Differences in Growth, and Nitrogen Uptake and Storage Between Two Container-Grown Cultivars of Rhododendron[1]

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Abstract

One-year-old liners of an evergreen rhododendron (*Rhododendron* L. 'H-1 P.J.M') and a deciduous azalea (*Rhododendron* L. 'Cannon's Double') were used to determine nitrogen (N) uptake, remobilization, and storage in relation to plant growth from May to September. Plants were grown in a substrate of equal parts (by vol) vermiculite, pumice, and sandy loam soil and received liquid fertilization with or without N. Rate of N uptake was correlated with the rate of plant growth and maximum uptake occurred during July [azalea, >4 mg/day (1.4E – 04 oz/day)] and August [rhododendron, >2 mg/day (7.1E – 05 oz/day)]. Compared to the rhododendron used in this study, the azalea cultivar grew faster and had a greater rate of N uptake and uptake efficiency (azalea, 12 to 33%; rhododendron, 8 to 16%). The old leaves of the rhododendron remobilized N for new growth. New azalea leaves exported approx. 40% of their N by September when the stems and roots were actively accumulating biomass. The roots, stems and new leaves of the rhododendrons were still accumulating biomass by September. Our results suggest that transplanted 1-year-old liners of rhododendron and azalea contained sufficient N reserves in both the plant and substrate to support initial plant growth and that increasing availability of N in the substrate during the period of rapid growth can significantly increase N uptake while improving vegetative growth and the N status of both rhododendron and azalea.

Index words: nitrogen uptake, plant growth, rhododendron, azalea, container-grown, mineral nutrition.

Significance to the Nursery Industry

Nitrogen (N) is an essential macronutrient required for plant growth and development. Nitrogen fertilizer inputs based on optimal plant needs (performance) and optimal time of application (uptake) will reduce overall fertilizer use, minimize point source pollution to groundwater and enhance plant performance. Data obtained from this study with transplanted liners of *Rhododendron* will provide growers with information on the timeframe for active N uptake and use in deciduous and evergreen cultivars grown in containers. This information will aid in the development of fertilizer management strategies for container nursery production practices that will decrease fertilizer use and production costs, improve plant quality, while minimizing N losses to the environment.

Introduction

To achieve production goals, producers of container-grown nursery crops favor practices that maximize plant growth and reduce production time. However, to ensure maximal productivity, production methods commonly use fertilizers and water inefficiently, resulting in restricted yields and elevated production costs (4). Nitrogen (N) is frequently cited as the most limiting factor to plant growth and crop productivity (13). Given the importance of N to plant growth, growers of nursery stock often provide a high level of N hoping to reduce the potential of N deficiency (4). In many woody plant species, early growth is supported mainly by remobilization of stored N before substantial root uptake occurs in the spring, resulting in a close correlation between stored N and plant initial growth (3, 15). Compared to annual field crops, woody plants generally are less efficient in N uptake (22). Many plants exhibit an asymptotic growth response with increasing N supply, resulting in unnecessary and excessive fertilization (1).

Increasing concerns about N runoff resulting from nursery production have increased the need for fertilization practices that increase efficiency of N use and decrease the potential for environmental contamination, without affecting crop productivity or quality (24). Over the last 3 decades, better substrate components and formulations of controlled-release fertilizers (CRFs), increased capture and reuse of runoff and increased understanding of the relationships between water use and mineral nutrient mobility, have improved nursery practices. Unfortunately, low recovery of N is still commonly reported for container-grown nursery plants (4, 5). This low efficiency of N uptake suggests fertilizer management could be improved by better understanding when plants take up available N most efficiently (1).

Published literature on N use in nursery production of *Rhododendron* spp. (L.) has been based primarily on trials assessing plant growth with different types or formulations of fertilizer or based on in-ground field production (23). Knowledge of the biological aspects of N uptake and use by *Rhododendron* is available from natural ecosystems (9, 10, 11, 12, 19), however this information may not be applicable to container-grown plants where horticultural practices as well as the physical and chemical environment of container production may alter plant N dynamics. There are also many

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different cultivars of commercially grown *Rhododendron* with variations in growth rates and morphology that may influence N utilization. The different leaf characteristics (e.g., deciduous vs. evergreen, lepidote vs. elipidote) found within this genus could alter resource use as reported for other genera (7). Information concerning the relationship between growth, N uptake, and N use in container-grown *Rhododendron* will aid in the development of fertilizer management strategies based on plant demand.

To produce 2-year-old plants, many growers transplant 1-year-old liners into larger containers during April and May, commonly applying CRFs during transplanting. The objective of this study was to characterize the timing of N uptake and use in a deciduous and an evergreen cultivar of *Rhododendron* from May through September after transplanting liners into larger containers.

