Budset and Growth of Eastern White Pine Following Application of 6-Benzylaminopurine to Seedlings Fertilized with Different Levels of Nitrogen[1]

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Abstract

Eastern white pine (*Pinus strobus* L.) were potted and solution fed once weekly during 2 growing seasons with 5 levels of N in the irrigation water: 50, 100, 200, 300 and 400 ppm. Leaders were treated with 750 ppm 6-benzylaminopurine (BA) in late June of the first year. The higher N levels resulted in greater stem diameter, greater foliage dry weight, longer and heavier needle fascicles, better foliage color, greater budset after application of BA, and more and longer branches on the BA-treated leader the second growing season. BA should be applied to trees with N concentration ≥ 1.5% in one-year-old foliage.

Index words: Christmas trees, nutrition, cytokinin, nursery crops

Introduction

The cytokinin 6-benzylaminopurine (BA) induces fascicular buds in many conifers (1, 4, 7, 9, 13), and offers Christmas tree growers the potential to produce denser trees with less shearing. Factors influencing budset following treatment with BA include: application rate (2, 4, 7), time of application (2, 7), point of application (7), and type and concentration of additives and surfactants (7, 10).

Little is known about the effects of nutrition on budset following application of BA. Erratic field results in past years led us to suspect N nutrition as an important factor influencing budset after applications of BA. Good fertility produced greater budset by balsam fir (*Abies balsamea* L.) in response to BA (7). Growers need to know if there are nutritional standards to maintain for acceptable budset and growth when the chemical is applied. Our study was conducted to determine how N nutrition affects growth of eastern white pine and budset following application of BA.

Materials and Methods

On October 24, 1984, vigorous three-year-old (3-0) eastern white pine seedlings were harvested from nursery beds in the North Carolina Forest Service Nursery, Morganton. Plants were graded for uniformity, heeled into boxes containing soil, and taken to Raleigh the same day. The next day, lateral branches on the lower stem were removed, and roots pruned to fit 7-liter (2-gal) pots. Plants (n = 144) were potted into a medium of 3 composted pine bark:1 peat:1 sand (by vol) amended with 3.5, 1.3 and 2.9 kg/m^3 (6, 2.2 and 5 lb/yd^3), respectively, of dolomitic lime, gypsum and triple super phosphate (0N-20P-0K). Plants were watered immediately after potting, placed outdoors in a nursery production area, and watered thereafter as needed by means of an overhead irrigation system. Each pot was topdressed with 7 g (0.25 oz) of sulfur coated 14-14-14 (14N-6.4P-11.5K).

Plants were overwintered under frames covered by white polyfilm from December 1984 to March 1985. They were randomly assigned to 5 nitrogen treatments on April 17, 1985: 50, 100, 200, 300 and 400 ppm N (NH_4NO_3 source), applied as 800 ml solution once weekly. The solution also contained 50 ppm P and 150 ppm K, with pH adjusted to 5.5. Trace elements (Peters STEM, W. R. Grace Co., Fogelsville, PA) were applied once monthly as recommended by manufacturer. Pots were arranged in a randomized complete block design, and watered daily.

On June 25, 1985, the terminal leader of each plant was sprayed to runoff with a distilled water solution containing 750 ppm BA and 0.25% Buffer-X (Abbott Laboratories, North Chicago, IL). This concentration was used because it produced good fascicular budset in our previous experiments (unpublished). The chemical was applied with a hand spray bottle from a distance of about 5 cm (2 in). Lateral branches were shielded. Most fascicle sheaths were open at that time. Nontreated plants were deemed unnecessary because fascicular buds do not form on eastern white pine when the terminal and subterminal buds are present (1), regardless of nutrient status. The terminal and subterminal cluster of buds was removed from each leader on August 20, 1985 to encourage growth of fascicular buds already present on the leader. Fertilizer treatments were terminated on September 15, 1985.

Foliage samples for chemical analysis were taken on October 3, 1985 by collecting needles from each plant at the mid-section of current-year growth on lateral branches in the 1984 whorl. Foliage was dried to constant weight at 60°C (140°F) and ground to pass a 20-mesh screen. Total N was determined by a modified Micro-Kjehldahl procedure (11).

