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Breaking Bud Rest on Detached Apple Shoots: Effects of Wounding and Ethylene[1]

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Abstract. **Buds of 'Golden Delicious' apple (*Malus domestica* Borkh.) were in deep rest between October 25 and December 20, 1976, although the uppermost bud on decapitated (apical and basal cuts) shoot segments developed at all sampling dates. Increases in rate of development of the uppermost bud, as well as in % break of lower buds after completion of deep rest, were both linear with time. Increases in % budbreak with time occurred in a basipetal direction, suggesting a gradient of increasingly deep rest from shoot apex to base. Development of the uppermost bud on decapitated shoots during deep rest was a result of the apical cut, and was not influenced by the original position of that bud on the intact shoot. The bud above the basal cut developed only on shoots with the terminal bud intact (basal cut only). Notching above a second bud on decapitated shoots did cause that bud to develop in addition to the uppermost bud. Results are discussed on the basis of a 2 stage rest model, rest gradient and apical dominance. There was no evidence for a role of ethylene in wounding or dinitro-ortho-cresol-stimulated budbreak. In addition, neither ethylene-releasing chemicals nor silver or cobalt had any influence on budbreak when applied to shoots at 2 dates during the rest period.**

Leaf stripping (12), stem wounding (18), the application of certain chemicals (17, 7), and high temperature (5) all effectively increase budbreak. However, in most studies there was little attempt to correlate results with stage of rest. Wounding is an established but little-documented treatment for breaking rest (18). In apple, girdling or notching directly above a bud during "late dormancy" has been shown to stimulate its development (22).

Stress, one result of many rest-breaking treatments, is known to generally increase ethylene levels in plants (1). Although there is no information on changes in ethylene levels after imposition of rest-breaking treatments, rest was broken in 14 woody plant species including apple following exposure to 1000 ppm of ethylene or propylene (21). Vapors of ethylene chlorhydrin, ethylene dichloride, and ethyl iodide effectively broke rest of lilac buds (6), and the ability of apple gas to break rest of beech and birch buds increased with increasing cold accumulation (4). These reports were all previous to 1940. Many reports on wounding or ethylene and dormant buds dealt not with rest but with correlative inhibition. There are reports of ethylene breaking dormancy of other organs including seeds (20, 13, 8) (but not cold-requiring seeds of woody plants), tubers and corms (21), and bulbs (10). As with buds, many of these responses were not specific for ethylene and could be duplicated with other gases or treatments i.e. heat treatment (1).

In this report we examine the effect of wounding by cutting, and the role of ethylene in the stimulation of apple budbreak on detached shoots during rest.

Materials and Methods

Uniform shoots were collected from mature 'Golden Delicious' apple trees at the Purdue Horticulture Farm. Unless stated otherwise, 30 cm segments, taken by cutting each shoot at 10 and 40 cm back from the terminal bud, were treated and then held individually with their bases in distilled H_2O in 25 ml vials completely randomized in a growth chamber at 26°C, 16 hr photoperiod and 43 klx. Five replicate shoots were used per treatment. Exposed cuts were sealed with grafting wax, and water in the vials was changed every 3 days. Unless stated otherwise, these shoots were used for all subsequent experiments. Rest was arbitrarily considered broken when buds reached level 1 (Fig. 1). Our observations indicated that once at level 1 buds would continue further development. Rate of further development was dependent on the amount of additional chilling. Data, collected at 15 days, was expressed as the % break of the 8 lateral buds on the shoots above the tops of the vials. The rate of development of the uppermost bud, another measure of rest intensity, was expressed as the level of that bud's development at 15 days. 'Golden Delicious' was sampled on 10 dates and the crabapples *Malus* MR #454 and *Malus* × *arnoldiana* on 9 dates, between Sept. 9, 1976 and March 5, 1977, to follow rest intensity.

