Nitrification in a Pine Bark Medium

by

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(Abstract)

The influence of nitrification on the "soil" solution of container media has not been documented. The investigation of this influence is justified since the ionic form of N in a soil solution has a significant influence on plant tissue nutrient content and growth. Three genera of woody plants were grown in one-liter containers filled with pine bark, treated with and without a nitrification inhibitor and fertilized with 210 ml of a 100 ppm NH_4-N solution. Without the inhibitor and over time, "soil" solution NH_4-N concentrations and pH decreased and NO_3-N concentrations increased. " Soil" solution and tissue cation concentrations were generally greater without the inhibitor.

In a second experiment, pine bark in one-liter containers was treated with either 0, 3 or 6 kg lime m^{-3}. "Soil" solution data and NO_3-N accumulation rate (NAR) data showed an earlier nitrification of NH_4-N at the 6 kg lime compared to the 3 kg lime treatment whereas NO_3-N was not found at the 0 kg lime treatment.

In a 3rd experiment, pine bark in one-liter containers was treated with 210 ml of either 25, 100 or 200 ppm NH_4-N. Over time "soil" solution NO_3-N concentrations were greatest and pH values were lowest at the 200 ppm N treatment. The NAR of the 25 ppm N treatment was less than the 100 and 200 ppm N treatment which were not different. The lack of correspondence between the "soil" solution NO_3-N data and the NAR data for the 100 and 200 ppm N treatments was explained on the basis of NH_4-N supply.

In a 4th experiment, pine bark in one-liter containers were subjected to either 10°, 20°, 30° or 40° C for 24 days. "Soil" solution NH_4-N concentrations decreased over time at 10°, 20° and 30°. "Soil" solution NH_4-N and NO_3-N concentrations at 40° were considerably higher and lower, respectively, than at other temperatures. Over time the general order of NAR was: 20° = 30° > 10° > 40°. Results of these experiments indicate that nitrification is an important consideration in the nutrition of container-grown plants.

DEDICATION

This dissertation is dedicated to my family.

ACKNOWLEDGEMENTS

Words are not enough to express my gratitude to my parents for their support. The same sentiments are expressed to Robert Wright, my advisor, who has trained me well. I thank my committee members Richard Johnson (whose quote on the correction sheet of my dissertation will always be smilingly rermembered), Lee Daniels, Ray Reneau (whose questions during my defense will always be remembered), and Ron Morse for their time and assistance. A note of appreciation goes to . A special expression of thanks is communicated to the unique .

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Chapter I

INFLUENCE OF NITRIFICATION ON THE "SOIL" SOLUTION AND GROWTH OF AZALEA, HOLLY, AND JUNIPER IN A PINE BARK MEDIUM

ABSTRACT

The influence of nitrification on the "soil" solution of a container medium has not been documented. The investigation of this influence is justified since the ionic form of N in the "soil" solution has a significant effect on plant tissue nutrient content and growth. Rooted cuttings of Rhododendron obtusum Planch., Ilex crenata Thunb., and Juniperus chinensis L. were grown in a 100% pine bark medium amended with the nitrification inhibitor nitrapyrin (NI) at 0, 41 and 82 μg g^{-1} bark in Expt.1, and 0 and 82 μg g^{-1} bark in Expt. 2. Ammoniacal-N was the only source of fertilizer N. Results of both experiments were similar. Without NI and over time "soil" solution NH_4-N concentrations decreased and NO_3-N concentrations increased. " Soil" solution pH at 0 NI decreased 1.0 and 0.6 units during the periods of rapid NO_3-N accumulation in Expts. 1 and 2, respectively, while during the same periods in the 82 NI treatment there was a respective 0.2 unit decrease and a 0.3 unit increase. The lower pH of the 0 NI treatment resulted in "soil" solution Ca, Mg, and Mn concentrations which were several times

greater than at 82 NI. However, only minor differences in tissue Ca, Mg and Mn concentrations occurred between 0 and 82 NI treatments. With the exception of holly plants in Expt. 1, no differences in shoot dry weight occurred in response to treatment. The results of this research indicate that nitrification is an important consideration in the nutrition of container-grown plants.

INTRODUCTION

Nitrification is a bacterially-mediated oxidation of NH_4^+ to NO_3^- and occurs in a 2 step process:

$$NH_4^+ + 1.5\ O_2 \longrightarrow NO_2^- + 2H^+ + H_2O$$

$$NO_2^- + .5\ O_2 \longrightarrow NO_3^-$$

Nitrification decreases the soil solution pH and the NH_4-N to NO_3-N ratio. As seen in the first step of these reactions 2 H^+ ions are produced for each molecule of NH_4^+ oxidized. Nutrient availability is affected by this change in soil pH (1, 23). Within this text the word "soil" is used parenthetically in the term soil solution since pine bark is not a true soil.

Pine bark, a lumber and pulp industry by-product, is a commomly used medium for container-grown woody plants in the southeastern U.S. An increased production trend of container-grown plants relative to field-grown plants has resulted in an increased use of pine bark. Container-grown plants are intensively fertilized and NH_4-N is a common component of the nutrients applied. A few studies (4, 5, 20, 22) have either reported or implied that nitrification occurs in a pine bark medium. However, no characterization of how this process influences the "soil" solution and growth have been made for any container medium. Such an effort is justified since the ionic form of N in the soil solution has a significant influence on plant nutrient content and growth (13, 14, 19).

Nitrapyrin (NI), 2-chloro-6-(trichloromethyl) pyridine, is a nitrification inhibitor which has been extensively used in the study of nitrification. NI was used to study the influence of the ionic form of N on tomato in pine bark (20), however, the efficacy of NI over time was not elucidated. Thus, the purpose of this research is to investigate the influence of nitrification on the" soil" solution, plant growth and plant nutrient content in a pine bark medium. Also to determine the efficacy of NI rates in a pine bark medium. Three commonly grown genera, Rhododendron, Ilex and Juniperus were used in this study.

MATERIALS AND METHODS

Expt. 1. Pine bark used in this experiment had a particle size distribution of 38% less than 0.05 mm (U.S. Sieve series #35), 28% between 0.5 and 1.2 mm (U.S. Sieve series #16), 20% between 1.2 and 2.4 mm (U.S. Sieve series #8) and 14% between 2.4 and 6.3 mm (U.S. Sieve series #3). This bark had a bulk density of 0.35 g cc^{-1} and was primarily from *Pinus taeda* L..

Pine bark was moistened and amended with 0.58 kg urea m^{-3} and 6 kg dolomitic lime m^{-3} ($CaCO_3$ equivalent = 100). Urea was added (preplant) to the bark in order to stimulate the estasblishment of nitrifier populations. Bark was treated with NI (Dow Chemical Co., Midland, MI) at either 0, 41 or 82 µg NI g^{-1}. On 21 July 1983 rooted cuttings of *Rhododendron obtusum* Planch. 'Hino Crimson', *Ilex crenata* Thunb. 'Helleri' and *Juniperus chinensis* L. 'Pfitzeriana' were transplanted into one-liter plastic bark-filled containers and grown in a glasshouse with day/night temperatures of 24°/18° C. Plants were arranged in a randomized complete block design with 3 plants per treatment in each of 5 blocks.

Plants were irrigated with 210 ml of nutrient solution containing 100 ppm N as $(NH_4)_2SO_4$, 10 ppm P as H_3PO_4, 25 ppm K as K_2SO_4, 5 ppm Fe as NaFeEDTA and other micronutrients

according to Hoagland and Arnon (15). After every 2 nutrient solution irrigations plants were irrigated with 420 ml distilled water to prevent excessive salt accumulation in the bark. The "soil" solution was periodically tested 4 to 6 hr after the second nutrient solution irrigation. Testing employed the pour-through technique (26) by applying 75 ml of distilled water onto the surface of the bark and collecting the leachate. These leachates were analyzed for NH_4-N, NO_3-N and pH using ion selective electrodes. "Soil" solutions from the last 2 sampling dates were also analyzed for K, Ca, Mg, Fe, Mn and Zn using an atomic absorption spectrophotometer.

On 28 October 1983 the most recently matured leaves of all plants were rinsed in distilled water and removed for tissue analysis. Plants shoots were harvested, dried for 48 hr at 70° C and weighed. Weight of leaves removed for tissue analysis were added to the respective shoot weights. Leaf tissue was analyzed for N using the micro-Kjeldahl technique (21), P using a colormetric procedure (25) and K, Ca, Mg, Fe, Mn, and Zn by atomic absorption spectroscopy.

Expt. 2. Pine bark in this experiment had a particle size distribution of 22% less than 0.5 mm (U.S. Sieve series #35), 22% between 0.5 and 1.2 mm (U.S. Sieve series #16), 18% between 1.2 and 2.4 mm (U.S. Sieve series #8), 24% bet-

ween 2.4 and 5.6 mm (U.S. Sieve series #3.5) and 14% greater than 5.6 mm. This bark had a bulk density of 0.32 g cc^{-1} and was primarily from Pinus taeda.

Pine bark was moistened and amended with 0.58 kg urea m^{-3}, 6 kg dolomitic lime m^{-3} and 1 kg Micromax (Sierra Co., Milpitas, CA) m^{-3}. Bark was then treated with 0 or 82 µg NI g^{-1}. On 23 March 1984 rooted cuttings of R. obtusum 'Coral Bells', I. crenata 'Helleri' and J. chinensis 'Sea Green' were planted into one-liter plastic bark-filled containers and grown in a glasshouse. Plants were arranged in a randomized complete block design with 2 plants per treatment in each of 7 blocks. The nutrient solution and the irrigation schedules were the same as in Expt. 1 with the exception that micronutrients were not added to the nutrient solution. Soil solutions were periodically tested as in Expt. 1 and analyzed for NH_4-N, NO_3-N, P, K, Ca, Mg, Fe, Mn and Zn. On 17 July 1984 tissue samples were taken and plants harvested as in Expt. 1.

RESULTS

"Soil" solution data for the 3 genera studied and of both experiments were similar thus results of both experiments will be addressed collectively using holly data (unless otherwise noted). Without NI, "soil" solution NH_4-N

concentrations decreased rapidly during the first 20 days and remained relatively low despite periodic NH_4-N fertilizations (Figs. 1, 2). Nitrate-N concentrations peaked during the first half of the experiments and subsequently decreased (Figs. 1, 2).

In the 41 NI treatment (Expt. 1) significant amounts of NO_3-N accumulated in the "soil" solution during the latter half of the experiment (Fig. 1). "Soil" solution NH_4-N concentrations consistently decreased during the period of NO_3-N accumulation. "Soil" solution NH_4-N concentrations at the 82 NI treatment remained relatively high with the exception of the last 15 days in Expt. 2 in which the inhibitory influence of NI most likely diminished. Ten days prior to this NH_4-N decrease significant amounts of NO_3-N were presentn the "soil" solution.

"Soil" solution pH for the 0 NI treatment decreased 1.0 and 0.6 units during the first 45 days in Expt. 1 and 56 days in Expt. 2, respectively (Figs. 3, 4).

