GROWTH OF JAPANESE HOLLY AS AFFECTED BY NITROGEN AND GROWTH REGULATORS

by

Charles Homer Gilliam

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APPROVED:

R. D. Wright, Chairman

J. A. Barden

S. W. Bingham

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Introduction

Many woody ornamental plants are vegetatively propagated in the summer and carried through the following winter as liners. Following propagation, insignificant growth occurs on these liners before the following spring. Vegetative propagation may also be done in the winter, and is normally followed by an immediate flush of growth. After the first full growing season following propagation, little difference in size can be seen between summer and winter propagated cuttings. However, a flush of growth on summer propagated cuttings immediately after rooting would result in a much larger plant at the end of the first full growing season, since growth would resume in the spring from a multiple branched cutting rather than from a single stem.

Hormonal imbalance (28) and nutritional deficiency (8, 16) have been shown to differentially regulate plant growth. Nitrogen (N), a critical element needed in large amounts, has been shown to increase the number of laterals on many plants (8, 11, 16, 17). Cytokinins and gibberellins have also been shown to stimulate the growth of laterals (10, 26, 37). However, to this author's knowledge little research has been done to determine if lateral buds on summer propagated liners can be stimulated to break before the onset of dormancy.

This study was designed to determine whether N and growth regulators, alone or in combination, would enhance vegetative growth of *Ilex crenata* cv. Helleri immediately after propagation.

Review of Literature

Dormancy may be considered an inherent property of woody plants. These plants possess the ability to respond to certain combinations of environmental factors. The specific effects of these external factors are determined by the genetic make-up of the plant and the physiological stage of development. Thus the same external conditions which are optimal for one phase of growth may not necessarily be favorable for another. Doorenbos (6) used the term dormancy in its widest sense to apply "to any case in which tissue predisposed to elongate does not do so." This usage was also used by Wareing (33), and Richardson (23).

We may reasonably assume that the distribution of nutrients, auxins, gibberellins, and cytokinins, together with other types of hormones such as abscisic acid and ethylene, are vital factors in the overall control of growth and differentiation (30, 41). Nevertheless, the patterns of distribution of growth hormones in the plant with regard to location, time, and concentration are controlled by interactions among the environment, the genetic make-up of the plant, and the physiological stage of development. Thus growth hormones serve as influential agents in the overall integration and coordination of growth and differentiation.

Auxins. The young tissues in the shoot apex region are sites of auxin synthesis, but it appears that some auxin synthesis also occurs in the roots and older leaves (30). Auxin is transported from the center of production in the shoot in a predominantly basipetal direction (35). This basipetal movement of auxins is believed to be a prime

inhibitor of axillary shoot growth (31).

Gibberellins. Gibberellins appear to by synthesized in growing leaves, fruits, and roots, and to be freely transported in all directions via the phloem and xylem (34). Gibberellic acid (GA) has been shown to overcome dormancy in a number of plants (24). The characteristic vegetative response of woody plants to GA is the stimulation of stem elongation (1, 15, 23, 40). GA also speeds the growth rate in a number of woody plants (1, 15, 41) and has been shown to induce favorable growth responses when applied to Japanese holly (40).

Cytokinins. It appears that cytokinins are synthesized primarily in the roots and transported via the xylem throughout the plant (14). They have been shown to cause bud initiation in tobacco callus and stem tissue cultures (29) and to release buds that are inhibited by auxins (37). Miller (19) indicated cytokinins to be involved in cell division, cell enlargement, shoot initiation, bud elongation, leaf growth, and possibly root growth.

Cytokinins have been shown to overcome apical dominance and stimulate axillary shoot development in a number of plant species (25, 26, 38, 39). Wright (39) reported that the synthetic cytokinin, N^6benzylaminopurine (BA), increased axillary shoot number and decreased stem length in two holly species. This increase in axillary shoot numbers may have been partially due to the accumulation of nutrients in areas where cytokinins have been applied (27).

Hormone-directed transport. The possibility that hormone-directed nutrient transport plays an important part in the correlative inhibition of axillary buds and shoots was originally proposed by Went (36).

