

ABSTRACT

Conden, Peter John. Propagation of *Castanopsis sclerophylla* and *Lindera umbellata* by Stem Cuttings and Nitrogen Nutrition of Containerized *Ternstroemia gymnanthera*. (Under the direction of Frank A. Blazich).

Stem cuttings of *Castanopsis sclerophylla* (Lindley & Paxton) Schottky were taken on three dates representing three growth stages (softwood, semi-hardwood, or hardwood). Semi-hardwood and hardwood cuttings were treated with 0, 2500, 5000, 7500, or 10,000 ppm of the free acid of indolebutyric acid (IBA) dissolved in 50% isopropyl alcohol, whereas softwood cuttings were treated with the same concentrations of the potassium (K) salt of IBA (K-IBA) dissolved in distilled water. Cuttings were placed in a raised greenhouse bench under intermittent mist. After 2 weeks, cuttings taken at the semi-hardwood and hardwood stages began to drop their leaves and the majority eventually died, with negligible rooting of surviving cuttings. Response of softwood cuttings to K-IBA was quadratic with the greatest rooting (63%) at 7500 ppm K-IBA. However, root number and root length were not significantly affected by K-IBA.

Softwood, semi-hardwood, or hardwood stem cuttings of *Lindera umbellata* Bl. (Chinese spicebush) were taken on three dates, each representing a different growth stage. Semi-hardwood and hardwood cuttings were treated with 0, 2500, 5000, 7500, or 10,000 ppm of the free acid of IBA dissolved in 50% isopropyl alcohol, whereas softwood cuttings were treated

with the same concentrations of K-IBA dissolved in distilled water. All cuttings were placed in a raised greenhouse bench under intermittent mist. After 12 weeks the cuttings were harvested and data recorded. The majority of the hardwood cuttings died, with none of the survivors rooting. Softwood cuttings survived but only 4.5% rooted. Response of the semi-hardwood cuttings to IBA was quadratic with the greatest rooting (73%) for cuttings treated with 7500 ppm IBA. However, root number and root length were not significantly affected by IBA.

Rooted stem cuttings of Japanese ternstroemia (*Ternstroemia gymnanthera* Thunb.) were grown in 3.8-L (1 gal) plastic containers utilizing 8 pine bark : 1 sand (by vol.) amended with micronutrients and dolomite. Plants were fertilized every other day with a solution consisting of P ($K_2H_2PO_4$) at a constant rate of 30 ppm, K (K_2SO_4) at a constant rate of 60 ppm, and a variable rate of N (NH_4NO_3) at 0, 10, 20, 40, 80, 160, or 320 ppm. Leaf area and shoot (stems and leaves) dry weight increased with increasing N application rate (NAR) until a plateau was reached at 117 ppm. Root : shoot ratio was 0.8 with N at 0 ppm, increased to 0.9 with N at 10 ppm, then decreased to ≈ 0.1 with N at 104 ppm. Root dry weight and root area increased in response to increasing NARs, reaching a plateau with N at 86 and 70 ppm respectively. Leaf weight ratio (leaf dry weight / total plant dry weight) increased from 0.2 with N at 0 ppm to a plateau of ≈ 0.6 with N at 109 ppm. Stem weight ratio (stem dry weight / total plant dry weight) was 0.4 with N at 0 ppm then decreased to a plateau of ≈ 0.3 with N at 52 ppm. Root

weight ratio (root dry weight / total plant dry weight) decreased steadily from 0.4 with N at 0 ppm to ≈ 0.1 with N at 117 ppm. Shoot N, P, K, and S concentrations increased with increasing NARs, reaching plateaus at 117, 23, 124, and 183 ppm, respectively, while Mg was unaffected by NAR. Calcium concentrations increased to 0.75% with a NAR of 40 ppm, and decreased to a plateau of 0.6 % with N at 107 ppm. Root mineral nutrient concentrations of N, P, K, and S increased with increasing NARs, reaching plateaus of 287, 53, 39, and 195 ppm respectively, whereas Ca and Mg were not affected by NAR.

**Propagation of *Castanopsis sclerophylla* and *Lindera umbellata* by Stem
Cuttings and Nitrogen Nutrition of Containerized
*Ternstroemia gymnanthera***

by

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A thesis submitted to the Graduate Faculty of
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Approved by:

Chair, Advisory Committee

Dedication

To

Marcia K. Sachs

My loving wife who followed me to North Carolina and without whose support, patience, and understanding none of this would have been possible

and to

my family, friends, and fellow graduate students for
making this all that much more enjoyable.

BIOGRAPHY

Peter J. Conden was born in Staten Island, New York on April 15, 1962, where he attended primary and secondary school. After graduation from high school in 1980, Peter enrolled in the Computer Technology Program at The College of Staten Island, and he graduated with an AAS degree in Fall 1983. In Spring 1984, Peter continued his education as a student in the Electrical Engineering Program at Trenton State College, Trenton, N.J. Prior to completing his degree, Peter accepted the position of Radar Technician with GE Aerospace, Moorestown, N.J., where he assisted in development of the United States Navy's Aegis Radar System. After 5 years, he realized that electronics was not the field for him, so in Spring 1990, Peter enrolled in the department of Ornamental Horticulture at Delaware Valley College, Doylestown, Pa. In Spring 1993, Peter graduated magna cum laude with a BS degree in Ornamental Horticulture. During his last semester at Delaware Valley College, he met Marcia Sachs, and they were married 1 year later. After graduation, Peter moved to northern New Jersey and worked for a bedding plant grower and two landscape nurseries before starting his own landscape contracting business, The Perennial Gardener, in 1995. In Spring 2000, began graduate studies at North Carolina State University to work toward a MS degree in Horticultural Science. Upon completion, Peter will remain at North Carolina State University to pursue a PhD in Horticultural Science. Peter hopes to secure an academic position at a university upon completion of the doctorate.

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Other people deserving thanks and recognition include William Reece and Juan Acedo for their invaluable technical assistance, and Dr. William Swallow for guidance in statistical analysis.

Last but not least, I extend my deepest thanks to my fellow graduate students, as well as the entire staff and faculty of the Horticultural Science Department for their friendship and support.

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General Introduction

With a few exceptions such as particular species of *Ilex* L. (holly) and *Magnolia* L. (magnolia), broadleaf evergreen trees are uncommon in the nursery industry, yet are highly prized for their year-round interest. One relatively unknown broadleaf evergreen tree species having several attributes that may be desirable for landscapes in the southeast United States is *Castanopsis sclerophylla* (Lindley and Paxton) Schottky.

Castanopsis sclerophylla, a close relative of the oaks (*Quercus* L. spp), is an evergreen tree in the Fagaceae, growing to a height of 9 to 12 m (29.5 to 39.4 ft). It is indigenous to broadleaf-evergreen forests covering a wide area of central to eastern China where the nuts are gathered and eaten by humans (7). With 10 to 18 cm (3.9 to 7.1 in) long by 4 to 8 cm (1.6 to 3.1 in) wide, glossy evergreen leaves and white flowers covering the tree in spring, this is truly a desirable ornamental plant. At maturity, *C. sclerophylla* becomes a rounded, medium height tree with dense evergreen foliage and attractive exfoliating gray bark. The plant's versatility is demonstrated by the success of 50+ year-old trees growing in full sun and sandy loam soil at the University of Georgia Bamboo Farm, Savannah, GA and a 15-year-old tree thriving in shade and heavy clay soil at the JC Raulston Arboretum, Raleigh, NC, proving it to be suitable for the varied landscape of the southern United States.

No information has been reported on propagation of *C. sclerophylla*, however, Bob McCartney of Woodlanders Nursery, Aiken, SC, grows it from

seed (personal communication), as is the case for propagating most oak species (2). Related evergreen members of the Fagaceae, Ubame oak (*Quercus phillyreoides* A. Gray) (6) and Chinese evergreen oak (*Q. myrsinifolia* Bl.) (5), have been propagated in high percentages utilizing softwood cuttings treated with indolebutyric acid (IBA). Thus, it may be possible to propagate *C. sclerophylla* by stem cuttings which would allow cloning of select genotypes.