This decrease in solution pH corresponded to periods of considerable NO_3-N accumulation. In comparison there was only a 0.2 unit decrease (Expt. 1) and a 0.3 unit increase (Expt. 2) at the 82 NI treatment during the same time periods. The reason for the unexpected 0.3 unit increase is not understood. In Expt. 1 differences in solution pH between 41 NI

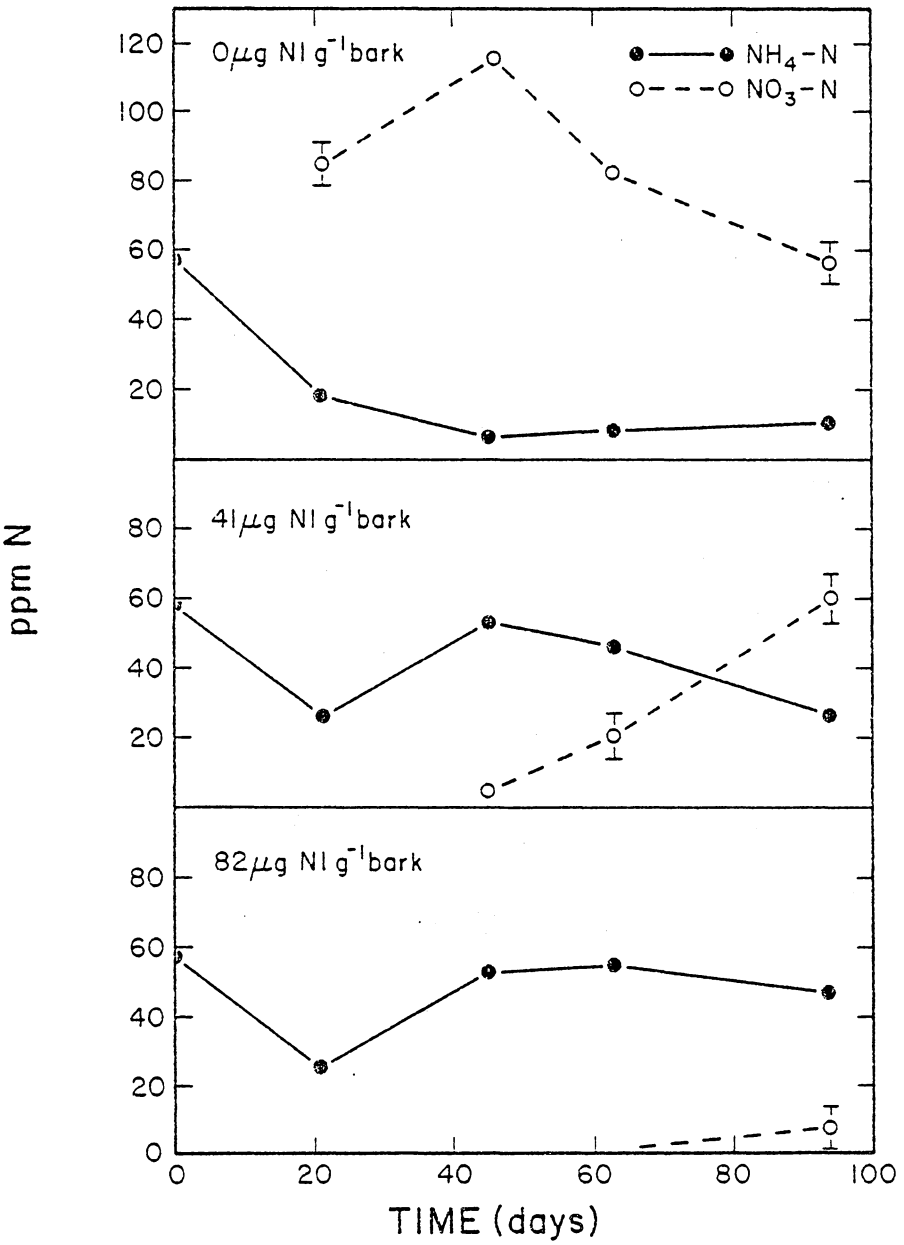

Fig.1. Influence of NI treatment on "soil" solution NH_4-N and NO_3-N concentrations (holly plants, Expt. 1).

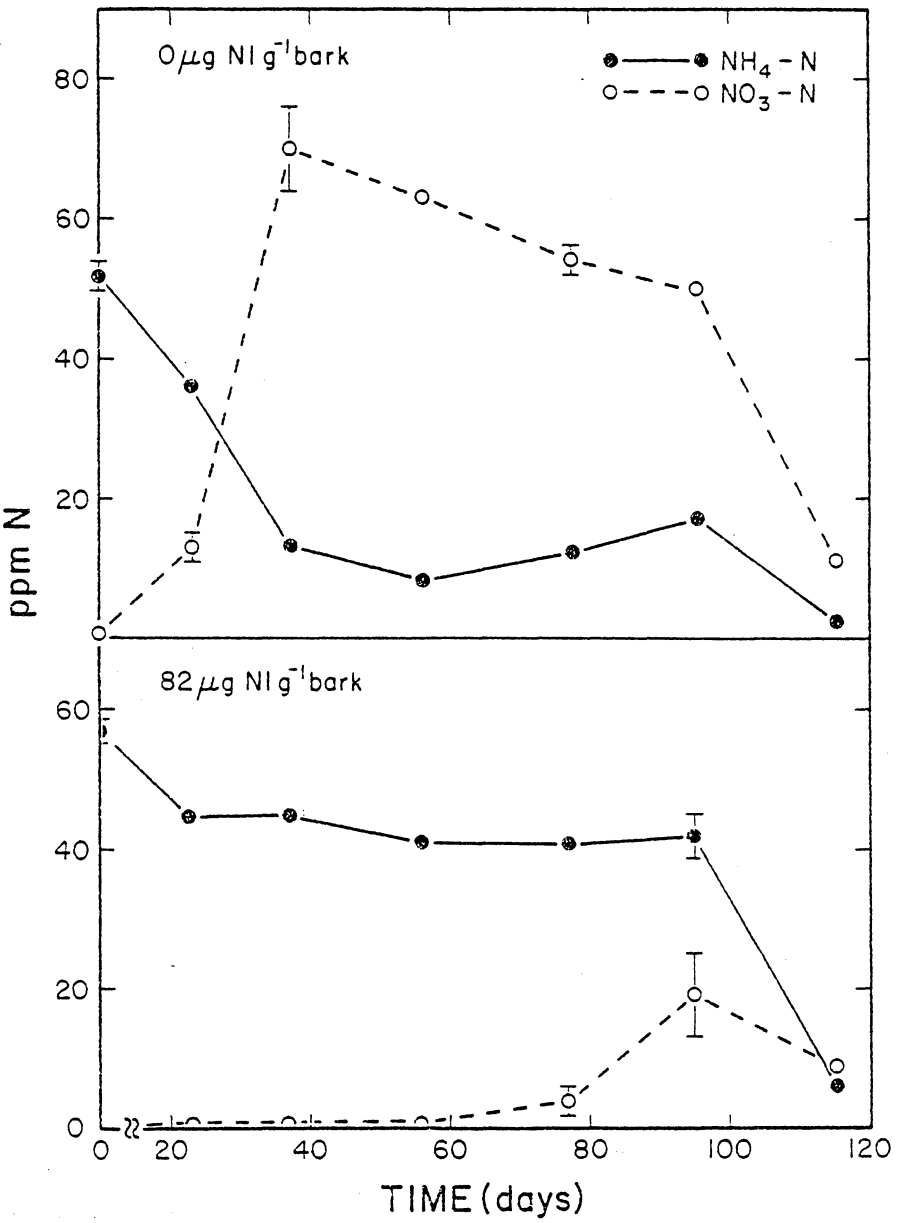

Fig.2. Influence of NI treatment on "soil" solution NH_4-N and NO_3-N concentrations (holly plants, Expt. 2).

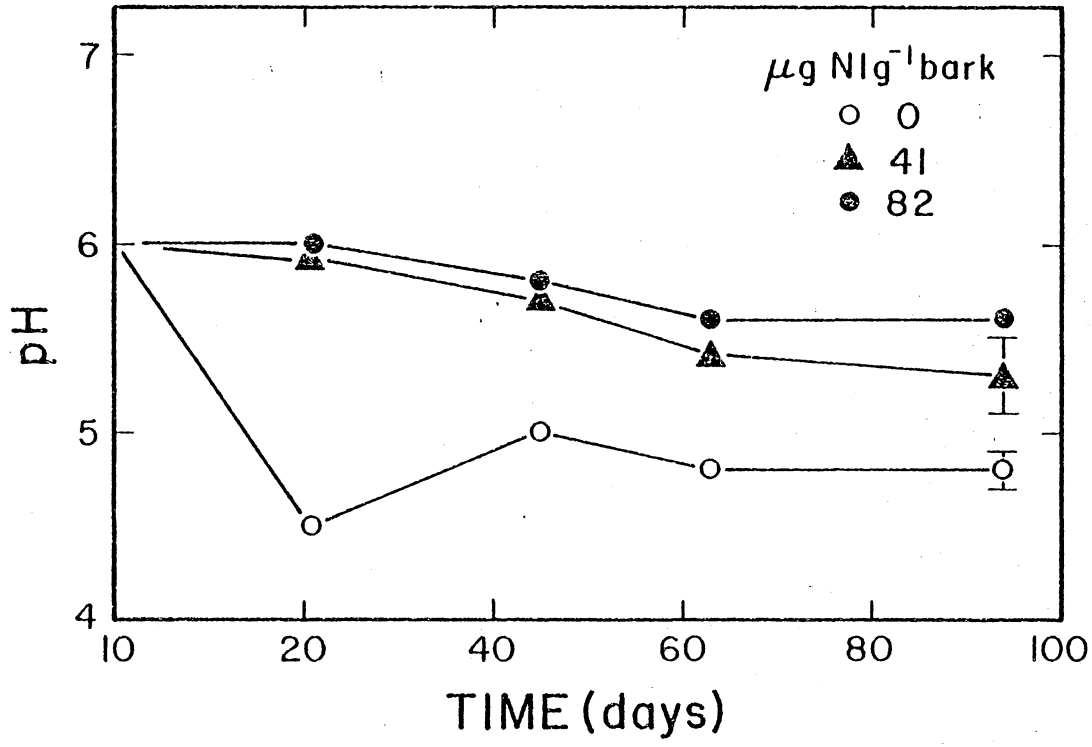

Fig.3. Influence of NI treatment on "soil" solution pH (holly plants, Expt. 1).

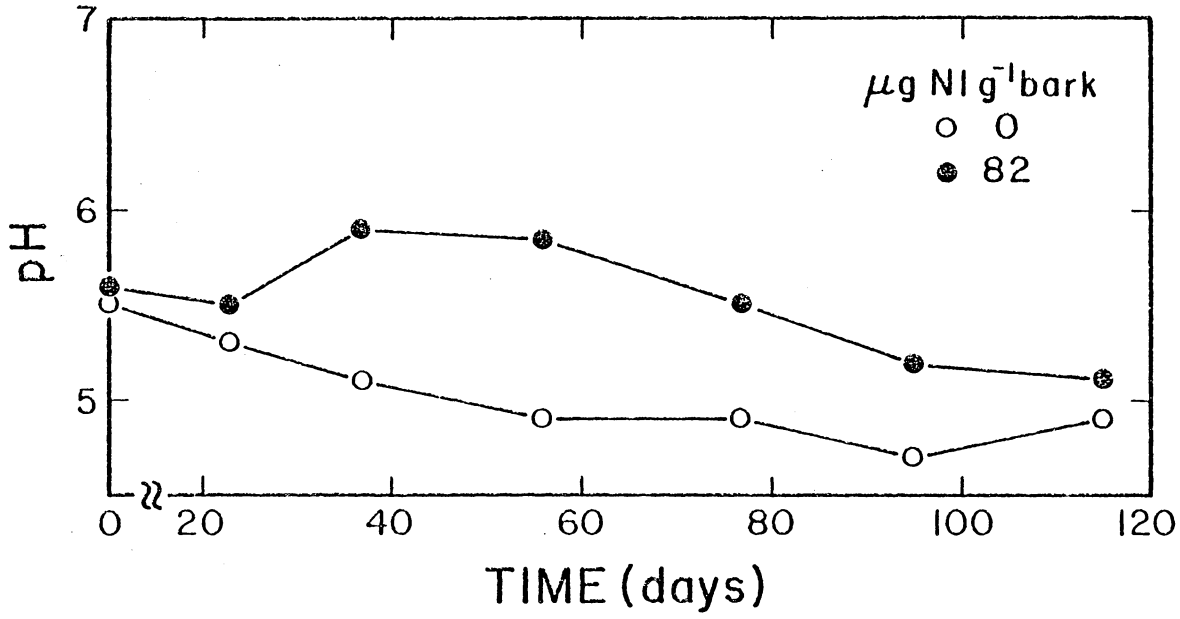

Fig.4. Influence of NI treatment on "soil" solution pH (holly plants, Expt. 2).

and 82 NI became evident at day 63, the time at which rapid NO_3-N accumulation occurred. At the 82 NI treatment the solution pH slowly decreased from 6.0 at day 1 to 5.6 at day 94.

"Soil" solution Ca and Mg concentrations were considerably greater at the 0 NI treatment than at the 81 NI treatment (Tables 1, 2). Manganese (and Zn in Expt. 2) showed a similar response to treatment. With the exception of day 94 in Expt. 1, solution Fe concentrations, in contrast to Ca, Mg, Mn and Zn concentrations, were unexpectedly greater at the 82 NI treatment than at 0 NI.

Greater shoot dry weight occurred at 82 NI with holly in Expt. 1 (Table 3), however, no such differences occurred in Expt. 2 (Table 4). In most cases tissue N concentrations of the 82 NI treatment were greater than at 0 NI (Tables 3, 4). In contrast to tissue N, cation tissue concentrations were generally greater at the 0 NI treatment than the 82 NI treatment.

DISCUSSION

NI was effective in controlling NO_3-N accumulation for approximately 40 days at 41 µg NI g^{-1} bark and for 90 days at 82 µg NI g^{-1} (Expt. 1) (Fig. 1). The efficacy of NI is related to rate of application (12) and to the rate of NI

Table 1. Influence of NI rate on soil solution nutrient concentrations at days 63 and 94 (holly plants, Expt. 1).