More recently other workers have recorded preferential movement of ^{14}C-assimilates, ^{14}C-sucrose, and ^{32}P to regions of high exogenous auxin concentrations (2, 4, 5). Consequently, it has been proposed that correlative inhibition of lateral buds is due to an impedance in the supply of nutrients to these buds, brought about by the preferential movement of nutrients to regions of high endogenous auxin concentration in the apical buds of an intact plant, or to the point of exogenous auxin application in a decapitated plant (37).

Nutrient availability. Further evidence indicated that nutrient availability is an important factor in apical dominance. This has been demonstrated with inorganic nutrient supply, particularly N, and to a lesser extent K and P (7, 8, 16, 17, 22, 32).

Essentially, the hormone-directed metabolite transport or nutrient diversion theory requires that necessary available metabolites accumulate in the growing apical bud. That such hormone-stimulated nutrient accumulation can occur has been shown repeatedly (references cited above). Some scientists believe this to be a direct effect (36); however, many feel that it is only an indirect effect (22). McIntyre (16) showed that N supply can influence correlative inhibition, but that it appeared to be an indirect effect. This effect of nutrition was well illustrated in a recent investigation on the influences of N supply on growth and development of *Agropyron repens* (17). It was found that the inhibiting influence of the rhizome apex on growth of lateral buds could be quite precisely controlled by varying the N level. At low N levels lateral bud growth was completely inhibited, but could be induced by removing the apex. However, at high N levels lateral bud

dormancy was eliminated. All the buds developed as lateral rhizome branches on the intact plant (17).

In a number of woody plants, high N levels have been shown to increase the number of laterals (11, 13). This suggests that high N levels may partially control apical dominance in some woody plants.

McIntyre (16) suggested as a working hypothesis that stimulation of lateral bud activity when the stem apex is removed is due to increased nutrient availability to these buds. Overwintered stems, which are normally high in nutrients and carbohydrates (18), have a higher percentage of lateral bud breaks than stems from current growth, which are low in nutrients and carbohydrates (12). Pruning is often necessary in mid-summer to stimulate lateral bud development on new growth. This suggests that high nutrition may stimulate lateral bud development either directly by increasing nutrient availability, or indirectly by increasing the concn of hormones necessary for the stimulation of growth.

Materials and Methods

Expt. 1. Single stem 'Helleri' holly cuttings (7 cm long) were taken March 17, 1975, and placed in 5 cm peat pots containing a medium of peat and perlite (1:1, v/v). Six peat pots, each containing 1 cutting, were placed in a 15x10 cm plastic tray and placed under intermittent mist (10 second burst/10 minutes). These plants were grown in a greenhouse maintained at a day-night temperature of $28^{o}/21^{o}$ under natural photoperiod. Pest control was routinely carried out.

On June 4, 1975, after the development of a uniform root system, 3 N levels and 4 growth regulator treatments were applied. Nutrients were supplied with a Hoagland and Arnon (9) nutrient solution lacking nitrogen and a Hoagland (9) micronutrient solution in which 5 ppm of iron was supplied in the form of NaFeEDTA.

The basic nutrient solution was supplemented with N at 50, 150, and 300 ppm, accounting for the 3 treatments. Potassium nitrate was used to supply 50 ppm N for all treatments. Ammonium nitrate was added to increase the levels of N to 150 and 300 ppm. Twenty ml of the basic nutrient solution containing either 50, 150, or 300 ppm was added weekly to each individual plant.

Gibberellic acid (GA_3) was dissolved in 2-3 ml of ethanol and then diluted to 1 liter with distilled water. A few drops of 1N KOH was added to benzyladenine (BA) before dissolving in 2-3 ml of ethanol which was then diluted to 1 liter with distilled water. The plants were treated with a fine foliar spray on June 4 and 11, 1975, using a hand atomizer. The following growth regulators containing Tween 80

(polyoxyethylene sorbitan monooleate at .05%) were applied to plants growing at each nitrogen level:

(1) Control-distilled water + Tween

(2) Gibberellic acid (GA_3) 400 ppm

(3) Benzyladenine (BA) 600 ppm

(4) GA_3 (400 ppm) + BA (600 ppm) sprayed separately 2 hours apart.