Another species with potential for southern landscapes is Chinese spicebush (*Lindera umbellata* Blume), a deciduous shrub in the Lauraceae. It is indigenous to “scrubland” up to elevations of 3000 m (9850 ft) in China from Kiangsi to West Sichuan, and in Honshu, Shikoku, and Kyushu, Japan (9). In its native habitat the plant will reach 6 m (19.7 ft), but a mature specimen growing at the JC Raulston arboretum in Raleigh measured 4 m (13.1 ft) tall with an equal spread. Its narrow oblong leaves with silvery undersides turn a striking orange and yellow color in the fall. Glossy black berries also develop in the fall and the foliage persists providing winter interest. These attributes suggest this species may have considerable landscape potential for the southeastern United States. No research has been reported on propagation of Chinese spicebush by stem cuttings, however Japanese spicebush (*L. obtusiloba* Bl.) has been propagated by semi-hardwood cuttings treated with IBA, as well as by seed (1).

On the other hand, Japanese ternstroemia (*Ternstroemia gymnanthera* Thunb.), an evergreen shrub in the Theaceae, has become a staple in the nursery industry of the southeastern United States. The species is valued for its

ease of propagation, both by seed and stem cuttings (1,8), its adaptability to sun and shade, its 7 to 10 cm (2.8 to 3.9 in) long by 1.3 to 4 cm (0.5 to 1.6 in) wide, rich green glossy leaves, and bright red fruit in the fall. Japanese ternstroemia is native to Japan, India, and Malaysia where it grows as a large shrub to small tree and reaches a height of 9 m (29.5 ft). This plant is often mislabeled in the nursery trade as *Cleyera japonica* Thunb., also a member of the Theaceae, which has larger leaves and black fruit (3). Although Japanese ternstroemia is common in the nursery trade, no information has been reported regarding mineral nutrition of containerized plants.

Nitrogen (N) affects plant growth more than any other mineral nutrient and increases total plant dry weight following a quadratic response (4). Once maximum growth is achieved, increasing N concentrations provide no additional growth and a point of toxicity is reached where growth is retarded. Excessive N application results in substrate leaching and may be a source of ground water contamination, currently a topic of great concern to the nursery industry (10).

Therefore, the following research was conducted with two objectives. The first objective was to develop protocols for propagation of *C. sclerophylla* and *L. umbellata* by stem cuttings. A second objective was to determine the influence of fertilizer N concentration on growth and mineral nutrient status of containerized Japanese ternstroemia.

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Chapter 1

Propagation of *Castanopsis sclerophylla* by Stem Cuttings

(In the format appropriate for submission to the
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Propagation of *Castanopsis sclerophylla* by Stem Cuttings¹

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Abstract

Stem cuttings of *Castanopsis sclerophylla* (Lindley & Paxton) Schottky were taken on three dates representing three growth stages (softwood, semi-hardwood, or hardwood). Semi-hardwood and hardwood cuttings were treated with 0, 2500 (0.25%), 5000 (0.5%), 7500 (0.75%), or 10,000 (1%) ppm of the free acid of indolebutyric acid (IBA) dissolved in 50% isopropyl alcohol, whereas softwood cuttings were treated with the same concentrations of the potassium (K) salt of IBA (K-IBA) dissolved in distilled water. Cuttings were placed in a raised greenhouse bench and rooted under intermittent mist. After approximately 2 weeks, cuttings taken at the semi-hardwood and hardwood stages began to drop their leaves and the majority eventually died, with negligible rooting of surviving cuttings. The response of the softwood cuttings to K-IBA was quadratic with the greatest rooting (63%) at 7500 ppm K-IBA. However, root number and root length were not significantly affected by K-IBA treatment.

Index words: auxin, indolebutyric acid, adventitious rooting, Fagaceae

Significance to the Nursery Industry

Castanopsis sclerophylla is a medium sized, rounded, evergreen tree, with considerable landscape potential for the southeastern United States. Although this plant can be readily propagated by seed, propagation by stem cuttings would allow cloning of desirable genotypes. Results of this study demonstrated that stem cuttings of *Castanopsis sclerophylla* can be rooted at percentages > 60% when taken at the softwood stage and treated with 7500 ppm K-IBA.

Introduction

Castanopsis sclerophylla is an evergreen tree in the Fagaceae and is indigenous to broadleaf-evergreen forests covering a wide area of central to eastern China. In its native habitat, the species grows to a height of 9 to 12 m (30 to 40 ft), and the nuts are gathered and eaten by humans (12). In addition, the ornamental characteristics of this plant may pique the interest of plant enthusiasts and the nursery industry alike. With 10 to 18 cm (3.9 to 7.1 in) long by 4 to 8 cm (1.6 to 3.1 in) wide, glossy evergreen leaves and white flowers covering the tree in spring, this is truly a desirable ornamental plant with potential to become a popular landscape species.

At maturity, *C. sclerophylla* becomes a rounded, medium height tree with dense evergreen foliage and attractive exfoliating gray bark. The species is adaptable to a wide range of environmental conditions, as demonstrated by the success of 50+ year-old trees growing in full sun and sandy loam soil at the

University of Georgia Bamboo Farm, Savannah, GA and a 15-year-old tree thriving in shade and heavy clay soil at the JC Raulston Arboretum, Raleigh, NC. Related evergreen oaks have also been used successfully as ornamentals for the southeast United States (6), and are gaining popularity in the nursery trade.

Although there are over 120 species of *Castanopsis* (D. Don) Spach growing in China, south and east Asia, and one in North America, only a few species are in cultivation (8), with *C. sclerophylla* not even listed among those few. *Castanopsis sclerophylla* is very rare in the nursery industry, and no information has been reported on its propagation. However, Bob McCartney of Woodlanders Nursery, Aiken SC, propagates it from seed (personal communication), as is the case for propagating most oak (*Quercus* L.) species (4), which are closely related to *C. sclerophylla*. Related evergreen members of the Fagaceae, ubame oak (*Quercus phillyreoides* A. Gray) (10) and Chinese evergreen oak (*Q. myrsinifolia* Bl.) (9), have been propagated in high percentages utilizing softwood cuttings treated with indolebutyric acid (IBA). Thus, it may be possible to propagate *C. sclerophylla* by stem cuttings, which would allow cloning of select genotypes. Sexual propagation often results in progeny with unpredictable phenotypes as a result of heterozygosity, whereas propagation by cuttings would ensure the grower clones of desirable genotypes. Therefore, the following research was conducted to develop a protocol for propagating *C. sclerophylla* by stem cuttings.

Materials and Methods

Terminal stem cuttings were taken at the hardwood (February 24, 2000), softwood (May 25, 2000), or semi-hardwood (September 28, 2000) stages from a 15-year-old tree in the adult growth phase growing at the JC Raulston Arboretum, Raleigh. For the softwood stage, cuttings were taken when the new leaves were fully expanded and the stems were just firm enough to snap when bent.

Following collection of cuttings, they were placed in plastic bags and kept on ice while being transported to the Horticulture Science Greenhouses, Raleigh. The cuttings were then trimmed from the bases to a 13 cm (5.1 in) length, and all leaves were removed from the lower third of each cutting. The basal 1 cm (0.4 in) of each cutting was treated for 1 sec with 0, 2500 (0.25%), 5000 (0.5%), 7500 (0.75%), or 10,000 (1%) ppm IBA. Semi-hardwood and hardwood cuttings were treated with the free acid of IBA dissolved in 50% isopropyl alcohol whereas the softwood cuttings were treated with the potassium (K) salt of IBA (K-IBA) dissolved in distilled water. After auxin treatment, the cuttings were air dried for 20 min before insertion into a raised greenhouse bench containing a steam pasteurized medium of 1 peat : 1 perlite (by vol.). Greenhouse air temperatures ranged from 18C (65F) to 29C (85F) and natural photoperiod and irradiance were provided. Bottom heat was utilized to maintain the temperature of the rooting medium at a minimum of 21C (70F). Intermittent mist operated 5 sec every 5 min from sunrise to dusk, and chlorothalonil (Daconil, Syngenta, Inc.,

Greensboro, NC), a preventative fungicide treatment, was applied weekly as a spray to runoff, at a concentration of 4 ml/liter (1.0 tsp/gal).

