

Apical dominance and apical control in multiple flushing of temperate woody species

Morris G. Cline and Constance A. Harrington

Abstract: In young plants of many woody species, the first flush of growth in the spring may be followed by one or more flushes of the terminal shoot if growing conditions are favorable. The occurrence of these additional flushes may significantly affect crown form and structure. Apical dominance (AD) and apical control (AC) are thought to be important control mechanisms in this developmental response. A two-phase AD – AC hypothesis for the factors controlling a subsequent flush is presented and evaluated on the basis of currently known studies. The first, very early phase of this additional flush consists of budbreak and the very beginning of outgrowth of the newly formed current buds on the first flushing shoot. There is evidence that this response often involves the release of AD, which is significantly influenced by the auxin:cytokinin ratio as well as by other signals including nutrients and water. This first phase is immediately followed by a second phase, which consists of subsequent bud outgrowth under the influence of apical control. Although definitive data for hormone involvement in this latter process is sparse, there is some evidence suggesting nutritional mechanisms linked to possible hormone activity. Stem-form defects, a common occurrence in multiple-flushing shoots, are analyzed via the AD – AC hypothesis with suggestions of possible means of abatement.

Résumé : Chez les jeunes plants de plusieurs espèces ligneuses, la première poussée de croissance printanière peut être suivie par une ou plusieurs poussées de croissance des pousses terminales si les conditions de croissance sont favorables. Ces poussées de croissance additionnelles peuvent affecter de façon significative la forme et la structure de la couronne. La dominance apicale et la régulation apicale sont considérées comme des mécanismes de contrôle importants dans cette réponse de croissance. Une hypothèse impliquant deux phases, soit la dominance apicale et la régulation apicale, les facteurs qui régiraient une poussée de croissance subséquente, est présentée et évaluée sur la base des études connues. La première et très précoce phase de cette poussée de croissance additionnelle est l'éclosion des bourgeons et la croissance initiale des bourgeons nouvellement formés sur les pousses issues d'une première poussée de croissance. Il y a lieu de penser que cette réponse implique souvent le relâchement de la dominance apicale qui est influencée de façon significative par le rapport entre les auxines et les cytokinines, aussi bien que par d'autres signaux incluant les nutriments et l'eau. Cette première phase est immédiatement suivie par la seconde phase qui se manifeste par une croissance subséquente des bourgeons sous l'influence de la régulation apicale. Bien qu'il existe peu de données qui démontrent une implication hormonale dans ce dernier processus, certains indices permettent de penser que des mécanismes nutritionnels pourraient être reliés à une activité hormonale. Des défauts dans la forme de la tige, une situation courante dans le cas des poussées de croissance multiples, sont analysés via l'hypothèse de la dominance apicale et de la régulation apicale et des moyens qui pourraient les atténuer sont suggérés.

[Traduit par la Rédaction]

Introduction

Subsequent to the initial flush of new growth following budbreak in the spring (fixed or determinate growth; Table 1), the terminal shoots of many temperate woody species will start to form bud scales; if growing conditions are favorable in early summer, young plants will move from this bud scale-forming stage into free growth (Fig. 1). Free growth can be of two forms: continuous growth, if the apex quickly resumes initiating and elongating leaf primordia

without setting a bud, or additional flushes of active terminal growth, if the apex pauses long enough to form multiple bud scales to cover the apex, then it resumes forming leaf primordia, which will be extended shortly. The obvious new flush of growth visible in the middle or late stage in the growing season has been referred to as "second flushing" or "summer flushing" wherein the newly formed (i.e., current) buds on the recently flushed spring shoot will open and grow out to varying degrees (Fig. 1, Table 1). Such repeated flushing is an example of multiple flushing and may occur a number of times during the growing season in some temperate species.

Shoot elongation in first or in multiple flushings may involve both cell division and cell enlargement with probably a predominance of the former, depending upon the species. Owens and Molder (1973) found the highest mitotic activity in cells of vegetative shoot apices of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) to occur in the stages of late bud scale initiation, rapid apical enlargement, and early, rapid leaf initiation. It was subsequently deter-

Received 13 February 2006. Accepted 10 August 2006.
Published on the NRC Research Press Web site at cjfr.nrc.ca on 29 March 2007.

M. Cline.¹ Department of Plant Cellular and Molecular Biology, Ohio State University, Columbus, OH 43210, USA.
C. Harrington. USDA Forest Service, Pacific Northwest Research Station, 3625 93rd Avenue SW, Olympia, WA 98512–9193, USA.

¹Corresponding author (e-mail: cline.5@osu.edu).

Table 1. Definitions of terms used to describe some of the processes, stages, or structures in shoot growth.

Term	Definition	Reference
Apical control	Growth suppression of an existing subdominant branch by a higher dominating shoot that functions to maintain crown dominance by a central stem; it is concerned with the regulation of growth after budbreak and, thus, is one step removed from apical dominance	Suzuki 1990; Wilson 1990, 2000
Apical dominance	Control exerted by an actively growing shoot apex over the outgrowth of lateral buds; it focuses mainly on the mechanisms that determine whether or not an inhibited bud begins to grow out	Wilson 1990; Cline 1996
Continuous growth	Uninterrupted formation and extension of leaf primordia during the growing season, also referred to as indeterminate growth	Kozlowski et al. 1991
Endodormancy	Winter dormancy resulting from processes internal to the bud that restrict growth	Lang et al. 1987
Fixed growth	Extension of stem units present in an overwintered bud, also referred to as determinate growth	Kramer and Kozlowski 1979
Free growth	Shoot growth from nonoverwintered bud primordia, formed and expanded in the same season; free growth can be divided into continuous growth and multiple flushing	Jablanczy 1971; Kozlowski et al. 1991
Multiple flushing	Episodic (polycyclic) outgrowths from current buds on a flushing shoot, which may occur one or more times during the same season (e.g., second, third flushing, etc.); second flushing shoots are sometimes referred to as "lammas growth;" multiple flushing refers to both terminal and lateral shoots and to both new branches originating from the central shoot and lateral extension of older branches; also referred to as rhythmic growth	Kozlowski et al. 1991; Barthélémy and Caraglio 2007
Proleptic branching	Outgrowth of shoots from overwintered buds formed during the preceding growing season	Halle et al. 1978
Sylleptic branching	Outgrowth of shoots from current lateral buds on flushing shoot with little or no intervening rest period between bud formation and outgrowth; for some situations, sylleptic branching and multiple flushing could be used interchangeably to refer to branches originating from a central shoot the season the bud was formed (see comment under multiple flushing)	Halle et al. 1978

mined by Owens and Simpson (1988) in Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) that "... lateral shoot elongation resulted primarily from cell divisions before vegetative bud flush and cell elongation following bud flush" with the bulk of shoot elongation occurring "as the mitotic index decreased and cell elongation increased."

