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Endogenous Gibberellins in Flushing Buds of Three Deciduous Trees: Alder, Aspen, and Birch

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Abstract. Endogenous gibberellins (GAs) were extracted from flushing (expanding) vegetative buds of river alder (*Alnus tenuifolia*), European white birch (*Betula pendula*), and aspen (*Populus tremuloides*) and identified by gas chromatography-mass spectrometry with full scans and/or selected ion monitoring. Five 13-hydroxylated GAs were detected from the three trees: GA_1, $_8$, and $_{20}$ from alder, GA_1, $_8$, $_{19}$ and $_{20}$ from aspen and GA_1, $_8$, $_{19}$, $_{20}$, and $_{29}$ from birch. Thirteen other GAs previously detected in *Salix* or common in other plants were specifically investigated but not detected. The presence of GA_1, its probable precursors GA_{19} and GA_{20}, and its probable metabolite, GA_8, suggests that the early 13-hydroxylated GA biosynthetic pathway is dominant in vegetative buds of these trees. Abundant endogenous GAs of these trees are similar to the principal GAs of willows (various *Salix* spp.) and poplars (various *Populus* spp.). This suggests similarities in the GA physiology and is consistent with a common role of GA_1 as a regulator of shoot growth in woody angiosperms.

Gibberellins (GAs) have been identified from both angiosperm and gymnosperm trees (Davies et al. 1985, Dunberg and Odén 1983, Koshioka et al. 1985, Rood et al. 1988). Nonhydroxylated GAs and 3-hydroxylated GAs such as GA_4 and GA_9 are abundant in conifers (Pharis and Kuo 1977, Odén et al. 1987), while 13-hydroxylated GAs, such as GA_{19} and GA_{20} are more abundant in angiosperm trees including apple (Koshioka et al. 1985), poplar (Rood et al. 1988) and *Salix* (willow) (Davies et al. 1985, Junttila and Jensen 1988).

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Phinney (1985) has suggested that GA_1 is the primary effector GA for stem elongation in maize and probably other plants. Among woody plants, GA physiology is probably best understood in *Salix* in which GA_1 is apparently the bioactive GA that regulates shoot elongation (Junttila and Jensen 1988). In *Salix*, GA_1 probably originates principally from GA_{20} (Rood and Junttila 1989), which in turn probably principally originates from GA_{19}, the common precursor of GA_{20} in numerous crop plants (Graebe, 1987). Junttila et al. (1992) have also shown that in *Salix* GA_1 can also be formed from GA_9 via hydroxylation through GA_{20}. Shoot growth in *Salix* is partly controlled by photoperiod (Junttila 1980) which apparently influences the conversion of GA_{19} to GA_{20} (Junttila and Jensen 1988) but not GA_{20} to GA_1 (Rood and Junttila 1989).

The principal GAs of a fast-growing interspecific poplar hybrid are GA_1, GA_{19}, and GA_{20} (Rood et al. 1988), the same as in *Salix*. Levels of GA-like substances were positively correlated with height in different poplar species and hybrids, supporting a regulatory role of GAs in the control of shoot elongation in another member of the Salicaceae (Bate et al. 1988).

Shoot growth of other fast-growing angiosperm trees is also under photoperiodic control, and this control has been proposed to be through the regulation of GA metabolism (Junttila 1993). Junttila (1993) recently demonstrated that exogenously applied GA_1 was more effective than GA_{20} or particularly, GA_{19} at overcoming shoot growth cessation in birch (*Betula pubescens*) and alder (*Alnus glutinosa*) caused by short days. This result as well as the recovery of shoot growth following the application of two growth retardants lead Junttila (1993) to propose that GA_1 is the bioactive GA regulating shoot elongation in alder and birch, as it apparently is in *Salix*.