NI treatment (μg NI g^{-1} bark)	Soil solution concentration (ppm)											
	Day 63						Day 94					
	Ca	Mg	K	Fe	Mn	Zn	Ca	Mg	K	Fe	Mn	Zn
0	94a[z]	130a	37a	2.4b	.47a	.1c	96a	111a	34a	2.5a	.92a	.12a
41	34b	52b	35a	2.5ab	.06b	.13b	66b	91b	36a	2.5a	.15b	.11a
82	25b	42b	36a	2.8a	.02b	.16a	28c	34c	31a	2.6a	.04b	.13a

[z]Mean separation within columns by Tukey's test, 5% level.

Table 2. Influence of NI rate on soil solution nutrient concentration over time (holly plants, Expt. 2).

NI treatment (μg NI g^{-1} bark)	Day 10	23	37	56	77	95	115	LSD[z]
			ppm K in solution					
0	43	40	47	34	26	22	10	4
82	45	41	37	30	22	21	10	3
t-test[z]	NS	NS	*	*	*	NS	NS	
			ppm Ca in solution					
0	23	24	116	67	68	92	57	9
82	26	20	42	24	33	49	47	6
t-test	NS	NS	*	*	*	*	*	
			ppm Mg in solution					
0	23	28	61	61	59	74	45	8
82	25	23	23	22	32	42	41	5
t-test	NS	NS	*	*	*	*	NS	
			ppm Fe in solution					
0	.54	.22	.07	.08	.08	.17	.09	.03
82	.54	.29	.18	.22	.16	.22	.13	.05
t-test	NS	*	*	*	*	*	*	
			ppm Mn in solution					
0	.76	.2	.36	.22	.24	.49	.26	.12
82	.87	.1	.05	.03	.03	.07	.09	.08
t-test	NS	*	*	*	*	*	*	
			ppm Zn in solution					
0	.19	.04	.09	.18	.16	.27	.13	.05
82	.14	.02	.02	.04	.01	.09	.09	.02
t-test	NS	*	*	*	*	*	*	

[z]Least significant difference within rows, 5% level.

NS, * - Nonsignificant, means significantly different within columns at 5% level by t-test.

Table 3. Influence of NI rate on shoot dry weight and leaf nutrient content (Expt. 1).

Genus	NI treatment (μg NI g^{-1} bark)	Shoot dry wt (g)	Leaf content (dry basis) N (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)
Azalea	0	10.4a[z]	2.7b	.99a	1.2a	.48ab	58a	136a	52a
	41	11.1a	2.9a	.95a	1.2a	.51a	56a	97b	62a
	82	11.3a	2.9a	.94a	1.0a	.45b	53a	101b	47a
Holly	0	2.6b	2.9a	1.1a	.76a	.48a	65b	433a	150a
	41	3.2a	3.2a	1.1a	.66b	.50a	74ab	306b	149a
	82	3.1a	3.6a	1.1a	.56c	.49a	80a	288b	131a
Juniper	0	2.9a	1.8b	1.2a	.99a	.38a	46a	210a	30a
	41	2.9a	2.5a	1.1ab	.79b	.39a	50a	189ab	28ab
	82	2.8a	2.7a	1.1b	.66c	.31a	44a	150b	25b

[z]Mean separation within columns by genus by Tukey's test, 5% level.

Table 4. Influence of NI rate on shoot dry weight and leaf nutrient content (Expt. 2).

Genus	NI treatment (μg NI g^{-1} bark)	Shoot dry wt (g)	Leaf content (dry basis) N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)
Azalea	0	10.0	1.3	.16	.95	.76	.33	50	93	39
	82	9.3	1.4	.15	.99	.67	.28	41	82	31
t-test		NS	*	NS	NS	*	*	*	*	*
Holly	0	8.0	1.7	.11	.94	.61	.45	44	256	182
	82	8.3	1.8	.12	.86	.65	.50	43	252	177
t-test		NS	NS	NS	*	NS	*	NS	NS	NS
Juniper	0	8.6	1.4	.20	1.2	.64	.33	18	196	49
	82	9.1	1.6	.16	1.1	.58	.30	15	158	37
t-test		NS	*	NS	*	*	NS	NS	*	NS

NS, * - Nonsignificant, means significantly different within columns at 5% level by t-test.

hydrolysis (17). Hydrolysis mitigates the inhibitory affect of NI with temperature being the major factor influencing hydrolysis.

Results of both experiments indicate that at 0 NI nitrification was responsible for the rapid decrease in NH_4-N and the rapid increase in NO_3-N (Figs. 1, 2). Ammoniacal-N concentrations remained relatively low despite periodic NH_4-N fertilizations. Nitrifier activity as well as plant uptake and possibly NH_4-N adsorption to bark particles (10) were factors responsible for these low NH_4-N concentrations. Following the peak NO_3-N concentrations which occurred in the first half of the experiments, NO_3-N concentrations continually decreased. This decrease may be attributed to a low level of nitrifier activity and increased plant uptake. Nitrifier activity is directly related to soil pH and the substrate (NH_4-N) supply.

The growth and metabolism of nitrifiers are optimal in the pH range of 7.0 to 8.0 (9). Gilmour (11), working with a mineral soil, showed a linear relationship between soil pH and NO_3-N production. Gilmour (11) and others (3, 7) using nitrification models predicted a zero nitrification rate at pH 4.1. At 0 NI the "soil" solution pH during the second half of Expts. 1 and 2 was less than 5.0 and most likely limited nitrification (Figs. 3, 4). During this same period

solution NH_4-N concentrations were relatively low and may have also limited nitrifier activity. Since plant size and therefore plant absorption of N increased with time, the amount of NO_3-N as well as NH_4-N removed from the solution most likely increased. Thus nitrifiers and plant roots were competing for the limited amounts of NH_4-N.

"Soil" solution pH values were consistently lower at 0 NI compared to 82 NI (Figs. 3, 4). This lower pH at 0 NI was primarily due to the acidifying effect of nitrification. The source of H^+ responsible for this acidification is the oxidation of NH_4^+, the first reaction of the nitrification process. In view of the inhibitory influence of a relatively low pH on nitrifier activity, nitrifiers impose a feedback inhibition on their activity via H^+ ion production. Another source of acidity occurs when H^+ ions are excreted from the root into the soil solution in exchange for plant absorbed NH_4^+. Since NH_4-N was the predominant N form at the 82 NI treatment, the pH decrease in this treatment is primarily attributed to the NH_4-N absorption.

Calcium and Mg were supplied via preplant incorporation of dolomitic lime into the bark in both experiments. The solution concentrations of Ca and Mg at 0 NI were considerably greater than at 82 NI (Tables 1, 2). Similar results have been shown with an acid mineral soil (23). This find-

ing can be attributed to the increased solubility of lime (2) at the relativley low solution pH of the 0 NI treatment (Figs. 3, 4). Also fewer cation exchange sites would exist on the bark at a lower pH and greater amounts of Ca and Mg would be contained in the soil solution and less adsorbed onto the bark. Foster et al. (10) showed that the adsorption of cations to pine bark increased as the pH increased. Correlation coefficients (Table 5) indicate that approximately 60% and 80% of the variance in Ca and Mg concentrations, respectively, can be attributed to the variance in pH. Data from day 10 was not included in the correlations since differences in pH were not yet manifested.

Treatment differences in "soil" solution micronutrient concentrations were found in Expt. 1 with Mn and Fe, and in Expt. 2 with Fe, Mn and Zn (Tables 1, 2). In Expts. 1 and 2, Mn (and Zn in Expt. 2) concentrations were several fold greater at the 0 NI treatment than at the 82 NI treatment. Smith and Weeraratna (23) showed similar results in an acid mineral soil. Mn^{2+} and Zn^{2+} are the predominant ionic species in a soil solution and their availability is pH dependent. The solubility of each species decreases 100 fold for each unit increase in pH (24). Additionally, these ions form unavailable complexes with organic matter at higher pH values (24). Correlation coefficients in Table 5 indicate

Table 5. Correlation coefficients between the soil solution pH and soil solution nutrient concentrations and coefficients between soil solution Fe and NH_4-N concentrations over time (Expt. 2).

Genus	Soil solution element							NH_4-N
	P	K	Ca	Mg	Fe	Mn	Zn	
Azalea	.18	-.13	-.55 ***	-.75 ***	.44 ***	-.55 ***	-.61 ***	.78 ***
Holly	.1	.35 **	-.65 ***	-.83 ***	.52 ***	-.75 ***	-.80 ***	.78 ***
Juniper	.04	.26 *	-.69 ***	-.82 ***	.14	-.76 ***	-.82 ***	.64 ***

*, **, *** Significance at .01, .001, and .0001, respectively.

the close association between pH and Mn and Zn concentrations.

Iron was supplied in a chelated form (5 ppm) with each nutrient solution application in Expt. 1 and as a component of a dry sulfate formulation which was preplant incorporated into the bark in Expt. 2. In contrast to other micronutrient cations in Expts. 1 and 2 solution Fe concentrations were greater at the 82 NI treatment. Iron is characteristically more available at a lower pH and the reason for this unexpected result is unknown. Iron and NH_4-N were closely correlated (Table 5) thus, a possible Fe-NH_4-N interaction may have occurred.

In general tissue K, Ca, Mg, Mn and Zn concentrations were greater at 0 NI than at 82 NI and thus reflected the solution composition as affected by treatment (Tables 3, 4). Ingram and Joiner (16), using a pine bark medium, fertilized Quercus shumardii Buckl. with NH_4-N and found an inverse relationship between NH_4-N nutrition and medium pH and a negative correlation between medium pH and tissue Fe, Mn and Zn concentrations. The lack of differences in shoot dry weight between treatments in the present experiments implies that the lower tissue concentrations of K, Ca, Mg, Mn and Zn at 82 NI were not limiting to the growth of the size of plants used in this study.

With the exception of holly plants in Expt. 1, shoot dry weights were not affected by treatment (Tables 3, 4). However, tissue N concentrations of plants grown at 82 NI were consistently greater than at 0 NI. Chrustic and Wright (5), working with pine bark and the same 3 genera used in these experiments, reported greater dry weight and tissue N contents as the NH_4-N to NO_3-N ratio increased. Other work with woody plants has shown similar results (6, 8, 16, 18).

In summary these experiments indicate that nitrification significantly lowers the NH_4-N to NO_3-N ratio when a NH_4-N fertilizer is applied to a pine bark medium. Despite the lack of dry weight differences due to treatment, the influence of the ionic form of N on dry weight accumulation is well established (13, 14, 19). Since nursery plants are sold on the basis of size, providing a container-grown plant with a soil solution NH_4-N to NO_3-N ratio which maximizes growth would result in increased profits. If a species grows maximally at a high ratio then nitrification should be controlled. The use of NI in controlling nitrification would be economically feasible in a nursery operation, however, application of this chemical may be difficult. Nitrification could be controlled by amending the medium with lower amounts of lime and thus inhibit nitrification via a low pH. The influence of lime rate on nitrification is discussed in chapter 2.

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Chapter II

EFFECT OF LIMING RATE ON NITRIFICATION IN A PINE BARK MEDIUM

ABSTRACT

Liming a pine bark medium is a common nursery practice, however, the influence of lime on nitrification has not been documented. A 100% pine bark medium was amended with dolomitic lime at 0, 3 or 6 kg m^{-3} and periodically fertilized with 210 ml of a nutrient solution containing 100 ppm N as $(NH_4)_2SO_4$. At the 3 and 6 kg lime treatments, "soil" solution NH_4-N concentrations decreased rapidly while NO_3-N concentrations increased. At 0 kg lime, the NH_4-N decrease was slower and NO_3-N was not found. In a 2nd experiment, similarly treated bark was used to determine a NO_3-N accumulation rate (NAR). NAR was greatest at 6 kg lime except at the last two sampling dates when NAR did not differ between 3 and 6 kg lime. This lack of difference was attributed to a limiting NH_4-N supply at 6 kg lime. A 3rd experiment used a bark amended with 6 kg lime m^{-3} and was fertilized with 100 ppm NH_4-N. At days 42 and 48 some containers were treated with 300 ppm NH_4-N instead of 100 ppm NH_4-N. At the 100 ppm N treatment, the NAR increased, peaked and decreased. However, when the 300 ppm NH_4-N was applied the

NAR increased 3 fold. These results indicate that liming a pine bark medium greatly stimulates nitrification. Additionally, nitrification can be controlled by not adding lime to the medium.

