

Hormonal control of second flushing in Douglas-fir shoots

MORRIS CLINE,^{1,4} MARK YODERS,¹ DIPTI DESAI,¹ CONSTANCE HARRINGTON² and WILLIAM CARLSON³

¹ Department of Plant Cell and Molecular Biology, Ohio State University, 318 W. 12th Ave., Columbus, OH 43210, USA

² USDA Forest Service, Pacific Northwest Research Station, 3625 93rd Ave., SW, Olympia, WA 98512-9193, USA

³ Weyerhaeuser, Propagation of Higher Valued Trees, 32901 Weyerhaeuser Way South, Federal Way, Olympia, WA 98001, USA

⁴ Corresponding author (cline.5@osu.edu)

Received September 13, 2005; accepted December 23, 2005; published online June 30, 2006

Summary Spring-flushing, over-wintered buds of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) produce new buds that may follow various developmental pathways. These include second flushing in early summer or dormancy before flushing during the following spring. Second flushing usually entails an initial release of apical dominance as some of the current-season upper lateral buds grow out. Four hypotheses concerning control of current bud outgrowth in spring-flushing shoots were tested: (1) apically derived auxin in the terminal spring-flushing shoot suppresses lateral bud outgrowth (second flushing); (2) cytokinin (0.5 mM benzyladenine) spray treatments given midway through the spring flush period induce bud formation; (3) similar cytokinin spray treatments induce the outgrowth of existing current lateral buds; and (4) defoliation of the terminal spring-flushing shoot promotes second flushing. Hypothesis 1 was supported by data demonstrating that decapitation-released apical dominance was completely restored by treatment with exogenous auxin (22.5 or 45 mM naphthalene acetic acid) (Thimann-Skoog test). Hypothesis 2 was marginally supported by a small, but significant increase in bud number; and Hypothesis 3 was strongly supported by a large increase in the number of outgrowing buds following cytokinin applications. Defoliation produced similar results to cytokinin application. We conclude that auxin and cytokinin play important repressive and promotive roles, respectively, in the control of second flushing in the terminal spring-flushing Douglas-fir shoot.

Keywords: apical dominance, auxin, branching, cytokinin, defoliation, lateral and terminal bud outgrowth, spring flushing, terminal shoot.

Introduction

The control of current bud growth in spring-flushing shoots of temperate woody species is critical in the determination of the subsequent developmental pathway, whether it be continuous free growth, second flushing in early summer or dormancy before flushing during the following spring. Second flushing usually entails an initial release of apical dominance because

some of the current-season upper lateral buds grow out. Although hormones are known to play dynamic roles in the control of branching in herbaceous species, there have been few hormonal studies on bud development and apical dominance in conifers.

Auxin and cytokinin are known to prevent and to promote bud outgrowth, respectively (Skoog and Miller 1957, Tamas 1995, Coenen and Lomax 1997). The role of auxin in bud growth is thought to involve apical dominance wherein auxin produced in an actively growing shoot apex moves basipetally down the stem and inhibits the outgrowth of the lower lateral buds (Tamas 1995). When this model of bud growth in herbaceous plants is extrapolated to mature trees, however, it is necessary to restrict the concept of auxin operating in apical dominance to current growth of single elongating branches rather than collectively to all perennial branches of a tree (Brown et al. 1967, Wareing 1970, Cline 2000).

The presence of endogenous auxin in conifer tissue including Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) has been established (DeYoe and Zaerr 1976, Chiwocha and von Aderkas 2002), and its basipetal transport down the shoot has been documented in Scots pine (*Pinus sylvestris* L.) (Sundberg and Ugglå 1998). However, evidence for the role of auxin in apical dominance in conifers is largely indirect (Ross et al. 1983, Little and Pharis 1995). There have been only a few studies in which the classical, Thimann-Skoog, auxin-replacement apical dominance experiment (described in the Materials and methods section) has been directly replicated in woody species (House et al. 1998, Cline 2000). The presence of cytokinins in buds has been documented in various conifers (Morris et al. 1990 in Douglas-fir, Qamaruddin et al. 1990 in Scots pine, Bollmark et al. 1995 in Norway spruce (*Picea abies* (L.) Karst), Zhang et al. 2003 in Monterey pine (*Pinus radiata* D. Don)). Cytokinins have been found to promote bud formation in Scots pine (Kossuth 1978) and in blue spruce (Mulgrew and Williams 1985), and bud outgrowth in longleaf pine (*Pinus palustris* Mill.) (Hare 1984), balsam fir (*Abies balsamea* (L.) Mill.) (Little 1985), Scots pine (Whitehill and Schwabe 1975), blue spruce (*Picea pungens* Engelm.) (Mazzola and Costante 1987) and in loblolly pine (*Pinus taeda* L.)

