

Gibberellin Content of Immature Apple Seeds from Paclobutrazol-Treated Trees over Three Seasons

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Abstract. Seeds from heavily fruiting (“on-year”), mature untreated, and paclobutrazol-treated apple trees (*Malus domestica* Borkh. cv. Spartan) were sampled in mid-June 1987, mid-July 1987, and mid-July 1990. After seeds were freeze-dried, gibberellins (GAs) were extracted, purified, and fractionated via C₁₈ reversed-phase high-performance liquid chromatography (HPLC). Nine GAs (GA₁, GA₃, GA₄, GA₇, GA₈, GA₉, GA₁₉, GA₂₀, and GA₅₃) were quantified by the use of deuterated GA internal standards. Paclobutrazol trunk drench treatments reduced vegetative shoot elongation in the seasons that seeds were sampled by 55% or more. Between June 17, 1987 and July 15, 1987, the dry weight of seeds from both untreated and treated trees increased about 2.5 times and there were reductions, on a per seed basis, of GA₄ in seeds from both untreated and treated trees, of GA₇ in seeds from treated trees, and of GA₉ in seeds from untreated trees. However, GA₉ increased in seeds from treated trees. Changes in levels of some of the early-13-hydroxylation pathway GAs (GA₁, GA₃, GA₈, GA₁₉, GA₂₀, and GA₅₃) also occurred during the month. For mid-July harvested seeds, the pattern, with some exceptions, was that 2 years after paclobutrazol treatment (1987), levels of early-13-hydroxylation pathway GAs in seeds from treated trees were lower compared to controls but after 5 years (1990) their levels tended to increase. For the non-13-hydroxylated GAs (GA₄, GA₇, and GA₉), 2 years after paclobutrazol treatment, GA₄ levels were equal in seeds from untreated and treated trees, GA₇ decreased in seeds from treated trees compared with controls, but GA₉ levels increased.

Levels of these three GAs were higher in seeds from treated trees 5 years after treatment (1990) but levels of GA₄, GA₇, and GA₉ dramatically increased in seeds from treated trees 4 years after treatment (1989), as we previously reported.

Floral induction in apple is thought to be closely associated with gibberellin (GA) metabolism and to be directly influenced by GAs that move from developing seeds to adjacent buds of bearing trees (Grochowska and Karaszewska 1976, Hoad 1978, 1984, Luckwill 1970, Pharis and King 1985). Applications of plant growth retardants, such as paclobutrazol, a GA biosynthesis inhibitor, to bearing apple trees can increase flower bud formation the season after treatment in some cases (Elfving and Cline 1990, Lever 1986, Steffens and Zimmerman 1989). To provide information on the role GAs may play in flower bud initiation in apple, we identified 21 GAs (Lin et al. 1991) and compared the levels of eight GAs (Steffens et al. 1991b) in immature (approximately 75 days after full bloom) 1989 ‘Spartan’ apple seeds from mature untreated trees vs. trees treated with paclobutrazol. Levels of early-13-hydroxylation pathway GAs (GA₁, GA₃, GA₁₉, GA₂₀, and GA₅₃) were unaffected by paclobutrazol treatment, whereas levels of GA₄, GA₇, and GA₉ (non-13-hydroxylated GAs) increased 13.4, 6.5, and 3.8 times, respectively. We now provide additional information comparing GA levels (nine GAs, the eight previously determined plus GA₈) in immature (approximately 75 days after full bloom) seeds harvested in 1987 and 1990 from heavily fruiting, untreated, and paclobutrazol-treated cv. Spartan apple trees from this same study. These studies allow for comparisons of GA levels in apple seeds har-

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vested in mid-July over three seasons—1987, 1989 (Steffens et al. 1991b), and 1990. This report also compares levels of the nine GAs in less mature seeds (approximately 47 days after full bloom) to those in seed harvested a month later (approximately 75 days after full bloom) from untreated and paclobutrazol-treated trees.

