

# Vegetative Propagation of Oconee Azalea (*Rhododendron flammeum*) by Stem Cuttings and Mound Layering<sup>1</sup>

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## Abstract

Deciduous azaleas (*Rhododendron* L.) offer a range of desirable ornamental characteristics and can be valuable nursery crops. Availability in the nursery trade, however, can be limited by the lack of effective propagation protocols. Therefore, the objectives of this research were to develop and optimize vegetative propagation protocols for Oconee azalea, *Rhododendron flammeum* (Michx.) Sarg., utilizing stem cuttings or in-field mound layering. An optimal method for producing rooted stem cuttings with large root systems (> 20 cm<sup>2</sup>) was to collect softwood stem cuttings from hedged stock plants followed by treatment with 10,000 ppm of the potassium salt (K-salt) of indolebutyric acid (K-IBA). Mound layering was also effective. Fifty percent of the stems on each mound resulted in rooted layers and approximately six layers were produced per mound regardless of mounding season (March or June), with or without wounding, or application of 5000 ppm K-IBA to stems prior to mounding in June. Both softwood cuttings and mound layering can be utilized to produce high quality plants.

**Index words:** deciduous azalea, stem cutting, mound layering, hedging, K-IBA, timing, auxin, wounding.

## Significance to the Nursery Industry

Propagation by stem cuttings is the principal method for mass production of clonally derived plant material (7); however, this method has been difficult for many of the deciduous azaleas native to the eastern United States (6). The availability of these deciduous azaleas for the landscape on a commercial scale is dependent upon reliable, productive propagation protocols. Vegetative propagation of Oconee azalea (*Rhododendron flammeum*) by stem cuttings or mound layering was successful, and both systems may be utilized to produce high quality plants.

## Introduction

Native eastern North American deciduous azaleas (*Rhododendron* L. sp.) offer tremendous ornamental diversity in habit, flower size and color, fragrance, and time of bloom. The presence of fine to medium textured foliage also aids in imparting a subtle, natural beauty to the landscape (6). These natives serve as outstanding landscape plants because different species flower throughout the spring into late summer. Unfortunately, several native azaleas can be difficult to propagate by stem cuttings (6, 17, 21, 23) and tremendous variability exists both among and within species for the ability to form adventitious roots on stem cuttings.

Oconee azalea (*Rhododendron flammeum*) is an early-flowering, medium-sized shrub with flower color ranging yellow-orange to red. Oconee azaleas are native to parts of northwestern and southern South Carolina and western Georgia and is valued for its heat tolerance. Oconee azaleas have

been noted as being moderately difficult to root from stem cuttings (9, 23). This difficulty in propagation has greatly limited the potential for selecting, breeding, and producing superior cultivars (e.g., *R. flammeum* 'Hazel Hamilton') (Ray Head, Jr., member, Southeastern Chapter, American Rhododendron Society, personal communication).

Propagation by stem cuttings is dependent upon the competence to form adventitious roots and proper maintenance and manipulation of the stock plant can have profound effects on this process. Maintaining stock plants for propagation provides a supply of cutting material and can maximize rootability while maintaining healthy, uniform, correctly identified stock blocks (7, 8). Several stock plant management techniques exist, including severe winter pruning (hedging), that have been identified as increasing rooting potential among difficult-to-root taxa (3, 8). Cameron et al. (3) noted that hedging stock plants of winter-hazel (*Corylopsis* Siebold & Zuccarini) and lilac (*Syringa* Mill.) greatly increased rooting percentage. Stock plants of *Rhododendron* have been manipulated successfully to stimulate adventitious root formation on stem cuttings (1).

