

The possible roles of nutrient deprivation and auxin repression in apical control

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Abstract Apical control is the suppression of growth in lower branches by a higher dominant branch or leader shoot. We investigated possible mechanisms involved in this developmental response in three widely diverse species (Japanese morning glory, *Ipomoea nil*, hybrid poplar, *Populus trichocarpa*, x *P. deltoides*, and Douglas-fir, *Pseudotsuga menziesii*). The following two hypotheses were tested: (1) the mineral nutrient-deprivation hypothesis, which is that the continued growth of the lower branches is repressed by the diversion of nutrients to the upper dominating branch or shoot, and (2) the auxin-repression hypothesis, which is that auxin produced in the upper dominating branch or shoot moves down to the lower branches where continued growth is repressed. The results of experiments involving the manipulation of available nutrients by dominant branch removal and fertilization were consistent with the first hypothesis for morning glory, poplar, and for second- or third flushing of lateral branches in Douglas-fir. The results of the experiments involving auxin (NAA, 1-naphthalene acetic acid) replacement treatments on decapitated shoots bearing growing lateral branches were inconsistent with the second hypothesis in morning glory, poplar and in first-flushing Douglas-fir. However, despite concerns about possible NAA toxic effects, there was

evidence of auxin repression of second flushing in Douglas-fir. Overall, the data supported a significant role for nutrient availability but not for auxin repression in apical control of morning glory and poplar. In Douglas-fir, apical control in first-flushing lateral branches from over-wintered buds was largely insensitive to both nutrient availability and auxin repression; however, second flushing was sensitive to both.

Keywords Apical control · Branching · Lateral buds · Nutrients · Auxin

Introduction

Apical control has been defined as "...the suppression of existing branch growth imposed by the growth of a higher dominating branch or shoot" (Cline and Sadeski 2002; Leaky and Longman 1986; Suzuki 1990; Wilson 1990, 2000). The rationale for discriminating between "apical control" and the more familiar "apical dominance" has been reviewed (Brown et al. 1967; Cline and Sadeski 2002; Wilson 2000). Based mostly on work with herbaceous plants, the term "apical dominance" historically has been employed to describe the repression by the growing shoot apex over the outgrowth of the lower lateral buds. However, in the case of woody species with decurrent and excurrent perennial branching, it became evident to Brown et al. (1967) that this term was inadequate to describe the dominance of a leader shoot over the growth of lower subdominant branches. Hence, the term "apical control" was introduced.

Physiologically, the definitions employed in the present study envision apical dominance as focusing on the regulation by the growing shoot apex on the very early processes of lateral bud outgrowth, and apical control as

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that by the growing shoot apex (or upper dominant branch) on the subsequent processes of an already-growing lower branch. Hence, apical dominance is concerned with how the growing shoot apex inhibits a lateral bud from starting to grow out whereas apical control is concerned with how the upper growing shoot represses the extension of a lower branch after its growth is already under way. Suzuki (1990) suggested that apical control is "one step removed" from apical dominance and Wilson (2000) concluded that "the key problem (of control) is what inhibits further growth of lower, proximal lateral shoots". One of the significant outcomes of apical control is that it provides for mechanisms that result in the continued dominance of the leader shoot over the lower lateral branches. Apical control may also involve alterations in branch angle and diameter (Wilson 2000). Although apical control has been mostly studied in woody species, it also may occur in herbaceous species (Thimann and Skoog 1934; Saks and Ilan 1984).

Relatively little is known about the mechanisms of apical control (Cline and Harrington 2007). Because some similarities do exist between apical control and apical dominance and because of the evidence for a strong indirect role for auxin as a repressor of lateral bud outgrowth in the latter (Ongaro and Leyser 2008), it has been presumed by many workers that auxin also plays a repressor role in apical control. However, there have been very few studies to test for such a role for auxin in apical control (Wilson 2000). In one such study with herbaceous *Ipomoea nil*, clear evidence was found for auxin inhibition of bud growth in apical dominance but not in apical control (Cline and Sadeski 2002).

Wilson and Gartner (2002) found data suggesting possible nutritional (carbohydrate) involvement in apical control based on girdling experiments in several conifers. Other indirect evidence for a role for nutrition in apical control in woody species has been recently reviewed (Cline and Harrington 2007).

The hypotheses tested in the present study were that the higher dominant branch or leader shoot exercised apical control over the subdominant branch or lower branches by suppressing their elongation by either (1) depriving the lower subdominant branches of mineral nutrients via diversion to the higher dominant branch or (2) controlling subdominant branches via auxin produced by the dominant branch wherein apically derived auxin is transported down the stem basipetally and over to the subdominant branches where it is acropetally transported up to repress elongation in the growing region. These two sub-hypotheses were tested in herbaceous *I. nil* (Japanese Morning Glory) and in woody *Populus trichocarpa* × *P. deltoides* (hybrid poplar) and *Pseudotsuga menziesii* (Douglas-fir). Since we previously have evaluated the role of auxin in apical control of *Ipomoea* (Cline and Sadeski 2002), the results of that study

are simply referenced here. In Douglas-fir, the influence of nutrients and auxin also were evaluated for their effect on apical control of second flushing (flushing of buds initiated in the spring) and lateral branch angle.

Materials and methods

Morning glory

Seeds of *I. nil* L. Roth. Strain Violet (syn. *Pharbitis nil*) from Marutane, Kyoto, Japan were scarified in concentrated sulfuric acid for 35 min, rinsed in water and germinated in Petri dishes for several days before planting in 10.2-cm diameter pots filled with Pro-mix (Premier, BX, Premier Horticulture Inc., Quakertown, PA), a general purpose peat-vermiculite growing medium. The growing plants were maintained in a greenhouse at 25–28°C, under 16-h photoperiods with supplementary General Electric (Hendersonville, NC, USA) 400 W mercury vapor lamps. Specified fertilizer (N, P₂O₅, K₂O; 20, 10, 20; 180 mg l⁻¹) treatments in water were given every day or so as needed. Experiments were generally started when plants were several weeks old.

