Influence of Nitrogen and Phosphorus Fertility on Cold Acclimation of Roots and Stems of Two Container-Grown Woody Plant Species[1]

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Abstract. Nitrogen fertility levels during the summer and fall had little effect on cold acclimation of root or stem tissues of container-grown plants of *Forsythia intermedia* Zab. cv. Lynwood by mid-November 1969. Tissue N levels of roots decreased from August 1 to September 3, but had increased again on October 30. Nitrogen fertilization rates affected tissue levels of P and K in *Forsythia* roots. Nitrogen and P levels that resulted in acceptable plants had limited effect on cold acclimation of *Cornus alba* L. var. *sibirica* Loud and *Forsythia* roots and stems in late October or early November 1970. Increased N and P fertilization affected tissue levels of N, P, K, Ca, and Mg.

Traditional practices for limiting N fertilization during the late summer and early fall to enhance the cold acclimation of woody plants are not supported by adequate research. An earlier study showed that 2 levels of N fertilizer applied in early September to container-grown plants of *Juniperus chinensis* cv. Hetzi did not result in differential cold acclimation (7). Coultas observed that winter injury of juniper and arborvitae was not necessarily greater at increasing N levels alone, but showed statistical interactions in responses to N-P-K treatments[3]. High N treatments resulting in 25 to 50% higher total leaf and stem N in some cases showed less winter injury than low N treatments. Meyer noted that high N rates of fertilizer resulted in increased winter injury to *Forsythia* when associated with high P levels[4].

Fall N fertilization has been observed by several workers to inhibit cold acclimation[5] (1, 2, 9). Many studies have failed to define or control the nutritional and moisture environment in which N has been observed to limit or delay cold acclimation.

Our objectives were: 1) to determine whether plants grown at several N levels through the summer and fall under ideal soil moisture and P and K fertility conditions differ in cold acclimation, and 2) to determine effect of N and P fertilization levels on cold acclimation and on tissue levels of N, P, and K for roots and stems of 2 woody plant species that differ in fall growth cessation.

Materials and Methods

Rooted cuttings of *Forsythia intermedia* Zab. cv. Lynwood were potted May 1969 in a mixture of loamy sand, sphagnum peat moss, and horticultural grade perlite of 1:1:1 (v/v/v). Each bushel of growing medium was supplemented with 103 g of 20% superphosphate and 206 g of dolomitic limestone. Ten uniform plants in 2 quart plastic pots were randomly assigned to each of 16 experimental plots representing 4 blocks (locations) with 4 N treatments per block in a randomized block design. The 4 N treatments received approx 25, 100, 200, and 300 ppm N as $Ca(NO_3)_2$ at each watering.

Cold acclimation of *Forsythia* root and stem segments was determined on Nov. 12, 1969; percent tissue N of root and stem segments, on Aug. 1, Sept. 3, and Oct. 30; and total Ca, K, and P on Oct. 30. Two cm internodal stem segments cut from the middle third of the current season's growth and root segments, 2 cm long varying in diam from 2 to 3 mm, were used for analyses.

The samples were cut from 2 randomly selected plants from each experimental plot on each sampling date. Stem and root segments were cut from each plant, each pooled, and randomly assigned to tissue analysis or temp exposures for determination of cold hardiness.

To determine hardiness, root and stem segments were frozen in test tubes submerged in an ethylene glycol bath in a Revco Model SZR-509 low temp chest. Test tubes with root or stem segments from each experimental plot were placed at 1°C for 2 hr prior to freezing. Temperature of the chest was lowered at rates not exceeding 5°C per hr for stem and 3°C for root tissues. Stem pieces were frozen at 7 temp ranging from -21.1 to -30.6° and roots were exposed to 5 temp between -9.4 and -15.9°. Tissues were thawed at 4°; stem tissues were incubated under intermittent mist in the greenhouse for 4 weeks. Roots were incubated in test tubes covered with polyethylene at room temp for 1 week. Tissue pieces were split longitudinally and browning was rated as 1) dead, 2) some injury, and 3) no apparent injury.

Total N determinations were made using the *micro-Kjeldahl* method (11). Samples were oven-dried for 48 hr at 70°C and ground in a Wiley mill using #20 mesh screen. Tissue P was determined colorimetrically (6). Calcium and K were determined by a Perkin-Elmer 303 atomic absorption spectrophotometer (8).