Hedging of young plants or serial hedging of older plants can be utilized to maintain juvenile-type vegetative characteristics or reinvigorate stock. Cuttings from juvenile plants are typically easier to root from stem cuttings than are cuttings from older, mature plants in the adult growth phase (19). After hedging, adventitious budbreak occurs at the base of plants, thereby producing an abundance of vigorously growing, nonflowering shoots, similarly to the juvenile growth phase (12). These shoots tend to grow faster and longer into the growing season than those on non-hedged individuals, while also yielding relatively smaller diameter cuttings with the capacity to root faster (7). Thus, hedging allows for preservation of the juvenile growth phase, which is correlated to an enhanced effect on rooting (7, 8, 12).

Auxins have positively impacted adventitious root formation and root growth of deciduous azaleas. Nawrocka-Grzeskowiak and Grzeskowiak (16) observed an increase in rooting percentages and root ball diameters among azaleas with the use of auxins [in the form of indolebutyric acid (IBA)]; however optimal concentrations were variable.

<sup>1</sup>Received for publication March 18, 2009; in revised form December 28, 2009. This research was funded in part by the North Carolina Agricultural Research Service, Raleigh, NC 27695-7643, and the Southeastern Chapter of the American Rhododendron Society. Technical assistance of Ray Head, Jr., Bill Klippel, Tom Eaker and Joe Conner is gratefully appreciated.

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Skinner (22) reported rooting success among Ghent and Mollis hybrids regardless of using auxins, but noted use of IBA increased rooting percentages to 100%, improved root system quality, and generally shortened the time required for rooting. For deciduous azaleas, Dirr and Heuser (5) suggested an auxin concentration of 4000 ppm IBA, while Sommerville (24) recommended a higher concentration of 8000 ppm IBA. Knight et al. (9) studied the influence of auxin concentration on the rooting of Florida flame azalea [*R. austrinum* (Small) Rehder] and sweet mountain azalea [*R. canescens* Michx.] but found no differences in rooting percentage associated with auxin concentrations from 0 to 10,000 ppm of the potassium salt (K-salt) of IBA (K-IBA). The response to K-IBA was variable for both species and this was attributed to genetic and physiological differences associated with collecting stem cuttings from wild plant populations.

Timing of collection of stem cuttings may also play a critical role in propagation success (11, 16, 23), and recommendations for the optimum time to collect cuttings of deciduous azaleas vary from April to June and again in the winter months (1, 2, 5, 9, 24, 25). Rather than adhere to strict calendar dates, timing may be classified according to the physiological growth stage of the stock plant from softwood to semi-hardwood to hardwood. The degree of lignification in the secondary xylem is closely associated with the growth stage and the date of collection, resulting in pronounced effects on the degree of rooting (16). Burton and Webster (2) suggested it was best to collect stem cuttings of azaleas after cessation of growth, when the tissue was most lignified (hardwood stage).

Mound layering, or stooling, is a method of in-field vegetative propagation, drawing upon the principles of serial pruning and etiolation to encourage adventitious root formation. Stock plants are hedged annually and the bases of emerging shoots are covered with substrate providing a suitable environment for root formation. Subsequent roots grow into the surrounding substrate and rooted stems (layers) can then be severed from the stock plant. Hedging aids in the maintenance of vegetative, juvenile growth that typically has a higher capacity for adventitious root formation (12). Covering the basal portions of the shoots creates an etiolated environment, which can decrease light-induced breakdown of endogenous indole-3-acetic acid (IAA) and retard tissue differentiation, resulting in more parenchyma cells with greater potential for root initiation and development (10). Etiolation has been utilized previously as a stock plant pretreatment to increase rooting potential by 25% in elepidote rhododendrons (13). Wounding, application of auxin, or a combination of both have been shown to increase rooting percentages when combined with mound layering (14). This form of propagation is a viable option for difficult-to-root plants and is utilized extensively with temperate fruit trees and to a lesser extent with bottle brush buckeye (*Aesculus parviflora* L. sp.) (15, 18). Mound layering has the added advantage of requiring minimal facilities and the technique can lend itself to mechanization in field situations. Upright growth habit and the ability to produce many new shoots following pruning are characteristics of plants that could be propagated successfully by mound layering (12).

