J Plant Growth Regul (1993) 12:123–127

Journal of
Plant
Growth
Regulation

Interaction of Growth Retardants, Daylength, and Gibberellins A_{19}, A_{20}, and A_1 on Shoot Elongation in Birch and Alder

Olavi Junttila

Department of Plant Physiology and Microbiology, University of Tromsø, N-9037 Tromsø, Norway

Received April 5, 1993; accepted July 14, 1993

Abstract. Gibberellins A_{19}, A_{20}, and A_1 were applied to seedlings of birch (*Betula pubescens* Ehrh.) and alder (*Alnus glutinosa* (L.) Gaertn.) in order to test their ability to counteract growth inhibition induced by growth retardants (ancymidol and BX-112) or short day (SD, 12 h) photoperiod. Ancymidol inhibits early and BX-112 inhibits late steps in gibberellin biosynthesis. BX-112 inhibited stem elongation in both species while ancymidol, applied as a soil drench, was effective in alder only. Growth retardants affected stem elongation mainly by inhibiting elongation of internodes. All three gibberellins were equally active when applied to seedlings treated with ancymidol; however, only GA_1 was able to counteract the growth inhibition induced by BX-112. SD-induced cessation of elongation growth in birch was counteracted by GA_1, and to some degree, by GA_{20}, while GA_{19} was inactive. SD treatment did not induce cessation of apical growth in alder. These results are consistent with the hypothesis that of gibberellins belonging to the early C-13 hydroxylation pathway, GA_1 is the only active gibberellin for stem elongation.

Based on studies on GA-deficient mutants of maize, Phinney (1984) has suggested that GA_1 is the effector GA for stem elongation. Gibberellins are central in the regulation of stem elongation in willow, *Salix pentandra*, and results suggest that GA_1 is the main active gibberellin for elongation growth in this species (Junttila 1976, Davies et al. 1985, Junttila and Jensen 1988, Junttila et al. 1991). Several earlier studies have demonstrated a significant enhancement of stem elongation in different hardwood species by GA, primarily GA_3 (Melchior and Knapp 1962). Although GA_3 is endogenous in some species (Rood et al. 1987), its physiological role is uncertain. The early C-13 hydroxylation pathway can be the primary pathway for biosynthesis of GA_1 in vegetative tissues of monocots and dicots, including hardwoods (Graebe 1987, Junttila 1991). With the exception of willow, biological activity of various GAs belonging to this pathway has not been studied on different tree species.

The purpose of this study was to compare biological activity of three GAs from the early C-13 hydroxylation pathway, GA_{19}, GA_{20}, and GA_1, on seedlings of birch and alder. Two growth retardants, ancymidol and BX-112, were used to inhibit seedling growth prior to GA application. Ancymidol blocks GA biosynthesis at an early step, *ent*-kaurene oxidation (Coolbaugh and Hamilton 1976), and BX-112 inhibits 3β-hydroxylation, including conversion of GA_{20} and GA_1 (Nakayama et al. 1990a,b, 1991, Kamiya et al. 1991, Sponsel and Reid 1991, Rademacher et al. 1992, Martinez-Garcia and Garcia-Martinez 1992). Kamiya et al. (1991) have suggested that 3β-hydroxylation is a necessary step in the biosynthesis of gibberellins that promote shoot elongation in rice. In addition, the three GAs were also tested for their ability to induce stem elongation in seedlings grown under short day conditions.

Materials and Methods

Plant Material

One seed lot of alder (*Alnus glutinosa* (L.) Gaertn., from 54° N lat.) and two seed lots of birch (*Betula pubescens* Ehrh., from 59° and 70° N lat.) were used. Seeds of birch were stratified at 4°C for 5–6 weeks before sowing. Seeds were germinated on fertilized peat, seedlings were transplanted in fertilized peat in 12 cm pots, four plants in each, and grown at 21°C in 24 h photoperiod to a size of about 5 cm. Natural light was supplemented with fluorescence tubes (Philips TL 65W/83) to give minimum 100 $\mu mol\ m^{-2}s^{-1}$ within 400–750 nm. Temperature in the phytotron was controlled to ±0.5°C, and water vapor pressure deficit was adjusted to 0.5 kPa.

