutrition studies have been performed on boxwood that identify fertility regimes to follow in order to attain optimal growth. For instance, Hefley (1979) found that nitrate nitrogen was found to induce higher levels of growth than ammonium nitrogen. Bilderback and Cartwright (1980) found that of several slow release fertilizers applied to two different media, Osmocote 18-6-12 incorporated into composted hardwood bark: sand (2:1,v: v) at 4.1 kg m^{-3} were most

effective in encouraging growth of *Buxus microphylla* Sieb. and Zucc var. *japonica*. Dickey (1974) performed an experiment that examined the effect of nitrogen application levels and two fertilization intervals on the growth and chemical composition of Japanese boxwood. The rooted cuttings grown in a peat moss/ sand media that were fertilized twice a month had a higher number of bud breaks and were of higher visual quality than those fertilized at the same rate once a month. In addition, it was found that the number of bud breaks increased as nitrogen levels increased from 556 to 1116 kg/ha per year. Huett (1997) conducted a study in which five plant species, including *B. sempervirens*, were grown in a 5:3:2 (pine bark: hardwood sawdust: sand, by volume) media, and received fertilizer treatments of Osmocote 19-12-10, Nutricote NPK 16-16-24, Dynamic Lifter 3-10-6, and a liquid fertilizer that consisted of a carnation formulation developed by the author. The liquid fertilizer treatment produced the highest growth rates and was applied at an electrical conductivity (EC) of 2.0 dS m^{-1} every six hours through a trickle irrigation line.

By establishing the proper nutritional regime for boxwood, not only will it be possible to produce a healthier, more attractive plant, but also it is possible that with proper nutrition, potential nutritional ecodormancy of boxwood may be overcome. Studies have been performed on plants other than boxwood that show that nitrogen applications can increase plant shoot growth and reduce time between growth flushes. *Ilex crenata* Thunb. 'Helleri' liners fertilized weekly with high rates of ammonium nitrate (NH_4NO_3) produced the following vegetative flush more rapidly than those plants treated with lower rates (Yeager and Wright, 1981). In Niemiera and Wright's (1982) work on *I. crenata* 'Helleri', they found that plants grown in sand culture that were fertilized daily with applications of N ranging from 87 to 100 ppm had the greatest shoot dry weight, and the increased shoot dry weight was attributed to the initiation of shoot elongation that followed treatment applications. Gilliam et al. (1980) found that a second growth flush occurred on *Acer rubrum* L. when plants grown in a pine bark/sand media were fertilized with 150 to 300 ppm N weekly. *Thuja* D. Don. x 'Green Giant' grown in containers was found by Griffin et al. (1999) to have maximum shoot dry weight at N application rates of 100 ppm applied weekly. Gilliam et al. (1984) showed that after two years, *Pyrus*

calleryana Decne. 'Bradford' grown in containers had the greatest caliper after receiving application of 300 ppm N weekly.

Phosphorus's most essential function is energy transfer and storage, and its presence in the soil is associated with good root growth, early crop maturity, and resistance to lodging (Havlin et al., 1999). The phosphorus range of uppermost mature leaf tissue of *B. sempervirens* 'Suffruticosa' is from 0.13% to 0.15% (Mills and Jones, 1996), a range significantly lower than that of nitrogen. Yeager and Wright (1982) showed that *Ilex crenata* 'Helleri' grown in pine bark achieved optimal shoot dry weight at 10 ppm P. Havis and Baker (1985) found *Cotoneaster adpressa praecox* Bois. grown in a perlite-peat medium achieved maximum shoot dry weight at applications of 10 ppm P applied three times weekly. They also demonstrated that *Rhododendron* L. 'Victor' obtained maximum shoot dry weight at applications of 2.5 ppm P. In a study by Gouin and Link (1966) on *Taxus media* Rehd. 'Hatfieldi', plants grown in a peat moss/sand media attained maximum growth at 75 ppm P applied twice monthly. *Vaccinium ashei* Reade. grown in sand culture had increasing shoot dry weight with increasing P levels, and dry weight decreased in plants that had received less than 4.5 ppm P when treatments were applied daily (Spiers, 1984). Wright and Niemiera (1985) established that *Ilex crenata* 'Helleri' obtained optimal growth at 5 to 15 ppm P when plants were fertigated.

Potassium is found in many enzymes involved in photosynthesis (Foth and Ellis, 1997) is required for enzyme function, water relations, energy relations, translocation of assimilates, and nitrogen uptake and protein synthesis (Havlin et al., 1999). The uppermost mature leaves of most of *B. sempervirens* 'Suffruticosa' have potassium ranging from 0.87 to 0.97 % K (Mills and Jones, 1996). Spiers (1984) work on *V. ashei* revealed that with increased K concentration in daily applications, leaf tissue K increased linearly, and increased K fertilization resulted in increased shoot dry weight. Potassium deficiency developed at leaf tissue levels below 0.35%. Gouin and Link's (1966) work with *Taxus media* 'Hatfieldi' showed that plants grown in a peat moss/sand medium achieved maximum growth at 135 ppm K when fertilized twice monthly. The study also revealed an antagonistic relationship between N and K where as leaf tissue N increased, leaf tissue K levels decreased. Fertigated *Ilex crenata* 'Helleri' grown in sand achieved optimal growth at 25 ppm K, and as P application concentration increased, increased N

and K application rates were required (Wright and Niemiera, 1985). Sinha and Singh (1982) also discussed this relationship between P and K in their work on *Mentha arvensis* L. var. *piperascens*. With K deficiency, metabolism of P is restricted and a higher level of P is accumulated in plant tissue. In a study by Cannon et al. (1960), sub-irrigated *Gleditsia triacanthos* var. *inermis* 'Moraine' (L.) Zab. grown in sand obtained optimal growth at 30 ppm K.

It is necessary to ascertain the appropriate levels of N, P, and K application for boxwood in order to achieve optimal growth. If any of these three primary macronutrients are limiting, boxwood could experience nutrient deficiency, a lack of growth, and even decline. It is also possible that with limited nutrition, boxwood could undergo summer "dormancy" as a reaction to deficiency (nutritional ecodormancy).

II. Plant Hormones.

A second potential cause of boxwood summer "dormancy" may lie in the plant's hormonal level and hormonal activity. Phytohormones regulate and coordinate plant metabolism, growth, and morphogenesis (Taiz and Zeiger, 1998). The five plant hormones that are known to participate in plant dormancy are auxin, cytokinin, gibberellin, abscisic acid and ethylene. Auxin plays a role in such plant activities as stem elongation, root initiation, and apical dominance. In Cline's (1997) review of apical dominance concepts and terminology, apical dominance is defined as "the control exerted by the shoot apex over lateral bud outgrowth." Four developmental stages were used to describe apical dominance: Stage 1 (lateral bud formation), Stage 2 (imposition of inhibition on lateral bud growth), Stage 3 (release of apical dominance following decapitation), and Stage 4 (branch shoot development). During Stage 3, initial bud outgrowth is promoted by cytokinin and inhibited by auxin while during Stage 4, auxin and gibberellin both promote subsequent bud outgrowth.

Cytokinin is synthesized in the root and moves acropetally to the shoot (Torrey, 1976). In addition to its role in reversing apical dominance during Stage 3, cytokinin also participates in cell division, cell enlargement, and leaf expansion (Taiz and Zeiger, 1998). In a study on plant growth regulation by cytokinins, genetically engineered cytokinin-deficient *Arabidopsis thaliana* Thal. developed stunted shoots with reduced apical meristems relative to wild type plants and developed highly branched, faster growing

roots with enlarged meristems relative to wild type plants. The results suggested that cytokinin plays a regulatory role in plant meristem activity with opposite functions in the shoots and roots (Werner et al., 2001).

Gibberellin, in addition to its role in Stage 4 of apical dominance, is responsible for coordinating many plant activities such as increasing shoot length through internode elongation and stimulation of cell division (Taiz and Zeiger, 1998). Considerable elongation of the first internode of *Triticum aestivum* L. var. Hong Mang Mai was induced by application of GA₃ to culture media (Chen et al., 2001).

A fourth plant hormone, abscisic acid, has been found to accumulate in dormant buds and is thought to interact with other plant hormones such as gibberellins and cytokinins to regulate bud dormancy and growth (Taiz and Zeiger, 1998). Piola et al. (1998) found that abscisic acid levels in micro cuttings with dormant buds were higher than those in micro cuttings with growing buds of in-vitro-propagated cuttings of *Cedrus libani* at 24 °C.

A fifth plant hormone, ethylene, is also closely associated with dormancy. Stress conditions such as drought, flooding, chilling, and mechanical wounding can increase ethylene biosynthesis. Biosynthesis of ethylene is most active in meristematic and nodal regions (Taiz and Zeiger, 1998). Ethephon, an ethylene-releasing compound, has been shown to reduce apical dominance in grape (*Vitis* spp. L.) by stimulating lateral budbreak (Bautista et. al, 1987). Gemma (1995) suggests that ethylene may be a rest-breaking hormone for grape.

The level and activity of plant hormones can be manipulated endogenously through such cultural practices as pruning and defoliation and exogenously through the application of plant growth regulators. Modification of boxwood's hormone level and activity could potentially lift summer "dormancy."

Shoot pruning can be an effective method of inducing lateral shoot growth by removing the shoot from apical dominance. In an experiment performed by Fare et al. (1988), shoot dry weight of several woody plant species, including *B. microphylla* var. *koreana* Nak., that were either pruned at potting or pruned 6 weeks after potting was lower than plants that received no pruning treatment. In addition, the Korean boxwood had more root development with the non-pruned plants than with either pruned treatment.

Despite the loss in dry weight due to removal of shoot biomass, visual assessment of plants implied no difference in plant quality. When Williams and Moser (1974) examined the effects of a pinching agent (4% Off-Shoot-O: 45% methyl esters of fatty acids, 4% C6, 56% C8, 38% C10, 2% C12) and hand pruning on several woody species including *Cotoneaster divaricata* Rehd. & Wils. and *Pyracantha coccinea* Roem. 'Lalandi', they found that unpruned plants had less shoots per plant than plants that were hand pruned or received applications of the pinching agent. In a study performed by Malek et al. (1992) on *Rhododendron calendulaceum* Michx., it was found that unpinched plants produced the fewest number of lateral shoots, and plants with only the first two nodes removed produced the greatest number of new shoots. In the second experiment, plants that were pinched produced an average of 3.9 lateral shoots while those that received no pinching produced an average of 1.7 lateral shoots.

While shoot pruning for the plants mentioned above may reduce plant height and shoot dry weight, shoot pruning these plants can also induce lateral budbreak. Pruning may result in a slightly lower shoot dry weight due to the removal of shoot biomass, but pruning may also result in increased lateral budbreak by removing apical dominance. Pruning boxwood could potentially release lateral buds from apical dominance and eliminate apical paradormancy.

In addition to altering the plants hormonal function by pruning (overcoming apical dominance), defoliating a plant can also affect its organs' hormonal level and activity. In the tropics and subtropics, treatments of manual defoliation have been used to overcome floral bud dormancy of apples (*Malus* spp. Mill.) (Edwards, 1985; Edwards, 1987; Theron et al., 1987), peach (*Prunus persica* (L.) Batsch) (Edwards, 1987; George and Nissen, 1993), grape (*Vitis* spp. L.) (Edwards, 1987), and mango (*Mangifera indica* L.) (Nunez-Elisea et al., 1996) in order to produce two fruit crops in a given year without fulfilling the typical chilling requirement.

Edwards (1985) showed with apples that following defoliation, the closed apical buds had three times the usual gibberellin concentration, a decrease in abscisic acid, and an increase in cytokinin compounds. It was suggested that by removing old leaves from the shoot, the source of the abscisic acid is removed, and gibberellin concentration then increases in the shoot apex. Bud burst ensues, and then the leaves on the following new

growth can use the existing cytokinins for expansion. The practice of defoliation has been shown to stimulate lateral bud break in apple and may also remove the dormancy of lateral buds in boxwood. By manipulating the balance of growth inhibiting hormones and growth inducing hormones, the dormancy of lateral buds may be lifted.

Ethylene may play a role in overcoming dormancy via pruning or defoliation as ethylene is produced in response to wounding. In a study reported by Ievinsh et al. (1995), rye (*Secale cereale* L.) seedlings showed an increase in ethylene and ethylene precursor synthesis following coleoptile removal. In addition, exogenously applied ACC, an ethylene precursor, promoted bud break in grape (Mizutani, 1995). Bautista (1987) found that Ethephon, an ethylene releasing compound, applied at 4000 ppm stimulated budbreak in lateral buds and appeared to reduce apical dominance. As pruning and defoliation are both types of wounding, increase in ethylene production may result from such treatments as a wounding response.

