Age, Growing Temperatures, and Growth Retardants Influence Induction and Length of Dormancy in *Malus*

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Abstract. Apple seedlings have a shallow dormancy, as has been observed in many other species. The length of bud dormancy in high-chilling-requirement seedlings does not reflect their genetic constitution well if dormancy is induced before they are 200 days old. Seedling populations sprayed with paclobutrazol and/or ethephon displayed bud dormancy periods resembling those of older populations of similar genetic constitution. Terminal bud formation and dormancy could not be induced by continuously exposing apple seedlings to low temperature ($8 \pm 1^\circ\text{C}$) and short photoperiod, even after extended periods. Stomate operation may not be completely functional under these conditions. Terminal bud formation was induced by holding apple seedlings above 20°C . Additional exposure to low temperature ($8 \pm 1^\circ\text{C}$) induced leaf fall. These findings suggest the existence of an active regulatory factor that induces terminal bud formation and dormancy and is either turned on or synthesized above 15 to 17°C . Chemical names used: β -[(4-chlorophenyl)methyl]- α -(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol (paclobutrazol); (2-chloroethyl)phosphoric acid (ethephon).

Developing apple cultivars adapted to the subtropics is the central objective of breeding programs in Israel and Brazil. Breeding efficiency could be significantly improved by selecting for adaptation in the early seedling stage. However, it is not known how well bud dormancy of young seedlings is correlated with that of mature clones.

Growth cessation, terminal bud formation, leaf senescence, and defoliation make up the sequence of dormancy development in most woody plants that become dormant, including *Malus*. In mature apple trees, bud formation and dormancy intensification start early in the summer (Hauagge and Cummins, 1991 b), when daylength and average temperature are still increasing. The dormancy induction process in *Malus* may differ from that in other woody species in which both short photoperiods and low temperatures play major roles. However, maximum dormancy intensity occurs after low temperatures are observed in the field (Hauagge and Cummins, 1991 b), and low temperature plays a role in development of the maximum dormancy intensity.

At least in some species, such as *Abies fraseri*, seedlings have a more shallow dormancy than do mature clones (Hinesley, 1982). Young apple seedlings and nonbearing trees tend to continue growing through the fall. Terminal buds, which are needed for dormancy establishment, may not be formed. Within a given set of apple seedlings, the relative order of spring budbreak is maintained throughout the years (Oppenheimer, 1962; Oppenheimer and Slor, 1968).

Water stress and some growth retardants, such as ethephon and paclobutrazol, may favor growth cessation and terminal bud formation in apple and other species (Lever, 1986). In raspberry (*Rubus idaeus*) plants, paclobutrazol induces earlier onset of

dormancy (Maage, 1986). Paclobutrazol inhibits gibberellic acid (GA) synthesis in cell-free homogenates of *Cucurbita* and may also decrease GA in perennial plants (Hedden and Graebe, 1985). Summer and fall applications of ethephon are known to delay spring budbreak in *Prunus* spp. (Coston et al., 1986; Funt and Ferree, 1986; Proebsting and Mills, 1976), possibly by accentuating or extending dormancy.

The purpose of our study was to determine 1) how bud dormancy in mature apple clones is related to bud dormancy in seedling populations of various ages and 2) how foliar sprays of paclobutrazol and ethephon affect this relationship.

Materials and Methods

Seed collection. Open-pollinated (OP) seeds of 'Anna' and 'Golden Delicious', as well as some other apple cultivars, were used in this experiment. Almost all apple cultivars in the orchard where seeds were collected have high standard bud chilling requirements (CR). Consequently, OP seedling populations resulted from crosses between low-CR and high-CR parents. In addition, both parents would have high CR in populations where the female parent had high CR. 'Anna' is self-incompatible.

Effects of temperature on terminal bud formation and leaf senescence. 'Golden Delicious' OP seedlings were grown in the greenhouse in 16 h light and at 20 to 26°C . Seeds were planted in trays spaced 6×6 cm, with 360 cm^3 of soil volume per seedling. Seedlings were subdivided into two populations 25 days after emergence from soil; one group remained in the same environmental conditions, while the other was placed in a growth chamber at $8 \pm 1^\circ\text{C}$ and 12 h light. The seedlings that remained in the greenhouse were brought to $8 \pm 1^\circ\text{C}$ and 12 h light 6 months later. Five hundred seedlings subdivided into five replications were used for each treatment. The dates for terminal bud formation and leaf senescence were determined.

Bud dormancy relationship between mature clones and seedling populations. OP and cross-pollinated seedling populations, varying in age from 70 to 560 days, were induced into dormancy

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Abbreviations: CR, chilling requirement; CU, chill unit; GDH, growing degree hours; OP, open-pollinated.

Table 1. Effects of temperature on growth and terminal bud formation in 'Golden Delicious' OP seedlings. Treatments started 25 days after seedling emergence from soil. Five hundred seedlings were used for each treatment.