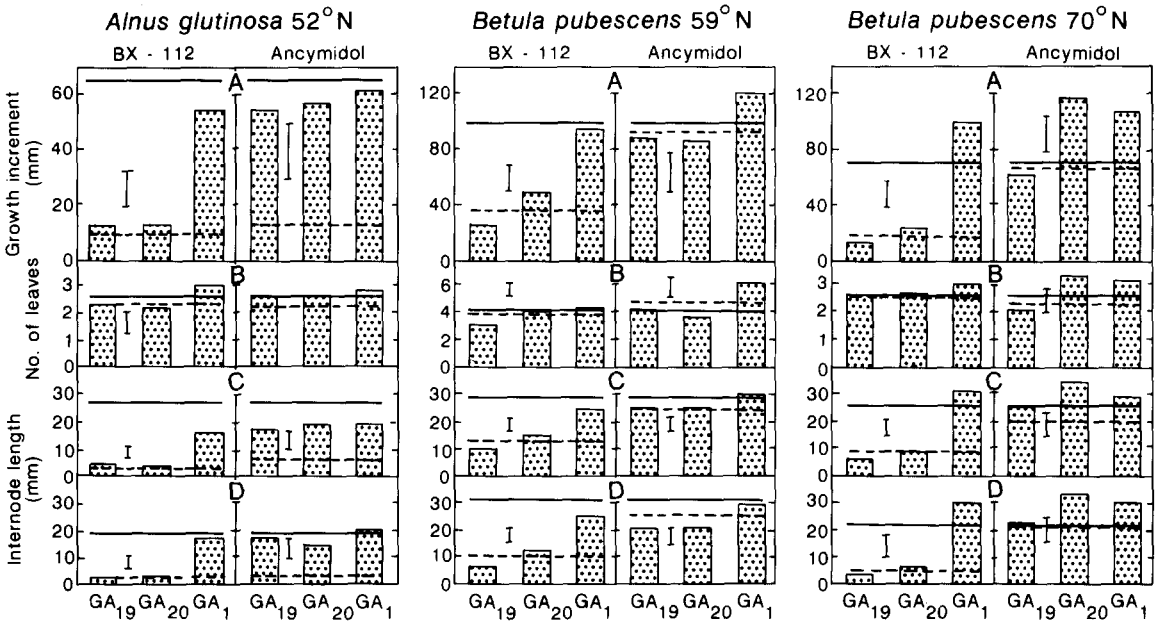

Fig. 1. Effects of growth retardants (BX-112, ancymidol) and gibberellins A_{19}, A_{20}, and A_1 on shoot elongation (**A**), development of new leaves (**B**), and length of the first (**C**) and the second (**D**) internode formed after application of GAs in seedlings of *Alnus glutinosa* and *Betula pubescens*. Growth was recorded 2 weeks after application of GAs. Solid line: untreated control; broken line: growth retardant alone. Vertical bars give $LSD_{0.05}$.

Treatments

Experiments were done at 18°C. Short day (SD) treatment consisted of 12 h light period, as described above, and 12 h darkness. Seedlings treated with ancymidol [α-cyclopropyl-α-(4-methoxyphenyl)-5-pyrimidine methanol, "Reducymol," Elanco Products Div., Eli Lilly & Co., IN, USA] or BX-112 (calcium 3,5-dioxo-4-propionylcyclohexane carboxylate, Kumiai Chemical Industry, Co., Ltd., Tokyo, Japan) were grown under 24 h photoperiod, 12 h daylight and 12 h light from incandescent lamps (Osram 75W, 11 umol $m^{-2}s^{-1}$, 400–750 nm). Ancymidol was applied as a soil drench, 50 ml of 0.02 mM solution per pot. BX-112 was sprayed on the pants as a 2.5 mM aqueous solution four times a week. Gibberellins (from Prof. L. N. Mander, Canberra, Australia) were dissolved in ethanol and applied once as a 1 μl microdrop to the apex. Gibberellins were applied 1 week after treatments with growth retardants were started, and 10 days after the initiation of SD treatment. Stem elongation, number of new leaves, and length of the two first internodes developed after GA application were observed after a growth period of 14 days at 18°C. There were a minimum of 8 seedlings in each treatment.

Statistics

Statistical analyses were carried out using Stat View SE + Graphics program (Abacus Concepts, Inc.). $LSD_{0.05}$ values or standard deviations are presented.

Results

BX-112 effectively inhibited stem elongation both in alder and in birch, while ancymidol had a significant effect on alder only (Fig. 1). Growth inhibition, caused by both growth retardants, was mainly due to an inhibition of internode elongation, and the number of new leaves developed during the experiment was not significantly affected (Fig. 1).

The growth inhibition induced by ancymidol was effectively counteracted by all three gibberellins—GA_{19}, GA_{20}, and GA_1, although in some cases GA_{20} and GA_1 tended to have a stronger effect than GA_{19}. However, only GA_1 was active in seedlings treated with BX-112, and the effects of GA_{19} and GA_{20} were not significantly different from BX-112 treatments (Fig. 1).

