SOME ASPECTS OF NITROGEN NUTRITION ON SELECTED ILEX

by

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Dedication

The completion of this manuscript would not have been possible without the patience, understanding and support of my wife, Gail. Also, to our wonderful parents we are indebted for their love, encouragement and support. Finally, I would recognize my grandparents, who have taught me so much. This dissertation is dedicated to all of these individuals.

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Chapter I

Effects of Four Nitrogen Levels on Soil, Soil Solution and Tissue Nutrient Levels in Three Container-grown *Ilex* Cultivars

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Abstract. Three holly cultivars, Ilex crenata, Thunb. 'Helleri' and 'Rotundifolia' and Ilex cornuta Lindl. et Paxt. 'Burfordi', were grown in 3 liter containers at 200, 300, 400 and 500 ppm nitrogen (N). Significant correlation coefficients were present between plant growth (dry wt accumulation) and both leaf N content and soil solution nitrates, while low correlation coefficients were present between soil nitrates and plant growth. Shoot growth of 'Helleri' and 'Burfordi' was not increased by N levels higher than 300 ppm while 400 ppm was optimal for 'Rotundifolia'.

Additional index words. holly, plant nutrition, soil testing, tissue analysis, pine bark.

Introduction

Production of nursery plants has increased substantially in the last 10-15 years. Recently, the trend has been to grow an increasingly larger portion of these plants in containers with amended soil or soilless media. The transition from field-grown plants to container-grown plants has been accompanied by many problems. Since the root systems are confined to a limited volume, frequent irrigation and fertilizer applications are needed to sustain plant growth. The restricted volume, altered physical characteristics and compositional properties of the media have contributed to nutritional deficiencies and excesses. One major problem in the area of nutrition is the lack of information on the rates of nitrogen (N) fertilizers necessary for maximization of plant growth in containers. Compared with other woody perennial crops, limited detailed information is available on the optimum levels of N for maximum growth, optimal endogenous N levels and the determination of the nutrient status by leaf analysis. Also most of the more common soil test procedures now in use are considered unacceptable for the soilless mixes. A soil test is needed to accurately monitor nutrient levels in the soil and to predict the response to applied nutrients.

The purpose of this study was to investigate the possibility of developing a systematic approach to predicting plant growth and plant nutrient requirements through soil, soil solution or tissue analysis, and to evaluate the effects of 4 N levels on the growth of 3 Ilex cultivars.

Review of Literature

Meyer and Splittstoesser (14) showed nitrogen to be the most important tissue component for spring growth of Syringia vulgaris. Plants which were grown under high nitrogen levels had faster growth rates and utilized more nitrogen and carbohydrates in the spring flush of growth than the plants grown under low nitrogen regimes. Gouin and Link (6) have shown maximum growth of Taxus to occur with the following combination of nutrients: N, 224 ppm; P, 75 ppm; and K, 135 ppm. In later studies with Pyracantha coccinia 'Loboy', Ilex crenata, Prunus laurocerasus, Juniperus horizontalis and Weigela florida, Gouin and Link (7) reported that osmocote 18-6-12 at 113.4 g and 226.8 g per 35.5 liters of media produced growth equal to plants receiving 100 to 150 ppm N at each irrigation from a water soluble fertilizer. Higher levels of osmocote reduced plant growth. Kelly (9) investigated the effects of different rates of application of nitrogen and potassium on growth of Pyracantha coccinea 'Lalandi' and Ilex crenata 'Rotundifolia'. He showed that nitrogen applied at 'moderate' rates (1.57 and 2.34 g of NH_4NO_3 per liter of water) produced the best growth response as determined by the number of laterals and total growth per plant. Higher rates produced limited or no additional growth. Dickey et al. (2) determined that high nitrogen (900 kg/ha/yr) combined with potassium at rates of 150 or 300 kg/ha/yr and phosphorus at 34 kg/ha/yr resulted in superior quality plants when applied to Rhododendron indicum 'Formosa' and Viburnum suspensum. Sanderson and Martin (17) grew Ilex cornuta 'Burfordi', Thuja occidentalis and Viburnum burkwoodii in two medium sources

with 9 nitrogen sources. The greatest growth resulted with weekly or biweekly liquid application compared to slow release fertilizers and dry fertilizer applications.

Data on nitrogen nutrition in container fertilization are limited not only with respect to nitrogen rates and growth responses, but also with respect to the determination of the nutrient status of the plant through tissue analysis. The leaf is the most commonly used mode for determining the nutritional status of plants. In 1960, Davidson (1) employed tissue analysis to determine the nutrient composition of 7 species of woody ornamentals. He noted that the nutrient composition between species of the same genus did not vary greatly. The samples were taken in June, July and August from 5 nursery locations, and determinations were made for 9 essential elements. Results indicated that N and K decreased with time, Ca, Mg, Fe and Mn increased, and P, B and Cu did not change, with time. Kelly and Shier (10) collected samples from Taxus media throughout the year, and concluded that the period from September to December gave the most accurate results because of steady levels of nutrient concn during this period.

Meyer and Tukey (15), working with Taxus media 'Hicksii' and Forsythia intermedia 'Spring Glory', reported that when various levels of N, P and K were added to the plants during the growing season, plant analysis during the dormant period was highly correlated with plant growth produced the following spring. Application of N and P increased the content of these nutrients measured during the dormant period and the amount of growth produced the following spring was directly related to nutrient content present in the dormant period. As a result of their

findings, Meyer and Tukey (15) suggested nutrient reserves could be used to predict growth of plants during the following year.

A number of studies have sought to determine the relationship between applied N and tissue N. In 1962, Kelly (8), using urea-formaldehyde as an N source and potassium frit as a K source, reported both N and K increased in leaves of 3 species as nutrient application rates were increased. Similar results were found by Dickey et al. (2) with Rhododendron indicum 'Formosa' and Viburnum suspensum. The critical N concn for growth of Rhododendron indicum was 2.0%.

