# Intermittent Short Days and Chilling, and Benzylaminopurine Affect the Growth and Morphology of Fraser fir Seedlings[1]

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## Abstract

Fraser fir (*Abies fraseri* (Pursh.) Poir.) seedlings were either grown continuously under long days (16 hr) or intermittently exposed to short days (8 hr) to induce bud set followed by a chilling period. Additionally, half the seedlings in each treatment received a foliar application of 444 uM benzylaminopurine (BAP). Seedlings that were allowed to set buds, followed by chilling, obtained the greatest height, but this treatment in combination with BAP reduced seedling height. BAP applied to seedlings grown continuously under long days stimulated height growth and prevented long dormant periods typical for non-chilled seedlings. Continuously grown, BAP-treated seedlings had 1.3 times larger root collar diameter, and 1.7 times more biomass, compared to other treatments. Root/shoot ratios for continuously grown, BAP-treated seedlings were comparable to intermittently chilled, non-BAP treated seedlings. These results suggest that either intermittent short days followed by chilling, or foliar BAP applications can be used to accelerate growth of containerized Fraser fir seedlings. However, the combination of chilling and BAP resulted in poorer development than either treatment alone.

**Index words:** Christmas trees; benzyladenine; growth regulators; chilling

**Species used in this study:** Fraser fir (*Abies fraseri* (Pursh.) Poir.)



### Significance to the Nursery Industry

Application of these findings to operational seedling production gives the grower of containerized greenhouse stock two potential options. If a grower can artificially chill seedlings during the warmer seasons, an accelerated production scheme could be employed as outlined in Table 1. Alternatively, foliar application of BAP to seedlings stimulates apical bud growth and circumvents dormant periods that are normally overcome by chilling. Regardless of which production system is adopted, either method produces a marketable seedling for the establishment of transplant beds.

### Introduction

Fraser fir (*Abies fraseri* (Pursh.) Poir.) is a valuable Christmas tree species produced primarily in Virginia, North Carolina, and Tennessee. Production of Fraser fir seedlings for plantation establishment and replacement of mature harvested trees is hampered by slow seedling growth. Nursery-grown seedlings require four to five years to obtain a reasonable size for plantation establishment.

Typically, Fraser fir seedlings kept under long photoperiods produce several short flushes of growth the first year after seed germination, followed by a period of dormancy and bud (apical) formation (5). This dormant state is normally broken by an exposure to cold temperatures (5, 9). Hinesley's (5) work outdoors, concluded that four to six weeks of artificial chilling was adequate to break Fraser fir seedling dormancy.

Alternative methods (ie. containerization and accelerating growth cycles) which would circumvent dormancy have been investigated by several researchers. Seiler and Kreh (9) demonstrated that growing containerized Fraser fir in a greenhouse with supplemental lighting, adequate water and nutrients, and fulfillment of the chilling requirement (using either natural or artificial conditions) following bud set, greatly reduced the time needed to produce commercially acceptable seedlings. Their study resulted in fifteen-month-old seedlings obtaining heights greater than four-year-old seedlings grown conventionally in outdoor beds. They also concluded that natural chilling resulted in greater stem elongation compared to artificial, but that both stimulated apical bud break.

Bryan et al. (2) successfully grew Fraser fir seedlings through several artificial cycles of long and short days to induce bud formation, followed by six weeks of artificial chilling (3°C (37°F)) to break dormancy. Foliar application of benzylaminopurine (BAP) prevented the long periods of dormancy and abnormal apical bud development typical of seedlings grown continuously under a 16-hour photoperiod without chilling (3). Seedlings treated with BAP exhibited almost continuous apical growth.

A growth comparison of seedlings intermittently placed on short days and chilled, with seedlings grown continuously on long days with BAP application, has not been conducted. It is not known how BAP-treated seedlings compare morphologically with seedlings allowed to set bud and flush following chilling. We hypothesized that the combination of short days, chilling, and BAP would result in growth superior to that produced by either treatment alone. Our objectives were to evaluate how the foliar application of BAP in combination with intermittent short days followed by chilling affects the growth of Fraser fir. Several morphological traits were examined to determine if this type of production produces seedlings that are commercially acceptable.

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## Materials and Methods

Seeds collected from Fraser fir trees in the Mount Rogers Recreational Area, VA (elevation 1746 m) were soaked in water for twenty-four hours and stratified at 4°C for thirty days to insure optimal germination (4). Stratified seeds were then sown (April 18, 1988) in flats filled with moistened sphagnum peat amended with 1.5 kg/m$^3$ (2.4 lb/yd$^3$) dolomitic limestone to adjust the pH (2). Seedlings emerged within fourteen days. Approximately forty-five days from sowing, 320 randomly selected seedlings (at the cotyledon stage of development) were transplanted into 160 cm$^3$ (9.7 in$^3$) super cell (Ray Leach Cone-tainer Nursery, Canby,OR) filled with the same planting medium described above.

