Apical Dominance in Apple (*Malus domestica* Borkh): The Possible Role of Indole-3-Acetic Acid (IAA)

Shiow Y. Wang,¹ Miklos Faust,¹ and Michael J. Line²

Beltsville Agriculture Research Center, Agriculture Research Service, U.S. Department of Agriculture, Beltsville, MD 20705

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Abstract. The effect of IAA on apical dominance in apple buds was examined in relation to changes in proton density (free water) and membrane lipid composition in lateral buds. Decapitation induced budbreak and enhanced lateral bud growth. IAA replaced apical control of lateral buds and maintained paradormancy. Maximal inhibition was obtained when IAA was applied immediately after the apical bud was removed; delaying application reduced the effect of IAA. An increase in proton density in lateral buds was observed 2 days after decapitation, whereas the change in membrane lipid composition occurred 4 days later. Removing the terminal bud increased membrane galacto- and phospholipids and the ratio of unsaturated to corresponding saturated fatty acids. Decapitation also decreased the ratio of free sterols to phospholipids in lateral buds. Applying thidiazuron to lateral buds of decapitated shoots enhanced these effects, whereas applying IAA to the terminal end of decapitated shoots inhibited the increase of proton density and prevented changes in membrane lipid composition in lateral buds. These results suggest that change in water movement alters membrane lipid composition and then induces lateral bud growth. IAA, presumably produced by the terminal bud, restricts the movement of water to lateral buds and inhibits their growth in apple.

The phenomenon of correlative inhibition of axillary buds by apical buds in certain plants is well established (Rubinstein and Nagao, 1976). The degree of dominance is a function of genetic loci, environmental factors, physiological processes, and plant age (Martin, 1987). Many fruit trees exhibit strong apical dominance in individual branches growing from the main trunk (Martin, 1987). Thimann and Skoog (1933, 1934) demonstrated the presence of auxin in apical buds. IAA can, to some degree, substitute for the apex in preventing the outgrowth of lateral buds (Thimann and Skoog, 1933), and the amount of inhibition is correlated with auxin concentration in apical buds (Thimann and Skoog, 1934).

IAA transport inhibitors triiodobenzoic acid (TIBA), morphactin, naptalan, DPX 1840, and phenacylidine phthalide hydrate can release buds from apical dominance (Hillman, 1984). These results suggest that IAA plays a direct role in correlative inhibition; i.e., high concentrations inhibit lateral bud growth. However, Gocal et al. (1991) and Hillman et al. (1977) reported a consistent rise in endogenous IAA content in lateral buds of decapitated *Phaseolus* plants 2 to 24 h after decapitation. Findings such as these have led to the concept that apically derived IAA may play an indirect role by modifying factors such as hormone-directed transport of nutrients and water and inhibition of vascular connection, which in turn modifies bud growth.

Magnetic resonance imaging (MRI) is a powerful tool to visualize water content in living organisms. This technique has been used successfully to study biological systems such as water distribution in fruit (Wang et al., 1988b) and water flow in plant stems and roots (Bottomley et al., 1986; Brown et al., 1986; Omasa et al., 1985; Pretty, 1978; Wang and Chang, 1986). Recently, Liu et al. (1993) examined intact paradormant apple buds by MRI and

found a higher ratio of free to bound water in the bud upon release from dormancy. Additional water moved into the buds as a result of treatment with thidiazuron (TDZ), a cytokinin-type growth regulator that activates lateral bud growth (Wang et al., 1986). Thus, MRI provides quantitative data on the state of water during paradormancy and thereafter.

Biological membranes are the fundamental active sites for many specific enzyme activities; membranes transport ions and act as hormonal receptors (Brenner, 1984). Low temperatures or TDZ increased the degree of unsaturation of fatty acids in the membrane lipids of apple buds, changing polar head group composition, increasing membrane phospholipid content, and changing sterol levels and composition. The ratio of sterols to phospholipids decreased during budbreak and bud growth (Wang and Faust, 1990; Wang et al., 1988a), indicating that increased membrane fluidity is concomitant with budbreak and rapid bud growth. Because movement of water into cells upon induction of growth in lateral buds may involve changes in membrane structure, evaluating membrane structure during growth induction seemed pertinent. The primary purpose of this study was to determine the inhibitory effect of IAA on lateral bud growth of apple in relation to the state of water and changes in membrane composition.