Materials and Methods

Plant culture and treatments. One-year-old liners [10 cm (4 in) containers] of an evergreen rhododendron 'H-1 P.J.M' (*Rhododendron* L. 'H-1 P.J.M') and a deciduous azalea 'Cannon's Double' (*Rhododendron* L. 'Cannon's Double') were transplanted into #1 (1 gal) polyethylene pots (1 plant/pot) containing a medium of equal parts (by vol) vermiculite, pumice, and sandy loam soil in late May 2004 and grown outdoors in a lathe house in Corvallis, OR (45° 59' 04" N, 123° 27' 22" W). The substrate for this study was chosen to minimize N availability to plants. Forty plants from each cultivar were randomly assigned to one of two groups and fertilized two times per week for 12 weeks starting June 3, 2004. One group of plants (–N treatment) received 250 ml of N-free modified Hoagland's solution (8) at each fertilization; the other group of plants (+N treatment) received 250 ml modified Hoagland's solution with 10 mM (140 ppm) N from NH_4NO_3. All plants were hand-watered as needed throughout the growing season.

Approximately every 2 weeks from June 3 through September 1, 2004, five plants of each cultivar by N treatment combination were randomly selected, the substrate was removed from the roots by washing and the plants were separated into roots, stems, and leaves. For both cultivars, stems were further separated by growing season and for the rhododendron, leaves were also separated by growing season (e.g., 2003 and 2004). Total leaf area [cm^2 (in^2)] of each plant was measured using a LI-COR Model LI-3100C Area Meter (LI-COR Environmental, Lincoln, NE). Stem length and the number of leaves were recorded. All samples were washed in doubled distilled (DD) water, placed into an –80C freezer then freeze-dried. The dry weight of each plant structure (e.g., roots, stems, and leaves) was recorded and samples were taken for mineral nutrient analyses.

Rate of biomass accumulation or growth rate (mg/day) was estimated by calculating the average change in biomass of each plant structure between harvest dates. Nitrogen concentration (mg/g) and content (mg) in each plant structure was determined using the methods of Bi et al. (3). Total plant N content was calculated as the sum of the N content in each plant structure. Rate of N uptake between harvests (mg/day) was calculated by determining the change in N content of each plant structure between harvest. Nitrogen uptake from fertilizer between harvests was estimated by subtracting the average N uptake of –N plants from the N uptake of +N plants. Uptake efficiency of N from fertilizer between harvests (%) was calculated as the proportion of total N applied accounted for by N uptake of plants in the +N treatment. The location of N in different plant structures was used to characterize the locations of N use and storage for each harvest date. Changes in N content (mg/day) in the different plant structures were used to indicate the times of net N import and export (13).

Experimental design and statistical analyses. The experiment was a completely randomized design with each treatment unit (pot) replicated five times for each N fertilization treatment (–N, +N), harvest date (7), and cultivar (rhododendron, azalea). Data were subjected to analysis of variance (ANOVA) procedures with cultivar, fertilization treatment, and harvest date as main effects. Data on growth rate, rate of N uptake, uptake efficiency of N from fertilizer, and change in N content were transformed prior to analysis to correct for unequal variance and achieve best model fit. Back-transformed least square means of actual data are reported in the figures. Where indicated by ANOVA, means were separated at $P < 0.05$ using Tukey Honestly Significant Difference procedure or Tukey Honestly Significant Difference procedure for unequal N ($THSD_{0.05}$). Rates of biomass and N accumulation between +N and –N plants within and between cultivars were compared using specific contrasts at $P < 0.05$. Differences in seasonal patterns of biomass and N accumulation between cultivars were compared using contrasts at $P < 0.05$. All analyses were performed using Statistica® (Statsoft, Inc., Tulsa, OK).

Results and Discussion

Plant growth. Increasing N-availability did not influence total plant biomass until July (Fig. 1A). The deciduous azalea (*Rhododendron* L. 'Cannon's Double') accumulated biomass at a faster rate than the evergreen rhododendron (*Rhododendron* L. 'H-1 P.J.M') ($P < 0.05$) and the effects of increased N-availability on total biomass were observed earlier with azalea (early July) than rhododendron (late July). Others have reported that new growth of young almond [*Prunus dulcis* (Mill) D. A. Webb] and peach (*Prunus persica* L. Batsch) trees is dependent on N from both plant reserves and fertilizer application early during the growing season (April through June) (3, 21). In comparison to these tree species, *Rhododendron* has a much slower growth rate which could result in a slower demand for N from reserves to be used for initial growth. Thus, reliance on N from fertilizer for *Rhododendron* may be later in the growing season than reported for these faster growing species such as almond and peach. With Alpen Rose (*Rhododendron ferrugineum* L.) and ericaceous plants such as Bilberry (*Vaccinium myrtillus* L.) and Lingonberry (*V. vitis idaea* L.), N availability has no influence on the timing or amount of growth up to the end of May, only altered the growth patterns after a second flush of leaf growth occurred (7, 12).