While endogenous plant hormones can be manipulated through the cultural practices described, exogenous plant hormones can also be applied to alter plant activity through the use of plant growth regulators (PGRs). For instance, hydrogen cyanamide has been shown to overcome the chilling requirement in kiwifruit (*Actinidia* spp. Sieb. & Zucc.) (Powell et al., 2000; Paulin and McHattie, 1989), peaches (George and Nissen, 1993; Diaz et al., 1987) and nectarines (*P. persica*) (Dozier et al., 1990; George and Nissen, 1993). Exogenous gibberellin applications have been used to promote fruit set in apples, to induce bolting in cabbage, and to increase the stalk length on seedless grapes (Taiz and Zeiger, 1998). Gibberellin and cytokinin applied separately or as blends have been applied to horticultural plants such as Japanese holly (*I. crenata*) (Gilliam and Wright, 1977; Wright and Aung, 1975) apple (Volz et al., 1994; Quinlan and Tobutt, 1990), sweet cherry (*Prunus avium* L.) (Quinlan and Tobutt, 1990), golden pothos (*Epipremnum aureum* Linden & André) (Al-Juboory and Williams, 1991a), and English ivy (*Hedera helix* L.) (Al-Juboory and Williams, 1991b) in order to achieve increased lateral bud break, lateral branching, and shoot elongation.

Promalin (Abbott Laboratories, North Chicago, Ill.) is a PGR that contains equal parts cytokinin (N-(phenylmethyl)-1H-purin-6-amine) and gibberellin (GA₄₊₇) (Theron et al., 1987). Promalin has been used to induce either lateral shoot formation, shoot

elongation, or both in many woody ornamentals including roses (*Rosa* spp. L.) (Wisniewska-Grzeszkiewicz and Treder, 1989), *Vinca minor* L. (Foley and Keever, 1989), pear (*P. calleryana*) (Jacyna et al., 1994; Keever and Foster, 1990), *Forsythia x intermedia* Zab. (Grzesik and Rudnicki, 1985; Grzesik and Rudnicki, 1987), *Boronia megastigma* Nees ex Bartl. 'Lutea' (Lewis and Warrington, 1988), Algerian ivy (*H. canariensis* L.) (Al-Juboory and Williams, 1990), hypericum (*Hypericum calycinum* L.) (Thomas et al., 1992), apples (Curry and Williams, 1983; Basak and Soczek, 1986, Theron et al., 1987), pecan (*Carya illinoensis* (Wangenh.) C. Koch) (Herrera et al., 1987), *Photinia x fraseri* Dress., *Nandina domestica* Thunb. 'Harbour Dwarf', and Formosa azalea (*Rhododendron* L. x 'Formosa') (Keever and Foster, 1990). Promalin has also been used in combination with such cultural practices as defoliation, pruning, and nutrient application to induce shoot growth in horticultural plants. Theron (1987), in his work on peach, found that defoliation and Promalin both induced lateral bud break, and that the effects were stronger when the treatments were applied together than when applied separately. The paper suggests that the two sources of axillary bud inhibition were caused by the subtending leaf and by the bud's distance from the apex. The effects of these two sources reduced with leaf or bud age respectively. Ouellette (1996) showed that Promalin applications increased budbreak and branching of apple and a combination of leaf removal and Promalin induced uniform branch distribution.

Some PGR work has been performed with boxwood. Sabatinos (1966) conducted a study on *B. sempervirens* that found that treatment with gibberellic acid significantly increased plant height, stem length, total dry weight, shoot: root ratio, and shoot production as compared to the control. McVey and Wittwer (1958) conducted a field study on several woody ornamental species, including *B. microphylla* var. *koreana* Nak., and found that plants treated with 1000 ppm yielded an open, leggy growth habit compared to those plants treated with 10 or 100 ppm, which were more uniform in their growth. Midsummer, the plants that had received single or weekly 10 ppm treatments and the plants that had received a single 1000 ppm treatment began a second flush. The second flush from the single or 10 ppm treatments formed terminal buds a 4 weeks later, but the flush from the plants that had received the 1000 ppm treatment set terminal buds after 7 or 8 weeks. This difference indicates that there may be a delay involved with the

higher treatment levels. The most notable lateral growth occurred on plants that had been treated weekly with 100 ppm or singly with 100 or 1000 ppm, the later group being most significant.

Applications of PGRs, most notably the gibberellin and cytokinin blends such as Promalin, have been used extensively to overcome dormancy of lateral buds and induce growth of many horticultural species. A blend such as Promalin may be ideal in overcoming the dormancy of lateral buds on boxwood by encouraging lateral bud break and shoot growth and elongation.

III. Summary

With new interest being shown in boxwood use in the landscape, boxwood production is in need of improved efficiency. By determining the plant's ideal nutritional regime, by modifying the canopy through pruning or defoliation, and by resolving the effect of exogenously applied hormones, summer dormancy may be eliminated in boxwood, thereby potentially reducing production time and potentially improving plant quality.

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Chapter Two

Fertilizer Requirements for Boxwood

Abstract

The objective of this study was to determine the nutritional regime for container grown boxwood and to determine if summer dormancy of boxwood can be removed via nutrition. *Buxus sempervirens* L. 'Suffruticosa', *B. sempervirens* L. 'Vardar Valley', and *B. sinica* var. *insularis* Nakai 'Justin Brouwers' were used for these studies. Various levels Osmocote 15-9-12 and a liquid fertilizer (10-4-6) were applied to boxwood in a pine-bark substrate. Optimal shoot dry weight (OSDW) was achieved at 12 to 16 g Osmocote and 100 to 125 N for the 10-4-6 fertilizer. Leachate EC corresponding to OSDW ranged from 0.5 to 0.7 dS/m and 0.7 to 1.5 dS/m for Osmocote and the liquid fertilizer respectively. Leaf tissue N levels corresponding to OSDW weight ranged from 3.1% to 4.3% for Osmocote and 5.0% to 5.5% for the liquid fertilizer. While the fertilizer requirements for boxwood OSDW were determined, additional flushes did not occur.

Chapter Two

Fertilizer Requirements for Boxwood

Introduction

Boxwood typically produces a single flush of growth in the spring, and then shoot elongation and bud break cease. The plant remains dormant for the rest of the year, producing only slight, erratic growth at best. Dormancy is defined as “a temporary suspension of visible growth in any plant structure containing a meristem.” When that dormancy is due to a stimulus from outside the plant (such as temperature, moisture, nutrient availability), it is considered ecodormancy (Lang et al., 1987). Some plant species like *Ilex crenata* Thunb. ‘Helleri’ produce only one flush of growth in natural landscape settings; however, when given optimal fertility and water, multiple flushes can be induced (Yeager and Wright, 1981). Whether multiple flushes can be induced with boxwood given optimal nutrition and irrigation is questionable.

In fact, definitive nutrition studies with boxwood are limited. Bilderback and Cartwright (1980) found that of several slow release fertilizers applied to two different media in a single application, Osmocote incorporated into composted hardwood bark: sand (2:1, v:v) media at 4.1 kg m^{-3} were most effective in encouraging growth of *Buxus microphylla* Sieb. & Zucc var. *japonica*. Dickey (1974) performed an experiment that examined the effect of nitrogen application levels and two fertilization intervals on the growth and chemical composition of Japanese boxwood. The rooted cuttings grown in a peat moss/ sand media that were fertilized twice a month had a higher number of bud breaks and were of higher visual quality than those fertilized at the same rate once a month. In addition, it was found as the number of bud breaks increased as nitrogen levels increased from 556 to 1116 kg/ha per year. Huett (1997) conducted a study in which five plant species, including *B. sempervirens*, were grown in a 5:3:2 (pine bark: hardwood sawdust: sand by volume) substrate, and received fertilizer treatments of Osmocote 19-12-10, Nutricote NPK 16-16-24, Dynamic Lifter 3-10-6, and a liquid fertilizer that consisted of a carnation formulation developed by the author. The liquid fertilizer treatment produced the highest growth rates and was applied at an electrical conductivity

(EC) of 2.0 dS/m every six hours through a trickle irrigation line. Even with these studies, adequate information on the fertilizer requirement of boxwood does not exist. Therefore, the purpose of this research was to establish the minimum levels of slow release and liquid fertilizer to produce optimal growth of boxwood.

Materials and Methods

Experiment 1. Rooted cuttings of *B. sempervirens* L. ‘Suffruticosa’, *B. sempervirens* L. ‘Vardar Valley’ and *B. sinica* var. *insularis* Nakai ‘Justin Brouwers’ were potted 2 March, 2000, 1 per pot, into 3 L black plastic containers using a pine bark media amended per m³ with 3.6 kg dolomitic limestone and 0.9 kg Micromax (O.M. Scott, Marysville, Ohio). Osmocote 15-9-12 (15N-3.9P-9.8K) (O.M. Scott, Marysville, Ohio) was surface applied March 8, 2000 at 0, 2, 4, 8, 12, 16, 20, or 24 g /pot. Plants were grown for 35 weeks under natural photoperiod and day/night temperature of approximately 26/21° C. There were 8 replications per treatment for *B. sinica* var. *insularis* ‘Justin Brouwers’ and *B. sempervirens* ‘Vardar Valley’ and 11 replications per treatment for *B. sempervirens* ‘Suffruticosa’ or English boxwood. Treatments were completely randomized by cultivar. The plants were hand irrigated as needed. At 24 weeks following treatment initiation, nutrients were extracted by the pour-through method (Yeager et al., 1983) from 6 subsamples of English boxwood per treatment. Solutions were analyzed for EC, ammonium-N, nitrate-N, phosphorus, potassium and pH. Nitrogen was determined by colorimetric flow injection analysis, and phosphorus and potassium was determined by inductively coupled plasma (ICP) spectrometry. The experiment was terminated 35 weeks after treatment initiation, and at this time, approximately 2.0 g of uppermost mature leaf tissue and all remaining shoot growth was removed from each plant and dried in a 65.6° C drying oven and total shoot dry weights were recorded. Leaf tissue was ground in a 40 mesh Cyclone Sample Mill (U.D. Corp., Boulder, Colorado) and weighed. Tissue was digested as described by the Kjeldahl-Block Digester Method (Peterson and Chesters, 1964) and analyzed for total nitrogen by colorimetric flow injection analysis. All data were submitted to regression analysis (SigmaPlot for Windows, Version 5.0). EC, nitrate-N, ammonium-N, phosphorus and potassium and pH in leachate data and nitrogen in leaf tissue data were transformed using a natural log transformation (Sokal and Rohlf, 1995).

Experiment 2. Liter sized liners of *B. sinica* var. *insularis* ‘Justin Brouwers’ were potted on 22 March, 2001, one per pot, into 3 L black plastic containers with a pine bark media amended per m³ with 3.6 kg dolomitic limestone and 0.9 kg Micromax. A 10N-1.7P-4.9K liquid fertilizer was applied with each irrigation (250 ml/pot) at a rate of either 0, 25, 50, 75, 100, 125, or 150 ppm N. Nitrogen was applied as NH₄NO₃, phosphorus as H₃PO₄, and potassium as KCL. Treatments were assigned in a completely randomized design with 4 replications per treatment and 2 plants per experimental unit beginning 29 March, 2001, and continued for 28 weeks under natural photoperiod and day/night temperatures of approximately 26/21° C (80/70° F). At 16 weeks following treatment initiation, nutrients were extracted via the pour-through method (described above). Leachate was filtered and analyzed for electrical conductivity, nitrate-N, ammonium-N, phosphorus, potassium and pH as above. At the termination of the experiment, 28 week following treatment initiation, approximately 2.0 grams uppermost mature leaf tissue and all remaining shoot growth was removed and dried in 65.5° C drying oven. Total shoot dry weights were recorded and leaf tissue was prepared and analyzed for N as above or ashed in a muffle furnace for 4 hours and analyzed for total phosphorus and potassium by inductively coupled plasma spectrometry. All data were submitted to regression analysis (SigmaPlot for Windows, Version 5.0). EC, nitrate-N, ammonium-N, phosphorus and potassium in leachate data, and pH data were transformed using a natural log transformation (Sokal and Rohlf, 1995).

Results and Discussion

Experiment 1. Despite an increase in shoot dry weight in response to increasing fertilizer levels, no budbreak or shoot growth and elongation was visible following the first spring flush for English boxwood and ‘Vardar Valley’. However, at higher fertility levels ‘Justin Brouwers’ continued to exhibit budbreak and shoot elongation throughout the length of the experiment indicating this species does not exhibit summer “dormancy.” The three cultivars reached optimal shoot dry weight at Osmocote 15-9-12 applications of 12 to 16 g per 4.4 L pot (Figure 1). This range is consistent with the manufacturers recommendation for nursery stock, 12 to 21 g per 3 L pot. As testing substrate leachate for EC and nitrate-N levels is frequently done to assess nutrient availability, the leachate and plant tissue analysis from these experiments can provide guidance to commercial

growers in assessing the nutrient status of container-produced boxwood. Leachate EC levels corresponding to optimal shoot dry weight ranged between 0.5 to 0.7 dS/m (Figure 2). These EC levels are lower than the EC level of 2.0 dS·m⁻¹ used by Huett (1997), but consistent with other broad leaf evergreens. *I. crenata* 'Helleri' was shown to require reapplications of liquid fertilizer when E.C. values fell below 0.5 to 1.0 dS·m⁻¹ (Schiflett, 1994). Nutrient levels in leachate corresponding to optimal shoot dry weight were 20 to 50 ppm nitrate-N (Figure 3a), 1.1 to 2.2 ppm ammonium-N (data not shown, $r^2 = 0.88$, $y = -1.4184 + 0.0945x + 0.0028x^2$), 12 to 15 ppm phosphorus and 70 to 100 ppm potassium, (Figure 3b, and 3c, respectively).