Experiments were conducted to determine the effect of shoot length and notching, and the difference between intact (basal cut only with terminal bud intact) and decapitated (apical plus basal cuts) shoots on the pattern of bud development. Notching consisted of removal of a bark strip 0.5 mm wide and 5.0 mm long from immediately above a bud. On Dec. 5, uniform, 40 cm long shoot segments with intact terminal buds were further shortened by removing the distal shoot ends at 10, 18, 22, 26, 30 and 34 cm below the terminal bud. The pattern of budbreak was observed at 30 days. Additional shoots, collected on Dec. 4, 20, Jan. 14, and Feb. 5, were cut to 30 cm lengths starting 10 cm below the terminal bud, and notched above bud no. 6. In these experiments % budbreak was determined at 15 days.

Fig. 1. Arbitrarily defined levels of bud development. Level 1 or higher signified budbreak.

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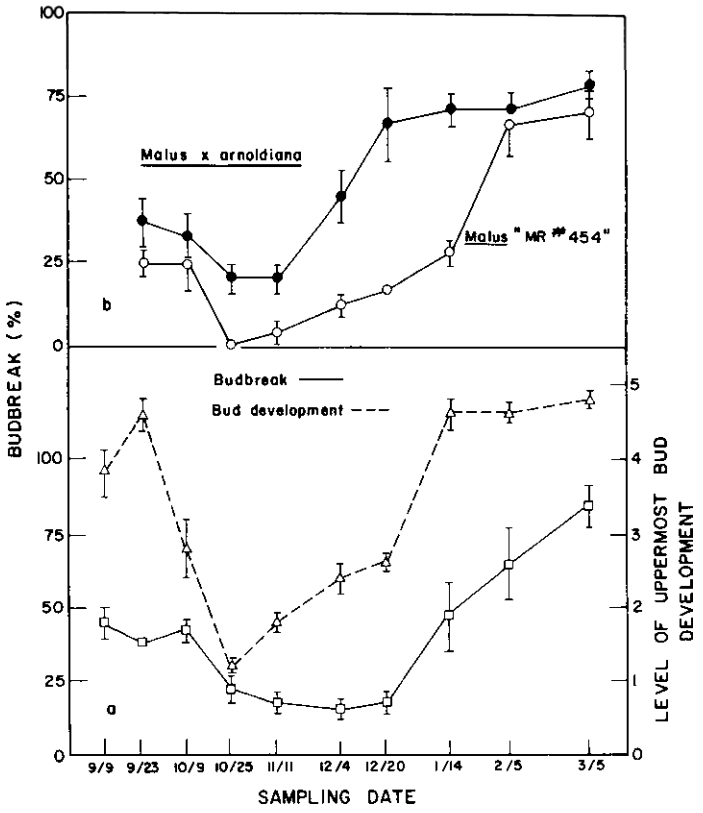

Fig. 2. a.) Percent budbreak and level of uppermost bud development on 30 cm long detached, decapitated, 'Golden Delicious' shoots collected on 10 sampling dates during the rest period. b.) Percent budbreak on 30 cm long detached, decapitated shoots of 2 crabapples collected on 9 sampling dates during the rest period.