Increasing N-availability had no influence on root biomass of either cultivar (Fig. 1B). Others have reported low N-availability can increase, decrease, or have no effect on root biomass depending on the plant species and the amount and duration of N deficiency (1, 4, 7, 16). In the present study, plants in the +N treatment had a smaller proportion of total plant biomass in roots (~20%) than plants in the –N treatment (~25%). This is consistent with the theory that when soil N is low, plants allocate more biomass to the roots to maximize uptake of available nutrients (16). Since roots were

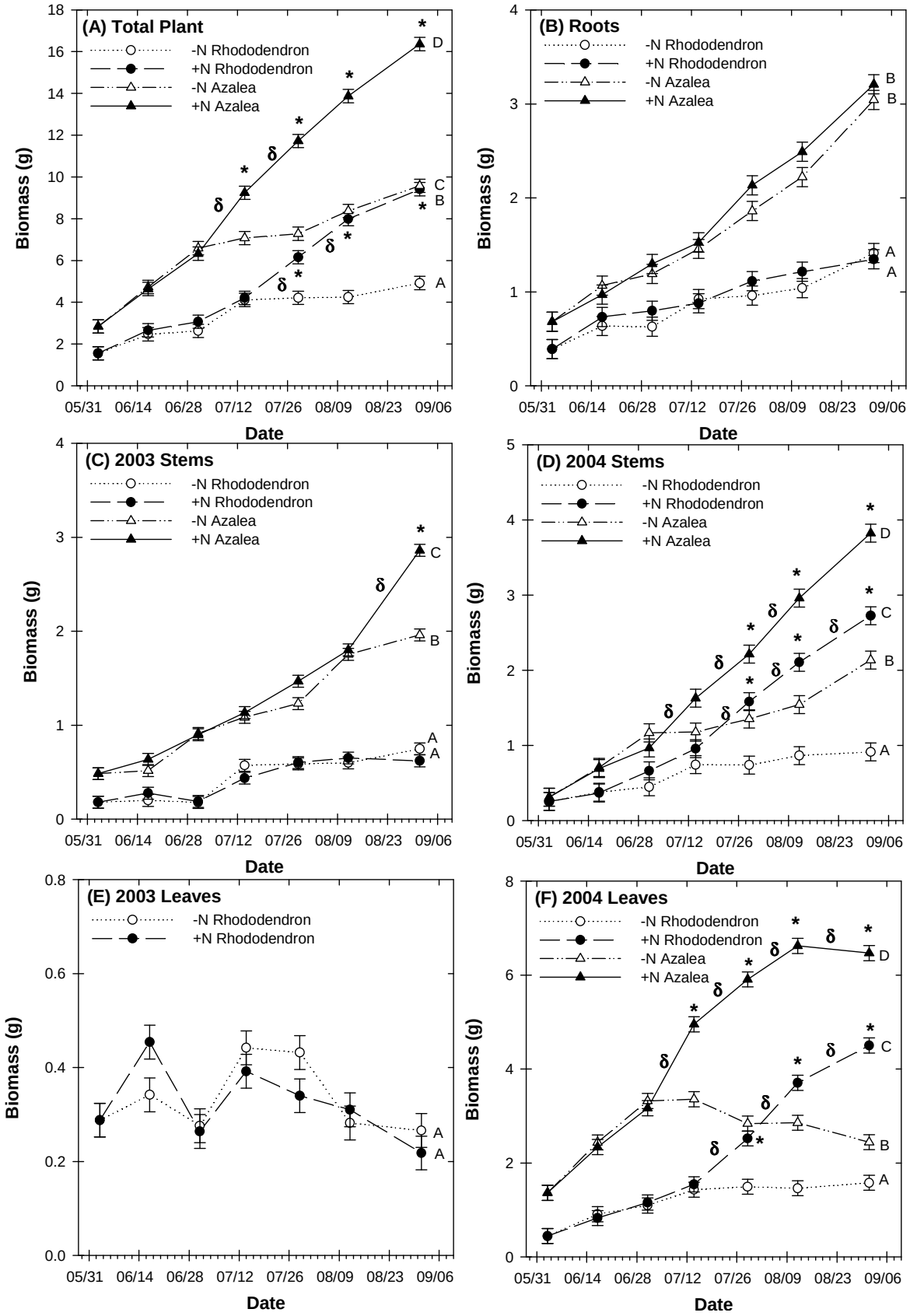

Fig. 1. **Change in total plant biomass (A), and biomass of roots (B), 2003 stems (C), 2004 stems (D), 2003 leaves (E), and 2004 leaves (F) of two *Rhododendron* cultivars grown in containers with (+N) or without (–N) additional nitrogen (N) from May through September 2004. Rhododendron = *Rhododendron* 'H-1 P.J.M'; Azalea = *Rhododendron* 'Cannon's Double'. Data points represent means and vertical bars = ± 1 SE (n = 5). Asterisks (*) by +N data points denote significant differences between +N and –N treatments within a cultivar and date ($THSD_{0.05}$). The delta (δ) symbols by segments of +N lines denote significant differences in rate of change between +N and –N treatments within a cultivar (contrasts at $P < 0.05$). Uppercase letters to the right of lines denote significant differences in response over time (contrasts at $P < 0.05$).**