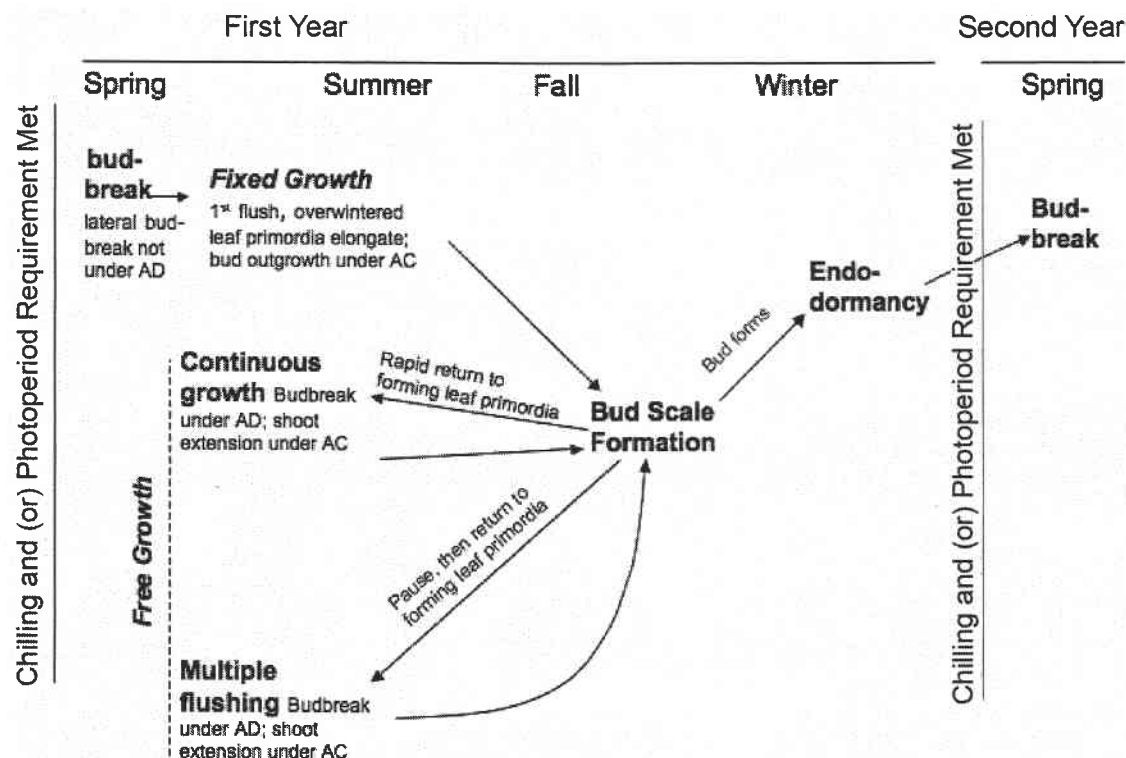
Multiple flushing is much easier to observe than continuous growth because of the obvious visibility of a new bud and the subsequent new shoot. Thus, most observations of free growth pertain to multiple (or second) flushing, which can include the outgrowth of both terminal and lateral buds. Multiple flushing has been reported to be enhanced by defoliation (Romberger 1963; Champagnat 1989; Borchert 1991; Collin et al. 1994), weed control (Roth and Newton 1996), or by environmental influences such as extended photoperiods and rain following a period of drought (Rudolph 1964). Multiple flushing generally decreases with tree age but loblolly pine (*Pinus taeda* L.) between ages 30 years and 35 years produced two to five annual flushes (Harrington 1991). Multiple flushes have also been analyzed in lodgepole pine (*Pinus contorta* Dougl. ex P. Laws. & C. Laws.) (Lanner and Van Den Berg 1973; O'Reilly and Owens 1989) but have not been observed in *Nothofagus pumilio* (Poepp. & Endl.) Krasser (Souza et al. 2000).

The primordia of determinate buds (both terminal and lateral) that eventually second flush are usually formed during

the previous growing season. These reside on shoot axes inside buds that overwinter and open during the following spring. There are many variations of this developmental pathway in different species (Cannell et al. 1976; Lanner 1976). For example, in Douglas-fir, in which the first or fixed phase of shoot growth can be characterized as determinate, the current lateral buds are not formed during the previous growing season, but their primordia are initiated in March or early April (Allen and Owens 1972). Depending on environmental conditions, these dynamic buds can exhibit continuous free growth, second flush, or go into endodormancy (Fig. 1, Table 1). Indeterminate species such as poplar (*Populus*), willow (*Salix*), and alder (*Alnus*) can gradually continue to add leaves throughout the growing season and may not second flush. Their lateral buds may exhibit sylleptic branching (Table 1). Although additional flushing is fairly common in some species, the basic control mechanisms, like those of the first flush, are not well understood.

Although the additional shoot growth associated with later flushes is usually not as great as that of the first flush, it can be significant (Kramer and Kozlowski 1979) and add substantially to height growth as in southern conifers. For other species, including Douglas-fir, continuous growth can be much more significant than that of multiple flushing in terms of total shoot length for the season. However, multiple flush-

Fig. 1. The phases in shoot growth and bud outgrowth in Douglas-fir. The stages of free growth are not exclusive; a tree can move into continuous growth, then pause, and reflush. A second flush can be followed by another phase of continuous growth, another flush, or the formation of the winter bud. By definition, all shoot growth during the year of germination is free growth. AD, apical dominance; AC, apical control.



ing is of interest, because it is often associated with deleterious stem forms such as additional clusters of branches, which lead to knots, and to changes in apical dominance (AD) – apical control (AC) caused by greater growth of lateral as opposed to terminal buds. Multiple flushing can also expose the shoot to increased risk of injury from early fall frosts because of decreased time for winter hardening (Kramer and Kozlowski 1979).

Although this review has general application to all temperate woody species, the following discussion will give emphasis to Douglas-fir with respect to the role of AD – AC in multiple flushing and includes a brief mention of possible ways of minimizing stem defects associated with multiple flushing.

Apical dominance and apical control

Apical dominance and apical control are among the central factors that determine branching patterns and shoot architecture (Sterck 2005). Although AD has long been a familiar term to plant scientists, credit must be given to Brown et al. (1967) for introducing the use of AC with respect to decurrent and excurrent woody species in a broad context and to Wilson (2000) for further amplification. For purposes of our present discussion, we have utilized rather narrow definitions of AD and AC (Table 1) to aid in sharply differentiating the roles of these two developmental processes in multiple flushing. This has been done because of an inadvertent tendency on the part of some workers to blur their distinctions and their differing underlying physiological

bases (Cline and Sadeski 2002). It is also recognized that an extremely wide variation exists in the expression of AD and AC among different woody species.

We suggest and evaluate a two-phase (AD–AC) hypothesis for second or subsequent flushing. We also attempt to analyze the efficacy of hormonal and (or) nutritional involvement in these two phases. As will subsequently be discussed (Fig. 1), AC plays the predominant role during the first flush of overwintered buds with little or no AD involvement. However, both AD and AC play important roles in the development of subsequent flushes of multiple-flushing shoots.