Nutrient status of containerized plants may also be assessed based on plant tissue analysis. Leaf tissue nitrogen levels for the three cultivars corresponding to optimal shoot dry weight spanned from 3.1 to 4.3 % (Figure 4). *I. crenata* 'Helleri' showed optimal growth correlated with shoot dry weight at 2.3% N in leaf tissue by Schiflett et al. (1994) and at 2.4% N by Wright and Niemiera (1991). Relative to the requirements of 'Helleri' holly, boxwood nitrogen requirements appear quite high.

Experiment 2. Optimal shoot dry weight of 'Justin Brouwers' was reached by fertigating at 100 to 150 ppm N (Figure 5a). These liquid fertilizer application levels are typical of those that produce optimal growth of many woody ornamentals. *Ilex crenata* 'Helleri' produced maximum shoot dry weight in sand culture at 75 to 100 ppm N (Niemiera and Wright, 1982) and in pine bark media:sand (9:1, v:v) substrate, optimal growth occurred at 50 to 100 ppm N (Schiflett et al., 1994).

Electrical conductivity levels from media leachate corresponding to optimal shoot dry weight ranged from 0.7 to 1.5 dS·m⁻¹ (Figure 5b). The differences in the EC levels from Experiment 1 and Experiment 2 is consistent with Catanazaro's (1998) findings that leachate EC was lower in a peat-based media fertilized with two slow release fertilizers (14N-1.7P-4.9K tablet and 12N-4.3P-14.1K resin-coated) than in the same media fertilized with two liquid fertilizers (15N-4.3P-24.9K alternating with tap water irrigation and 15N-4.3P-24.9K applied with each irrigation). Leachate nutrient levels corresponding to optimal shoot dry weight ranged from 30 to 115 ppm nitrate-N, phosphorus levels, and 70 to 120 ppm potassium (Figure 6a, 6b and 6c, respectively).

Ammonium-N levels in substrate leachate corresponding to optimal shoot dry weight were considerably lower than nitrate-N levels at 1 to 4 ppm (data not shown, $r^2 = 0.84$, $y = -1.4354 + 0.0076x + 0.0001x^2$). The media for Experiments 1 and 2 was amended with lime, and, as nitrification occurs more rapidly at alkaline pH (Focht and Verstraete, 1977; Niemiera and Wright, 1986; Walden and Epelman, 1988), it is logical that higher amounts of nitrate-N were available than ammonium-N. In Experiment 2, leachate collected from 'Justin Brouwers' boxwood had pH ranging from 7.4 at the lowest fertilizer application levels to 6.4 at the highest fertilizer application levels (data not shown).

Leaf tissue nutrient levels corresponding to the 100 to 150 ppm application level had nitrogen at 5 to 5.5%, phosphorus at 0.48 to 0.57%, and potassium at 0.8 to 1.4% (Figure 7). These tissue nitrogen levels are higher than those from the previous experiment. Levels were also double those of other nursery crops like *I. crenata* 'Helleri' (Niemiera and Wright, 1982; Schiflett et al., 1994).

Leachate EC, nitrate-N, ammonium-N, phosphorus and potassium, and leaf tissue N levels as associated with optimal plant growth were lower for plants receiving Osmocote than plants receiving liquid fertilizer. It is suggested that this difference in nutrient level may be a result of the fact that controlled release fertilizers such as Osmocote deliver nutrients to the substrate on a continuous basis, and thus a relatively low nutrient level in substrate solution is sufficient to induce optimal growth. As nutrients provided by liquid fertilizers are applied periodically, a relatively high level of nutrients is required in substrate solution to prevent depletion of nutrients between fertilizer applications. As nutrients in substrate solution from an Osmocote source may be maintained at lower levels relative to those from a liquid fertilizer source, Osmocote may be a preferable nutrient source to liquid fertilizer in terms of limiting environmental contamination from nitrate leaching.

This research demonstrates that *B. sinica* var. *insularis* 'Justin Brouwers' does not appear to exhibit characteristics of summer "dormancy," and that summer "dormancy" of *B. sempervirens* 'Suffruticosa' and *B. sempervirens* 'Vardar Valley' is not related to fertility regime. These findings indicate that *B. sempervirens* and likely other boxwood cultivars that produce only one growth flush each year are not nutritionally ecodormant

because, despite providing plants with the appropriate nutritional environment, they did not produce additional growth flushes. However, despite a lack of subsequent shoot elongation and budbreak from *B. sempervirens*, commercial growers may use the findings of this work to assess the nutrient status of boxwood in order to ensure optimal growth (dry mass accumulation) during the current year and promote a more vigorous flush the following spring (Meyer and Tukey) Furthermore, by providing plants with appropriate but not excessive fertility regime, nursery induced environmental contamination may be reduced, higher quality plants can be produced, and greater profits may result.

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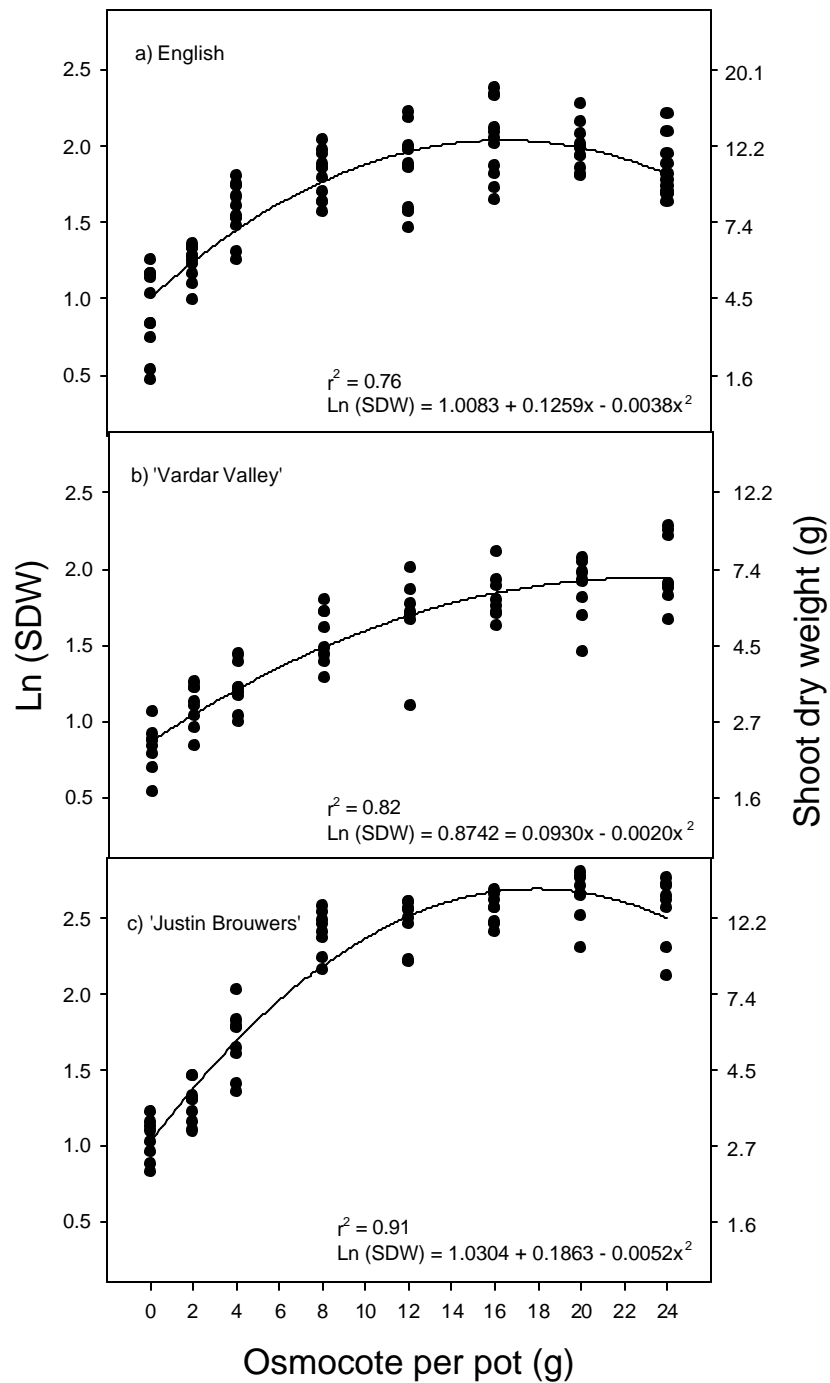


Figure 1. Influence of Osmocote application rate on shoot dry weight (SDW) of a) English, b) 'Vardar Valley,' and c) 'Justin Brouwers' boxwood 35 weeks following treatment initiation ($p < 0.0001$).

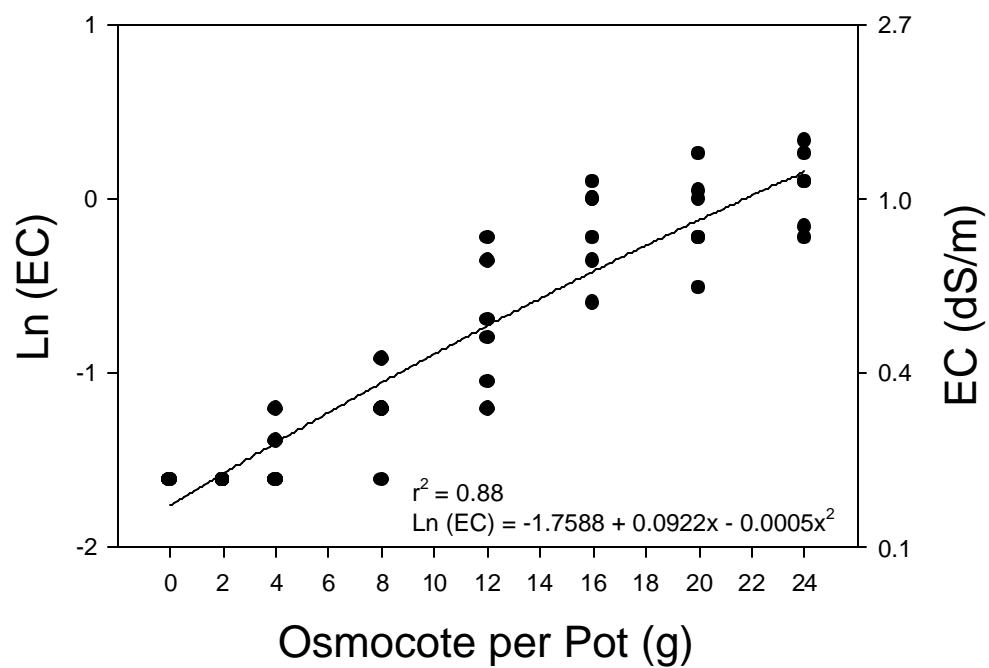


Figure 2. Influence of Osmocote application rate on electrical conductivity (EC) in leachate collected from 'Vardar Valley' boxwood 24 weeks following treatment initiation from ($p < 0.0001$).