still accumulating biomass by September, it would be interesting to know when roots stop growing in *Rhododendron* to better understand the implications of fall transplanting and cold hardiness development of this genus. Others have reported roots of heather (*Calluna* spp. L.) in temperate climates can continue growing and absorbing N through the autumn and winter (2).

Increasing N-availability had no influence on biomass of 2003 stems (old stems) on rhododendron and increased biomass of 2003 stems on azalea in September (Fig. 1C). Biomass of 2004 stems (new stems) increased from June through September and increasing N-availability increased biomass of 2004 stems on both cultivars at a similar time (late July) (Fig. 1D). Increasing N-availability had no influence on elongation of 2004 stems on azalea and prolonged stem elongation on rhododendron for approximately 1 month (data not presented). These results indicate reliance of new stem biomass on N-availability is similar between the two cultivars while the effects on plant form, e.g., stem elongation, are different.

Biomass, area, and number of rhododendron 2003 leaves (old leaves) were not influenced by N-availability [Fig. 1E; area, 24 ± 3 cm^2 (3.7 ± 0.5 in^2); 5–9 leaves per plant]. From June to September the 2003 leaves of rhododendron lost dry weight, on average 1.7 mg/day (5.9E – 05 oz/day). Increasing N-availability increased biomass (Fig. 1F) and area of 2004 leaves (new leaves) after new leaf production had ceased (azalea, July; rhododendron, mid-August). The 2004 leaves on +N plants accumulated biomass and area through September. The maximum area of 2004 leaves on +N plants was ~2 to 3 times greater than on –N plants [+N azalea, 709 cm^2 (110 in^2); +N rhododendron, 438 cm^2 (68 in^2)]. Increased N-availability increased 2004 leaf production in rhododendron by ~15 leaves but had no effect on the number of 2004 leaves on azalea. The maximum number of 2004 leaves on +N plants was 76 (azalea) and 56 (rhododendron). These results indicate the reliance of leaf production on N-availability occurs earlier for azalea than rhododendron and reliance of rhododendron on N for leaf production lasts until later in the year. Others have reported the greatest growth rate of new leaves on Lapland rosebay [*R. lapponicum* (L.) Wahlenb.] occurs between July and October (10). Loss of 2004 leaf biomass in –N azalea after July without loss of leaves suggests leaves on –N plants started to senesce earlier than leaves on +N plants. Nitrogen deficiency can cause early leaf senescence and abscission in other deciduous species (6).

Our results indicated that liners of 1-year-old *Rhododendron* contain enough N in the liner substrate or as reserves in the plant to satisfy plant growth demands until July. Differences between rhododendron and azalea in timing of the reliance on N fertilizer applications after transplanting are a function of differences in growth rate. Increasing N supply either via CRFs or liquid fertilizers in July and August could be used to maximize growth. However, since high N in the late summer can delay development of cold hardiness in some plant species, the effects of increased soil N availability in the late summer on plant cold hardiness need to be addressed.

Nitrogen concentration. Increased N-availability increased N concentrations 2 to 4 weeks earlier than observed effects on total plant biomass (Fig. 2A). The concentrations of N in +N plants were similar to results reported for field grown *R. ferrugineum* (12). The lag-time between the effects of N-availability on N concentration and measurable effects on plant growth highlights the sensitivity of using routine mineral nutrient analyses as a predictive fertility management tool, rather than just a diagnostic tool after deficiencies are observed in production.

During late August, the N concentration in the +N azaleas decreased (Fig. 2A) as the result of increased biomass and decreased N concentrations in roots (Fig. 2B) and decreased biomass and N concentration in 2004 leaves (Fig. 2F). Increased root biomass and decreased N concentration in roots suggested that the roots were actively growing during this time and not acting as a location of N storage. Root growth and N uptake by other ericaceous plants can occur throughout the winter when environmental conditions are conducive for root growth (2). Therefore, if roots act as a location for N storage, they may be a sink for N after August. Decreased biomass and N concentration in 2004 leaves suggest that export or loss of N from leaves started before September. In other deciduous species, reserves of carbohydrates and nutrients are moved from leaves to other organs prior to leaf abscission (15) and in temperate climates some species start exporting reserves as early as August, while other species do not export N until later in the autumn (15, 18). Increased N concentrations and biomass in both old and new stems (Fig. 2C and D) of azalea in late August indicates plants were starting to store reserves prior to leaf abscission and stems were important sinks for N. Older stems can act as primary locations of reserve storage in deciduous plants while newer stems or roots provide a secondary role in N storage (6, 14). If stems of azalea are sheared to improve plant form after mid-July, the loss of N could be substantial and negatively influence plant growth and increase plant reliance on N from fertilizer the following spring.