Materials and Methods

Seed Samples

All seeds, both untreated and treated, were from fruit produced on *Malus domestica* cv. Spartan own-rooted apple trees propagated via tissue culture techniques (Zimmerman and Fordham 1985). The trees were planted in the orchard in 1979 at Beltsville, Maryland, and treated by pouring 500 ml of an aqueous solution containing paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol] around the trunk of each tree at the soil line (trunk drench). All treated trees received 38 mg paclobutrazol/cm² trunk cross-sectional area (TCSA) on November 8, 1983 (average of 0.9 g/tree) and 76 or 152 mg/cm² TCSA on May 15, 1985 (average of 3.6 or 7.3 g/tree). Untreated trees served as controls.

Seeds were sampled on two dates in 1987, June 17, and July 15, and were from the same five untreated and six paclobutrazol-treated trees (three treated at 76 mg and three treated at 152 mg/cm² TCSA in 1985) described in our earlier report on the GA content of 1989 seeds from 'Spartan' trees (Steffens et al. 1991b). The 1990 seeds were harvested on July 12 from different trees in the same experiment (see Fig. 1): five untreated and four treated (all treated at 152 mg paclobutrazol/cm² TCSA in 1985). In all cases, harvested fruits were from heavily fruiting, "on-year" untreated, and paclobutrazol-treated trees. Seeds were removed from the harvested fruit, pooled into treated and untreated lots, frozen at -70°C, and then freeze-dried.

Gibberellin Extraction and HPLC

Gibberellins were extracted from 2-g lots of the freeze-dried seeds harvested in 1987 and 1990. Extracts were purified and fractionated via HPLC as outlined previously (Lin et al. 1991, Steffens et al. 1991b). In addition to the eight internal standards previously listed for the 1989 seeds, 100 ng of [17,17-²H₂]GA₈ were added to each methanol extract of the 1987 and 1990 seeds.

Derivatization and GC-MS

Each HPLC fraction was dried, methylated with excess ethereal diazomethane, and redissolved in 10 µl of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA). Five microliters were analyzed by GC-MS using a Finnegan (San Jose, CA, USA) ion trap mass spectrometer interfaced with a Hewlett-Packard (Palo-Alto, CA, USA) 5890 gas chromatograph containing a 15 m × 0.32 mm i.d. column with a 0.25 µm 5% phenyl methyl silicone stationary phase (J & W Scientific, Folsom, CA, USA). Samples were introduced onto the column via a split-splitless injector and

Table 1. Effect of 1983 and 1985 paclobutrazol trunk drench treatments on shoot elongation of cv. Spartan apple trees at the end of the 1987, 1989, and 1990 seasons.

Treatment	1987 (cm)	1989 (cm)	1990 (cm)
Control	23.5 ± 2.1	27.6 ± 3.3	26.2 ± 2.7
Paclobutrazol	6.4 ± 2.4	12.5 ± 3.4	9.0 ± 1.4

Mean length of 10 randomly selected lateral shoots per tree, ±SEM.

chromatographed in the splitless mode (Hazebroek and Metzger 1990). Absolute levels of endogenous GAs for which there were ²H-labeled internal standards (GA₁, GA₃, GA₄, GA₇, GA₈, GA₉, GA₁₉, GA₂₀, and GA₅₃) were estimated (Metzger and Hazebroek 1990).

Results and Discussion

To evaluate data available for the three seasons, some of the data from our earlier 1989 study (Steffens et al. 1991b) will be referenced and discussed along with the 1987 and 1990 data presented here.

Tree Growth and Flowering

Paclobutrazol treatment reduced terminal shoot elongation by 55% or more each of the three seasons under discussion (Table 1). All treated trees from which fruit samples were taken showed triazole growth inhibition characteristically typical for apple trees (Steffens et al. 1985). All trees, both untreated and treated, had developed a biennial-bearing pattern when sampled. The number of flowers decreased the season after the 1987 (as well as the 1989) samples were taken (see Steffens et al. 1991b) as is usual for biennial-bearing trees (Steffens et al. 1991a); however, the number of flower clusters decreased little in 1991, the season after the 1990 samples were obtained (Fig. 1).