(Zimmerman and Brown 1971).

Ross et al. (1983) reported cytokinin spray retarded stem elongation and induced adventitious bud formation in Douglas-fir seedlings. Goldfarb et al. (1991) found that a liquid cytokinin pulse induced adventitious shoot formation from Douglas-fir cotyledons. Pilate et al. (1989) observed changes in cytokinin concentration in Douglas-fir buds during bud quiescence release. Lavender and Zaerr (1967) mentioned that treatment of 2-month-old Douglas-fir seedlings with a cytokinin foliar spray resulted in a "proliferation of lateral buds near apices." Mazzola and Costante (1987) found that whole-tree (but not terminal leader) foliar benzyladenine (BA) applications promoted both bud formation and second flushing in 6-year-old Douglas-fir seedlings.

Although there is general evidence that pest defoliation of woody species reduces shoot growth (Kramer and Kozlowski 1979), axillary and young terminal buds usually grow out following defoliation in early summer (Romberger 1963). Spruce budworm defoliation has been reported to induce epicormic branch production in Douglas-fir (Johnson and Denton 1975). Because of possible interactions between hormonal- and defoliation-induced mechanisms of bud growth promotion, we included a defoliation treatment in our study of second flushing shoots.

The primary focus of our study was to analyze the effects of auxin and cytokinin on the outgrowth of the newly formed buds that develop during second flushing of Douglas-fir shoots. Specifically, we proposed and tested four hypotheses. (1) Apically derived auxin in terminal spring flushing shoots suppresses lateral bud outgrowth. This was tested by exogenous auxin treatments to decapitated terminal shoot apices to determine if apical dominance could be restored. (2) Cytokinin promotes new current bud formation in the terminal spring flushing shoot. This was tested by determining whether cytokinin spray treatments caused an increase in the number of current lateral buds in the terminal spring flushing shoot. (3) Cytokinin promotes the outgrowth of current buds in the terminal spring flushing shoot. This was tested by determining whether cytokinin spray treatments to the leader shoot caused an increase in the number of outgrowing current buds (second flushing). (4) Defoliation of needles on the terminal spring flushing shoot promotes current lateral bud outgrowth. This was tested by complete needle removal to determine if the current lateral buds could be induced to grow out.

Materials and methods

Two-year-old, bare-root seedlings of Douglas-fir, were lifted from the Mima Weyerhaeuser nursery near Olympia, Washington and shipped to Columbus, Ohio, by overnight courier. They were potted in a general purpose peat and vermiculite growing medium, in 4-liter Deepots. The potted seedlings were maintained in a greenhouse with weekly N,P,K fertilization (20,10,20; 180 mg l⁻¹) and watered every two or three days as needed. The temperature-controlled greenhouse was set for 20–25 °C; however, extreme outdoor temperatures

sometimes caused fluctuations (18.1–29.1 °C for 2005) beyond these limits. Supplementary overhead light in the greenhouse was provided by 400-W mercury vapor lamps when irradiance fell below 600 µmol m⁻² s⁻¹. The photoperiod was 16 h.

After the seedlings had been in the greenhouse for several weeks, the over-wintered buds began to swell and begin flushing. Some of the lateral buds were pruned to balance crown shape and to reduce competition with the elongating terminal bud. Hormone treatments were started midway during spring flushing. Separate sets of plants with their controls were used for each experiment and hormone treatment.