Lumis (12) collected tissue samples from 12 woody species from 8 locations in an attempt to determine the foliar nutrient content of healthy 'Ontario grown' plants, and to establish norms from which fertilizer recommendations could be based. Nitrogen content was found to range from 1.5-3.4% N. In 1973, Smith (19) surveyed the foliar mineral element content of 30 species of nursery-grown ornamentals and presented average foliar nutrient levels.

Data on nitrogen fertilization are also limited with respect to the determination of the medium nutrient status. Soil testing procedures used to determine soil nutrient levels for field crops are not well adapted for container-grown nursery crops where artificial soil mixes are used (11). Soil tests for field crops usually measure plant nutrients in the soil solution plus reserve soil nutrients that may not be readily available for plant growth. The amount of nutrients in the soil solution is the primary interest in container-grown woody ornamentals, due to the limited reserve potential of the various artificial mixes.

A soil testing procedure was proposed by Lucas in 1972 (11). This procedure, the saturated soil extract, was a more reliable test for available nutrients in greenhouse mixes than the soil test presently used. Limited correlation data on this method of analysis are presently available for container-grown plants.

The unreliability of many of the more common soil testing procedures is underscored by low correlation coefficients determined by a number of workers when correlating soil nutrients to growth of container-grown plants. Flint and McGuire (4) attempted to determine the optimum levels of N and K in the soil for maximum growth of Forsythia intermedia 'Lynwood Gold' and Viburnum pliccatum tomentosum. The Spurway extracting solution (.018 N acetic acid) was used for extraction of the individual elements. Although ranges were given, no clear optimum ranges were found. Similar results were reported by Dickey et al. (2) and Kelly (8).

Materials and Methods

Single stem rooted cuttings of Ilex cornuta 'Burfordi' were potted in 3 liter containers in a pine bark and sandy loam medium (6:1 v/v) on March 26, 1976. To each 0.76 m^3 (1 yd^3) of the medium 2.7 kg of dolomitic limestone, 0.9 kg gypsum and 1.4 kg of 20% superphosphate were added. The plants were grown in a greenhouse at 28°C (day)/21°C (night) under natural photoperiod. On April 2, a uniform level of N, P and K (150, 65 and 125 ppm) was applied to all plants. Two weeks later applications of 4 N levels were initiated. A base level of nutrients was supplied with a 20 N - 8.7 P - 16.7 K nutrient solution containing 150 ppm N, 65 ppm P and 125 ppm K. Ammonium nitrate was used to increase the levels of N to 200, 300, 400 and 500 ppm. Two hundred ppm N was chosen as a lower limit based on previous work (5). Nutrient solutions were applied weekly with a Syfonex applicator. A randomized block design with 12 plants per treatment and 4 replicates was used.

On July 7, following the initial flush of growth, 2 plants per replication were used to determine fresh and dry wt of shoots and roots. Tissue samples of the most recently matured leaves were used to determine Ca, Mg, K, P and N. Samples were oven dried for 48 hours at 70°C, ground in a Wiley mill through a 20 mesh screen and total N determined by a modified micro-Kjeldahl method (16). Calcium, Mg and K were determined spectrophotometrically and tissue P colorimetrically (13).

Soil and soil solution samples were taken at the same time for Ca, Mg, P, K, NO_3, soluble salts and pH determinations. For the soil analysis, 4 ml of soil was mixed with 20 ml of dilute 0.05N HCl-0.025N

H_2SO_4 extracting solution, shaken for 5 min and filtered. Calcium, P, K and Mg levels of the filtrate were determined according to procedures employed by the VPI & SU Soil Testing Laboratory (3). Nitrates were extracted with 0.02N $CuSO_4$ solution for 10 min and filtered. Nitrate determinations were then made using an Orion nitrate specific ion electrode. Soil and soil solution samples were taken at the same time for pH and soluble salts. For the soil pH, 20 ml of soil was mixed with 20 ml of distilled H_2O, stirred, allowed to set 15 minutes and stirred immediately prior to reading. Determinations for pH were then made using an Orion model 601 pH meter. Soluble salts were determined by adding 20 ml of distilled H_2O to the 1:1 soil-water solution for pH determination and reading conductivity on a Solu Bridge RD 15. Soil solution samples consisted of the combined leachates from 2 containers collected after the addition of 100 ml of H_2O per 3 liter (1 gal) container. Calcium, P, K, Mg, NO_3, soluble salts and pH of the leachates were analyzed according to the procedures outlined above. Similar data were determined for soil, soil solution and tissue samples at the end of the second flush of growth (Aug. 22). For all cultivars, Time 1 (T1) indicates the sample taken after the first flush of growth in early summer, and Time 2 (T2) indicates sampling after the second flush of growth in late summer. At T2, data also included a 'growth index' $\left(\frac{\text{Height} + \text{Width}}{2}\right)$ measurement.

Ilex crenata 'Rotundifolia' and I. crenata 'Helleri' were handled similarly to I. cornuta 'Burfordi' with the following exceptions: (a) Treatments applied to 'Helleri' began May 13, 1976 and (b) Samples of 'Rotundifolia' were taken on May 7 and August 11, 1976 at the end of

each flush of growth.

Results

Plant Growth. Neither initial shoot growth measured at T1 nor root growth at either T1 and T2 were affected by N rates (data not shown). At T2 the shoot dry wt of the 3 cultivars was greater at 300, 400 and 500 ppm N compared to plants grown at 200 ppm N (Table 1). With the exception of the cultivar Rotundifolia, where the dry wt of the shoots increased with each N increment up to 400 ppm N, there were no differences among the 3 highest treatments. Growth index responses at T2 were similar to dry wt data at T2 (Table 2).