For each growth stage, the experimental design in the mist bed was a randomized complete block with eight cuttings per treatment and six replications. After 10 weeks, the cuttings were harvested and various data recorded to include percentage rooting, and number and length of primary roots ≥ 1 mm (0.04 in). A cutting having one root ≥ 1 mm (0.04 in) was considered rooted. Data for each growth stage were subjected to analysis of variance and regression analysis.

Results and Discussion

After 2 weeks, hardwood and semi-hardwood cuttings began to drop their leaves, and the majority of the cuttings died by the end of 10 weeks. Total rooting across all treatments was negligible at both the hardwood and semi-hardwood stages, with 3% and 1% rooting, respectively; therefore, only data from the softwood stage are presented. Softwood cuttings rooted at much higher percentages with auxin treatment being essential (Table 1). Without K-IBA treatment, rooting was negligible. Auxin treatment stimulated rooting and the response was quadratic with the greatest rooting (63%) occurring for cuttings treated with 7500 ppm K-IBA. Although root number appeared to increase from 2.0 for cuttings treated with 2500 ppm K-IBA to 4.1 for cuttings treated with 10,000 ppm K-IBA, root number was not significantly affected (Table 1).

Similarly, average root length was not influenced by auxin treatment and ranged from 4.6 to 8.0 cm (1.8 to 3.1 in).

The question could be raised that the isopropyl alcohol used to dissolve the free acid of IBA, or the IBA itself, was toxic to the semi-hardwood and hardwood cuttings, resulting in high mortality and negligible rooting. However, the nontreated semi-hardwood and hardwood cuttings also died during the course of the experiment, suggesting this was not the case. To further test this, another experiment was conducted in September 2001 in which semi-hardwood cuttings were treated with 0, 2500 (0.25%), 5000 (0.5%), 7500 (0.75%), or 10,000 (0.1%) ppm K-IBA, and placed in a propagation bed having intermittent mist. These cuttings responded in a similar manner to semi-hardwood and hardwood cuttings treated with the free acid of IBA dissolved in alcohol, with leaf drop and onset of necrosis of the basal portions by the second week, and death of most cuttings by 5 weeks, indicating that the auxin and/or isopropyl alcohol were not toxic to the cuttings.

Results clearly indicate that rooting stem cuttings of *C. sclerophylla* can best be achieved with softwood cuttings and that auxin treatment is imperative. These results agree with previous reports by McGuigan et al. (9,10) in which evergreen oaks were also propagated in high percentages using softwood cuttings. Although in the present investigation, the best rooting (63%) was achieved with 7500 ppm K-IBA, the authors feel rooting can be increased further by manipulating various environmental conditions [e.g., water relations (mist)]

during rooting. In addition, roots of the rooted cuttings were observed to be brittle and were easily broken by routine handling. Based on this, the authors recommend rooting *C. sclerophylla* cuttings in small individual containers or in flats with individual cells to avoid damage to roots during transplanting which can result in transplant shock.

Following rooting, the softwood cuttings were transplanted into individual 3.8-L (1gal) containers using a medium of 8 pine bark : 1 sand (by vol.). Each container was top-dressed with an 8-9 month controlled-release fertilizer having an analysis of 18N-6P₂O₅-12K₂O plus micronutrients (Wilbro, Inc., Norway, SC) at the rate of 9.0 g (0.32 oz) per container, and placed in a lathe house at the Horticulture Field Lab, Raleigh, where they received daily overhead irrigation. Approximately 90% of the plants produced a flush of new growth during the summer, and overwinter survival percentages were high (≈80%). Whether or not a growth flush following rooting is essential for overwinter survival of rooted stem cuttings of *C. sclerophylla* is unknown, although other studies of various woody species have reported increased over-winter survival of rooted cuttings which produced a flush of growth following rooting (1,9,13,14).

Results of this study were based on stem cuttings taken from a single tree (clone) of *C. sclerophylla*. However, the possibility exists that tree-to-tree variation in rooting may exist for this species in which case rooting would vary depending on the particular clone from which cuttings were taken. This would not be surprising since clonal differences in rooting have been reported for many

woody species (2,3,5,7,10,11), and based on these reports, there is a high probability that the same phenomenon exists for *C. sclerophylla*.

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Table 1. Effects of K-IBA treatments on rooting of softwood cuttings of *Castanopsis sclerophylla*.

Treatment (ppm K-IBA)	Rooting ^z (%)	Mean root no. ^y	Mean root length ^y (mm)
Nontreated ^x	2.5	2.0	3.5
2500	15.0	2.0	80.4
5000	37.5	2.1	46.2
7500	62.5	3.5	60.7
10,000	47.5	4.1	63.8
Linear	NS	NS	NS
Quadratic	*	NS	NS

^zEach value is based on 48 cuttings.

^yEach value is based on the number of cuttings which rooted for a particular treatment.

^xNontreated cuttings were not included in the statistical analysis.

NS, * Nonsignificant or significant at $P < 0.05$, respectively

Chapter 2

Propagation of *Lindera umbellata* by Stem Cuttings

(In the format appropriate for submission to the
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Propagation of *Lindera umbellata* by Stem Cuttings¹

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Abstract

Stem cuttings of *Lindera umbellata* Bl. (Chinese spicebush) were taken on three dates representing three growth stages (softwood, semi-hardwood, or hardwood). Semi-hardwood and hardwood cuttings were treated with 0, 2500 (0.25%), 5000 (0.5%), 7500 (0.75%), or 10,000 (1%) ppm of the free acid of indolebutyric acid (IBA) dissolved in 50% isopropyl alcohol, whereas softwood cuttings were treated with the same concentrations of the potassium (K) salt of IBA (K-IBA) dissolved in distilled water. All cuttings were placed in a raised greenhouse bench and rooted under intermittent mist. After 12 weeks, the cuttings were harvested and various data recorded. The majority of the hardwood cuttings died, with none of the survivors rooting. Softwood cuttings survived but with overall rooting of only 4.5%. Response of the semi-hardwood cuttings to IBA was quadratic with the greatest rooting (73%) observed for those cuttings treated with 7500 ppm IBA. However, root number and root length were not significantly affected by IBA treatment.

Index words: auxin, indolebutyric acid, adventitious rooting, Lauraceae

Significance to the Nursery Industry

Lindera umbellata (Chinese spicebush) is a large deciduous shrub distinguished by extraordinary fall color with persisting, silvery beige foliage and glossy black fruit (drupes), providing winter interest. The species is also resistant to environmental stresses, pests, and various diseases, and is therefore an excellent choice for a low maintenance, ornamental shrub. This study demonstrated that *L. umbellata* can be propagated by stem cuttings in high percentages with best results (73%) obtained from semi-hardwood cuttings treated with 7500 ppm IBA. However, overwinter survival of the rooted cuttings was extremely poor.

Introduction

There are many species of *Lindera* Thunb. suitable for landscapes in the southeast United States. These plants exhibit resistance to environmental stress, insect pests and disease and are adaptable to a wide range of growing conditions (12).

Lindera umbellata (Chinese spicebush) is an attractive deciduous species in the Lauraceae. It is indigenous to “scrubland” up to elevations of 3000 m (9850 ft) in China from Kiangsi to West Sichuan, and in Honshu, Shikoku, and Kyushu, Japan (11) where it grows as a spreading, large shrub. In its native habitat, the plant can reach 6 m (19.7 ft), and a mature specimen growing at the JC Raulston Arboretum, Raleigh, NC measured 4 m (13.1 ft) tall

with an equal spread. Its 7.6-to 12.7-cm (3-to 5-in) long by 2.5-cm (1-in) wide, narrow oblong leaves with silvery undersides turn a striking orange and yellow color in the fall. Glossy, round black fruit (drupes), 8 mm (0.31 in) in diameter (7), also mature in the fall and the foliage turns silvery beige in winter and persists, providing year round interest. There are no known insect or disease problems associated with Chinese spicebush, and it has exhibited drought tolerance at the JC Raulston Arboretum, where it is grown in full sun with no supplemental irrigation. These attributes suggest this species may have considerable landscape potential for the southeastern United States.