Rates used were based on data by Wright (39, 40).

A split plot design with 4 replicates was used. The 3 N levels were assigned to main units, which were split to accommodate the 4 growth regulator treatments. Six plants per treatment were used. All means were separated by Duncan's multiple range test.

On July 14, 1975, after the initial flush of growth had ceased, data on the number of initial shoots and shoot length were taken. After this date, data were taken biweekly on the number of secondary shoots (those which grew from the initial shoots), length of the secondary shoots, and length of the initial shoots until August 25, 1975. When axillary buds expanded to 2 mm in length, they were counted as new shoots. Both initial and secondary shoot lengths were determined by measuring the 3 longest shoots per plant, and taking an average. Final data, taken August 25, also included maximum plant height, maximum plant width, fresh and dry weights. Three plants per replication were used to determine fresh and dry weights and N content. These samples were oven-dried for 48 hours at 70°C, ground in a Wiley mill using 20 mesh screen and total N determinations made using a modified microKjeldahl method (42).

Expt. 2. 'Helleri' holly cuttings were taken July 9, 1975, and

handled similarly to Expt. I with the following exceptions: (a) propagation media was Weblite[1]; (b) N treatments were begun September 25, 1975, 2 weeks before growth regulator treatments which were applied October 9 and 16, 1975; (c) long day conditions were maintained by providing supplemental light with incandescent lamps at approx. 15 ft-c from 11 p.m. until 2 a.m.; (d) data were taken weekly for 6 weeks on initial shoot number and length.

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[1]Webster Brick Company, Roanoke, Va. 24012

Results

Nitrogen: effects on number and length of initial shoots. The number of initial shoots that occurred on the single stem liners tended to increase with N levels in Expt. 1, but not significantly at the 5% level (Table 1). There was a greater response in Expt. 2 with 300 ppm N increasing initial shoot numbers by 30% over 50 ppm N.

Initial bud break at 300 ppm N began approx. 3-5 days before 150 ppm N and 2 weeks before 50 ppm N in both experiments. Plants in Fig. 1, photographed at 3 weeks after the first N treatment, demonstrate this effect. The early bud break under high N resulted in 50 and 60% of the growth at 150 and 300 ppm N respectively, occurring within the first 3 weeks, whereas only 5% of the growth at 50 ppm N occurred during the first 3 weeks (Table 2).

Initial shoot length was increased in both experiments by 300 ppm N compared to 50 ppm N. Also, shoot length at 300 ppm N was greater than at 150 ppm N in Expt. 2 (Table 1).

Growth regulators: effects on number and length of initial shoots. In Expt. 1, only BA significantly increased the number of initial shoots, while GA decreased shoot numbers (Table 1). The length of initial shoots in both Expt. 1 and 2 was increased by GA_3, and was decreased by BA (Table 1). These differences were not persistent and were not detected in data taken after the 7th week. Since no differences were apparent in subsequent measurements, these data are omitted.

Growth rate effects by growth regulators were not detected (Table 2).

Table 1. Effects of N rate and growth regulator applications on number and length of initial shoots of 'Helleri' holly.[z]

	Expt. 1		Expt. 2	
	No. of initial shoots/ plant	Avg length[y] of initial shoots (cm)	No. of initial shoots/ plant	Avg length of initial shoots (cm)
Nitrogen ppm				
50	5.2a[x]	3.9b	3.5b	1.9b
150	5.6a	4.2a	4.4a	2.1b
300	6.0a	4.2a	4.6a	2.4a
Growth regulators				
Control	5.7b	4.1b	4.2a	2.1b
BA (600 ppm)	6.6a	3.6c	4.3a	2.0b
GA_3 (400 ppm)	4.9c	4.6a	3.9a	2.4a
GA_3 + BA[w]	5.3b	4.1b	4.1a	2.2ab

[z]Data taken at the end of the first flush of growth. Expt. 1, 7 weeks. Expt. 2, 6 weeks.

[y]Means of the 3 longest shoots/plant.

[x]Means within a column not followed by a letter in common are significantly different at the 5% level.

[w]GA_3 (400 ppm) was applied until run-off. Two hours later BA (600 ppm) was applied until run-off.