Materials and Methods

Plant materials and growth substance treatments. Summer-dormant 'Anna' apple buds were used. Buds were paradormant (correlative inhibition), and removing the terminal bud or TDZ treatment could induce lateral budbreak. Shoots (25 cm long) were collected in August 1992 and defoliated (blade removed, petiole retained). Various amounts of IAA (2, 10, 20, and 100 nmol) were injected directly with a syringe 2 mm into the terminal ends of decapitated shoots immediately after apical buds were removed. To study the effect of delayed IAA application, IAA (20 nmol) was applied at different times following decapitation. To study the effect of IAA and TDZ, shoots were divided into five groups.

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¹Fruit Laboratory.

²Environmental Chemistry Laboratory.

Group 1 was used as a control and not decapitated. Groups 2 to 5 were decapitated and treated as follows: group 2, no chemical was applied; group 3, IAA (20 nmol) in water was injected into the tip; group 4, TDZ [100 μ M in 2.5% (v/v) dimethyl sulfoxide plus 0.5% (v/v) Tween-20] was applied to the upper 10 lateral buds; group 5, IAA (20 nmol) was injected into the tip and TDZ (100 μ M) was applied to the upper 10 lateral buds. Shoots (30/treatment) were placed with their basal ends in glass jars containing distilled water at 22 to 24C for 2 weeks to observe budbreak and bud growth. The basal ends were recut and water was changed at 2-day intervals.

MRI analysis. MRI analysis was performed 48 h after shoots were treated by determining the T_2 times (Millard et al., 1993). Shoots were placed in 10-mm MRI tubes for imaging. A 15-mm-diameter insert was placed in a MRI imaging probe (400 MHz, 9.4T) (Bruker Instrument Co., Billerica, Mass.). Single-slice, single-echo images were used with a matrix size of 256×256 pictured elements (pels) and a spatial resolution of $20 \times 20 \times 500 \mu$ m. Data acquisition time was 2 h, echo delay time (TE) was 11.2 ms, and repetition time (TR) was 1.6 sec. Image slices were taken perpendicular to the axis of the shoot through the midpoint of the bud (Faust et al., 1991). Proton density and T_2 images were created, and the number of pixels with T_2 relaxation times for <15, 15 to 25, and >25 ms were determined. Images were obtained using a video graphic printer (Up-811; Sony, Tokyo). T_2 times for stems and buds were calculated by plotting the energy decay of the measurable signal through eight echoes and using a single component fit analysis, $y = K_0 e^{-t/T_2} + K_a$, where y refers to the intensity of the signal, t refers to the time, and k is a constant (Bevington, 1969). The distribution of proton signals in buds and stems was measured by the number of pixels in nuclear magnetic resonance (NMR) imaging.

Extraction, fractionation, and analysis of lipids. The top three buds of each shoot from each treatment were used in this study. Triplicate samples of 0.5 g fresh weight were collected from these shoots 2 and 4 days after treatment. The extraction, fractionation, and analysis of lipids in apple buds were as described previously (Wang et al., 1988a; Wang and Faust, 1990). Purified lipids were separated into neutral, glyco-, and phospholipid fractions by silicic acid column chromatography on 100- to 200-mesh Bio Sil

A (Bio Rad Laboratories, Richmond, Calif.). The glyco- and phospholipid fractions were further separated by thin layer chromatography on 20×20 -cm glass plates precoated with 250- μ m-thick silica gel (EM Reagents, Darmstadt, Germany) using 100 acetone : 2 acetic acid : 1 water (by volume) and 85 chloroform : 15 methanol : 10 acetic acid : 3.5 water (by volume). Individual galacto- and phospholipids were identified by chromatography using authentic standards (Sigma Chemical Co., St. Louis; Supelco, Bellefonte, Pa.) and spray reagents specific for hexose sugars or phosphate. Individual lipid bands were scraped off and eluted in 2 chloroform : 1 methanol (v/v). Total fatty acids were derivatized to fatty acid methyl esters (FAMES) for flame ionization detection–gas chromatography (FID–GC) analysis (Wang and Faust, 1990; Wang et al., 1988a). n-Heptadecanoic acid was included in all samples as an internal standard and methyl heptadecanoate was used as an external standard. Individual FAMES were identified by comparing retention times with those of authentic standards (Supelco). This tentative identification of major polar lipid fatty acids was corroborated by further analysis using GC–mass spectrometry (GC–MS) (Wang et al., 1988a).