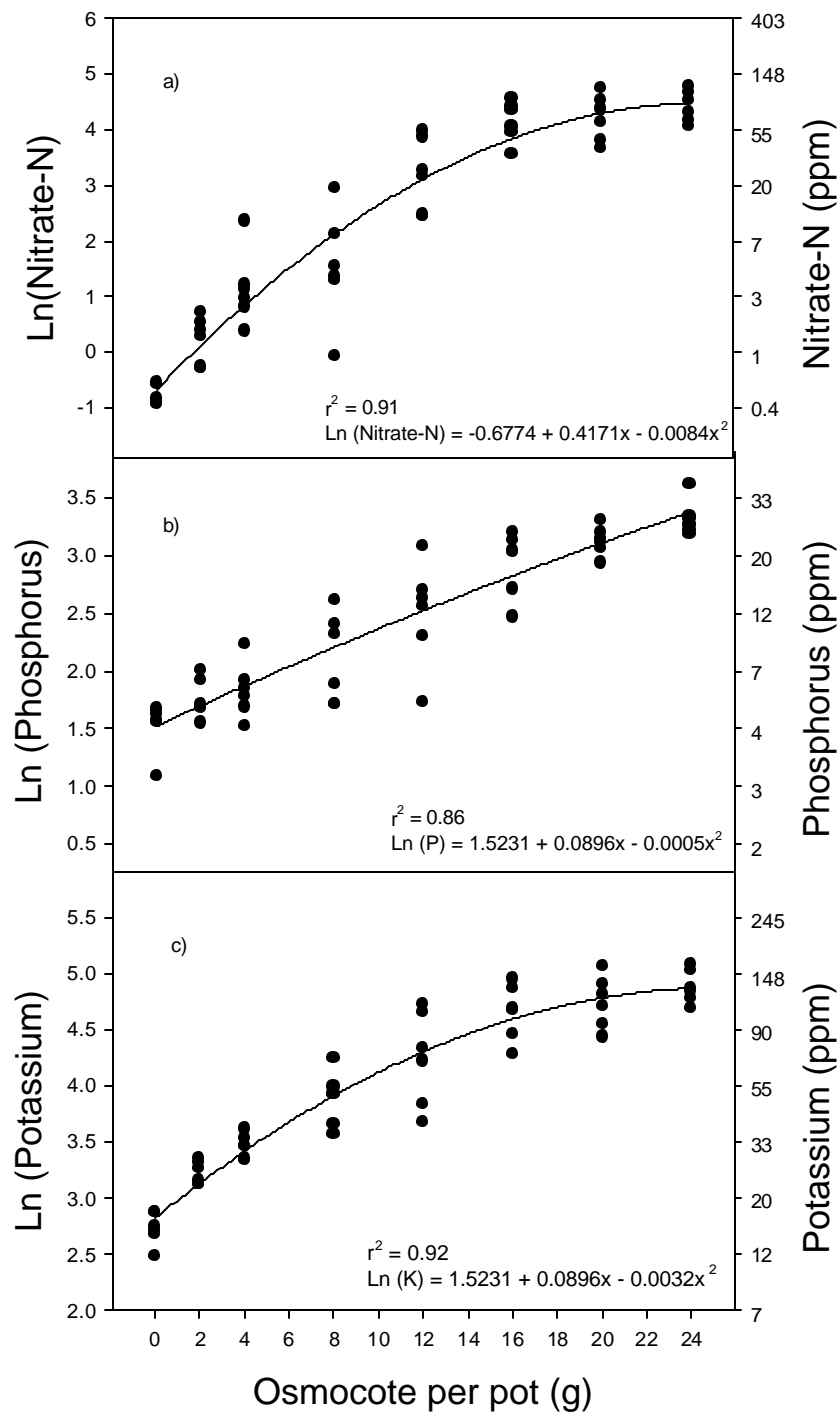


Figure 3. Influence of Osmocote application rate on a) nitrate-N, b) phosphorus, and c) potassium in leachate collected 24 weeks following treatment initiation from 'Vardar Valley' boxwood ($p < 0.0001$).

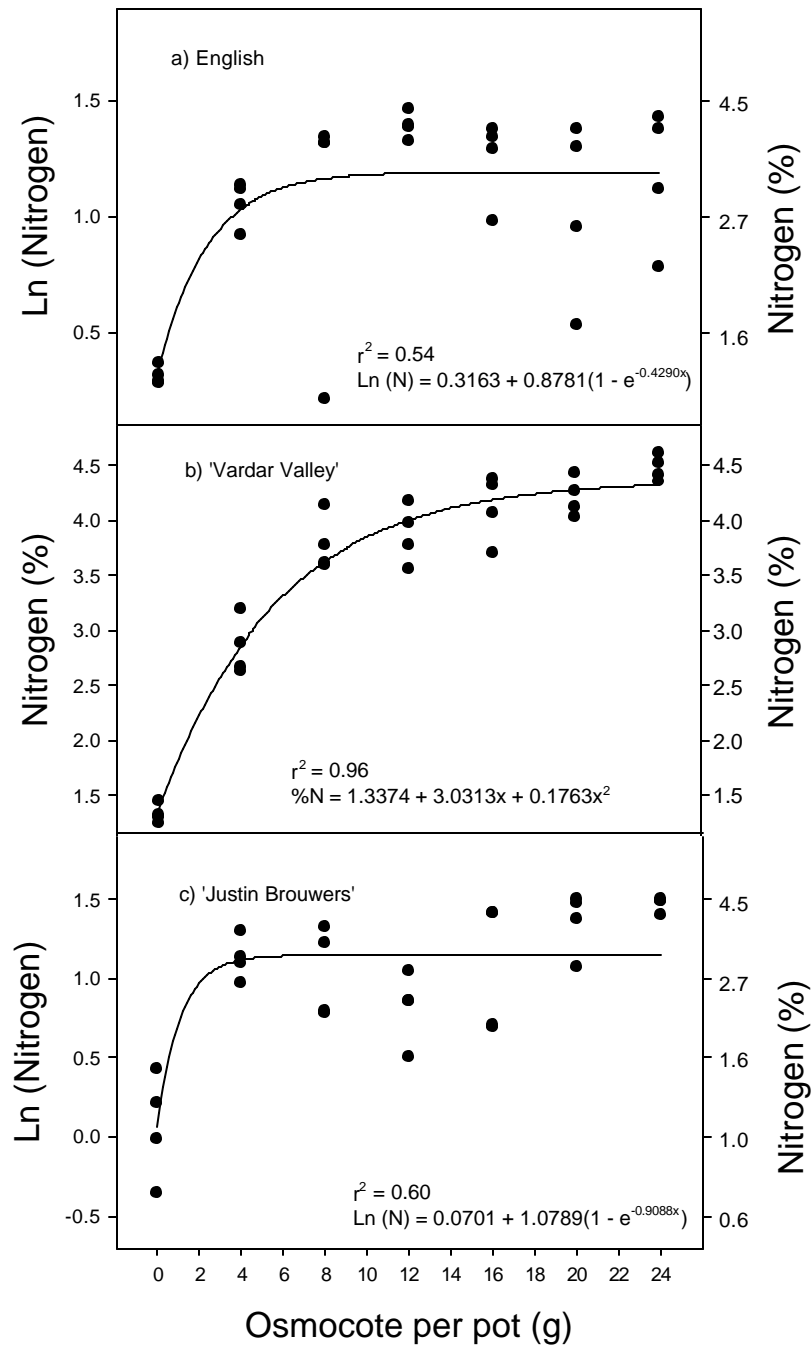


Figure 4. Influence of Osmocote application rate on nitrogen level in leaf tissue samples collected 35 weeks following treatment initiation from a) English, b) 'Vardar Valley,' and c) 'Justin Brouwers' boxwood ($p < 0.0001$).

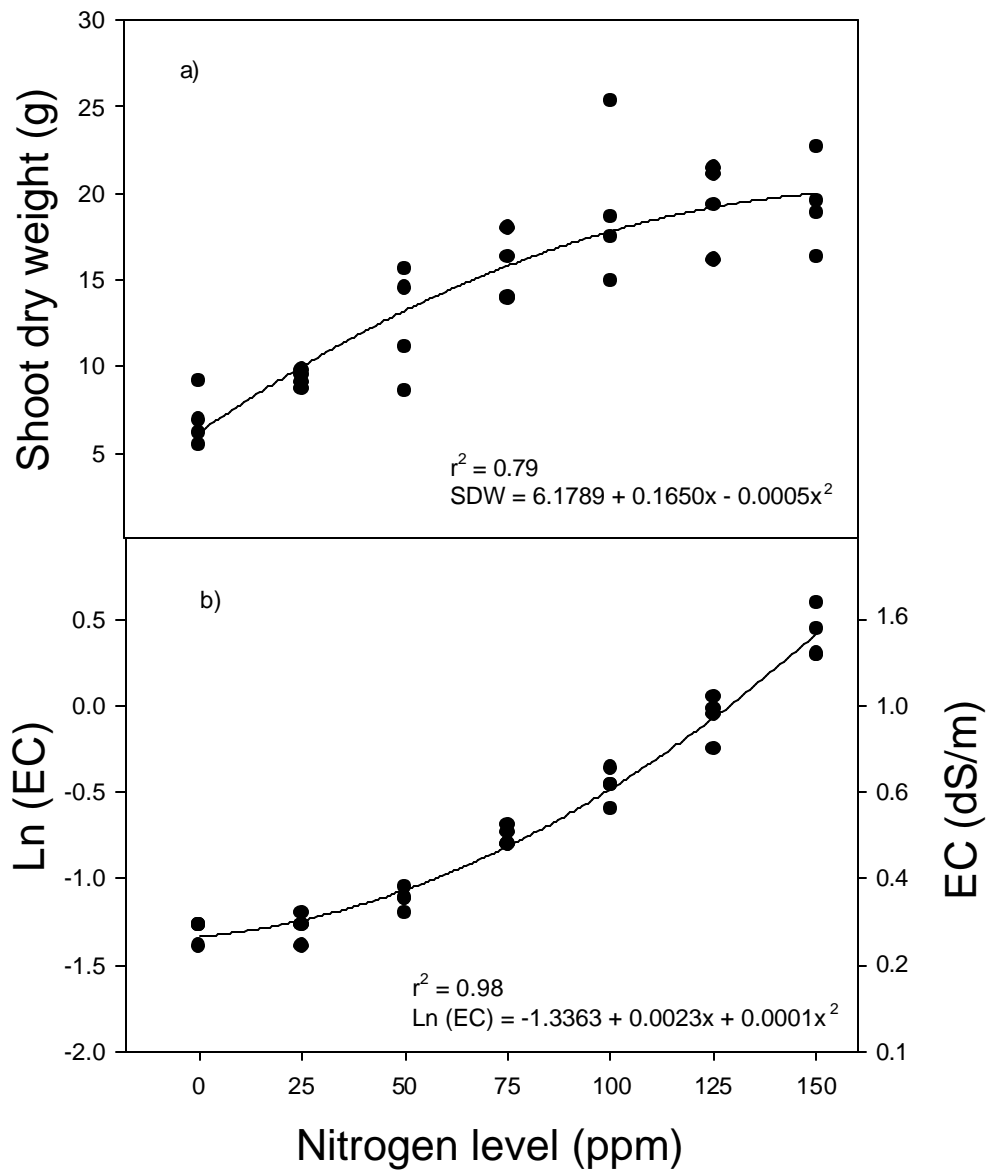


Figure 5. Influence of fertilizer level as ppm N in irrigation water on a) shoot dry weight determined 28 weeks following treatment initiation and on b) electrical conductivity (EC) in leachate collected 16 weeks following treatment initiation from 'Justin Brouwers' boxwood ($p < 0.0001$).

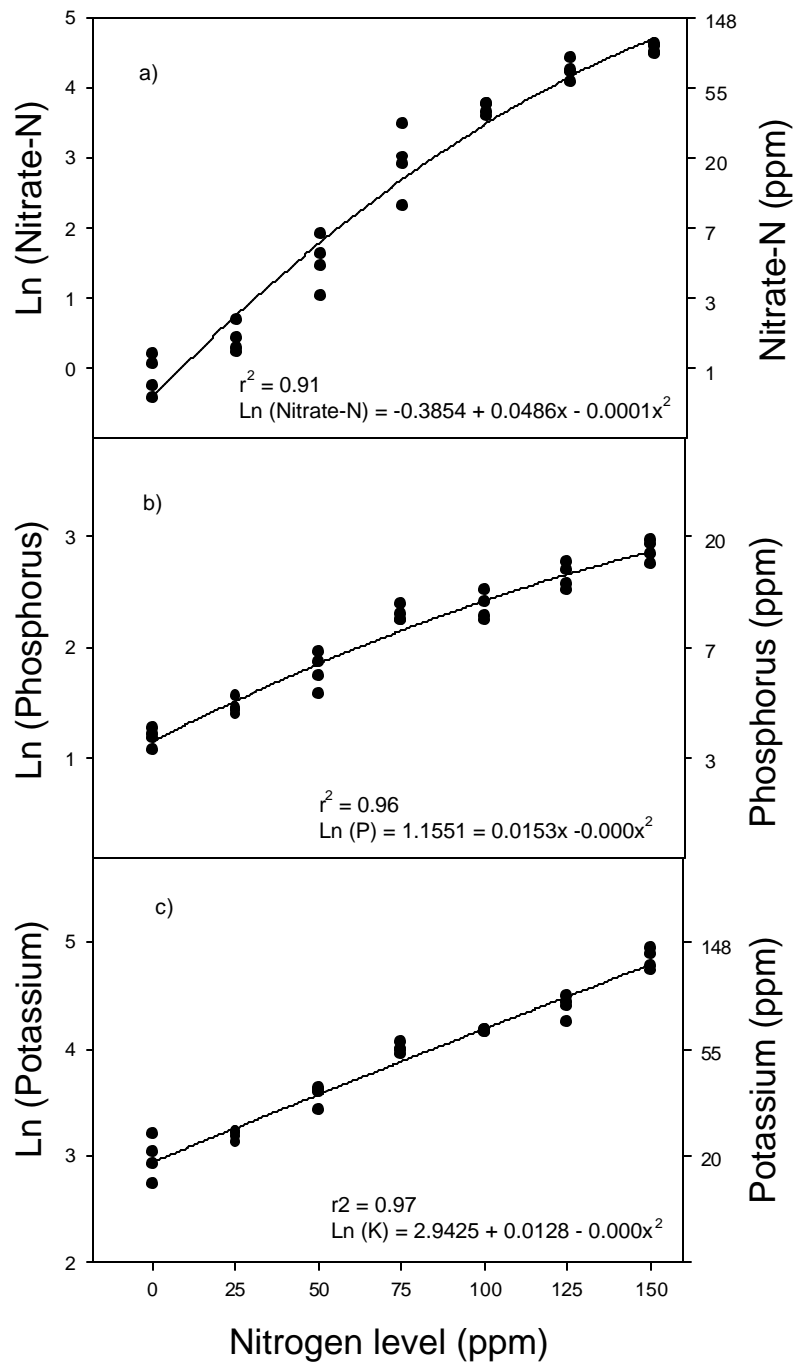


Figure 6. Influence of fertilizer level as ppm N in irrigation water on a) nitrate-N, b) phosphorus, and c) potassium in leachate collected 16 weeks following treatment initiation from 'Justin Brouwers' boxwood ($p < 0.0001$).