Junttila's (1993) studies suggest the common importance and regulation of GA_1 in the shoots of alder, birch, and willow. However, this proposed role of GA_1 and importance of conversion from GA_{19} and GA_{20} is somewhat speculative since the endogenous GAs of birch and alder have not been identified. Junttila's (1993) hypothesis would result in two testable predictions. Firstly, GA_1, and two of its biosynthetic precursors, GA_{19} and GA_{20}, should be abundant in the shoots of other angiosperm trees. Secondly, these GAs should be particularly abundant in flushing vegetative buds, the shoot structures that respond to increasing day length with the commencement of growth. Consequently, the present study investigated the endogenous GAs of flushing vegetative buds of alder, aspen, and birch.

Materials and Methods

Plant Materials

Branches of alder, *Alnus tenuifolia* Nutt. [syn. *A. incana* (L.) Moench, (Kuijt 1982)], and aspen, *Populus tremuloides* Michx., were collected from the eastern slopes of the Canadian Rockies near the University of Lethbridge Westcastle field station (49°29'N and 114°25'W) on May 1, 1993, during the early stages of bud flushing. Branches from cultivated birch trees, *Betula pendula* Rott, with unexpanded vegetative buds were harvested at Lethbridge (latitude 49°41'N and longitude 112°51'W) on May 2, 1993. Branches were placed in water at the University of Lethbridge greenhouse with a temperature of about 22°C. After 48 h (aspen and birch) or 96 h (alder), expanding vegetative buds were excised, frozen in liquid nitrogen, and lyophilized for 7 days.

Analyses of Endogenous GAs

Expanding vegetative buds (with dry weights ranging from 1.42 to 4.67 g) were ground in cold 80% aqueous methanol (MeOH) using a mortar and pestle and subsequently homogenized using a polytron probe. After 12 h of extraction at 4°C, samples were filtered under vacuum. After the addition of 0.1 M phosphate buffer (pH 8.0), the MeOH was removed *in vacuo* at 35°C, the aqueous extract was adjusted to pH 9 using 2 N KOH, and the extract was partitioned twice against water-saturated diethyl ether, which was discarded. The aqueous extract was adjusted to pH 7 using 1 N HCl, slurried with poly-N-polyvinylpyrolidine, and vacuum filtered. The filtrate was acidified to pH 3 and partitioned three times against water-saturated ethyl acetate (EtOAc). The EtOAc extract was frozen and filtered to remove water, and the EtOAc was removed *in vacuo*.

Prior to further purification by step-elution silicic acid partition chromatography (Durley et al. 1972, Rood et al. 1983) and C_{18} reversed-phase HPLC (Koshioka et al. 1983), standards of [1,2-^{3}H]GA_1 and [1,2-^{3}H]GA_4 (0.25 KBq each, specific activity: 1.2TBq/mmol, Amersham, Oakville, Ontario) were added to the extracts. Fractions were grouped based on the retention times of authentic ^{3}H-GA standards, published reports (Koshioka et al. 1983) and the elution of endogenous GAs from other tissues previously analyzed (Dobert et al. 1992, Zanewich and Rood 1993). After drying under vacuum, HPLC samples were methylated and silylated (Rood et al. 1987) and analyzed using GC-MS with both full scan and selected ion monitoring (SIM) programs on a Hewlett Packard 5890 Series II gas chromatograph (containing a 15 m × 0.25 mm J&W Scientific DB5MS fused capillary column [Chromatographic Specialties, Brockville, Ontario]) and 5970 series mass spectrometer (Zanewich and Rood 1993).

For SIM analyses, appropriate fractions were probed for those GAs indicated in Table 1 in addition to $GA_{3,\ 4,\ 5,\ 7,\ 9,\ 17,\ 24,\ 27,\ 34,\ 36,\ 44,\ 51,}$ and $_{53}$, other GAs that have been found in woody angiosperms or are metabolically related GAs. GA identifications were based on comparison with Kovats Retention Indices (KRI) (Gaskin et al. 1971) and ion abundances of authentic GA standards, published data (Crozier and Durley 1983, Hedden 1986, Takahashi et al. 1986), and the GC-MS behavior of GAs previously identified from other tissues (Dobert et al. 1992, Zanewich and Rood 1993).