INTRODUCTION

Liming container media is a routine practice in the nursery industry. Liming increases media pH, supplies Ca (and Mg in the case of dolomitic lime) and influences nutrient availability. An increased media pH stimulates nitrification, the biological oxidation of NH_4^+ to NO_3^-. Thus plant response to lime rate is at least in part a response to the ionic form of N in the soil solution. Chrustic and Wright (2) treated pine bark with dolomitic lime in the range 0 to 8 kg lime m^{-3} and reported that the 8 kg lime treatment had the lowest solution NH_4-N to NO_3-N ratio. These authors attributed this result to increased nitrifier populations and an increased adsorption of NH_4-N to bark particles. However, bark was fertilized with NH_4NO_3 in their experimentation and the application of NO_3-N to the bark makes the influence of nitrification on the "soil" solution difficult to discern. Niemiera and Wright (11) treated pine bark with 6 kg lime m^{-3} and fertilized with $(NH_4)_2SO_4$ and found a depletion of NH_4-N and an accumulation

of NO_3-N in the "soil" solution. A nitrification inhibitor, nitrapyrin (NI), was used in this experiment and the NH_4-N concentrations remained relatively high indicating that NH_4-N depletion without NI was unequivocally a result of nitrification.

The ionic form of N has a significant influence on plant nutrient composition and growth (6, 7, 10). Chrustic and Wright (2) found that the growth of 3 woody genera was greatest at the 0 or 2 kg lime m^{-3} bark treatments and attributed this response to a greater NH_4-N to NO_3-N ratio. Other researchers working with woody plants (8, 9, 13) have shown a similar response to such a ratio. Growers of container-grown crops commonly fertilize with NH_4-N and amend their media with lime. However, lime rate varies greatly within the industry depending on the species being grown and grower preference. The purpose of this research is to determine how varying lime rates affects nitrification in a pine bark medium.

MATERIALS AND METHODS

Expt. 1. Pine bark used in this experiment had a particle size distribution of 22% less than 0.5 mm (U.S. Sieve series #35), 22% between 0.5 mm and 1.2 mm (U.S. Sieve series #16), 18% between 1.2 and 2.4 mm (U.S. Sieve series #8),

24% between 2.4 and 5.6 mm (U.S. Sieve series #3.5) and 14% greater than 5.6 mm. This bark had a bulk density of 0.32 g cc^{-1} and was primarily from Pinus taeda L..

Pine bark was moistened and amended with 0.58 kg urea m^{-3} and 1 kg Micromax (Sierra Co., Milpitas, CA) m^{-3}. Bark was then treated with either 0, 3 or 6 kg dolomitic lime m^{-3}. This lime contained 51% $CaCO_3$, 40% $MgCO_3$ and had a $CaCO_3$ equivalent of 100. On 24 January 1984 rooted cuttings of Ilex crenata Thunb. 'Convexa' were transplanted into one-liter plastic containers and grown in a glasshouse with day/night temperatures of 24°/18° C. Containers were arranged in a randomized complete block design with 3 containers per treatment in each of 5 blocks.

Plants were irrigated with 210 ml nutrient solution containing 100 ppm N as $(NH_4)_2SO_4$, 10 ppm P as H_3PO_4 and 25 ppm K as K_2SO_4. Irrigation frequency was dependent upon plant need for water. After every 2 nutrient solution irrigations plants were irrigated with 420 ml tap water to prevent excessive salt accumulation in the bark. Tap water pH was 8.0 and Ca and Mg concentrations were 16 and 6 ppm, respectively. The "soil" solution was periodically tested 4 to 6 hr after the second nutrient solution irrigation. Testing employed the pour-through technique (15) by applying 75 ml distilled water onto the surface of the bark and col-

lecting the leachate. Leachates were analyzed for pH and NH_4-N and NO_3-N using ion selective electrodes.

Expt. 2. Pine bark was treated and irrigated as in Expt. 1, however, no plants were planted into the containers. A sufficient number of containers were prepared to allow for 1 container per treatment in each of 5 blocks to be periodically sampled.

Periodic determinations of the NAR were made following the second nutrient solution irrigation. Following irrigation, containers were allowed to drain for 1 hr and a uniform subsample of bark was removed. The containers with remaining bark were enclosed in a plastic bag and stored in a incubator at 25° c for 96 hr. The subsample was put and put into a PVC tube (2.5 x 15.2 cm) with one end covered with cheesecloth to retain the bark during the leaching process. Bark-filled tubes were leached with 210 ml distilled water at a rate of 70 ml hr^{-1} and the leachates collected (hr 0 NH_4-N content). This process was repeated 96 hr later with another bark subsample from the same containers. After the leaching process, bark from each tube was dried and weighed. Leachates were analyzed for NH_4-N and NO_3-N. The volume of leachate collected was multiplied by the NH_4-N and NO_3-N concentrations to determine the total amount of leachable NH_4-N and NO_3-N in the bark sample. This amount was then

divided by the weight of the bark to arrive at an amount of N g^{-1} bark. The hr 0 amount of NO_3-N was then subtracted from the amount at hr 96 and the difference was divided by 96 to get a NAR expressed as μg NO_3-N g^{-1} bark hr^{-1}.

Expt. 3. Methodology in this experiment was similar to that in Expt. 2 with the exception that NAR was determined every 6 days and at days 42 and 48 some of the containers previously fertilized with 100 ppm NH_4-N were treated with with 300 ppm NH_4-N.

RESULTS

Expt. 1. Figure 5 depicts the changes in "soil" solution NH_4-N and NO_3-N concentrations over time in response to lime rates. Ammoniacal-N concentrations decreased over time in all treatments, however, the decrease occurred earlier, to a greater extent and was more rapid in the 6 kg lime treatment compared to the 3 kg lime treatment. Ammoniacal-N concentrations at 0 kg lime declined gradually over time. Periods of increasing NO_3-N concentration in the "soil" solution coincided with the decline in NH_4-N. These periods occurred between days 29 and 70 and days 56 and 84 for the 6 and 3 kg lime treatments, respectively. Nitrate-N was not detected at any time in the 0 kg lime treatment.

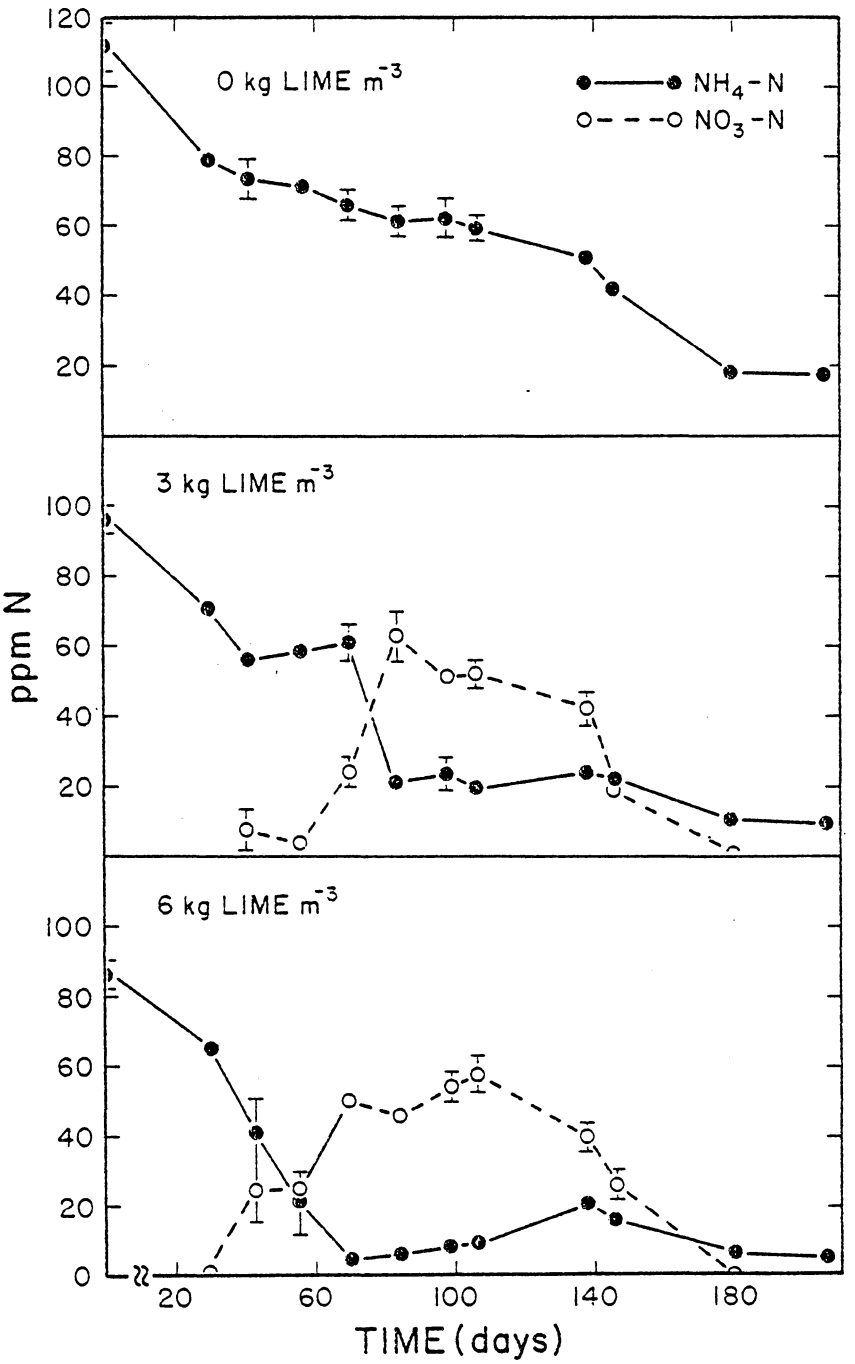

Fig.5. Influence of lime treatment on "soil" solution NH_4-N and NO_3-N concentrations.

"Soil" solution pH decreased over time for all treatments (Fig. 6). Greater decreases in pH occurred during the period of rapid NO_3-N accumulation at 3 and 6 kg lime compared to similar periods at 0 kg lime. Thus, during days 29 through 70 and days 56 through 84 in the 6 and 3 kg lime treatments, respectively, there was a 0.9 pH unit decrease for each treatment. During these periods at 0 kg lime there was a respective 0.2 and 0.6 unit decrease.

Expt. 2. Measurable NAR first occurred at days 41 and 84 for the 6 and 3 kg lime treatments, respectively while NO_3-N accumulation did not occur at 0 kg lime (Fig. 7). NAR were greatest at 6 kg lime until after day 84 when there were no significant differences between NAR at 3 and 6 kg lime.

The amount of leachable NH_4-N g^{-1} bark at hr 0 beyond day 41 and the amount of this N remaining after 96 hr was inversely related to the liming rate (Table 6). For example, The percent of the hr 0 leachable N remaining after 96 hr at day 107 was 90, 38 and 12% for the 0, 3 and 6 kg lime treatments.

Expt. 3. This experiment was conducted to determine if the NH_4-N supply of the 100 ppm NH_4-N treatment limited nitrification over time. NAR of the 100 ppm N treatment increased until maximum values occurred at days 19 and 25 aft-

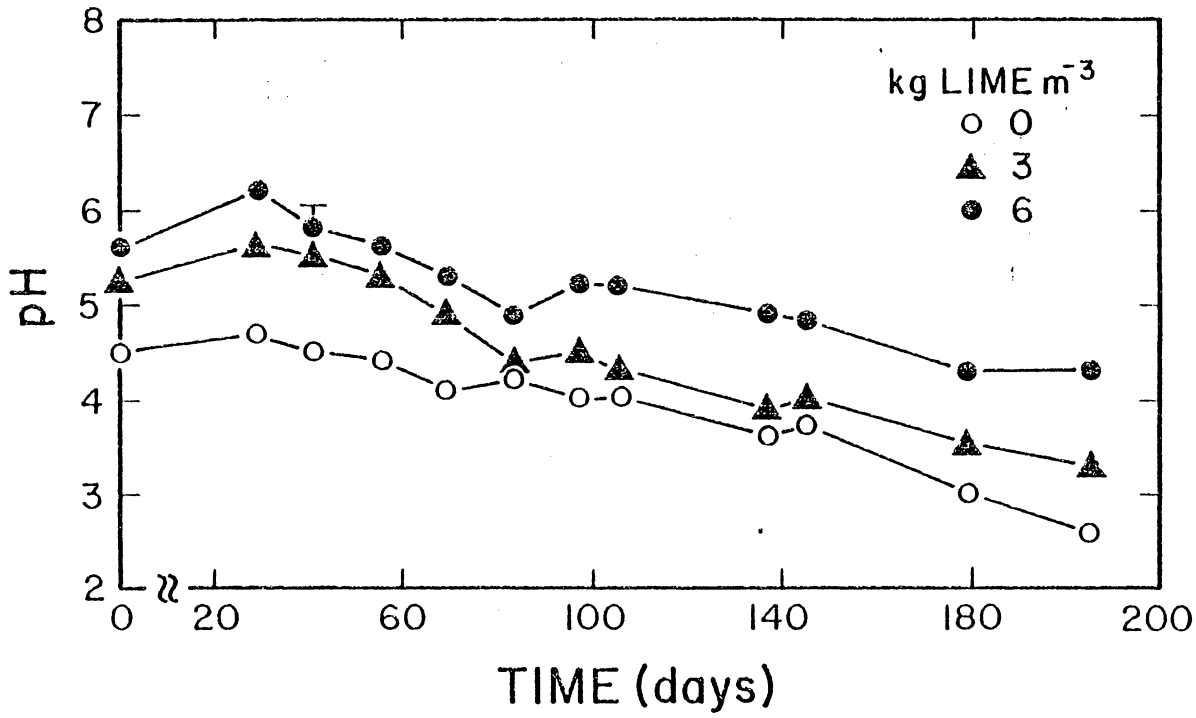

Fig.6. Influence of lime treatment on the "soil" solution pH.