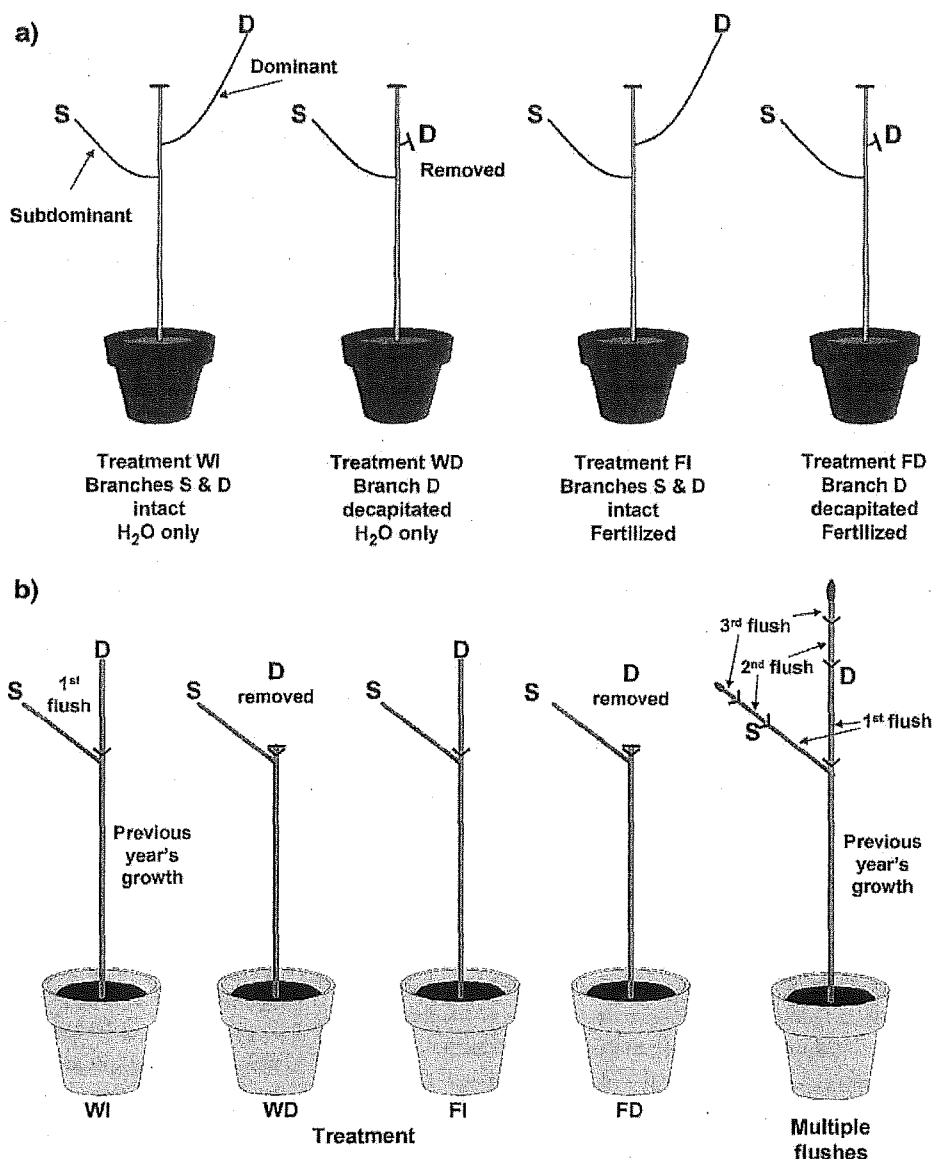
Poplar

Two clones of hybrid poplar, *Populus trichocarpa*, Torr. & Gray × *P. deltoides*, Bartr. ex Marsh, with contrasting degrees of sylleptic branching were utilized: clone 11-11 with a high amount of sylleptic branching and clone 47-174 with a low amount of sylleptic branching (clonal material developed by the University of Washington-Washington State University *Populus* breeding program, syllepticity described by Ceulemans 1990). These clones were propagated from greenwood cuttings under a periodic water spray with greenhouse lighting supplemented by General Electric 400-W mercury vapor lamps. The cuttings were subsequently transplanted to 3.8-l pots in the above-mentioned Pro-mix growing medium with the above-described greenhouse growing conditions. The 11-11 clone was used in the fertilization studies and the 47-174 clone was employed in the auxin studies. Specified fertilization treatments were given as described above for morning glory. With the auxin experiments, plants were fertilized once a week.

Douglas-fir

Two-year-old (1 + 1), bare-root *Pseudotsuga menziesii* Mirb. (Franco) seedlings were lifted from the Weyerhaeuser Company Mirna Nursery near Olympia, Washington and shipped overnight to Columbus, Ohio where they were

Fig. 1 a Nutrition tests for morning glory and poplar. Branch D, upper dominant; branch S, lower subdominant. Treatments: *WI* = water only, branches S and D intact; *WD* = water only, branch D removed; *FI* = fertilized, branches S and D intact; and *FD* = fertilized, branch D removed. b Nutrition tests for Douglas-fir. Treatments as above except D = leader. Multiple flushes: branch S and leader shoot D with second and third growth flushes



potted in the above-mentioned Pro-mix growing medium in 4-l DeepotsTM and maintained in the greenhouse at 20–25°C, under the above-described light conditions. Fertilizer treatments were similar to those for morning glory and poplar. Two half-sib Douglas-fir families were utilized for the greenhouse trials; the families differed substantially in past evaluations of their tendency to produce ramiform (RC) branches. RC branches are high-angle branches which form when an upper lateral bud close to the terminal bud (TB) grows out the same season it is initiated but the TB does not grow out until the following spring. The RC

branch can compete with the leader shoot for dominance and even if the leader shoot maintains dominance, the presence of the RC branch can reduce log quality as it usually results in a large-diameter branch separate from the ones at the annual whorl. The over-wintered buds of these

seedlings began to swell and subsequently to burst in their first flush of growth several weeks after potting and being moved into the greenhouse. A second, and sometimes a third, flushing of the lateral and TBs commonly followed in the late spring and early summer. One auxin experiment was carried out outside on an approximately 10- to 15-year-old sapling located about 30 km southeast of Columbus.

Nutrient studies

In order to detect whether mineral nutrient deprivation in subdominant branch S might account for its repression in apical control by dominant branch D (Fig. 1a, b), the following four nutrient or fertilizing treatments were given to growing seedlings of all three species: (1) water only with

branches D and S intact (hence referred to as treatment WI); (2) water only with branch S intact but with the removal of branch D (treatment WD); (3) continuous fertilization with both branches intact (treatment FI); (4) continuous fertilization with branch S intact but with the decapitation of branch D (treatment FD). The upper, rapidly elongating, dominant shoot or branch was designated as D, whereas the lower, more slowly elongating subdominant branch as S (Fig. 1a, morning glory and poplar; Fig. 1b, Douglas-fir). An initial dominance test for each of the species was carried out to determine whether or not the higher branch D was dominant over the lower branch S; this was accomplished by demonstrating that the elongation of the branch D in treatment WI, was more rapid than the elongation of branch S.