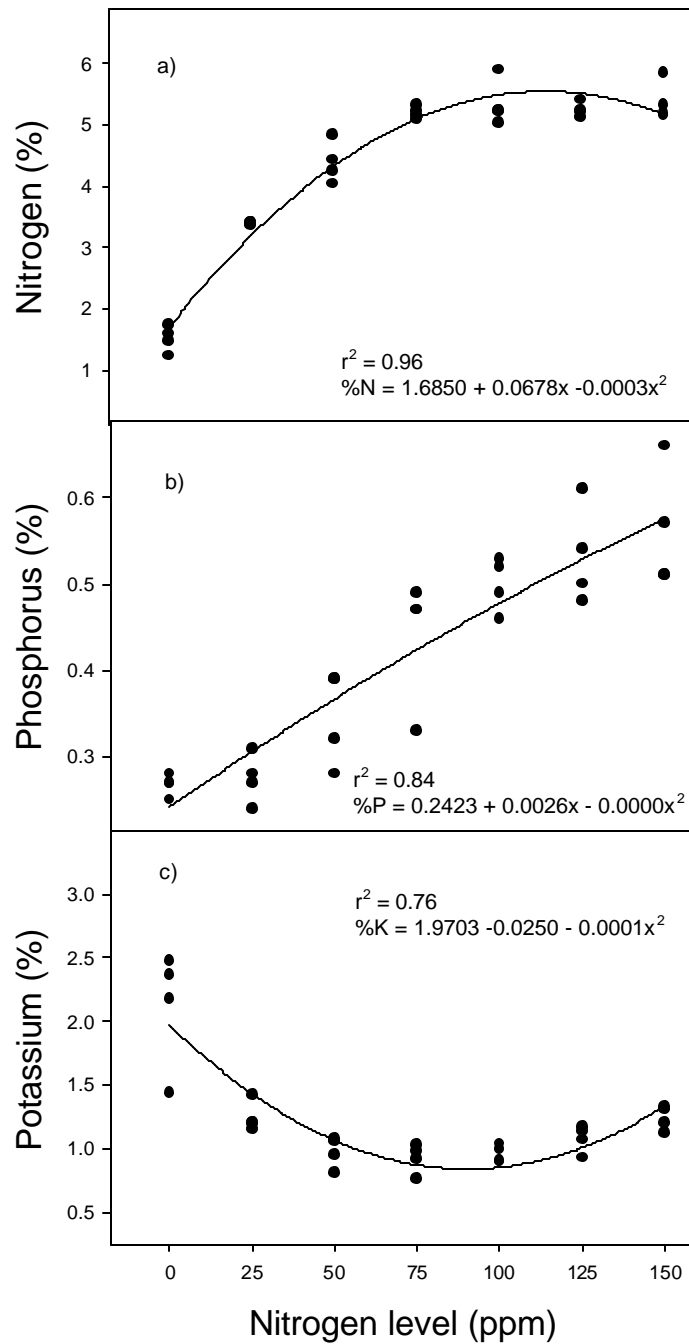


Figure 7. Influence of fertilizer level as ppm N in irrigation water on a) nitrogen, b) phosphorus, and c) potassium levels in leaf tissue collected 28 weeks following treatment initiation from 'Justin Brouwers' boxwood ($p < 0.0001$).

Chapter Three

Effect of Pruning, Defoliation, and Promalin on Growth of *Buxus* spp.

Abstract

The objective of this study was to if summer dormancy of boxwood could be overcome via pruning, defoliation and growth regulator applications. *Buxus sempervirens* L. 'Suffruticosa', *B. sempervirens* 'Vardar Valley' *B. sinica* var. *insularis* Nakai 'Justin Brouwers' were used in this study. Promalin alone and in combination with pruning was shown to be somewhat effective in promoting new shoot growth. Results were not consistent from experiment to experiment. Neither did pruning alone (shearing or tip removal) seem to be consistently effective in inducing new shoot growth. Defoliation (removal of new spring growth), however, caused dramatic increase in new shoots especially when applied ten weeks after budbreak in comparison to application closer to budbreak.

Chapter 3

Effect of Pruning, Defoliation, and Promalin on Growth of *Buxus* spp.

Introduction

Boxwood typically produces a single flush of growth in the spring, and then shoot elongation and bud break cease. The plant remains dormant for the rest of the year, producing only slight, erratic growth at best. Lang et al. (1987) has defined dormancy as “a temporary suspension of visible growth in any plant structure containing a meristem.” When the dormancy is due to a stimulus that exists within dormant plant structure, it is considered endodormancy. For example, seeds of some plant species remain dormant until they have met the necessary chilling requirement (cryogenic endodormancy). Paradormancy is regulated by physiological factors outside (para-) of the dormant structure, as with apical dominance (apical paradormancy). Accumulation of auxin in the apical bud prevents the lateral buds from breaking. In this situation, the lateral bud is dormant and is under the external control of the apical bud (Lang et al., 1987).

A potential cause of boxwood summer dormancy may lie in the plant’s hormonal level and hormonal activity since plant hormones regulate and coordinate plant metabolism, growth, and morphogenesis (Taiz and Zeiger, 1998). Shoot pruning can be an effective method of inducing lateral shoot growth by releasing the shoot from apical dominance. In a study reported by Malek et al. (1992) on *Rhododendron calendulaceum* Michx., it was found that plants that were pinched produced an average of 3.9 lateral shoots while those that received no pinching produced an average of 1.7 lateral shoots.

Defoliating, as well as pruning, a plant can also affect its organs’ hormonal level and activity. In the tropics and subtropics, treatments of manual defoliation have been used to overcome floral bud dormancy of apple (*Malus* spp. Mill.) (Edwards, 1985; Edwards, 1987; Theron et al., 1987), peach (*Prunus persica* (L.) Batsch) (Edwards, 1987; George and Nissen, 1993), grape (*Vitis* spp. L.) (Edwards, 1987), and mango (*Mangifera indica* L.) (Nunez-Elisea et al., 1996) in order to produce two fruit crops in a given year without fulfilling the typical chilling requirement. Edwards (1985) showed that following defoliation of apple trees, the closed apical buds had three times the usual gibberellin concentration, a decrease in abscisic acid, and an increase in cytokinin compounds. It was suggested that by removing old leaves from the shoot, the source of

the abscisic acid is removed, and gibberellin concentration then increases in the shoot apex. Bud burst ensues, and then the leaves on the following new growth can use the existing cytokinins for expansion.

Ethylene may play a role in overcoming dormancy via pruning or defoliation as ethylene is produced in response to wounding. In a study reported by Ievinsh et al. (1995), rye (*Secale cereale* L.) seedlings showed an increase in ethylene and ethylene precursor synthesis following coleoptile removal. In addition, exogenously applied ACC, an ethylene precursor, promoted bud break in grape (Mizutani, 1995). Bautista (1987) found that Ethephon, an ethylene releasing compound, applied at 4000 ppm stimulated budbreak in lateral buds and appeared to reduce apical dominance. As pruning and defoliation are both types of wounding, increase in ethylene production may result from such treatments as a wounding response.

While endogenous plant hormones can be manipulated through the cultural practices described, exogenous plant hormones can also be applied to alter plant activity through the use of plant growth regulators (PGR). Promalin (Abbott Laboratories, North Chicago, Ill) is a PGR that contains equal parts cytokinin (N-(phenylmethyl)-1H-purin-6-amine) and gibberellin (GA₄₊₇) (Theron et al., 1987). Promalin has been used to induce either lateral shoot formation, shoot elongation, or both in many woody ornamentals including roses (Wisniewska-Grzeszkiewicz and Treder, 1989), *Vinca minor* (Foley and Keever, 1989), pear (Jacyna et al., 1994; Keever and Foster, 1990), *Forsythia x intermedia* (Grzesik and Rudnicki, 1985; Grzesik and Rudnicki, 1987), *Boronia megastigma* 'Lutea' (Lewis and Warrington, 1988), Algerian ivy (Al-Juboory and Williams, 1990), Hypericum (Thomas et al., 1992), apples (Curry and Williams, 1983; Basak and Soczek, 1986, Theron et al., 1987), pecan (Herrera et al., 1987), *Photinia x fraseri*, *Nandina domestica* Thunb. 'Harbour Dwarf', and Formosa azalea (Keever and Foster, 1990). Promalin has also been used in combination with such cultural practices as defoliation, pruning, and nutrient application to induce shoot growth in horticultural plants. Theron's (1987) work on peach revealed that treatments of defoliation and Promalin both induced lateral bud break, and that the effects were stronger when the treatments were applied together than when applied separately. The paper suggests that the two sources of axillary bud inhibition were caused by the subtending leaf and by the

bud's distance from the apex. Ouellette (1996) showed that Promalin applications increased budbreak and branching of apple and a combination of leaf removal and Promalin induced uniform branch distribution.

Some PGR work has been performed with boxwood. Sabatinos (1966) conducted a study that found that treatment of gibberellic acid significantly increased plant height, stem length, total dry weight, shoot:root ratio, and shoot production as compared to the control. McVey and Wittwer (1958) conducted a field study on several woody ornamental species, including *B. microphylla koreana*, and found that plants treated with 1000 ppm gibberellin yielded an open, leggy growth habit compared to those plants treated with 10 or 100 ppm, which were more uniform in their growth. Currently, additional data is needed to establish the way in which boxwood dormancy may be altered by hormonal manipulation. Therefore, the purpose of this research was to determine the effects of pruning, defoliation, and Promalin on boxwood growth.

Materials and Methods

Experiment 1. Rooted cuttings of *Buxus sempervirens* 'Vardar Valley' were potted on 2 March, 2000 into liter-sized pots in a pine bark media. Incorporated per m³ into the pine bark were 3.6 kg dolomitic limestone, 0.9 kg Micromax (O.M. Scott, Marysville, Ohio), and 4.7 kg Osmocote plus 15-9-12 (15N-3.8P-9.8) (O.M. Scott, Marysville, Ohio). Plants received treatments composed of either pruning, 0, 500, 1000, 1500, or 2000 ppm Promalin (Abbott Laboratories, North Chicago, Ill.) applied at three application timings: Time 1) immediately after bud break, Time 2) at the end of the first growth flush, and Time 1/2) at both Time 1 and Time 2. A surfactant, 19 % Tween 20, was combined with all Promalin treatments at 1 ppm and to all remaining plants as a control. The different treatments of five Promalin rates and pruning applications at each of the three stages resulted in eighteen treatments in a completely randomized design with eight single plant replications. On 8 March, 2000, time 1 and time 1/ time 2 combination plants were sprayed with Promalin, and on 18 April, 2000 the pruning treatment was given and Promalin was sprayed on time 2 plants and time 1/ time 2 combination plants. Plants were grown under natural photoperiod with day/night temperatures of approximately 26/21° C (80/70° F) and hand watered as needed. On 22 August, 2000, at the termination of the experiment, final shoot number was taken. All

data were submitted to analysis of variance (SAS Institute Inc., Cary, N.C., Release 8.2), and all rate data were submitted to regression analysis (SigmaPlot for Windows, Version 5.0).

Experiment 2. Rooted cuttings of *B. sempervirens* 'Vardar Valley', *B. sinica* var. *insularis* 'Justin Brouwers', and *B. sempervirens* 'Suffruticosa' were potted on 2 March, 2000, into liter-sized pots in a pine-bark media. The media was amended as above. Each cultivar received treatments of pruning or Promalin applications in 3 species x 2 Promalin treatments x 2 pruning treatments factorial arrangement, resulting in twelve treatments. The pruning treatment was performed on 16 June, 2000, by pruning all plants to approximately 10 cm in height. On 20 June, 2000, Promalin was applied at a rate of 1500 ppm as above. Initial number of shoots was taken for all plants on 30 June, 2000. Plants were grown under natural photoperiod with day/night temperatures of approximately 26/21° C (80/70° F) and hand watered as needed. A completely randomized design with ten replications and one plant per experimental unit was used. On 20 October, 2000, at the termination of the experiment, a shoot count was taken for all plants, and the initial shoot number was subtracted from final shoot number revealing the number of new shoots. Top growth of all plants was harvested November 2, 2000, oven dried at 65.6°C, and final dry weight was taken. All data were submitted to analysis of variance with mean separation by Duncan's multiple range test (SAS Institute Inc., Cary, N.C., Release 8.2). All count data was transformed using the natural log transformation (Sokal and Rohlf, 1995).