In addition to its ornamental qualities, *L. umbellata*, as well as other *Lindera* spp., have been studied for their potential roles in modern medicine (1,8,9), as well as having a history of traditional medicinal use. Recent research has found that bark extracts from *L. umbellata* inhibit growth of melanoma cells (16). If this species proves to be useful in the treatment of certain diseases, asexual propagation will be essential for generating clones of desired genotypes, with propagation by stem cuttings being the simplest and least expensive method.

Sexual (seed) propagation has been reported for several species of *Lindera*, although at the risk of unpredictable plant-to-plant variation (6). Propagation by stem cuttings, however, would facilitate cloning of desirable genotypes, possibly leading to named cultivars. No research has been reported on propagation of *L. umbellata* by seed or stem cuttings, although *L. obtusiloba*

Bl. (Japanese spicebush) has been propagated sexually and by semi-hardwood cuttings treated with indolebutyric acid (IBA) (4). The native North American *L. benzoin* (L.) Bl., is reported to have been rooted from “half-ripe” stem cuttings, but in low percentages, and can also be propagated by seed (4). With the aforementioned reports supporting the possibility of vegetative propagation of *L. umbellata*, the following research was conducted to investigate the feasibility of propagating the species by stem cuttings.

Materials and Methods

Terminal stem cuttings were taken on June 22, 2000 (softwood), August 3, 2000 (semi-hardwood), or December 9, 2000 (hardwood), from a single plant in the adult growth phase growing at the JC Raulston Arboretum. Following collection, cuttings were placed in plastic bags and kept on ice while being transported to the Horticultural Science Greenhouses, Raleigh. Cuttings of all growth stages were then trimmed from the bases to 12 cm (4.7 in), and leaves of the softwood and semi-hardwood cuttings were removed from the lower third of each cutting. Cuttings taken at the hardwood stage were stripped of flower buds prior to treatment. The basal 1 cm (0.4 in) of each cutting was treated for 1 sec with 0, 2500 (0.25%), 5000 (0.5%), 7500 (0.75%), or 10,000 (1.0%) ppm IBA. Semi-hardwood and hardwood cuttings were treated with the free acid of IBA dissolved in 50% isopropyl alcohol while the softwood cuttings were treated with the potassium (K) salt of IBA (K-IBA) dissolved in distilled water. After auxin

treatment, the cuttings were air dried for 15 min before insertion into a raised greenhouse bench containing a steam pasteurized medium of 1 peat : 1 perlite (by vol.). Greenhouse air temperatures ranged from 18C (65F) to 29C (85F) and natural photoperiod and irradiance were provided. Bottom heat was utilized to maintain the minimum temperature of the rooting medium at 21C (70F). Intermittent mist operated 5 sec every 5 min from sunrise to dusk, and chlorothalonil (Daconil; Syngenta, Inc., Greensboro, NC), a preventative fungicide treatment, was applied weekly as a spray to runoff, at a concentration of 4 ml/liter (1.0 tsp/gal).

For each growth stage, the experimental design in the mist bed was a randomized complete block with eight cuttings per treatment and six replications. After 12 weeks, the cuttings were harvested and various data recorded to include percentage rooting, and number and length of primary roots ≥ 1 mm (0.04 in). A cutting having one root ≥ 1 mm (0.04 in) was considered rooted. Data for each growth stage were subjected to analysis of variance and regression analysis.

Results and Discussion

The majority of the hardwood cuttings (87%) died by the end of the 12-week rooting period, and none of the surviving cuttings rooted. On the other hand, 75% of the softwood cuttings survived, although only 4.5% rooted, mostly with one short root ≈ 10 mm (0.4 in) in length. Each of the surviving softwood cuttings developed large masses of callus on the basal portion, each being an

average of 1.9 cm (0.75 in) in diameter. Auxin treatment had no effect on rooting of softwood cuttings, although callus formation increased with higher auxin concentrations. It is unknown whether the callus blocked adventitious roots from emerging, or if adventitious roots would have eventually developed had the cuttings been left in the bench for a longer period. Stem cuttings of various genera such as oak (*Quercus* L.) and olive (*Olea* L.) have also been reported to produce callus but no roots during rooting experiments (3,15).

The best results were observed at the semi-hardwood stage, with auxin treatment having a quadratic effect on percentage rooting (Table 1). Cuttings across all treatments rooted, with the highest percentage from cuttings treated with 7500 ppm IBA (73%). Although auxin treatments significantly affected rooting, the nontreated cuttings also rooted (23%), indicating that auxin treatment was not absolutely essential for rooting at the semi-hardwood stage. Callus began to develop on the basal ends of the semi-hardwood cuttings by week 3 of the experiment, and increased in diameter by week 12 [\approx 1.9 cm (0.75 in)].

Mean root length appeared to exhibit a quadratic trend to auxin treatment, however it was not significant ($P=0.06$) and ranged from 30.6 mm (1.2 in) to 53.9 mm (2.1 in) (Table 1). Although mean root number also appeared to follow a quadratic response to IBA treatment, the differences were very small and it was also not statistically significant ($P=0.51$).

Following evaluation, 70 rooted cuttings from the semi-hardwood growth stage were transplanted into individual 0.9-L (1 qt) containers using a medium of

8 pine bark : 1 sand (by volume), and topdressed with 3 g of a 3-4 month, controlled-release fertilizer having an analysis of 15N-9P₂O₅-12K₂O plus micronutrients (Osmocote, Scotts-Sierra Hort. Products Co., Marysville, OH).

The containers were placed on a gravel pad at the Horticulture Field Laboratory, Raleigh, where they received daily overhead irrigation.

None of the transplanted cuttings produced a flush of new growth before being placed in an unheated greenhouse for overwintering. The following spring, only six of the 70 plants produced a flush of growth, and of these six, none survived the first growing season. This suggests that *L. umbellata* may require a flush of growth before overwintering in order to survive. Poor overwinter survival is not uncommon for cuttings which have not produced a flush of new growth following rooting, and various strategies have been investigated in attempts to overcome this problem (2,5,10,13,14,17). Although results herein demonstrate that semi-hardwood stem cuttings of *L. umbellata* can be rooted in percentages > 70%, additional research is necessary to improve overwinter survival of rooted cuttings. In actuality this problem must be overcome before rooting stem cuttings can be regarded as a successful means to propagate *L. umbellata*.

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Table 1. Effects of IBA treatments on rooting of semi-hardwood cuttings of *Lindera umbellata*.

Treatment (ppm IBA)	Rooting ^z (%)	Mean root no. ^y	Mean root length ^y (mm)
Nontreated	23.0	2.6	23.6
2,500	43.7	2.7	42.2
5,000	56.2	3.5	53.9
7,500	73.0	3.8	38.4
10,000	58.3	3.0	29.4
Linear	NS	NS	NS
Quadratic	*	NS	NS

^zEach value is based on 48 cuttings.

^yEach value is based on the number of cuttings which rooted for a particular treatment.

NS, * Nonsignificant or significant at $P < 0.05$, respectively

Chapter 3

Nitrogen Nutrition of Containerized *Ternstroemia gymnanthera*

(In the format appropriate for submission to the
Journal of Environmental Horticulture)

Nitrogen Nutrition of Containerized *Ternstroemia gymnanthera*¹

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Abstract

Rooted stem cuttings of Japanese ternstroemia (*Ternstroemia gymnanthera* Thunb.) were grown in 3.8-L (1 gal) plastic containers utilizing a substrate of 8 pine bark : 1 sand (by vol.) amended with micronutrients and dolomitic limestone. Plants were fertilized every other day with a solution consisting of P ($K_2H_2PO_4$) at a constant rate of 30 mg/L (ppm), K (K_2SO_4) at a constant rate of 60 mg/L (ppm), and a variable rate of N (NH_4NO_3) at 0, 10, 20, 40, 80, 160, or 320 mg/L (ppm). Leaf area and shoot (stems and leaves) dry weight increased with increasing N application rate (NAR) until a plateau was reached at 117 mg/L (ppm). Root : shoot ratio was 0.8 with N at 0 mg/L (ppm), increased to 0.9 with N at 10 mg/L (ppm), then decreased to ≈ 0.1 with N at 104 mg/L (ppm). Root dry weight and root area increased in response to increasing NARs, reaching a plateau with N at 86 and 70 mg/L (ppm) respectively. Leaf weight ratio (leaf dry weight / total plant dry weight) increased from 0.2 with N at 0 mg/L (ppm) to a plateau of ≈ 0.6 with N at 109 mg/L (ppm). Stem weight ratio (stem dry weight / total plant dry weight) was 0.4 with N at 0 mg/L (ppm) then decreased to a plateau of ≈ 0.3 with N at 52 mg/L (ppm). Root weight ratio (root dry weight / total plant dry weight) decreased steadily from 0.4 with N at 0 mg/L (ppm) to ≈ 0.1 with N at 117 mg/L (ppm). Shoot N, P, K, and S concentrations increased with increasing NARs, reaching plateaus at 117, 23, 124, and 183 mg/L (ppm), respectively, while Mg was unaffected by NAR. Calcium concentrations increased to 0.75% with a NAR of 40 mg/L (ppm), and decreased to a plateau of 0.6 % with N at 107 mg/L (ppm).