Seedlings were randomly divided into two groups. Half were grown continuously in a greenhouse under high pressure sodium vapor lighting (150 umol/m$^2$/sec after sunset) to extend the photoperiod to 16-hours. Greenhouse temperatures were maintained at a minium of 20°C (68°F) and occasionally reached 35°C (95°F). Water was supplied daily to avoid moisture stress. Seedlings were fertilized once weekly with 10 ml of an aqueous solution containing a 0.5 g/L (.067 ounces/gallon) 20-20-20 (Peter's General Purpose, Fogelsville, Pa.) and 1.43 g/L (0.19 ounces/gallon) 21-7-7 (Peter's Azalea Neutral) to yield a final solution concentration of 400 ppm N, 134 ppm P, and 332 ppm K. The remaining seedlings were grown under the same environmental conditions and then subjected to a series of short days (to induce budset) followed by chilling (Table 1). Short days, (8-hour photoperiod), were achieved by moving the seedlings (within the greenhouse) to an area which was covered with a PAK Nolite Photo Period Fabric (A.H. Hummert Seed CO., St. Louis, Mo) for 16-hours daily to promote terminal bud development. Seedlings were subjected to an 8-hour photoperiod for thirty to forty days, based on development of the terminal bud. After this group of seedlings exhibited a well-developed terminal bud, they were chilled either by natural (outdoors in a lathe house) or artificial (cold room, 2°C (36°F)) conditions (depending on the time of year). Henceforth, this treatment will be referred to as "chilled" and the treatment of the first group (continuous long days), "non-chilled".

In combination with the above treatments, an aqueous solution of BAP (Product No. B-6750, Sigma Chemical Co., St. Louis, Mo.) was sprayed once monthly on all the foliage (until runoff occurred) of half the seedlings in each treatment. The concentration was 444 uM (100 ppm) with 0.1% Tween 80 (Fisher Scientific, Springfield, N.J.) as a surfactant (3). The remaining seedlings from each treatment were not treated. Previous research established the 444 uM concentration to be the best promoter of continual apical meristem growth and that Tween 80 in no way affected Fraser fir seedling growth (1, 3). BAP treatments began five weeks after initial seedling emergence and were discontinued on the chilled seedlings when they were placed on short days.

At the conclusion of the third growing cycle, when the chilled treatment no longer showed any active terminal growth, all treatments were measured and harvested. Root collar diameter (RCD), total height, number of primary and secondary lateral branches, and total number of buds were tabulated. Seedlings were then separated at the root collar and oven dried (60°C (140°F)) to a constant weight for dry matter determination.

The experiment was a randomized compete block consisting of five replications with a combination of two BAP treatments (0, and 444 uM) and the chilling treatments (chilled and non-chilled). Sixteen seedlings per treatment combination were sub-sampled and the average used as the experimental unit. Initial analysis of variance indicated that the interaction between the BAP and chilling treatment was significant for all variables ($p<.0005$). As a result, analysis of variance was performed across all treatments. The means of the four treatments were compared using Duncan's multiple range test (alpha = .05).

## Results and Discussion

Chilling, without BAP, resulted in the greatest height growth (Table 2). BAP, when applied to chilled seedlings, reduced height growth. It was hypothesized that this combined treatment might have resulted in the greatest overall height, because of the separate benefits of chilling (5, 9), and BAP (3). However, when placed on short days, it is likely that the continual apical growth as a result of BAP application (3) delayed terminal bud formation, resulting in smaller buds and less elongation following chilling.

Although foliar application of BAP to non-chilled seedlings improved their height by 3.3 cm (1.3 in.), total height was still significantly less than the chilled, control seedlings (Table 2). This supports the findings by Bryan and Seiler (3) who demonstrated that foliar application of BAP avoided the long periods of apical bud dormancy typical of continuously grown, non-chilled Fraser fir seedlings. They observed, when BAP was applied to the shoot, that it continually stimulated apical meristem cell division resulting in semi-continuous shoot elongation.

The least total height growth was observed for non-chilled, non-BAP treated seedlings. These seedlings lost apical dominance, had deformed or aborted terminal buds, were stunted