Tissue Analysis. The greatest increase in leaf N for 'Helleri' at T1 and T2 occurred with plants treated at 300 ppm N vs 200 ppm N (Table 3). Less increase in leaf N occurred beyond 300 ppm N for this cultivar. Leaf N content of 'Burfordi' and 'Rotundifolia' increased as a result of both 300 and 400 ppm applied N. Values for leaf N concn at each N treatment for the 3 cultivars were in similar ranges at both sampling dates. Significant r values were present between leaf N concn at T1 and plant growth (Table 4). Nitrogen levels at T1 were used for the correlation since it represented the nutrient status of the tissue preceding the second flush of growth.

Tissue levels of Ca, Mg and K tended to decrease with increasing leaf N concn at T2 (Table 5) for the Ilex crenata cultivars. With 'Burfordi' consistent trends were not evident.

Soil Solution. In general, increases in NO_3 soil solution levels occurred at fertilizer rates up to 400 ppm N (Table 3). These differences occurred at both T1 and T2, although nutrient levels were greater

Table 1. The effects of four N levels on total shoot dry wt (g) of three Ilex cultivars.

N ppm	'Rotundifolia' T1	'Rotundifolia' T2	'Helleri' T1	'Helleri' T2	'Burfordi' T1	'Burfordi' T2
200	2.0a	3.3c[z]	2.2a	4.5b	3.2a	5.2b
300	2.1a	3.9bc	2.2a	5.7a	3.7a	6.3a
400	2.2a	5.0ab	1.6a	5.4a	3.3a	6.3a
500	2.2a	5.4a	2.1a	5.6a	3.7a	6.8a

[z] Mean separation within columns by Duncan's multiple range test 5% level.

Table 2. The effects of weekly N applications on the 'growth index'[z] at the end of the first full season following propagation.

N ppm	'Rotundifolia'	'Helleri'	'Burfordi'
200	14.6c[y]	19.4b	15.6c
300	15.6c	25.2a	17.7a
400	17.6b	20.5b	16.4b
500	20.5a	25.2a	17.7a

[z] Growth Index = $\frac{\text{Height + Width}}{2}$, data taken at T2.

[y] Mean separation within columns by Duncan's multiple range test 5% level.

Table 3. Effects of four N levels on leaf N concn, soil solution and soil nitrate values of three container-grown Ilex cultivars following two successive flushes of growth (Time 1 and Time 2).[z]

N ppm	Time 1 Leaf N	Time 1 Soil solution NO_3-N	Time 1 Soil NO_3-N	Time 2 Leaf N	Time 2 Soil solution NO_3-N	Time 2 Soil NO_3-N
	(%)	(ppm)	(ppm)	(%)	(ppm)	(ppm)
			Rotundifolia			
200	1.82b	26b	12b	1.95c	6c	5a
300	2.28ab	103ab	18b	2.06bc	15bc	7a
400	2.41a	135a	25b	2.28ab	22ab	5a
500	2.34a	134a	48a	2.34a	30a	20a
			Helleri			
200	1.73c	7c	5b	1.74b	5b	5a
300	2.15ab	35b	9b	2.16a	14a	13a
400	2.29a	79a	30a	2.24a	12a	11a
500	2.40a	80a	40a	2.31a	18a	19a
			Burfordi			
200	1.42c	30d	12b	1.58c	7c	5c
300	1.65b	92c	46b	1.74b	24c	14c
400	2.01a	185b	82ab	1.95a	60b	35b
500	1.96a	235a	136a	1.92a	93a	61a

[z] Mean separation within columns by Duncan's multiple range test 5% level.

Table 4. Correlation coefficients between shoot dry wt accumulation of three Ilex cultivars and tissue N, soil solution NO_3 and soil NO_3 values.[z]

Variable	Dry wt increase between T1 and T2		
	Rotundifolia	Helleri	Burfordi
Tissue N[y]	0.73**	0.69**	0.67**
Soil Solution NO_3	0.52*	0.69**	0.71**
Soil NO_3	0.37	0.12	0.30

[z] N = 16 pairs

[y] T1 data used for all parameters correlated with plant growth.

** Indicates significant at 1% level and * indicates significant at 5% level.

Table 5. Effects of four N levels on tissue levels of Ca, Mg and K of three container-grown Ilex cultivars.[z]

N ppm	Time 1			Time 2		
	Ca	Mg	K	Ca	Mg	K
	(%)	(%)	(%)	(%)	(%)	(%)
			Rotundifolia			
200	.87a	.59a	1.75a	.98a	.61a	1.79a
300	.97a	.61a	1.78a	.84a	.59a	1.62a
400	.92a	.60a	1.81a	.84a	.59a	1.65a
500	.92a	.61a	1.76a	.87a	.58a	1.63a
			Helleri			
200	.54a	.38a	1.75a	.73a	.57a	1.99a
300	.54a	.37a	1.63b	.70a	.55a	1.77b
400	.55a	.39a	1.57b	.67a	.55a	1.62c
500	.55a	.39a	1.58b	.65a	.53a	1.48c
			Burfordi			
200	.90a	.42a	1.44a	.64b	.44a	1.94a
300	.93a	.39a	1.30b	.65b	.42a	1.81a
400	.88a	.37a	1.25b	.79a	.44a	1.86a
500	.88a	.37a	1.25b	.73a	.43a	1.81a

[z] Mean separation within columns by Duncan's multiple range test 5% level.

at T1 than at T2. Significant r values were found between soil solution nitrates at T1 and plant growth (Table 4).

Soil Analysis. Differences in soil analysis values for nitrates exhibited trends similar to soil solution nitrates at the various fertilizer rates (Table 2). Potassium values were approx the same in both soil and soil solution analyses. All soil test values for Ca, Mg and P were much higher than soil solution values for the same nutrients. Also pH was generally lower in soil test values compared to soil solution values, and decreased with increasing application of N (Table 6).