Table 2. Effects of N rate and growth regulator applications on percent of total growth occurring between 2-3, 3-4 and 4-5 weeks in Expt. 2.

	Weeks			Mean shoot length (cm)[z]
	2-3	3-4	4-5	
Nitrogen ppm				
50	5	51	44	1.4b[y]
150	50	30	20	2.0a
300	60	30	10	2.0a
Growth regulators				
Control	41	40	19	1.7a
BA (600 ppm)	43	42	15	1.6a
GA_3 (400 ppm)	39	34	27	2.1a
GA_3 + BA[x]	39	35	26	1.8a

[z]Means of the 3 longest shoots/plant.

[y]Means within a column not followed by a letter in common are significantly different at the 5% level.

[x]GA_3 (400 ppm) was applied until run-off. Two hours later BA (600 ppm) was applied until run-off.

Nitrogen: effects on secondary shoot number and length. In Expt. 1, secondary shoot number and length were increased by 300 and 150 ppm N compared to 50 ppm N (Table 3 and Fig. 2).

Expt. 2 was terminated at the end of the initial flush of growth; therefore, secondary shoot numbers were not evaluated.

Growth regulators: effects on secondary shoot number and length. Secondary buds on GA_3 treated plants broke approx. 3-5 days before any other treatment and produced the greatest number of secondary shoots (Table 3).

Secondary shoot length was slightly increased by GA_3 over the control, but not at a significant level (Table 3).

Nitrogen: effects on fresh and dry weights and plant size. Fresh and dry weights were greater at 150 and 300 ppm N than at 50 ppm N (Table 4). Only with dry weights in Expt. 1 was there no significant difference between 300 and 150 ppm N.

Plants grown at 300 and 150 ppm N were 1.3x larger in Expt. 1, and 1.8x larger in Expt. 2 than those grown at 50 ppm N (Fig. 3). Plant sizes were approx. equal at 150 and 300 ppm N in both experiments.

Growth regulators: effects on fresh and dry weights. Growth regulators did not significantly affect fresh or dry weights (Table 4).

Nitrogen-growth regulator interactions. There were no N-growth regulator interactions.

Table 3. Effects of N rate and growth regulator application on secondary shoots, plant height and width of 'Helleri' holly.

	Nitrogen ppm			Growth regulators			
	50	150	300	Control	BA (600 ppm)	GA_3 (400 ppm)	GA_3+BA[z]
Expt. 1[y]							
Secondary shoot no./plant	4.2b[x]	9.7a	11.0a	7.7b	7.2b	10.0a	8.3ab
Secondary shoot length (cm)	1.4b	3.0a	3.1a	2.3a	2.4a	2.7a	2.6a
Height (cm)	8.3b	10.0a	10.1a	9.5a	9.6a	9.5a	9.4a
Width (cm)	8.8b	12.7a	12.8a	11.5a	12.2a	11.1a	11.1a
Expt. 2[w]							
Height (cm)	5.5b	7.9a	8.5a	---	---	---	---
Width (cm)	4.4b	9.0a	10.1a	---	---	---	---

[z]GA_3 (400 ppm) was applied until run-off. Two hours later BA (600 ppm) was applied until run-off.

[y]Expt. terminated at 13 weeks.

[x]Means within a row not followed by a letter in common are significantly different at the 5% level.

[w]Expt. terminated at 6 weeks.

Table 4. Effects of N rate and growth regulator applications on N concn, total N, fresh wt, and dry wt of 'Helleri' holly.

	Nitrogen ppm			Growth regulators			
	50	150	300	Control	BA (600 ppm)	GA_3 (400 ppm)	GA_3+BA[z]
Expt. 1[y]							
Fresh wt (g)	5.9c[x]	9.1b	10.3a	8.2a	9.1a	7.8a	8.8a
Dry wt (g)	2.1b	2.8a	3.0a	2.6a	2.8a	2.5a	2.7a
N-% of dry wt	2.1c	2.2b	2.6a	---	---	---	---
Total N (mg)	44.0c	62.0b	78.0a	---	---	---	---
Expt. 2[w]							
Fresh wt (g)	0.6c	0.8b	0.9a	0.8a	0.7a	0.8a	0.8a
Dry wt (g)	0.2c	0.3b	0.4a	0.3a	0.3a	0.3a	0.3a
N-% of dry wt	1.4b	2.0a	1.6b	---	---	---	---
Total N (mg)	2.8b	5.0a	5.2a	---	---	---	---

[z]GA_3 (400 ppm) applied until run-off. Two hours later BA (600 ppm) was applied until run-off.

