The Influence of Three Plant Growth Regulators on Susceptibility to Cold Injury Following Warm Winter Spells in Fraser Fir [Abies fraseri (Pursh) Poir] and Colorado Blue Spruce (Picea pungens)

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Abstract. The effects of three plant growth regulators (PGRs) (prohexadione–calcium, paclobutrazol, and flurprimidol) on the resistance of Fraser fir (Abies fraseri) and Colorado blue spruce (Picea pungens) to cold injuries were investigated. Treated and untreated seedlings were first allowed to harden before exposure to warm temperatures in a greenhouse. The seedlings were then subjected to an artificial freezing test to simulate the return to normal winter conditions. Chlorophyll fluorescence, relative electrolyte leakage, bud survival, and posttreatment plant survival were recorded to evaluate the effectiveness of the treatments. Results showed that treatments with paclobutrazol and flurprimidol maintained the photosynthetic ability of the plants and reduced the extent of needle cold injuries. There was no effect on bud and plant survival, possibly as a result of the timing of the PGR application. Further studies with adjustment of the timing and rates of PGR treatments are needed to validate these results.

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During the past few years, unusual winter temperature patterns have caused serious damage to Colorado blue spruce (Picea pungens) and Fraser fir [Abies fraseri (Pursh) Poir] in mid-Michigan nursery and Christmas tree plantations. Damages expressed after bud flushing were cold desiccation and burn and were the most serious on trees located on the south-facing areas of the field. The phenomenon was hypothesized to have been caused by several days of warm winter temperatures. It is believed that as temperatures increased over 50°F for several days in January and February, plants responded by rehydrating plant tissues, losing their winter-hardiness and progressively triggering growth. Such a phenomenon has been observed and studied in several landscape and horticultural plants. Jones and Cregg (2006) evaluated budbreak and winter injury in exotic firs and concluded that trees that broke bud early were more prone to late spring frost damage. Similar phenomena have been reported in blueberry (Ehlenfeldt et al., 2006), pecan (Smith, 2002), apples (Forney et al., 2000), apricot (Gunes, 2006), and several grass species (Busey, 2003; Iriarte et al., 2005; Webster and Ebdon, 2005). The warmer the temperatures and the greater the duration of the warm spell, the greater the loss in hardiness observed. Consequently, sudden subfreezing temperatures after those warm days are likely to cause severe injuries to buds and needles, resulting in serious loss in growth (Kang et al., 1998) as a result of reduced plant winter-hardiness.

Plant winter-hardiness is well studied in plant physiology. It is reported to be genetically controlled and driven by temperature, photoperiod, and water stress, which induce the metabolic hardening mechanism and produce an increase in cold-hardiness in the fall and a decrease in the spring (Beuker et al., 1998; Jones and Cregg, 2006; Wisniewski et al., 2003). The process is initiated by the continuous exposure to temperatures below 5°C (chilling requirement) necessary to prepare apical meristems of temperate perennial plants to resume growth when temperatures become favorable in the spring. In boreal conifers, chilling is reported to be the main factor promoting rest completion and growth when warm temperatures become prevalent (Hamner and Westin, 2000). Knowledge of chilling requirements is critical to the success of conifer plantations, and the suitability of species to a given site is largely dictated by the number of days of low temperatures at that location (Hällgren and Öquist, 1990). In regions with mild winters, warm temperatures in late fall and winter regularly cause chilling deficit and delay bud burst when conditions become favorable in the spring. In regions with cold winters, however, chilling requirements are far exceeded by winter temperatures, and climatic warming would force bud burst at an earlier date and increase the risk of frost injuries (Hamann et al., 2001). This appears to be the case in Michigan where the chilling requirement of most plant species is largely met by December, and warm winters cause dehardening and growth initiation resulting in serious frost injury when temperatures returned to normal winter subfreezing levels. This practical issue creates a challenge for researchers to develop treatments or approaches that will improve tree resistance to frost damage in case of warm winter temperatures. One potential solution is the use of plant growth regulators (PGRs) to influence the natural hormonal processes that triggered dormancy and growth.