Changes in Seeds Between Mid-June 1987 and Mid-July 1987

Although dry weight per seed increased nearly 2.5 times from June 17, 1987 to July 15, 1987 (Table 2), GA₁ and GA₈ content per seed did not change; GA₃ doubled in untreated seeds but remained the same in treated seeds; and GA₁₉ did not change in untreated seeds but decreased in treated seeds (Table 3). GA₂₀ and GA₅₃ of both untreated and treated seeds increased but changes in levels of these and the other early-13-hydroxylation pathway GAs ap-

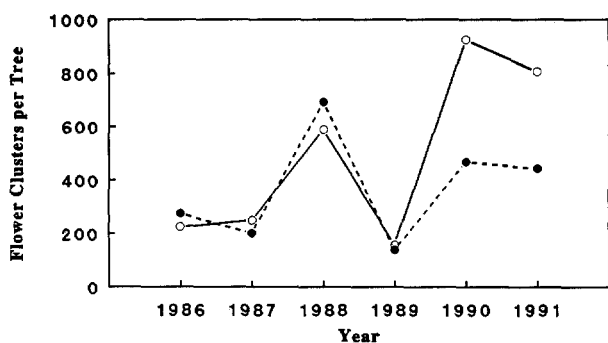


Fig. 1. Mean number of flower clusters on untreated (○—○) and paclobutrazol-treated (●--●) own-rooted cv. Spartan apple trees from which seeds were obtained in 1990. Flower clusters per tree were counted in 1987, 1988, 1990, and 1991, whereas estimates of the number of flower clusters were made in 1986 and 1989.

Table 2. Weight of freeze-dried cv. Spartan apple seeds harvested June 17, 1987, July 15, 1987, July 11, 1989 (Steffens 1991b), and July 12, 1990 from untreated and paclobutrazol-treated trees.

	June 17, 1987	July 15, 1987	July 11, 1989	July 12, 1990
	(mg seed ⁻¹)			
Untreated	10.9	26.9	25.1	28.5
Treated	10.2	26.6	23.5	27.2

Table 3. Endogenous GAs in cv. Spartan apple seeds harvested June 17, 1987 and July 15, 1987 from untreated and paclobutrazol-treated trees.

GAs	June 17, 1987		July 15, 1987	
	Untreated (ng seed ⁻¹)	Treated (ng seed ⁻¹)	Untreated (ng seed ⁻¹)	Treated (ng seed ⁻¹)
GA ₁	0.14	0.13	0.18	0.16
GA ₃	0.28	0.28	0.66	0.31
GA ₄	4.19	5.43	3.19	3.15
GA ₇	4.40	3.70	4.63	1.49
GA ₈	0.29	0.29	0.38	0.19
GA ₉	3.17	1.97	1.78	3.04
GA ₁₉	0.11	0.54	0.09	0.11
GA ₂₀	0.07	0.01	0.61	0.21
GA ₅₃	0.03	0.05	1.49	0.17

peared to follow no consistent pattern and reasons for changes are not apparent.

Between June 17 and July 15 there were losses, on a per seed basis, of GA₄ from seeds of both untreated (24%) and treated (42%) trees; of GA₇ from seeds of treated (42%) trees; and of GA₉ from seeds of untreated (42%) trees (Table 3). Decreases

in concentration of these GAs may have been due to diffusion of GAs out of the seeds and/or metabolism to other GAs or bound forms of GA (Pharis and King 1985). In contrast, GA₉ increased in seeds from treated trees (35%) between mid-June and mid-July, possibly due to the biosynthesis of GA₉, transport of GAs into seeds, conversion from other GAs, or release of bound forms.