Hormonal interaction

In discussing hormonal control of bud dormancy, Romberger (1963) made the following interesting comment: “Our knowledge of endogenous growth regulators ... and their interactions under various conditions is so inadequate that intelligent discussion of the subject is not yet possible.” Borchert (1991) added a further caution to oversimplifying interpretations of hormone treatment and extraction data on the control of shoot growth periodicity. Whether there has been sufficient progress since the time of Romberger’s pronouncement to justify further discussion could be debated, but it is timely to summarize what is known, to propose a hypothesis, and to test it with the available evidence.

Although the plant hormones cytokinin, auxin, and gibberellin (GA) may promote both cell division and cell elongation (the components of shoot elongation) under cer-

tain circumstances, cytokinin primarily promotes cell division, whereas auxin and GA primarily promote cell enlargement (Srivastava 2001; Taiz and Zeiger 2002). Even though the major focus of the present review is on auxin and cytokinin effects, there is also evidence of GA promotion of shoot elongation in some conifers (Hare 1984a; Owens et al. 1985; Graham et al. 1994).

General auxin effects on apical dominance

Auxin, which is produced in actively growing shoot apices, is thought to play a major role in AD by moving down the stem via polar transport and inhibiting the outgrowth of the lower lateral buds by some indirect mechanism that probably also involves other signals (Beveridge 2000; Leyser 2003; Schmitz and Theres 2005). The evidence in most but not all plant species suggests that auxin acts as a repressor of bud outgrowth and restores AD when applied to the cut stem surface of a decapitated shoot in the classic Thimann-Skoog test (Thimann and Skoog 1933; Cline 1996, 2000). Although many workers have presumed a similar line of action for auxin in AC as in AD, the precise role of auxin in AC, if any, is much less clear. There is substantial evidence for the presence of endogenous auxin in woody plant species, including the identification of auxin-related genes (Andersson-Gunneras et al. 2006). It should also be pointed out that bud sensitivity to hormones is probably as important as bud hormone content (Trewavas 1987).

General cytokinin effects on apical dominance

AD is also very sensitive to the plant hormone cytokinin, which often can cause vigorous outgrowth if applied directly to buds in some species (Pillay and Railton 1983; Cline and Dong-II 2002). The primary promoting effect on bud growth is focused on the very early stages of outgrowth, i.e., cytokinin promotes budbreak but does not promote subsequent elongation, and additional treatments can be inhibitory to further growth in some instances. The bulk of cytokinin, which is thought to be produced in the roots, is transported acropetally up the xylem to the shoots, where it has a counteracting influence on the repressive effect of the apically derived auxin. Endogenous cytokinin has been found in a wide variety of herbaceous and woody species.

Presently, the auxin:cytokinin ratio is a widely accepted working model for explaining hormonal control of AD (Stafstrom 1993; Klee and Romano 1994; Coenen and Lomax 1997). However, other signals, nutrients, and environmental factors are probably also involved and should not be ignored.

Hormone effects on apical control

Wilson (2000) stated that, although it seems likely that hormones play an important role in AC, there has been relatively little research done on this topic. After reviewing some of the pertinent hormone studies including possible effects on branch orientation as summarized by Timell (1986) and suggesting a possible hormonal role in maintaining "the strength of the stem sink for branch-produced assimilate,"

Wilson points out that the mechanism of action of hormones in AC remains unknown.

In our studies, when auxin was applied to the cut stem surface of decapitated growing shoots of rapidly growing Japanese morning glory (*Ipomoea nil* (L.) Roth), the initial outgrowth of the lower lateral buds was completely inhibited and AD was restored (Cline and Sadeski 2002). On the other hand, in a wide variety of treatments, there was no evidence of growth inhibition in the lower, dominated growing branches and no restoration of AC when auxin was applied to decapitated, dominant, already-growing shoots. The problem of long-distance acropetal transport, which presumably is required for auxin-mediated AC, appears to be formidable. Hence, in this plant system, auxin does not seem to play a role in AC similar to that in AD. Whether these results in this herbaceous plant can be validly extrapolated to woody species remains to be demonstrated.

Since a primary role for cytokinin in the release of AD in bud outgrowth is only to initiate this outgrowth, there seems less likelihood for its involvement in the later AC stages of bud outgrowth, where its effects might even be inhibitory. On the other hand, cytokinin is also known to induce nutrient mobilization and, hence, may be capable of creating metabolic sinks (Taiz and Zeiger 2002), which could play a positive role in AC.

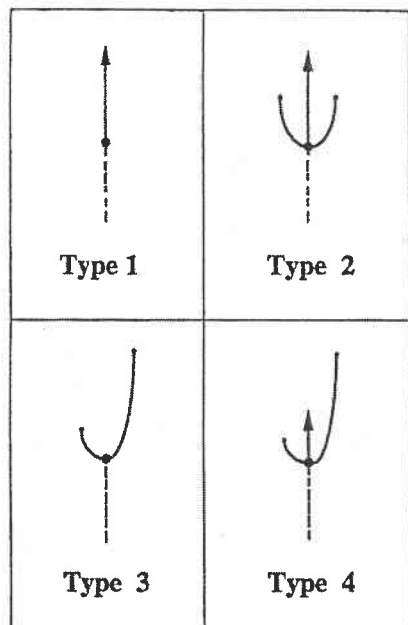
The AD-AC hypothesis for multiple flushing

For purposes of physiological and developmental analysis, the early portion of a second or a subsequent flush may be conveniently divided into two phases. The first phase will be referred to as the AD phase. It is concerned with whether or not the newly formed current buds on the first flushing shoot grow out at all. If growing conditions are favorable and the buds do begin to grow out, this first phase occurs at the very beginning of their outgrowth, near the end or shortly after the end of the first flush of the terminal shoot. It is usually, although not always, accompanied by the release of AD. Varying degrees of AD may be expressed during this first phase of second flushing. If AD is strong, there will be little or no lateral branching and vice versa, if it is weak. The terminal and (or) some of the upper lateral buds will begin to grow out in this first AD phase. Hence, as shown in De Champs (1971) classification of Douglas-fir second flushing stem types (Fig. 2), this first phase will involve either the beginning of outgrowth of the terminal bud alone (type 1), of both the terminal and lateral buds (type 2), or of the lateral buds alone (type 3).

The second phase (AC) begins sometime after the first phase is underway. It is concerned with whether or not the buds keep elongating. It is usually characterized by subsequent and continued outgrowth of the terminal and (or) lateral buds. It may or may not involve competitive elongation between these shoots and the possible loss of AC.