Based on the data from these four treatments, the following three tests were applied to the nutrient deprivation hypothesis for the sub-dominance of branch S:

1. Did the removal of branch D in the WD treatment result in an acceleration of growth in branch S as compared to its growth in WI treatment with branch D intact? If so, this could be interpreted as an interruption to the diversion of nutrients away from branch S to branch D and, hence, a restoration of nutrient availability and of growth to branch S. Alternatively, branch D decapitation might also result in the removal of the source of a growth inhibitor, possibly auxin.
2. Did the presence of added fertilizer in treatment FI promote greater elongation in branch S than in branch D compared to that observed in the WI treatment? If so, this result would be consistent with the hypothesis that the nutrient-starved subdominant branch S would be more responsive to the added fertilizer than the nutrient-monopolizing dominant branch D.
3. Did the presence of fertilizer in treatments PI and FD have a reducing effect on the promotion of branch S growth by removal of branch D as compared to the water-only treatments WI and WD? If nutrient availability was the primary limitation to outgrowth of branch S, then removal of branch D would presumably have a greater promotive effect on branch S elongation in the WI treatment, where nutrient availability was more limiting, than in treatment FD.

Because morning glory and poplar had moderately strong apical dominance in our greenhouse environment, they exhibited little or no lateral branching. In order to evaluate the effects and role of nutrients and auxin on elongating branches as described above, it was necessary to decapitate the main shoot of these seedlings to promote the outgrowth of branches S and D (Fig. 1a). However, this was not necessary with the spring flushing Douglas-fir leader shoot wherein there appeared to be little or no apical

dominance and nearly all of the lateral buds grew out. All the outgrowing lateral buds below branch S and the main shoot D in the upper 8–10 cm of the Douglas-fir shoot were removed (Fig. 1b).

Elongation of branches S and D were measured with a ruler during and following the treatments over a period of 7–13 days for morning glory, 11–21 days for poplar and 95–115 days for the slower growing Douglas-fir.

The effects of the four nutrient treatments in Douglas-fir on second and third flushing, when it occurred (Fig. 1b, multiple flushing), were also determined in four replicate experiments. Elongation of TBs of branches S and D was monitored as well as the total number of lateral buds which grew out.

The effects of the nutrient treatments on the angle to the vertical for branch S were measured with a protractor in three of the Douglas-fir experiments. An imaginary vertical line was employed for measurement of angle S in cases where shoot D had been removed.

Auxin studies

To determine whether auxin transported from the higher dominant shoot might function as a repressor of elongation in the lower subdominant branch(es), the classic Thimann and Skoog (1934) auxin-replacement test was carried out on the decapitated dominant shoot with subsequent measurements of these branches. Periodic fertilization treatments were given.

With poplar, two procedures were employed. The first procedure (Fig. 2a) was similar to that of treatment WD in the nutrient investigation, except that immediately following the decapitation of branch D about 0.5 cm from the main shoot, auxin (1% 1-naphthalene acetic acid, NAA) in lanolin was applied directly to the stem stump to determine whether repression of branch S elongation occurred 7–11 days later.

In the second procedure (Fig. 2b), two sub-tests were run—the first subtest to demonstrate the efficacy of 1% NAA in lanolin to inhibit the release of apical dominance and the second, to test its efficacy to inhibit continued branch elongation in apical control. The first sub-test, with three experiments of 55 plants, was carried out on shoots with unexpanded lateral buds by applying the NAA-lanolin mixture to the upright shoot stump immediately following decapitation 1–2 cm above the bud with measurements 9–15 days later. The second sub-test with four experiments of 61 plants was done on the upright shoot by delaying the NAA-lanolin application until after a second decapitation which occurred 9–13 days after the first decapitation, thus allowing time for the highest lateral bud to start growing out as defined for apical control. Bud length measurements were made 2–6 days after the second decapitation to test

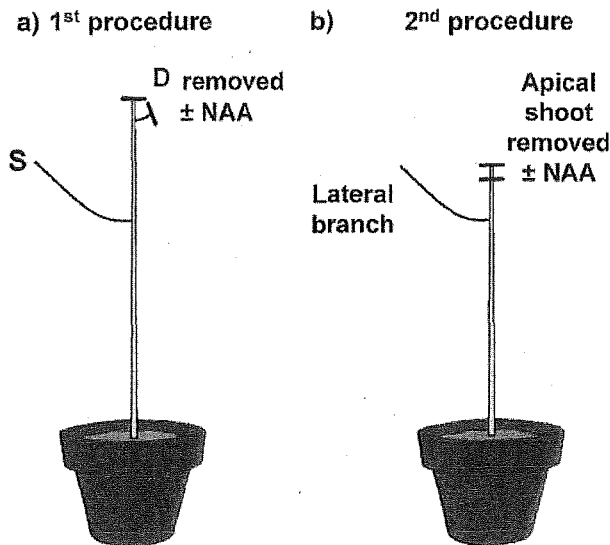


Fig. 2 a Auxin tests for poplar: First procedure with decapitated stump of dominant branch D treated with lanolin and with or without 1% NAA. b Second procedure with the stem stump of the apical shoot treated with lanolin and with or without 1% NAA following the second decapitation

the bud's sensitivity to the possible inhibitory effects of the apically applied auxin. The advantage of this second procedure was that it only involved basipetal auxin transport straight down the main shoot to the subdominant branch without having the extra transport step of moving across from another branch. For this second procedure, the terms "lateral branch" and "apical shoot", respectively, were employed rather than the designated branches "S" and "D",

For Douglas-fir, the effects of auxin on apical control were analyzed in the leader shoots both for first (Fig. 3a) and second flushing (Fig. 3b) and for third flushing when it occurred. The timing and method of auxin application were based on extensive preliminary trials carried out during the previous year (2006). All lateral buds/branches were allowed to remain intact before and after decapitation of the leader shoot TB and NAA application. This is in contrast to the nutrition experiments described above where all lateral buds in the vicinity of lateral branch S were removed except for branch S.

In first-flushing leader shoots of both low- and high RC branch producing families, a sub-test was initially carried out in two combined overlapping experiments involving 106 seedlings to detect possible inhibitory effects of auxin (0.5 or 1% NAA in lanolin), when the treatment was applied to the base of the decapitated TB of the leader shoot just prior to lateral bud outgrowth. This would test primarily for the role of auxin in apical dominance and indirectly for apical control. The percentage of lateral buds growing out as well as the mean length of all emerging lateral buds on

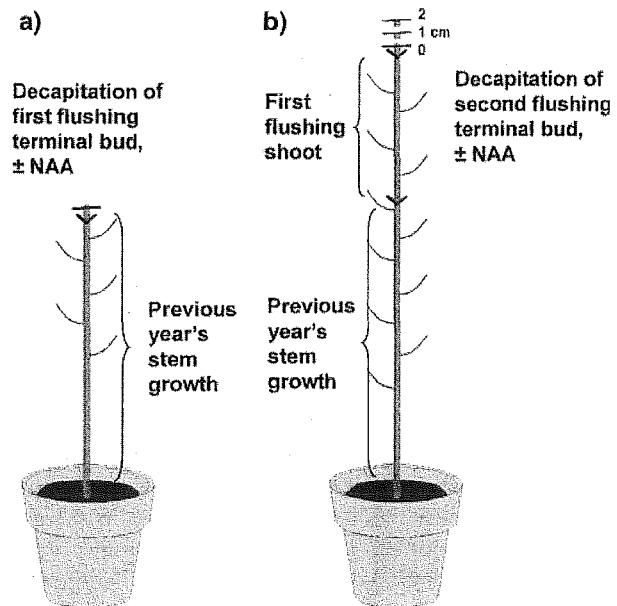


Fig. 3 a Auxin tests in Douglas-fir with and without 0.5 or 1% NAA in lanolin applied to the stem stump of the decapitated first-flushing terminal bud of the leader shoot. b Auxin tests in Douglas-fir with and without 0.5 or 1% NAA in lanolin applied to the stem stump of the second-flushing terminal bud of the leader shoot decapitated at 0, 1 or 2 cm above the base of the bud

the first-flushing leader shoots were measured after 31–32 days. In order to evaluate the possibility of toxic effects associated with NAA on locally positioned lateral buds, the mean distance between the point of NAA treatment on the decapitated stump and the emergence of the first outgrowing bud below was also determined.