Table 6. Effects of four N levels on soluble salts and pH in three container-grown Ilex cultivars. [z]

N	Time 1				Time 2			
	Soil		Soil solution		Soil		Soil solution	
ppm	ss[y]	pH	ss	pH	ss	pH	ss	pH
				Rotundifolia				
200	758a	--	1305a	--	140a	6.2a	93c	7.0a
300	771a	--	1564a	--	198a	6.2a	128b	6.9a
400	819a	--	1772a	--	144a	6.2a	158ab	6.9a
500	777a	--	1753a	--	220a	6.0a	181a	6.9a
				Helleri				
200	412b	5.7a	463b	6.5a	240a	6.0a	192a	6.7a
300	406b	5.6ab	670ab	6.2b	236a	5.9a	191a	6.8a
400	461ab	5.4b	741a	6.2b	222a	5.9a	154a	6.9a
500	659a	5.2c	834a	6.2b	244a	5.8a	176a	6.8a
				Burfordi				
200	358b	6.0a	432c	6.6a	324a	6.2a	195c	6.6ab
300	464b	5.8ab	635bc	6.4ab	275a	6.2a	224c	6.8a
400	595ab	5.7bc	1105ab	6.2b	329a	6.0b	344b	6.5bc
500	938a	5.4c	1552a	6.1b	419a	5.8c	509a	6.4c

[z] Mean separation within columns by Duncan's multiple range test 5% level.

[y] ppm

Discussion

This study indicates that a systematic approach to fertilization of container-grown woody ornamentals is feasible through a combination of tissue analysis and soil solution analysis. Significant correlation values for tissue N with plant growth indicate that leaf N concn of the most recently matured leaves may be more closely related to future growth than any other factor studied. This suggests future plant growth may be estimated (predicted) based on leaf N analysis before growth begins. Samples taken at the end of the spring flush would indicate the relative intensity of growth to occur on the following flush. Earlier studies (5,15) showed that samples taken in late summer reflected the relative intensity of growth that occurred the following spring. Critical leaf N levels for maximum growth of the 3 cultivars were: Helleri - 2.20%, Rotundifolia - 2.4% and Burfordi - 1.8-2.0%. Kelly (9) reported a leaf N concn of 2.65% was the optimum for growth of 'Rotundifolia' based on correlation values between total shoot length and leaf N concn. The fact that our data are based on shoot dry wt could account for the difference. Values of leaf N concn were at similar levels at both T1 and T2 for each respective treatment indicating a consistency of testing with this procedure.

Significant correlation values between soil solution nitrates and plant growth indicate the feasibility of using this method to predict plant growth and to monitor media nutrient levels during the current season. The low soil solution NO_3 levels at T2 compared to T1 were probably due to leaching by frequent irrigation during the summer months.

The use of this method of soil analysis may necessitate a systematic sampling procedure where samples would be taken regularly (biweekly or monthly) after the same no. of waterings following fertilization. Application of soluble fertilizer with each watering or use of a slow release fertilizer would alleviate variation in NO_3 levels with the exception of periods with frequent rainfall.

Soil analysis was the least useful of methods tested for predicting fertility requirements. This is in agreement with work done by other authors (2,4). When soil nitrates were correlated with plant growth, low r values were present (Table 4). Also, all soil test values for Ca, Mg and P were very high compared to soil solution test values, yet all Ca tissue levels were lower than other workers have reported (19). This indicated nutrients unavailable for plant growth were being extracted in the soil testing procedure.

This study also showed no advantage to weekly application of N higher than 300 ppm during the first full growing season following propagation of the 'Helleri' and 'Burfordi' (Table 1). 'Rotundifolia', however, may require higher levels of N fertilization to maximize growth.

With any of the 3 species tested there were no differences in plant dry wt between N treatments at T1 (Table 1). The lack of a growth response to the N applications indicates plant growth responded to prior fertilizer applications. Thus, the first flush of growth was a result of uniform fertilization following propagation. This is in agreement with work done previously by these authors (5) and by Meyer and Tukey (15) which demonstrates initial plant growth in the spring

to be a function of fertilizer applications the previous fall.

The concept of plant growth responding primarily to prior N application and not to application made during a flush is important to growers who buy plants and step them up to larger containers. Their first flush of growth depends primarily on the previous owner's fertilization program. Consideration should therefore be given to the fertilization program of the seller when buying plants.

This study also shows that pH ranged from 5.2 - 6.2 for soil and 6.1 - 7.0 for soil solution (Table 6). This should indicate to nurserymen that when adequate nutrients and water are available, acceptable plant growth will occur under a wide range of pH. Furthermore, when high levels of N are applied, a lowering of the pH will occur when acid forming N fertilizers are used (Table 6). Thus growers with a good N fertilization program, if they are using acid forming N fertilizers, may have lower pH's than growers with poor N fertilization practices.

Also, pH of the soil solution was approximately .5 units higher than the corresponding soil pH. This difference of .5 units should be taken into consideration when soil solutions are used.

Soil soluble salt values ranged from 150 - 950 ppm while soil solution soluble salts ranged from 100 - 1700 ppm (Table 6). There was no apparent plant damage from soluble salt accumulation within this experiment.

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Chapter II

Effects of Three Nitrogen Levels on Tissue Nitrogen Fluctuation During a Flush of Growth on 'Helleri' Holly

Abstract. The effects of 3 nitrogen (N) levels on tissue N fluctuations of Ilex crenata, Thunb. 'Helleri' were studied. For all N levels, tissue N levels were shown to increase following the cessation of shoot elongation until a level of tissue N was reached where a new flush of growth began. The N levels at which new growth began were approx the same for all N treatments. The time necessary to reach this level was dependent on the level of N added; 5 weeks for 300 ppm, 13 weeks for 150 ppm and 18 weeks for 50 ppm N. Once new growth began, tissue N levels began to decrease.

Additional index words. holly, plant nutrition, tissue analysis.