Rooted cuttings for *Forsythia* and *Cornus alba* L. var. *sibirica* Loud were potted June 25, 1970 in the same soil mixture as in 1969, but amended with dolomitic limestone at the rate of 150 g/bu. The basic design was a factorial arrangement of treatments in randomized block. Nine uniform plants were randomly assigned to each of 24 experimental plots. These represented 4 blocks (locations) of all possible combinations of 2 N (N_1, N_2) and 3 P (P_1, P_2, P_3) fertility levels as 6 treatments. Phosphorus was applied at the rate of 0, ½, and 2 tsp of 20% superphosphate to the top of the soil in each 2 quart plastic pot on July 1, July 30, Aug. 18, and Sept. 21. The 2 levels of N were maintained by applying $Ca(NO_3)_2$ at each watering with approx 25 and 200 ppm N. Potassium as KCl was supplied to all pots at each watering with 200 ppm K. Potted plants were grown under natural outdoor conditions as in 1969.

Cornus stems were sampled Oct. 26 and roots on Nov. 17. *Forsythia* stems were sampled on Nov. 9; and roots, on Nov. 11.

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[3]Coultas, C. L. 1965. The influence of fertilizers on the nutrition and performance of certain container grown evergreens. Ph.D. Thesis, University of Minnesota, St. Paul.

[4]Meyer, M. M., Jr. 1965. The influence of nutrition and root temperature on the dormant season nutrient content and spring growth of *Taxus media* and *Forsythia intermedia*. Ph.D. Thesis, Cornell University, Ithaca, New York.

[5]Way, R. D. 1953. Orchard factors affecting winter hardiness in apple tissue. Ph.D. Thesis, Cornell University, Ithaca, New York.

Samples were prepared for determination of cold hardiness and tissue mineral levels as in 1969. Low temp exposures are given in Table 3. Tissue levels of total N, P, K, Ca, and Mg, were determined. *Forsythia* shoot measurements were made Nov. 3 as total length of all shoots on each of 5 plants in each experimental plot.

Analysis of variance and Duncan's multiple range test (12) were used to determine statistical significance of all treatment differences for tissue mineral levels in both studies. For injury ratings as a measure of cold acclimation, Friedman's 2-way analysis of variance (12) was used as a nonparametric procedure to determine statistical significance of differences for all treatments in both studies.

Results

Forsythia at the 25 ppm N treatment in 1969 were less vigorous, had chlorotic foliage, and abscised most of their leaves earlier in the fall than plants under higher N levels. No differences in size and foliage color were readily discernible among plants in the 100, 200, and 300 ppm N treatments. Plant ht varied from 75 to 120 cm on Sept. 3.

Cold acclimation, 1969. Cold hardiness was determined on Nov. 12, a date selected to represent a period of rapid cold acclimation near the midpoint in hardiness development. Stems were not injured at -24.1°C, but at the 3 lower temp, means of injury ratings decreased with increased N fertility (Table 1). Ratings of stem injury after -26.0, -27.4, and -29.2° and root injury after -11.1, -12.8, and -14.4° exposures were not significantly different among treatments at the .05 level using Friedman's 2-way analysis of variance (12).

If injury rating means at each temp were ranked 1 to 4 and the ranks summed across temp, rank totals for stems and roots were progressively lower at increased N fertility levels (Table 1). At any temp, increased N fertility resulted in mean injury differences that were nearly equal to ratings caused by temp differences of about 1.4 to 2°C.

Table 1. Injury ratings[z] of *Forsythia* stems and roots after exposure to low temp, Nov. 12, 1969.

N	Stems				Roots				
treatment	Low temp exposures °C							Sums of ranks[y]	
ppm	-24.1	-26.0	-27.4	-29.2	-11.1	-12.8	-14.4	Stem	Roots
25	3.00	2.63	2.00	1.88	2.75	2.00	1.25	14.0	10.5
100	3.00	2.63	1.63	1.50	2.50	1.38	1.60	12.0	9.0
200	3.00	2.00	1.38	1.38	1.38	1.50	1.25	8.0	6.5
300	3.00	2.00	1.25	1.25	2.00	1.25	1.00	6.5	4.0

[z]Injury rating where 1 = dead, 2 = some injury, and 3 = no injury. Means are of 4 replicates with 2 samples per temp exposure. Means are not significantly different at the .10 probability level at any temp as determined by Friedman's 2-way analysis of variance.