Root mineral nutrient concentrations of N, P, K, and S increased with increasing NARs, reaching plateaus of 287, 53, 39, and 195 mg/L (ppm) respectively, whereas Ca and Mg were not affected by NAR.

Index Words: mineral nutrition, container production, fertilization

Significance to the Nursery Industry

Japanese ternstroemia (*Ternstroemia gymnanthera* Thunb.) is an upright evergreen shrub that is valued for its versatility as a specimen plant or as an informal hedge for both sun and shade. However, no information has been reported on the mineral nutrition requirements for this species. Results of this research indicate that maximum growth during containerized culture can be attained by applying ammonium nitrate at a rate of 117 mg/L (ppm), with a corresponding substrate solution electrical conductivity (EC) of 600 $\mu\text{S/m}$, or a 3-4 month controlled-release fertilizer (19N-6P₂O₅-12K₂O, Osmocote, Scotts-Sierra Hort. Prod. Co., Marysville, OH) at a rate of 12 g (0.4 oz) per pot, with a substrate solution EC of 690 $\mu\text{S/m}$.

Introduction

Japanese ternstroemia, an evergreen shrub in the Theaceae, has become a staple in the nursery industry of the southeastern United States. The species is valued for its ease of propagation, both by seed and stem cuttings (5,12,13), and its adaptability to sun and shade. The plant's ornamental features include 7 to 10 cm (2.8 to 3.9 in) long by 1.3 to 4 cm (0.5 to 1.6 in) wide, dark-green glossy leaves with purple-red new growth, creamy white drooping 2 cm (0.8 in) wide flowers, and bright red fruit in the fall. Compounds isolated from Japanese ternstroemia are also being studied for use as anti-HIV drugs (9).

The species is native to Japan, India, and Malaysia, where it grows as a large shrub to small tree and reaches a height of 9 m (29.5 ft). The plant is often mislabeled in the nursery trade as *Cleyera japonica* Thunb., also a member of the Theaceae, which has larger leaves and black fruit (10). Although Japanese ternstroemia is common in the nursery trade, no information has been reported regarding mineral nutrition of container-grown plants.

Nitrogen affects plant growth more than any other mineral nutrient and increases total plant dry weight following a quadratic response (11). Once maximum growth is achieved, increasing N concentration provides no additional growth and a point of toxicity is reached where growth is retarded. Excessive N application results in substrate leaching and may be a source of ground water contamination, currently a topic of great concern to the nursery industry (18). Therefore, the following research was conducted to determine the influence of fertilizer N concentration on growth and mineral nutrient status of containerized Japanese ternstroemia.

Materials and Methods

On December 14, 2000, 150 rooted stem cuttings of Japanese ternstroemia were potted in 3.8-L (1 gal) containers utilizing a medium of 8 pine bark : 1 sand (by vol.) amended with 0.68 kg /m³ (1.5 lbs /yd³) Micromax (Scotts-Sierra Hort. Prod. Co., Marysville, OH) and 0.9 kg /m³ (2 lbs /yd³) dolomitic limestone. The plants were placed in an unheated greenhouse to receive 3 months of chilling. Seven N

application rates (NAR) were begun on March 26, 2001. At treatment initiation, 10 plants were harvested, roots washed free of substrate, and separated into leaves, stems, and roots. Initial leaf area and root area were measured prior to the plant tissue being dried at 70C (158F) for 96 hr. Leaf area was measured with a LI-COR 3100 Area Meter (LI-COR, Inc., Lincoln, NE). Root area was measured using a Monochrome Agvision System 286 Image Analyzer (Decagon Devices Inc., Pullman WA). Initial leaf area and root area were 16.0 cm² and 18 cm², respectively. Initial dry weights of leaves, stems, and roots were 0.28 g, 0.16 g, and 0.28 g, respectively.

Plants were grown in a glass greenhouse under natural photoperiod and irradiance with greenhouse air temperatures ranging from 18C (65F) to 29C (85F). A fertigation system was designed to deliver the seven N treatments using two Dosatron 16l proportional injectors (Dosatron, Inc., Clearwater, FL) connected in series. One injector was set to provide a constant rate of P (K₂H₂PO₄) at 30 mg/L (ppm) and K (K₂SO₄ and K₂H₂PO₄) at 60 mg/L (ppm), while the other was set to provide N (NH₄NO₃) at 0, 10, 20, 40, 80, 160, or 320 mg/L (ppm). The stock solutions were premixed in containers, the injectors adjusted for a 64 : 1 dilution ratio, and the solutions applied every other day as a constant feed through individual 11.4 L/hr (3 gal /hr), 23 cm (9 in) radius spray stakes (Acu-spray Stick 35, Wade Mfg. Co., Fresno, CA). After each treatment, the lines were flushed with tap water. Initial fertigation volume was 200 mL (6.8 oz) per pot. Leaching fraction was monitored bi-weekly and fertigation volume

was adjusted to maintain leaching fractions of 0.25 (leachate volume ÷ irrigation volume). No additional irrigation was provided.

Substrate solution samples were collected bi-weekly using the pour through extraction method (21). These samples were analyzed for EC and pH using a combination EC/pH meter (Accumet 50, Fisher Scientific Co. Pittsburgh, PA).

Three additional treatments of a 3-4 month controlled-release fertilizer (19N-6P₂O₅-12K₂O Osmocote, Scotts-Sierra Hort. Prod. Co., Marysville, OH) were included as a top dressing at 6, 12, or 18 g (0.2, 0.4, or 0.6 oz) per pot for comparative purposes and were not included in statistical analysis. These plants were irrigated with tap water at the same volume and frequency as the liquid feed plants. The experimental design was a randomized complete block with 10 treatments and 10 single plant replications. Six weeks after initiation of the study, several plants in replication 10 were injured during greenhouse maintenance, therefore nine replications were used in analysis.

After 20 weeks, the study was terminated and all plants harvested. Substrate was washed from the roots and all plants separated into stems, leaves, and roots. Prior to drying, four replications were chosen at random and measurements taken to include leaf area, root area, and root length. Root length was measured using a Monochrome Agvision System 286 Image Analyzer. All plant parts were then dried at 70C (158F) for 96 hr and weighed. Prior to preparation (17) for mineral nutrient (N, P, K, Ca, Mg, and S) analysis, stems and

leaves (shoots) were combined. Mineral nutrient analysis was conducted at the Analytical Service Laboratory, Department of Soil Science, North Carolina State University, Raleigh.

Leaf weight ratio (LWR), stem weight ratio (SWR), and root weight ratio (RWR) were calculated by dividing the dry weight of the respective plant part by total plant dry weight to illustrate that plant part's percentage of the total dry weight in response to changing NAR. Root : shoot ratio (R : S) was calculated as root dry weight ÷ shoot dry weight. Data were subjected to analysis of variance, regression analysis, and a quadratic plateau was fit to the data using PROC NLIN (6,15)

Results and Discussion

Shoot dry weight and leaf area increased with increasing NAR until a plateau was reached at 117 mg/L (ppm) at which point no noticeable growth occurred with increasing NAR (Fig. 1). R : S ratio was 0.8 with N at 0 mg/L (ppm), increased to 0.9 at 10 mg/L (ppm), then decreased to ≈ 0.1 with N at 104 mg/L (ppm) and remained at that level for the remaining NARs. Typically, at low available N, a higher concentration of nitrate is reduced in the roots, while at higher available concentrations, more N is translocated to shoots (11).