From mid-July to mid-August the N concentration in the +N rhododendrons increased (Fig. 2A) primarily as the result of increased biomass and N concentrations in roots (Fig. 2B) and 2004 leaves (Fig. 2F). The concentration of N in the stems (Fig. 2C and D) of the rhododendrons changed little from June to September indicating these structures are not locations of reserve storage for this cultivar or reserve storage occurs later in the year. In *Rhododendron lapponicum* (10) and other ericaceous and nonericaceous evergreen genera [*Picea sitchensis* (Bong.) Carr., *V. vitis-ideae*] stems do not act as primary storage locations for N (7, 17). Increased N concentration and biomass in 2004 leaves in mid-July and August indicates 2004 leaves are an important sink for N during this time. Since the rhododendrons were still actively accumulating biomass during August, maintaining high N concentrations in these leaves would help improve plant carbon gain through photosynthesis (11).

Our results indicated that concentrations of N in both the stems and leaves of rhododendron changed little from June to September when N in the growing substrate is not limiting, while N concentrations in stems and leaves of azalea exhibited more dynamic changes. This suggests that sampling to estimate plant N status from June to September will have a greater predictive ability with rhododendron than azalea. Since both cultivars were still accumulating biomass by September, a longer study which tracks nutrient and carbohydrate storage by *Rhododendron* later into the autumn is needed to clarify our understanding of reserve N storage.

Nitrogen content. Increased N-availability increased total plant N content by early July (Fig. 3A). The maximum N