For standard determinations of apical control in the first-flushing leader shoots, auxin was applied to the base of the decapitated TB of the main flushing shoots after lateral bud outgrowth had begun and was under apical control. The lengths of all emerging lateral buds on the shoots were measured at the time of the decapitation/auxin treatment (Fig. 3a) and again 14–15 days later in replicated studies of both high and low RC families.

A separate first-flushing apical-control experiment was done on 45 branches of an approximately 12-year-old Douglas-fir sapling growing in an open field about 30 km SE of Columbus, OR. The randomly assigned branch treatments were 15 intact, 15 decapitated at the base of the first-flushing TB and treated with lanolin, and 15 decapitated and treated with 1% NAA. After 52 days, 5 May–26 June 2007, measurements were made of each of the growing lateral buds which ranged in number from 6 to 20 per branch and, initially, were less than 4 cm in length and finally, about 5–20 cm.

The effects of auxin on the angle to the vertical of the highest designated lateral branch of the first flush were

analyzed in a greenhouse trial involving 60 Douglas-fir seedlings from the low RC family. Branch angle was measured with a protractor at the beginning and at the end of treatments after 91 days involving decapitation of the TB of the main shoot followed by immediate application of auxin.

To study the effects of auxin on Douglas-fir second flushing, shortly after the beginning of the second flushing of the TB of the main shoot, located at the apex of the first-flushing shoot, this TB was decapitated at its base (0 cm) or at 1 or 2 cm above its base and immediately treated with auxin (Fig. 3b). At this time, the length of the emerging second-flushing lateral buds were generally less than 3 cm.

These treatments were carried out on 245 seedlings, 137 from the low RC and 108 from the high RC family. The variable NAA concentrations and shoot distances of NAA treatment were employed in an attempt to minimize the possible problems associated with NAA toxicity and those of auxin transport distance. It was hoped that this approach would aid in discriminating between the repressive physiological effects of auxin and those simply due to its toxicity.

The lengths of all lateral buds growing out (usually about three to five buds per seedling) on each of the first-flushing leader shoots of all 245 seedlings were measured, initially at the time of NAA treatment, when they were generally less than 3 cm and finally after 48–81 days, when they ranged from 6 to 8 cm in length. The considerable lack of synchrony in the timing of outgrowth of second-flushing lateral buds between different seedlings in Douglas-fir did not lend itself to utilizing precisely uniform growth periods for the various treatments.

Statistical analysis

Values are reported as the mean for all plants $\pm$ the standard error. Following a test of data normality by normal probability plots, two-sample *t* tests and ANOVA were carried out using Minitab 15 (Minintab Inc., PA) statistical computing software with significance specified as $\alpha = 0.05$. Percentage values given for the comparison of various treatments were calculated from the means.

Results.

Nutrition studies

I. nil (Japanese morning glory)

The upper dominant branch D exhibited apical control over the lower subdominant branch S; this was demonstrated by a significant increase ($P < 0.05$) in elongation of branch D

over that of branch S during the 11-day water-only treatment WI (Figs. 1a, 4).

The nutrient-deprivation hypothesis was strongly supported by the following evidence from the three nutrition tests described previously in the materials and methods section:

1. In treatment WD, when the dominant branch D was removed, the elongation of subdominant branch S was significantly ($P < 0.05$) greater than that of branch S in treatment WI where branch D remained intact (Figs. 1a, 4).
2. Comparisons of results from treatment WI to those in treatment FI revealed that elongation of branch S was much more enhanced (481%) by fertilization than was branch D (185%) (Figs. 1a, 4; $P < 0.05$).
3. In treatment PD, the increase in elongation of the subdominant branch S due to the removal of dominant branch D with fertilization was only 45% above that in treatment FI, whereas in treatment WD, the promotion of elongation of branch S due to the removal of branch D without fertilization was 236% above that in treatment WI (Figs. 1a, 4).

Populus trichocarpa $\times$ *P. deltoides* (Hybrid Poplar, clone 11-11)

The upper dominant branch D exhibited significant ($P < 0.05$) apical control over the lower subdominant branch S by reducing branch elongation during a water-only treatment WI (Figs. 1a, 5).

The nutrient deprivation hypothesis was supported in the three nutrition tests by the following evidence:

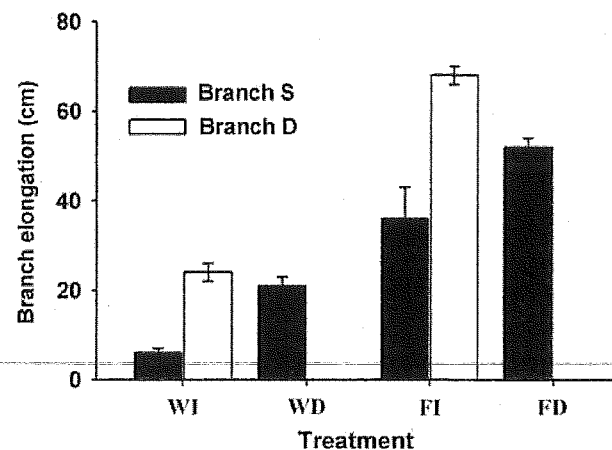


Fig. 4 Effect of nutrition treatments on elongation of subdominant branch S and dominant branch D in morning glory. Treatments as defined in Fig. 1a

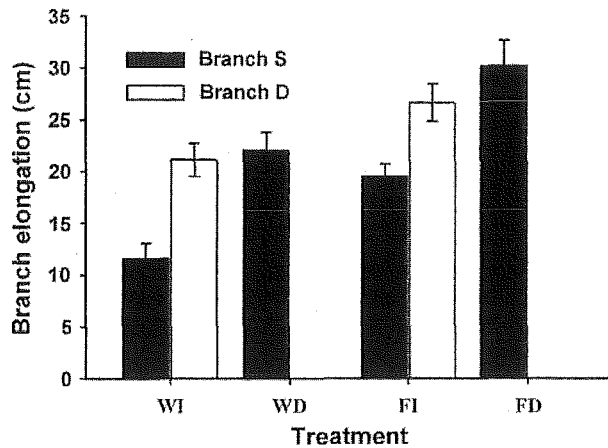


Fig. 5 Effects of nutrition treatments on elongation of subdominant branch S and dominant branch D in poplar. Treatments as defined in Fig. 1a. Values are mean $\pm$ SE

1. The elongation of branch S was significantly greater ($P < 0.05$) (Figs. 1a,S) in treatment WD than in treatment WI.
2. Elongation of branch S was increased 68% by fertilization when treatment PI is compared to treatment WI; whereas elongation of branch D was increased by only 26% (Figs. 1a,S).
3. In treatment FD, the relative enhancing effect of removal of branch D on the elongation of branch S was lessened from 90% with the water-only treatment to 54% with fertilization (Figs. 1a,S). This difference was not statistically significant.