Estimates of relative endogenous GA content were made by first finding the abundance of the molecular ions or base peak (m/z 508, 596, 436, and 420, respectively) from a mixture of 10 ng of each of [$^2H_2$]$GA_{1,\ 8,\ 19,}$ and $_{20}$, and expressing these relative to the abundance of the molecular ion of [$^2H_2$]GA_{20}. [$^2H_2$]GAs were obtained from L. N. Mander, Australian National University. These values were then used to estimate the relative quantities of endogenous GAs, relative to GA_{20}, in analyses of each of the trees. This comparison should account for differences in derivatization and fragmentation following ionization but does not consider differential purification recovery.

Results and Discussion

Fractions from C_{18} reversed-phase HPLC were combined in eight different groups of GC-SIM analyses. Ions for eighteen different GAs, including common 13-hydroxylated-, nonhydroxylated-, and 3-hydroxylated-GAs were monitored from the appropriate HPLC groupings. Five different GAs, $GA_{1,\ 8,\ 19,\ 20,}$ and $_{29}$, were detected from alder, aspen, and/or birch buds (Table 1), with identifications being based on combined information including appropriate HPLC retention time, KRI values, and mass spectra or selected ion abundances similar to authentic GA standards and published values. Gibberellins A_1, A_8, and A_{20} were identified from the vegetative buds of all three trees. GA_{19} was present in birch and aspen tissue but undetectable in the alder sample. A common 2β-hydroxylated metabolite of GA_{20}, GA_{29}, was detected only from the birch buds.

The relative quantities of the GAs detected were apparently $GA_8 > GA_{20} > GA_1$ in alder, $GA_8 > GA_{20} > GA_{19} > GA_1$ in aspen, and $GA_{20} > GA_1 > GA_{19} > GA_8$ in birch (Table 2). Consistent with this assessment, quantities of GA_{20} were sufficient for identification by full scan GC-MS. However, the comparison of GA abundances across the trees may be confounded since alder and aspen were har-

Table 1. Me-TMSi gibberellins (GAs) identified by capillary gas chromatography-selected ion monitoring (GC-SIM) or mass spectrometry (GC-MS) from alder, aspen, and birch vegetative buds.

GA	HPLC fraction	KRI[a]	Ion m/z and (relative abundance)						
Alder									
GA_1	24–26	2649	506 (100)	491 (16)	448 (21)	447 (9)	416 (6)	390 (4)	377 (24)
GA_8	14–18	2768	594 (100)	579 (6)	565 (2)	547 (2)	553 (2)	535 (5)	448 (22)
				379 (12)	375 (33)				
GA_{20}	30–32	2499	418 (100)	403 (10)	387 (trc)[b]	375 (20)	359 (20)	301 (4)	
Aspen									
GA_1	22–24	2648	506 (100)	491 (11)	448 (21)	447 (23)	416 (5)	390 (20)	377 (25)
GA_8	14–17	2771	594 (100)	579 (4)	565 (1)	553 (12)	547 (1)	535 (4)	448 (26)
GA_{19}	31–33	2601	462 (4)	434 (100)	447 (23)	431 (20)	402 (43)	375 (58)	
GA_{20}	28–30	2486	418 (100)	403 (16)	387 (6)	375 (76)	359 (19)	301 (11)	
Birch									
GA_1	22–24	2649	506 (100)	491 (9)	448 (27)	447 (20)	416 (4)	390 (8)	377 (19)
GA_8	14–17	2768	594 (100)	579 (6)	565 (2)	553 (2)	535 (7)	519 (15)	448 (31)
				379 (12)					
GA_{19}	31–33	2603	462 (10)	434 (100)	447 (trc)[b]	431 (5)	402 (44)	375 (40)	374 (60)
				345 (56)					
GA_{20}	28–31	2489	418 (100)	403 (15)	387 (2)	375 (75)	359 (16)	301 (2)	
GA_{29}	14–17	2651	506 (100)	491 (10)	477 (trc)[b]	447 (7)	389 (20)	375 (68)	

[a] Kovats Retention Index. Samples were analyzed on a DB5MS capillary column.
[b] Trace ion abundance.