The classical Thimann and Skoog (1933) apical dominance test was implemented by applying 0.5% (22.5 mM) or 1% (45 mM) α -naphthaleneacetic acid (NAA, potassium salt; Sigma, St. Louis, MO) in lanolin (anhydrous, lab grade; Carolina Supply Co., Burlington, NC) to the cut stem surface immediately following shoot decapitation, about 1 cm below the apex of the flushing leader shoot when it was 5–10 cm in length. Measurements of any outgrowth that occurred in the lateral buds were made 5–10 weeks following treatment. The five separate replicate experiments yielded essentially similar results. Experiment 1, carried out in May–June 2004, consisted of four intact controls, five decapitated controls and four decapitated plants treated with 22.5 mM NAA. Measurements were made 38 days after treatment. Inhibition of lateral bud outgrowth by 22.5 mM NAA was complete in all seedlings. The remaining four experiments were carried out with 45 mM NAA for 58, 52 and 47 days at different times in April, May and June 2005 in the same greenhouse as was used in 2004.

In Experiment 5, the usual pre-experiment pruning of some of the sub-terminal lateral buds of the first flush was omitted. This appeared to have had little or no effect on the final results measured on Day 56 of treatment. The total number of seedlings in Experiments 2–5 were 24 (4 intact, 10 decapitated, and 10 decapitated and NAA-treated), 25 (6 intact, 9 decapitated, and 10 decapitated and NAA-treated), 23 (5 intact, 9 decapitated, and 9 decapitated and NAA-treated) and 18 (4 intact, 7 decapitated, and 7 decapitated and NAA-treated). For analysis, data from all five experiments were combined (see Table 1). At the time of experimentation, it was deemed important to have a larger number of seedlings in the decapitated, and decapitated and NAA-treated groups than in the control group; however, in retrospect, the use of equal numbers of intact seedlings would have been preferable. The results, nonetheless, were definitive. Although the treatment time in the first experiment (38 days) was substantially shorter than in the remaining four experiments, the difference had little or no effect on the final response. In general, outgrowth of buds in response to decapitation of Douglas-fir shoots occurs slowly.

For the cytokinin treatments, 0.5 mM benzyladenine (BA, 6-benzylamino purine; Sigma, St. Louis, MO) foliar spray in 0.1% Aromax (C/12, AKZO; Nobel, McCook, IL) was applied daily for 1–2 weeks to the entire flushing shoot beginning about midway through the flushing period when the leader shoot was 5–10 cm in length. Six replicate experiments, each with equal numbers of control and BA-treated seedlings, were

carried out in the same greenhouse. In Experiment 1, in May–July 2004, there was a total of 29 seedlings (15 water controls and 14 BA-treated plants). The BA spray treatment was given for 2 weeks and measurements were made 5 weeks later. In the remaining five experiments carried out at different times (March through June 2005), there was a total of 22, 15, 38, 28 and 34 seedlings (including water controls) receiving a 2-week BA spray treatment except for the last experiment in which seedlings received a 1-week treatment. For analysis, data were combined for all the 2-week spray experiments (see Table 2). Lateral buds were counted as growing out if the bud had clearly opened up (visibly exhibiting green pigment) or had started flushing. Length and fresh mass of the first flushing terminal shoot as well as number and fresh mass of second flushing terminal and lateral buds were measured 5–6 weeks following treatment.

Six defoliation experiments (without hormone treatments) of terminal shoots were carried out midway during the flushing period. Each experiment consisted of nine seedlings per treatment: control (intact flushing terminal shoots) and defoliated (terminal shoot defoliated by plucking all needles). In two of the experiments, we covered the terminal shoot in the control and defoliation treatments with plastic bags to prevent desiccation and die-back of the defoliated shoots. The data in Table 3 represent the combined data of these two experiments. The bagging procedure caused no deleterious side effects. Because of desiccation and die-back, only one of the other four defoliation experiments without bagging or with only partial bagging yielded sufficient valid data for analysis.

Statistical analyses

The effects of treatments on the dependent variable (e.g., number of plants breaking bud, length of shoot, number of buds that grew out) were tested by analyses of variance (ANOVAs) for unbalance design with individual experiments in each set of trials as blocks. Two proportion tests were used to determine significant differences in percentage analyses.

Results

Auxin

Thimann-Skoog apical dominance experiments (Thimann and Skoog 1933) were carried out on current buds of terminal spring-flushing shoots. Decapitation of the terminal bud followed by treatment of the cut stem surface with lanolin resulted in the outgrowth of several of the highest current lateral buds below the point of decapitation within a month (Figure 1; Table 1). Decapitation-induced lateral bud outgrowth (second flushing), or release of apical dominance, was detected ($P = 0.001$) despite the confounding effects of simultaneous natural second flushing. Second flushing was observed in 93% of the decapitated seedlings and only 35% of the intact seedlings.