Several studies have investigated the influence of growth regulators on growth and stress resistance of ornamental and fruit plant species. For instance, Zhou and Leul (1998) used uniconazole to attenuate the hormonal balance and induce freezing injury in winter rape. A number of research projects have investigated the use of paclobutrazol on drought resistance in Douglas fir (Pseudotsuga menziesii), lodgepole pine (Pinus contorta), white spruce (Picea glauca), and jack pine (Pinus banksiana) (Marshall et al., 1991). Paclobutrazol has also been reported to affect shoot and root growth in Douglas fir (Wheeler, 1987) and loblolly pine (Pinus taeda) (Barnes and Kelley, 1992). Another study conducted by Albrecht et al. (2004) investigated the use of prohexadione–calcium, vitamin E, and glycerin to reduce frost injury in apple (Malus domestica) and reported a significant reduction in frost injury resulting from the application of prohexadione-Ca. Similar studies conducted by Rossi and Buelow (1997) and Burnell (2005) explored the use of PGRs to reduce winter injury on blue grass (Poa annua L.). Results indicated enhanced freezing stress tolerance with low rates of trinexap-ethyl, flurprimido- dol, and paclobutrazol. No published study reports on the influence of PGR treatments on the susceptibility of ornamental and Christmas tree species to cold injuries after warm winter spells.

The objective of this study was to evaluate the influence of three PGRs on the susceptibility of Fraser fir and Colorado blue spruce species to cold damage after simulated warm winter temperature conditions.

Material and Methods

Two species, Fraser fir [Abies fraseri (Pursh) Poir] and Colorado blue spruce (Picea pungens), were included in the study. Four-year-old seedlings (2-2) were obtained from a commercial nursery in southwestern Michigan in Oct. 2005. The seedlings were potted in containers with a potting mixture of peatmoss and vermiculite (4:1 v/v) and acclimated in a greenhouse with daily irrigation for 2 weeks before treatments.
Table 1. Treatment labels and concentrations.

| Treatment description | Label      | Concentration (ppm) |
|----------------------|------------|---------------------|
| Control              | CTRL      | 0 mg L⁻¹ (0 ppm)    |
| Apogee–low (prohexadione-Ca) | ProH-Ca-1 | 1100 mg L⁻¹ (1100 ppm) |
| Apogee–high (prohexadione-Ca) | ProH-Ca-2 | 2200 mg L⁻¹ (2200 ppm) |
| Piccolo–low (0.12 g paclobutrazol) | Pacl-1 | 200 mg L⁻¹ (200 ppm) |
| Piccolo–high (0.12 g paclobutrazol) | Pacl-2 | 400 mg L⁻¹ (400 ppm) |
| Cutless–low (50% flurprimidol) | FlurP-1 | 50 mg L⁻¹ (50 ppm) |
| Cutless–high (50% flurprimidol) | FlurP-2 | 100 mg L⁻¹ (100 ppm) |

**Dehardening and Artificial Freezing Test**

After 8 weeks, seedlings were moved for conditioning into the greenhouse under spring-like growing conditions to initiate the dehardening process. The average daily maximum and minimum temperatures in the greenhouse for this period were 18 °C (64.1 °F) and 15 °C (59.4 °F), respectively. The average daily temperature at 0800 hr was 16 °C (61.1 °F). After the week-long conditioning, seedlings were subjected to an artificial freezing test (AFT). A low-temperature programmable freezer (SciTemp model 40–12A, Adrian, MI) equipped with a programmable CAL controller was used for the study. A program was created using CALgrafix programming software (Lesman Instrument Co., Bensenville, IL) to allow the freezer to start at the ambient temperature of 20 °C, decrease at 5 °C per/hour to 0 °C, then hold at 0 °C for 4 h (Fig. 1). After the hold period, the temperature was decreased at the same rate to −20 °C and maintained for 4 h. After 4 h at −20 °C, the temperature was increased to 0 °C at the same rate and maintained 4 h for slow thawing until plants were removed from the freezer. Seedlings were then moved back into the greenhouse to resume their growth (under fluorescent lights providing an 18-h photoperiod). The plant materials from all treatments were watered with injected well water (pH = 5.5), twice a week, with Peters 20–10–10 Peat-Lite Special fertilizer (Peter Chemical Co., Hawthorne, NJ), providing near optimal macro- and micronutrient levels.

Morphological attributes (plant height, shoot height, and number of buds) were recorded for each treatment group.

**Data Analysis**

Morphological attributes, CF, and REL data collected were analyzed for normality and outliers using EDA on SAS (SAS Institute, Cary, NC). For CF and REL, values obtained for PGR treatments were compared with the untreated control using a one-way analysis of variance (PROC GLM, SAS version 9.1). Treatments means were separated using Fisher’s least significant difference procedure (α = 0.05).