Plants were placed under white polyfilm in December 1985. Twelve plants were randomly selected from each treatment on March 17, 1986, and fascicular buds ≥ 1 mm in length were counted on the 1985 leader.

On April 2, 1986, remaining plants were repotted in 11-liter (3-gal) plastic pots containing a medium of 5 composted

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pine bark:1 sand (by vol) amended as previously described. Trace elements (Micromax, 14 g, 0.5 oz; Sierra Chemical Co., Milpitas, CA) were topdressed on each pot on April 6, 1986, and liquid fertilizer treatments used the previous year were initiated and continued at weekly intervals until October 30, 1986. Pots were watered as needed during the growing season.

On November 3, 1986, the number and length of branches on the 1985 leader was recorded for each plant. Final diameter of the central leader was measured 2 cm (0.8 in) above the 1984 branch whorl. Fifty current-year needle fascicles from the 1984 branch whorl were dried to constant weight at 60°C (140°F) to determine average fascicle weight, and 5 fascicles were measured for length. All current-year foliage (1986) was removed from each plant and dried to constant weight at 60°C (140°F), and analyzed for N as previously described.

Polynomial regressions (Table 1) were fitted to relate N status to the various growth parameters. A nonlinear S-shaped curve (14) was fitted to budset data using the 'NLIN' option of SAS (12). The independent variable in all equations was N concentration (ppm) in the nutrient solution.

Results and Discussion

Nitrogen levels accounted for about 60% of the variation in fascicular budset in 1985 (Table 1). Plants fertilized during the 1985 growing season with 50 and 100 ppm N averaged 22 to 25 fascicular buds on the leader (Fig. 1). Bud number increased sharply at 200 ppm, and reached a maximum of 96 buds at 300 ppm. Foliar N increased commensurate with applied N levels, with the maximum bud number at approximately 1.5%. Although only about half these buds resulted in branches during 1986 (Fig. 2), the influence of the N treatment on branch number was evident. For example, 36 branches developed on leaders of plants grown at 400 ppm N the previous year compared to only 11 at 50 ppm N. Branch length on the terminal leader averaged 41 mm (1.6 in) for the highest N level, compared to 22 mm (0.9 in) for the lowest (Fig. 2). Even though the relationship between N levels and branch number and length was significant (Table 1), there was great variation among individual plants within treatments.

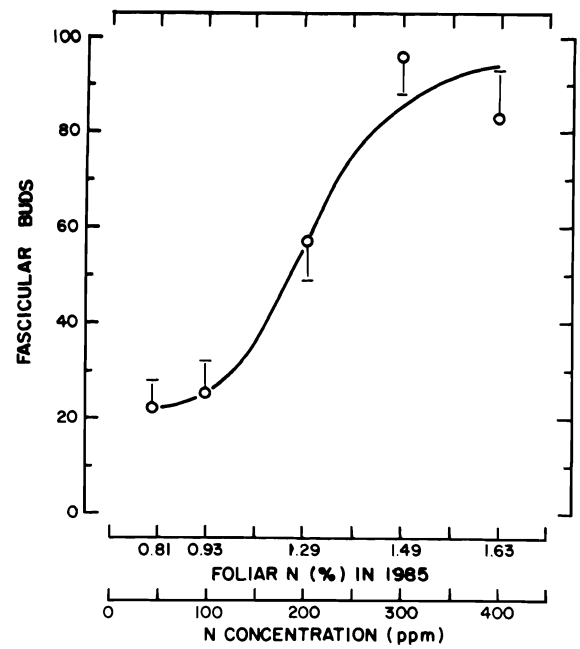

Fig. 1. Number of fascicular buds formed in 1985 on the leaders of potted eastern white pine fertilized with different levels of N. Plants received an early summer application of BA on the leader. Each point is mean ± s.e.; n = 12. Axis labelled "Foliar N (%) in 1985" for current-year foliage is not to scale.