This AD-AC hypothesis for second or additional flushes has been largely fashioned from direct observations of shoot growth in Douglas-fir, ash (*Fraxinus*), and northern red oak (*Quercus rubra* L.) as well as information on other species from the literature. A wide spectrum of variation undoubtedly exists in multiple flushing (including sylleptic branching) of the thousands of known temperate woody species

Fig. 2. Second-flushing types according to De Champs (1971; cited by Adams and Bastien 1994): type 1, only the terminal bud of the leading shoot second flushes; type 2, both the terminal and one or more lateral buds at the base of the terminal bud second flush, but the lateral shoots are subdominant to the terminal shoot at the end of the growing season; type 3, the terminal bud doesn't second flush, but one or more lateral buds do; and type 4, both the terminal and lateral buds second flush, but one or more laterals are dominant at the end of the growing season. Broken lines indicate growth prior to second flushing, and solid lines indicate growth following second flushing. (Used with permission from Dr. Bernd Degen, Editor of *Silvae Genetica* (Adam, W.T., and Bastien, J.-C. 1994. *Silvae Genetica*, 43(5-6): 345–352).



(Rudolph 1964; Cannell et al. 1976; Lanner 1976; Powell 1987). The extent of applicability of this hypothesis remains to be determined.

Evaluation of hormone activity in multiple flushing within the context of the AD-AC hypothesis

Sensitivity to auxin in apical dominance

In temperate woody species, AD operates mainly in the newly formed buds of the current year's growth. It generally does not function in the overwintered buds formed during the previous growing season nor in the shoots formed in previous years (Brown et al. 1967; Wareing 1970; Zimmerman and Brown 1971; Cline 2000). Hence, for the most part, auxin plays no repressive role in overwintered buds. In spring-flushing Douglas-fir leader shoots, the overwintered terminal buds and a majority of the upper overwintered lateral buds are irrepressible. They flush in the spring more or less simultaneously (Cline et al. 2006). The response is similar in hardwoods, although the proportion of irrepressible buds may be less. These irrepressible lateral buds will grow out regardless of any auxin treatments except for possible toxic effects in the close vicinity of auxin application. The

rest period and (or) winter exposure of these buds appears to have somehow negated the inhibitory effect of auxin and the operation of AD in this spring first flushing response.

An exception to this is known to occur in some woody species such as white (*Fraxinus americana* L.) and green ash (*Fraxinus pennsylvanica* Marsh.) as well as northern red oak, where some overwintered latent buds lower on the shoot that normally would not grow out in the spring flush unless the terminal bud is decapitated are under the control of AD and can be repressed by auxin treatments (Cline 2000).

Our studies of Douglas-fir seedlings have demonstrated strong evidence for current lateral bud sensitivity to auxin and AD (Cline et al. 2006). These newly formed buds remain repressed during much of the first flushing period and will not grow out unless the flushing shoot is decapitated, defoliated, or treated with cytokinin midway through this first period. Decapitation can release AD, as demonstrated by visible evidence of lateral bud outgrowth, within a week or less in hybrid poplar (data not shown) or within a month or less in Douglas-fir (Cline et al. 2006). This release may be completely inhibited by immediate auxin application to the stump of the decapitated shoot via the classic Thimann and Skoog (1933) protocol.

This sensitivity to auxin and AD by the current lateral buds appears to be more prevalent in seedlings and saplings than in mature trees (Cline and Deppong 1999). Hence, it appears in the young and critical years of shoot architecture formation that AD via auxin plays a significant controlling role in the early outgrowth of the current terminal and lateral buds in multiple flushing.

In Zimmerman and Brown's (1971) statement concerning the insensitivity to auxin and AD of "... lateral buds on the previous year's twig following a period of winter dormancy or rest," the use of the term "rest" in this context appears to refer to the time between the first and second flush. Later in the same chapter as they describe AC in decurrent species, they state, "... after a period of dormancy or occasionally during the current season, if lammas shoots are formed, one or more of the uppermost lateral buds elongate as rapidly as, or more rapidly than, the terminal bud giving rise to repeatedly branching stems." An obvious implication here is that this "rest" period between the two flushings eliminates the sensitivity of the lateral buds to repression by auxin in AD as does the winter dormancy period. Hence, their outgrowth in a second growth flush is facilitated.

There might be some question as to whether the "rest" period between the first and second flushing is long enough to be justifiably equated with a winter dormancy period and concomitant desensitization to auxin and AD. In some species, the gap may be only 10 days or 15 days. In Douglas-fir, there is often some overlap in time between the ending of the first flush and the beginning of the second flush. Although this would seem to eliminate the existence of a distinct rest period between flushes, it is possible that there may be a gradual diminishment of sensitivity to auxin and AD in the latter part of the first flush. Alternatively, a reduction in shoot growth also might result in a reduction in bud growth suppressors. Some combination of these might effectively allow for such a rest period.

Sensitivity of the first phase (AD) of second flushing to cytokinin and to the auxin:cytokinin ratio

As indicated above, a cytokinin spray treatment given to repressed current buds in Douglas-fir midway through the first flush of a terminal shoot will promote the beginning of outgrowth (second flushing). This response will occur in both lateral and terminal buds and also has been confirmed by other workers in Douglas-fir (Lavender and Zaerr 1967; Mazzola and Costante 1987). The promotive effects of cytokinin on bud outgrowth in the control of AD, as well as the repressive effects of auxin, however, are confined to the first phase of the AD-AC second flushing.

There also is substantial evidence of cytokinin promotion of bud outgrowth in a variety of woody species: Norway spruce (*Picea abies* (L.) Karst.; Bollmark et al. 1995), long-leaf pine (*Pinus palustris* Mill.; Hare 1984b), balsam fir (*Abies balsamea* (L.) Mill.; Little 1985), Scots pine (*Pinus silvestris* L.; Whitehill and Schwabe 1975), blue spruce (*Picea pungens* Engelm.; Mazzola and Costante 1987), and loblolly pine (Zimmerman and Brown 1971). The particular distribution and precise location of the lateral buds in relation to the terminal bud on the flushing shoot together with the particular sites of auxin and cytokinin availability may be critical in determining their selective outgrowth. It also must be reemphasized that the possible effects of other signals, nutrients, or environmental factors cannot be overlooked.

Sensitivity of the second phase (AC) of second flushing to hormone and nutritional signals

Lanner and Van Den Berg (1973) propose that the leading shoots of lodgepole pine maintain their height advantage (or AC) over the shorter lateral branches by producing more stem units (i.e., internodes) because of "... obscure reasons, possibly connected with biochemical gradients of growth substance concentration." They also add that AC "... appears to be intimately connected with the peculiar delay in the maturation of lateral branch buds borne at the end of the terminal bud's last cycle."

With respect to possible nutritional involvement in the AC mechanism, Wilson's group has made progress in understanding the mechanisms involved by imposing girdling treatments in several conifers wherein branches released from AC elongate and bend upwards (Wilson and Gartner 2002). Their results give some support for a competitive-sink hypothesis between the branch and the subadjacent stem for branch-produced carbohydrates. These data do not exclude the possibility of some involvement or interaction with auxin.