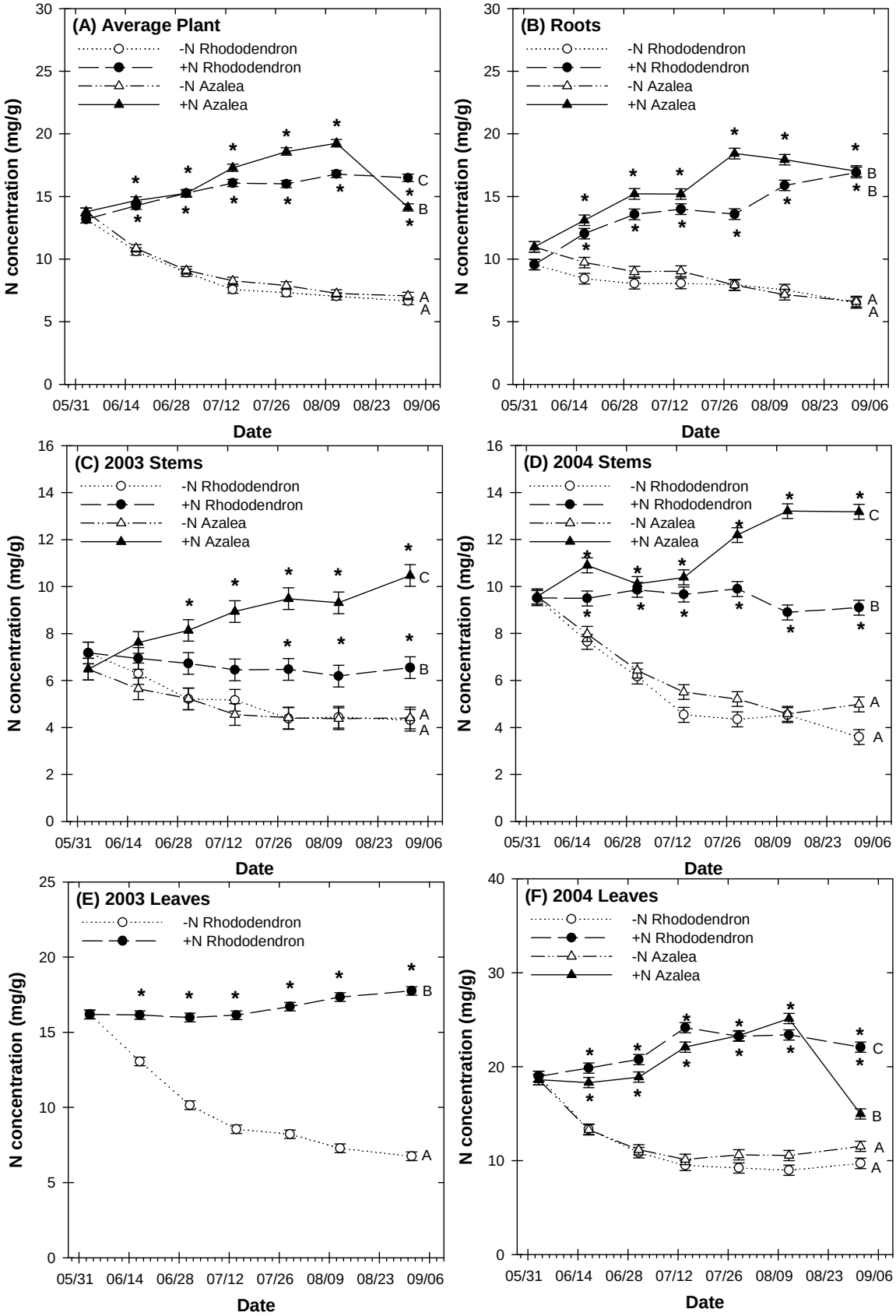

Fig. 2. **Change in average plant nitrogen (N) concentration (A) and N concentration of roots (B), 2003 stems (C), 2004 stems (D), 2003 leaves (E) and 2004 leaves (F) of two *Rhododendron* cultivars grown in containers with (+N) or without (–N) additional N from May through September 2004. Rhododendron = *Rhododendron* 'H-1 P.J.M'; Azalea = *Rhododendron* 'Cannon's Double'. Data points represent means and vertical bars = ± 1 SE (n = 5). Asterisks (*) by +N data points denote significant differences between +N and –N treatments within a cultivar and date ($THSD_{0.05}$). Uppercase letters to the right of lines denote significant differences in response over time (contrasts at $P < 0.05$).**