The objectives of this project were to develop and optimize protocols for vegetative propagation of *R. flammeum* utilizing both stem cuttings and in-field mound layering. The effects of hedging stock plants and applying a range of auxin

concentrations to both softwood and semi-hardwood stem cuttings were evaluated to provide a protocol for propagation by stem cuttings. Effects of timing of mounding and the effect of wounding or applying an auxin to the emerged stems prior to mounding were also evaluated to determine the potential usefulness of mound layering as an in-field propagation method.

## Materials and Methods

**Plant material.** In Fall 2005, 100 Oconee azaleas (50 plants per experiment) in 12 liter (#3) containers (East Fork Nursery, Sevierville, TN) were planted on center 1 × 2.5 m (3 × 8 ft) in field beds at the Mountain Horticultural Crops Research Station, Mills River, NC. For each experiment, plants were arranged in five blocks of 10 plants each in a randomized complete block design.

**Stem cutting experiment.** Treatments included two growth stages, two hedging treatments, and five concentrations of K-IBA. In March 2006 each block of 10 plants was divided in half to produce two experimental units each with five subsamples. In each block, one experimental unit was chosen randomly and all five subsamples were hedged to 15 cm (6 in) above the root collar (stem-root junction), leaving the remaining experimental unit in each block as the nonhedged control. Terminal stem cuttings approximately 7.6 cm (3 in) or longer were collected in June (softwood cuttings) or September (semi-hardwood cuttings) 2006 and 2007. Thirty stem cuttings were collected from each experimental unit per block for a total of 300 cuttings (the 30 cuttings were collected evenly among the subsamples in each experimental unit). Stem cuttings were recut from the bases to 7.6 cm (3 in) and the basal 1 cm (0.4 in) dipped for 3 sec in either 0, 2500, 5000, 7500, or 10,000 ppm K-IBA and inserted immediately in mass into 38 × 38 × 15 cm (15 × 15 × 6 in) trays (Anderson flats, Anderson Die & Manufacturing Co., Portland, OR) containing a substrate of peat:perlite (2:3 by vol) and placed under intermittent mist in a propagation greenhouse. Intermittent mist was applied using a Superior Controller (Superior Controls, Co., Inc. Valencia, CA). Between 6:00 AM and 10:00 AM mist was applied every 20 min for 8 sec; between 10:00 AM and 6:00 PM mist was applied every 10 min for 8 sec; between 6:00 PM and 10:00 PM mist was applied every 20 min for 8 sec and every 240 min for 8 sec between 10:00 PM and 6:00 AM. The rooting studies conducted for each growth stage were randomized complete block designs with five blocks and each treatment contained six stem cuttings within a block. Stem cuttings were spaced 4 cm (1.6 in) × 4 cm (1.6 in) in open trays for approximately 7 weeks prior to recording data. Data recorded included total number of terminal stem cuttings ≥ 7.6 cm (3 in) (recorded on each subsample per experimental unit prior to removing cuttings), rooting percentage, and root system size (expressed as the height × width of the overall root system measured at the largest points). Root system size was used as an estimation of the total root system to avoid destructive harvests. Plants were immediately potted after each experiment for future evaluation.

**Mound layering experiment.** In March 2006, 50 plants were hedged to 15 cm (6 in) above the root collar. Each block of 10 plants was sub-divided into five experimental units each containing two plants (subsamples). After hedging in

