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Response of Apple Buds to Pressure Injection of Abscisic Acid and Cytokinin[1]

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Abstract. **A pressure injection method was used with abscisic acid (ABA) and 6-benzylamino purine (BA) to control bud break of one-year-old trees of 'Yellow Delicious' apple (*Malus domestica* Borkh). When dormant trees were injected with 3 ml of 250 ppm (wt/vol) ABA, 58% of buds were inhibited after 28 days compared to 31% of the buds of the controls; injections of 3 ml of 200 ppm (wt/vol) BA above the ABA-injection site, induced 52% of the inhibited buds to open. Radioassays of apple stems collected 11 and 9 days after injection, respectively, with ^{14}C-ABA or ^{14}C-BA indicated highly significant increases in radioactivity in the phloem, buds and new shoots compared to radioassays taken immediately after injection.**

Control of bud break in fruit trees is especially valuable for prevention of spring frost damage. Wareing and Saunders (14) postulated that bud break was controlled by a balance of endogenous hormones including ABA and cytokinin. Phillips (11), in a review of apical dominance, concluded that the cytokinin deficiency in the lateral buds induced by auxin in the apical bud foliage, inhibited lateral bud break and that more information is needed regarding the roles of other growth hormones including ABA.

It is well documented that exogenously applied ABA effectively inhibits bud break (5, 7, 8, 12) and that BA stimulates the growth of dormant buds of several woody species (2, 4, 15). Previously ABA and BA have been applied directly to the buds or taken up through the vascular system by gravitational or transpirational forces (4, 8, 9). In this study an attempt was made to control bud break by injecting ABA and BA solutions into the vascular systems of one-year-old dormant apple trees. Also, translocation and metabolism experiments were performed after injection with ^{14}C-labeled ABA and BA.

Materials and Methods

Injection technique. Aqueous ethanolic solutions of ABA and BA were injected into young apple trees 3 and 5 cm, respectively, above the graft with a miniature pressure injector. The injector consisted of a modified Vise-Grip[4] locking pliers, a tapered stainless steel injector barrel, and a disposable syringe designed specifically to introduce solutions into the vascular systems of woody stems ranging from 6 to 36 mm in diameter. The injector is described in detail by Sterrett and Creager (13). A 2 mm diameter hole was drilled three-quarters of the way through the stem with an electric drill. The tapered tip of the injector was inserted into the hole and wedged into place by closing the attached locking pliers. A prefilled disposable, plastic syringe was then inserted into the injector and the liquid was forced into the hole by hand.

Injection distance. To determine the distance that chemical solutions could be forced by the injector into the dormant stems of the apple trees used in this study, 3 ml of an aqueous solution of 0.5% azo sulfamide dye were injected 3 cm above the graft in a manner similar to that of an earlier study (13). The direction and maximum distances the dye was forced were determined immediately after injection by pruning off small sections of the stem well above and below the injection site until the dye was detected in the tissues of the vascular system. The mean ± SE of 5 apple transplants was determined.

Bud response. Young apple trees were removed from cold storage (5°C) after completion of rest and planted in 4-liter pots in the greenhouse in April. Immediately after potting, the trees, except control ones, were injected 3 cm above the graft with 3 ml of 250 ppm ABA in 3% ethanol. Plants were shortened to 80 cm above the graft at the time ABA was injected and the number of buds located between the injection site and the cut surface was recorded. Further treatments were as follows: Seven days after ABA injection the number of unopened buds was recorded and the stem re-injected 2 cm above the ABA injection site with 3 ml of 200 ppm BA in 12% ethanol or with 3 ml of 12% ethanol to determine any ethanol effect. A second removal of the top was made (without injection) above the highest unopened bud above the injection site to remove apical dominance. On the 28th day the number of unopened buds was again recorded and their percentage determined. Treatments were replicated 5 times in duplicate experiments. The data were analyzed with Duncan's multiple range test.

Translocation. Apple seedlings were removed from cold storage (5°C) and planted in 4-liter pots in April and grown in the greenhouse. Immediately after planting a group of trees was injected 3 cm above the graft with 3 ml of 250 ppm ^{14}C-ABA (specific activity 11.3 mCi/mmole; 0.6 μCi/3 ml); an equal number was injected with 3 ml of 250 ppm of unlabeled ABA. After injection the stem was excised 75 cm above the graft union at the time of injection and divided into 5 – 15 cm sections. The wood, bark, and buds from the 5 sections of the stem were separated and assayed for radioactivity. The wood and bark of a section of the root stock below the graft union (section 1) was also harvested and assayed. Wood and bark from the upper 3 cm of each section and all buds from each section were oven dried at 60°C for 48 hr and then ground in a Wiley Mill and oxidized according to the method described by Peterson, et al. (10). The amount of $^{14}CO_2$ evolved was quantitated by liquid scintillation using Reich solution (10). One lot of the plants treated with ^{14}C-ABA was harvested immediately after injection and the other half after 11 days. Twenty-eight days after injecting with unlabeled ABA, 3 ml of 200 ppm ^{14}C-BA (specific activity 13.4 mCi/mmole; 0.6 μCi/3 ml)