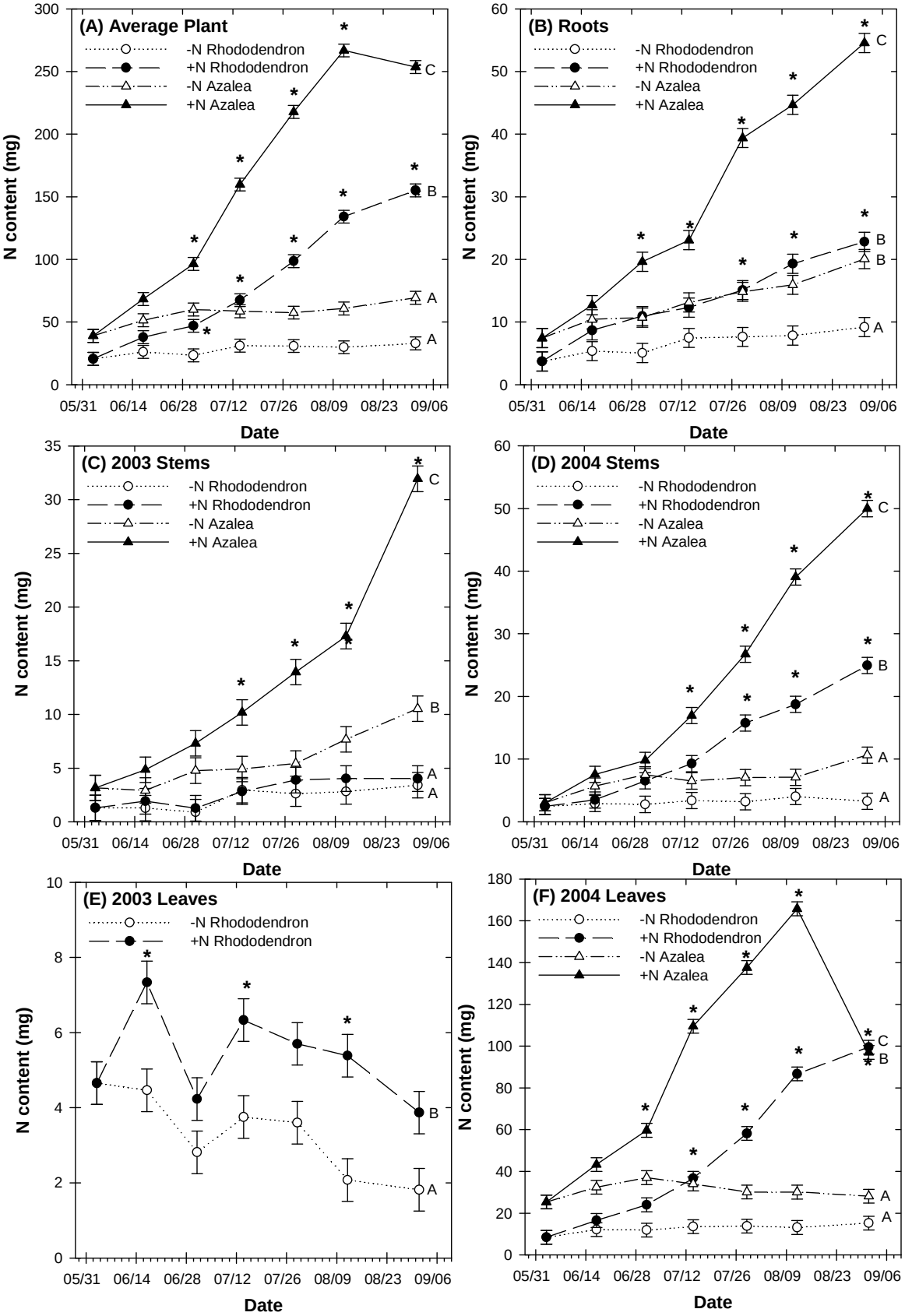

Fig. 3. **Change in total plant nitrogen (N) content (A) and N content of roots (B), 2003 stems (C), 2004 stems (D), 2003 leaves (E), and 2004 leaves (F) of two *Rhododendron* cultivars grown in containers with (+N) or without (–N) additional N from May through September 2004. Rhododendron = *Rhododendron* 'H-1 P.J.M'; Azalea = *Rhododendron* 'Cannon's Double'. Data points represent means and vertical bars = ±1 SE (n = 5). Asterisks (*) by +N data points denote significant differences between +N and –N treatments within a cultivar and date ($THSD_{0.05}$). Uppercase letters to the right of lines denote significant differences in response over time (contrasts at $P < 0.05$).**

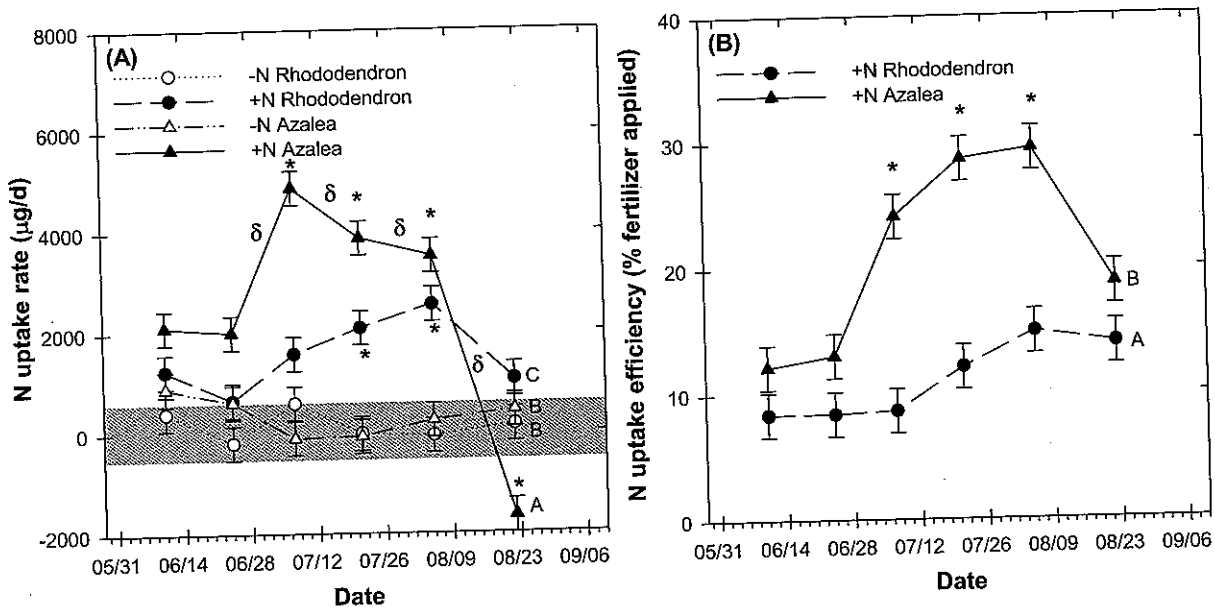

Fig.4. Nitrogen (N) uptake rate (A) and N uptake efficiency (B) of two *Rhododendron* cultivars grown in containers with (+N) or without (-N) additional N from May through September 2004. Rhododendron = *Rhododendron* 'H-1 P.J.M'; Azalea = *Rhododendron* 'Cannon's Double'. Data points represent means and vertical bars = ±1 SE (n = 5). Asterisks (*) in (A) by +N data points denote significant differences between +N and –N treatments within a cultivar and date and (*) in (B) denote significant differences between cultivars ($THSD_{0.05}$). The delta (δ) symbols by segments of +N lines (A) denote significant differences in N uptake rate between cultivars (contrasts at $P < 0.05$). Uppercase letters to the right of lines denote significant differences in response over time (contrasts at $P < 0.05$). Data points within grey blocks (A) are not significantly different ($P < 0.05$) from a rate of zero.

accumulation in the +N rhododendrons was 134 mg N (4.7E – 03 oz) and in +N azalea was 228 mg N (8.0E – 03 oz). Rhododendron in the –N treatment accumulated 13 mg N (4.6E – 04 oz) and –N azalea accumulated 32 mg N (1.1E – 03 oz). By September, 60% of the N in +N azalea was in roots and stems and 70% of the N in +N rhododendron was in 2004 new leaves (Fig. 3B, C, D, E, and F).