[y]Sums of treatment rank over all temp where treatment means were ranked from lowest to highest at each temp. Chi-square = 6.00 for stems and 5.01 for roots where $P_{.20}$ = 4.64 and $P_{.10}$ = 6.25 with 3 degrees of freedom.

Main effects of N fertilizer levels and temp exposures on injury ratings of stems were compared. Injury ratings of each replicate were summed across all temp for each level of N (data not shown), the sums ranked 1 to 4 across N levels, and the ranks summed at each N level to determine the main effect of N. The differences of sums of injury ratings due to N treatment were not statistically significant at the .05 level using Friedman's 2-way analysis of variance. Similarly, main effects of temp on injury ratings resulted in significance at the .05 level. Hence, injury rating differences due to temp exposures were greater than differences due to N treatment.

Tissue mineral levels, 1969. Total tissue N measured on Aug. 1, Sept. 3, and Oct. 30 allowed comparisons of changes from the period of active growth through cold acclimation (Fig. 1). Total N was highest in roots and stems at the 200 ppm N level on Aug. 1, although not significantly different than the 300 ppm N treatment. The 300 ppm N treatment resulted in equal or higher total N on the later sampling dates.

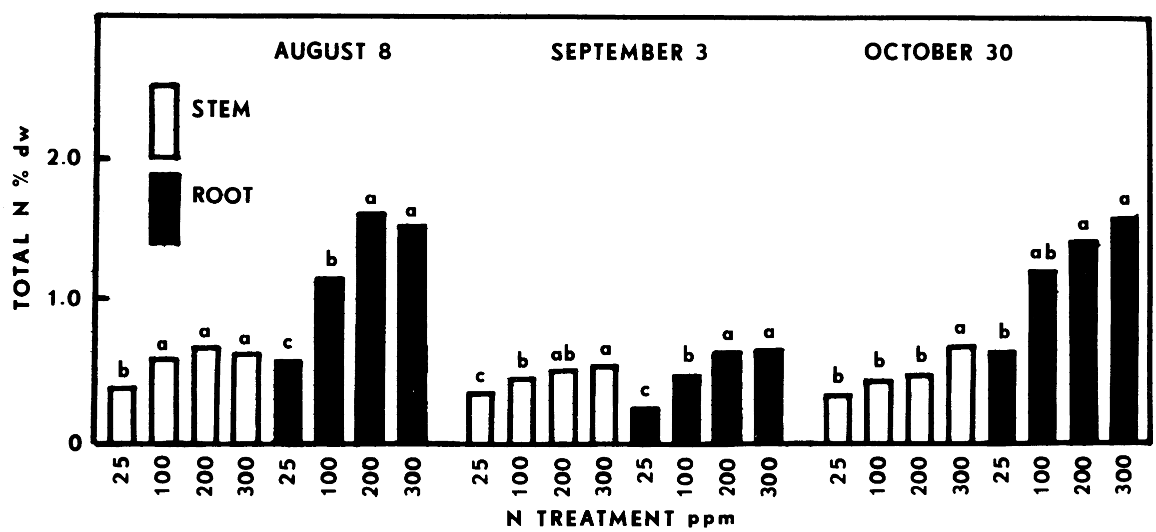

Fig. 1. Relation of N fertility to *Forsythia* tissue levels of N. Means are of 4 replicates. Bars for the same tissue with different letters are significantly different at the .05 probability level using Duncan's multiple range test.