The neutral lipid fraction from apple buds was dried under a stream of N_2 and redissolved in 2 ml hexane. Silicic acid column chromatography (100- to 200-mesh Bio Sil A) was used to separate the steryl ester and free sterols by elution with 10 hexane : 1 ether and 2 hexane : 1 ether, respectively. After adding 20 μ g of cholesterol or cholesteryl myristate as an internal standard, steryl ester was saponified in 1.0 ml of 1 N KOH in 85% ethanol for 1 h at 80C (under N_2). Free sterols were precipitated with digitonin (Sigma Chemical Co.) (Wang and Faust, 1990). The digitonides were collected on a glass-fiber filter (Reeve Angel, Clifton, N.J.) and washed with 1 acetone : 2 diethyl ether (v/v). Free sterols were liberated from the digitonides by refluxing in 1 ml pyridine for 30 min at 100C. After cooling, 1 ml deionized, distilled water was added and the free sterols were recovered by extraction with 2 ml of hexane. Sterol composition was determined by FID–GC and further confirmed by GC–MS (Wang et al., 1988a). Data values are the means of three replicate samples. Standard errors of means are reported in the tables.

Fig. 1. Effects of various concentrations of IAA applied to the tip of decapitated apple shoots on the length and fresh weight of the most distal lateral bud. Samples were taken 14 days after treatments. Vertical bars represent the mean of three replications $\pm$ SE.

Results

Bud growth and proton profile. Decapitation induced budbreak and lateral bud growth. Buds started to swell 5 days after decapitation (data not shown). IAA reduced lateral bud growth (Figs. 1–3), the effect increasing with concentration (Fig. 1); 20 nmol was the lowest concentration effective in keeping apple buds paradormant (Fig. 1).

Lateral bud growth was inhibited when IAA was applied to the cut end of the shoot immediately after the apical bud was removed, but delaying this application beyond 3 h after decapitation reduced the effect (Fig. 2). Lateral bud growth was inhibited the most in the two most distal lateral buds, whereas the other buds were inhibited less (Fig. 3). Treating lateral buds with TDZ without removing the

terminal bud activated all buds. The treated buds developed rapidly and, after 14 days, the two most distal lateral buds were larger than buds in similar positions on shoots induced by decapitation (data not shown). Among the TDZ-treated buds of the decapitated shoots, growth of the most distal lateral bud was inhibited the most by IAA (Fig. 3). Bud growth was inhibited up to the seventh lateral bud with decreasing degree in a basipetal direction with IAA (Fig. 3), a result suggesting a gradient of decreasing IAA inhibition from shoot apex to base.

MRIs of stems and the first and tenth lateral buds were taken within 48 h after treatment (Fig. 4 a–d). The stem sections (Fig. 4 b and d) always contained more free water than the buds (Fig. 4 a and c), and their free water content was greater at node 10 than at node 1 (Fig. 4 b and d), as expressed by number of pixels with >15

Fig. 2. Effects of application time of IAA (20 nmol) to decapitated apple shoots on the length and fresh weight of the most distal lateral buds. Samples were taken 14 days after treatments. Vertical bars represent the mean of three replications $\pm$ SE.

Fig. 3. Effects of decapitation (D), IAA (20 nmol injected into tip), and thidiazuron (TDZ) (100 μ M applied to 10 distal buds) on the growth of the 10 lateral buds. Samples were taken 14 days after the various treatments. The position of the lateral bud was counted from the apical tip. Vertical bars represent the mean of three replications $\pm$ SE. C = control (no treatment).