**Results and Discussion**

**Chlorophyll fluorescence.** The chlorophyll fluorescence data measured before and after the artificial freezing test are summarized in Figure 2. Before seedlings were...
subjected to the AFT, the treated and untreated needle Fv/Fm ratios ranged from 0.78 to 0.82 and 0.67 to 0.77 for *A. fraseri* and *P. pungens*, respectively, and there were no statistically significant differences (*P* > 0.05) between treated and untreated plants, indicating that the three PGR treatments did not adversely affect their photosynthetic abilities.

After the AFT, the average Fv/Fm ratios ranged between 0.77 to 0.80 and 0.65 to 0.75 for *A. fraseri* and *P. pungens*, respectively (Fig. 2). For both species, there were no significant differences (*P* > 0.05) in Fv/Fm between untreated samples and plants treated with the two rates of prohexadione-Ca.

*Abies fraseri* seedlings treated with paclobutrazol and flurprimidol had higher Fv/Fm values (0.60 to 0.62) compared with control samples (0.51). A similar trend was observed on *P. pungens* with Fv/Fm values of 0.60 to 0.65 for treated samples compared with 0.52 for control samples. These differences were statistically significant (*P* < 0.05) for *P. pungens* and *A. fraseri* treated with paclobutrazol and flurprimidol. These results clearly indicate that seedlings treated with paclobutrazol and flurprimidol had a smaller reduction in their photosynthetic abilities after the AFT compared with untreated specimens.

Both paclobutrazol and flurprimidol are PGRs having a N-containing heterocycle and act as inhibitors of monoxygenases catalyzing the oxidative steps from ent-kaurene to ent-kaurenoic (Rademacher, 2000). Normally, as reported by Häggren and Òquist (1990), the exposure of untreated plants’ photosynthetic apparatus to low temperature likely altered the thylakoid membrane and the transfer of electrons connected with photosystem II (PS2). Treatment with N-containing heterocycles helped stabilize the chloroplast thylakoid membrane retarding the chlorophyll degradation and stimulating photosynthetic processes in the plant. Such processes have been previously reported in other horticultural crops (Smith et al., 1985).

The reasons for the lack of response from the prohexadione-Ca are not known. However, under practical conditions, the growth-retarding effect of a given compound is not uniquely determined by its type of interaction with the Gibberilins (GA) metabolism (Rademacher, 2000), but can also be affected by factors such as plant responsiveness, moisture uptake, chemical translocation and persistence, or several other incidental effects (Rademacher, 2000; Schott and Walter, 1991). Any combination of these factors could explain the lack of CF response to prohexadione-Ca treatments.

Relative electrolyte leakage. Measuring the electrolyte leakage after a simulated freezing stress is a rapid and reliable screening technique for assessing freezing tolerance. Higher relative electrolyte leakage percentages are a good indicator of increased cell damage (Campos et al., 2003). The results obtained in this study (Fig. 3) indicated that before the AFT, all seedlings from all PGRs treatments had REL values very similar to those of untreated specimens for both species, ranging from 34% to 41% (± 2% to 4%) for *A. fraseri* and 34% to 41% (± 3% to 5%) for *P. pungens*. Before the AFT, there were no statistical differences between controls and PGR treatments, indicating that the treatments did not affect the REL in both species. In *A. fraseri* control, the AFT produced a 28% increase in REL (Fig. 3). In the low and high prohexadione-Ca treatments, the AFT resulted in REL increases of 10% and 18%, respectively. Samples treated with paclobutrazol at concentrations of 400 ppm and those treated with flurprimidol at 50 and 100 ppm had REL values comparable to those obtained before the AFT, indicating lower cell electrolyte leakage after the freezing procedure. Untreated *P. pungens* samples also had a higher REL (73% ± 3%), indicating a high level of cell damage after the AFT.

Similarly, prohexadione-Ca-treated samples also had REL readings much higher (69% ± 5% and 77% ± 5%, respectively) compared with values before the AFT. This level of cell electrolyte leakage was not statistically different from the control, indicating that this PGR treatment did not improve the resistance to freeze damage. Samples treated with the two concentrations of paclobutrazol and flurprimidol had REL values ranging from 42% ± 4% to 45 ± 5%. The values obtained for these two PGRs were all statistically not different from those obtained before the AFT, indicating a positive effect of the treatment on freezing resistance.