Temp (°C)	Seedlings with terminal buds formed at		Seedling ht ^z (cm)	No. leaves ^z	Internode length ^z (cm)
	180 days (%)	370 days (%)			
8 ± 1	0.0 ± 0.0	0.0 ± 0.0	16.9 ± 2.5	18.8 ± 3.3	0.9 ± 0.1
20–26	100 ± 0.0	---	35.0 ± 10.4	35.9 ± 11.1	1.0 ± 0.2

^zEstimations based on a 40-seedling sample made 370 days after seedling emergence.

(Hauagge and Cummins, 1991 b), overwintered, and forced. Seedlings 560 days old were in their second winter cycle in this experiment. Plants were grown under 16 h light and at a 20C minimum. Hardening and dormancy were induced by a process modified from Stushnoff (personal communication) at various seedling ages after terminal buds had been established. Temperature was maintained at 14 ± 2C, 8 ± 2C, and 14 ± 2C for 20, 40, and 14 days, respectively (Hauagge and Cummins, 1991a). Both chill units (CU) (Shaltout and Unrath, 1983) and growing degree hours (GDH) (Anderson et al., 1986) were calculated based on hourly recorded temperature and started to accumulate at the end of the dormancy induction treatment. At this time, 80% to 95% of the leaves on all plants had abscised or were in an advanced stage of senescence.

Overwintering and forcing were done either at constant temperature (5 ± 1C and 20 ± 2C, respectively) or simultaneously in an alternating temperature regime, similar to what normally occurs in the winter in subtropical areas (Hauagge and Cummins, 1991a), where bud dormancy in standard apple cultivars is not completely broken. In these conditions, both CU and GDH accumulate simultaneously. The GDH accumulated between leaf fall and budbreak (at least 1 bud reaching green-tip stage) under alternating-temperature overwintering conditions is related to CR as determined under subtropical conditions (Hauagge and Cummins, 1991a). Alternating-temperature overwintering

was accomplished by maintaining 5 ± 2C for 9 h at night and 14 ± 3C during the day. Day temperatures as high as 24C occurred a few times for short periods (< 2 h).

GDH between leaf fall and budbreak for seedlings (under alternating-temperature overwintering) or days for budbreak after forcing (under constant-temperature overwintering) was compared with that of grafted female parents subjected to similar conditions. The index Δ :seedlings/ Δ parents was created to relate the length of dormancy period in two OP seedling populations having different CR to the length of the dormancy period of their female parents (a and b) at different seedling ages. This index was calculated by: $[m(GDH)_{sa} - m(GDH)_{sb}] / [(GDH)_{pa} - (GDH)_{pb}]$, where $m(GDH)_s$ is equal to the population mean GDH accumulated between leaf fall and budbreak, and $(GDH)_p$ is equal to the mean GDH accumulated between leaf fall and budbreak of the respective grafted female parent growing in the same conditions.

Effects of ethephon and paclobutrazol on the GDH accumulated between leaf fall until budbreak in two OP seedling populations. OP seedlings of 'Anna' and 'Golden Delicious' were grown in the greenhouse in 16 h light and at a 20C minimum. Seedlings were sprayed with ethephon (500 ppm a.i.; seedlings 110 days old) or paclobutrazol (300 ppm a.i.; seedlings 80 days old) plus ethephon (500 ppm a.i.; seedlings 110 days old) to runoff in three treatment combinations. Each treatment plot in-

Table 2. Days to budbreak in OP apple seedlings and grafted seed parents at 20 ± 2C and a 16-h photoperiod following 960 CU under alternating-temperature overwintering. Seedlings were 70 days old at time of dormancy induction.

Female parent	N ^z	Days to budbreak				Grafted female parents (mean ± SE)	Chilling requirement of the female parent ^y
		Seedling classes					
		<15	15–28	29–38	39–53		
<i>Seedlings in class (%)</i>							
Anna	40	3	90	7	0	15 ± 3	Very low
Ein Shemer	54	0	68	5	17	38 ± 4	Low
Khashabi	48	5	80	10	5		Low
Zabaoani	50	0	76	22	2		Low-medium
Hume	47	0	42	47	1		Medium
Winter Banana	53	0	38	38	24	67 ± 8	Medium
Beverly Hills	52	0	11	57	32		Medium-high
Leyda	42	0	42	42	16		Medium-high
Gala	42	0	59	24	17	79 ± 9	High
Golden Delicious	94	0	51	32	17	83 ± 9	High
McIntosh	47	0	10	55	35		High
Nova Easygro	46	0	9	59	32		High
Priscilla	52	0	51	17	24		High
Marin Oufroy	34	0	35	44	31		Very high

^zNumber of seedlings in the population.

^yEstimated chilling requirement of female parent under subtropical conditions.

eluded 24 seedlings per cultivar; there were four replications. Dormancy induction began at 150 days when the seedlings were moved into an alternating-temperature regime (Hauagge and Cummins, 1991a), as described previously. Maximum CU (Shaltout and Unrath, 1983) accumulation reached 1010 CU. Time of budbreak was monitored, and GDH between "leaf fall and budbreak was then calculated.