Nitrogen accounted for 60 to 90% of the variation in stem diameter, total foliage dry weight, and average fascicle length and weight (Table 1). In December 1986, the diameter of the central leader for the 400 ppm N treatment was approximately 70% greater than for 50 ppm, and dry weight of all current-year foliage was 8 times greater (Fig. 3). Average length and dry weight of current-year fascicles increased sharply as N application rate increased to 200 ppm N (foliar N concentration = 1.3%), but increased less thereafter (Fig. 4). Plants which received 300 or 400 ppm N produced fascicles 92 to 93 mm (3.6 in) in length during 1986, compared to 58 mm (2.3 in) at 50 ppm N. Dry weight

Table 1. Regressions to estimate various plant dimensions following applications of 6-benzylaminopurine to plants grown at different nitrogen levels.

Parameter	Equation	R^2
YEAR 1985		
Fascicular buds on leader	$Y = 74\left(\frac{1}{0.0099923 - 9.0201190\ X}\right)^{393.24} + 22$	0.58**
YEAR 1986		
a) Number of branches on 1985 leader	$Y = 3.38 + 0.006623\ X$	0.20**
b) Average branch length on 1985 leader (mm)	$\ln(Y) = 3.09 + 0.001363\ X$	0.14**
c) Diameter of central leader (mm)	$Y = 2.82 + 0.004773\ X - 0.000005206\ X^2$	0.62**
d) Total dry weight of all current-year foliage (g)	$Y = 2.27 + 0.03235\ X - 0.00003560\ X^2$	0.89**
e) Average fascicle length (mm)	$Y = 6.95 + 0.0157\ X - 0.00002272\ X^2$	0.73**
f) Average fascicle dry weight (mg)	$Y = 0.69 + 0.003604\ X - 0.000005712\ X^2$	0.65**

[z]The variable 'X' equals N concentration (ppm) in the nutrient solution; the variable 'Y' is the specified parameter; n = 81; ** significant at $P \leq 0.01$.

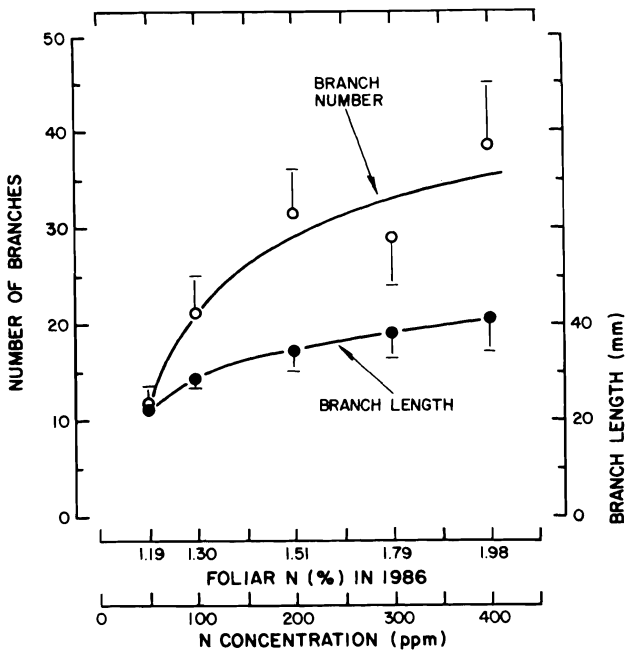

Fig. 2. Average number and length of branches in Nov. 1986 on the leader of eastern white pine fertilized with different levels of N. Branches were derived from fascicular buds which were formed on the leader in 1985 after an early summer application of BA. Each point is mean ± s.e.; n = 16. Axis labelled "Foliar N (%) in 1986" for current-year foliage is not to scale.