Table 2. Estimates of relative content of endogenous GAs from alder, aspen, and birch vegetative buds.

	Relative GA amount		
Gibberellin	Alder	Aspen	Birch
GA_1	0.6×	0.4×	0.9×
GA_8	2.8×	1.7×	0.2×
GA_{19}		0.6×	0.6×
GA_{20}	1×	1×	1×

Note. Amounts are normalized relative to the abundance of GA_{20} with detection efficiencies based on prior analyses of $[^2H_2]GA_{1,3,8,19,20}$. Relative contents are not comparable between tree species.

vested from a montane site, whereas birch tissue was from a cultivated tree growing in an adjacent prairie region. It must also be recognized that the present assessment of comparative GA abundances is imprecise since internal standards were not included during initial GA extraction and purification. Instead, prior to and following endogenous GA analyses, deuterated GA standards were analyzed to derive correction factors that were then used to assess relative endogenous GA content.

The presence of GA_1 and its probable precursor, GA_{20} (Rood and Junttila 1989), suggests that the early 13-hydroxylation biosynthetic pathway was dominant in these three tree species, at least with respect to the vegetative buds. GA_8 is a common metabolite from GA_1 and its occurrence in buds from the three trees is consistent with the abundance of GA_1. GA_{44} and GA_{53}, two other 13-hydroxylated GAs that are probable precursors of GA_{19}, were not detected in any samples. The apparent abundance of GA_{1}, $_{19}$, and $_{20}$ but scarcity of the earlier biosynthetic precursors such as GA_{44} or GA_{53} suggest that vigorously growing flushing buds might represent a site of GA_1 action where GA biosynthesis is not limited prior to GA_{19}. However, alternate explanations such as GA_{19} translocation are possible.

The abundance of GA_{1}, $_{19}$, and $_{20}$ in the flushing buds of these three trees is consistent with previous reports based on actively growing young shoots and seedlings of hybrid poplar, *Populus balsamifera X P. deltoides* (Rood et al. 1988), and *Salix* (Davies et al. 1985). It is thus likely that there are commonalities of GA physiology in these fast-growing angiosperm trees.

The abundance of GA_{20} and GA_1 also suggests that these GAs are physiologically important and could regulate bud flushing and shoot growth in these three trees. The apparently greater abundance of GA_{20} than GA_{19} suggests that the conversion of GA_{19} to GA_{20} is not blocked, a conclusion that is consistent with the proposal that long-day conditions induce bud flushing and shoot growth in some woody angiosperms at least partially by promoting the conversion of GA_{19} to GA_{20} (Junttila and Jensen 1988; Junttila 1993).

The apparent absence of non-13-hydroxylated

GAs is noteworthy. GAs including GA_4, $_9$, and $_{24}$, are abundant in some tissues of other plants (Zanewich and Rood 1993), and GA_4 could provide an alternate biosynthetic precursor for GA_1 (Junttila 1993, Rood and Hedden 1994). Their scarcity in the vegetative buds suggest that the 13-hydroxylated GAs are more important for leaf and branch growth in alder, aspen, and birch. The abundance of 13-hydroxylated GAs and scarcity of non-13-hydroxylated GAs in alder, aspen, and birch is similar to *Salix* (Davies et al. 1985) and poplar (Rood et al. 1988).

In conclusion, five 13-hydroxylated GAs were identified from flushing vegetative buds of alder, aspen, and birch. This suggests that the early 13-hydroxylation biosynthetic pathway is prominent and physiologically important in growing leaves and branches of these trees. The occurrence of these GAs and the apparently high levels of GA_1 and GA_{20} are consistent with the proposal that bud flushing and shoot elongation are controlled by GA_1 and suggests that there are commonalities of GA physiology in the fast-growing angiosperm trees, alder, aspen, birch, poplar, and *Salix*.

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