In all five trials, repression of lateral bud outgrowth by exogenous auxin (22.5 or 45 mM NAA) treatment to the decapitated shoots was absolute and complete (Figure 1; Table 1).

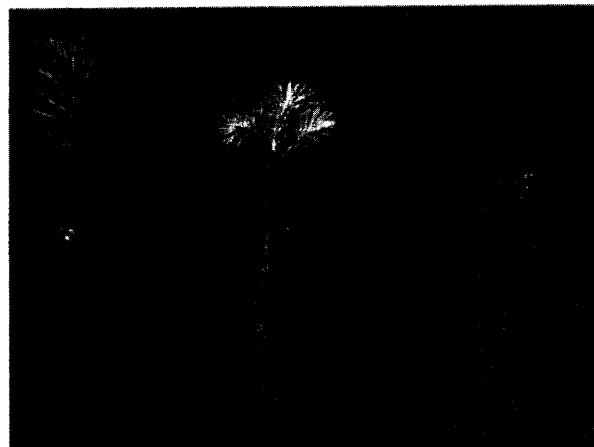


Figure 1. Effect of auxin on current lateral bud outgrowth 36 days after decapitation and treatment. Left: intact terminal shoot with a secondary flushing terminal bud and a small lower lateral bud. Center: three flushing lateral buds released just below the point of decapitation. Right: apical cut stem surface of shoot treated with 45 mM α -naphthaleneacetic acid (NAA) in lanolin.

That is, outgrowth of all lateral buds due to natural second flushing and decapitation-induced release was prevented by the NAA treatment. Some swelling of the NAA-treated decapitated stumps was observed.

Cytokinins

At 5–6 weeks following 2 weeks of cytokinin (0.5 mM BA) foliar spray treatments of the terminal spring-flushing leader shoots, there was a small, but significant ($P = 0.029$) increase in the total number of lateral buds (Table 2) and a large and significant ($P < 0.001$) increase in current bud outgrowth (Figure 2; Table 2). Furthermore, the stimulatory effect of BA on second flushing was more pronounced in the lateral buds than in the terminal buds. However, the finding that second terminal bud flushing occurred in only 28.4% of the control seedlings in the five experiments compared with 81.5% of the BA-treated seedlings indicates that BA substantially enhanced second flushing of the terminal buds.

Table 1. Restoration of apical dominance by auxin (22.5 or 45 mM α -naphthaleneacetic acid (NAA) in lanolin) treatment. Measurements were made 38–58 days after decapitation and NAA treatment of terminal spring-flushing shoots. Values (means $\pm$ SE) are pooled from five experiments. Overall, $P < 0.001$.

	Intact	Decapitated control	Decapitated + NAA
No. of seedlings	23	40	41
No. of outgrowing lateral buds	0.87 ± 0.5	3.04 ± 0.4	0.02 ± 0.4
Percent of seedlings with flushing lateral buds	34.8	92.5	0

Table 2. Effects of cytokinin on lateral and terminal bud outgrowth in flushing terminal shoots by daily foliar spray treatments with 0.5 mM benzyladenine (BA). Measurements were made 5–6 weeks after the end of a 2-week spray treatment. Values (means $\pm$ SE) are pooled from five experiments.

	Water control	BA treatment	P value
No. of seedlings	67	65	
No. of second-flushing lateral buds	1.7 $\pm$ 1.1	14.8 $\pm$ 1.1	< 0.001
No. of current buds ¹	15.9 $\pm$ 1.4	18 $\pm$ 1.4	0.029
Flushing terminal shoot length (cm) at beginning of treatment	7.4 $\pm$ 0.5	7.5 $\pm$ 0.4	0.717
Flushing terminal shoot length (cm) 5–6 weeks after end of treatment	15.4 $\pm$ 0.9	11.5 $\pm$ 1.0	< 0.001
Terminal shoot fresh mass (g)	3.9 $\pm$ 0.6	5.4 $\pm$ 0.6	< 0.001
Fresh mass of second-flushing lateral buds (g)	0.6 $\pm$ 0.3	1.8 $\pm$ 0.4	< 0.001
Fresh mass of second-flushing terminal bud (g)	0.4 $\pm$ 0.2	0.3 $\pm$ 0.1	0.309
Length of second-flushing terminal bud (cm)	2.5 $\pm$ 0.9	0.6 $\pm$ 1.0	0.010
Percent second-flushing terminal buds	28.4	81.5	< 0.001

¹ Includes both dormant and outgrowing buds observed 5–6 weeks after spray treatment.