Comparisons of GA Levels in Mid-July Seeds Over Three Seasons

Early-13-hydroxylation pathway GAs (GA₁, GA₃, GA₈, GA₁₉, GA₂₀, and GA₅₃) in the seeds sampled in mid-July over the three seasons ranged from 1.1 to 55.5 ng/g (Table 4) and were, in general, at lower concentrations than GA₄, GA₇, and GA₉, which ranged from 15 to 278 ng/g seed.

The early-13-hydroxylation pathway GAs, including GA₁, GA₃, GA₈, GA₁₉, GA₂₀, and GA₅₃, have been more closely associated with vegetative growth than with flowering in plants (Phinney and Spray 1990, Reid 1990). GA₁ is generally recognized as the physiologically active GA in vegetative tissues for stem elongation (i.e., it needs no further metabolism to achieve biological activity) (Phinney 1985). Levels of this GA were nearly the same for untreated and paclobutrazol-treated seeds all three seasons. On the other hand, treatment effects on levels of GA₅₃, a C₂₀-GA which can be converted to biologically active C₁₉-GAs (Graebe 1987), were inconsistent (Table 4). GA₄ and GA₇ are among the main GAs in apple seeds (Hoad 1978) and may be closely associated with apple flower bud initiation and development (Pharis and King 1985). GA₉ has previously been shown to occur in apple seed (Hoad 1978), although it has not been directly associated with flowering in apple.

Two years after the last paclobutrazol treatment (1987), four of the six GAs belonging to the early-13-hydroxylation pathway decreased in seeds from treated trees (Table 4). The level of GA₇ also decreased, but GA₄ did not change, and the GA₉ level increased compared with controls. We reported (Steffens et al. 1991b) that 4 years after the last paclobutrazol treatment, in 1989, levels of most of the early-13-hydroxylation pathway GAs in seeds of treated trees were unchanged but levels of GA₄, GA₇, and GA₉ increased dramatically. Five years after treatment, in 1990, levels of none of the GAs determined were lower in seeds from paclobutrazol-treated trees (Table 4). Levels of early-13-hydroxylation pathway GAs in 1990 seeds from treated trees increased (some only a very little).

Table 4. Endogenous GAs in cv. Spartan apple seeds harvested July 15, 1987, July 11, 1989 (from Steffens et al. 1991b), and July 12, 1990 from untreated and paclobutrazol-treated trees.

GAs	July 15, 1987		July 11, 1989		July 12, 1990	
	Untreated (ng g ⁻¹ DW)	Treated	Untreated (ng g ⁻¹ DW)	Treated	Untreated (ng g ⁻¹ DW)	Treated
GA ₁	6.5	6.0	8.0	8.0	5.0	5.6
GA ₃	24.5	11.5	28.0	24.0	11.0	13.1
GA ₄	118.5	118.5	15.0	201.0	73.5	104.5
GA ₇	172.0	56.0	39.0	249.0	72.0	152.0
GA ₈	14.0	7.0	ND	ND	1.1	12.5
GA ₉	66.0	114.5	73.0	278.0	130.5	164.0
GA ₁₉	3.5	4.0	13.0	14.0	25.5	44.7
GA ₂₀	22.5	8.0	27.0	18.0	11.9	12.1
GA ₅₃	55.5	6.5	29.0	27.0	5.6	34.7

ND, not determined.

Levels of GA₄, GA₇, and GA₉ also increased in seeds from treated trees in 1990 but increases for these GAs were more dramatic in 1989 seeds from treated trees (13.4, 6.5, and 3.8 times for GA₄, GA₇, and GA₉, respectively).