Pseudotsuga menziesii (Douglas-fir)

Leader shoot D from the first spring flush exhibited apical control over the lower subdominant lateral branch S; this was demonstrated by the significant ($P < 0.05$) increase in the elongation of shoot D over that of the branch S in the water-only treatment WI (Figs. 1b, 6a).

The nutrient-deprivation hypothesis for the first spring flush of this slower-growing conifer was not strongly supported in the three nutrition tests.

1. In treatment WD, there was a relatively small (28%) increase in the elongation of branch S over that in treatment WI where the leader shoot D was intact (Figs. 1b, 6a).
2. The promotive effects of treatment PI on the elongation of shoot D (70%) were slightly greater (and significantly so) than those on shoot S (61%) (Figs. 1b, 6a).
3. Growth of branch S was somewhat greater (40%) in treatment FD than in treatment WD (28%; Figs. 1b, 6a).

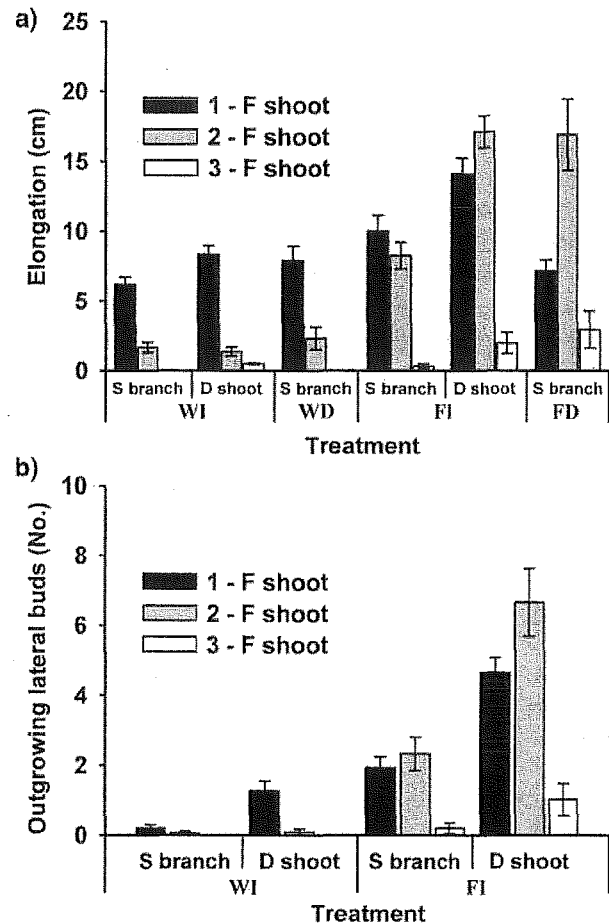


Fig. 6 a Effects of nutrition treatments on branch S and leader shoot D elongation in first flushing (1-F), second flushing (2-F), and third flushing (3-F) Douglas-fir seedlings. b Effects of fertilization on the number of outgrowing lateral buds on first (1-F), second (2-F) and third (3-F) flushing branch S and leader shoot D in Douglas-fir. Treatments as defined in Fig. 1b. Values are mean $\pm$ SE

Second flushing occurred in a majority of seedlings, particularly in those receiving fertilizer treatments. In treatment WI, the second-flushing TB of leader shoot D did not elongate any more rapidly than did the second-flushing TB of branch S (Figs. 1b, 6a). The removal of the first-flushing leader shoot D in the WD treatment did promote second-flushing terminal-bud elongation of branch S to a small extent. However, the PI treatment vigorously promoted second-flushing in the TBs of both S and D and particularly in D (Figs. 1b, 6a). Hence, fertilization did enhance second-flushing apical control by the higher dominant shoot D over the lower branch S.

Third flushing of shoot D and branch S was essentially non-existent in the water-only treatments WI and WD but was exhibited by some of the seedlings in fertilized treatments PI and FD (Figs. 1b, 6a).

Fertilization also strongly promoted lateral bud outgrowth, not only in the first-flushing lateral branch S and

leader shoot D but also, particularly, in the second-flushing and to a lesser extent in the third-flushing TBs of the dominant shoot D (Figs. 1b, 6b).

Nutrient effect on lateral branch angle to the vertical Removal of the upper dominant leader shoot D by decapitation caused the lower subdominant lateral branch S to bend upwards in replacement of leader shoot D and, hence, to reduce its angle to the vertical. Data from experiment(s) taken periodically during the growth period demonstrated that fertilization did not effectively alter this response (Table 1).

Auxin studies

Populus trichocarpa × *P. deltoides* (hybrid poplar, clone 47-174)

The results from the WI treatments (without auxin) demonstrated in poplar that the upper dominant branch D exerted apical control (i.e., more rapid growth) over the lower subdominant branch S (23.1 ± 1.0 cm Branch D, 17.6 ± 0.9 cm Branch S; $P < 0.05$) in two experiments (wherein the decapitated leader shoot gives rise to branches Sand D as described in materials and methods section, Fig. 2a).

When branch D was decapitated and immediately treated with auxin (Fig. 2a), there was no significant repression of subsequent elongation of branch S as compared with that of the control which was treated with lanolin (20.5 ± 1.5 cm elongation when treated with 1% NAA, 21.0 ± 1.7 cm with treated with lanolin only; ns).

A second procedure (Fig. 2b) was employed which allowed for a simultaneous test on the efficacy of the role of NAA as a repressor in apical dominance and/or in apical control. In three experiments, the 1% NAA treatment applied immediately to the decapitated shoot apex was effective in repressing the outgrowth of the highest lateral bud and hence, in demonstrating its role as a repressor in apical dominance (3.7 ± 0.5 cm Branch D, 7.5 ± 0.6 cm Branch S; $P < 0.05$). However, in the same experiments,

when NAA was not applied to the re-decapitated stem stump until 9–13 days after the first decapitation, thus giving time for the lateral bud to grow out to some extent before the NAA treatment, there was no significant auxin repression of the subsequent lateral branch outgrowth after 2–4 days (4.1 ± 0.3 cm when treated with 1% NAA, 4.5 ± 0.4 cm when treated with lanolin only; ns). Only a small significant inhibition was observed after 4–6 days (6.2 ± 0.4 cm when treated with 1% NAA, 7.4 ± 0.4 cm when treated with lanolin only; $P < 0.05$). Hence, even though auxin appeared to function normally as a repressor in apical dominance in this *Populus* system, its function in apical control was marginal at best.