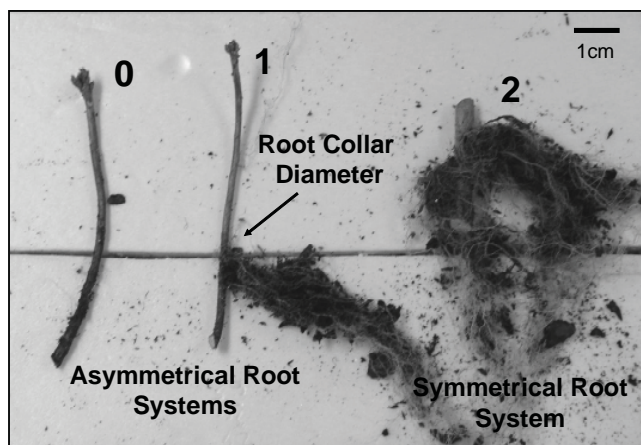


Fig. 1. Root system quality of layers of *Rhododendron flammeum* illustrating relative root score (0, 1, or 2), root collar diameter (RCD), and determination of root system symmetry (symmetrical root systems had two or more roots at least 130 degrees apart).

March, one experimental unit in each block was mounded (March mounding). The remaining four experimental units in each block were mounded in June (June mounding) after being randomly assigned four treatments in a  $2 \times 2$  factorial arrangement. All stems on each plant in the June mounding treatment were either wounded or not wounded using a grafting knife to expose 2.5 cm (1 in) of the vascular cambium on one side of a stem 20 cm (8 in) above the root collar, and treated or not treated with 5000 ppm K-IBA using a spray bottle to mist stems acropetally from the root collar to 20 cm (8 in). Mounding for both seasons consisted of covering plants with 46 cm (18 in) of aged pine bark held in place by a 60 cm (24 in) diameter cylinder constructed from hardware cloth. The experimental design was a split-plot with five blocks. Mounding time was the main plot and wounding and K-IBA were the sub-plot in the June mounding only (March mounding contained one experimental unit). Although mounding times were split between March and June, each experimental unit was arranged randomly within the block and all treatments were re-assigned randomly when the experiment was repeated the following year.

All shoots were harvested in March of the following year and evaluated for rooting percentage, number of rooted layers produced per mound, root collar diameter (RCD), relative root score, and root system symmetry. Rooting was defined as the presence of visible roots  $\geq 1$  cm (0.4 in). Root collar diameter was measured at the stem to root interface using a caliper. Because rooted stems were harvested in winter and loosed of mounding substrate, a relative root score was based visually on size of the root systems with small ( $< 1$  cm diameter) (0.4 in) root systems receiving a 0, intermediately sized (1–3 cm diameter) (0.4–1.2 in) root systems receiving a 1, and large ( $> 3$  cm diameter) (1.2 in) root systems receiving a 2 (Fig. 1). Symmetrical root systems had at least two roots  $130^\circ$  apart around the stem (Fig. 1). Overall assessments of root system quality were based on the measurements of RCD, root score, and root system symmetry.

**Data analysis.** Data for rooting percentage and root system size were transformed using arcsine square root and the

natural logarithm, respectively, when they were not normally distributed. Data for stem cutting propagation were analyzed as a split-split plot design with year as the main plot, growth stage as a sub-plot, and IBA and hedging as sub-sub-plots. Data for mounding were analyzed similarly as a split-split plot with year as the main plot and mounding time as the sub-plot, and wounding and K-IBA as the sub-sub-plot. Data from both experiments were subjected to analysis of variance using general linear models (PROC GLM), and regression analysis (PROC REG) was used to determine the relationship between rooting variables and the concentration of K-IBA for stem cutting propagation (20). Both experiments were conducted twice in a 2-year period and data represents harvests from both years.

## Results and Discussion

**Propagation by stem cuttings.** Rooting percentage of stem cuttings of *R. flammeum* was affected by growth stage ( $P < 0.01$ ), hedging ( $P < 0.01$ ), K-IBA concentration ( $P < 0.10$ ), and the interactions of growth stage by rate ( $P < 0.01$ ) and growth stage by hedging ( $P < 0.01$ ) (ANOVA not presented). Overwhelmingly, utilization of softwood cuttings had the greatest influence on improving rooting percentage (Fig. 2). For softwood cuttings, rooting percentage increased linearly with increasing concentrations of K-IBA ( $r^2 = 0.81$ ,  $P < 0.01$ ). Rooting percentage of semi-hardwood cuttings, however, was not affected by K-IBA concentration (Fig. 2). Hedging stock plants did not increase rooting percentage for softwood cuttings, but hedging did improve rooting percentage for semi-hardwood cuttings. Softwood cuttings had mean rooting of 85%, regardless of hedging. Semi-hardwood cuttings from hedged stock plants had mean rooting of 35%, whereas, rooting of cuttings from nonhedged plants was negligible (0.01%). Treatment of semi-hardwood cuttings with K-IBA, collected from either hedged or nonhedged stock plants, did not improve rooting percentages.