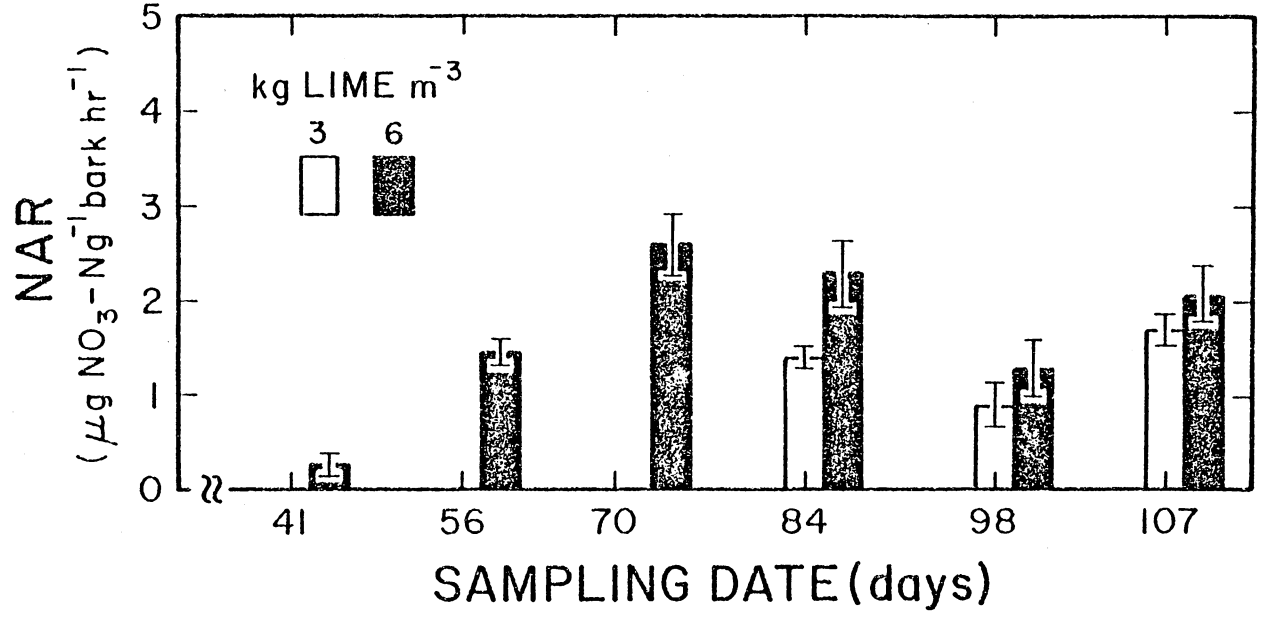

Fig.7. Influence of lime treatment on NAR.

Table 6. Influence of lime treatment on the amount of leachable NH_4-N measured at hr 0 and hr 96 during periodic NAR determinations (Expt. 2).

Lime treatment (kg lime m^{-3} bark)	Days						
	41	56	70	84	98	107	LSD[y]
	Hr 0 leachable NH_4-N (ug NH_4-N g^{-1} bark)						
0	290	356	362	363	386	324	59
3	275	279	324	292	303	235	43
6	265	278	206	163	142	123	45
LSD[z]	44	44	54	51	59	60	
	Leachable NH_4-N remaining after 96 hr (ug NH_4-N g^{-1} bark)						
0	290	376	362	364	341	291	62
3	275	278	305	275	195	87	38
6	265	264	22	17	13	14	33
LSD	45	74	38	44	42	38	

[z]Least significant difference within columns, 5% level.

[y]Least significant difference within rows, 5% level.

er which rates decreased (Table 7). The hr 0 amount of leachable NH_4-N decreased from 229 µg NH_4-N g^{-1} bark at day 1 to 28 µg NH_4-N g^{-1} at day 48 (Table 7). The amount of this N remaining after 96 hr decreased from 208 µg NH_4-Ng^{-1} bark at day 1 to 19 µg NH_4-N g^{-1} bark at day 25. Bark which had been previously treated with 100 ppm NH_4-N was treated with 300 ppm NH_4-N at days 42 and 48. In response to this increase in N supply NAR increased at least 3 fold. Bark of the 300 ppm N treatment contained at least 3 times as much hr 0 leachable NH_4-N than bark of the 100 ppm N treatment. In the 300 ppm N treatment only 6% of the hr 0 NH_4-N was found after 96 hr for both sampling dates.

DISCUSSION

The 6 kg lime treatment stimulated an earlier and greater nitrification of NH_4-N and an earlier appearance of NO_3-N in Expts. 1 and 2 (Figs. 5, 7) This earlier activity is in agreement with the fact that nitrifier growth and metabolism are pH dependent (4). Yet similar accumulations of NO_3-N occurred at 3 and 6 kg between days 70 and 145 in Expt. 1 and no differences in NAR occurred at the last 2 sampling dates in Expt. 2 between these treatments. The reason for the lack of differences between treatments may be explained by a difference in substrate availability. Ammo-

Table 7. Influence of N treatment on NAR and on the amounts of NH_4-N measured at hr 0 and hr 96 during periodic NAR determinations (Expt. 3).

Measurement	N treatment (ppm)	Days									LSD[z]
		1	7	13	19	25	31	37	43	49	
NAR	100	.21	.44	1.5	2.1	2.1	1.1	.81	.78	.47	.31
(μg NO_3-N g^{-1} bark hr^{-1})	300								2.3	1.7	.3
t-test									*	*	
Hr 0 leachable NH_4-N	100	229	159	174	141	92	58	42	31	28	13
(μg NH_4-N g^{-1} bark)	300								114	104	28
t-test									*	*	
Leachable NH_4-N remaining after 96 hr	100	208	122	150	99	17	7	8	4	3	11
(μg NH_4-N g^{-1} bark)	300								7	6	3
t-test									NS	NS	

[z]Least significant difference within rows, 5% level.

NS, * - Nonsignificant, means significantly different within columns at 5% level by t-test.

niacal-N concentrations in Expt. 1 were generally lower during the period of considerable NO_3-N accumulation at 6 kg compared to the 3 kg lime treatment. Beyond day 70 in Expt. 2 the hr 0 NH_4-N contents of bark at 6 kg lime were significantly less than other treatments (Table 6). Furthermore, only 10% of this hr 0 NH_4-N content remained after 96 hr beyond day 56. Thus, the relatively low "soil" solution NH_4-N concentrations in Expt. 1, the relatively low hr 0 NH_4-N contents in Expt. 2 and the low amount of this leachable NH_4-N remaining after 96 hr indicates that nitrifier activity at 6 kg lime was apparently limited by a low NH_4-N supply. Therefore, a nitrifier population exposed to a favorable pH and a limiting NH_4-N supply could over time produce an amount of NO_3-N similar to that produced by a population exposed to a less favorable pH and a greater NH_4-N supply.

Results of Expt. 3 support the above hypothesis. When 100 ppm NH_4-N applications were made to bark treated with 6 kg lime m^{-3}, the NAR increased, peaked and decreased (Table 7). However, when 300 ppm NH_4-N was applied the NAR increased 3 fold. Also the amount of hr 0 NH_4-N on the bark was 3 times as great as at 100 ppm N and only 6% of this N remained after 96 hr. Thus nitrifiers were capable of oxidizing more NH_4-N than was supplied by the 100 ppm N treatment.

"Soil" solution NO_3-N concentrations of the 3 and 6 kg lime treatments decreased to 0 during the 2nd half of Expt. 1 (Fig. 5). A similar observation was reported in earlier work with a pine bark medium (11). This lack of NO_3-N accumulation may be ascribed to a relatively low "soil" solution NH_4-N concentration and low pH since both of these factors limit nitrifier activity. Additionally, since plant size increased with time, plant absorption of N increased. Thus roots were in direct competition with nitrifiers for NH_4-N. Nitrate-N absorption by roots most likely occurred and thus the evidence for nitrifier activity was removed.

Expt. 2 was conducted without plants in the bark-filled containers so that a NAR could be obtained without root absorption of nitrifier substrate or product. Since root function has a significant effect on the soil solution pH and removal of substrate, results of Expts. 1 and 2 are not distinctly parallel. However, the similar methodology of the 2 experiments makes the relationships found within each experiment relevant to each other.

Nitrate-N was not detected in the "soil" solution at 0 kg lime in either experiment (Fig. 5, Table 6). In Expt. 1 solution pH ranged from 4.7 to 2.6 at 0 kg lime. Thus establishment of nitrifier populations in both experiments was apparently inhibited by the acidic environment of the un-

limed bark. In agreement with the present experimentation Chrustic and Wright (2) amended pine bark with a range of lime and noted higher NH_4-N to NO_3-N ratios at low lime rates. Gilmour (5), working with an acid soil, showed NO_3-N accumulation to be linearly related to pH in the range of 4.9 to 7.2. Gilmour (5) and others (1, 3) constructed mathematical descriptions which predicted a nitrification rate of zero at pH 4.1. Yet, NO_3-N accumulations have been found in soils with a pH of 4.1 (14).

The "soil" solution pH of all treatments in Expt. 1 declined over time (Fig. 6). As noted in the results a greater decrease in pH occurred during the periods of NO_3-N increase at 3 and 6 kg lime compared to the corresponding times at 0 kg lime. This greater pH reduction is attributable to H^+ ions released during the oxidation of NH_4^+ to NO_2^-, the first reaction of the nitrification process. Ammoniacal-N was the sole form of N in solution at 0 kg lime. Thus the 2.1 pH unit decrease over time is primarily attributed to root exudation of H^+ ions in response to absorption of NH_4^+.

In summary, this work has characterized the "soil" solution N and pH status of a pine bark medium treated with different rates of lime. In general the stimulative influence of lime on nitrification increased as the rate of lime

increased and resulted in a low solution NH_4-N to NO_3-N ratio. The influence of this ratio on dry weight accumulation is well established (6, 7, 10) and is apparently species specific. Nursery plants are sold on the basis of size, hence a ratio which maximizes growth would increase profits. Such a ratio could be effected by manipulating the amount of lime added to the medium. Chrustic and Wright (2) showed that the growth of azalea, holly and juniper plants to be greater at 0 or 2 kg lime m^{-3} bark than at higher rates of lime and attributed this growth response in part to the relatively high NH_4-N to NO_3-N ratio. Thus a growth response to lime treatment is in part a response to the ionic form of N as affected by nitrification and not a response to the increased pH or Ca availability. In agreement with this statement Starr and Wright (12) showed no growth response to soil solution Ca concentrations greater than 10 ppm.

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Chapter III

THE INFLUENCE OF NH_4-N APPLICATION RATE ON NITRIFICATION IN A PINE BARK MEDIUM

ABSTRACT

Fertilizing container-grown plants with NH_4-N is a common nursey practice, however, the influence of this practice on nitrification has not been documented. A 100% pine bark medium was periodically fertilized with 210 ml of either 25, 100 or 200 ppm NH_4-N. A rapid decrease in "soil" solution NH_4-N concentrations occurred in the first half of the experiment for each treatment. Rapid increases in NO_3-N concentrations coincided with these NH_4-N decreases and greater NO_3-N concentrations were found at the higher N treatments. During the periods of NO_3-N increase, "soil" solution pH decreased 0.3, 0.7 and 1.3 units for the 25, 100 and 200 ppm N treatments, respectively. In a 2nd experiment similarly treated bark was used to determine a NO_3-N accumulation rate (NAR). NAR of the 100 and 200 ppm N treatments were greater than at 25 ppm N with no consistent differences between the 100 and 200 ppm N treatments. These results indicate that the solution concentration of NO_3-N is directly related to the concentration of applied NH_4-N.