It is possible that a metabolic sink in the apex of a terminal shoot could account for AC. The generation of such sinks can be facilitated by various factors or processes including increased water conductance (McIntyre and Hsiao 1990; Sellin 1988; Ewers and Zimmerman 1984), high photosynthetic rates (Livingston et al. 1998), or altered hormone activity. Mor et al. (1981) demonstrated that the darkening of the dominant shoot of a decapitated rose (*Rosa*) branch shifted dominance and ^{14}C -assimilation to a lower previously dominated shoot. However, the treatment of the darkened upper shoot with cytokinin reversed the dominance and the ^{14}C -assimilation back to the upper dark shoot, thus

suggesting possible hormone or nutrient mechanisms for generating AC sinks. The ability of cytokinins to transport and to mobilize nutrients is another possible mechanism for sink-directed AC.

Interestingly, Brown et al. (1967) essentially equate second flushing in decurrent hardwoods (oak, hickory, (*Carya*), and maple (*Acer*)) with the typical decurrent response, wherein several of the uppermost, large vigorous lateral buds that are completely repressed by the flushing terminal shoot in one season will often outgrow this shoot during the following season resulting in a multiple forked shoot without a central leader. This also results in the change of the conically shaped crown of a young hardwood seedling or sapling to a rounded crown as it matures. These authors appear to attribute the success of the laterals (originating from large buds) in outgrowing the terminal shoot during the following season as basically a nutritional consequence of a compensatory process, wherein the laterals suppress the growth of the terminal bud via a predominating competition for nutrients, food, and water "so that apical control is lost".

Whereas Harmer and Baker (1995) found little correlation between bud size and shoot length in sessile oak (*Quercus patraea* (Mattuschka) Liebl.), Bollmark et al. (1995) do find a positive relationship among bud size, zeatin riboside concentration, and the crown height of terminal buds in *Picea abies*. Likewise, Little (1970) finds a substantial correlation between bud length and the length of the shoot formed by that bud in eastern white pine (*Pinus strobus* L.) as do Kozłowski et al. (1973) in red pine (*Pinus resinosa* Ait.).

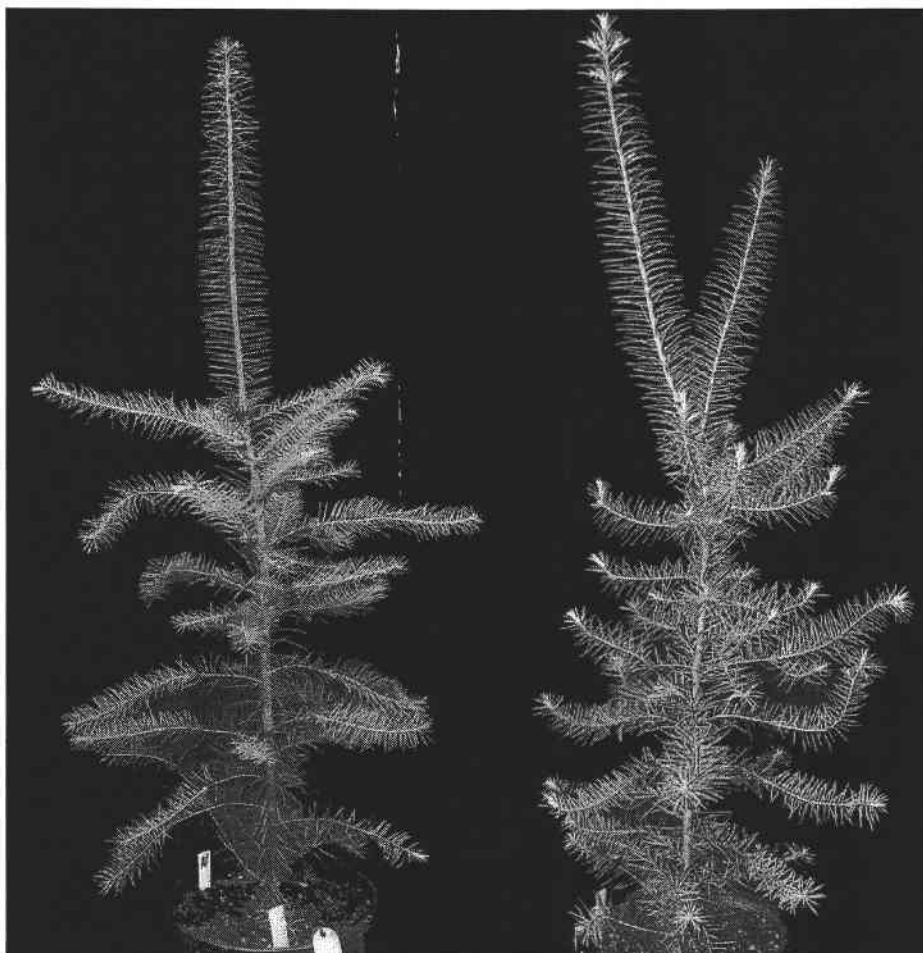
Rasmussen et al. (2003) determined that removal of buds and branches from seedlings of Nordman fir (*Abies nordmanniana* (Steven) Spach) enhanced leader shoot elongation and, hence, AC via growth allocation. Leaky and Longman (1986) report that lower shoots of obeche (*Triplochiton sderoxylon* K. Schum.), amply supplied with nutrients can be protected from AC by larger shoots. Jankiewicz and Stecki (1976) have emphasized the role of nutrients and their possible interaction with auxin in correlative relationships between conifer buds. Presuming the mechanisms of AC are similar in both first and multiple flushing, it would seem likely that in the second phase (AC) of the second or subsequent flushes of woody species, nutrient availability with possible auxin-cytokinin interaction may play a critical secondary, if not a primary role.

Analysis of stem-form defects associated with multiple flushing via the AD-AC hypothesis

Defects in stem form are often associated with multiple flushing, apparently because of the loss of AD and AC in the terminal shoot (Rudolph 1964; Kramer and Kozłowski 1979). These anomalies in stem form can be found in a wide spectrum of woody species but are particularly common in conifers. One or more of the lateral shoots will outgrow the terminal shoot resulting in a forklike, multiple or ramicorn terminal shoot (Fig. 3, right) that usually diminishes future wood value (Campbell 1965).

The De Champs classification of second flushing terminal stem types in Douglas-fir (Fig. 2; De Champs 1971; Adams and Bastien 1994) also generally may typify those of other conifers and some hardwoods. Type 1 exhibits very strong AD in the elongating terminal bud without lateral bud out-

Fig. 3. (Left) A Douglas-fir seedling with a normal single terminal shoot. (Right) A seedling with a forklike multiple (ramicorn) terminal shoot consisting of a dominating lateral shoot on the left that has outgrown the shorter terminal shoot on the right. The lateral shoot originated from second flushing during the previous growing season (similar to type 4 in Fig. 2).



growth. The more common type 2 demonstrates the normal central leader with subdominant laterals. Although these two types of second flushing do not give rise to forklike multiple apical shoots, short internodes in type 2 may still reduce future wood value for lumber (although not for Christmas trees) with increased branching and knottiness (Rudolph 1964).