Pseudotsuga menziesii (Douglas-fir)

Possible auxin effects on apical control in first-flushing lateral buds In a sub-test of first flushing where the auxin treatment was given to the decapitated stump of the leader shoot just prior to lateral bud flushing, it was evident that the NAA treatments did have an inhibitory effect on the percentage of lateral buds that had grown out (Fig. 3a, Table 2). Local toxic effects associated with NAA application were observed; i.e., some of the uppermost lateral buds were severely injured or killed. Thus, it is possible that part of the treatment effects were due to local injury of tissues in the apical region of the shoot rather than to physiological effects associated with auxin. The injury hypothesis is also substantiated by the greater mean distance between the highest outgrowing lateral bud and the decapitated shoot stump when treated with NAA as compared to the lanolin controls (Table 2). Over a period of weeks, the exogenous auxin treatment also resulted in a dramatic thickening of the upper stem which was sometimes visible throughout the flushing leader shoot.

In the standard apical control experiments involving first flushing (Fig. 3a), the NAA treatments were delayed until the outgrowth of a number of the lateral branches was already underway. After 14 or 15 days, there was essentially no evidence of auxin repression of this first flush in

Table 1 Effect of water and fertilizer application upon upward branch angle in first-flushing Douglas-fir shoots

Treatment	Status of shoot D	Branch S angle (degrees) by days after treatment				
		0	34	91	101	123
Water	Intact	37.90	48.70	49.30	47.50	
	Decapitated	40.30	28.90	13.80	16.90	
Fertilizer	Intact	44.13	53.25	50.38	56.38	57.25
	Decapitated	40.11	38.13	29.00	32.25	26.50

Initial measurements were made on subdominant branch S following the 27 April 2007 decapitation of dominant branch D with subsequent measurements during the growing season

N = 11–15 seedlings per treatment. Mean (in degrees)

Table 2 Effects of auxin (NAA) application on release of apical dominance following decapitation of first-flushing shoots of Douglas-fir from two families, one with a high and the other with a low tendency to produce ramicorn (RC) branches

Family and variable	Decapitated + lanolin	Decapitated + 0.5% NAA	Decapitated + 1% NAA
Low RC plants			
Lateral buds			
Total number	138	138	115
Percent inhibition of bud outgrowth	2	41	34
Mean distance between shoot tip and the highest outgrowing lateral bud below	0.56 ± 0.65	3.20 ± 1.9	2.70 ± 1.9
High RC plants			
Lateral buds			
Total number	126	143	114
Percent inhibition of bud outgrowth	2	41	46
Mean distance between shoot tip and the highest outgrowing lateral bud below	0.38 ± 0.55	3.14 ± 2.45	2.82 ± 1.97

Mean distance (SD ± cm) is the distance between the shoot tip and uppermost lateral bud which grew out

N = 16–18 per treatment and family combination

either of the two RC families (lateral bud outgrowth ranged from 5.17 to 5.77 cm across treatments in the low RC family and from 4.59 to 5.32 cm across treatments in the high RC family; ns). Furthermore, decapitation of the leader shoot TB had no promotive effect on the elongation of the outgrowing lateral branches (lateral bud outgrowth ranged from 5.18 to 5.69 cm across treatments in the low RC family and from 4.70 to 4.84 across treatments in the high RC family; ns). In an outside experiment involving first-flushing branches of a sapling, there was no repressive effect of NAA on apical control (5.25 ± 0.16 cm intact control, 5.81 ± 0.19 cm decapitated plus lanolin, 5.80 ± 0.16 cm decapitated plus 1% NAA in lanolin; ns).

Auxin effects on lateral branch angle of first-flushing shoots Removal of the upper dominant main shoot in the low RC family by decapitation caused a lower subdominant lateral branch to bend upwards in replacement of the removed upper shoot and, hence, to reduce the angle of the lateral branch to the vertical from 61° to 19.3° (Table 3). Significant auxin inhibition of this decapitation-induced vertical bending of the lateral branch was observed following the treatment with NAA. There was also some evidence of auxin-induced stem swelling and possible toxic effects at the site of NAA treatment adjacent to the stem/branch junction.

Auxin effects on second-flushing lateral buds The second-flushing lateral branches grew out mostly from near the apical region of the first-flushing leader shoot. When NAA treatments were made following 0, 1, or 2 cm decapitations above the base of the TB of the already second-flushing leader shoot, it was determined from lateral branch length measurements (of all outgrowing branches) that there was

Table 3 Effect of auxin (NAA) application upon upward branch angle in first-flushing Douglas-fir shoots

Treatment	Branch S angle to the vertical by date		
	27 April 2007	07 June 2007	27 July 2007
Branch D intact	59.5 ± 8.1	69.5 ± 10.2	69.7 ± 10.5
Decapitated + lanolin	61.0 ± 8.1	38.4 ± 14.6	19.3 ± 17.0
Decapitated + 0.5% NAA	56.7 ± 10.5	56.2 ± 8.4	55.7 ± 10.6
Decapitated + 1% NAA	55.7 ± 11.9	52.2 ± 10.8	48.5 ± 12.8

Initial measurements were made on subdominant branch S following the 27 April 2007 decapitation of dominant branch D and NAA treatment with subsequent measurements on 07 June 2007 and 27 July 2007

N = 11–15 seedlings per treatment. Mean (in degrees) ± SD

some significant repressive NAA effect on apical control. The decrease in lateral branch elongation ranged from a high of 64% in the low RC family with 0.5% NAA treatment following decapitation at 0 cm above the base of the TB to a low of 33% at 2 cm above the base. The decreases were slightly higher for the 1% NAA treatments. There appeared to be only minor differences in response to NAA between the low and high RC families (Fig. 7; data shown averaged for both families). In the lanolin controls, there was more evidence of some release of apical control following decapitation at 0 cm above the base of the second-flushing leader-shoot TB than at the 1- or 2-cm decapitation points.