Fig. 4. Effects of decapitation (D), IAA (20 nmol), and thidiazuron (TDZ) (100 μ M) on proton density. Proton density was measured by pixel number in nuclear magnetic resonance (NMR) imaging for the top (first) lateral bud (a), first internode stem section (b), the tenth lateral buds (c), and tenth internode stem section (d) of apple shoots with various treatments. NMR was performed 48 h after treatment. An increase in T_2 time indicates less binding of water. T_2 values <6 ms did not show images. T_2 values >15 ms indicate free water in the tissues. C = control (no treatment).

ms T_2 time on the MRI. Decapitation and TDZ increased the pixel number with higher T_2 times, indicating more free water in buds and stems due to these treatments (Fig. 4 a–d). Applying IAA reduced the effect of decapitation or TDZ in the first internode stem section (Fig. 4b) but was much less effective in the tenth internode stem section (Fig. 4d). In the first lateral buds of shoots with intact terminals, proton signals were weak (Fig. 4a), with few pixels showing free water. Free water increased in the lateral buds 48 h after decapitation (Fig. 4a). TDZ was the most effective treatment in breaking dormancy in top lateral buds (Fig. 3) and in increasing free water in these buds, judged by the increased number of pixels with high T_2 times (Fig. 4a). Treating decapitated shoots with IAA inhibited TDZ action on growth (Fig. 3) and pixel number (Fig. 4a), with the top lateral buds retaining weak proton signals and short T_2 times. The T_2 signals obtained from the tenth lateral bud showed about the same number of pixels with high T_2 in the TDZ-treated buds of decapitated shoots and those treated with TDZ and IAA (Fig. 4c). There was a positive correlation ($r = 0.981$) between free water content (pixel number in NMR imaging) and bud growth (Fig. 5).

Galacto- and phospholipids. Monogalactosyl diglyceride (MG) was the major galactolipid, and phosphatidylcholine (PC) and

phosphatidyl ethanolamine (PE) were the major phospholipids found in the buds, along with small amounts of phosphatidylglycerol (PG) and phosphatidylinositol (PI) (Fig. 6). Polar lipid fatty acids included mainly palmitate (C16:0), stearate (C18:0), oleate (C18:1), linoleate (C18:2), and linolenate (C18:3). Linolenic acid was the dominant fatty acid in MG and the other main galactolipid, digalactosyl diglyceride (DG). Linoleate, linolenate, and palmitate were the major fatty acids in PG and PI, while palmitate, linoleate, and linolenate were dominant in PE and PC. There were no significant changes in galacto- and phospholipid content, their relative percentage of fatty acids, and the degree of fatty acid saturation in polar membrane lipids 2 days after decapitation and treatment with IAA and TDZ (data not shown). However, several changes in plasma membrane lipid levels were found 4 days after treatment (Fig. 6). The amount of change was different for each lipid. There was an increase in galacto- and phospholipid content in lateral buds of decapitated shoots. These changes were more pronounced if buds from decapitated shoots were treated with TDZ. IAA applied to the terminal tip of decapitated shoots inhibited the increase of membrane polar lipids in lateral buds (Fig. 6).

The individual fatty acids—palmitate, stearate, oleate, linoleate, and linolenate—for control buds on nondecapitated shoots ac-

Fig. 5. Relationship between bud proton density (total pixel number) and bud fresh weight. Numbers inside figure indicate various treatments. C = control (no treatment), D = decapitation, TDZ = thidiazuron.

Fig. 6. Effect of decapitation (D), IAA (20 nmol), and thidiazuron (TDZ) (100 μ M) on glycolipids and phospholipids in apple buds. Buds were collected 96 h after treatment. Vertical bars represent the mean of three replications $\pm$ SE. C = control (no treatment), MG = monogalactosyl diglyceride, DG = digalactosyl diglyceride, PC = phosphatidylcholine, PE = phosphatidylethanolamine, PG = phosphatidylglycerol, PI = phosphatidylinositol.

counted for $\approx 10\%$, 6% , 15% , 16% , and 53% , respectively, for MG; $\approx 12\%$, 8% , 15% , 11% and 54% , respectively, for DG; $\approx 42\%$, 5% , 7% , 15% and 32% , respectively, for PI; $\approx 26\%$, 8% , 19% , 23% , and 25% , respectively, for PC; $\approx 27\%$, 7% , 10% , 32% , and 24% , respectively, for PE; and $\approx 47\%$, 3% , 12% , 8% , and 31% , respectively, for PG. The relative percentages of C18:3 in galacto- and phospholipids increased, while the relative percentage of C16:0 decreased, 4 days after decapitation or decapitation plus TDZ (Table 1). The other fatty acids remained relatively constant among various treatments.