Root system size was affected by growth stage ( $P < 0.01$ ), hedging ( $P < 0.01$ ) and K-IBA treatment ( $P < 0.01$ ). Softwood

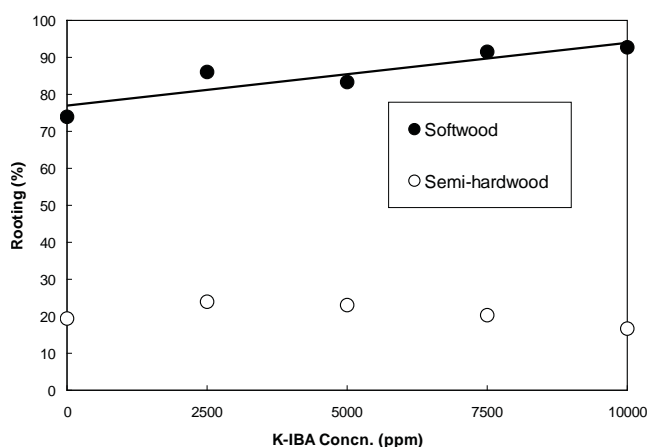


Fig. 2. Rooting percentage of softwood or semi-hardwood cuttings of *Rhododendron flammeum* treated with five concentrations K-IBA and rooted for seven weeks. Rooting percentage (softwood) =  $77.0 + 0.0017(\text{K-IBA})$ ;  $r^2 = 0.81$ ,  $P = 0.01$ . The relationship between semi-hardwood cuttings and K-IBA treatment was not significant (mean rooting was 20%).



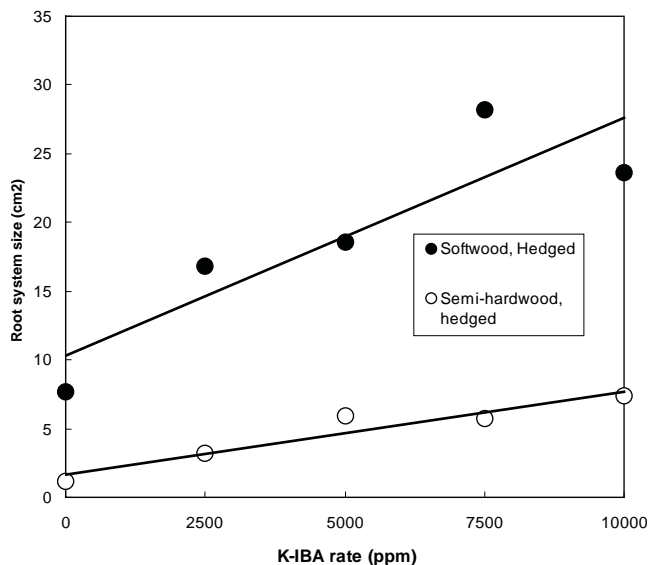


Fig. 3. Mean root system size index (length  $\times$  width of the root ball) for softwood and semi-hardwood cuttings collected from hedged stock plants of *Rhododendron flammeum*, treated with K-IBA, and rooted for seven weeks. Root system size index of rooted softwood cuttings =  $10.4 + 0.0018(\text{K-IBA rate})$ ,  $r^2 = 0.78$ ,  $P = 0.04$ . Root system size of rooted semi-hardwood cuttings =  $1.7 + 0.0006(\text{K-IBA rate})$ ;  $r^2 = 0.91$ ,  $P = 0.01$ .