SD treatment did not induce cessation of apical growth in alder, probably due to the southern origin of the seed lot. Two separate experiments were carried out with alder. In one experiment no significant effect of SD could be observed, but in the other experiment internode elongation was reduced by SD. This effect was counteracted most effectively by GA_1, although GA_{20} and GA_{19} were also active (Table 1).

Seedlings of both ecotypes of birch stopped growing and formed apical bud during the 10 day SD treatment. Thus, untreated SD plants did not develop any new leaves during the experiment. Application of GA_1 induced new growth, both production of nodes and elongation of internodes, under SD conditions, comparable to growth under LD (Fig. 2). GA_{20} was significantly less active than GA_1, and in the northern ecotype even 30 nmol of GA_{20} had a minor influence only. GA_{19} was, with

Table 1. Effect of photoperiod and gibberellins on internode length in seedlings of *Alnus glutinosa*.

	Internode length (mm)			
LD	SD Contr.	SD GA_{19}	SD GA_{20}	SD GA_1
20.4 ± 4.3	9.3 ± 2.6	16.1 ± 3.5	19.9 ± 5.5	28.5 ± 5.2

Length of the first internode developed after start of the experiment was measured. Temperature 18°C, photoperiod 24 h (LD) or 12 h (SD). Ten plants per treatment. Six nmol GA was applied per plant. Mean ± standard deviation. Photoperiod of 12 h was not short enough to induce bud set within 24 days in the southern (54° N lat.) ecotype of *Alnus* used.

the amounts used, completely inactive in the northern ecotype, but in the southern ecotype a small response was observed with 30 nmol of GA_{19} (Fig. 2).

Discussion

Lack of biological activity of GA_{19} and GA_{20} in seedlings of alder and birch treated with BX-112 (Fig. 1) strongly indicates that these GAs are active in these species only due to their conversion to GA_1. In accordance with this, all three GAs were equally active in seedlings treated with ancymidol which prevents metabolic steps before formation of GA_{19}. These results give further support to the suggestion that GA_1 can be a universal effector GA for stem elongation in higher plants (Phinney 1984), although also GA_4 is expected to be active per se in certain species (MacMillan 1985). However, it should be stressed that our knowledge of endogenous GAs, their metabolism and physiological effects, is restricted to very few species, and thorough studies on various new taxa are needed before too many generalizations can be made. Endogenous GAs in *Betula* and *Alnus* are not yet known, but unpublished results (S. B. Rood, personal communication) indicate the presence of the early-13-hydroxylation biosynthetic pathway in paper birch.

Short photoperiod has been suggested to inhibit the conversion of GA_{53} to GA_{44} and GA_{19} to GA_{20} (Gilmour et al. 1986, Zeevaart et al. 1991). In *Salix pentandra*, both GA_{20} and GA_1 are equally active in stimulation of stem elongation under SD conditions, while GA_{19} and earlier members of this pathway are inactive, suggesting a SD-induced block between GA_{29} and GA_{20} (Junttila and Jensen 1988). This seems to be the case in birch as well (Fig. 2). In addition, contrary to the results with willow, GA_{20} was significantly less active than GA_1 in birch. This indicates that the mechanism of photoperiodic regulation of stem elongation is not necessarily similar