Among major lipid components, the galactolipids contained the highest proportion of polyunsaturated fatty acids and had the highest ratio of unsaturated to saturated fatty acids among all polar membrane lipids (Table 1). Decapitation and decapitation plus TDZ increased the ratio of the unsaturated to corresponding saturated fatty acids in galacto- and phospholipids of apple bud

tissue 4 days after treatment (Table 1). Applying IAA, with or without TDZ, to the cut end of decapitated shoots inhibited the increase of 18:3, the overall unsaturated to saturated fatty acid ratio (Table 1), and bud growth (Fig. 3).

Sterols. β -Sitosterol, campesterol, stigmasterol, and sitosterol esters were found in lateral buds (Table 2); β -sitosterol and sitosterol esters were dominant. Other sterols and sterol esters were present in trace amounts. Two days after decapitation, sterol content, composition, and ratio of free sterol to phospholipids in lateral buds had not changed significantly among treatments (data not shown). Four days after decapitation, sterol content had increased and the ratio of free sterols to phospholipids had decreased. β -Sitosterol, campesterol, and stigmasterol content increased in response to decapitation and decapitation plus TDZ (Table 2). Applying IAA to the cut ends of decapitated shoots suppressed the increase of β -sitosterol, campesterol, and stigmasterol content and

Table 1. Effect of decapitation, IAA (20 nmol), and thidiazuron (TDZ) (100 μ M) on fatty acid, palmitic, and linolenic acid content (weight percent of total) of galacto- [monogalactosyl diglyceride (MG) and digalactosyl diglyceride (DG)] and phospholipids [phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidyl-glycerol (PG)] and ratio of unsaturated to saturated fatty acids in apple lateral buds 4 days after treatment.

Fraction	Fatty acid	Intact control	Treatment			
			Decapitated			
			+IAA	+TDZ	+IAA and TDZ	
MG	16:0(%)	9.5 $\pm$ 0.7	5.1 $\pm$ 0.6	10.2 $\pm$ 0.8	4.3 $\pm$ 0.4	7.6 $\pm$ 0.7
	18:3(%)	52.8 $\pm$ 3.1	58.6 $\pm$ 1.8	51.7 $\pm$ 2.6	61.8 $\pm$ 1.2	52.2 $\pm$ 3.9
	U : S ²	5.5 $\pm$ 0.3	9.8 $\pm$ 0.6	5.1 $\pm$ 0.4	11.1 $\pm$ 0.5	6.6 $\pm$ 0.7
DG	16:0(%)	12.0 $\pm$ 0.8	5.0 $\pm$ 0.2	12.6 $\pm$ 1.0	4.6 $\pm$ 0.1	11.1 $\pm$ 0.7
	18:3(%)	54.0 $\pm$ 2.7	65.7 $\pm$ 1.1	49.6 $\pm$ 2.7	67.5 $\pm$ 0.6	53.6 $\pm$ 1.2
	U : S	4.0 $\pm$ 0.3	10.1 $\pm$ 0.5	3.7 $\pm$ 0.2	10.6 $\pm$ 0.4	4.1 $\pm$ 0.2
PI	16:0(%)	41.6 $\pm$ 0.9	40.4 $\pm$ 0.2	42.8 $\pm$ 1.2	38.2 $\pm$ 0.7	43.2 $\pm$ 1.0
	18:3(%)	32.0 $\pm$ 1.6	33.7 $\pm$ 0.8	30.4 $\pm$ 1.3	36.4 $\pm$ 1.7	30.3 $\pm$ 1.5
	U : S	1.2 $\pm$ 0.0	1.3 $\pm$ 0.0	1.1 $\pm$ 0.1	1.4 $\pm$ 0.1	1.1 $\pm$ 0.1
PC	16:0(%)	25.5 $\pm$ 0.5	22.2 $\pm$ 0.7	25.5 $\pm$ 0.7	20.2 $\pm$ 1.1	25.0 $\pm$ 0.8
	18:3(%)	24.5 $\pm$ 1.2	26.6 $\pm$ 0.8	22.5 $\pm$ 0.8	35.0 $\pm$ 2.8	23.1 $\pm$ 1.0
	U : S	2.0 $\pm$ 0.1	2.4 $\pm$ 0.0	1.9 $\pm$ 0.1	2.7 $\pm$ 0.2	2.0 $\pm$ 0.0
PE	16:0(%)	27.4 $\pm$ 0.5	20.7 $\pm$ 1.5	27.9 $\pm$ 0.6	20.3 $\pm$ 1.1	27.5 $\pm$ 0.7
	18:3(%)	24.0 $\pm$ 1.3	28.4 $\pm$ 1.7	20.8 $\pm$ 1.4	31.8 $\pm$ 1.5	24.0 $\pm$ 1.2
	U : S	1.9 $\pm$ 0.2	2.7 $\pm$ 0.0	1.9 $\pm$ 0.1	2.9 $\pm$ 0.1	2.0 $\pm$ 0.3
PG	16:0(%)	46.5 $\pm$ 1.2	30.6 $\pm$ 3.1	49.0 $\pm$ 1.2	23.2 $\pm$ 2.8	46.5 $\pm$ 1.3
	18:3(%)	30.7 $\pm$ 1.5	9.4 $\pm$ 0.2	27.8 $\pm$ 1.4	49.9 $\pm$ 0.2	24.0 $\pm$ 1.7
	U : S	1.0 $\pm$ 0.1	2.0 $\pm$ 0.2	0.9 $\pm$ 0.1	2.8 $\pm$ 0.3	1.0 $\pm$ 0.1