In types 3 and 4, it appears that the lack of sufficient outgrowth of the terminal bud combined with excessive outgrowth of the lateral buds characterize the defective stem forms that lack AC. In type 3, AD is not released for the terminal bud but is released for the two lateral buds that grow out and do not appear to be under any AC by the completely inhibited terminal bud. The less common type 4 could be due to a slow AD release of the terminal bud and (or) to weak AC by the slow-growing terminal shoot over the excessive growth by the laterals.

Rudolph (1964) showed that the forked condition in jack pine (*Pinus banksiana* Lamb.) was often temporary and did not persist for more than 2 years or 3 years. A lateral shoot took over as the terminal shoot. However, a residue of large knots still remained. He also found that late shoots tended to occur repeatedly in the same tree. Schermann et al. (1997) found a moderate correlation among early season budburst, second flushing, and stem defects.

It is interesting to note that the branches on the terminal shoot of Douglas-fir that form just below the base of the second or subsequent flush have enhanced development and become larger in diameter compared with other branches that formed from buds during the current season. Hence, in terms of sink strength, the branches just below the base of a second flush seem to have more in common with the branches that form the following year than with the branches that form only a few weeks following the formation of the second flush. Thus, it is possible that proximity to the apex or the formation of bud scales somehow plays a role in future bud outgrowth and branch development.

Because evidence in the case of our Douglas-fir studies (Cline et al. 2006) indicated that the first phase of second flushing is under the control of AD which in turn is strongly dependent upon the auxin:cytokinin ratio, it is possible that the lack of lateral bud outgrowth in type 1 second flushing might be due to high auxin and low cytokinin levels at the site of hormone action in the spring flushing shoot. Likewise, the beginning of the excessive outgrowth of the current laterals in type 3 second flushing might be due to low auxin and high cytokinin levels at the appropriate sites.

The foregoing explanation of possible hormone involvement in AD in second flushing applies only to the first phase at the very beginning of bud outgrowth and not to the subse-

quent bud elongation of the second phase, wherein the loss of AC may occur and the development of the defective stem form becomes obvious. Nevertheless, the occurrences in the first phase and how they are influenced are critical for what follows. Whether the terminal and (or) lateral buds break or not in the first phase will largely determine their subsequent development into one of the four flushing types and, hence, whether their stem-form defects will develop.

McCabe and Labisky (1959) attributed the forking problem (similar to types 3 and 4) in eastern white pine to insufficient auxin production in the terminal bud to prevent lateral bud competition during second flushing.

Harmer (1992a) has pointed out that about 25% of sessile oaks with crooked stem form lose their leaders because the terminal buds do not grow out for a variety of causes, including frost damage, pest damage, and aborted second flushing shoots. In addition, "... terminal buds often fail to develop for reasons that are not obvious." In a subsequent paper (Harmer 1992b), he further explains that, compared with spring flushing shoots, there are fewer viable shoots produced by the second flush from terminal buds: "As recurrent flushing is more likely to occur on the leading shoot of young trees, and the terminal bud or shoot tip on second flush shoots often dies, then young trees that show a strong tendency to produce a second flush may grow into trees with worse form than those that usually flush once." Defective stem forms in all woody species occur with a much higher frequency during subsequent flushing than during first flushing.

Considerations for attempting to minimize these stem-form defects could include appropriate environmental, physiological, or genetic modifications of either of the two phases (AD or AC) of second flushing. Lanner (1972) concluded that the second-flushing forking problem in Taiwan red pine (*Pinus taiwanensis* Hayata) was primarily heritable and could best be resolved by careful genetic selection of seed source. However, Roth and Newton (1996) found that seed source had no effect on occurrence of second flushing occurrence in Douglas-fir. Adam's group (Adams and Bastien 1994; Temel and Adams 2000; Vargas-Hernandez et al. 2003) has pointed out the challenge in making genetic improvements in stem growth without also increasing stem defects. However, progress is occurring in this regard. Silvicultural manipulations such as high plant densities, thinning, and pruning also can do much to reduce stem defects, but the cost is high (Schermann et al. 1997). The second (AC) phase of a subsequent flush can be modified by selective pruning. It may also be possible on some sites and with some genotypes to keep trees in continuous growth until the overwintered bud is formed; thus, silvicultural operations that keep conditions favorable for the formation and extension of leaf primordia, such as promoting high soil moisture conditions, could prevent or reduce the occurrence of multiple flushing and the ramicorn branches or branch whorls associated with it.

One could also envision possible transgenic measures (Herschback and Kopriva 2002) to reduce multiple flushing via appropriate endogenous hormonal manipulations (e.g., auxin:cytokinin ratio in the AD phase). Efficient gene transfer systems that might facilitate these efforts have been employed in hybrid poplar (Bradshaw et al. 2000) and are being

developed for conifers (Merkle and Dean 2000). Quantitative genetic selection is presently being used quite effectively to improve needed traits in Douglas-fir.

Conclusions

A two-phase AD-AC hypothesis for analyzing hormonal and nutritional effects on the developmental control of multiple flushing of temperate woody species has been proposed and evaluated. Although this is simply one preliminary approach to a very complex natural phenomenon, substantial evidence does exist for auxin-cytokinin control of the first phase (AD) along with other possible hormonal and nutrient signals. Present evidence for a hormonal role in the second phase (AC) is much less compelling. However, there is fragmentary but tantalizing indications of a nutritional role in this phase.

Exciting new advances utilizing genetic and molecular approaches in the study of the branching process provide significant hope for increased understanding of the involved controlling mechanisms involved in bud growth. There also remains a great neglected need for basic physiological-environmental studies of these mechanisms, both those relating to AD and, particularly, to AC. To minimize stem form defects associated with multiple flushing, silvicultural and genetic selection methods have been found useful. Hopefully, future corrective transgenic techniques also will be available.

Acknowledgements

We thank Dr. William C. Carlson, Weyerhaeuser, Propagation of Higher Valued Trees, for his valuable insight and encouragement on this project and Dr. Brayton F. Wilson, Department of Natural Resources Conservation, University of Massachusetts, for his very helpful suggestions on the manuscript.