The presence of localized effects such as NAA-induced localized tissue necrosis and swelling were observed most frequently on the stumps of the stems decapitated and treated with NAA at the base of the TB on the second-flushing leader shoot. The evidence of NAA repression of lateral bud elongation was significantly less in three out of

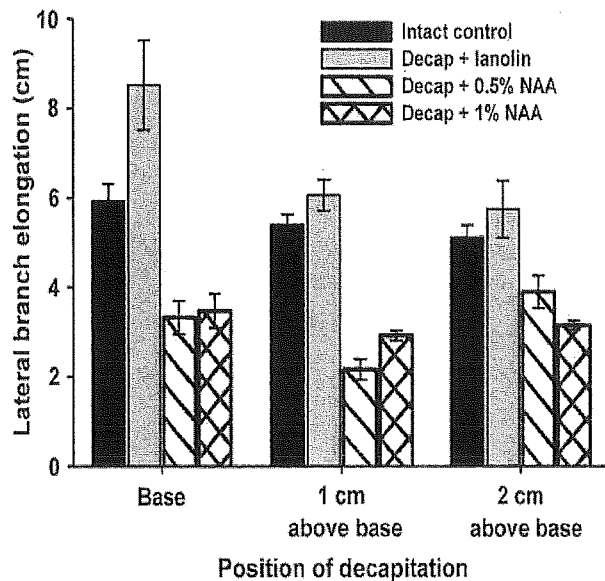


Fig. 7 Aa, apical control test on the repressive effects of auxin (0.5 or 1% NAA in lanolin) on continued lateral branch outgrowth in Douglas-fir decapitated at 0, 1 or 2 cm above the base of the second-flushing terminal bud of a leader shoot. Values are mean $\pm$ SE

four treatments when the decapitation NAA treatment was 2 cm above the base of the TB. Hence, the possibility that these small repressive effects of auxin on apical control were simply due to toxicity rather than to a physiological effect cannot be completely discounted.

Discussion

Nutrient-deprivation hypothesis

The results observed in rapidly growing morning glory and poplar were consistent with the nutrient-deprivation hypothesis for apical control (Table 4). Sub-dominance of branch S due to "monopolization" and diversion of nutrients to the leader shoot or upper dominant branch D was

suggested by the following: (1) significant and substantial promotion of elongation of branch S following the removal of branch D suggestive as being due to the interruption of nutrient diversion from branch S to branch D and, hence, the enhancement of nutrient availability to branch S; (2) fertilization had a greater growth-promoting effect on the presumed nutrient-starved subdominant branch S than on the dominant, nutrient "monopolizing" branch D; and (3) the presence of added fertilizer appeared to have a diluting influence on the promotive effect of removal of branch D on the elongation of branch S.

Similar or related results have been reported by others. Rasmussen et al. (2003) reported that bud removal in *Abies nordmanniana* enhanced the growth of other shoots and Kaitaniemi and Ruohomäki (2003) discussed the significance of source-sink interactions with allometry in the control of resource allocation in formation of crown in *Betula pubescens*.

The first flushing of buds on over-wintered branches on the slower growing Douglas-fir was much less sensitive to the three nutrient tests mentioned above than was observed for morning glory and poplar. However, second and third flushing were very strongly enhanced by the fertilization treatment as demonstrated in test 2. This difference in nutrient sensitivity between the first and second flushes might be explained by the fact that the first flushing of over-wintered buds is partially dependent upon the utilization of nutrients stored during the previous growing season. Hence, the effectiveness of any of the present nutrient-manipulating treatments to test the nutrient-deprivation hypothesis for the first flush may have been minimal. However, second flushing which occurred later in the growing season and originated from currently formed buds with a more limited supply of stored nutrients, would likely be more sensitive to current nutrient availability and would be less affected by conditions in the previous growing season. Hence, the high sensitivity of lateral branch second flushing to fertilization is consistent with the nutrient-deprivation hypothesis for their apical control.

Table 4 Summary of tests of hypotheses to explain apical control in three species

Species	Type of hypothesis and method of testing	
	Nutrient deprivation as tested by manipulating nutrient availability via removal dominant branch and fertilization	Auxin repression as tested by auxin (NAA) replacement on decapitated branches or leader shoots
Morning glory (<i>Ipomoea nil</i>)	Supportive	Non-supportive (Cline and Sadeski 2002)
Poplar (<i>Populus</i>)	Supportive	Non-supportive
Douglas-fir (<i>Pseudotsuga menziesii</i>)		
First flush	Equivocal results	Non-supportive
Second flush	Supportive	Partially supportive
Third flush	Partially supportive	Not tested

Branch orientation in Douglas-fir

As expected, the removal of the dominant shoot D caused branch S to orient itself more vertically; however, fertilization of seedlings in the present study did not restore this response which is often identified with apical control. Among possible interpretations, it could be postulated that: (1) nutrient availability plays no direct role in apical control, (2) the upward-branch-bending response does not constitute a significant part of the apical control process, or (3) there are multiple mechanisms operating in apical control, one of which is continuous shoot elongation under the control of many factors including nutrient availability and another which is shoot orientation that is not primarily under the control of nutrient availability.

Removal of the terminal shoot or upper dominating branch allows the next lower branch to respond to negative gravitropism via differential growth on upper and lower sides of the branch to bend the branch toward a more upright position. Plant bending, as a response to light or gravity, results from redistribution of auxin (Taiz and Zeiger 2006). Wilson (2000) has pointed out that the upward bending of a woody branch after the release from apical control requires the formation of new wood.

RC branching

Ramicorn branching, wherein lateral buds outgrow the TB, is generally known to have a higher frequency in second than in first flushing (Adams and Bastien 1994). Thus, we thought that the comparison of the 2 families with low and high tendency toward production of RC branches would be instructive. The results of the present study suggest that the unbalanced sequestering of nutrients to the growing lateral buds during this highly nutrient-sensitive time in apical control might play a significant role. Likewise, the demonstrated sensitivity of bud outgrowth to auxin during second flushing may also be pertinent to this branching phenomenon.

Conclusion

The nutrient-deprivation hypothesis for apical control of lateral branch elongation was supported in the present study by the results of the trials with morning glory and poplar; for Douglas-fir, it was strongly supported only in the results of trials involving branches associated with second or third flushing.

Auxin repressor hypothesis

Although exogenous auxin treatments were clearly shown to play a strong repressive role in apical dominance in

morning glory (*I. nil*), there was no evidence in this species that auxin plays a similar role in apical control (Cline and Sadeski 2002). That is, the application of auxin to the stump of a decapitated shoot before the initiation of lateral bud outgrowth strongly repressed its subsequent outgrowth. However, auxin treatment of the stump after lateral bud outgrowth was already underway had little or no repressive effect. The results in the present study with poplar, although not as dramatic, were similar. Auxin (NAA) was efficacious in maintaining apical dominance but not in apical control (Table 4). Hence, these data were consistent with the generally observed basipetal movement of polar auxin transport in the restoration of apical dominance and the apparent lack of acropetal transport that is presumably required for auxin to function in repressive role in apical control. Much still remains to be understood concerning the precise mechanism of auxin transport and there is some evidence for non-polar transport in the phloem with assimilates from source leaves to sink (Hoad 1995; Taiz and Zeiger 2006).