INTRODUCTION

The rate of nitrification is considered to follow first order kinetics under limiting amounts of NH_4-N and zero order kinetics when the NH_4-N is non-limiting (2, 3, 8). However, conditions within a plant-soil system such as soil pH can decrease the correspondence between NH_4-N supply and nitrification.

Nitrifier growth and metabolism are related to pH with maximum activity occurring in the range of 7.0 to 8.0 (3). When nitrifiers oxidize a molecule of NH_4^+, 2 H^+ ions are liberated thereby acidifying the soil solution. Thus nitrifiers impose a feedback inhibition on their activity via H^+ ion liberation.

Nitrifier activity and plant absorption of NH_4-N reduce the soil solution NH_4-N supply. Niemiera and Wright (11, 12) showed that NH_4-N applied to pine bark of container-grown plants was rapidly nitrified resulting in a relatively low "soil" solution NH_4-N to NO_3-N ratio. These authors (12) also demonstrated that 90% of the leachable NH_4-N in a pine bark sample was nitrified within 96 hr. Since plant size and therefore plant absorption of N increases with time, the competition between plant and nitrifier for NH_4-N increases with time.

Container-grown plants are intensively fertilized by various methods of nutrient application. The amount or concentration of N in the "soil" solution depends on the particular method of fertilization. If a dry fertilizer is used, then the amount of N in solution depends on the amount applied and the solubility characteristics of the fertilizer. The use of slow-release fertilizers supplies the "soil" solution with a relatively low N concentration (9). If N is applied via the irrigation system, then the amount of N in the solution depends on the concentration applied and the frequency of application.

Ammoniacal fertilizers are widely used in the nursery industry, however, the impact of varying amounts of NH_4-N on nitrification has not been elucidated for container-grown crops. Such an effort is justified since the ionic form of N in a soil solution has a significant influence on plant nutrient composition and growth (5, 6, 10). Additionally, nitrification has a significant impact on the soil solution pH which influences cation availability (1, 11, 13). Therefore, the purpose of this paper is to determine how varying rates of NH_4-N affects nitrification in a pine bark medium.

MATERIALS AND METHODS

Expt. 1. Pine bark used in this experiment had a particle size distribution of 22% less than 0.5 mm (U.S. Sieve series #35), 22% between 0.5 and 1.2 mm (U.S. Sieve series #16), 18% between 1.2 and 2.4 mm (U.S. Sieve series #8), 24% between 2.4 and 5.6 mm (U.S. Sieve series #3.5) and 14% greater than 5.6 mm. This bark had a bulk density of 0.32 g cc^{-1} and was primarily from Pinus taeda L..

Pine bark was moistened and amended with 0.58 kg urea m^{-3}, 6 kg dolomitic lime m^{-3} and 1 kg Micromax (Sierra Co., Milpitas, CA) m^{-3}. On 24 January 1984 rooted cuttings of Ilex crenata Thunb. 'Convexa' were transplanted into one-liter bark filled plastic containers and grown in a glasshouse. Plants were arranged in a randomized complete block design with 3 containers per treatment in each of 5 blocks.

Plants were irrigated with 210 ml of nutrient solution containing either 25, 100 or 200 ppm N as $(NH_4)_2SO_4$, 10 ppm P as H_3PO_4 and 25 ppm K as K_2SO_4. Irrigation frequency was dependent upon plant need for water. After every 2 nutrient solution irrigations, plants were irrigated with 420 ml of tap water to prevent excessive salt accumulation in the bark. The "soil" solution was periodically tested 4 to 6 hr after the 2nd nutrient solution irrigation. Testing employed the pour-through technique (14) by applying 75 ml of

distilled water onto the surface of the bark and collecting the leachate. Leachates were analyzed for pH and NH_4-N and NO_3-N using ion selective electrodes.

Expt. 2. Pine bark was treated and irrigated as in Expt. 1, however, no plants were transplanted into these containers. A sufficient number of containers were prepared to allow for 1 container per treatment in each of 5 blocks to be periodically sampled throughout the experiment.

Periodic determinations of the NAR were made following the 2nd nutrient solution irrigation. Following irrigation, containers were alloed to drain for 1 hr and a a uniform subsample of bark was removed. The containers with remaining bark were enclosed in plastic bags and stored in an incubator at 25° C for 96 hr. The subsample was put into a PVC tube (2.5 x 15.2 cm) with one end covered with cheesecloth to retain bark during the leaching process. Tubes were leached with 210 ml distilled water at a rate of 70 ml hr^{-1} and leachates collected (hr 0 NH_4-N content). This process was repeated 96 hr later with another bark subsample from the same containers. After leaching, bark from each tube was dried and weighed. Leachates were analyzed for NH_4-N and NO_3-N. The volume of leachate collected was multiplied by the NH_4-N and NO_3-N concentrations to determine the total amount of leachable NH_4-N and NO_3-N in the bark

sample. This amount was then divided by the weight of the bark to arrive at an amount of N g^{-1} bark. The hr 0 µg NO_3-N g^{-1} bark was subtracted from the amount at hr 96 and the difference was divided by 96 to get the NAR expressed as µg NO_3-N g^{-1} hr^{-1}.

RESULTS

By day 56 the concentration of NH_4-N in the "soil" solution for all treatments decreased by at least 80% (Fig. 8). A rapid increase in NO_3-N concentration coincided with the decline in NH_4-N for each treatment. Peak NO_3-N concentrations were 46, 76 and 150 ppm for the 25, 100 and 200 ppm N treatments, respectively. Following these peak values, NO_3-N concentrations decreased for all treatments. By day 56 there was a 0.7 and 1.3 pH unit decrease in the "soil" solution at the 100 and 200 ppm N treatments, respectively (Fig. 9). At the 25 ppm N treatment there was a 0.3 pH unit decrease between days 10 and 47 (the period of NH_4-N decline).

Expt. 2. With the exception of day 41, NAR for the 100 and 200 ppm N treatments were greater than the 25 ppm N treatment (Fig. 10). The hr 0 amount of leachable NH_4-N was greatest at 200 ppm N and least at 25 ppm N for all sampling dates (Table 8). Relatively low amounts of leachable NH_4-N

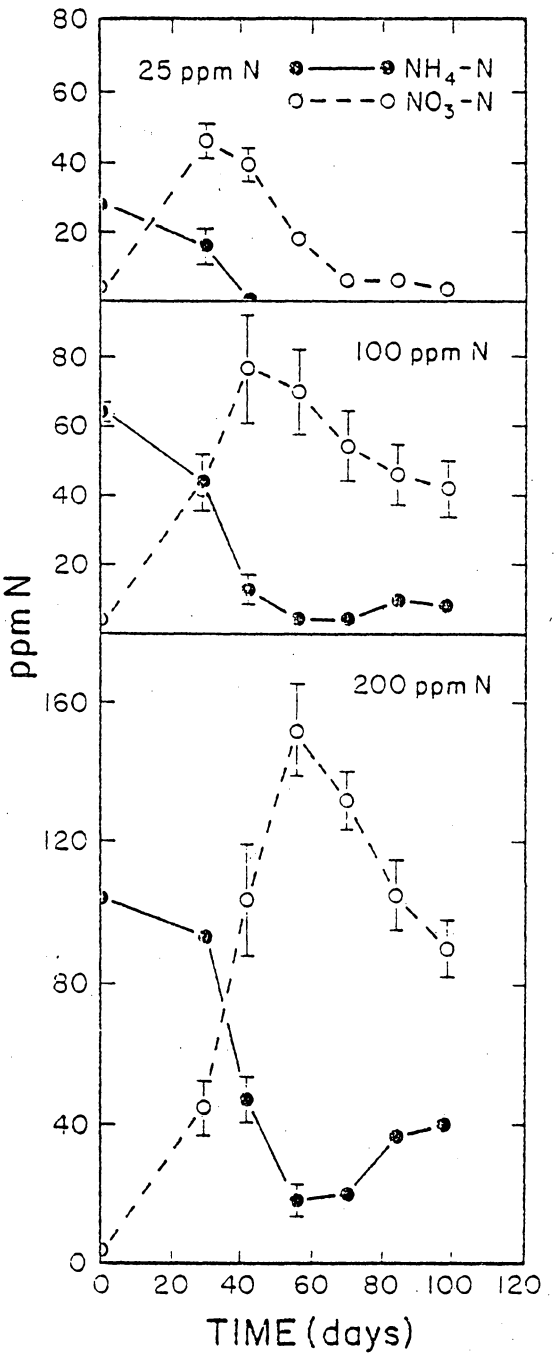

Fig.8. Influence of N treatment on the "soil" solution NH_4-N and NO_3-N concentrations (Expt. 1).

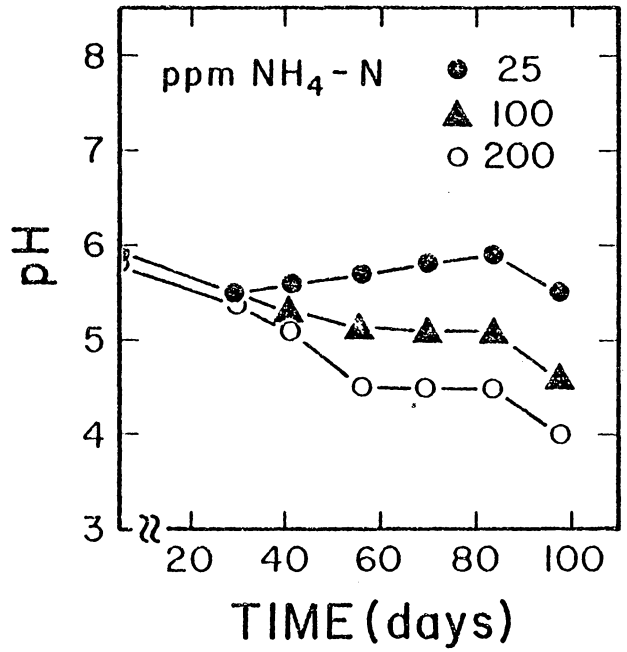

Fig.9. Influence of N treatment on the "soil" solution pH (Expt. 1).

remained after 96 hr for the 25 ppm and 100 ppm N treatments.

DISCUSSION

The concentration of NO_3-N in the "soil" solution (Expt. 1) was positively related to concentration of the applied NH_4-N (Fig. 8). Thus apparent nitrifier activity was related to the amount of NH_4-N in the "soil" solution. However, the NH_4-N supplied by the N treatments was not totally available to nitrifiers since plants were most likely absorbing a portion of the solution NH_4-N.

The NAR of the 25 ppm N treatment (Expt. 2) was significantly lower than the 100 and 200 ppm N treatments at each sampling date with the exception of day 41 (Fig. 9). Relatively low amounts of leachable NH_4-N at hr 0 as well as hr 96 indicates that the NH_4-N supply of the 25 ppm treatment was limiting. In most cases the NAR of the 200 ppm N treatment were not greater than the 100 ppm N treatment. Yet higher NO_3-N concentrations were existed in the solution at 200 ppm N than at 100 ppm N (Expt. 1) (Fig. 8). This apparent anomaly can be explained on the basis of substrate availability. NAR in Expt. 2 was established during a 96 hr period following fertilization and hence a period of maximum NH_4-N supply. The amount of leachable NH_4-N remaining after

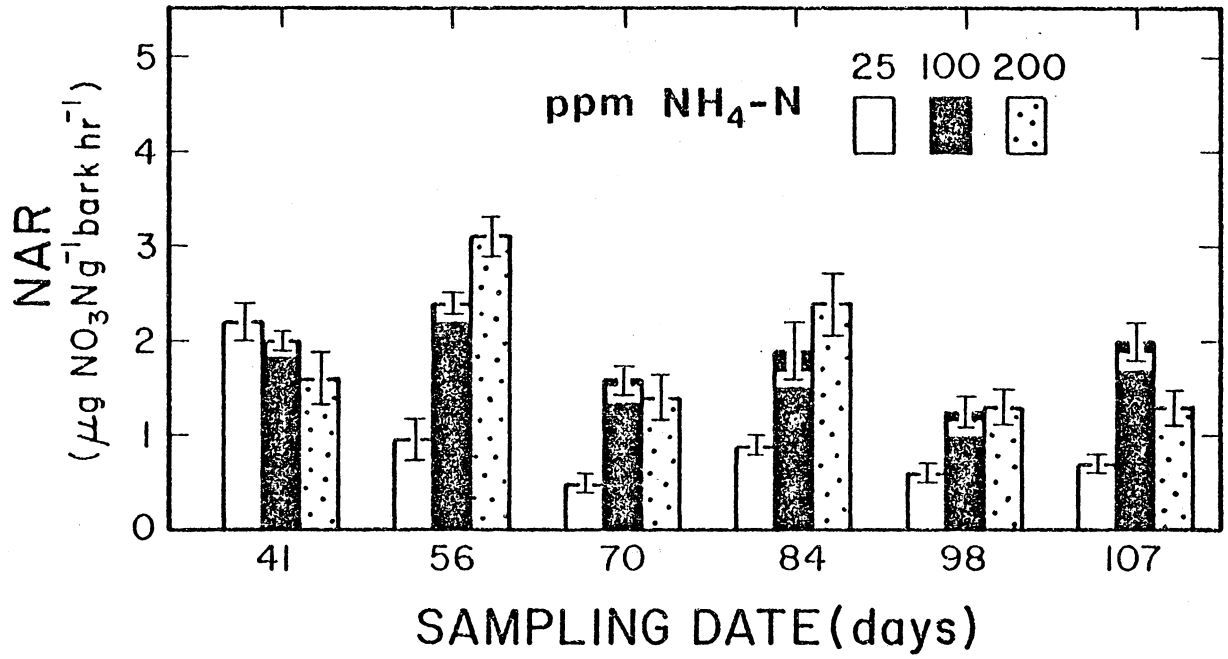

Fig.10. Influence of N treatment on NAR (Expt. 2).