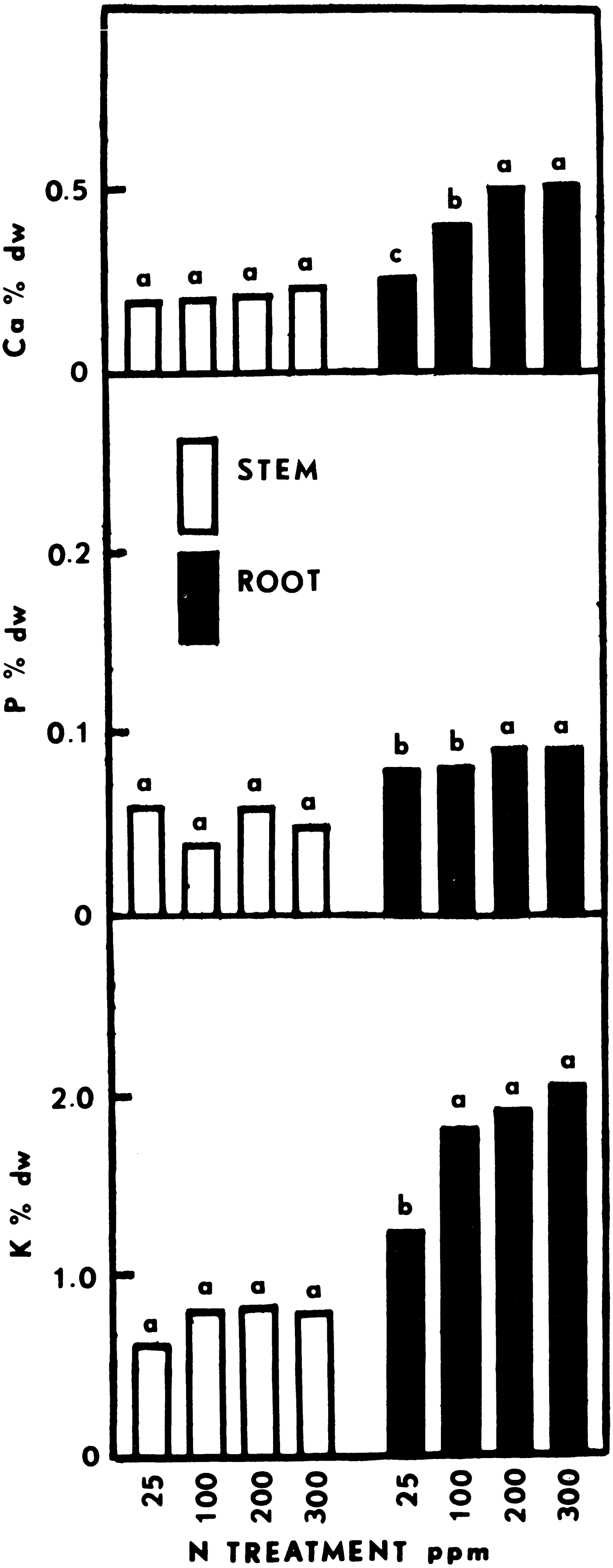

Fig. 2. Relation of N fertility to *Forsythia* tissue levels of Ca, P and K on Oct. 30, 1969. Means are of 4 replicates. Bars for the same tissue with different letters are significantly different at the .05 probability level using Duncan's multiple range test.

Table 2. Total length of stems (cm) of *Forsythia* as influenced by N and P fertilization, Nov. 3, 1970.[z]

N levels	P levels: P_1	P_2	P_3	$\bar{N}$
N_1	68.05b	76.65b	174.15a	106.27a
N_2	43.10b	193.35a	216.53a	150.99a
$\bar{P}$	55.58	135.00	195.34	

[z]Means are of 4 replicates, 5 plants per replicate. Means followed by the same letter are not significantly different at the .05 probability level, using Duncan's multiple range test.

Total N of roots considerably decreased at all N treatments between Aug. 1 and Sept. 3 (Fig. 1). The decrease was less pronounced in stems. The observations were made during a period with the onset of physiological rest, leaf abscission, cold acclimation, and reduction of growth rate for some plants. However, *Forsythia* at the 3 highest N levels appeared to grow actively until frost injured the foliage. Total N in roots decreased from Aug. 1 to Sept. 3, but increased by Oct. 30 (Fig. 1).

Tissue P and K were increased at higher N fertility levels in roots, but not stems, on Oct. 30 (Fig. 2). Increased Ca differences at higher N levels may have come from $Ca(NO_3)_2$ which was used as a source of N fertilizer (Fig. 2). Ca has been reported to increase hardiness (4) and may have had a confounding influence on the effect of N treatments. However, all levels of Ca were considered to be above those necessary for max growth and development, and no statistically significant differences occurred in stem Ca where hardiness differences among N levels were the greatest (Table 1).

Table 3. Injury ratings[z] of *Cornus* and *Forsythia* stems and roots after exposure to low temp °C, 1970.