Introduction

The use of tissue analysis as a diagnostic tool for maintaining an efficient fertilizer program for container-grown woody ornamentals is increasing. Although limited information is available on tissue analysis and growth of woody ornamentals, the tree fruit crops have been extensively investigated. Work with deciduous fruit crops have shown the concn of mobile plant elements to generally decrease throughout the summer months. Koo and Young (11) working with avocado showed N, P and K content of new, fully expanded leaves to decrease from June to December. Similar results were reported by other workers with avocado (2,6). Pistachio leaflets were initially high in N, P and Zn concn, but then dropped rapidly during leaf expansion, reaching a steady state during the early summer when stem elongation ceased (15). Other elements reached constant levels later in the season. Similar results with seasonal changes in nutrient concn were reported for peach (1) and apple (13).

The effect of the second flush of growth on mineral composition of the spring flush of Valencia orange leaves was discussed by Smith (14). He found significantly less N, K and Mg in leaves subtending the additional growth, indicating that these elements had been transported into new growth. This illustrates that the mobile elements from leaves near the origin of new growth tend to move into new growth. It was also suggested that the presence, absence or extent of subsequent growth be given consideration in tissue sampling and interpretation of results, especially with N and K.

For both deciduous and evergreen species of woody ornamentals, Davidson (5) has shown that the seasonal nutrient trends resembled those reported for deciduous fruit trees. Nitrogen and K decreased throughout the summer; Ca, Mg, Fe and Mn increased, while P, B and Cu did not change. This concurred with results reported by Cannon et al. (4) and Boonstra et al. (3).

Little information is available on the fluctuations of nutrients in woody ornamentals, which may exhibit 2 or more flushes of growth during the summer months as has been reported with citrus crops (14). Until this information becomes available, the use of tissue analysis as a means of predicting fertility requirements for these plants is limited.

The purpose of this study was to investigate the fluctuation of tissue N during a flush of growth on Ilex crenata 'Helleri' grown at 3 N levels and to determine tissue N concn at which new growth occurs.

Materials and Methods

Single stem 'Helleri' holly cuttings (7 cm long) were taken March 9, 1976 and propagated in 7 cm rose pots containing a Weblite[1] medium. Plants were subsequently grown in a greenhouse at 28°C (day)/21° (night) under natural photoperiod.

On May 23, 1976, 3 N treatments were initiated. Nutrients were applied with a Hoagland and Arnon (9) nutrient solution lacking N and a Hoagland and Arnon micronutrient solution in which 5 ppm of iron was supplied in the form of NaFeEDTA. The basic nutrient solution was supplemented with N (ammonium nitrate) at 50, 150 and 300 ppm. Twenty ml of the nutrient solution was added weekly to each plant. A randomized block design with 20 plants per treatment and 4 replicates was used.

Eight weeks later, after shoot elongation for the first flush of growth had ceased on plants grown at 300 ppm N, 2 plants per replication were used to determine fresh and dry wt of shoots and roots. Tissue samples of shoots (stems and leaves combined) were used to determine tissue N by a modified micro-Kjeldahl method (12). For plants receiving 150 and 50 ppm N initial samples were taken on August 31, and October 7, 1976 respectively, since it took this long for shoot elongation to cease on these treatments. Weekly sampling continued for all treatments for 9-10 weeks after shoot elongation for the first flush of growth had ceased until stem elongation had ceased on the second flush.

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[1] Webster Brick Corp., Roanoke, VA

Results

Once shoot elongation and leaf expansion were completed, tissue N concn began to increase for all 3 N treatments, until similar levels of 2.1%, 2.0% and 1.9% were reached for 50, 150 and 300 ppm respectively (Fig. 1). A new flush of growth began when tissue N concn reached the above mentioned levels, after which a drop in the tissue N concn occurred with shoot elongation. An important difference between the fertilizer treatments was that plants grown at 300 ppm N reached the level of N at which a new flush of growth occurred 9 and 13 weeks before plants grown at 150 and 50 ppm N respectively. Also, sporadic growth occurred on plants grown at 50 ppm N, resulting in tissue N levels remaining relatively high during the second flush, whereas, plants grown at 300 ppm N had a rapid uniform flush of growth resulting in a more dramatic fluctuation of tissue N.