²Ratio of unsaturated fatty acids (18:1 + 18:2 + 18:3) to saturated fatty acids (16:0 + 18:0).

Table 2. Effect of decapitation, IAA (20 nmol), and thidiazuron (TDZ) (100 μ M) on sterol content and ratio of free sterols to phospholipids in apple lateral buds 4 days after treatment.

Treatment	Sterol content (μ g·g ⁻¹ dry weight)				Free sterols : phospholipids
	β -Sito-sterol	Campe-sterol	Stigma-sterol	Sitosteryl ester	
None	204 $\pm$ 6	23 $\pm$ 4	12 $\pm$ 2	920 $\pm$ 27	1.45
Decapitation (D)	367 $\pm$ 15	52 $\pm$ 7	24 $\pm$ 3	773 $\pm$ 18	0.99
D + IAA	199 $\pm$ 9	20 $\pm$ 4	11 $\pm$ 2	1008 $\pm$ 31	1.62
D + TDZ	396 $\pm$ 11	59 $\pm$ 8	28 $\pm$ 4	669 $\pm$ 20	0.76
D + IAA + TDZ	198 $\pm$ 10	24 $\pm$ 2	10 $\pm$ 2	1001 $\pm$ 29	1.51

inhibited the decrease of the ratio of free sterols to phospholipids (Table 2). Applying TDZ to lateral buds of decapitated shoots enhanced these effects, whereas applying IAA to the cut ends of decapitated shoots prevented them (Table 2).

Discussion

Apical dominance is exerted by the shoot apex over the out-growth of lateral buds in apple. Lateral bud growth on decapitated shoots was inhibited by applying auxin to the cut end of the shoot, and 20 nmol is the lowest concentration effective in keeping apple lateral buds dormant (Fig. 1). White et al. (1975) reported that the concentration of exogenous IAA needed to prevent lateral bud growth was 1000 times that found in the apex. The IAA content of 'Anna' apical buds is not known, but we applied $\approx$ 5000 times the amount (20 nmol) that White et al. (1975) reported to be present in beans (0.7 ng/shoot tip). Applying a quantity one magnitude less (500 times the amount reported for beans) did not effectively maintain apple bud dormancy.

Distal IAA application restricted the movement of water to lateral buds and inhibited bud growth. Delaying IAA application beyond 3 h after decapitation reduced the effect. This result

suggests that IAA inhibits the primary stage of lateral bud growth; i.e., after growth had been induced by decapitation, IAA was less capable of inhibiting bud growth.

TDZ effectively overcomes apical dominance and enhances lateral bud growth. TDZ increased the pixel number with higher T_2 times in stems and buds, indicating more free water in these tissues. This is consistent with a previous finding (Liu et al., 1993) and indicates that TDZ promotes water transport into the buds. This also agrees with the finding that breaking endodormancy in winter apple buds by chilling resulted in longer T_2 times in the buds (Faust et al., 1991). TDZ is most effective in breaking dormancy in the most distal lateral buds. This gradient in apple bud growth response to TDZ has been observed previously (Wang et al., 1986).