Cornus	Stems, Oct. 26						Roots, Nov. 17		
Fertilizer treatment	-34°	-37°	-39°	-42°	-49°	Sum of ranks[y]	-7°	-10°	Sum of ranks[y]
$N_1 P_1$	3.00	2.63	2.13	1.63	1.38	22.0	1.25	1.50	7.0
$N_2 P_1$	2.50	2.88	2.25	1.88	1.68	26.0	1.38	1.25	9.0
$N_1 P_2$	2.25	2.38	2.00	1.50	1.25	11.0	1.50	1.13	9.5
$N_2 P_2$	2.75	2.00	1.88	1.63	1.50	17.0	1.38	1.13	7.5
$N_1 P_3$	2.67	2.38	2.00	1.75	1.50	20.0	1.38	1.00	5.5
$N_2 P_3$	2.67	1.75	1.38	1.25	1.38	9.0	1.32	1.00	3.5
Probability level[x]	.30	.10	.50	.50	.80	.05	.30	.99	.80

Forsythia	Stems, Nov. 9					Roots, Nov. 11			
	-14°	-16°	-19°	-22°	Sum of ranks[y]	-4°	-7°	-10°	Sum of ranks[y]
$N_1 P_1$	2.08	1.42	1.18	1.42	8.5	1.25	1.00	1.41	9.0
$N_2 P_1$	2.25	1.50	1.33	1.08	9.5	1.25	1.17	1.00	6.5
$N_1 P_2$	2.50	1.92	1.67	1.34	21.0	1.67	1.42	1.25	14.5
$N_2 P_2$	2.56	2.00	1.57	1.78	21.5	1.67	1.08	1.50	12.5
$N_1 P_3$	2.25	1.84	1.18	1.25	12.0	1.25	1.25	1.00	7.5
$N_2 P_3$	2.20	1.78	1.57	1.22	11.5	1.33	1.30	1.33	13.5
Probability level[x]	.50	.90	.50	.10	.20	.20	.50	.20	.30

[z]Injury ratings are means of 4 replicates where 1 = dead, 2 = some injury, 3 = no injury.

[y]Sum of treatment ranks over all temp where treatment means are ranked from lowest to highest at each temp and summed across temp.

[x]Probability of a greater chi-square for differences among treatment means at a temp and sum of ranks as determined by the Friedman's 2-way analysis of variance.

Increasing fertility levels of N and P in 1970 resulted in significantly greater total length of *Forsythia* stems (Table 2). *Cornus* and *Forsythia* plants at the low P level were stunted and unsatisfactory in appearance. The 6 treatments representing 2 N and 3 P levels did not result in significant differences in injury ratings of *Forsythia* and *Cornus* roots and stems at any given temp (Table 3). If means of injury ratings for each temp exposure were ranked 1 to 6, from lowest to highest, and the ranks summed across temp, significant differences occurred between treatment on *Cornus* stems. Similar, but nonsignificant differences occurred with *Forsythia* stems.

Injury differences from fertilizer treatment were not consistent from temp to temp, but P levels evidently influenced cold acclimation. When comparing sums of ranks across temp for *Cornus* stems, treatments with no P (P_1) had higher rankings (greater hardiness) than the 2 higher P levels (Table 3). *Forsythia* stems had the highest hardiness rankings for the intermediate P (P_2) level.

There was little evidence that N levels alone influenced hardiness, although the nonparametric statistical methods used did not separate N and P effects (Table 3). The higher N treatment resulted in lower injury ratings (more injury) of *Cornus* stems at the P_3 fertility level for 4 of the 5 low temp exposures, but had higher ratings at P_1 for 4 of the 5 temp (Table 3). The same trends were noted for *Forsythia* stems at 3 of 4 exposure temp. Low P levels may have resulted in increased cold acclimation at high N fertility. High P levels may have resulted in less cold acclimation with high N fertility; however, sample size was limited and the differences may not be real.

Table 4. Treatment means of tissue mineral levels, Fall 1970.[z]