In the spring flushing of previously formed, over-wintered buds of most woody species, auxin and apical dominance are thought to play little or no role (Brown et al. 1967; Wareing 1970; Zimmerman and Brown 1971; Cline 2000). A majority of these over-wintered buds simply grow out. Auxin treatments have little inhibitory influence other than some local toxic effects near the point of application (Cline 2000). With the exception of some probable toxic NAA effects, these results were reaffirmed in the present Douglas-fir apical dominance test where the majority of the lower-situated over-wintered lateral buds did grow out in auxin-treated shoots. However, our previous Douglas-fir studies (Cline et al. 2006) of decapitation and auxin treatments on the stumps of these shoots which had currently formed lateral buds demonstrated the presence of apical dominance and a strong auxin repression of their outgrowth.

We tested for possible repressive effects of auxin on apical control on already-growing, first-flushing, and over-wintered Douglas-fir lateral buds; based on studies of branch growth with greenhouse seedlings and with an outside sapling, we concluded that there was little or no repression associated with auxin application. However, for second-flushing lateral buds of Douglas-fir, auxin treatments given on or near the base of the decapitated second-flushing TB after bud growth was already underway did exhibit some inhibition of apical control. It is possible that this apparent repression was caused by local and difficult-to-define NAA toxic effects because the degree of inhibition for most seedlings in a majority of treatments was reduced more by NAA treatment applied at 2 cm above versus at the base of the TB on the second-flushing shoot (Fig. 7). Nevertheless, it appears that some inhibition did occur.

These results are consistent with, but do not prove the existence of, some type of repressive role for auxin in apical control of second flushing in Douglas-fir. The forking response in the leader shoot of eastern white pine was suggested by McCabe and Labisky (1959) to be due to insufficient auxin production in the TB at the time of second flushing. Further investigation on the possible repressive role of auxin as well as the promotive role of cytokinin in second flushing is needed.

Despite possible complications associated with toxicity of NAA, the fact that NAA treatments applied to decapitated stumps of first-flushing dominant terminal shoots counteracted the upward bending of designated upper lateral branches suggests some type of auxin sensitivity in this bending response. Veierskov et al. (2007) demonstrated in *A. nordmanniana* that although decapitation/auxin treatments had no effect on branch orientation, auxin treatments to the leader bud scar did counteract the effects of decapitation on plagiotropic bud arrangement.

Conclusion

The evidence from our previous studies (Cline and Sadeski 2002) with morning glory and from present studies with poplar does not support a repressive role for auxin in apical control of lateral branching in these two species nor in first flushing of Douglas-fir branches that originated from previously formed, over-wintered buds (Table 4). That auxin treatment in Douglas-fir did appear to inhibit vertical reorientation of a first-flushing subdominant lateral branch following the removal of the upper dominant branch requires further study. There was equivocal evidence for some auxin inhibition of apical control of second flushing of lateral branches in Douglas-fir. As with nutrient availability, there were no significant differences in the auxin effects between the two families with varying propensity to produce RC branches. Although endogenous branch tissue levels of nutrients and auxin in apical control were not evaluated in the present study, such determinations are greatly needed for further testing of the nutrient/auxin hypotheses.

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References

Adams W, Bastien J-C (1994) Genetics of second flushing in a French plantation of coastal Douglas-fir. *Silvae Genet* 43:345–352

- Brown CR, McAlpine M, Kormanik P (1967) Apical dominance and form in woody plants: a reappraisal. *Am J Bot* 54:153–162. doi: 10.2307/2440793
- Ceulemans R (1990) Genetic variation in functional and structural productivity determinants in poplar. Amsterdam Thesis, Amsterdam
- Cline M (2000) Execution of the replacement apical dominance experiment in temperate woody species. *Am J Bot* 87:182–190. doi: 10.2307/2656904
- Cline M, Harrington C (2007) Apical dominance and apical control in multiple flushing of temperate woody species. *Can J Res* 37:1–10. doi:10.1139/X06-199
- Cline M, Sadeski K (2002) Is auxin the repressor signal of branch growth in apical control? *Am J Bot* 89:1764–1771. doi: 10.3732/ajb.89.11.1764
- Cline M, Yodel's M, Desai D, Harrington C, Carlson W (2006) Hormonal control of second flushing in Douglas-fir shoots. *Tree Physiol* 26:1369–1375
- Hoad G (1995) Transport of hormones in the phloem of higher plants. *Plant Growth Regul* 16:173–182. doi:10.1007/BF00029538
- Kaitaniemi P, Ruohomäki K (2003) Factors controlling resource allocation in mountain birch. *Perspect Plant Ecol Syst* 5(4):231–249. doi:10.1078/1433-8319-00037
- Leaky R, Longman K (1986) Physiological, environmental and genetic variation in apical dominance as determined by decapitation in *Triploclitron scleroxylon*. *Tree Physiol* 1:193–207
- McCabe R, Labisky R (1959) Leader forking of red and white pines in plantations. *J For* 57:94–97
- Ongaro V, Leyser O (2008) Hormonal control of shoot branching. *J Exp Bot* 59:67–74. doi:10.1093/jxb/erm134
- Rasmussen H, Soerensen S, Andersen L (2003) Bud set in *Abies nordmanniana* Spach. influenced by bud and branch manipulations. *Trees (Berl)* 17:510–514. doi:10.1007/s00468-003-0268-9
- Saks Y, Ilan I (1984) Hormone-mediated regulative action of the sunflower shoot apex on growth and cation level in the cotyledons—an additional manifestation of apical control. *Plant Physiol* 74:408–412
- Suzuki T (1990) Apical control of lateral bud development and shoot growth in mulberry (*Morus alba*). *Plant Physiol* 90:350–356
- Taiz L, Zeiger E (2006) Plant physiology, 4th edn. Sinauer Associates, Inc, Sunderland
- Thimann K, Skoog F (1934) On the inhibition of bud development and other functions of growth substances in *Vicia faba*. *Proc R Soc Lond B Biol Sci* 114:317–339
- Veierskov B, Rasmussen H, Eriksen B, Hansen-Møller J (2007) Plagiotropism and auxin in *Abies nordmanniana*. *Tree Physiol* 27:149–153
- Wareing P (1970) Growth and its coordination in trees. In: Luckwill L, Cuttings C (eds) *Physiology of tree crops*. Academic Press, New York, pp 1–21
- Wilson B (1990) The development of tree form. *Hortic Sci* 25:52–54
- Wilson B (2000) Apical control of branch growth and angle in woody plants. *Am J Bot* 87:601–607. doi:10.2307/12656846
- Wilson B, Gartner B (2002) Effects of stem girdling on conifers: apical control of branches, growth and air in wood. *Tree Physiol* 22:347–353
- Zimmerman M, Brown C (1971) *Trees: structure and function*. Springer, New York