Root length and root area had similar responses to NAR, therefore, only root area data are presented. Root dry weight and root area increased in response to increasing NARs, reaching a plateau at 86 and 70 mg/L (ppm)

respectively (Fig. 2). In contrast, other researchers reported decreasing root production in response to increasing available N (3,6,22). Andrews (1) reported that partitioning of N between roots and shoots of tropical and subtropical species can remain fairly constant with increasing external nitrate concentration, which may explain our results with Japanese ternstroemia, which is native to tropical regions. Root dry weight is often used as an indicator of fertilizer response since it is simple to weigh roots. However, root area may be a better response indicator since a greater root surface area allows for increased water and mineral nutrient uptake (14). Root dry weight can be accounted for by a few large roots with relatively little surface area while root area can include an extensive fine root system with lighter weight.

LWR increased from 0.2 with N at 0 mg/L (ppm) to a plateau of ≈ 0.6 with N at 109 mg/L (ppm) (Fig. 3A), which was also reflected in the R : S ratio (Fig. 1C). This was contrasted by SWR, which was 0.4 with N at 0 mg/L (ppm) then decreased to a plateau of ≈ 0.3 with N at 52 mg/L (ppm) (Fig. 3B), indicating that leaf production continued to increase after stem production reached a plateau. RWR decreased steadily from 0.4 with N at 0 mg/L (ppm) to ≈ 0.1 with N at 117 mg/L (ppm). A lower RWR could translate to decreased transplant survivability and growth; however, Cabrera and Devereaux (4) reported this not to be the case for crape myrtle (*Lagerstroemia indica* L. x *L. fauriei* Koehne 'Tonto').

Shoot mineral nutrient (N, P, K, S) concentrations increased with increasing NAR, whereas Ca concentration decreased (Fig. 4). Shoot Mg

concentration, averaging 0.4%, was unaffected by NAR (data not presented).

All shoot mineral nutrient concentrations were in the range of those reported by Jones et al. (7).

Shoot N concentrations increased with increasing NAR until reaching a plateau of 2.1% at NAR of 287 mg/L (ppm) (Fig. 4A). This response was consistent with previous reports (2,6,8,17). Nitrate will be continually absorbed by plants as long as it is present in the soil, with excess nitrate being stored when supply exceeds demand for growth (6). Maximum shoot dry weight was observed at a NAR of 117 mg/L (ppm), with corresponding shoot N concentration of 1.75%. However, since stems and leaves were pooled for tissue analysis in this study, some nutrient concentrations may be lower than those one may find when analyzing leaves alone.

Increasing NARs can suppress uptake of P, K, S, Ca, and Mg (7,8,19,20). In the present investigation, however, shoot P, K, and S concentrations (Fig. 4B,C, and E) increased with increasing NAR, reaching plateaus of 0.6%, 1.9%, and 0.27% at NARs of 23, 124, and 183 mg/L (ppm), respectively. Magnesium was unaffected by NAR (data not presented). Calcium concentrations (Fig. 4D) increased to 0.75% at a NAR of 40 mg/L (ppm), and decreased to a plateau of 0.6% at 107 mg/L (ppm). This may have been caused by dilution of Ca in shoot tissue due to growth (11).

Root mineral nutrient concentrations of N, P, K, and S increased with increasing NAR (Fig. 5), whereas Ca (0.17%) and Mg (0.2%) were not affected

by NAR (data not presented). Shoot mineral nutrient concentrations were similar to those found in the roots, except the root nutrient concentrations for N, P, and S reached plateaus at higher NARs than the shoots. The concentration of K in the roots reached a plateau of 1.5% at a NAR of 39 mg/L (ppm) while the shoot concentration of K reached a plateau of 1.9% with N at 124 mg/L (ppm).

Maximum growth was predicted with N at 117 mg/L (ppm) with a corresponding substrate solution EC of 600 $\mu\text{S}/\text{m}$, which is within the desired range for liquid fertilizer treatments (16). For the CRF treatments, growth was maximized at 12 g (0.4 oz) per pot, with a substrate solution EC of 690 $\mu\text{S}/\text{m}$. Throughout the experiment, substrate solution pH measurements for all N treatments were within acceptable range, with the exception of N at 320 mg/L (ppm), which decreased below pH 4 at week 8 and remained low for the remainder of the experiment.

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List of Figures

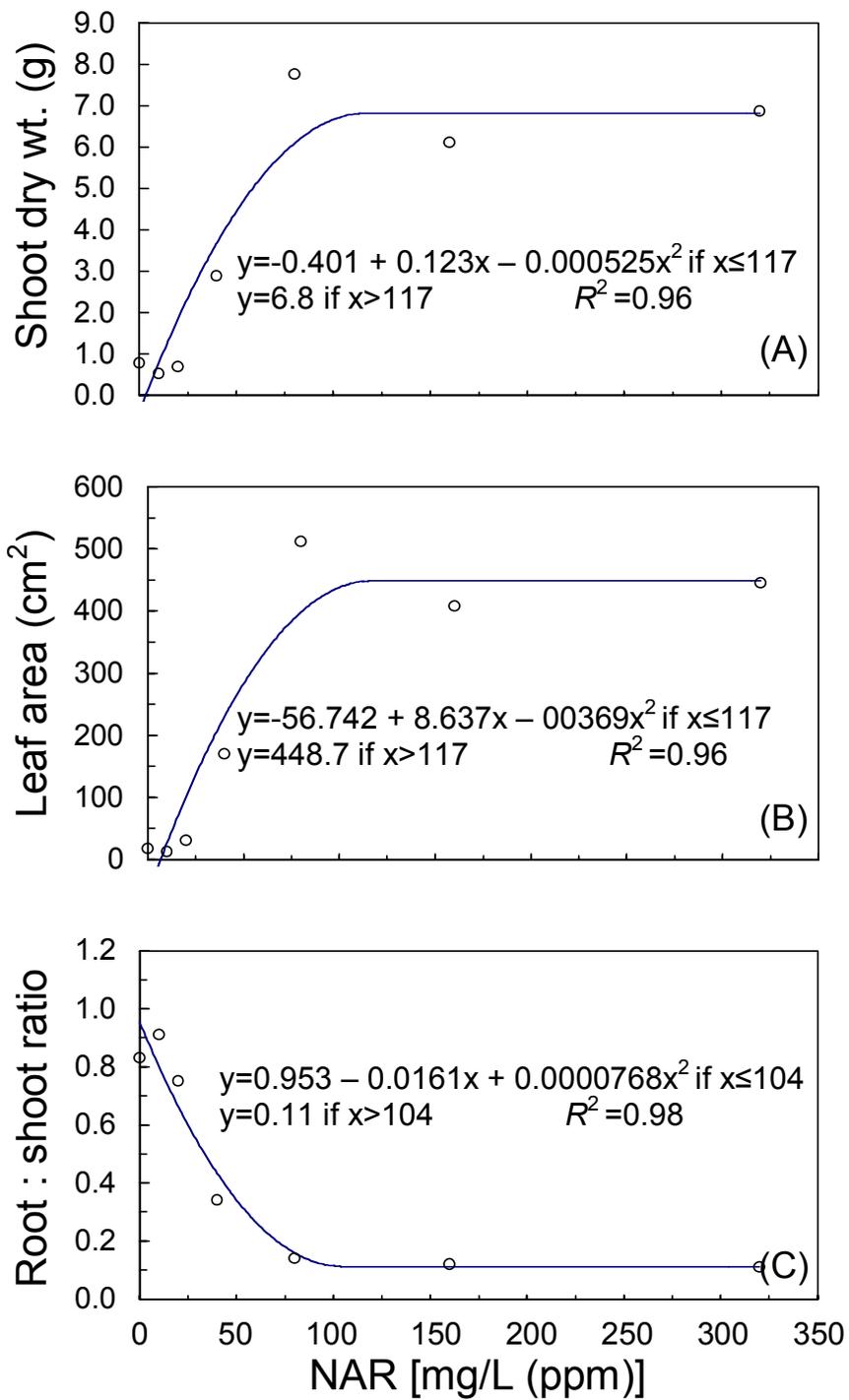
Fig. 1. Effect of nitrogen application rate (NAR) on (A) shoot dry weight, (B) leaf area, and (C) root : shoot ratio of containerized Japanese ternstroemia.