	Cornus						*Forsythia*					
	Stems			Roots			Stems			Roots		
N % dw	P_1	P_2	P_3	P_1	P_2	P_3	P_1	P_2	P_3	P_1	P_2	P_3
N_1	.84bc	.76d	.78cd	1.80b	1.29c	1.34c	.57b	.48c	.51c	.88d	.44e	.54e
N_2	.91ab	.80cd	.94a	2.71a	2.77a	2.58a	.69a	.67a	.74a	1.28c	2.21b	2.45a
P % dw												
N_1	.090e	.153d	.170b	.140d	.340c	.533b	.083d	.080e	.172a	.087c	.121c	.398b
N_2	.080f	.157c	.180a	.120d	.380c	.644a	.072f	.097c	.167b	.065c	.146c	.497a
K ppm												
N_1	3217a	3019a	3056a	10831e	14725b	15392a	7483d	7413e	9606a	16325d	16219e	22031b
N_2	2987a	2713b	2825a	6444f	11194d	11883c	6702f	7500c	8958b	14638f	16656c	23125a
Mg ppm												
N_1	592bc	622ab	632ab	1700b	2881a	3091a	396ab	410ab	366b	1013c	1053bc	1532a
N_2	566c	659a	654a	1622b	2500b	2329ab	393ab	434ab	550a	1050bc	1447ab	1471ab
Ca ppm												
N_1	4169e	5144a	3931f	5300f	5750e	7300b	2322c	2375c	3038a	2737f	2906d	6600b
N_2	4327d	5100b	4650c	5925d	6363c	8967a	2642bc	2683b	3100a	2894e	5175c	8700a

[z]Means of 4 replicates. Means for each mineral for the 6 treatments followed by the same letter are not significantly different at the .05 probability level using Duncan's multiple range test.

Differences in injury rating means caused by N and P fertility were not greater than injury rating differences at 2 to 3°C temp differences (Table 3).

Forsythia roots showed more injury in 1970 at temp of -4 and -7°C than they did on approx the same date in 1969 when frozen at -11.1° (Tables 1, 3). The average daily temp in October was approx 2.6°C (4.7°F) lower in 1969 than in 1970. Lower soil temp may have resulted in more rapid cold acclimation in 1969.

The high N treatment in 1970 increased N in *Cornus* and *Forsythia* roots and stems (Table 4). Roots of both plants had higher N than stems, as reported for *Forsythia* during the fall (5). Increased P fertilization tended to decrease N in root tissues at the N_1 level. Increasing P fertility resulted in higher N in *Forsythia* roots at the N_2 level, but not in *Forsythia* stems or *Cornus* stems or roots.

The higher N fertilization resulted in higher tissue P in *Cornus* and *Forsythia* roots at the highest P fertility level (Table 4). P levels for *Cornus* and *Forsythia* roots appeared to respond directly to the amount of P applied.

The N_2 level decreased tissue K at all P fertility levels for *Cornus* stems and roots (Table 4). Tissue K increased between P_2 and P_3 at the N_1 level and between all P levels at the N_2 level for *Forsythia* stems and roots. Potassium increased between P_1 and P_2 at both N levels for *Cornus* roots, but no significant difference was noted between P levels for stems (Table 4).

Increasing P fertility resulted in higher tissue Mg at both N levels for *Cornus* stems and roots and *Forsythia* roots (Table 4). Nitrogen levels had little effect on tissue Mg. Tissue Ca increased in response to N levels for *Cornus* roots and *Forsythia* roots and stems (Table 4), perhaps because $Ca(NO_3)_2$ was the source of N fertilization. Calcium increased in *Cornus* roots and *Forsythia* roots and stems at both N levels in response to increasing levels of P. Calcium carbonate is present in superphosphate fertilizer used as a source of P. Increased P treatment may have increased Ca availability.

Discussion

Forsythia grew more actively in late summer and fall and retained its foliage longer than *Cornus.* Fertilizer treatments caused substantial growth differences of *Forsythia* stems in 1970.

Winter injury caused by late summer-early fall fertilization is usually attributed to late growth slowing cold acclimation (13). Measurements of cold hardiness differences related to soil fertility are best expressed in mid-autumn when early stages of cold acclimation are most likely to be inhibited by late growth. Higher P and N fertility levels maintained through late summer and fall resulted in small or no significant inhibition in the cold acclimation of these plants as measured in mid-autumn. The low temp required to injure stems or roots on the sampling dates would be unlikely to occur at Burlington. Based on weather records, there is only a 10% probability of -6.7°C (20°F) or lower on Nov. 1 or -8.9°C (16°F) or lower on Nov. 10 in Burlington, Vermont (3). *Forsythia* stems showed no injury on Nov. 12, 1969, at any N fertility level when exposed to -24°C (Table 1).