cuttings had larger root system sizes [ $15.5 \pm 1.6 \text{ cm}^2$  ( $2.4 \pm 0.25 \text{ in}^2$ )] than semi-hardwood cuttings [ $0.95 \pm 0.72 \text{ cm}^2$  ( $0.15 \pm 0.11 \text{ in}^2$ )]. Cuttings collected from hedged stock plants and rooted had larger root system sizes [ $11.8 \pm 1.6 \text{ cm}^2$  ( $1.8 \pm 0.25 \text{ in}^2$ )], than those from nonhedged stock plants [ $4.6 \pm 1.6 \text{ cm}^2$  ( $0.71 \pm 0.24 \text{ in}^2$ )]. Root system size of rooted cuttings increased linearly with increasing K-IBA concentration for both softwood ( $r^2 = 0.78$ ,  $P = 0.04$ ) and semi-hardwood ( $r^2 = 0.91$ ,  $P = 0.01$ ) cuttings when collected from hedged stock plants (Fig. 3).

Overall, the optimal responses were achieved using softwood cuttings treated with 10,000 ppm K-IBA resulting in 94% rooting and the largest root systems. Hedging, as a method of stock plant manipulation of *R. flammeum*, effectively increased the quality of the root system. When rooting semi-hardwood cuttings of the Oconee azalea, the auxin concentration was irrelevant in regards to rooting percentages; however, increasing the concentration resulted in larger root systems.

Hedging affected the total number of cuttings available for rooting ( $P < 0.01$ ). Hedged plants produced  $19 \pm 2$  stem cuttings at either softwood or semi-hardwood collection times, whereas nonhedged plants produced  $37 \pm 7$  stem cuttings at both growth stages. Although not an effective strategy for producing more stem cuttings per plant in *R. flammeum*, hedging produced longer, more uniform stem cuttings (personal observation) with larger root systems.

The effect of auxin treatment on percentage rooting and quality of rooting was observed both visually and statistically. Rooting may occur without use of auxin, but auxin treatment resulted in higher quality root systems and more rooted cuttings, especially for softwood cuttings. Nolde and Coartney (17) also observed a positive effect of auxin on rooting of flame azalea [*R. calendulaceum* (Michx.) Torr.].

Even in plant species with adequate levels of endogenous auxin for regeneration of roots, auxin application can dramatically increase root quality (4). Similarly, the use of higher concentrations of auxin in the present study was quite beneficial for *R. flammeum*. Knight et al. (9) observed rooting of softwood cuttings of *R. austrinum* at all rates applied; however, the highest rooting percentages (100%) were obtained using higher rates of K-IBA (7500 and 10,000 ppm K-IBA). Additionally, the authors noted root number and root length of rooted softwood cuttings was greatest when using 10,000 ppm K-IBA.

**Propagation by mound layering.** Mound layering of *R. flammeum* was successful for both mounding times of March and June. Rooting percentage and number of layers produced per mound was not significantly different for either mounding time (Table 1). Additionally, within the June mounding time, neither rooting percentage nor number of layers was affected by wounding, K-IBA treatment, or their interaction (Table 1). Regardless of mounding season or treatment, mound layering had a 50% success rate that produced nearly six rooted layers per mound.

Similar to rooting percentage and number of layers, there was no effect of timing, wounding, or K-IBA application on root system symmetry (Table 2). Approximately 60% of the root systems of successfully propagated plants were symmetrical. Significant differences were observed between the March and June mounding times for root collar diameter and relative root scores (Table 2). March mounding resulted in rooted plants with an average RCD of 5.3 mm (0.21 in) and a root score of 1.0. Plants in the June mounding treatment had a mean RCD of 4.6 mm (0.18 in) and a root score of 0.80. Within the June mounding time, RCD was affected by the interaction of wounding and K-IBA treatment. Wounding plus K-IBA treatment resulted in a mean RCD of 4.2 mm (0.17 in), which is 0.4 mm (0.02 in) less than the overall mean RCD of June mounded plants, and may not be operationally significant because wounding or K-IBA did not affect any other variables measured. Relative root score was unaffected.