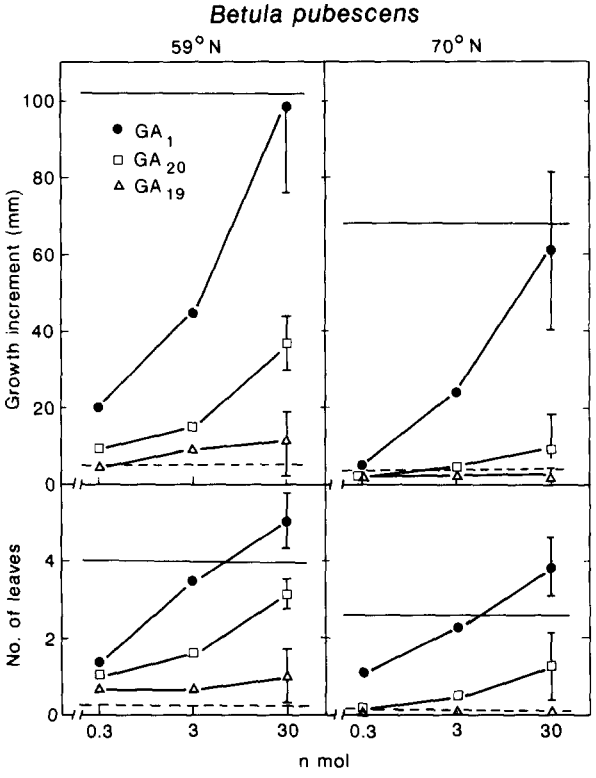

Fig. 2. Shoot elongation and development of new leaves in seedlings of two *Betula pubescens* ecotypes grown in SD (12 h photoperiod) as affected by gibberellins A_{19}, A_{20}, and A_1. Growth was recorded 2 weeks after application of GAs. Solid line: LD control (24 h photoperiod); broken line: SD control. The largest standard deviations are shown by vertical bars.

in *Salix* and *Betula*. A simple interpretation of the results in Fig. 2 is that both the step from GA_{19} to GA_{20} and the step from GA_{20} to GA_1 can be inhibited in SD. It has been suggested (Nishijima et al. 1991) that in cold treated seedlings of *Raphanus sativus* conversion of GA_{20} to GA_1 is activated in LD.

Results in Fig. 2 also indicate that there are quantitative differences between latitudinal ecotypes of birch in their responses to applied GAs under SD conditions. The southern ecotype was more sensitive than the northern one. This effect can be related to different critical photoperiods for growth between these ecotypes. In northern temperate zone tree species, the critical photoperiod increases with increasing latitude of the ecotype (Heide 1974, Håbjørg 1978, Junttila 1980), and for the used ecotypes the critical photoperiod is approximately 16 and 22 h for the southern and northern origin, respectively. Previous experiments with *Salix pentandra* have shown that the response to applied GAs increases when the photoperiod approaches the critical one (Junttila 1991). If the photoperiod is close to the critical photoperiod, even GA_{19} gives a

significant response. This conclusion is also supported by the present results with alder, where GA_{19}, GA_{20}, and GA_1 were active under SD conditions that did not induce complete bud set (Table 1). Photoperiodic ecotypes are known to exist in alder (Håbjørg 1974), and lack of clear photoperiodic response in 12 h photoperiod is most probably due to the very southern origin of the ecotype used (54° N, compared to 59° for the southern ecotype of birch). Physiological and biochemical bases of these quantitative differences between geographic ecotypes are not known at all.

The present results, like those with *Salix pentandra* (Junttila and Jensen 1988, Junttila et al. 1991), clearly show that growth inhibition by growth retardants is physiologically different from the effect of SD. SD leads both to an inhibition of development of new leaves and internode elongation, while growth retardants primarily prevent elongation of internodes (Figs. 1 and 2). Although both types of growth inhibitions can be completely antagonized by externally applied GA, there must be significant differences between these two types of treatments in respect to their physiological effects. These differences can be related to correlative influences between different parts of meristematic tissues, apical meristem proper and subapical meristem, as well as between meristems and elongating tissues.

In conclusion, of the three gibberellins, GA_{19}, GA_{20}, and GA_1, only GA_1 is active per se in stimulation of elongation growth in seedlings of alder and birch. Activity of GA_{19} and GA_{20} is due to their metabolic conversion to GA_1. Further, these results from exogenous applications indicate that photoperiodic control of shoot growth in birch involves regulation of GA_{19} to GA_{20} and, perhaps, GA_{20} to GA_1.

Acknowledgments. Kumiai Chemical Industry Co., Ltd. is acknowledged for the sample of BX-112. Thanks are due to the staff at the phytotron of the University of Tromsø for their assistance. Financial support from the Norwegian Research Council for Sciences and Humanities (NAVF) is also acknowledged.

References

Coolbaugh RC, Hamilton R (1976) Inhibition of ent-kaurene oxidation and growth by α-cyclopropyl-α-(p-methoxyphenyl)-5-pyrimidine methyl alcohol. Plant Physiol 57: 245–248

Davies JK, Jensen E, Junttila O, Rivier L, Crozier A (1985) Identification of endogenous gibberellins from *Salix pentandra*. Plant Physiol 78:473–476

Gilmour SJ, Zeevaart JAD, Schwenen L, Graebe JE (1986) Gibberellin metabolism in cell-free extracts from spinach leaves in relation to photoperiod. Plant Physiol 82:190–195

Graebe JE (1987) Gibberellin biosynthesis and control. Annu Rev Plant Physiol 38:419–465

Håbjørg A (1978) Photoperiodic ecotypes in Scandinavian trees and shrubs. Meld Norges Landbrhøgsk 51:1–27