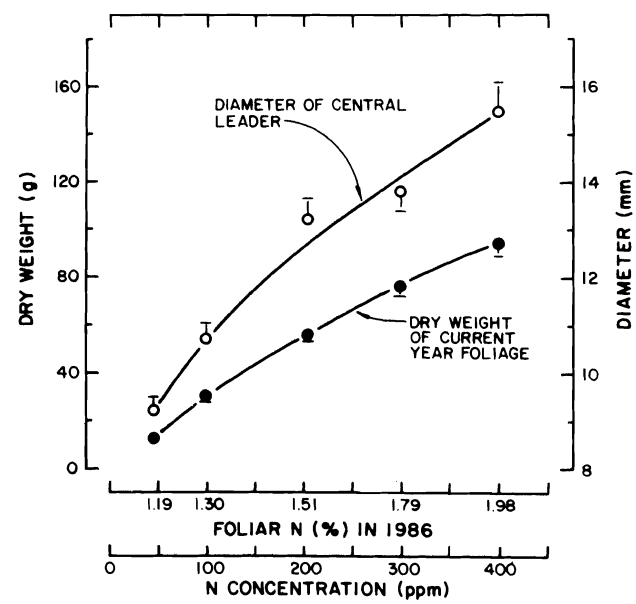

Fig. 3. Stem diameter of central leader and total dry weight of all current-year foliage for potted eastern white pine fertilized with different levels of N; measured in Nov. 1986. Each point is mean ± s.e.; n = 16. Axis labelled "Foliar N (%) in 1986" for current-year foliage is not to scale.

of individual fascicles in December 1986 was 56 to 63 mg at N levels ≥ 200 ppm, compared to 28 mg at 50 ppm.

Qualitatively, plants grown at 50 ppm N (foliar N concentration = 1.2% in 1986) were of poor vigor and chlorotic. They had abnormally short needles, and minimal branch elongation, which gave branches a tufted appearance. Fo-

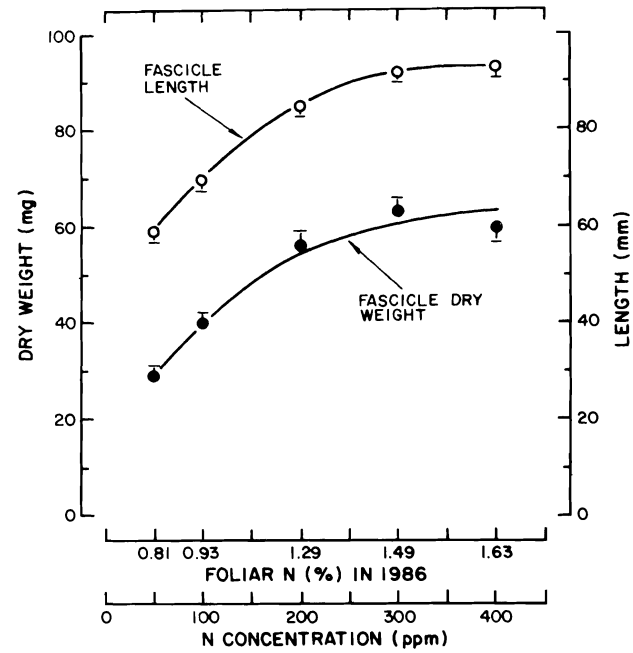

Fig. 4. Average length and dry weight of current-year fascicles for eastern white pine fertilized with different levels of N; measured in Nov. 1986. Each point is mean ± s.e.; n = 16. Axis labelled "Foliar N (%) in 1986" for current-year foliage is not to scale.

liage color was marginal at 200 ppm N (foliar N concentration = 1.5% in 1986) even though growth was relatively good. Plants which received 300 and 400 ppm N (foliar N ≥ 1.8%) had healthy green color as well as good vigor.

Increasing N application rates resulted in more foliage per plant, longer and heavier needle fascicles, greater stem diameter of the central leader, better foliage color, and greater budset following applications of BA (Figs. 1, 3, 4). More branches emerged the following year, and were of greater length, compared to N-deficient plants (Fig. 2).

Nutrition, especially N status, is perhaps the single most important factor in control of apical dominance (3). Good fertility generally results in larger buds, compared to poor fertility. In a species such as white pine, which normally produces one flush of growth per year, final shoot length is largely a function of initial bud size (6). Better nutrition, and consequently greater vigor, reduces correlative inhibition, which enables lateral branches to achieve greater length (3, 5, 6, 8). In this experiment, the absence of plants not treated with BA in each N treatment does not alter the conclusion that budset was affected by nutrition. The terminal bud cluster was removed late in the 1985 growing season and excluded from final counts. If it had been removed in late June, fascicular buds would have developed on the leader, even if BA had not been applied. Shearing during late June is customary for white pine Christmas trees in North Carolina. However, in August 1985 when the terminal cluster was removed, it was too late to induce fascicular buds. Therefore, fascicula buds that formed on the leader earlier in the summer while the terminal and subterminal buds were present, represented a response to BA. Our results confirm a strong relationship between BA-induced bud development and tissue N levels of white pine. Similar observations have been made for balsam fir under good versus poor fertility (7).