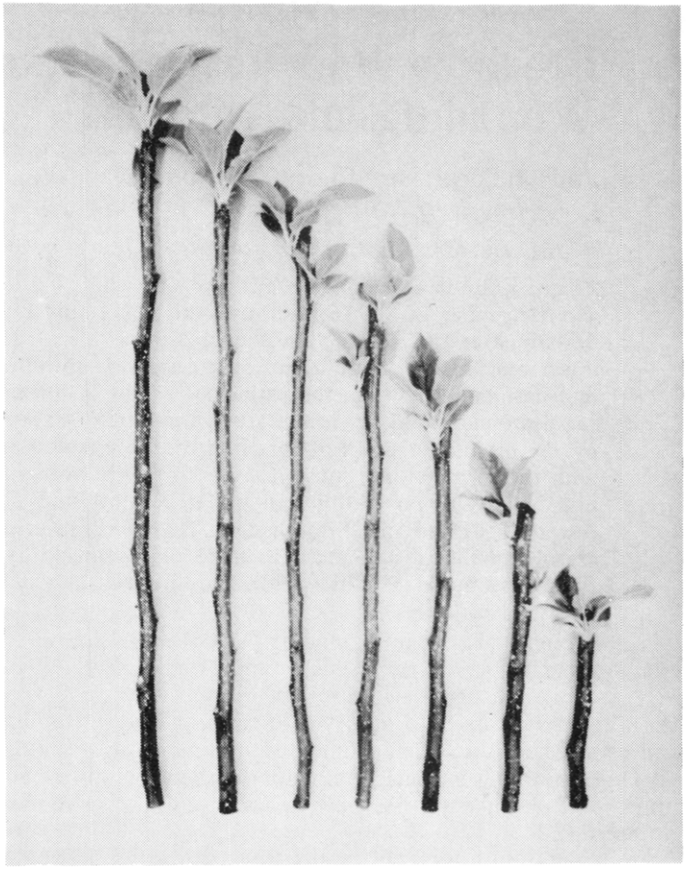

Fig. 3. Shoot segments, all made with basal cuts at the same distance from the terminal bud, indicating uniform break and development of the uppermost bud irrespective of that bud's original position on the intact shoot. Shoots were collected and segments cut on Dec. 5, and photographed Jan. 5.

Internal ethylene levels were measured on Dec. 22, 24 hr after shoots were cut and treated with 0.2% dinitro-ortho-cresol (DNOC). This DNOC concn was effective in breaking rest when applied at that time (16). That part of shoot segments above the vial tops was sub-divided into 3 sections; the top, middle, and bottom sections included buds no. 1 and 2, 4 and 5, and 7 and 8, respectively. Short sections including buds no. 3 and 6 were discarded. Top, middle, and bottom sections from 4 shoots were combined and evacuated together. Additional shoots remained 15 days in the growth chamber for budbreak analysis.

Shoots were treated on Jan. 4 with 1000 ppm of ethephon or CGA 15281. The latter chemical, a new ethylene releasing compound, very closely resembles 2-chloroethyl-tris-(2-methoxyethoxy)-silane (CGA 13586) in molecular wt, toxicity, and decomposition characteristics (Ciba-Geigy Corp. Technical Information Sheet). Internal ethylene concn were determined at 1, 24, and 120 hr. On March 7, CGA 15281 was applied at 2000, 1500, 1000, 500, 250, 100, 50 or 10 ppm. $AgNO_3$ (500 ppm) and $Co(NO_3)_2$ (150 ppm) were applied on Jan. 4, and internal ethylene was measued at 3 days. Applications of $AgNO_3$ at 1000, 500 or 200 ppm, $Co(NO_3)_2$ at 150, 100 or 10 ppm, and NH_4NO_3 at 1000 ppm were made on March 5. Ag(I) and Co(I) are thought to block ethylene action (2) and production (9), respectively. In all experiments shoots were submerged 5 min in appropriate solutions plus 0.1% Tween 20 (by vol). Percent budbreak was determined at 15 days.

Internal gases were collected and ethylene levels measured using the method of Leopold et al. (14). Ethylene concn obtained were not affected by amounts of tissue evacuated or length of evacuation time. Collected gases were sampled in 1 ml syringes for injection into a gas chromatograph fitted with an activated alumina column. The ethylene peak was identified by retention time and by co-chromatography with pure ethylene.

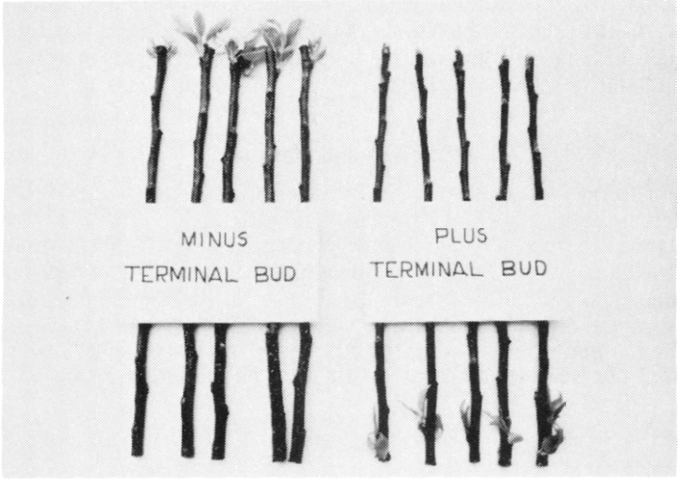

Fig. 4. Pattern of budbreak on 30 cm long detached shoots collected Dec. 4 and photographed Jan. 4. Left: decapitated shoots with apical cuts made 10 cm below the terminal bud. Right: shoots with intact terminal buds.