Table 8. Influence of N treatment on the amount of leachable NH_4-N measured at hr 0 and hr 96 during NAR determinations (Expt. 2).

N treatment (ppm)	Days						
	41	56	70	84	98	107	LSD[y]
	Hr 0 leachable NH_4-N (ug NH_4-N g^{-1} bark)						
25	122	57	41	27	23	14	18
100	268	154	126	121	124	129	40
200	468	370	334	377	452	372	67
LSD[z]	54	60	48	26	64	29	
	Leachable NH_4-N remaining after 96 hr (ug NH_4-N g^{-1} bark)						
25	59	7	4	7	4	6	16
100	202	20	14	42	28	53	16
200	430	296	151	303	432	338	47
LSD	38	30	35	13	41	24	

[z]Least significant difference within columns, 5% level.

[y]Least significant difference within rows, 5% level.

96 hr at 100 ppm N (Table 8) was relatively low and was not significantly different from the 25 ppm N treatment at days 56, 70 and 98. In contrast, the amount remaining after 96 hr at 200 ppm N was relatively high. After day 41 this leachable NH_4-N was 2 to 3 times greater at 200 ppm N than at 100 ppm N. Thus the NH_4-N supply of the 100 ppm N treatment was apparently limiting relative to the supply at 200 ppm N. Previous NAR determinations in pine bark (12) showed that over time the NH_4-N supply of a 100 ppm N treatment was limiting relative to the NH_4-N supply at a 300 ppm N treatment. Thus, despite the similar NAR of the 100 and 200 ppm N treatments, the greater NH_4-N supply at 200 ppm N allowed for a greater NO_3-N accumulation beyond the 96 hr period. This statement is supported by the fact that the peak NO_3-N concentration at 200 ppm N in Expt. 1 was twice as great as the peak concentration at 100 ppm N (Fig. 8).

The greater amount of NH_4-N oxidation at 200 ppm N resulted in a greater "soil" solution acidification as evidenced by the 0.6 unit difference in pH after day 41 between the 100 and 200 ppm N treatments in Expt. 1 (Fig. 9). Ingram and Joiner (7), working with a pine bark medium, also showed that medium pH decreased as the NH_4-N application rate increased. Since nitrifier activity is directly related to pH, the lower pH at 200 ppm N may have contributed to

the lack of difference in NAR between the 100 and 200 ppm N treatments. This lower pH can also be attributed to an increased H^+ ion excretion by roots as a result of an increased NH_4^+ absorption. However, previous work (11) has shown that the pH decrease due to nitrification is apparently greater than the decrease due to plant NH_4-N absorption. In Expt. 1 NO_3-N concentrations for all treatments decreased following the peak NO_3-N concentration. This occurrence has been reported in previous experiments with pine bark (11, 12) and may be related to a low NH_4-N supply, to a low pH or to plant uptake. Holly plants similar in size to the plants used in the present experiment absorbed approximately 25 mg N per day from a nutrient solution (unpublished data). If the "soil" solution concentrations of 3 one-liter bark-filled containers (at 100% gravimetric moisture holding capacity) were 25, 100 and 200 ppm N, then the 25 mg of absorbed N would be 312%, 75% and 37%, respectively, of the "soil" solution N content. Thus plant absorption of N was responsible for a significant reduction in the soil solution N supply. Since plant size and therefore absorption of N increases with time, the competition between plants and nitrifiers would also increase with time.

Expt. 2 was conducted without plants in the bark-filled containers so that an NAR could be obtained without root ab-

sorption of nitrifier substrate or product. Since root function has a significant influence on a soil solution pH and removal of substrate, results of Expts. 1 and 2 are not distinctly parallel. However, the similar methodology of the 2 experiments makes the relationships found within each experiment relevant to each other.

The concentrations of the applied NH_4-N solutions and the resulting "soil" solution N concentrations of this experiment are representative of these concentrations found in nursery-grown container crops. If the "soil" solution of a 3.8 liter bark-filled container (1250 g bark) was at 100 ppm NH_4-N and assuming that the bark was at 100% moisture holding capacity (gravimetric basis), then there would be 0.1 mg NH_4-N per g of bark. At a NAR of 2 µg NO_3-N g^{-1} bark hr^{-1} (as found in Expt. 2), the "soil" solution NH_4-N content would be oxidized in 50 hr. Thus a nitrifier population is capable of rapidly oxidizing amounts of NH_4-N which are commonly applied to a pine bark medium. If a species is known to prefer a relatively high NH_4-N to NO_3-N ratio then a grower could limit nitrification by the amount of lime added to the pine bark (12).

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Chapter IV

INFLUENCE OF TEMPERATURE ON NITRIFICATION IN A PINE BARK MEDIUM

ABSTRACT

Media of container-grown woody plants are exposed to a wide range of temperatures, however, the influence of temperature on nitrification in a container medium has not been documented. One-liter pine bark-filled containers were stored in a glasshouse for 50 days and periodically fertilized with 210 ml of 100 ppm NH_4-N. Containers were then subjected to either 10°, 20°, 30° or 40° C for 24 days. "Soil" solutions were tested for NH_4-N, NO_3-N and pH and a NO_3-N accumulation rate (NAR) was determined. "Soil" solution NH_4-N concentrations decreased over time at 10°, 20° and 30° with a greater decrease occurring at 20° and 30°. Ammoniacal-N concentrations at 40° were considerably greater than at other temperatures and increased over time. "Soil" solution NO_3-N concentrations were lower at 40° than concentrations at other temperatures. Over time the general order of NAR was: 20° = 30° > 10° > 40°.

INTRODUCTION

Nitrification, the biological oxidation of NH_4^+ to NO_3^-, has been shown to have a profound influence on the "soil" solution composition of a pine bark medium (8). The "soil" solution NH_4-N to NO_3-N ratio and pH decreased over time and cation availability increased. Container-grown woody ornamentals are exposed to a wide range of temperatures. Temperature has a significant affect on nitrifier activity within the range of 5° to 40° C (1). A great deal of research has been conducted on the influence of temperature on nitrification in mineral soils, however, nitrifier response to temperature in mineral soils may not necessarily be extrapolated to a pine bark medium since the physical and chemical properties of a soil markedly affect nitrifier activity (1). In addition fertilization practices for container-grown crops differ greatly from those employed in mineral soil systems. Thus, the purpose of this study is to determine how temperature influences nitrification in a pine bark medium.

MATERIALS AND METHODS

Pine bark used in this experiment had a particle analysis distribution of 22% less than 0.5 mm (U.S. Sieve series 35), 22% between 0.5 and 1.2 mm (U.S. Sieve series 16), 18% between 1.2 and 2.4 mm (U.S. Sieve series 8), 24% between 2.4 and 5.6 mm (U.S. Sieve series 3.5) and 14% greater than 5.6 mm. This bark had a bulk density 0.32 g cc^{-1} and was primarily from Pinus taeda L..

Pine bark was moistened and amended with 0.58 kg urea m^{-3}, 6 kg dolomitic lime m^{-3} and 1 kg Micromax (Sierra Co., Milpitas, CA) m^{-3}. Bark was placed into one-liter plastic containers and stored in a glasshouse for 50 days with day/night temperatures of 24°/18° C. Containers were periodically irrigated with 210 ml of a nutrient solution containing 100 ppm N as $(NH_4)_2SO_4$, 10 ppm P as H_3PO_4 and 25 ppm K as K_2SO_4 and occasionally irrigated with 420 ml tap water to avoid excessive accumulation of salts.

After the 50 day preincubation period containers for "soil" solution testing and NAR determinations were fertilized as above, and stored at either 10°, 20°, 30° or 40° C. Containers were enclosed in plastic while being subjected to these temperatures. Every 6 days for a period of 24 days the same 5 containers per temperature were removed from their environments for soil testing by applying 75 ml of

distilled water onto the surface of the bark and collecting the leachate (10). Containers were then fertilized, allowed to drain for 1 hr, and returned to the respective treatment. The values shown in table 9 represent the status of the soil solution 6 days after the previous fertilization.

At 6 day intervals all containers used for the NAR determinations were removed from their treatment, fertilized with 210 ml of the nutrient solution and allowed to drain for 1 hr. Five containers per temperature were selected at random for testing and the remainding containers were returned to their respective temperature. A uniform subsample of bark was removed from each of the 5 containers per temperature. These subsampled containers were enclosed in a plastic bag and returned to their tereatment for 48 hr. The bark subsample was put into a PVC tube (2.5 x 15.2 cm) with one end covered with cheesecloth. Tubes were leached with 210 ml distilled water at a rate of 70 ml hr^{-1} and leachate was collected (hr 0). This process was repeated 48 hr later with another bark subsample from the same containers. After leaching, bark from each tube was dried and weighed. Leachates were analyzed for NH_4-N and NO_3-N. The volume of leachate collected was multiplied by the NH_4-N and NO_3-N concentrations to determine the total amount of leachable NH_4-N and NO_3-N in the bark subsample. This amount was then

divided by the weight of the bark to arrive at the amount of N g^{-1} bark. The hr 0 µg NO_3-N g^{-1} bark was subtracted from the amount at hr 48 and the difference was divided by 48 to get a NAR expressed as µg NO_3-N g^{-1} hr^{-1}.

Bark-filled containers were kept in a glasshouse and periodically fertilized prior to being subjected to different temperatures. The purpose of this preincubation period was to allow nitrifier populations to become established. The presence of relatively high "soil" solution NO_3-N concentrations (Table 9) found during the treatment period are partly attributed to nitrifier activity during the preincubation period.

RESULTS

"Soil" solution NH_4-N concentrations decreased over time at the 10°, 20° and 30° temperatures with the lowest values generally occurring at 20° and 30° (Table 9). In contrast, NH_4-N concentrations at 40° were considerably greater and increased over time. Generally lower "soil" solution NO_3-N concentrations were detected at the last 2 sampling dates compared to the first 2 sampling dates for all treatments. Nitrate-N concentrations of the 40° treatment were notably less than other temperatures. Solution pH values were lower at day 24 than at day 6 for the 10°, 20° and 30° temperatures.

Table 9. Influence of temperature on soil solution NH_4-N and NO_3-N concentrations and pH over time.

Temperature (°C)	Days 6	12	18	24	LSD[Y]
		Soil solution NH_4-N (ppm)			
10	7	5	3	3	20
20	2	1	0.4	0.2	0.7
30	4	2	0.4	0.4	1
40	13	19	20	27	7
LSD[Z]	2	3	3	6	
		Soil solution NO_3-N (ppm)			
10	119	117	113	97	16
20	129	143	96	107	22
30	130	139	107	96	21
40	75	55	28	22	11
LSD	18	21	16	17	
		Soil solution pH			
10	4.7	4.8	4.5	4.5	0.11
20	4.8	4.8	4.7	4.6	0.17
30	4.9	4.9	4.7	4.6	0.10
40	5.0	4.9	4.9	4.9	0.18
LSD	0.1	0.1	0.1	0.2	

[Z]Least significant difference within columns, 5% level.

[Y]Least significant difference within rows, 5% level.