Significance to the Nursery Industry

BA is a potentially useful tool for increasing the density of white pine Christmas trees. In addition to the obvious effects of better growth and appearance, good fertility results in greater production of fascicular buds and branches following applications of BA. Growers should ensure that the N status of trees is adequate before attempting to increase the number of growing points with an application of BA.

Literature Cited

1. Cohen, M.A. 1978. Shoot apex development and rooting of *Pinus strobus* L. by dwarf shoots. J. Amer. Soc. Hort. Sci. 103:483–484.

2. Cohen, M.A. and J. Shanks. 1975. Effect of N^6-BA, GA_3, and removal of terminal buds on dwarf shoot development in *Pinus ponderosa*. J. Amer. Soc. Hort. Sci. 100:404–406.

3. Gregory, F.G. and J.A. Veale. 1957. A reassessment of the problem of apical dominance. Symp. Soc. Exp. Biol. 11:1–20.

4. Kossuth, S.V. 1978. Induction of fascicular bud development in *Pinus sylvestris* L. HortScience 13:174–176.

5. Kozlowski, T.T. 1973. Predictability of shoot length from bud size in *Pinus resinosa* Ait. Can. J. For. Res. 3:34–38.

6. Little, C.H.A. 1970. Apical dominance in long shoots of white pine (*Pinus strobus*). Can. J. Bot. 48:239–253.

7. Little, C.H.A. 1984. Promoting bud development in balsam fir Christmas trees with 6-benzylaminopurine. Can. J. For. Res. 14:447–451.

8. McIntyre, G.I. 1968. Nutritional control of the correlative inhibition between lateral shoots in the flax seedling (*Linum usitatissimum*). Can. J. Bot. 46:147–155.

9. Mulgrew, S.M. and D.J. Williams. 1985. Effect of benzyladenine on the promotion of bud development and branching of *Picea pungens*. HortScience 20:380–381.

10. Nauer, E.M. and S.B. Boswell. 1981. Stimulating growth of quiescent citrus buds with 6-benzylaminopurine. HortScience 16:162–163.

11. Peterson, H.C. and G. Chester. 1964. A reliable total nitrogen determination of plant tissue accumulating nitrate nitrogen. Agron. J. 56:89–90.

12. SAS Institute Inc. 1982. SAS user's guide: statistics. SAS Institute, Cary, N.C.

13. Stiff, C.M. and A.A. Boe. 1985. Effects of foliar-applied benzylaminopurine on fascicular bud development in Mugo pine. HortScience 20:285–287.

14. Van Genuchten, M.T. 1980. A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. J. Soil Sci. Soc. Am. 44:892–898.

Micropropagation of Flame Azalea[1]

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Abstract

Shoots tips excised from an actively growing stock plant of flame azalea [*Rhododendron calendulaceum* (Michx.) Torr.] were surface sterilized, the terminal portions were removed (decapitated) and the shoots placed horizontally on agar-solidified Woody Plant Medium (WPM) supplemented with 15 ppm 6-(γ, γ-dimethylallylamino)-purine (2iP). Within 4 to 6 months multiple shoot formation commenced. After 2 to 3 additional months of growth, axillary shoots were excised from the original explants. The shoots were decapitated and placed on WPM. After 2 subcultures, 8-node axillary shoots were excised, decapitated and cultured on agar-solidified WPM supplemented with 0, 4, 8, 12, 16, 24, and 32 ppm 2iP. The greatest number of shoots (microcuttings) ≥ 5 mm (0.2 in) were produced at 12 ppm 2iP. Microcuttings ≥ 10 mm (0.4 in) were rooted using *ex vitro* procedures. Enhancement of both axillary shoot multiplication and shoot length was achieved by addition to the medium of 80 ppm adenine sulfate and 200 ppm NaH_2PO_4.