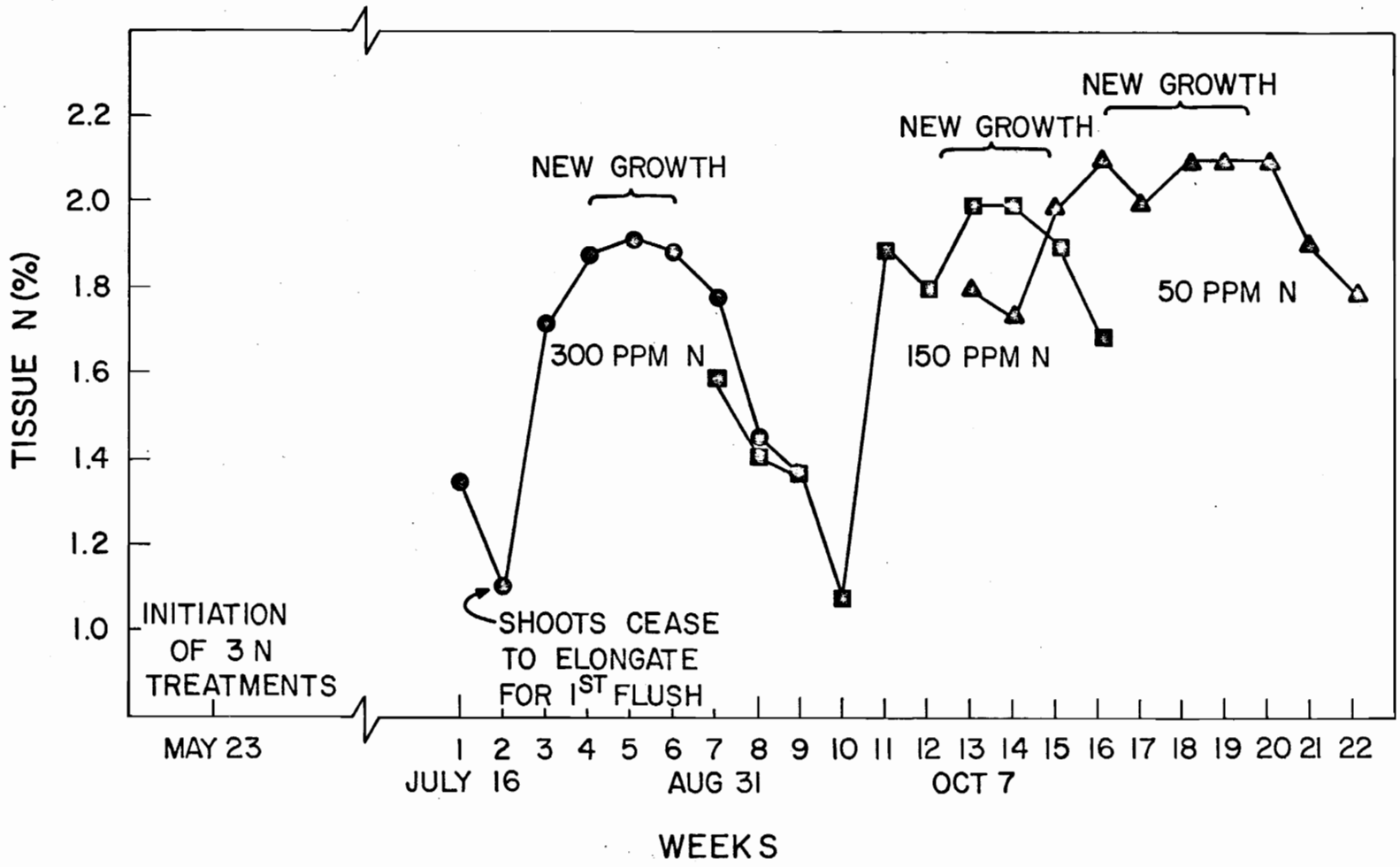

FIG. 1. THE EFFECTS OF 3 NITROGEN LEVELS ON TISSUE N FLUCTUATIONS DURING A FLUSH OF GROWTH ON 'HELLERI' HOLLY

Discussion

This study shows that 'Helleri' holly, which has more than 1 flush of growth, exhibited rapid fluctuations in tissue N during these flushes. These rapid changes in tissue N would limit the use of tissue analysis for N during the summer months to critical periods when little change in N concn is occurring -- normally just prior to a new flush. Other mobile elements not monitored in this experiment, P, K and Mg, may exhibit similar trends. Even then, sampling for tissue N just prior to a new flush is not very meaningful since, regardless of the rate of fertilizer added, shoots of 'Helleri' holly attained similar levels of N just before new growth occurs (Fig. 1). However, these data do not preclude the use of tissue analysis for diagnosis of nutrient deficiencies. For example, sampling of plants grown at low N levels (50 ppm), during mid to late summer should indicate a low N status because of the long interval between flushes and slow N accumulation. Consequently, the use of tissue analysis on plants exhibiting low vigor in the summer months may be valuable in detecting plant nutrient deficiency. Also, tissue analysis for N should be beneficial in the fall after the onset of dormancy since studies conducted by Kelly and Shier (10) reported the autumn months to be the most desirable sampling period for Taxus because of minimal changes in nutrient concn at this time. Plants having low tissue N could be supplemented with N during the fall or early spring when media temperatures are above freezing to increase tissue N to the desired concn (11). Nitrogen added before the spring flush would be available for growth during the spring flush. Tissue N

concn is important since studies have shown the intensity of the spring flush to be dependent upon tissue N levels at the time the flush begins (7,8).

Although the level of tissue N concn attained before new growth begins is independent of added fertility, the time necessary to reach this concn is dependent on the rate of applied fertility. Thus 1 advantage of the higher fertilizer rates is that the flushes of growth are more frequent, and thereby result in more total growth at the end of a growing season. Also, plants grown at higher N levels have a faster rate of growth and more total growth in a given flush than plants grown at lower N rates (7).

Acknowledgement. The authors wish to express appreciation to Lancaster Farms, Inc., Suffolk, Va. for providing 'Helleri' plants.

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Chapter III

Timing of Fertilizer Application in Relation to Multiple Flushes of Growth on 'Helleri' Holly

Abstract. The response of Ilex crenata Thunb. 'Helleri' to 15 fertilizer treatments consisting of different times and lengths was studied. Fertilizer applied during a period following the cessation of stem elongation and before the next flush resulted in greater total tissue N and shoot growth, regardless of whether it was also applied during other weeks or not. Root growth was suppressed by 3 or more fertilizer applications, regardless of the time of application.

Additional index words. holly, plant nutrition, tissue analysis.

Introduction

The correct timing of fertilizer applications has been shown to result in greater growth with woody plants having annual flushes of growth. De Werth and Chadwick (2) stated that for elm and maple, spring applications of fertilizer were more beneficial than fall applications. Autumn applications were shown to be as effective as spring applications when growth of Ligustrum was compared by Good and Tukey (5).

The previously mentioned papers were concerned with plants having an annual flush of growth. However, little information is available on the timing of fertilizer applications coinciding with multiple flushes of growth during the summer months. The relative intensity of a growth flush has been shown to be dependent upon the level of fertilizer added preceding the flush (3). Other work in Chapter II has shown that tissue N accumulates rapidly during the latter stage of a flush, between the cessation of stem elongation and the beginning of a new flush. These data suggest that correctly timed fertilizer applications to woody plants during a flush of growth may result in more efficient use of applied fertilizer.

The purpose of this study was to determine if a specific time existed during a flush of growth on Ilex crenata 'Helleri' when added fertilizer would be most efficiently utilized.