Experiment 3. Liter sized pots of *B. sempervirens* 'Vardar Valley' received treatments of Promalin applications, pruning, and defoliation, each at three stages of growth: Stage 1) the end of the spring growth flush (shoots fully elongated, leaves fully expanded and light green in color) on 3 April, 2001, Stage 2) three weeks following the end of the spring growth flush on 24 April, 2001, and Stage 3) ten weeks following the end of the spring growth flush (woody tissue hardened, leaves dark green in color) on 11 July, 2001. The different treatments of Promalin, pruning, or defoliation applications at each stage were in a factorial arrangement resulting in twenty-four treatments. Promalin was applied at a rate of 1000 ppm, and all plants received an application of a surfactant (19% Tween-20 at 1 ppm). The pruning treatment was performed by removing the apical

bud from each shoot. Defoliation was accomplished by removing all current season leaves from each shoot by hand. Leaves from the previous years' growth were not removed. On 18 April, 2001, all plants received a single application Osmocote plus 15-9-12 at 4 grams per pot, and all plants received an application of 100 ml liquid fertilizer (10-4-6) at 1000 ppm-N on 18 April, 2001 and 11 July, 2001. On the day of treatment for each stage, initial shoot number was taken. Plants were grown under natural photoperiod with day/night temperatures of approximately 26/21° C (80/70° F) and watered as needed with overhead irrigation. A completely randomized design with nine replications and one plant per experimental unit was used. On 2 October, 2001 at the termination of the experiment, a shoot count was taken for all plants, and the initial shoot number was subtracted from final shoot number revealing the number of total new shoots. Also on this date, the number of total dead shoots was recorded for all plants. Shoots from all plants were harvested 9 October, 2001, oven dried at 65.6°C, and shoot dry weight was taken. All data were submitted to analysis of variance (SAS Institute Inc., Cary, N.C., Release 8.2). All count data was transformed using a square root transformation (Sokal and Rohlf, 1995). All zero counts were removed from data set to allow for the square root transformation.

Results

Experiment 1. Promalin applications of 1000 and 1500 ppm slightly increased shoot number at the end of the first growth flush (Time 1), although variability was high (Figure 1). Similar trends occurred for plants treated with continued treatments. The pruning treatment did not increase plant shoot number or plant height (data not shown).

Experiment 2. The Promalin/pruned combination treatment produced more new shoots relative to the control treatment for all three cultivars (Figure 2). Pruning alone also produced more mean new shoots than the control for 'Vardar Valley' and English boxwood (Figures 2a and 2c), whereas Promalin alone produced more mean new shoots than the control treatment for only 'Justin Brouwers' boxwood (Figure 2b). Shoot dry weight was increased over the control by Promalin plus pruning for 'Vardar Valley' boxwood and by Promalin alone for 'Justin Brouwers' boxwood (Figure 3a and 3b, respectively). Shoot dry weight was also reduced compared to the control by pruning alone for 'Justin Brouwers' and English boxwood (Figure 3c).

Experiment 3. Defoliation increased new shoot numbers dramatically at Stage 3 and less so for Stage 1 and 2 (Table 1 and Figure 4). A three-way interaction was shown between stage of application, defoliation, and Promalin for number of new shoots (Table 1 and Figure 4). For example, the greatest number of new shoots was produced ten weeks following the end of the spring growth flush (Stage 3) with the defoliation only treatment (Figure 4). When Promalin was applied in this case and at the end of the spring growth flush (Stage 1), plants produced 76% and 49% less shoots respectively than defoliation alone (Figure 4) and increased the number of dead shoots (Table 1, Figure 5). Plants that did not receive defoliation treatments produced no more than 2 new shoots per plant (Figure 4, Table 1). Over all, pruning increased the number of new shoots from 9 to 11 (Table 1). Shoot dry weight was decreased by defoliation. In addition, without defoliation, pruned plants had lower shoot dry weights than unpruned plants; while with defoliation, pruned plants had higher shoot dry weights than unpruned plants (Figure 6). Shoot dry weight was also greater when Promalin was applied to non-defoliated plants but dry weight was lower when Promalin was applied to defoliated plants (Figure 7).

Discussion

Experiment 1 showed that Promalin can increase shoot number in ‘Vardar Valley’ boxwood, in keeping with the findings of McVey and Wittwer (1958). They found that GA (one of the components of Promalin) at 1000 ppm induced a second growth flush in boxwood when applied the end of the first flush. Experiment 2 showed that Promalin and pruning in combination can be effective in increasing new shoot number in ‘Vardar Valley’, ‘Justin Brouwers’, and English boxwood. However, in Experiment 3, Promalin alone was not effective in producing new shoots nor were Promalin and pruning in combination.

In Experiment 2, pruning alone increased shoot number of ‘Vardar Valley’ and English boxwood, but pruning decreased shoot dry weight of the three cultivars. In Experiment 3, pruning alone was significant in production of new shoots, and pruning did not reduce shoot dry weight. The different methods of pruning used (shearing for experiment 2 vs. tipping for experiment 3) may be responsible for the differences in results between the two experiments. In Experiment 1, pruning was not shown to be effective in producing new shoots, and this lack of evidence may be due to a lack of

initial data. Without an initial shoot count, it is difficult to determine the number of new shoots produced.

For Experiment 3, defoliation was most effective in producing new shoots. However, when Promalin was applied in combination with defoliation, the number on new shoots produced decreased dramatically and the number of dead shoots increased. It may be that a phytotoxic level of hormones within the plant was created by the combination treatment resulting in necrosis of new shoots. Edwards (1985) showed that following defoliation, abscisic acid accumulation is reduced, and gibberellin and cytokinin levels increase. In Experiment 3, if endogenous hormone levels increased due to defoliation at the same time that exogenous hormones are being applied, the gibberellin and/or cytokinin levels within the plant may have become so elevated as to induce phytotoxicity or shoot necrosis. However, in Theron's (1987) work on peaches, it was found that treatment combinations of Promalin and defoliation induced lateral budbreak more effectively than when applied separately.

Pruning and defoliation reduced the plant shoot dry weight relative to the control plants. Fare et al. (1988) found that pruned plants, including *B. microphylla koreana*, had shoot dry weights lower than the unpruned control. When pruning and defoliation applications are given, removing as little plant tissue as possible may eliminate reduced shoot dry weight. It may be that removing a few leaves rather than all the leaves from the new growth flush is adequate to induce shoot production.

While Promalin, pruning, and defoliation show promise in overcoming summer dormancy of boxwood, defoliation may not be feasible for the commercial grower as it can be time consuming and labor intensive. Additional research is necessary to determine if defoliant can be used on boxwood to induce the same results as manual defoliation. Paraquat and sodium chlorate have been used to defoliate cotton to improve harvest ease and quality (Snipes et al., 1995). Defoliant have also been applied to deciduous holly to determine their effectiveness at defoliating the plant without damaging the plant's fruit (Banko and Stefani, 1999). Manual defoliation remains an alternative to defoliant application providing that time and labor are available. Altering boxwood hormone level and function through pruning, defoliation, or Promalin application can lead to increased growth, and therefore summer dormancy of boxwood can be overcome.

The ultimate effect of the treatments on growth and development in subsequent years is yet to be determined.

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Table 1. Effect of stage of application, Promalin, pruning, and defoliation on 1) new shoot number 2) dead shoot number, 3) shoot dry weight (SDW) of ‘Vardar Valley’ boxwood. Untransformed means presented here.

Stage	Promalin	Prune	Defoliate	New shoot number	Dead shoot number	Shoot dry weight (g)
one	no	no	no	0	0	11.1
	yes			0.4	0.1	12.5
	no	yes		1.3	0	9
	yes			2.1	0	10.3
	no	no	yes	15.7	0.1	8.6
	yes			7.7	5.1	5.5
	no	yes		20.1	0	9.1
	yes			8.7	6.7	7.5
two	no	no	no	0.4	0	11.3
	yes			1.6	0	13.2
	no	yes		1	0	9.6
	yes			1	0	10.7
	no	no	yes	11.3	0	7
	yes			16.8	6.2	7.1
	no	yes		18.9	0	9.2
	yes			14.1	7	7.1
three	no	no	no	0.8	0	12.4
	yes			0.7	0	12.3
	no	yes		1.6	0	10.5
	yes			0.8	0.7	11.3
	no	no	yes	45	0.1	7.5
	yes			7.3	4.6	6.5
	no	yes		48.6	0	8.2
	yes			15	5	7.4
Pr > F						
Source			New shoot number	Dead shoot number	Shoot dry weight (g)	
Stage			0.0003	0.5989	0.7484	
Promalin			<.0001	<.0001	0.6188	
Stage*Promalin			<.0001	0.5109	0.5476	
Prune			0.0366	0.3706	0.2038	
Stage*Prune			0.7498	0.9784	0.983	
Promalin*Prune			0.3426	0.2286	0.8804	

Stage*Promalin*Prune	0.2943	0.9124	0.2898
Defoliation	<.0001	<.0001	<.0001
Stage*Defoliation	0.0002	0.2353	0.3416
Promalin*Defoliation	<.0001	<.0001	0.0001
Stage*Promalin*Defoliation	<.0001	0.1805	0.2816
Prune*Defoliation	0.6679	0.6175	<.0001
Stage*Prune*Defoliation	0.6286	0.6967	0.7497
Promalin*Prune*Defoliation	0.87	0.4185	0.8804
Stage*Promalin*Prune*Defoliation	0.435	0.6911	0.6213

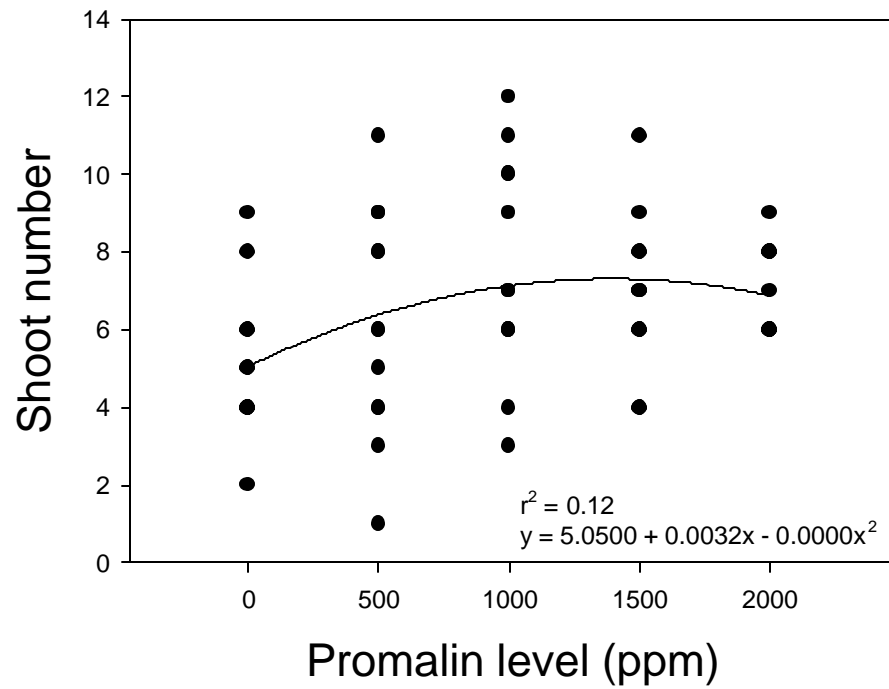


Figure 1. Influence of Promalin rate on final shoot number 24 weeks following treatment initiation of 'Vardar Valley' boxwood applied at the end of the spring growth flush (Time 2) ($p = 0.0242$).