Table 1. Percent break of uppermost buds on 30 cm detached shoots at 4 sampling dates. The uppermost bud was the terminal bud on intact shoots, or a lateral bud on shoots decapitated 10 cm below the terminal bud. Budbreak was determined at 15 days.

Uppermost bud	Bud break (%)			
	Sampling date			
	Dec. 4	Dec. 20	Jan. 14	Feb. 5
Terminal	0a[z]	40a	80a	100a
Lateral	100b	100b	100a	100a

[z]Mean separation in columns by Student-Newman-Keuls' multiple range test, 5% level.

Results

Deep rest in 1976–77, measured as % budbreak at 15 days, occurred between Oct. 25 and Dec. 20 (Fig. 2a). The uppermost bud on decapitated shoots developed at each sampling date, its rate of development being slowest on Oct. 25 before increasing to a max on Jan. 14. After Dec. 20, % budbreak increased linearly until March 5 when the rest influence had nearly disappeared. Increases in % budbreak as a function of time occurred in a basipetal direction, and when more than 1 bud broke it developed more slowly than the bud immediately above. At the end of rest all buds broke uniformly and reached developmental level 5 by day 15 (data not shown).

Both crabapples also entered deep rest on Oct. 25 (Fig. 2b). *Malus* x *arnoldiana* was similar to 'Golden Delicious', as the top 1 or 2 buds on decapitated shoots developed at each date. However, the deep rest period was shorter, and % budbreak increased faster, than for 'Golden Delicious'. *Malus* MR #454 did reach 0% budbreak on Oct. 25.

Development of the uppermost bud on decapitated shoots during deep rest was a result of cutting, and was not influenced by the original position of that bud on the intact shoot (Fig. 3). On intact shoots terminal buds, which responded to chilling, did not develop on Dec. 4 (Table 1). Instead, during deep rest, budbreak on intact shoots occurred immediately above the basal cut (Fig. 4). On decapitated shoots the basal cut did not induce budbreak even at 30 days. Notching above bud 6 on decapitated shoots stimulated that bud to grow (Table 2). The notched bud developed at the same rate as the uppermost bud. Notching above all buds was too severe a treatment and killed the shoots.

There was no difference in internal ethylene levels at 24 hr between top, middle, and bottom sections of decapitated, control shoots (Table 3), indicating that while cutting stimulated break of only the uppermost bud, it did not create differential ethylene levels in the shoot. DNOC treatment improved budbreak in the middle section but did not increase internal ethylene in that section at 24 hr. Ethephon and CGA 15281, each applied at 1000 ppm at the end of the deep rest period on Jan. 4, failed to influence budbreak despite raising internal shoot ethylene levels (Table 4). This is a period when GA_3 and DNOC very effectively increase budbreak (16). CGA 15281, applied at 8 concn at the end of rest on 3/5, again had no effect (data not shown). Ag(I) and Co(I) had no effect on either internal ethylene levels at 72 hr or budbreak when applied at the end of the deep rest period on Jan. 4. Applications of Ag(I) and Co(I) made at the end of rest on March 7 were also without effect.

Table 2. Effect of notching immediately above bud no. 6, on the % break of bud no. 6 at 4 sampling dates. The uppermost bud on 30 cm long detached shoots occurred just below the apical cut, made 10 cm below the terminal bud. Budbreak was determined at 15 days.

Bud	Notch	Bud break (%)			
		Sampling date			
		Dec. 4	Dec. 20	Jan. 14	Feb. 5
Uppermost		100a[z]	100a	100a	100a
No. 6	–	0b	0b	20b	80a
No. 6	+	80a	80a	100a	100a

[z]Mean separation in columns by Student-Newman-Keuls' multiple range test, 5% level.