Many species of woody plants tend to slow their growth rate in the summer under natural conditions as a response to moisture or nutrient limitation (10). Late summer N fertilization in presence of sufficient soil moisture may encourage vigorous growth, causing delay in cold acclimation (13). However, the nursery industry and certain other plant-growing enterprises try to maximize growth. Maximum growth usually depends on optimum fertility and moisture throughout the growth period.

The growth response of woody plants to increasing levels of nutrients during a single growing season may be limited (5). However, the availability and uptake of these nutrients during late summer and early fall has enhanced the subsequent spring growth of these plants, maximizing total growth. The increased N in *Cornus* and *Forsythia* roots caused by higher N application supplied a higher reserve which may have stimulated growth the following season. Low P levels resulted in lower N and K in *Forsythia* roots and lower K in *Cornus* roots in 1970. Proper balance of macroelements is desirable for maximizing uptake of nutrients and growth. High rates of fertilizer necessary for max growth may be of little practical importance in the cold acclimation of container-grown plants that are well within their hardiness limits. The inhibition of cold acclimation by only 2 to 3°C caused by high fertility may be of limited significance in production of most nursery plants under controlled conditions.

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Ammonium Reduction of Calcium and Magnesium Content of Tomato and Sweet Corn Leaf Tissue and Influence on Incidence of Blossom End Rot of Tomato Fruit[1]

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Abstract. Fertilization of tomato and sweetcorn seedlings with NH_4-N generally resulted in growth reduction as well as reduced contents of Ca and Mg in the tissues to deficient or nearly deficient levels. The effects were more pronounced on tomatoes than on corn. Fertilization of plants, with a combination of NO_3 + NH_4-N resulted in Ca and Mg levels in the tissue intermediate between that of plants receiving NH_4-N or NO_3-N. Ammonium N applied during fruiting of tomato plants resulted in rapid development of blossom-end rot of the fruit, probably due to the influence of NH_4-N on Ca uptake.

The form of N fertilizer has been shown to affect the chemical composition as well as growth and development of plants. Ammonium-N has resulted in lower levels of Ca and Mg (1, 2, 3, 5, 9, 10), and produced leaf and stem lesions (5, 13), reduced growth (1, 12, 13), and caused root injury (16). Apple fruits often show blemishes and reduced quality due to deficiencies in Ca (11). These changes in the Ca and Mg contents of the plant may have implications for animal health since Mg deficiency may result in grass tetany in ruminants or in Mg deficiency disease in humans (7).

This investigation was undertaken to study the effects of the source of N fertilization on the nutrient absorption by plants.

Materials and Methods

Seeds of tomato cv. Heizn 1370 were germinated in flats of vermiculite and seedlings were transplanted at the 2 leaf stage into 2 gal crocks containing "sand blast size" quarz sand. Sweetcorn cv. Seneca 110 was seeded directly into the quartz sand in 2 gal crocks and thinned to 1 plant per pot after emergence. The seedlings were cultured with 112 ppm N as NO_3 form in Hoagland's solution for 14 days prior to the initiation of N form treatments. Nitrogen was supplied at 112 ppm as NO_3, NH_4, or a combination of 56 ppm N each as NO_3 and NH_4 in a modified Hoagland's solution. The treatments were formulated as follows: 112 ppm N as NO_3 - M KH_2PO_4 1 ml, 0.5 M K_2SO_4 4 ml, M $Ca(NO_3)_2$ 4 ml, M $MgSO_4$ 2 ml; 112 ppm N as NH_4 - M KH_2PO_4 1 ml, 0.5 M K_2SO_4 4 ml. M $(NH_4)_2SO_4$ 4 ml, M $CaCl_2$ 4 ml, M $MgSO_4$ 2 ml; 56 ppm N as NO_3 + 56 ppm N as NH_4 - M KH_2PO_4 1 ml, 0.5 M K_2SO_4 4 ml, M $CaCl_2$ 4 ml, M NH_4NO_3 4 ml, M $MgSO_4$ 2 ml. The designated volumes of stock salt solutions were added per liter of solution. In the various solutions P, K, Ca, Mg, and minor elements were held constant and the chloride and sulfate concn were varied. The solutions were applied as a 1 liter drench every other day to completely renew the solution in the system. Each treatment was replicated 4 times. The tomato plants were harvested 15 days and the corn plants 21 days after start of treatment. Fresh wt were determined for the shoots and roots. The 2 topmost fully expanded leaves of the corn and tomato plants were dried in a forced air oven at 70°C and ground in a

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