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were injected 2 cm above the ABA injection site. Half of these plants was harvested immediately and the rest 9 days later, two days longer than the "bud response" experiment to insure bud inhibition by ABA.

Chromatographic analysis. Samples of the dried, ground xylem, phloem, buds, and new shoots from the ^{14}C-ABA-injected plants that exhibited radioactivity in the translocation experiment were extracted in methanol for 3 days at 25°C. Extracts were filtered and the filtrate reduced in volume under vacuum. The residue was spotted on Eastman Kodak silica gel TLC sheets, and the chromatogram developed for 10 cm in n-propanol:n-butanol:NH_4OH:H_2O (6:2:1:2 by volume). For reference, ^{14}C-ABA was added to extracts of xylem, phloem, buds and new shoots obtained from untreated plants. A known aliquot of the reference sample was co-chromatographed with all samples. The absorbent from the developed chromatograms was removed in ten 1-cm increments and radioassayed using liquid scintillation. The TLC procedure for the ^{14}C-BA injected plants was similar to that for ^{14}C-ABA injected plants except that the chromatograms were developed in 2-propanol: NH_4OH:H_2O (10:1:1 by volume).

Results

Injection distance. Dye was forced upward from the injection site of the stem in apple 63 ± 3.6 cm and downward into the root-stem transition region 27.1 ± 1.1 cm. The dye was quite apparent in the vascular system of the transition region and at 63 cm above the injection site but appeared to be more uniformly distributed in the vessels within 15 cm above and below the injection site. Dye was not detectable in the buds. Based on the results of this experiment, 3 ml of chemical solution were injected in the bud response and translocation experiments, and 15-cm sections were harvested in the translocation experiment beginning at a point 15 cm below the graft union.

Bud response. Injections of ABA resulted in 58% inhibition of bud break for 28 days compared to 31% inhibition from injections of water in the control (Table 1). This bud break inhibition caused by ABA was overcome by injection of BA. Removal of the apical shoot growth 7 days after injection above the uppermost inhibited bud also overcame the ABA inhibition. Injections of ethanol alone did not negate the ABA effect. The lateral shoot growth induced by BA remained small compared to that near the apex (Fig. 1).

Translocation. Immediately after injection, most of the radioactivity from both ^{14}C-ABA and ^{14}C-BA was detected in the xylem for a distance of about 30 cm above the injection site and in the root stock; some radioactivity could be detected in the phloem and buds located in sections near the injection site (Figs. 2 and 3). Radioassays made of stems treated with ^{14}C-ABA 11 days after injection and of stems treated with ^{14}C-BA 9 days after injection indicated highly significant increases in activity in the buds and phloem tissue compared to those harvested immediately after injection. The radioactivity detected in the upper stem sections (#5 and #6, Fig. 2) in trees injected with ^{14}C-ABA was over 20 times greater (126 dpm/mg) after 11 days than the radioactivity detected in comparable stem sections (Fig. 3) 9 days after injection with ^{14}C-BA (3 to 6 dpm/mg). BA was present in higher concentrations in the lower lateral shoots than in the apical shoots (Fig. 3).

Chromatographic analyses. Eleven and 9 days after injection, 73 to 100% of the ^{14}C-ABA and 83 to 100% of the ^{14}C-BA, respectively, were located in the xylem, phloem, buds and new shoots. These compounds had migrated the same distance as the original substance injected (Fig. 4 and 5).

Discussion

The inhibition of bud break resulting from injection of ABA can be correlated with the exogenous ABA that was translocated from the xylem to the buds. The negation of the ABA-induced bud inhibition after injection of BA was, no doubt, due to translocation of the injected BA into the buds from the xylem. Since inhibition of lateral bud break by exogenous ABA was overcome by removing apical shoots, this finding supports the conclusion of Phillips (11) that the amounts of ABA and cytokinin in the lateral buds are regulated by auxin in the apex of the stem. Apparently, 200 ppm BA could only partly overcome the inhibitory effect of 250 ppm ABA as was shown by

Table 1. Percentage of unopened apple buds after injection of 3 ml of ABA (250 ppm) followed by 3 ml of BA (200 ppm) in 12% ethanol. All plants were excised 80 cm above graft on first day of treatment.