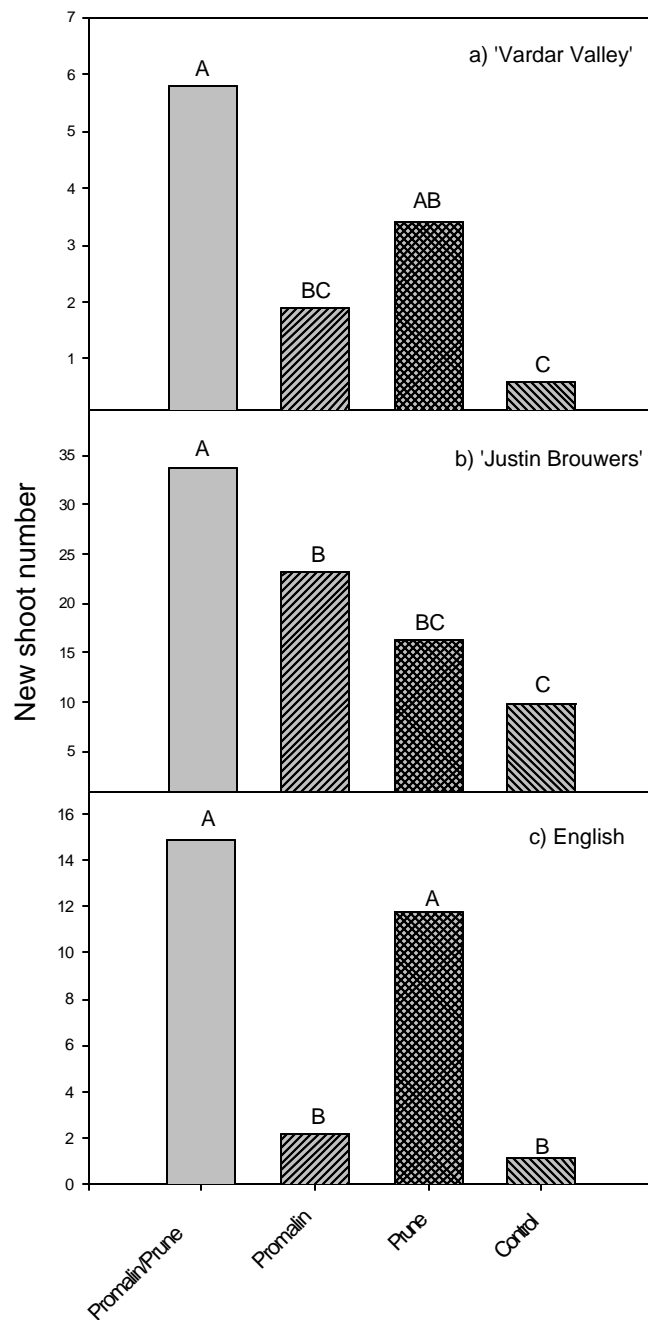


Figure 2. Influence of Promalin and pruning on number of new shoots produced by a) 'Vardar Valley,' b) 'Justin Brouwers,' and c) English boxwood 20 weeks following treatment initiation. Bars with same letters not significantly different at $\alpha = 0.05$ (Duncan's MRT) ($n = 10$).

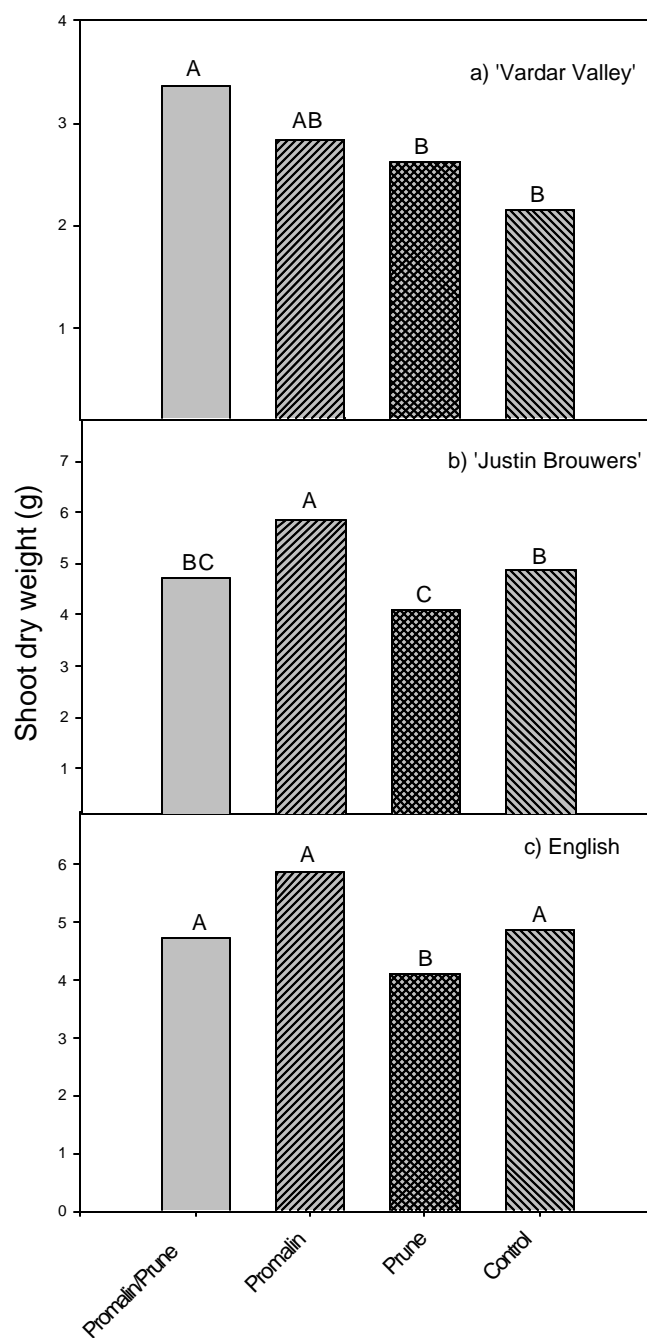


Figure 3. Influence of Promalin and pruning on shoot dry weight 20 weeks following treatment initiation of a) 'Vardar Valley,' b) 'Justin Brouwers,' and c) 'English' boxwood. Bars with the same letters not significantly different at $\alpha=0.05$ (Duncan's MRT) (n = 10).

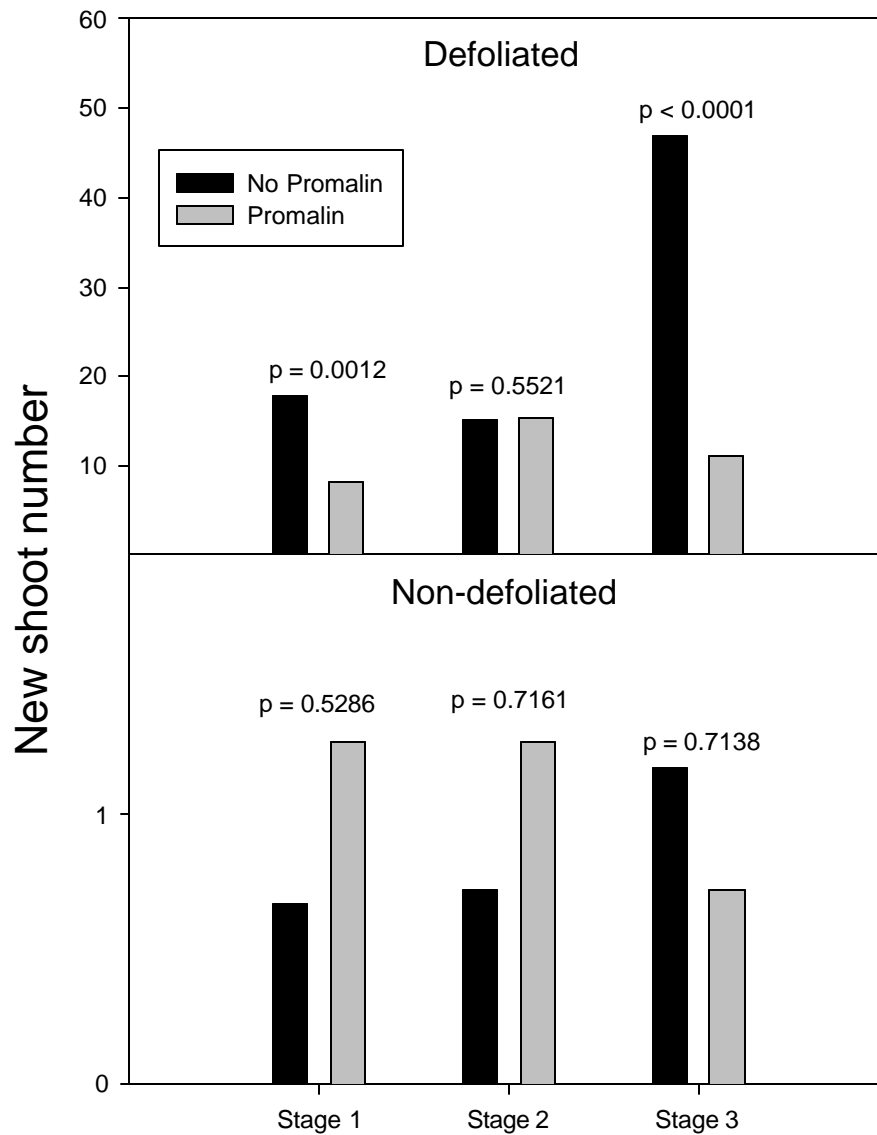
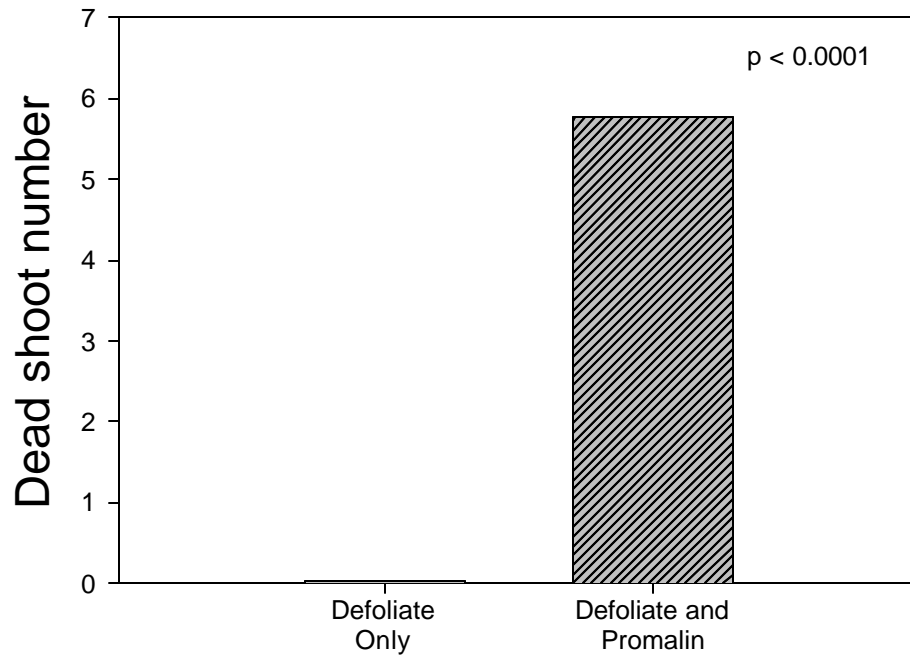


Figure 4. Influence of Promalin, Defoliation, and stage of application (Stage 1: end of the spring growth flush, Stage 2: three weeks following the spring growth flush, and Stage 3: ten weeks following the end of the spring growth flush on number of new shoots produced on 'Vardar Valley' boxwood 26 weeks following treatment initiation. Untransformed means presented here. P-values represent test for treatment effect within each stage (n = 9)



Promalin and defoliation applications

Figure 5. Influence of Promalin in combination with defoliation on number of dead shoots produced by 'Vardar Valley' boxwood 26 weeks following treatment initiation. Untransformed means presented here ($n = 9$).

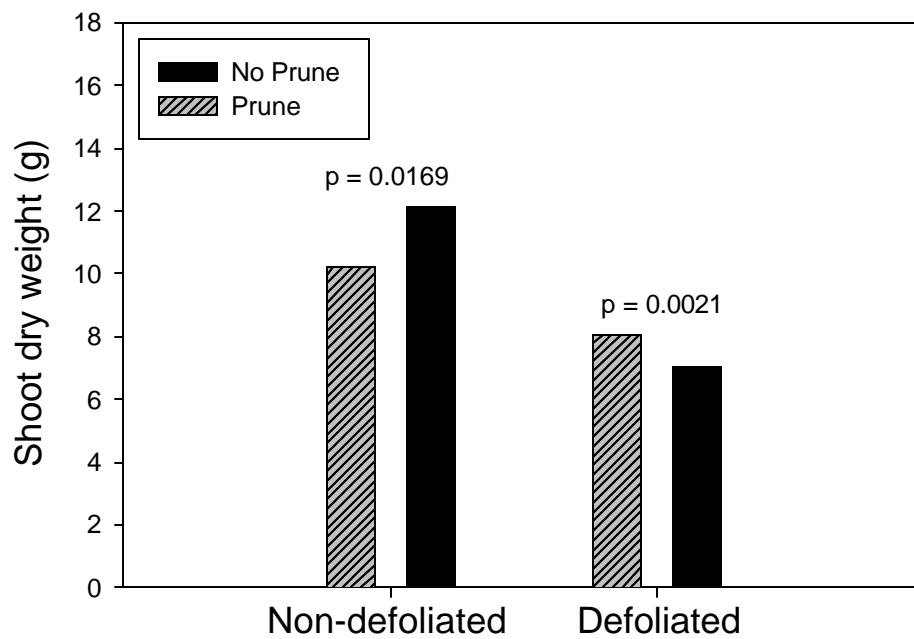


Figure 6. Effect of pruning and defoliation on shoot dry weight of 'Vardar Valley' boxwood 26 weeks following treatment initiation (n = 9).