Heide OM (1974) Growth and dormancy in Norway spruce ecotypes (*Picea abies*). I. Interaction of photoperiod and temperature. Physiol Plant 30:1–12

Junttila O (1976) Growth cessation and shoot tip abscission in *Salix*. Physiol Plant 38:278–286

Junttila O (1980) Effect of photoperiod and temperature on apical growth cessation in two ecotypes of *Salix* and *Betula*. Physiol Plant 48:347–352

Junttila O (1991) Gibberellins and regulation of shoot elongation in woody plants. In: Takahashi N, MacMillan J, Phinney BO (eds) Gibberellins. Springer-Verlag, Heidelberg, pp 190–210

Junttila O, Jensen E (1988) Gibberellins and photoperiodic control of shoot elongation in *Salix*. Physiol Plant 74:371–376

Junttila O, Jensen E, Ernstsen A (1991) Effects of prohexadione (BX-112) and gibberellins on shoot growth in seedlings of *Salix pentandra*. Physiol Plant 83:17–21

Kamiya Y, Kobayashi M, Fujioka S, Yamane H, Nákayama I, Sakura A (1991) Effect of a plant growth regulator, prohexadione calcium (BX-112), on the elongation of rice shoots caused by exogenously applied gibberellins and helminthosporol, Part II. Plant Cell Physiol 32:1205–1210

MacMillan J (1985) Gibberellins: Metabolism and function. In: Randall DD, Blevins DG, Larson RL (eds) Current topics in plant biochemistry and physiology, Vol. 4. University of Missouri, Columbia, Missouri, pp 53–66

Martinez-Garcia JF, Garcia-Martinez JL (1992) Effect of the growth retardant LAB 198 999, an acylcyclohexanedione compound, on epicotyl elongation and metabolism of gibberellins A_1 and A_{20} in cowpea. Planta 188:245–251

Melchior GH, Knapp R (1962) Gibberellin-Wirkungen an Bäumen. Silvae Genetica 11:29–39

Nakayama I, Kamiya K, Kobayashi M, Abe H, Sakurai A (1990a) Effects of a plant growth regulator, prohexadione, on the biosynthesis of gibberellins in cell-free systems derived from immature seeds. Plant Cell Physiol 31:183–190

Nakayama I, Miyazawa T, Kobayashi M, Kamiya Y, Abe H, Sakurai A (1990b) Effects of a new plant growth regulator, prohexadione calcium (BX-112), on shoot elongation caused by exogenously applied gibberellins in rice (*Oryza sativa* L.) seedlings. Plant Cell Physiol 31:195–200

Nakayama I, Miyazawa T, Kobayashi M, Kamiya Y, Abe H, Sakurai A (1991) Studies on the action of the plant growth regulators BX-112, DOCHC and DOCHC-Et. In: Takahashi N, MacMillan J, Phinney BO (eds) Gibberellins. Springer-Verlag, Heidelberg, pp 311–319

Nishijima T, Koshioka M, Katsura N, Yamaji H, Nakayama M, Yamane H, Yamaguchi I, Yokota T, Murofushi N, Takahashi N, Nonaka M (1991) Relationship between endogenous gibberellins and bolting of Japanese radish (*Raphanus sativus* L.). Abstracts 14th International Conference on Plant Growth Substances, Agricultural University, Wageningen, p 66

Phinney BO (1984) Gibberellin A1, dwarfism and the control of shoot elongation in higher plants. In: Crozier A, Hillman TR (eds) The biosynthesis and metabolism of plant hormones. Cambridge University Press, Cambridge, pp 17–41

Rademacher W, Temple-Smith KE, Griggs DL, Hedden P (1992) The mode of action of acylcyclohexanediones: A new type of growth retardant. In: Karssen CM, van Loon LC, Vreugdenhil D (eds) Progress in plant growth regulation. Kluwer Academic Publishers, Dordrecht, pp 571–577

Rood SB, Pearce D, Pharis RP (1987) Identification of endogenous gibberellins from oilseed rape. Plant Physiol 85:605–607

Sponsel VM, Reid JB (1991) Effects of two growth retardants and gibberellins A_1 and A_{20} on light and dark grown seedlings of tall and dwarf pea. Plant Physiol (Suppl.) 96:157

Zeevaart JDA, Talon M, Wilson TM (1991) Stem growth and gibberellin metabolism in spinach in relation to photoperiod. In: Takahashi N, MacMillan J, Phinney BO (eds) Gibberellins. Springer-Verlag, Heidelberg, pp 273–279