[y]Expt. terminated at 13 weeks.

[x]Means within a row not followed by a letter in common are significantly different at the 5% level.

[w]Expt. terminated at 6 weeks.

Fig. 1. Effects of 50, 150, and 300 ppm N (left to right) on shoot length which gives an indication of time of bud break of 'Helleri' holly.

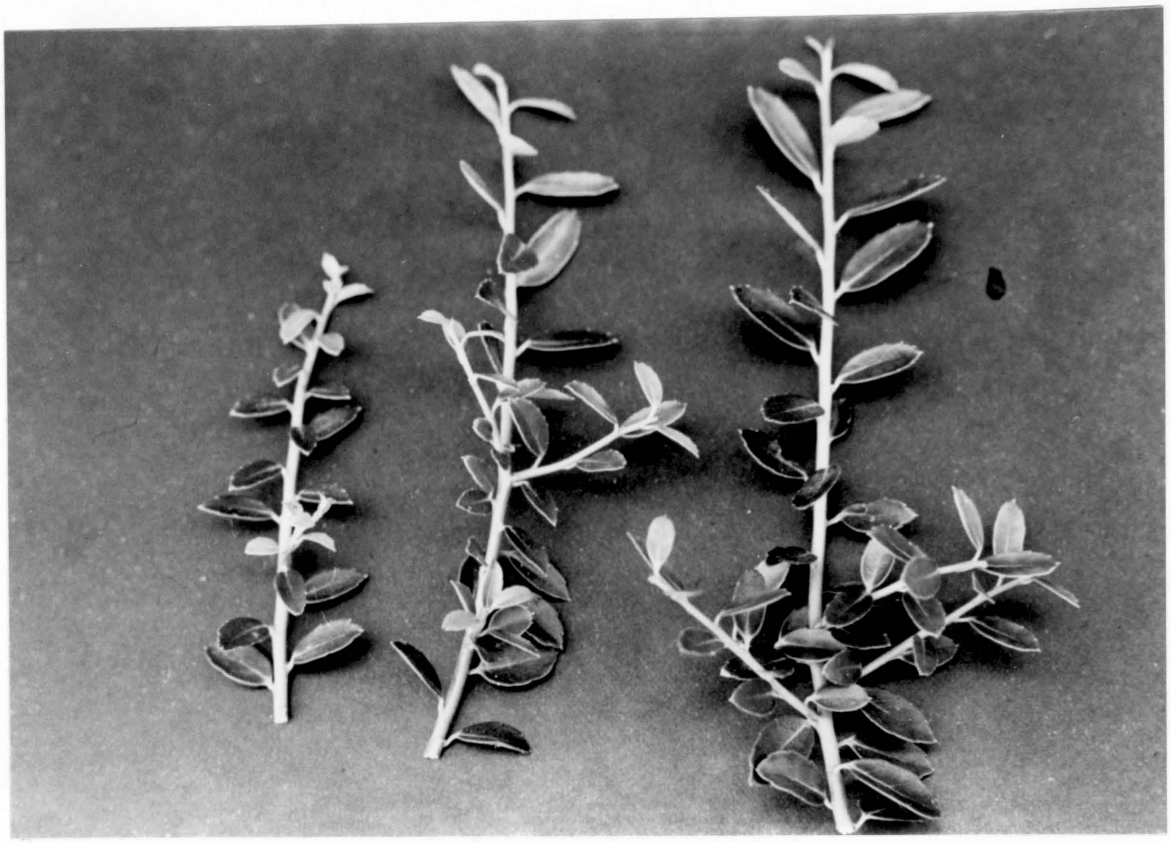

Fig. 2. Effects of 50, 150, and 300 ppm N (left to right) on secondary shoot number and length from a single stem of 'Helleri' holly.