**Table 1. Chronological timetable for Fraser fir seedlings which were intermittently exposed to short days and chilling.**

| Month/Date | Production status |
|---|---|
| June 29–Sept. 16, 1988 | Continual seedling growth in 16-hour photoperiod with terminal bud formation starting to occur September 1, 1988. |
| Sept. 16–Oct. 14 | Seedlings placed in short days (8-hour photoperiod). Fertilizer and BAP application discontinued. |
| Oct. 14–Dec. 14 | Seedlings placed outside in lathe house for chilling requirement. Average maximum and minimum temperature for this period were 11.7°C (53°F) and −2.2°C (28°F) respectfully. 87% of the days had minimum temperatures below 2C. |
| Dec. 14–Mar. 30, 1989 | Seedling placed back in 16-hour photoperiod and greenhouse conditions. Fertilizer and BAP application resumed. |
| Mar. 30–June 21 | Seedlings again placed in short days. Fertilizer and BAP application discontinued. |
| June 21–Aug. 17 | Seedlings placed in cold storage (2°C, 36°F)), watered and covered with plastic to avoid root and shoot dehydration. |
| Aug. 17–Nov. 28, 1989 | Seedlings placed back in 16-hour photoperiod and greenhouse conditions. On November 28, 1989 the majority of the chilled (control) seedlings terminal apex were determined not to be actively growing, (the next step normally would be placement in short days), therefore all treatments (seedlings) were measured and harvested. |

Table 2. Fraser fir (72-week-old) seedling morphology as influenced by foliar BAP application and chilling.[z]

| Treatment | BAP level (uM) | Total height (cm) | RCD[y] (mm) | Root dry wgt. (g) | Shoot dry wgt. (g) | Root/shoot ratio |
|---|---|---|---|---|---|---|
| Chill | 0 | 16.8 a | 4.99 b | 1.38 c | 2.99 b | 0.46 b |
| | 444 | 15.3 b | 4.24 c | 0.85 d | 2.65 b | 0.33 c |
| Non-chill | 0 | 11.6 c | 6.38 a | 1.96 b | 2.87 b | 0.69 a |
| | 444 | 14.9 b | 6.59 a | 2.20 a | 4.86 a | 0.45 b |

[z]Means within each column followed by the same letter are not significantly different (p = 0.05).

[y]Root collar diameter.

and lacked the symmetry common to Fraser fir. This is typical of seedlings which experience continuous optimum growing conditions without chilling (3, 5).

Root collar diameter (RCD) averaged, 29% less in chilled versus non-chilled seedlings (Table 2). This could be attributed to a continuation of cambial growth in non-chilled seedlings. BAP did not influence RCD of the non-chilled seedlings (Table 2). Bryan and Seiler (3) demonstrated that the apical, and not the cambial, meristem was affected by foliar BAP application.

Root and shoot dry weights for non-chilled, BAP-treated seedlings were substantially greater than for other treatments (Table 2). This resulted in a husky appearance. Despite the greater mass of non-chilled, BAP-treated seedlings, the root/shoot ratio was similar to that of chilled, control seedlings. A proper root/shoot ratio could be important to plant survival when the seedlings are out-planted (6). Performance following outplanting will have to be evaluated in future studies.

The mean number of primary lateral branches and mean total number of buds were significantly reduced when BAP was applied to both chilled and non-chilled seedlings (Table 3). Little (7, 8) found an increase in the total number of lateral buds formed on the shoot when BAP was applied to five- or six-year-old Balsam fir (*Abies balsamea* (L.) Mill.) trees. The discrepancy between Little's findings and our results is possibly linked to the tree/seedling age differences, the differences in apical and cambial meristematic activity and/or the much higher concentrations and more frequent applications used by Little. Young seedlings treated with BAP tended to maintain apical meristem activity and possibly suppress the formation of lateral branches. Non-chilled, control seedlings also had fewer branches and lateral buds, compared to the chilled, control treatment. This was probably due to the general shoot degradation caused by the absences of chilling as discussed previously.

Table 3. Mean number of primary and secondary lateral branches and mean total number of buds produced as affected by BAP and chilling treatments for Fraser fir (72-week-old) seedlings.[z]

| Treatment | BAP levels (uM) | No. of primary laterals | No. of secondary laterals | Total no. of buds |
|---|---|---|---|---|
| Chill | 0 | 8.2 a | 1.7 a | 23.1 a |
| | 444 | 3.7 b | 1.2 a | 19.0 b |
| Non-chill | 0 | 2.1 c | 0.2 b | 13.7 c |
| | 444 | 3.7 b | 1.6 a | 9.1 d |

[z]Means within each column followed by the same letter are not significantly different (p = 0.05).

The reduction in the mean number of buds and branches in our seedlings in response to foliar BAP applications might cause concern. However, preliminary observations of outplanted BAP treated seedlings show a resumption of pretreatment level of branching and bud proliferation the following year after a period of seedling dormancy. Furthermore, most buds on non-BAP-treated seedlings result in branches at the base ("handle") of a harvested Christmas tree. These limbs are usually removed before or after harvest so the tree base can accommodate a tree stand. This results in adding either extra handling and cost to the grower or inconvenience to the buyer. With fewer branches on the handle, a BAP-produced seedling would ultimately be easier to prune.

BAP application to the shoot of Fraser fir stimulated semicontinuous apical bud growth in non-chilled seedlings, resulting in taller seedlings. Exposing seedlings to short days to induce bud formation, followed by chilling, also improved growth. However, BAP applied in conjunction with short days and chilling actually decreased growth, especially roots.

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