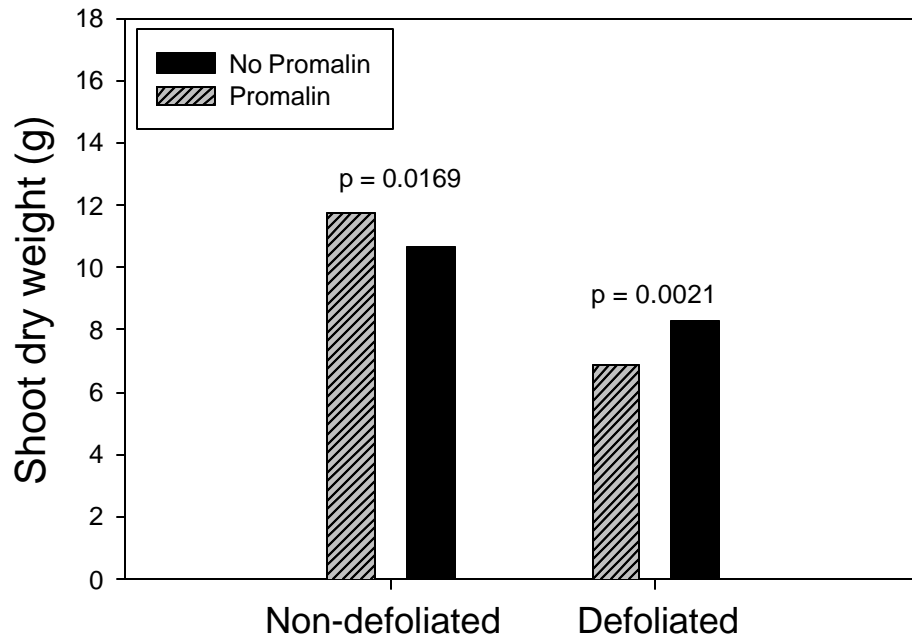


Figure 7. Influence of Promalin and defoliation on shoot dry weight of 'Vardar Valley' boxwood 26 weeks following treatment initiation (n = 9).

Appendix A

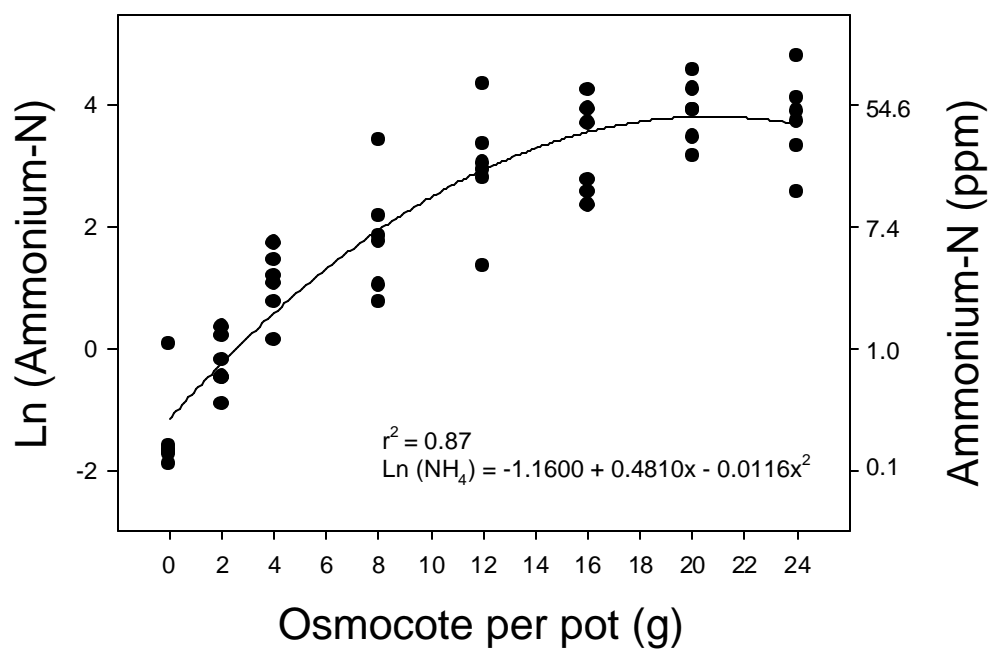


Figure 8. Influence of Osmocote application rate on ammonium-N in leachate collected from 'Vardar Valley' boxwood 24 weeks following treatment initiation ($p < 0.0001$)

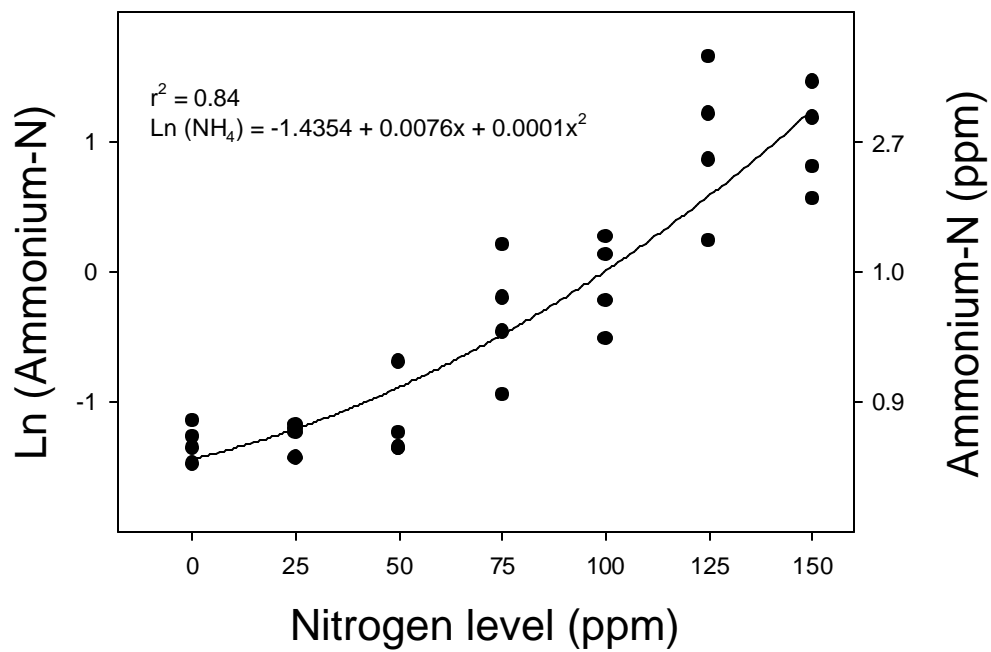


Figure 9. Influence of fertilizer level as ppm-n in irrigation water on ammonium-N in leachate collected from 'Justin Brouwers' boxwood 16 weeks following treatment initiation ($p < 0.0001$).

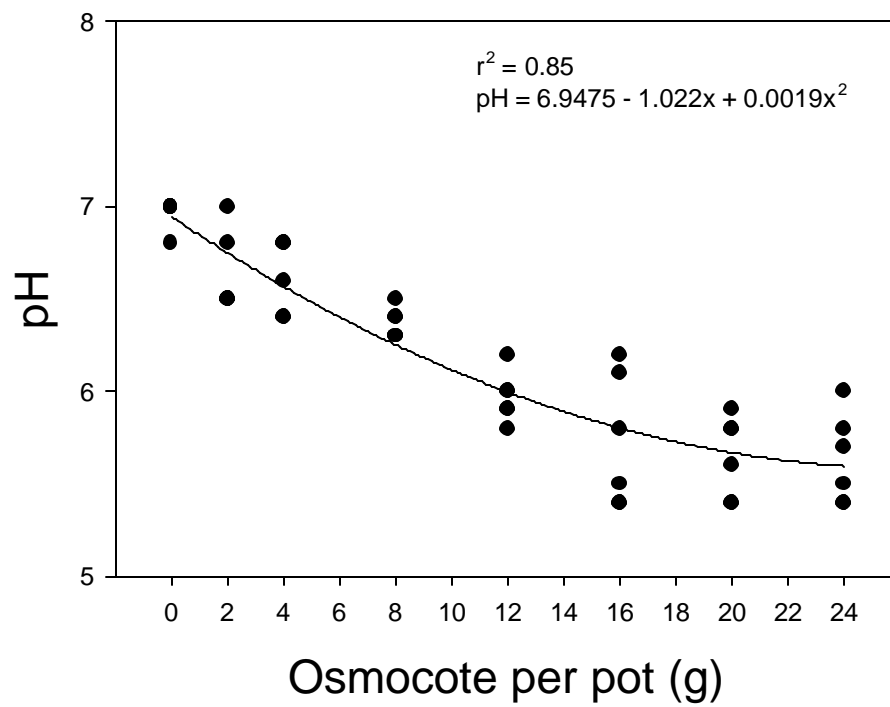


Figure 10. Influence of Osmocote application rate on pH in leachate collected from English boxwood 11 weeks following treatment initiation ($p < 0.0001$).

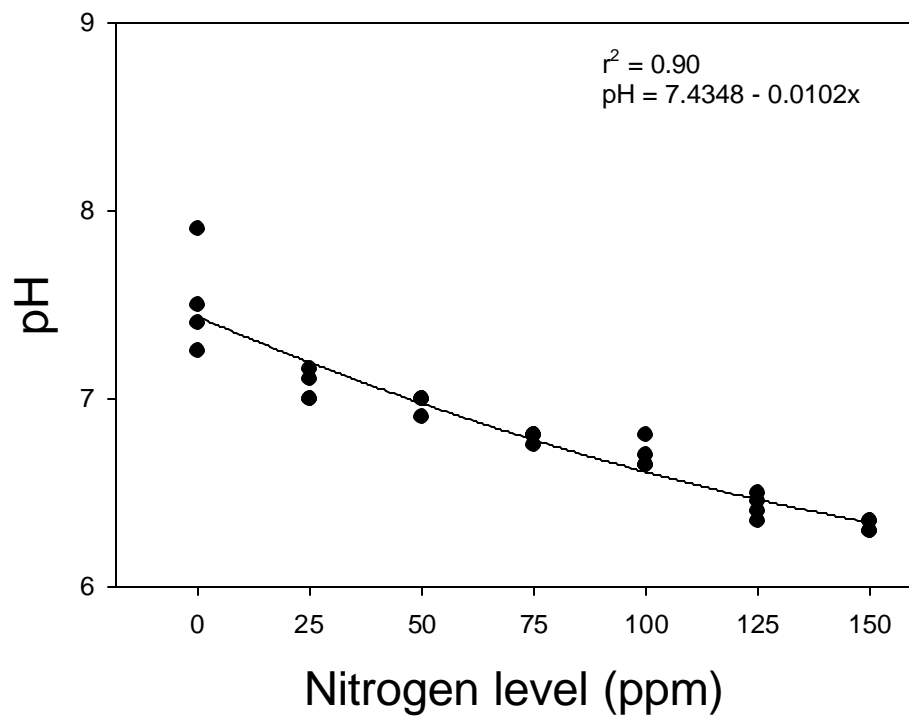


Figure 11. Influence of fertilizer level as ppm N in irrigation water on pH in leachate collected 16 weeks following treatment initiation from 'Justin Brouwers' boxwood ($p < 0.0001$).

Appendix B

	'Vardar Valley'		'Justin Brouwers'		<u>English</u>	
	New shoot number	Shoot dry weight	New shoot number	Shoot dry weight	New shoot number	Shoot dry weight
Source	<u>Pr > F</u>					
Treatment	0.0012	0.0084	<.0001	<.0001	<.0001	0.0016
Treatment	<u>Means</u>					
Prom+/Prun+	5.8	3.4	33.8	4.5	14.9	4.7
Prom+/Prun-	1.9	2.8	23.2	6.2	2.2	5.9
Prom-/Prun+	3.4	2.6	16.3	4.3	11.8	4.1
Prom-/Prun-	0.6	2.2	9.9	5.1	1.1	4.9

Table 2. Effect of Promalin and pruning on new shoot number and shoot dry weight of 'Vardar Valley,' 'Justin Brouwers,' and English boxwood 20 weeks following treatment initiation. Untransformed means presented here.

VITA

Sheri Ruth Musselwhite was born July 10, 1973 in Winston-Salem, North Carolina and was raised in Wilmington, N.C. She graduated from John T. Hoggard High School in May of 1991 and then went on to the University of North Carolina at Greensboro where she received a B.A. in Sociology in 1995. Following graduation, Sheri completed a year of service with Americorps, and she worked in retail horticultural sales until 1998 at which point she returned to college. While at North Carolina State University studying horticultural science, she received several academic scholarships and spent a semester with the National Student Exchange at New Mexico State University. She then began graduate work at Virginia Polytechnic Institute and State University in the summer of 2000. Sheri is a member of Pi Alpha Xi and the Golden Key National Honor Society. Sheri completed her Master of Science in Horticulture in May of 2002.