Fig. 2. Effect of nitrogen application rate (NAR) on (A) root dry weight and (B) root area of containerized Japanese ternstroemia.

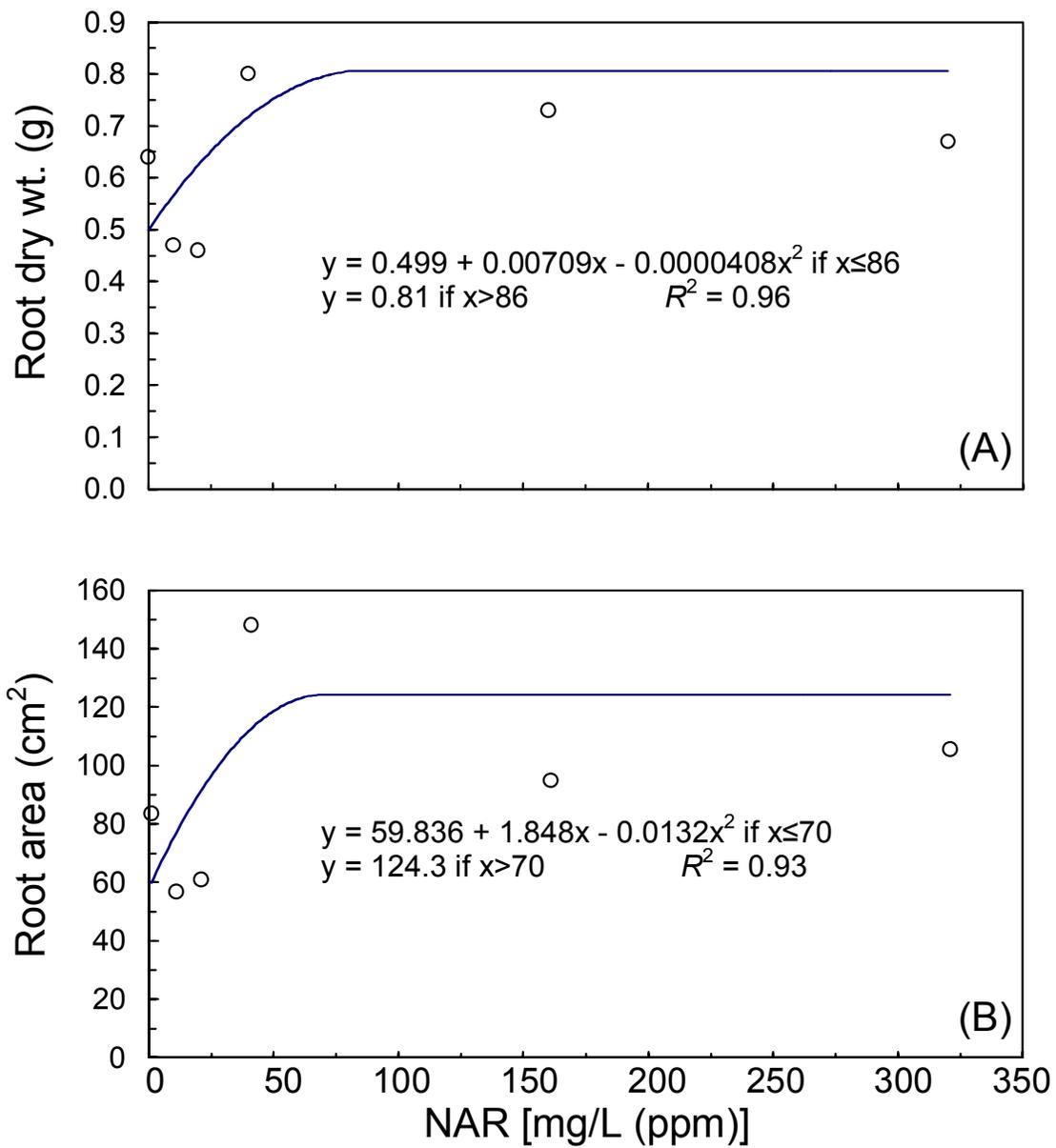
Fig. 3. Effect of nitrogen application rate (NAR) on (A) leaf weight ratio (leaf dry weight / total plant dry weight), (B) stem weight ratio (stem dry weight / total plant dry weight), and (C) root weight ratio (root dry weight / total plant dry weight) of containerized Japanese ternstroemia.

Fig. 4. Effect of nitrogen application rate (NAR) on shoot mineral nutrient concentration of containerized Japanese ternstroemia. (A) nitrogen, (B) phosphorous, (C) potassium, (D) calcium, and (E) sulfur.

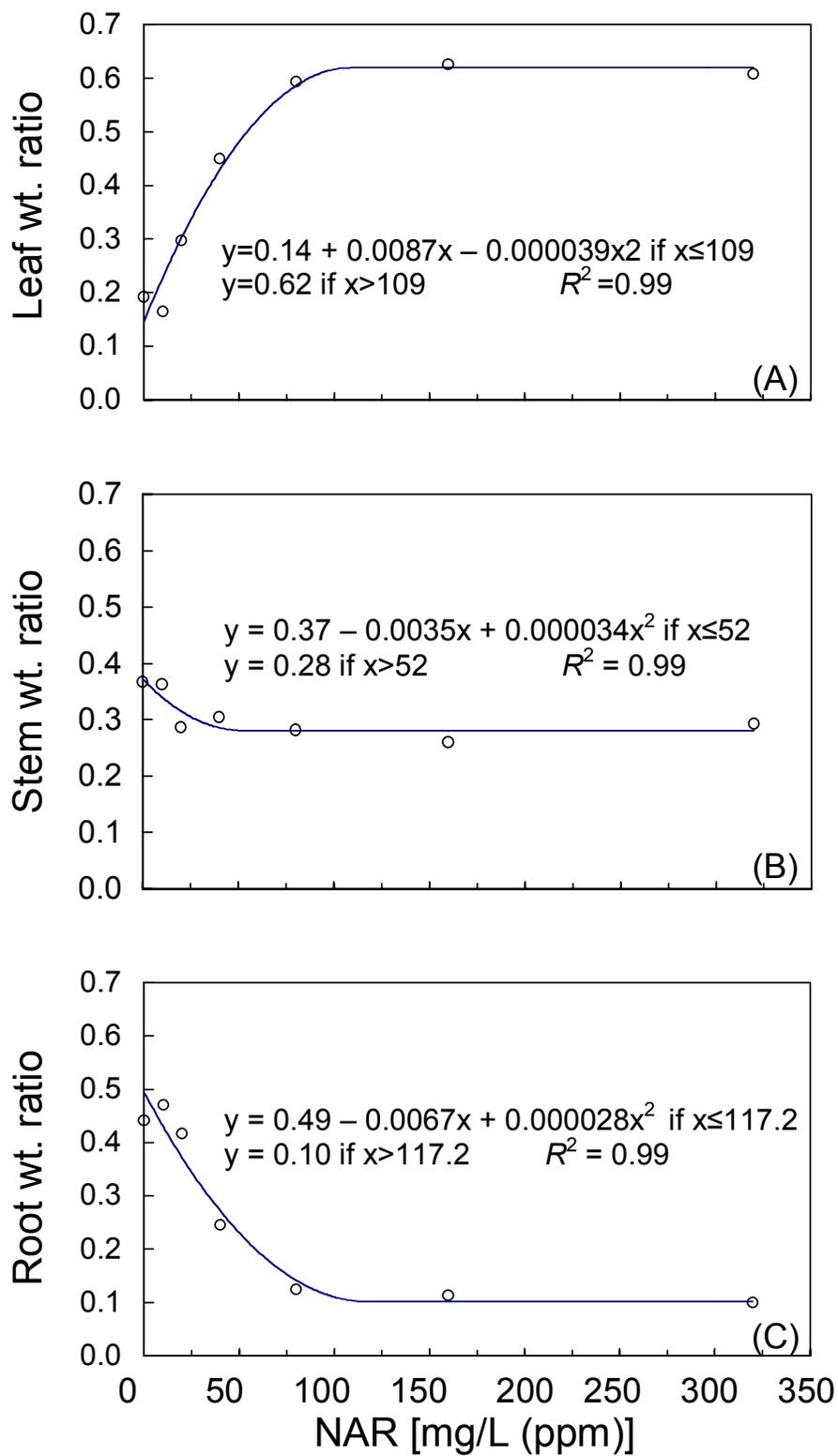
Fig. 5. Effect of nitrogen application rate (NAR) on root mineral nutrient concentration of containerized Japanese ternstroemia. (A) nitrogen, (B) phosphorous, (C) potassium, and (D) sulfur.