Index words: *in vitro* propagation, tissue culture, *Rhododendron calendulaceum*

Introduction

Flame azalea [*Rhododendron calendulaceum* (Michx.) Torr.] occurs naturally in the Appalachian region of the United States, extending from southwestern Pennsylvania and Ohio to northern Georgia (7,8). It blooms in late spring and is regarded as one of the most striking, native flowering shrubs with flower color ranging from orange-yellow to scarlet (8). The tremendous floral display coupled with the diversity of flower color provides incentives for selection and propagation of superior forms. However, before the horticultural virtues of this species can be exploited, techniques must be developed for vegetative propagation of desirable clones. One of the main reasons why this plant is not widely utilized is due to the lack of suitable procedures for asexual propagation.

One technique for vegetative propagation might involve the use of rooting stem cuttings. This would seem like a logical approach because stem cuttings of many *Rhododendron* species can be rooted without much difficulty. Unfortunately, stem cuttings of flame azalea, particularly those taken from plants in the adult growth phase, are extremely difficult to root (13,14). Pronounced clonal variation in the rooting response also places severe limitations on cuttings as a means to propagate selected clones (12,14).

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Corrigenda

In the article, "Budset and growth of eastern white pine following application of 6-benzylaminopurine to seedlings fertilized with different levels of nitrogen", by L. Eric Hinesley and Robert D. Wright (J. Environ. Hort. 6(2):42–45, June 1988), Tables 1 and Figure 4 were incorrect. The corrected Table 1 and Figure 4 with caption are printed below.

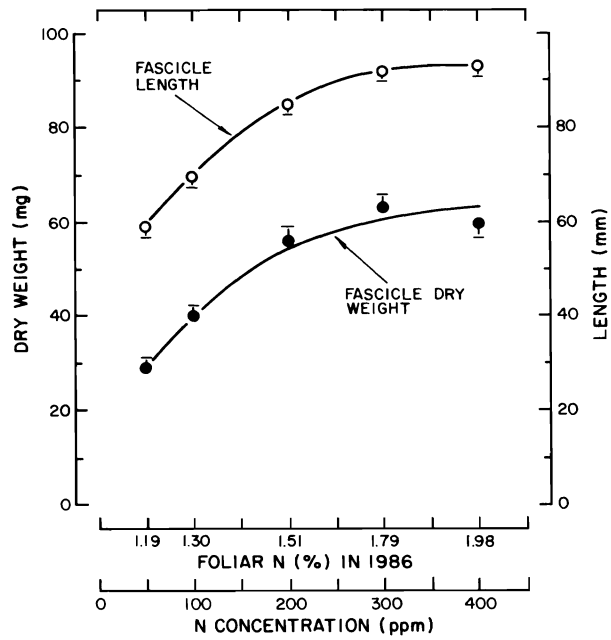

Fig. 4. Average length and dry weight of current-year fascicles for eastern white pine fertilized with different levels of N; measured in Nov. 1986. Each point is mean + s.e.; n = 16. Axis labelled "Foliar N (%) in 1986" for current-year foliage is not to scale.

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b) Average branch length on 1985 leader (mm)	$\ln(Y) = 3.09 + 0.0014\ X$	0.14**
c) Diameter of central leader (mm)	$\sqrt{Y} = 2.82 + 0.0048\ X - 0.0000052\ X^2$	0.62**
d) Total dry weight of all current-year foliage (g)	$\sqrt{Y} = 2.27 + 0.032\ X - 0.000036\ X^2$	0.89**
e) Average fascicle length (mm)	$\sqrt{Y} = 6.95 + 0.016\ X - 0.000023\ X^2$	0.73**
f) Average fascicle dry weight (mg)	$\sqrt{Y} = 4.36 + 0.023\ X - 0.000036\ X^2$	0.65**

[2]The variable 'X' equals N concentration (ppm) in the nutrient solution; the variable 'Y' is the specified parameter; n = 81; **significant at $P \leq 0.01$.