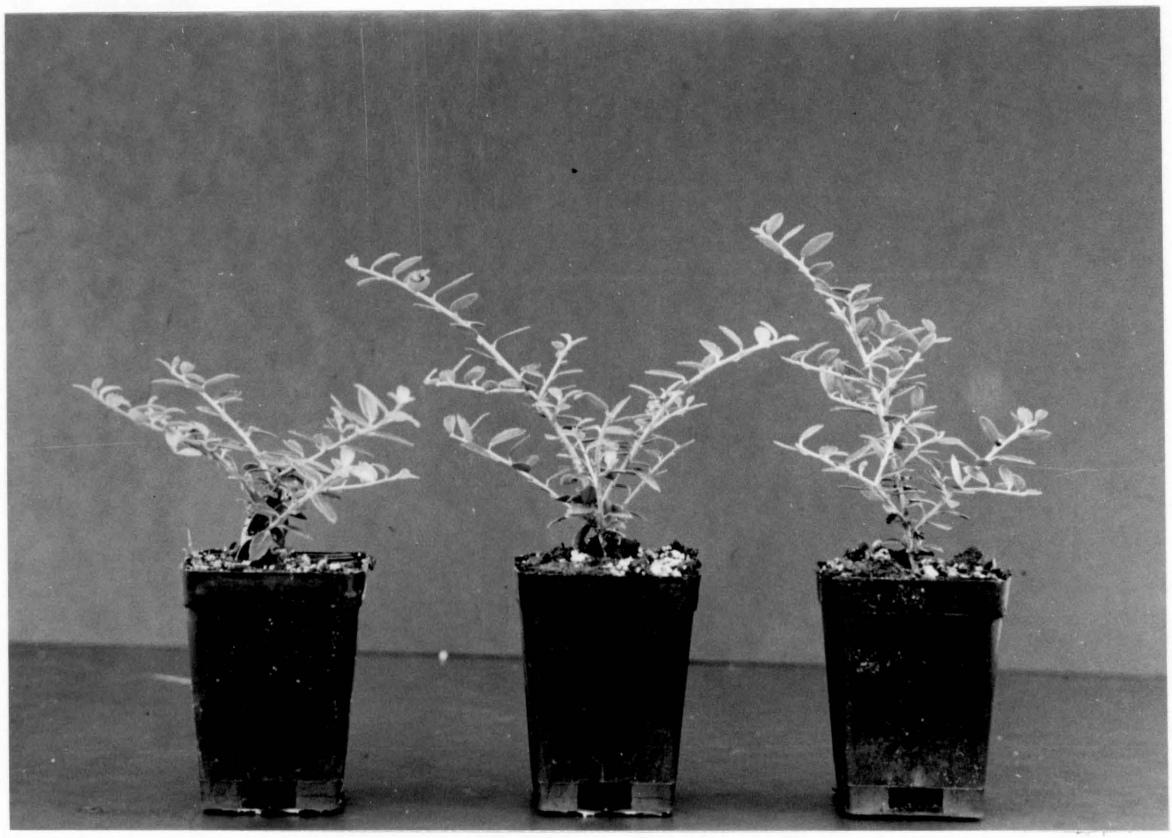

Fig. 3. Plant size at 13 weeks occurring on 'Helleri' holly grown at 50, 150, and 300 ppm N (left to right).

Discussion

This study indicates that summer propagated 'Helleri' holly cuttings (Expt. 2) can be induced to grow following propagation if adequate N is applied. These induced shoots would be a frame from which growth would occur the following spring. In comparison, growth from winter rooted cuttings must occur from a single shoot the following spring unless multiple branched cuttings are taken. Consequently, by the end of the first full growing season the summer propagated cutting should have a greater size and value.

Earlier bud break produced by 300 and 150 ppm N treated plants resulted in an early flush which grew rapidly and became dormant sooner than at 50 ppm N. Since lack of hardiness of new growth occurring late in the season is a major problem with summer rooted cuttings, the earlier growth at the high N levels may allow additional time for the cutting to 'harden off'.