The two most distal lateral buds retained weak proton signals and short T_2 times and growth was inhibited the most by IAA. The T_2 signals obtained from the tenth lateral bud showed about the same number of pixels with high T_2 in the TDZ-treated buds of decapitated shoots and those treated with TDZ and IAA. The decreasing effectiveness of IAA with distance from the application point may be due to IAA metabolism or stimulation of bud growth by TDZ, which takes place before IAA reaches lower buds. IAA transport in shoots is basipetal. The rate of IAA transport in pea

seedlings, pea sections, and corn coleoptile sections is 11 to 12 mm·h⁻¹ (Goldsmith, 1982; Morris and Thomas, 1978). We have not determined the IAA transport rate in apple shoots, but apparently IAA either lost effectiveness by turnover or was transported at a slower rate as it moved down the stem.

Decapitation and decapitation plus TDZ increased the ratio of the unsaturated to corresponding saturated fatty acids in galactolipids and phospholipids and decreased the ratio of free sterols to phospholipids in lateral buds. An accumulation of polar membranes and increased ratio of unsaturated to saturated fatty acids presumably enhanced the fluidity necessary for proper membrane function (Bishop et al., 1979; Thompson, 1979), which was in turn associated with budbreak and bud growth (Wang and Faust, 1990). Applying IAA, with or without TDZ, to the cut end of decapitated shoots inhibited the increase of 18:3, the overall unsaturated to saturated fatty acid ratio, and bud growth.

Sterol content increased and the ratio of free sterols to phospholipids decreased in response to decapitation and decapitation plus TDZ. The increase in free sterols may be, in part, the result of hydrolysis of reserve sterol in buds. Changes in sterol content and composition have been correlated with altered membrane permeability in higher plants (Goad, 1983; Grunwald, 1975) and also may be involved in inducing other biochemical or physiological processes related to bud growth. The sterol to phospholipid ratio is an index of the fluidity of membranes (Quinn, 1981). A decrease in this ratio upon decapitation-associated budbreak and bud growth indicates an increase in membrane fluidity and cell metabolism (Yoshida and Uemura, 1990). Applying TDZ to lateral buds of decapitated shoots enhanced these effects, whereas applying IAA to the cut ends of decapitated shoots prevented them.

Collectively, our data suggest that the increase of free water in apple buds is the first sign of budbreak. Changes in membrane lipid composition may be associated with the movement of water into cells and changes in cellular organizational processes that remove apical dominance. IAA in terminal buds seems to inhibit lateral bud growth by maintaining a bound state of water in the buds. Although the details of this control mechanism are still unclear, this effect of IAA on the state of water in connection to apical dominance in apple is apparently important in growth resumption.