Both mounding times were effective and use of wounding and K-IBA treatment are not necessary to improve rooting percentages, number of layers, or percentage of symmetrical root systems. The earlier March mounding time allowed for slightly larger root collar diameters and visually superior root systems. This effect may be attributed to a longer root development time throughout the year when compared to

Table 1. Rooting percentage and number of plants produced per mound for March and June mounding treatments of *Rhododendron flammeum*.

Treatment	Rooting % <sup>a</sup>	No. layers
March mounding	48.4 $\pm$ 8.3	7.3 $\pm$ 1.9
June mounding	50.5 $\pm$ 4.3	5.7 $\pm$ 0.7
Wounding + K-IBA	60.0 $\pm$ 9.1	5.6 $\pm$ 1.1
Wounding and no K-IBA	53.3 $\pm$ 9.1	7.3 $\pm$ 1.6
No wounding + K-IBA	45.4 $\pm$ 7.5	5.3 $\pm$ 1.2
No wounding and no K-IBA	44.7 $\pm$ 9.3	4.9 $\pm$ 1.5

<sup>a</sup>Values represent means  $\pm$  1 SE for 10 replications (5 replications  $\times$  2 years). Rooting percentage or number of layers was not affected significantly by mounding time or by the application of wounding or IBA treatments within the June mounding time at  $P < 0.05$ .

**Table 2. Root system quality of *Rhododendron flammeum* indicated by root system symmetry, root collar diameter, and relative root score (see Fig. 1 for visual descriptions).**

Treatment	Symmetry <sup>a</sup>	RCD <sup>b</sup> (mm)	Root score
March mounding	0.59 ± 0.05A	5.3 ± 0.3A	1.0 ± 0.1A
June mounding	0.60 ± 0.03A	4.6 ± 0.1B	0.8 ± 0.0B
Wounding + K-IBA	0.54 ± 0.05a	4.2 ± 0.2b	0.8 ± 0.1a
Wounding and no K-IBA	0.66 ± 0.04a	4.7 ± 0.2ab	0.9 ± 0.1a
No wounding + K-IBA	0.58 ± 0.06a	4.8 ± 0.3a	0.8 ± 0.1a
No wounding and no K-IBA	0.60 ± 0.05a	4.7 ± 0.2ab	0.7 ± 0.1a

<sup>a</sup>Values represent means ± 1 SE for 10 replications (5 replications × 2 years). Means followed by a different letter, within columns, represent significant differences at  $P < 0.05$ . Upper case (A) within a column denotes comparison between March and June mounding, whereas lower case (a) denotes comparisons among wounding and K-IBA treatments within June mounding. Means were separated using Fischer's LSD.

<sup>b</sup>RCD = root collar diameter.

the later June mounding time. For the grower, this information provides flexible timing for in-field propagation of *Rhododendron flammeum*. Because mounding in March on a yearly basis might prove stressful for plants, growers can alternate mounding times during production to allow plants to recover between mounding. Plants could be mounded in March during year one, but mounded in June the following year. In situations where propagation facilities are not readily available and field space is not limiting, mound layering is feasible for azalea propagation.

In conclusion, results herein indicate vegetative propagation is a viable approach for mass production of *Rhododendron flammeum*, and protocols have been developed for both rooting stem cuttings and mound layering. Treating softwood stem cuttings of *R. flammeum* with 10,000 ppm K-IBA produced the highest number of rooted cuttings with the largest root systems. Hedging stock plants improved the rooting percentage of semi-hardwood stem cuttings and also improved the size of the root systems of rooted softwood and semi-hardwood cuttings. Utilizing higher concentrations of auxin with softwood cuttings may improve vegetative propagation and increase the number of plants available commercially.

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