Winter propagated cuttings (Expt. 1) were not as responsive to N levels as summer propagated cuttings (Expt. 2). With the exception of fresh weight, growth parameters measured in Expt. 1 at 300 ppm N were not significantly greater than at 150 ppm. In Expt. 2 though, 300 ppm N resulted in a greater response over 150 ppm in a number of instances. This is probably due to a difference in the physiological state between summer and winter propagated cuttings. Cuttings taken in the winter have received their chilling requirement and normally have adequate nutrient and carbohydrate reserves to sustain growth (21). This is why they normally grow immediately after propagation even if no nutrients

are supplied. On the other hand, cuttings from current season's growth are lower in nutrients and carbohydrate reserves (14). Consequently 150 ppm N may be adequate for cuttings propagated in late winter and early spring, while 300 ppm N may be necessary for summer propagated cuttings.

In both experiments, plant size was greater at 150 and 300 ppm N (Fig. 3). If maintained at these nutrition levels for one growing season, the result would be a plant of significantly greater size and therefore a more salable item. The lack of major differences in growth response to 150 and 300 ppm N in Expt. 1 indicates that 150 ppm N may be adequate for rapid growth of a small liner. However, this does not rule out the possibility of a higher nutrient requirement as plant size increases (8).

Total N in plant tissue increased with increasing application of N in Expt. 1. This agrees with work done by several authors (11, 20, 21). However, in Expt. 2, N concn was significantly higher in plants grown at 150 ppm N in comparison to plants grown at 300 ppm N. This is thought to have occurred because of sampling procedure. Plants grown at 300 ppm N were beginning a new flush of growth resulting in a dilution of N concn within the plant. However, plants grown at 150 and 50 ppm N were approaching a new flush of growth, which likely contained a higher concn of N.

Initial shoot numbers were increased by BA, and decreased by GA_3. Shoot length was suppressed by BA while GA_3 had the opposite effect. BA has been shown to have these characteristic effects on other plants (3, 18, 24, 26, 39), while the most characteristic effect of GA has

been to increase stem length (1, 15, 22, 40, 41). The GA_3 treated plants had an open, widely branching appearance which provides a more favorable frame for future growth compared to BA treated plants.

In Expt. 2, the same trends were evident with initial shoot numbers and length, although not as pronounced. The reverse was expected as with N effects since endogenous levels of growth regulators in the summer cuttings would normally be low compared to winter cuttings and thereby respond more to the growth regulator applications. The reason for this lack of response on summer propagated cuttings may be that the growth regulators were applied 2 weeks later than the N treatment, consequently endogenous hormones may have reached adequate levels for lateral bud development before the growth regulators were applied.

In general the response to growth regulators was measurable for 6-7 weeks; after this the difference gradually disappeared. Therefore, repeated application of growth regulators may be necessary for a continuing response.

The results of this investigation have clearly shown that adequate N levels can promote rapid growth and lateral branching of 'Helleri' holly liners. However, there remain many unanswered questions on N nutrition of holly.

Furthermore it seems that GA merits a more intensive investigation involving other GA's, the number of applications, and timing necessary to elicit a favorable growth response over an entire growing season.

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GROWTH OF JAPANESE HOLLY AS AFFECTED

BY NITROGEN AND GROWTH REGULATORS

by

Charles Homer Gilliam

(ABSTRACT)

The effects of 3 nitrogen levels and 2 growth regulators on growth of winter and summer propagated cuttings of *Ilex crenata* cv. Helleri were studied.

Nitrogen applications promoted bud break after rooting of summer cuttings and also enhanced the growth of winter rooted cuttings. Generally, plants grown at 300 and 150 ppm N had greater initial shoot numbers and length, secondary shoot numbers and length, height, width, fresh weight, and dry weight compared to plants grown at 50 ppm N.

Initially, benzyladenine (BA) at 600 ppm increased the number of primary breaks and decreased the stem length, while gibberellic acid (GA) at 400 ppm decreased the number of primary bud breaks and increased stem length. The hormone responses persisted for approx. a month, starting a fortnight from the first application. GA_3 + BA caused very erratic responses. There were no N-growth regulator interactions.