References

- Adams, W., and Bastien, J.-C. 1994. Genetics of second flushing in a French plantation of coastal Douglas-fir. *Silvae Genet.* **43**: 345-352.
- Allen, G., and Owens, J. 1972. The life history of Douglas-fir. Environment Canada, Canadian Forest Service, Ottawa, Ont.
- Andersson-Gunneras, S., Mellerowicz, E., Love, J., Segerman, B., Ohmiya, Y., Coutinho, P., Nilsson, P., Henrissat, B., Moritz, T., and Sundberg, B. 2006. Biosynthesis of cellulose-enriched tension wood in *Populus*: global analysis of transcripts and metabolites identifies biochemical and developmental regulators in secondary wall biosynthesis. *Plant J.* **45**: 144-165.
- Barthélémy, D., and Caraglio, Y. 2007. Plant architecture: a dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. *Ann. Bot. (London)*, **99**: 375-407.
- Beveridge, C. 2000. Long-distance signaling and a mutational analysis of branching in pea. *Plant Growth Regul.* **32**: 193-203.
- Bollmark, M., Chen, H.-J., Moritz, T., and Eliasson, L. 1995. Relations between cytokinin level, bud development and apical control in Norway spruce, *Picea abies*. *Physiol. Plant.* **95**: 563-568.
- Borchert, R. 1991. Growth and periodicity and dormancy. In *Physiology of trees*. Edited by A. Raghavendra. John Wiley & Sons, New York. pp. 221-245.

- Bradshaw, H., Ceulemans, R., Davis, J., and Stettler, R. 2000. Emerging model systems in plant biology: poplar (*Populus*) as a model forest tree. *J. Plant Growth Regul.* **19**: 306–313.
- Brown, C., McAlpine, R., and Kormanik, P. 1967. Apical dominance and form in woody plants: a reappraisal. *Am. J. Bot.* **54**: 153–162.
- Campbell, R. 1965. Phenotypic variation and repeatability of stem sinuosity in Douglas-fir. *Northwest Sci.* **39**: 47–59.
- Cannell, M., Thompson, S., and Lines, R. 1976. An analysis of inherent differences in shoot growth within some north temperate conifers. In *Tree physiology and yield improvement*. Edited by M. Cannell and F. Last. Academic Press, New York. pp. 173–205.
- Champagnat, P. 1989. Rest and activity in vegetative buds of trees. In *Forest tree physiology*. Edited by E. Dreyer et al. *Ann. Sci. For.* **46**(Suppl.): 9s–26s.
- Cline, M. 1996. Exogenous auxin effects on lateral bud outgrowth in decapitated shoots. *Ann. Bot. (London)*, **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 Deppong, D. 1999. The role of apical dominance in paradormancy of temperate woody plants: a reappraisal. *J. Plant Physiol.* **155**: 350–356.
- Cline, M., and Dong-Il, K. 2002. A preliminary investigation of the role of auxin and cytokinin in sylleptic branching of three hybrid poplar clones exhibiting contrasting degrees of sylleptic branching. *Ann. Bot. (London)*, **90**: 417–421.
- Cline, M., and Sadeski, K. 2002. Is auxin the repressor signal of branch growth in apical control? *Am. J. Bot.* **89**: 1764–1771.
- Cline, M., Yoders, M., Desai, D., Harrington, C., and Carlson, W. 2006. Hormonal control of second flushing in Douglas-fir shoots. *Tree Physiol.* **26**: 1369–1375.
- Coenen, C., and Lomax, T. 1997. Auxin-cytokinin interactions in higher plants: old problems and new tools. *Trends Plant Sci.* **2**: 351–356.
- Collin, P., Badot, P.-M., and Millet, B. 1994. Control de l'allongement rythmique des tiges par defoliation chez le frêne commun (*Fraxinus excelsior* L.). *C.R. Acad. Sci. Paris Science Vie*, **317**: 1005–1011.
- De Champs, J. 1971. Pousses d'aout du Douglas. Rapport annuel. Association Forêt-Cellulose (AFOCEL), Paris. pp. 173–217.
- Ewers, F., and Zimmerman, M. 1984. The hydraulic architecture of eastern hemlock, *Tsuga canadensis*. *Can. J. Bot.* **62**: 940–946.
- Graham, J., Hobbs, S., and Zaerr, J. 1994. The effect of flurprimidol on bud flush, shoot growth, and on endogenous gibberellins and abscisic acid of Douglas-fir seedlings. *J. Plant Growth Regul.* **13**: 131–136.
- Halle, F., Oldeman, R., and Tomlinson, P. 1978. Tropical trees and forests. Springer-Verlag, Berlin.
- Hare, R. 1984a. EL-500: an effective growth retardant for dwarfing southern pine seedlings. *Can. J. For. Res.* **14**: 123–127.
- Hare, R. 1984b. Stimulation of early height growth in longleaf pine with growth regulators. *Can. J. For. Res.* **14**: 450–462.
- Harmer, R. 1992a. Relationships between shoot length, bud number and branch production in *Quercus petraea* (Matt.) Liebl. *For.* **65**: 61–72.
- Harmer, R. 1992b. The incidence of recurrent flushing and its effect on branch production in *Quercus petraea* (Matt.) Liebl. growing in southern England. *Ann. Sci. For.* **49**: 589–597.
- Harmer, R., and Baker, C. 1995. An evaluation of decapitation as a method for selecting clonal *Quercus petraea* (Matt.) Liebl. with different branching intensities. *Ann. Sci. For.* **52**: 89–102.
- Harrington, C. 1991. Retrospective shoot growth analysis for three sources of loblolly pine. *Can. J. For. Res.* **21**: 306–317.
- Herschback, C., and Kopriva, S. 2002. Transgenic trees as tools in tree and plant physiology. *Trees*, **16**: 250–261.
- Jablanczy, A. 1971. Changes due to age in apical development in spruce and fir. *Can. For. Serv. Bi-Mon. Res. Notes* **27**: 10.
- Jankiewicz, L., and Stecki, Z. 1976. Some mechanisms responsible for differences in tree form. In *Tree physiology and yield improvement*. Edited by M. Cannell and F. Last. Academic Press, New York. pp. 157–172.
- Klee, H., and Romano, C. 1994. The roles of phytohormones in development as studied in transgenic plants. *Crit. Rev. Plant Sci.* **13**: 311–324.
- Kozłowski, T., Torrie, J., and Marshall, P. 1973. Predictability of shoot length from bud size in *Pinus resinosa* Ait. *Can. J. For. Res.* **3**: 34–38.
- Kozłowski, T., Kramer, P., and Pallardy, S. 1991. The physiological ecology of woody plants. Academic Press, New York.
- Kramer, P., and Kozłowski, T. 1979. Physiology of woody plants. Academic Press, New York.
- Lang, G., Early, J., Martin, G., and Darnell, R. 1987. Endo-, para-, and ecodormancy: physiological terminology and classification for dormancy research. *Hortic. Sci.* **22**: 371–377.
- Lanner, R. 1972. Growth and morphogenesis of pines in the tropics. In *Selection and breeding to improve some tropical conifers*. Vol. 1. Edited by J. Burley and D. Nikles. Commonwealth Forestry Institute, Oxford, and Department of Forestry, Queensland, Australia. pp. 126–132.
- Lanner, R. 1976. Patterns of shoot development in *Pinus* and their relationship to growth potential. In *Tree physiology and yield improvement*. Edited by M. Cannell and F. Last. Academic Press, New York. pp. 223–243.
- Lanner, R., and Van Den Berg, D. 1973. The vegetative buds and shoots of lodgepole pine. In *Management of lodgepole pine ecosystems*. Vol. 1. Edited by D. Baumgartner. Washington State University, Pullman, Wash. pp. 68–85.
- Lavender, D., and Zaerr, J. 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.
- Leaky, S., and Longman, K. 1986. Physiological, environmental and genetic variation in apical dominance as determined by decapitation in *Triplochiton scleroxylon*. *Tree Physiol.* **1**: 193–207.
- Leyser, O. 2003. Regulation of shoot branching by auxin. *Trends Plant Sci.* **8**: 541–546.
- Little, C. 1970. Apical dominance in long shoots of white pine (*Pinus strobus*). *Can. J. Bot.* **48**: 239–253.
- Little, C. 1985. Increasing lateral shoot production in balsam fir Christmas trees with cytokinin application. *Hortic. Sci.* **20**: 713–714.
- Livingston, N., Whitehead, D., Kelliher, F., Grace, Y., Walcroft, A., Byers, J., McSeveny, T., and Millard, P. 1998. Nitrogen allocation and carbon isotope fractionation in relation to intercepted radiation and position in a young *Pinus radiata* D. Don tree. *Plant Cell Environ.* **21**: 795–803.
- Mazzola, M., and Costante, J. 1987. Efficacy of BA for the promotion of lateral bud formation on Douglas-fir and Colorado blue spruce. *Hortic. Sci.* **22**: 234–235.
- McCabe, R., and Labisky, R. 1959. Leader forking of red and white pines in plantations. *J. For.* **57**: 94–97.
- McIntyre, G., and Hsiao, A. 1990. The role of expanded leaves in the correlative inhibition of axillary buds in milkweed (*Asclepias syriaca*). *Can. J. Bot.* **68**: 1280–1285.