NAR were consistently greatest at 20° and 30° (Fig. 11). Rates at 10° increased until day 18 and were considerably lower at day 24. Rates at 40° were relatively low throughout the experiment with no measurable rate occurring at day 24. The hr 0 leachable NH_4-N contents for each sampling date were generally lower at day 24 than at day 6 for 10°, 20° and 30° (Table 10). Although not statistically different in each case, hr 0 leachable NH_4-N values were consistently less at 20° and 30° compared to the 10° treatment. Leachable NH_4-N contents at 40° were considerably greater than at other temperatures. The amount of the hr 0 leachable NH_4-N remaining after 48 hr was greatest at 10° and 40° and least at 20° and 30° (Table 10).

DISCUSSION

The fact that "soil" solution NH_4-N concentrations remained relatively low despite periodic NH_4-N fertilizations and that the solution pH decreased over time in the 10°, 20° and 30° treatments (Table 9) indicates that nitrification has a significant influence on the "soil" solution of a pine bark medium within a 10° to 30° range. These findings are consistent with generally accepted values obtained with mineral soils (1, 4, 6). The decrease in pH over time was expected since nitrification is an acidifying process.

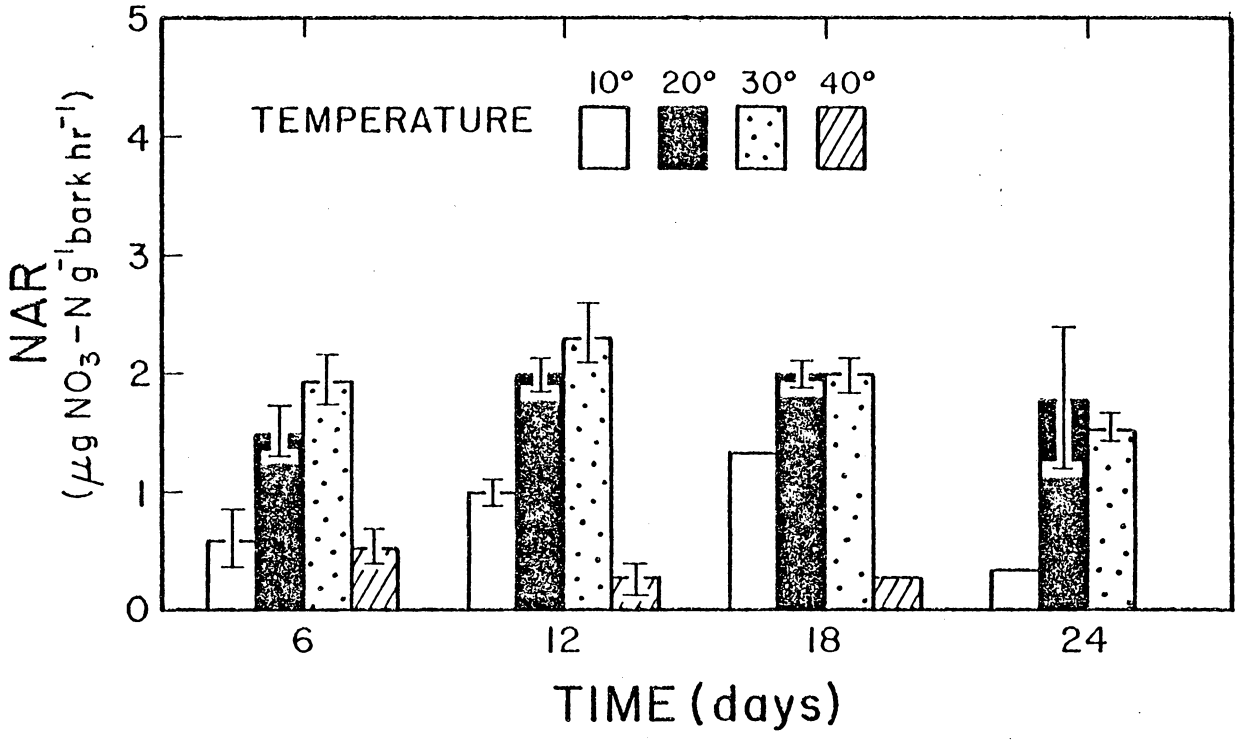

Fig.11. Influence of temperature on NAR.

Table 10. Influence of temperature on the amounts of leachable NH_4-N measured at hr 0 and hr 96 during NAR determinations (Expt. 2).

Temperature (°C)	Days 6	12	18	24	LSD[Y]
	Hr 0 leachable NH_4-N (μg NH_4-N g^{-1} bark)				
10	108	136	107	80	23
20	90	97	80	64	16
30	82	104	83	66	16
40	154	179	200	200	32
LSD[Z]	22	22	24	22	
	Leachable NH_4-N remaining after 96 hr (μg NH_4-N g^{-1} bark)				
10	86	86	71	54	17
20	17	25	10	10	7
30	13	18	10	12	4
40	146	154	176	180	18
LSD	14	22	11	13	

[Z]Least significant difference within columns, 5% level.

[Y]Least significant difference within rows, 5% level.

"Soil" solution NH_4-N concentrations indicate a greater nitrifier activity at 20° and 30° (Table 9) while NAR data show activity to be greatest at 20° and 30° (Fig. 11). However, solution NO_3-N concentrations generally decreased over time. This finding may be related to the low solution NH_4-N concentrations and the low hr 0 leachable NH_4-N values. The possibility exists that denitrification may have been responsible for the decrease in solution NO_3-N concentrations.

NAR was determined over a 48 hr period following a fertilization. Thus these rates do not reflect nitrifier activity during the 6 day period between samplings. Nitrifier activity during this interim period at 10°, 20° and 30° may have been limited by NH_4-N availability. Lower amounts of hr 0 leachable NH_4-N found in the NAR determinations (Table 10) and lower soil solution NH_4-N concentrations (Table 9) at the end of both experiments support this contention. Furthermore, the amount of the hr 0 leachable NH_4-N remaining after 48 hr at 20° and 30° was relatively low (Table 10). Therefore NH_4-N is rapidly oxidized and nitrification following this 48 hr period and prior to the next fertilization would be limited a by low NH_4-N availability. Niemiera and Wright (9) have demonstrated that after 25 days periodic 100 ppm NH_4-N fertilizations can supply a suboptimal amount of NH_4-N.

NAR at 10° ranged from 0.4 to 1.4 μg NO_3-N g^{-1} bark hr^{-1}. Thus, as indicated by the soil solution data and in agreement with other work on mineral soils (2, 3, 4) considerable nitrification occurs at 10°. NAR at 40° were low throughout the experiment with no measurable NAR occurring at day 24 (Fig. 11). This finding is consistent with other reports (5, 6) which indicated that this temperature inhibits nitrification. The lack of a measurable NAR at day 24 may be due to the 24 day exposure to 40°. This exposure may have reduced the growth and regeneration of nitrifiers which populated the bark during the preincubation period.

An NAR of 2 μg NO_3-N g^{-1} bark hr^{-1}, a rate measured at 20° and 30°, can be related to the relatively low "soil" solution NH_4-N concentrations found over time. The amount of N contained in a 100 ppm NH_4-N soil solution of a one-liter container (330 g bark at 100% gravimetric moisture holding capacity) is approximately 33 mg. This amount of N expressed per g of bark is 0.1 mg NH_4-N per g bark. At an NAR of 2 μg NO_3-N g^{-1} bark hr^{-1}, the "soil" solution NH_4-N would be oxidized in 50 hr. This calculation is in agreement with "soil" solution data (Table 9) which shows the NH_4-N concentrations to be less than 1 ppm at the last 2 sampling dates.

Container temperatures during the growing season encompass the range of temperatures to which the pine bark was exposed in this experiment. Thus if NH_4-N fertilizers are applied, container-grown plants are subject to low or high "soil" solution NH_4-N to NO_3-N ratios depending on the time of the growing season. Nitrate-N rather than NH_4-N fertilizers are generally recommended for the cooler times of the year for greenhouse as well as nursery-grown plants. This recommendation is based on the assumption that applied NH_4-N will not be nitrified at lower temperatures thus creating the potential for ammonium toxicity. However, results of this study indicate that considerable nitrification occurs at 10° and hence the potential for NH_4-N toxicity is relatively low. Ammonium toxicity could become a problem when medium temperatures exceed 30°.

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Chapter V

SUMMARY AND CONCLUSIONS

The influence of nitrification on the "soil" solution of container-grown plants has not been documented in the literature. The justification for an investigation of nitrification is based on the fact that the ionic form of N influences plant nutrient content and growth. Furthermore, container-grown plants are intensively fertilized and NO_3^- runoff from nurseries is an important environmental concern. Thus, a series of experiments were conducted to determine the influence of nitrification on the "soil" solution of container-grown plants and to determine the influence of such variables as lime and NH_4-N application rate and temperature on nitrification. Pine bark was selected for this research since this medium is used by the majority of nurseries in the southeastern U.S.

In chapter 1 the influence of nitrification on the "soil" solution was determined by using NI, a nitrification inhibitor, as a control. Over time nitrification was responsible for a low "soil" solution NH_4-N to NO_3-N ratio and a decrease in solution pH of at least 1.0 unit. Associated with this lower pH was a greater availability of solution K, Ca, Mg, Mn and Zn. The magnitude of the difference in the

availability of these cations between the NI treatment and the control treatment is of considerable interest. This finding contradicts the belief that the pH of a container media does not significantly influence the nutrition of container-grown crops (2). The increased availability of the aforementioned cations in the present study resulted in greater tissue contents of these cations. However, no difference in shoot dry weights occurred between the treatments despite the fact that with NI plants contained a greater amount of N. Previous work with pine bark (1) using the same genera as in the present study showed that greater shoot dry weights occurred at a relatively high NH_4-N to NO_3-N ratio. If there is a plant "preference" for NH_4-N, then a relatively high ratio as found in the control treatment should have resulted in greater shoot dry weights. However, significant NH_4-N may have been available to the plant following fertilization so that dry weight differences were not manifested. Thus growers fertilizing with NH_4-N, a common source of N in nursery fertilizers, can expect nitrification to significantly alter the "soil" solution NH_4-N to NO_3-N ratio, pH and cation availability.

Results of chapter 2 show that nitrification is related to the amount of lime added (preplant) to pine bark. Whereas no "soil" solution NO_3-N nor an NO_3-N accumulation rate

(NAR) were found at the 0 kg lime treatment, significant concentrations and a relatively high NAR occurred at the 3 and 6 kg lime treatments. At the 6 kg lime treatment, nitrification, as evidenced by "soil" solution and NAR data, occurred earlier and to a greater extent compared to the 3 kg lime treatment. Thus nitrification could be effectively inhibited or promoted by the amount of lime added to pine bark. Previous research with pine bark (1) has shown that the growth of 3 woody genera to be greater at 0 or 2 kg lime m^{-3} bark compared to higher lime rates. This growth response was attributed in part to a relatively high NH_4-N to NO_3-N ratio at the lower lime rates. Thus it appears that the culture of some woody plants benefit from the control of nitrification. Additionally, an umlimed medium would have a lower pH and hence a greater cation availability as demonstrated in chapter 1.

Results of chapter 3 show that the concentration of NO_3-N found in the "soil" solution was positively related to the concentration of the applied NH_4-N. In general, there were no differences in NAR between the 100 ppm and 200 ppm NH_4-N treatments yet this finding only reflected nitrification during a 96 hr period following fertilization. Data in chapter 2 as well as in this chapter implies that following this 96 hr period, the NH_4-N supply of the 100 ppm NH_4-N

treatment was limiting to nitrifiers. "Soil" solution pH is inversely related to the NH_4-N application rate. The lower pH of the 200 ppm NH_4-N treatment most likely inhibited nitrification, however, the extent of this inhibition is unknown. "Soil" solution and NAR data indicated that nitrification was greatest in the 20° to 30° C range (chapter 4). Considerable nitrification occurred at 10° while nitrifier activity was relatively low or not found at 40°. Thus a plant species known to be susceptible to ammonium toxicity should not be fertilized with NH_4-N when temperatures are less than 10° or exceed 30°.

This research was comprised of a series of experiments each of which investigated the influence of a single variable over time. This approach was taken to describe the general effects of each variable since such information has not been documented. Within each experiment the "soil" solution N and pH status were generally in a state of flux. Additionally, plant uptake of N increased over time since plant size increased over time. Thus, many factors interact at any given time and collectively influence nitrification. In this view, the "soil" solution of container-grown crops is a dynamic environment for plant roots and indicates the necessity for periodic soil testing to determine the supply capacity of the "soil" solution.

LITERATURE CITED

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