In azalea, maximum N import by 2004 leaves occurred early July [4.0 mg/day (1.4E – 04 oz/day)], root uptake or import was greatest during July [0.97 mg/day (3.4E – 05 oz/day)], maximum N import by 2004 stems occurred in early August [(0.88 mg/day (3.1E – 05 oz/day)], and maximum N import by 2003 stems occurred in late August [0.59 mg/day (2.1E – 05 oz/day)]. Conversely, in the rhododendrons, maximum N import by 2004 stems occurred in late-July [0.44 mg/day (1.6E – 05 oz/day)], root uptake or import was greatest in early-August [0.28 mg/day (1.0E – 05 oz/day)], maximum N import by 2004 leaves occurred in early-August [2.1 mg/day (7.4E – 05 oz/day)] and there was no net N import into 2003 stems.

From June through September, no net N export occurred from 2004 leaves of +N rhododendron or from roots or stems of +N plants of either cultivar. Nutrient 'remobilization', according to Marschner (13) can be estimated by the decrease in the net nutrient content of plant structures during a given period, less the losses caused by abscission or turnover. Although N could have been translocated between structures during this experiment, no net loss or 'remobilization' of N from stems or roots was detected. In contrast, the biomass and N content of 2003 leaves on rhododendron decreased from mid-June to September (Fig. 3E) without any decrease in the number of leaves on the plants (data not presented). Our results suggest that the 2003 leaves slowly exported and remobilized N regardless of external N availability. Similar reductions in old leaf biomass without loss of leaves occur in young evergreen *R. ferrugineum* (12). Old leaves on young *R. ferrugineum* (12) contribute a high proportion of N to current-year aboveground growth; however, reliance of N from old leaves in evergreen *Rhododendron* may change as plants mature (19). Old leaves on mature *R. lapponicum* (9) and *R. ferrugineum* (19) do not contribute directly to the nutrition of expanding new leaves. Instead the major N source for shoot growth is N stored in the woody tissues (stem and root).

During late August, leaves on the +N azaleas exported approximately 40% of their N [3.3 mg/day (1.2E – 04 oz/day)]. Since no leaf abscission occurred prior to September, this suggests N was translocated to other locations in the plant. This rapid export of N to storage is similar to the response of other deciduous plant species prior to leaf senescence in the autumn (14, 18).

Nitrogen uptake and fertilizer N uptake efficiency. Increasing N-availability increased N-uptake from late June to early August (azalea) and from mid-July to early August (rhododendron) (Fig. 4A). On average, N-uptake of +N plants was 1.6 mg/day (5.6E – 05 oz/day) (rhododendron) and 2.6 mg/day (9.2E – 05 oz/day) (azalea). Rate of N-uptake was correlated with rate of biomass accumulation (rhododendron: $r^2 = 0.8584$, $P < 0.001$; azalea: $r^2 = 0.6785$, $P < 0.001$). Nitrogen uptake by +N azalea was greatest in July [3.9–4.9 mg/day (1.4E – 04 to 1.7E – 04 oz/day)] and maximum N uptake in rhododendron occurred in August [2.1–2.6 mg/day (7.4E – 05 to 9.2E – 05 oz/day)]. This positive correlation between growth rate and N uptake is consistent with results reported for other plant species (18).

In our study, there was a significant N accumulation in –N plants. This N may have been a result of a carry-over of nutrients in the substrate from 1-year-old liners, or from the

sandy loam soil in the experimental substrate, or from the irrigation water. Interestingly, the –N azaleas were able to accumulate more N than the –N rhododendrons. Since the substrate and irrigation of the azaleas was the same as that used with the rhododendrons, the azaleas apparently had a higher efficiency of N uptake. Estimates of fertilizer uptake efficiency show that the +N azaleas took up 12 to 33%, while the +N rhododendrons took up only 8 to 16% of the N from the applied fertilizer (Fig. 4B).

Nitrogen uptake efficiency from fertilizer was correlated with rate of biomass accumulation (rhododendron: $r^2 = 0.6853$, $P < 0.001$; azalea: $r^2 = 0.5865$, $P < 0.007$). Efficiency of N uptake from fertilizer was greatest from July to mid-August. Interestingly, uptake efficiency of both cultivars was similar to the results of others (5, 20) until July when uptake and demand by both cultivars increased. These results suggest that N fertilizer application strategies for transplanted liners of the cultivars studied should include an N supply with low availability after transplanting followed by increased availability of N in the summer. This may improve N uptake efficiency and minimize N loss.

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