Although the BA treatment caused vigorous initial outgrowth of most of the terminal and lateral buds, these buds usually did not elongate beyond 1 cm (Table 2). Buds of the control leader shoots that grow out during natural second flushing elongated to a much greater extent than buds on the BA-treated shoots (Figure 2C; and cf. terminal bud lengths of control second-flushing leader shoots with those of the BA-treated leader shoots in Table 2).

The inhibitory effect of BA on leader shoot elongation was especially apparent when the BA treatment was given before the initiation of flushing or early in the flushing period. The BA treatment applied at this time caused almost complete inhibition of stem elongation as well as a relatively persistent twisting pattern of the upper shoot needles in some of the seedlings (Figure 3). The optimal time for initiation of the BA spray treatment was about midway during the flushing period when the length of the elongating shoot was between 5 and 10 cm. Even then, the BA treatment caused a 25% reduction in subsequent elongation of the flushing shoots (Table 2), indicating that the retarding effect of BA on shoot elongation affected both the current terminal and lateral shoots. In another experiment where the BA treatment was applied for only 1 week, the results were similar as those obtained in the 2-week treatment (data not shown). Application of gibberellic acid (GA4/7) to buds and shoots under various conditions had little or no significant effect on bud growth (data not shown).

Defoliation

Complete defoliation of the Douglas-fir leader shoots midway through flushing, at about the same developmental stage at which the BA treatment was given, significantly ($P < 0.001$) enhanced second flushing of lateral buds and to a lesser extent that of terminal buds (Figure 4; Table 3). Defoliation had no effect on total bud number.

Discussion

We obtained evidence that auxin and cytokinin are involved in the control of current bud growth (second flushing) in the terminal spring-flushing shoot of the conifer Douglas-fir. Decapitation of the terminal spring-flushing shoot resulted in the outgrowth of some lower lateral buds, indicating the release of apical dominance. Immediate treatment of the stump of the decapitated shoot with auxin completely restored apical dominance (Thimann and Skoog 1933), indicating a significant role for native auxin in the shoot apex in apical dominance. These data support Hypothesis 1 that endogenous auxin produced in the shoot apex moves down the shoot and inhibits the outgrowth of the lower lateral buds.

Zimmerman and Brown (1971) pointed out that "...lateral buds on the previous year's twig following a period of winter dormancy or rest are no longer under the auxin inhibition of

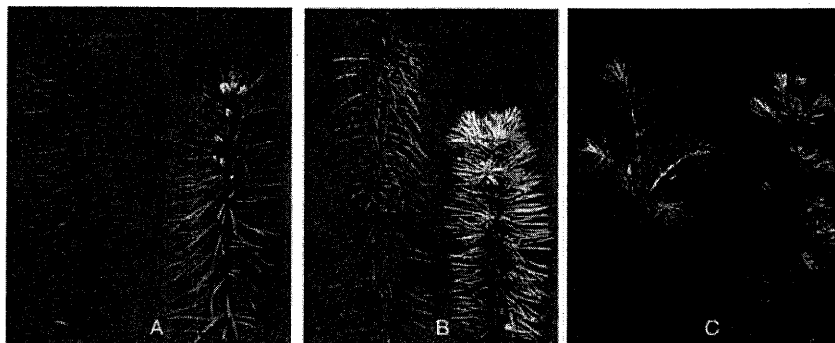


Figure 2. Effect of 0.5 mM benzyladenine (BA) on current lateral bud outgrowth (A) 18 days after the end of a 2-week BA treatment; (B) 43 days after the end of a 2-week BA treatment; and (C) 40 days after the end of a 1-week BA treatment. Left: control shoot. Right: BA-treated shoot.