Materials and Methods

Multiple stem liners of Ilex crenata 'Helleri' were potted on March 1, 1977 in 0.987 liter plastic containers with a 100% milled pine bark medium. To each 0.76 m^3 of the medium, 2.7 kg of dolomitic limestone, 0.9 kg gypsum and 1.4 kg of 20% superphosphate were added. The plants were grown in a greenhouse at 28°C (day) and 21°C (night) under natural photoperiod from March 1 to the end of the experiment. A randomized block design with 8 plants per treatment and 4 replicates was used.

Treatments were initiated March 15, 1977, using a 20.8 N - 8.7 P - 16.5 K nutrient solution containing 300 ppm N, 130 ppm P and 247 ppm K. Fifteen treatments (Table 1) consisting of different time and length of fertilization comprised the experiment. The first treatments began with the first flush of growth when new shoots were about 2 mm long and continued over a 5 week period. The last treatments were applied 1 week before the second flush of growth began. When 2 or more fertilizer applications comprised a treatment, fertilizer applications were made at weekly intervals.

At the beginning of the second flush of growth, 6 weeks after initial N applications, 2 plants per replication were used to determine fresh and dry wt of shoots and roots. Tissue N was determined on the most recently matured leaves using a modified micro-Kjeldahl method (6).

Twelve weeks after fertilization treatments were initiated, at the end of the second flush of growth, 2 plants per replication were

used to determine fresh and dry wt of shoots and roots. Plants were not fertilized during the latter 6 weeks.

Table 1. Weekly fertilizer treatments applied to 'Helleri' holly to investigate the relationship between time and length of fertilizer application on plant growth.

Number of fertilizer applications	Time of fertilizer application during 1st flush of growth[z] (wks)
1	1, 2, 3, 4, 5
2	1-2, 2-3, 3-4, 4-5
3	1-3, 2-4, 3-5
4	1-4, 2-5
5	1-5

[z] Treatments began at week 1 when shoots were just beginning to elongate.

Results

There were no differences in shoot or root growth among treatments at the end of the first flush of growth (data not shown). Nitrogen levels in the tissue were greatest when fertilizer applications were made during a period of growth between cessation of stem elongation - week 3, and the second flush of growth - week 6 (Table 2). For example, when applied for a 1 week period, plants receiving fertilizer during the fourth week had greater tissue N, or when applied for a 2 week period, plants receiving fertilizer weeks 4-5 had greater tissue N. Tissue N was also increased by increasing the number of fertilizer applications. However, little difference occurred between treatments applied for a 3, 4 or 5 week period if they included the fourth week.

Shoot growth (dry wt) during the second flush was generally greatest on treatments which accumulated the higher tissue N levels -- those fertilized following the cessation of stem elongation (Table 2). Again those treatments which included week 4 seemed to be the most effective in promoting growth during the next flush.

Regardless of the time of fertilizer application during a flush of growth, root dry wt was suppressed with increasing numbers of fertilizer applications (Table 2). For example, root wt averages between the numbers of applications showed plants receiving 4, 3, 2 and 1 fertilizer application(s) had 0, 11, 25 and 31% greater root wt than plants receiving 5 fertilizer applications.

Table 2. Effect of the time and no. of weekly fertilizer applications during a flush of growth on tissue N accumulation and subsequent shoot and root dry wt of 'Helleri' holly.[z]

Total N

Total no. of wk(s) fertilizer applied									
1		2		3		4		5	
wk	% N	wks	% N	wks	% N	wks	% N	wks	% N
4	2.27	4-5	2.45	2-4	2.58	1-4	2.69	1-5	2.59
5	2.04	3-4	2.26	3-5	2.38	2-5	2.55		
3	2.01	2-3	2.23	1-3	2.13				
2	1.99	1-2	2.10						
1	1.88								

LSD 5% = .26

Shoot dry wt

Total no. of wk(s) receiving fertilizer									
1		2		3		4		5	
wk	wt	wks	wt	wks	wt	wks	wt	wks	wt
4	6.2	3-4	6.9	2-4	7.1	1-4	6.5	1-5	7.0
2	5.3	4-5	6.1	3-5	6.7	2-5	6.5		
5	5.2	2-3	5.9	1-3	6.1				
1	5.1	1-2	5.5						
3	4.9								

LSD 5% = 1.24

Root dry wt

Total no. of wk(s) receiving fertilizer									
1		2		3		4		5	
wk	wt	wks	wt	wks	wt	wks	wt	wks	wt
3	2.9	2-3	2.4	1-3	1.9	1-4	1.8	1-5	1.7
1	2.4	3-4	2.4	2-4	1.9	2-5	1.6		
2	2.4	1-2	2.3						
4	2.4	4-5	2.0						
5	2.2								

LSD 5% = .50

[z] Plants were harvested 12 weeks after initial fertilizer treatments were applied -- at the end of the second flush of growth.

Discussion

This study shows no advantage to continuous weekly fertilization at 300 ppm N for optimum shoot growth of Ilex crenata 'Helleri'. The second flush of growth on plants receiving 2 or 3 weekly fertilizer applications after the cessation of stem elongation in the first flush was equal to growth on plants receiving 5 weekly fertilizer applications over the entire flush. One explanation for elevated growth with fertilizer treatments applied after the cessation of stem elongation is that the uptake of N, or other nutrients not monitored in this experiment, was more efficient during the period following the cessation of stem elongation. Higher tissue N levels of plants fertilized during the latter part of the flush supports this view. This coincides with data in Chapter II, which showed tissue N to accumulate rapidly during a period following cessation of shoot elongation.

Since 2 or 3 flushes occur during a growing season with 'Helleri' holly and some other woody species, concentrating fertilization during critical periods following the cessation of stem elongation would reduce the frequency of fertilization and consequently fertilizer cost without reducing shoot growth.

This study also shows that root growth of 'Helleri' holly was suppressed with 3 or more fertilizer applications. This agrees with work by Brouwer (1), which showed greater root growth when limited N was added. When 1 or 2 fertilizer applications were timed correctly, after the cessation of stem elongation on 'Helleri' holly, both root and shoot growth were at or near maximum for this experiment. These

results show that limited fertilization may yield more total growth (shoot + root) if fertilizer applications are timed correctly.