Injection treatment	Unopened apple buds (%)	
	Day 7	Day 28
Water on day 1	35 a[z]	31 a[z]
ABA on day 1	58 b	58 b
ABA on day 1 and second excision on day 7	58 b	33 a
ABA on day 1 and BA in ethanol on day 7	62 b	30 a
ABA on day 1 and ethanol on day 7	58 b	56 b

[z]Mean separation in columns by Duncan's multiple range test, 5% level.

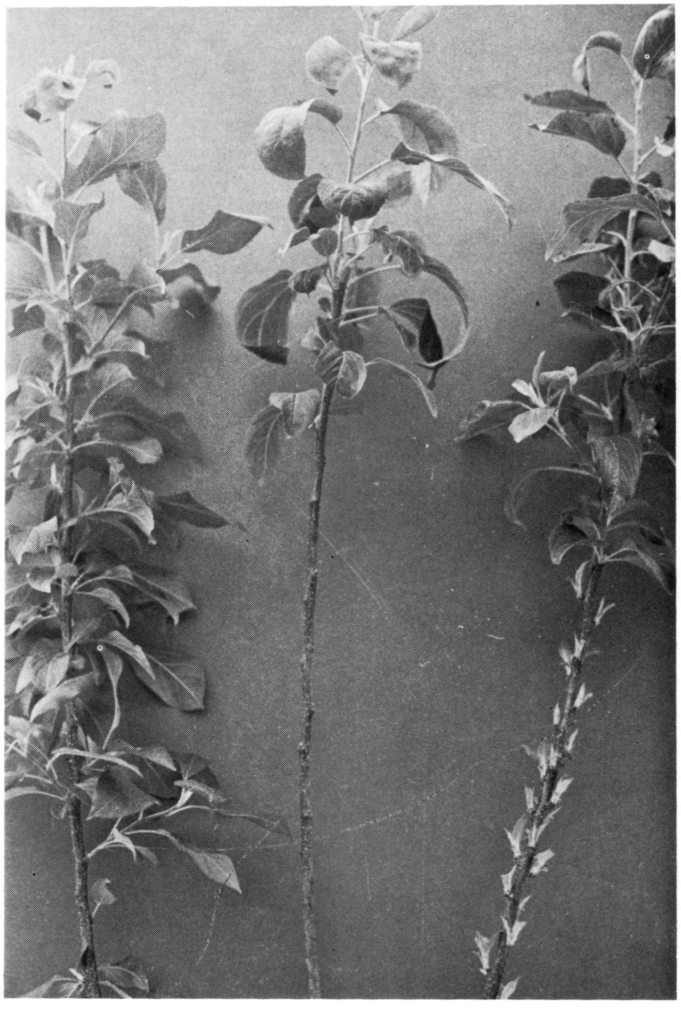

Fig. 1. 'Yellow Delicious' apple trees 28 days after injection with ABA. Left: Injected with water on day 1. Middle: Injected with ABA on day 1. Right: Injected with ABA on day 1 and BA on day 7.