**Buds and plant survival after the artificial freezing test.** Bud damage and mortality are the most serious injuries caused by plant exposure to cold temperatures. Cold-hardiness studies have shown that despite a normal external appearance, injured buds when dissected show necrotic tissues at their center and do not flush when exposed to growing conditions. Therefore, the total bud survival after the AFT is an indication of the ability of the PGR treatment to reduce bud damage after conditioning and exposure to cold temperatures. The data collected and summarized in Table 2 (*A. fraseri*) showed that for...
all PGR treatments, with the exception of prohexadione-Ca at 1100 ppm, significantly improved bud survival compared with the control.

*Picea pungens* displayed much higher bud damage than *A. fraseri* with bud survival rates of 54% or lower. Only the prohexadione-Ca at 2200 ppm treatment was statistically different from the control, and it resulted in lower bud survival (Table 3). The results suggest that *P. pungens* buds are more susceptible to fluctuations in winter temperature than *A. fraseri*. These results are similar to the findings of Bigras and Margolis (1996) who reported greater sensitivity to late fall freezing bud damage in white spruce. The frost hardness of buds is generally related to ambient temperature fluctuations, and short-term dehardening and rehardening has been reported in deciduous wood species (Beuker et al., 1998; Proebsting et al., 1989). It is possible that the short-term dehardening before the AFT could explain the lack of specific response to PGR treatments. Another possible explanation is the timing of the PGR treatments. It has been reported that frost-hardness is low after bud initiation while plants undergo intense physiological activity to store carbohydrates in preparation for the winter (Colombo, 1997). An indicator of this process is the rate of initiation of the needle primordia in the terminal bud (Colombo, 1997). Applying PGR treatments after bud set may have limited the translocation of a.i. to buds, leading to the lack of differential response after PGR treatments. This is not a contradiction with the positive response obtained for CF and REL, because plants were still photosynthetically active at the time of the treatments, thus allowing the translocation of PGR ingredients to the foliage.

There were no significant differences (*P* > 0.05) in seedling survival between the controls and any of the PGR treatments for *A. fraseri* (Table 2). However, *P. pungens* specimens treated with paclobutrazol at both low and high doses displayed statistically significantly higher survival rates (*P* = 0.01) compared with untreated samples. However, the results were not significant for flurprimidol and prohexadione-Ca (*P* > 0.05). These results are consistent with empirical observations in the field where most plants affected by the warm spell had their buds injured by cold temperatures, resulting in bud death and subsequent loss of growth.

### Conclusion

Results indicated that paclobutrazol and flurprimidol positively affected the photosynthetic activities of treated plants as measured by the chlorophyll fluorescence. There was no difference between specimens treated with prohexadione-Ca and untreated specimens. The REL confirmed the physiological data with specimens treated with paclobutrazol and flurprimidol having lower leakages after the freezing test, indicating a lower level of freezing injuries compared with untreated specimens. The evaluation of bud mortality indicated a lower responsiveness to the PGR treatments. We hypothesized that this lack of response may be the result of short-term dehardening or to the timing of the PGR treatments, which prevented the translocation of PGR chemical ingredients to buds during the bud set and hardening. With the exception of paclobutrazol treatments on *P. pungens*, the overall plant survival was also unaffected by the PGR treatments. It can be concluded that treatments with paclobutrazol and flurprimidol have the potential to improve *A. fraseri* and *Picea pungens* resistance to cold injuries after sudden warm winter temperatures. However, the PGR treatment should probably be applied early enough to allow translocation to all parts of the plant during bud formation. Further studies manipulating the application timing and rates of the PGRs...
Table 3. Picea pungens seedling and bud survival for untreated (controls) and treated plants with prohexadione-Ca, paclobutrazol, and flurprimidol at low and high doses.

| Treatments   | Avg stem diam (cm) | Avg ht (cm) | Seedlingsa | Buds |
|--------------|--------------------|-------------|------------|------|
| Control      | 21.03              | 34.16       | 52.63 b    | 43.41 ab |
| ProH-Ca-1    | 22.25              | 40.23       | 42.11 b    | 42.62 bc |
| ProH-Ca-2    | 21.39              | 25.40       | 47.37 b    | 35.68 c  |
| Pacl-1       | 24.03              | 37.24       | 84.21 a    | 43.68 a  |
| Pacl-2       | 32.51              | 28.60       | 89.47 a    | 50.35 a  |
| FlurP-1      | 21.62              | 25.81       | 63.16 ab   | 52.88 ab |
| FlurP-2      | 24.28              | 31.95       | 68.42 ab   | 54.05 ab |

*aMeans followed by the same letter are not statistically different (α = 0.05).

are needed to confirm these preliminary results and test the hypotheses discussed here.

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