Considering the data for the three seasons shown in Table 4, which includes previously published data for 1989 seeds (Steffens et al. 1991b), 2 years after paclobutrazol treatment, levels of early-13-hydroxylation pathway GAs in seeds from treated trees were lower compared to controls, after 4 years they were unchanged, and after 5 years they tended to increase. For the non-13-hydroxylated GAs, GA₄, GA₇, and GA₉, 2 years after treatment, GA₄ levels in seeds from untreated and treated trees were the same; GA₇ decreased in seeds from treated trees; but the level of GA₉ increased. Levels of GA₄, GA₇, and GA₉ were dramatically higher in seeds from treated trees 4 years after the final paclobutrazol application. Even though magnitudes of increase were not as great, levels for these GAs were also higher in seeds from treated trees 5 years after treatment.

Variations in the pattern of GA level between untreated and treated seeds, as well as across seasons, were not unexpected. In these studies, part of the variation may be attributable to differences in the physiological maturity of untreated vs. treated seed. Freeze-dried seed weight varied only slightly for the three seasons but weight per seed from untreated trees was greater than from treated trees each of the three seasons (Table 2). Earlier studies (Dennis 1976, Hedden and Hoad 1985) have shown that GA-like biological activity during apple seed development can change rapidly. Soil-applied paclobutrazol has been reported to advance date of apple flowering (Stinchcombe et al. 1984) and, be-

cause of increased sunlight on fruiting spurs, to advance fruit maturity (Williams 1984). Paclobutrazol application has also been shown to influence fruit ripening in apple (Luo et al. 1987), fruit maturity in peach (Yoshikawa et al. 1987), and time of anthesis in apricot, cherry, and peach (Proebsting and Mills 1985). Paclobutrazol has been found to influence the metabolism of plants in several ways (Hedden 1990), but a major affect is the inhibition of entkaurene oxidation which is an early step in the pathway of GA biosynthesis (Graebe 1982, Hedden and Graebe 1985). Paclobutrazol treatment would thus be expected to lower the GA level in tissues of treated plants; reasons for the increased levels of GA in seeds from treated apple trees are not apparent.

The fact that applications of GA₄ and C-3 epi-GA₄ to apple trees have been shown to promote flower bud development (Looney et al. 1985) and GA₇ application can reduce flowering (Tromp 1982) has generated interest for determining endogenous levels of these GAs in apple seeds. Seed-produced GAs, including GA₄ and GA₇, are thought to diffuse from attached fruit and move to spur buds where they could then influence flower bud formation or development (Hoad 1978, 1984, Pharis and King 1985). The presence of large numbers of seeded fruits has been implicated as the source of GAs, especially GA₄ and GA₇, which promote (GA₄) or inhibit (GA₇) flower bud initiation when biennial-bearing patterns develop (Looney and Pharis 1986, Marino and Greene 1981). Pharis and King (1985) suggest that GA₄ may be more rapidly metabolized than GA₇ in spur shoots, the consequence of which would be a prolonged influence of GA₇, thus producing decreased flower bud initiation the season after a heavy fruit load year. In addition to varia-

tions in GA metabolic rates, flower bud initiation may be influenced by differences in rate and/or duration of GA movement from fruit to shoots. Green (1987) investigated several aspects of the theory that hormones from apple seeds moving to buds inhibit floral initiation. Green found that high levels of several GAs, including GA₄ and GA₇, were present in apple seeds 7 to 10 weeks after full bloom, but that the hormone complement, both qualitatively and quantitatively, was similar in biennial trees compared with trees of the regular cultivar. In addition, no GA₄ or GA₇, and only a small amount of GA₁, could be identified by GC-MS in fruit diffusates. As shown here, however, paclobutrazol treatment of apple trees can increase levels of GA₄ and GA₇, as well as alter the ratio of GA₄ to GA₇, in immature apple seeds. Even though increases in flower bud formation and intensified biennial-bearing, which is often noted in apple trees treated with triazole plant growth regulators (Steffens and Zimmerman 1989, Tukey 1986), were not found in these studies, alterations in the GA level, such as those shown may, nevertheless, be related to these phenomena. If such relationships do exist, they appear to be quite complicated and require further clarification.

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