Literature Cited

- Bevington, P.R. 1969. Data reduction and error analysis for the physical sciences. McGraw-Hill, New York.
- Bishop, D.G., J.R. Kendrick, A.S. Macpherson, S.R. Johns, and R.I. Willing. 1979. The influence of fatty acid unsaturation on fluidity and molecular packing of chloroplast membrane lipids, p. 375–389. In: J.M. Lyons, D. Graham, and J.K. Raison (eds.). Low temperature stress in crop plants: The role of the membrane. Academic Press, New York.
- Bottomley, P.A., H.H. Rogers, and T.H. Foster. 1986. NMR imaging shows water distribution and transport in plant root systems in situ. *Proc. Natl. Acad. Sci.* 83:87–89.
- Brenner, R.R. 1984. Effect of unsaturated acids on membrane structure and enzyme kinetics. *Prog. Lipid Res.* 23:69–96.
- Brown, J.H., G.A. Johnson, and P.J. Kramer. 1986. In vivo magnetic resonance microscopy of changing water content in *Pelargonium hortorum* roots. *Plant Physiol.* 82:1158–1160.
- Faust, M., D. Liu, M.M. Millard, and G.W. Stutte. 1991. Bound versus free water in dormant apple buds—A theory for endo-dormancy. *HortScience* 26:887–890.
- Goad, L.J. 1983. How is sterol synthesis regulated in higher plants? *Biochem. Soc. Trans.* 11:548–552.
- Gocal, G.F.W., R.P. Pharis, E.C. Yeung, and D. Pearce. 1991. Changes after decapitation in concentrations of indole-3-acetic acid and abscisic acid in the larger axillary bud of *Phaseolus vulgaris* L. cv. Tender Green. *Plant Physiol.* 95:344–350.
- Goldsmith, M.H.M. 1982. A saturable site responsible for polar transport of indole-3-acetic acid in sections of maize coleoptiles. *Planta* 155:68–75.
- Grunwald, C. 1975. Plant sterols. *Annu. Rev. Plant Physiol.* 26:209–236.
- Hillman, J.R. 1984. Apical dominance, p. 127–148. In: M.B. Wilkins (ed.). *Advanced plant physiology*. Pitman Press, London.
- Hillman, J.R., V.B. Math, and G.C. Medlow. 1977. Apical dominance and the levels of indole acetic acid in *Phaseolus* lateral buds. *Planta* 134:191–193.
- Liu, D., M. Faust, M.M. Millard, M.J. Line, and G.W. Stutte. 1993. States of water in summer dormant apple buds determined by proton magnetic resonance imaging. *J. Amer. Soc. Hort. Sci.* 118:632–637.
- Martin, G.C. 1987. Apical dominance. *HortScience* 22:824–833.
- Millard, M.M., D. Liu, M.J. Line, and M. Faust. 1993. Method for imaging the states of water by nuclear magnetic resonance in low water containing apple bud and stem tissues. *J. Amer. Soc. Hort. Sci.* 118:628–631.
- Morris, D.A. and A.J. Thomas. 1978. Microautoradiographic study of auxin transport in the stem of intact pea seedlings (*Pisum sativum* L.). *J. Expt. Bot.* 29:147–157.
- Omasa, K., M. Onoe, and H. Yamada. 1985. NMR imaging for measuring root systems and soil water content. *Environ. Control Biol.* 23:99–102.
- Pretty, J.A. 1978. Fluid flow through the vessels of birch wood. *J. Expt. Bot.* 29:1463–1469.
- Quinn, P.J. 1981. The fluidity of cell membranes and its regulation. *Prog. Biophys. Mol. Biol.* 38:1–104.
- Rubinstein, B. and M.A. Nagao. 1976. Lateral bud growth and its control by the apex. *Bot. Rev.* 42:83–113.
- Thimann, K.V. and F. Skoog. 1933. Studies on the growth hormone on plants. The inhibiting action of the growth substance on bud development. *Proc. Natl. Acad. Sci. USA* 19:714–716.
- Thimann, K.V. and F. Skoog. 1934. On the inhibition of bud development and other functions of growth substance in *Vicia faba*. *Proc. Royal Soc. London B* 114:317–339.
- Thompson, Jr., G.A. 1979. Molecular control of membrane fluidity, p. 347–364. In: J.M. Lyons, D. Graham, and J.K. Raison (eds.). *Low temperature stress in crop plants: The role of the membrane*. Academic Press, New York.
- Wang, P.C. and S.J. Chang. 1986. Nuclear magnetic resonance imaging of wood. *Wood Fiber Sci.* 18:308–314.
- Wang, S.Y. and M. Faust. 1990. Changes of membrane lipids in apple buds during dormancy and budbreak. *J. Amer. Soc. Hort. Sci.* 115:803–808.
- Wang, S.Y., G.L. Steffens, and M. Faust. 1986. Breaking bud dormancy in apple with a plant bioregulator, thidiazuron. *Phytochemistry* 25:311–317.
- Wang, S.Y., T. Sun, B.D. Whitaker, and M. Faust. 1988a. Effect of paclobutrazol on membrane lipids in apple seedlings. *Physiol. Plant.* 73:560–564.
- Wang, S.Y., P.C. Wang, and M. Faust. 1988b. Non-destructive detection of watercore in apple with nuclear magnetic resonance imaging. *Scientia Hort.* 35:227–234.
- White, J.C., G.C. Medlow, J.R. Hillman, and M.B. Wilkins. 1975. Correlative inhibition of lateral bud growth in *Phaseolus vulgaris* L. Isolation of indole acetic acid from the inhibitory region. *J. Expt. Bot.* 26:419–424.
- Yoshida, S. and M. Uemura. 1990. Responses of the plasma membrane to cold acclimation and freezing stress, p. 293–319. In: C. Larsson and I.M. Moller (eds.). *The plant plasma membrane*. Springer-Verlag, Berlin.