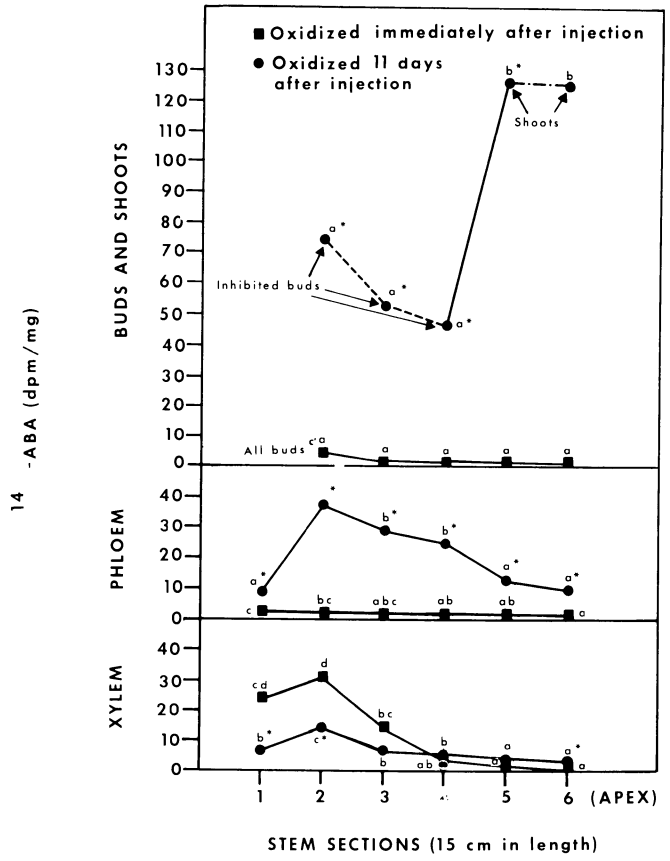

Fig. 2. The location of ^{14}C-activity in 'Yellow Delicious' apple trees immediately and 11 days after injection of 3 ml of ^{14}C-ABA (250 ppm). Only the buds on stem sections 5 and 6 became shoots 11 days after treatment. Letters indicate mean separation, within curves, by Duncan's multiple range test at the 5% level. Asterisks above letters indicate significant differences at the 5% level between oxidation periods within sections of tissue using the rank-sum test (6).

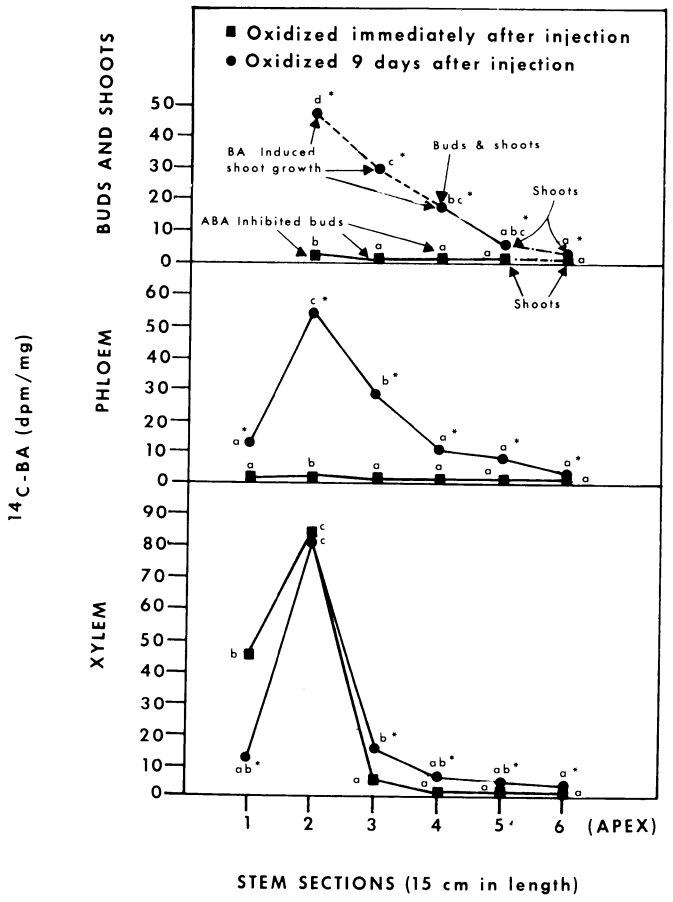

Fig. 3. The location of ^{14}C-activity in 'Yellow Delicious' apple trees immediately and 9 days after injection of 3 ml of ^{14}C-BA (200 ppm) 5 cm above the graft in the scion stem (injection occurred 28 days after injection of unlabeled ABA). Letters indicate mean separation, within curves, by Duncan's multiple range test at the 5% level. Asterisks above letters indicate significant differences at the 5% level between oxidation periods within sections of tissue using the rank-sum test (6).

the lack of full leaf expansion after bud break (Fig. 1).

Apparently no metabolites were produced by apple from both ABA and BA since the chromatographic peak on the TLC plates closely coincided. This indicates that a high percentage of the ^{14}C-activity detected represented either non-metabolized ABA or BA.

In a separate experiment in which the same procedures were used as in the bud response experiment, the break of apical bud on 1-year-old apple trees was not inhibited when 3 ml of 250 ppm ABA were injected 15 cm below the apex. This distance from the injection site to the apical bud was well within the range in which lateral bud break was inhibited in the bud response experiments. Apparently, the concentration of endogenous auxin and/or cytokinin in the apical bud was sufficiently high to antagonize (or reverse) the activity of the injected ABA. Borkowska (3) found that the endogenous cytokinins in apical apple buds were higher than in other buds. However, Bellandi, and Dörffling (1) were able to inhibit apical bud break by applying ABA directly to the apical bud of *Pisum sativum* L.