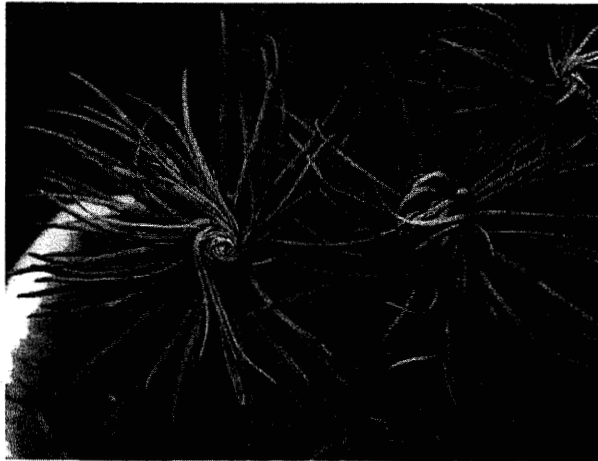


Figure 3. Twisting effect of 0.5 mM benzyladenine (BA) on needle growth 16 days after the end of a BA treatment.



Figure 4. Effect of complete defoliation of the entire terminal spring flushing shoot on current lateral bud outgrowth after 33 days. Left: control shoot. Right: previously defoliated shoot.

the terminal bud; rather some of the uppermost lateral buds usually release synchronously with the terminal bud (during spring flushing)...” Although it is not known how winter exposure and rest makes over-wintered lateral buds insensitive to apical dominance and auxin, we confirmed this phenomenon. Thus, applied auxin had little or no repressive effect on over-wintered lateral bud outgrowth in decapitated Douglas-fir shoots (data not shown).

However, the newly formed current lateral buds located on the terminal spring-flushing shoots were sensitive to apical dominance and auxin, as demonstrated by our finding that when these flushing leader shoots were decapitated midway during the flushing period (between the lengths of 5 and 10 cm and well before attaining their mean final length of 17–18 cm), the highest lateral buds below the point of decapitation flushed. However, if auxin (NAA) was applied according to the Thimann-Skoog protocol, lateral bud outgrowth was completely repressed. Thus, when discussing the role of auxin in bud development, it is important to distinguish between the auxin-sensitive current lateral buds of the flushing shoots and the auxin-insensitive over-wintered lateral buds.

As noted by Allen and Owens (1972), during early Douglas-fir bud development, removal of the terminal vegetative bud can cause latent axillary buds to grow out. It has also been demonstrated (Cline and Deppong 1999) that the probability that decapitation will release apical dominance in shoots of woody species is higher in seedlings than in mature trees. Furthermore, we found that 4–6 weeks was required for apical dominance release by decapitation in the conifer Douglas-fir compared with 1 week or less in Japanese morning glory (*Ipomoea nil* (L.) Roth.) (Cline 1996).

Our finding that cytokinin treatment of flushing Douglas-fir shoots promoted terminal and lateral bud outgrowth (second flushing) corroborates previous observations. For example, Lavender and Zaerr (1967) reported a cytokinin-induced “proliferation of lateral buds near apices” of 2-month-old seedlings; however, proliferation is a vague term that could refer to the promotion of bud formation or bud flushing, or both. Mazzola and Costante (1987) reported promotion of both bud formation and second flushing in 6-year-old Douglas-fir seed-

Table 3. Effect of defoliation on bud outgrowth in flushing terminal shoots. Measurements were made 33–35 days after defoliation. Values (means $\pm$ SE) are pooled from two experiments.

	Intact control	Defoliated	P value
No. of seedlings	14	16	
No. of second-flushing lateral buds	2.4 $\pm$ 0.7	7.0 $\pm$ 0.7	< 0.001
No. of current buds ¹	14.5 $\pm$ 1.1	15.1 $\pm$ 1.1	0.676
Flushing terminal shoot length (cm) at time of defoliation	8.1 $\pm$ 0.3	8.5 $\pm$ 0.3	0.317
Flushing terminal shoot length (cm) 33–35 days after defoliation	15.1 $\pm$ 0.8	14.8 $\pm$ 0.8	0.785
Terminal shoot fresh mass (g)	3.9 $\pm$ 0.6	5.4 $\pm$ 0.6	0.025
Fresh mass of second-flushing lateral buds (g)	0.1 $\pm$ 0.1	0.7 $\pm$ 0.1	0.001
Fresh mass of second-flushing terminal bud (g)	0.09 $\pm$ 0.06	0.3 $\pm$ 0.06	0.008
Length of second-flushing terminal bud (cm)	0.6 $\pm$ 0.3	2.2 $\pm$ 0.3	0.002
Percent second-flushing terminal buds	46.7	86.7	0.010

¹ Includes both dormant and outgrowing buds measured 33–35 days after defoliation.

lings. They observed growth promotion only when the whole tree was sprayed once with 1000 ppm BA and not when the treatment was confined to the terminal leader (as in our study).