Table 3. Internal ethylene concn and % budbreak in the top (buds 1 + 2), middle (buds 4 + 5) and bottom (buds 7 + 8) sections of 30 cm long detached shoots collected and treated with 0.2% DNOC on Dec. 22. Some shoots were sectioned for ethylene measurement at 24 hr, and others remained in the growth chamber for budbreak determination at 15 days.

Stem section	Ethylene (ppm)		Budbreak (%)	
	DNOC	Control	DNOC	Control
Top	2.7aA[z]	0.6aB	80A	80A
Middle	0.7bA	0.7aA	30A	0B
Bottom	1.6bA	0.9aA	10A	0A

[z]Mean separation in columns (lower case letters) and rows (upper case letters) by Student-Newman-Keuls' multiple range test, 5% level.

Discussion

We interpret the results of Fig. 2a as suggesting that rest occurs in 2 stages, both presumably mediated by cold. In stage 1 budbreak did not occur, even after shoots remained 30 days under favorable conditions. After accumulating sufficient cold, buds entered stage 2 where, after placement in a favorable environment, rate of development was dependent on the date of shoot collection. Manual leaf removal through Oct. 9 may have influenced results through that date, since leaf stripping has been used to circumvent rest and force apple shoots back into growth (12). The existence of both qualitative and quantitative rest stages was previously proposed (3). In that study buds in stage 1 (qualitative chilling period) did not develop regardless of how long shoots remained under favorable conditions.

Shoots in deep rest have all buds in stage 1. Buds complete stage 1 and progress through stage 2 in basipetal order, suggestive of a rest gradient down the shoot, with lower buds in deeper rest than those above. This may be a result of the finding that dormancy is a function of age of individual buds, and occurs earlier in the season in older buds and later in more

Table 4. Internal ethylene concn (ppm) and % budbreak in 30 cm long detached shoots collected and treated with 1000 ppm of ethephon and CGA 15281 on Jan. 4. Budbreak was determined at 15 days.

Treatment	Ethylene (ppm) at:			Budbreak (%)
	1 hr	24 hr	120 hr	
Control	0.5b[z]	0.6c	0.5b	29a
Ethephon	0.9b	4.7b	0.6b	31a
CGA 15281	8.9a	5.1a	1.2a	34a

[z]Mean separation in columns by Student-Newman-Keuls' multiple range test, 5% level.

distal buds (15). Results with crabapples suggest that the pattern of rest can vary considerably with different plants.

The first 1 or 2 buds immediately below a cut were stimulated out of stage 1; thus even during deep rest up to 25% budbreak occurred on decapitated shoots as a result of cutting (Fig. 2a). Stage 2 was not bypassed by cutting, because rate of bud development was still dependent on date of shoot collection. On decapitated shoots only the uppermost bud developed, possibly because, as a result of the proposed rest gradient, it reached stage 2 slightly ahead of the lowermost bud. A developing upper bud could, in a situation analogous to apical dominance, inhibit further development of a lower bud, even if that lower bud had been stimulated out of stage 1 by cutting and had the potential to develop. On intact shoots, the lowermost bud did develop in the absence of upper bud development. Notched buds did develop even on decapitated shoots, since notching provided the stimulus to complete stage 1 as well as intercepting the influence of the upper bud. Ringing and steam girdling were shown to release buds from correlative inhibition (11, 19). All buds which completed stage 1 were unaffected by correlative inhibition, as they all commenced growing simultaneously when placed under favorable conditions.

We found no evidence that ethylene is involved in emergence from rest. However, it is possible that our timing, or concn of ethephon, CGA 15281, Ag(I) or Co(I) were incorrect, that internal shoot ethylene levels do not reflect bud ethylene levels, or that there was inadequate uptake of Ag(I) and Co(I). DNOC did significantly increase internal shoot ethylene levels, although the increase was detected where the cut surfaces allowed chemical entry and not in the middle of the shoots where improved budbreak occurred. Further studies are needed. However, earlier studies generally involved exposure to high concn of gas. In these cases ethylene may simply have been another indirect stimulus acting in the same manner as DNOC and other chemicals, heat shock and cutting. How these treatments substitute for chilling during rest remains to be discovered.

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