The miniature pressure injector made possible the introduction of measured amounts of solutions into the vascular system of the dormant apple transplants, which, in addition to controlling bud break, provided support for the hormonal theory

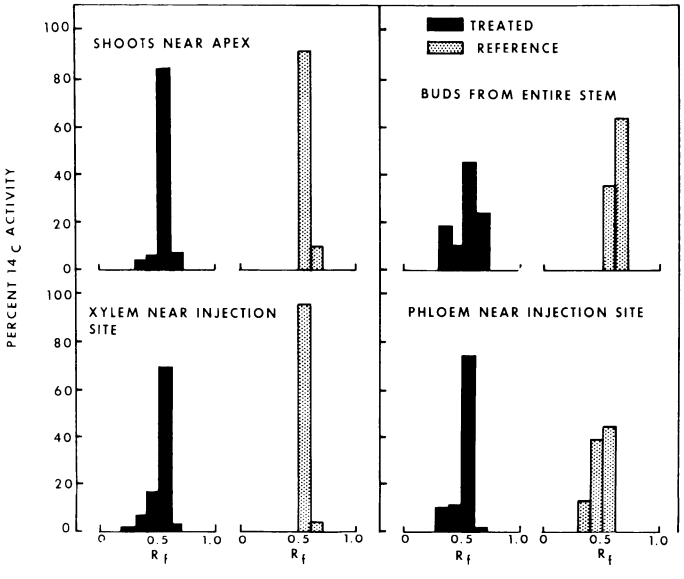

Fig. 4. Percent ^{14}C-activity in 1-cm sections of thin-layer chromatograms of methanolic extracts of apple stems 11 days after injection of ^{14}C-ABA compared with fortified reference extracts. Thin-layer chromatograms were developed in n-propanol:n-butanol:NH_4OH:H_2O (6:2:1:2, by volume).

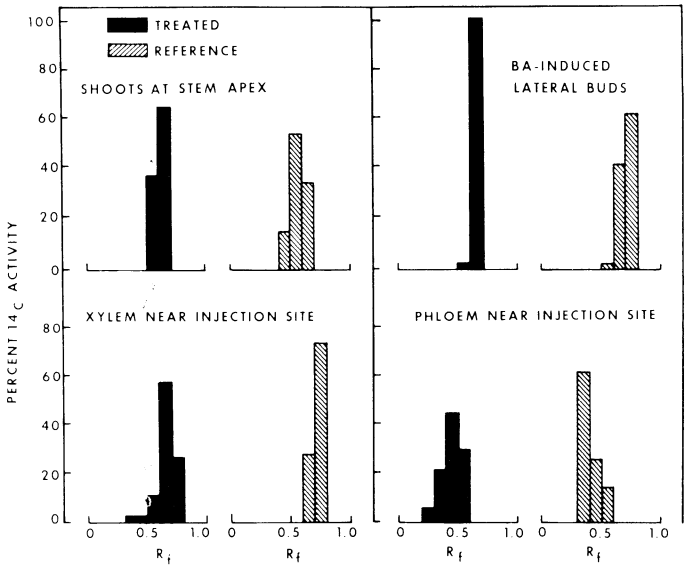

Fig. 5. Percent ^{14}C-activity in 1-cm sections of thin-layer chromatograms of methanolic extracts of apple stems 9 days after injection of ^{14}C-BA compared with fortified reference extracts. Thin-layer chromatograms were developed in 2-propanol:NH_4OH:H_2O (10:1:1, by volume).

of apical dominance. Although the miniature injector was designed to study growth regulators, it should be a useful tool for the evaluation of any chemical solution that can be introduced into the vascular system of woody plants.

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Seasonal Trends in Net Photosynthetic Potential, Dark Respiration, and Specific Leaf Weight of Apple Leaves as Affected by Canopy Position[1]

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Abstract. **Changes in photosynthetic parameters in 'Stayman'/Malling Merton (MM) 111 apple (*Malus domestica* Borkh.) trees measuring 5 m wide and 4 m high were studied for an entire growing season. Parameters investigated included penetration of photosynthetically active radiation (PAR), changes in spur leaf net photosynthetic (Pn) potential, dark respiration (Rd) and specific leaf weight (SLW). As measured by changes in PAR penetration, canopy development was generally complete by mid-May. Pn, Rd, and SLW were modified by canopy position. SLW was influenced by the previous light environment as peripheral canopy leaves had higher SLW's than interior leaves.**

Only limited data are available on seasonal trends of apple leaf Pn potential, Rd, and SLW as related to canopy position and PAR level. Heinicke (9, 10) measured light levels within large trees in late summer and found only 2 zones, a peripheral one with irradiance levels of approximately 65-119 klx and a shaded interior with irradiances of 4-8 klx. Peripheral shoot leaves exposed to these light levels had Pn rates of 26 and 7

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