The slight, but significant, increase in total bud count in response to the BA treatment could be a result of increased bud initiation or enlargement of latent buds. These marginal results contrast with the apparently strong promotive effects of BA on bud formation reported by Mazzola and Contante (1987) and with studies showing that cytokinins promote bud formation and the beginning of bud outgrowth in several plant systems (Grayburn et al. 1982, Cline 1991, Taiz and Zeiger 2002). Although detection of very small buds is difficult, a possible explanation for the discrepancy is that the bulk of lateral bud formation observed in our study occurred before the BA spray treatment was applied.

To observe promotion of bud outgrowth by BA, we found that the timing of application of the 1- and 2-week cytokinin treatments to the flushing shoot was critical. Treatment could not be initiated until midway through the flushing period. If given earlier, the elongation of the flushing shoot was inhibited, which would presumably interfere with lateral bud growth. A 2- to 4-day BA spray period would probably allow a greater and a more natural degree of lateral branch outgrowth in second flushing than occurred in our 1- and 2-week spray treatments. After testing BA applications in different concentrations, both as drops and sprays, 0.5 mM BA foliar spray was found to be optimal under our greenhouse conditions.

We were unable to determine if the defoliation-induced second flushing shared common mechanisms with the second flushing induced by cytokinin; however, the responses to both treatments were similar in several respects. Likewise, the restoration of apical dominance by exogenous auxin treatments is consistent with the hypothesis of an important role for auxin repression of second flushing in spring-flushing shoots of Douglas-fir.