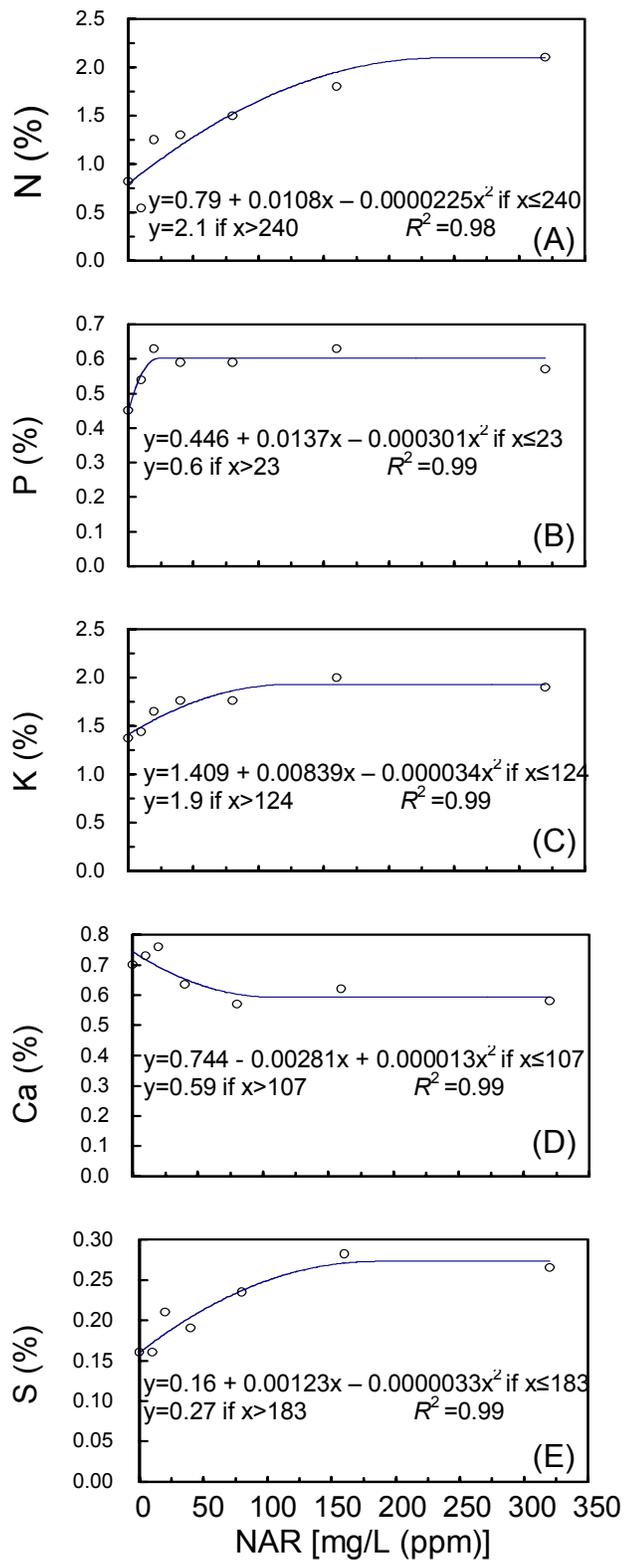
(Fig. 1)



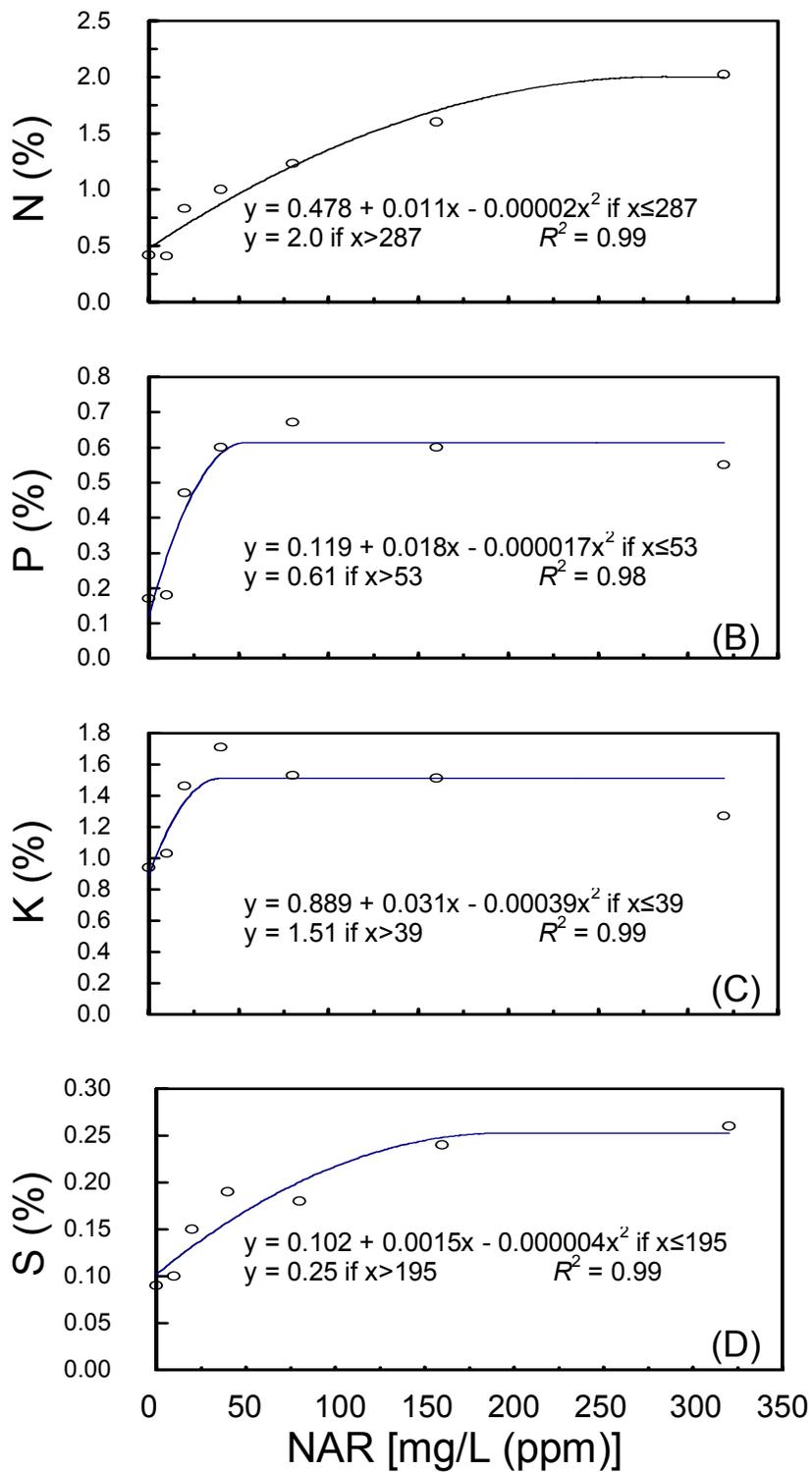
(Fig. 2)



(Fig. 3)



(Fig. 4)



(Fig. 5)