- Merkle, S., and Dean, J. 2000. Forest tree biotechnology. *Curr. Opin. Biotechnol.* **11**: 298–302.
- Mor, Y., Spiegelstein, H., and Halevy, A. 1981. Translocation of ^{14}C -assimilates in roses. II. The effect of shoot darkening and cytokinin application. *Physiol. Plant.* **52**: 197–200.
- O'Reilly, C., and Owens, J. 1989. Polycyclic growth and branching in provenances of lodgepole pine. *Can. J. For. Res.* **19**: 79–87.
- Owens, J., and Molder, M. 1973. A study of DNA and mitotic activity in the vegetative apex of Douglas-fir during the annual growth cycle. *Can. J. Bot.* **51**: 1395–1409.
- Owens, J., and Simpson, S. 1988. Bud and shoot development in *Picea engelmannii* in response to cone induction treatments. *Can. J. For. Res.* **18**: 231–241.
- Owens, J., Webber, J., Ross, S., and Pharis, R. 1985. Interaction between gibberellin $A_{4/7}$ and root-pruning on the reproductive and vegetative processes in Douglas-fir. III. Effects on anatomy of shoot elongation and terminal bud development. *Can. J. For. Res.* **15**: 354–364.
- Pillay, L., and Railton, L. 1983. Complete release of axillary buds from apical dominance in intact, light-grown seedlings of *Pisum sativum* L., following a single application of cytokinin. *Plant Physiol.* **71**: 972–979.
- Powell, G. 1987. Syllepsis in *Larix laricina*: analysis of tree leaders with and without sylleptic long shoots. *Can. J. For. Res.* **17**: 490–498.
- Rasmussen, H., Soerensen, S., and Andersen, L. 2003. Bud set in *Abies nordmanniana* Spach. influenced by bud and branch manipulations. *Trees*, **17**: 510–514.
- Romberger, J. 1963. Meristems, growth and development in woody plants. USDA Tech. Bull. No. 1293.
- Roth, B., and Newton, M. 1996. Role of lammas growth in recovery of Douglas-fir seedlings by weed control, fertilization, and seed source. *Can. J. For. Res.* **26**: 936–944.
- Rudolph, T. 1964. Lammas growth and prolepsis in jack pine in the Lake States. *For. Sci. Monogr.* **6**: 1–70.
- Schermann, N., Adams, W., Aitken, S., and Bastein, J.-C. 1997. Genetic parameters of stem for traits in a 9-year-old coastal Douglas-fir progeny test in Washington. *Silvae Genet.* **46**: 166–170.
- Schmitz, G., and Theres, K. 2005. Shoot and inflorescence branching. *Curr. Opin. Plant Biol.* **8**: 506–511.
- Sellin, A. 1988. Hydraulic architecture in the Norway spruce. *Fiziol. Rast. (Moscow)*, **35**: 1099–1107.
- Souza, M., Puntieri, J., Barthélemy, D., and Brion, C. 2000. Bud content and its relation to shoot size and structure in *Nothofagus pumilio* (Poepp. et Endl.) Krasser (Nothofagaceae). *Ann. Bot. (London)*. **85**: 547–555.
- Srivastava, L. 2001. Plant growth and development. Academic Press, New York.
- Stafstrom, J. 1993. Axillary bud development in pea: apical dominance, growth cycles, hormonal regulation and plant architecture. In *Cell communication in plants*. Edited by R. Aminsino. Plenum Publishing Corporation, New York. pp. 75–86.
- Sterck, F. 2005. Woody tree architecture. *Annu. Plant Rev.* **17**: 209–237.
- Suzuki, T. 1990. Apical control of lateral bud development and shoot growth in mulberry (*Morus alba*). *Physiol. Plant.* **80**: 350–356.
- Taiz, L., and Zeiger, E. 2002. Plant physiology. 3rd ed. Sinauer Associates, Inc., Mass.
- Temel, F., and Adams, W. 2000. Persistence and age-age genetic correlations of stem defects in coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). *For. Genet.* **7**: 145–153.
- Thimann, K., and Skoog, F. 1933. Studies on the growth hormones of plants. III. The inhibition action of growth substance on bud development. *Proc. Nat. Acad. Sci. U.S.A.* **19**: 714–716.
- Timell, T. 1986. Compression wood in gymnosperms. II. Springer-Verlag, New York.
- Trewavas, A. 1987. Growth substance sensitivity: the limiting factor in plant development. *Physiol. Plant.* **55**: 60–72.
- Vargas-Hernandez, J., Adams, W., and Joyce, D. 2003. Quantitative genetic structure of stem form and branching traits in Douglas-fir seedlings and implications for early selection. *Silvae Genet.* **52**: 36–44.
- Wareing, P. 1970. Growth and its coordination in trees. In *Physiology of tree crops*. Edited by L. Luckwill and C. Cuttings. Academic Press, New York. pp. 1–21.
- Whitehill, S., and Schwabe, W. 1975. Vegetative propagation of *Pinus sylvestris*. *Physiol. Plant.* **35**: 66–71.
- 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.
- Wilson, B., and 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., and Brown, C. 1971. Trees: structure and function. Springer-Verlag, New York.