References

- Allen, G. and J. Owens. 1972. The life history of Douglas-fir. Environment Canada, Forestry Service, Ottawa, 56 p.
- Bollmark, M., H.-J. Chen, T. Moritz and L. Eliasson. 1995. Relations between cytokinin level, bud development and apical control in Norway spruce, *Picea abies*. *Physiol. Plant.* 95:563–568.
- Brown, C., R. McAlpine and P. Kormanik. 1967. Apical dominance and form in woody plants: a reappraisal. *Am. J. Bot.* 54:153–162.
- Chiwacha, S. and P. von Aderkas. 2002. Endogenous levels of free and conjugated forms of auxin, cytokinins and abscisic acid during seed development in Douglas-fir. *Plant Growth Regul.* 36: 191–200.
- Cline, M. 1991. Apical dominance. *Bot. Rev.* 57:318–358.
- Cline, M. 1996. Exogenous auxin effects on lateral bud outgrowth in decapitated shoots. *Ann. Bot.* 78:255–266.
- Cline, M. 2000. Execution of the auxin replacement apical dominance experiment in temperate woody species. *Am. J. Bot.* 87:182–190.
- Cline, M. and D. Deppong. 1999. The role of apical dominance in paradormancy of temperate woody plants: a reappraisal. *J. Plant Physiol.* 155:350–356.
- Coenen, C. and T. Lomax. 1997. Auxin-cytokinin interactions in higher plants: old problems and new tools. *Trends Plant Sci.* 2: 351–356.
- DeYoe, D. and J. Zaerr. 1976. Indole-3-acetic acid in Douglas fir. *Plant Physiol.* 58:299–303.
- Goldfarb, B., G. Howe, L. Bailey, S. Strauss and J. Zaerr. 1991. A liquid cytokinin pulse induces adventitious shoot formation from Douglas-fir cotyledons. *Plant Cell Rep.* 10:156–160.
- Grayburn, W., P. Green and G. Steucek. 1982. Bud induction with cytokinin. *Plant Physiol.* 69:682–686.
- Hare, R. 1984. Stimulation of early height growth in longleaf pine with growth regulators. *Can. J. For. Res.* 14:459–462.
- House, S., M. Dieters, M. Johnson and R. Haines. 1998. Inhibition of orthotropic replacement shoots with auxin treatment on decapitated hoop pine, *Araucaria cunninghamii*, for seed orchard management. *New For.* 16:221–230.
- Johnson, P. and R. Denton. 1975. Outbreaks of the western spruce budworm in the American northern Rocky Mountain area from 1922 through 1971. USDA For. Serv. Gen. Tech. Rep. INT-20, 144 p.
- Kossuth, S. 1978. Induction of fascicular bud development in *Pinus sylvestris* L. *HortScience* 13:174–176.
- Kramer, P. and T. Kozlowski. 1979. Physiology of woody plants. Academic Press, New York, 811 p.
- Lavender, D. and J. Zaerr. 1967. The role of growth regulatory substances in the physiology of Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) seedlings. *Proc. Int. Plant Propagation Soc.* 17: 146–156.
- Little, C. 1985. Increasing lateral shoot production in balsam fir Christmas trees with cytokinin application. *HortScience* 20: 713–714.
- Little, C. and R. Pharis. 1995. Hormonal control of radial and longitudinal growth in the tree stem. In *Plant Stems: Physiology and Functional Morphology*. Ed. B.L. Gartner. Academic Press, San Diego, pp 281–319.
- Mazzola, M. and J. Costante. 1987. Efficacy of BA for the promotion of lateral bud formation on Douglas-fir and Colorado blue spruce. *HortScience* 22:234–235.
- Morris, J., P. Doumas, R. Morris and J. Zaerr. 1990. Cytokinins in vegetative and reproductive buds of *Pseudotsuga menziesii*. *Plant Physiol.* 93:67–71.
- Mulgrew, S. and D. Williams. 1985. Effect of benzyladenine on the promotion of bud development and branching of *Picea pungens*. *HortScience* 20:380–381.
- Pilate, G., B. Sotta, R. Maldiney, J. Jacques, L. Sossountzov and E. Miginiac. 1989. Abscissic acid, indole-3-acetic acid and cytokinin changes in buds of *Pseudotsuga menziesii* during bud quiescence release. *Physiol. Plant.* 76:100–106.
- Qamaruddin, M., I. Dormling and L. Eliasson. 1990. Increases in cytokinin levels in Scots pine in relation to chilling and budburst. *Physiol. Plant.* 79: 236–241.
- Romerger, J. 1963. Meristems, growth and development in woody plants. USDA Tech. Bull. 1293, US Govt. Printing Office, Washington, DC, 214 p.
- Ross, S., R. Pharis and W. Binder. 1983. Growth regulators and conifers: their physiology and potential uses in forestry. In *Plant Growth Regulating Chemicals*. Vol. 2. Ed. L. Nickell. CRC Press, Boca Raton, FL, pp 35–78.
- Skoog, F. and C. Miller. 1957. Chemical regulation of growth and organ formation in plant tissues in vitro. In *Symp. Soc. Exp. Biol.* XI. The Biological Action of Growth Substances. Academic Press, New York, 344 p.

- Sundberg, B. and C. Ugglå. 1998. Origin and dynamics of indoleacetic acid under polar transport in *Pinus sylvestris*. *Physiol. Plant.* 104:22–29.
- Tamas, I. 1995. Hormonal regulation of apical dominance. *In* Plant Hormones. Ed. P. Davies. Kluwer Academic Publishers, London, pp 572–597.
- Taiz, L. and E. Zeiger. 2002. Plant physiology. 3rd Edn. Sinauer Associates, MA, 690 p.
- Thimann, K. and F. Skoog. 1933. Studies on the growth hormones of plants. III. The inhibition action of growth substance on bud development. *Proc. Nat. Acad. Sci. USA* 19:714–716.
- Wareing, P. 1970. Growth and its coordination in trees. *In* Physiology of Tree Crops. Eds. L. Luckwill and C. Cutting, Academic Press, New York, pp 1–21.
- Whitehill, S. and W. Schwabe. 1975. Vegetative propagation of *Pinus sylvestris*. *Physiol. Plant.* 35:66–71.
- Zhang, H., K. Horgan, P. Reynolds and P. Jameson. 2003. Cytokinins and bud morphology in *Pinus radiata*. *Physiol. Plant.* 117: 264–269.
- Zimmerman, M. and C. Brown. 1971. Trees: structure and function. Springer-Verlag, New York, 336 p.