These data concur with earlier work (4) which showed that plants exhibiting episodic growth responded to prior N fertilization. The second flush of growth was a result of N accumulation during the first flush, since fertilization was withheld in the second flush of growth.

Acknowledgement. The authors wish to express appreciation to Lancaster Farms, Inc., Suffolk, Va. for providing 'Helleri' plants

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Appendix I

Plant N Procedure

Reagents

(1) Digestion mixture -

200 g K_2SO_4
20 g $CuSO_4 \cdot 5\ H_2O$ Mix - use 50 mg
2 g Selenium

(2) 10 N NaOH·400 g NaOH dissolved in distilled H_2O and brought to 1000 ml with H_2O.

(3) Boric acid indicator solution - Dissolve 20 g of pure H_2Bo_3 in approx 700 ml hot H_2O per liter of solution desired - cool and transfer to a volumetric flask containing 200 ml ethanol and 20 ml mixed indicator - (mixed indicator - 300 mg bromcresol green and 165 mg methyl red in 500 ml ethanol). After mixing the contents of the volumetric flask, add small amounts of 0.05 N NaOH (2 g NaOH in one liter H_2O) until 1 ml of water mixed with 1 ml of the boric acid indicator solution gives a pale green color - Check the color change using a spot plate after each addition of the 0.05 NaOH. Use 95% ETOH.

(4) Devarda Alloy - Ball mill until it will pass a 100 mesh screen and at least 75% passes a 300 mesh screen - mix and store in a tightly stoppered jar.

Plant N Procedure

(1) Weigh 50 mg dry sample in 100 ml Kjeldahl flask.

(2) Add 50 mg digestion mixture and mix.

(3) Add 2 ml concentrated H_2SO_4 and swirl.

(4) Put the samples on the digestion apparatus and turn the heat on low until frothing ceases.

(5) Cut the heat on high and boil for 1 hr after the sample clears.

(6) Cool samples and add 5-10 ml of water immediately prior to distilling.

Distillation

(1) Add 5 ml boric acid indicator to a 50 ml erlenmeyer flask marked at 30 ml and place it under the condenser tube.

(2) Add 7 ml, 10 N, NaOH to the distillation flask.

(3) Add 0.2 g Devarda alloy to the distillation flask and attach to the distillation apparatus immediately. (Unnecessary on tissue samples that do not accumulate nitrates.)

(4) Distill until 30 ml is collected in the flask containing the boric acid indicator.

Titration

Titrate the solution in the 50 ml erlenmeyer flask with 0.1 N H_2SO_4 - (made by using 100 ml of 1 N standardized H_2SO_4 brought to 1000 ml with distilled H_2O). Prepare blanks by the same procedure as for the samples; also run some water blanks using the same procedure as the distillation of the samples.

To Calculate

$$\% \text{ N in tissue} = \frac{(14 \text{ mg/meq})\ (0.1 \text{ N } H_2SO_4)\ (\text{ml acid for sample} - \text{ml acid for blank})\ (100\%)}{50 \text{ mg}}$$

$$= \frac{(14)\ (0.1)\ (100)\ (\text{ml titrated} - \text{ml for blank})}{50}$$

$$= 2.8\ (\text{ml acid for sample} - \text{ml for blank})$$

Example:

sample - 1.07 ml of 0.1 N HCl
blank - 0.07
1.00

1.00
x 2.8
2.800

therefore, the sample contains 2.80% N

Vita

Charles Homer Gilliam was born October 14, 1952, in Lexington, Tennessee. He received his primary and secondary education in the Henderson County, Tennessee school system, graduating in 1970. He entered the University of Tennessee at Martin in 1970, and was awarded a Bachelor of Science degree in Agricultural Education in 1974.

In March of 1974, he received his commission of Second Lieutenant in the United States Army, Signals Corps, serving active duty at Fort Gordon, Georgia from April to July, 1974, when he was honorably discharged from active duty into the United States Army Reserves.

He entered graduate school at Virginia Polytechnic Institute and State University in September 1974, and was awarded a Master of Science degree in Horticulture in 1976.

He was married to the former Gail Cross in September 1971, and they are expecting their first child in September 1977.

Charles H. Gilliam

SOME ASPECTS OF NITROGEN NUTRITION ON SELECTED *ILEX*

by

Charles Homer Gilliam

(ABSTRACT)

Three holly cultivars, *Ilex crenata*, Thunb. 'Helleri' and 'Rotundifolia' and *Ilex cornuta* Lindl. et Paxt. 'Burfordi' were grown in 3 liter containers at 200, 300, 400 and 500 ppm nitrogen (N). Significant correlation coefficients were found between plant growth (dry wt accumulation) and both leaf N content and soil solution nitrates, while low correlation coefficients were found between soil nitrates and plant growth. Shoot growth of 'Helleri' and 'Burfordi' was not increased by N levels higher than 300 ppm while 400 ppm N was optimal for 'Rotundifolia'.

The effects of 3 N levels on tissue N fluctuations during a flush of growth on 'Helleri' were also studied. For all N levels, tissue N levels were shown to increase following the cessation of stem elongation until a level of tissue N was reached where a new flush of growth began. These N levels were approx the same for all N treatments. The time necessary to reach this level was dependent on the level of N added; 5 weeks for 300 ppm, 13 weeks for 150 ppm and 18 weeks for 50 ppm N. Once new growth began, tissue N levels began to decrease.

The response of 'Helleri' to 15 fertilizer treatments consisting of different time and lengths was studied. Fertilizer applied during a period following the cessation of stem elongation and before the next flush resulted in greater total N and shoot growth, regardless

of whether it was also applied during other weeks or not. Root growth was suppressed by 3 or more fertilizer applications, regardless of the time of application.