Results and Discussion

Effects of temperature on terminal bud formation and leaf senescence. Terminal bud formation was completely induced within 180 days after seedling emergence in those growing above 20C (Table 1). In addition, limiting the volume of soil and forcing water "to become a limiting factor during some period of the day often resulted in terminal bud formation within 60 to 70 days after germination (data not shown). However, absolutely no terminal bud formation was induced at 8 ± 1 C. At this temperature, the seedlings continued to grow, although at a low rate, even after 370 days of treatment; no leaf senescence was observed. The seedlings that remained in the greenhouse and formed terminals were brought into 8 ± 1 C and 12 h light in a growth chamber 180 days after germination. Leaf senescence, as judged by leaf color, was first observed in these seedlings 2 weeks after exposure to 8 ± 1 C had started. Forty-five days later, $83\% \pm 6\%$ of these seedlings had abscised at least 90% of their leaves or they were in an advanced stage of senescence. This result suggests the possible existence of an active regulator factor that induces terminal bud formation at temperatures of 20C and above. Differences in terminal bud formation between the two treatments could not be attributed to differences in seedling height or juvenility because 16% of seedlings in both treatments were of similar height (data not shown). After terminal buds have formed, leaf senescence and deeper dormancy will occur as a result of low-temperature treatment (Hauagge and Cummins, 1991b). Jonkers' (1979) work supports this concept. He grew apple and pear plants at a range of constant temperatures from 9 to 25C for 16 weeks. They were then defoliated and stored for 6 weeks at 2C. Plants grown previously at temperatures below 13C grew normally after forcing; those grown previously between 15 and 17C were intermediate in behavior; and plants grown previously at higher temperatures remained dormant. Six weeks of constant chilling does not provide enough cold to break dormancy in 'Golden Delicious'. Consequently, growth potential was related to the state of dormancy before chilling treatment, which, in turn, was related to

Table 3. Days to budbreak in open- and cross-pollinated apple seedling populations at 20 ± 2 C and a 16-h photoperiod following 700 CU at 5 ± 1 C. Seedlings were 150 days old at the time of dormancy induction.

Parentage	No. seedlings	Classes (days to budbreak)					
		0-6	7-13	14-20	21-27	28-35	>35
<i>Seedlings in class (%)</i>							
Anna OP ^z	31	12	61	12	0	0	12
Golden Delicious OP	38	0	21	21	10	0	48
Northern Spy x Anna	40	23	46	0	4	8	19
Northern Spy x Ein Shemer	54	11	46	11	7	0	25
Northern Spy OP	38	0	28	23	13	0	36

^aGrafted 'Anna' plants broke dormancy (50% of the plants) in < 20 days, but all 'Golden Delicious' remained dormant even after 50 days.

the growing temperature. The fact that secondary dormancy induction in seeds, chilling negation, and the onset of bud dormancy are all achieved at temperatures higher than 15 to 18C suggests that a common mechanism is shared in the production of these phenomena. In addition to apple (Hauagge and Cummins, 1991a, 1991b, 1991c), temperature effects on dormancy development in *Cornus sericea* vary with the growth stage as well (Kobayashi et al., 1982).

After 370 days, the seedlings that were continuously maintained at 8 ± 1 C were subjected to light water stress, but no terminal buds were formed. The seedlings wilted, desiccated quickly, and died. It seems that the mechanism controlling leaf stomata opening does not operate under this low-temperature condition.

Seedling population age and dormancy. Seedlings of high-CR cultivars, such as 'Golden Delicious', have been reported to have high CR (Miller and Sherman, 1980). However, wide variation for CR can be expected in seedlings from low-CR

Fig. 1. Frequency class distribution for the logarithm of GDH accumulated from 'leaf fall to the time of budbreak in 70- to 560-day-old 'Anna' and 'Golden Delicious' OP seedling populations. (I-L) Populations were treated with ethephon (500 ppm a.i.) and/or paclobutrazol (300 ppm a.i.) at 80 and 110 days of age, respectively. Arrows point to the mean time of budbreak of the female parent. Solid dark classes are present only in 'Anna' seedlings. Each treatment/population is composed of at least 65 seedlings. Seedlings were overwintered under alternating-temperature regime. GDH accumulated between leaf fall and budbreak under alternating-temperature overwintering conditions is related to CR determined in subtropical conditions (Hauagge and Cummins, 1991a).

Table 4. Effects of seedling age on inducing variation in the relationship between the length of dormancy period in 'Anna' and 'Golden Delicious' OP seedling populations vs. that of grafted female parents estimated by the $\Delta\text{seedlings}/\Delta\text{parents}$ index.

Seedling age at dormancy induction time (days)	CU ^z	$\Delta\text{Seedling}/\Delta\text{parents}^y$
70	980	0.24
150	960	0.55
200	1380	0.72
360	960	0.72
• 560	1010	0.68

^zCU accumulated under alternating-temperature regime (Hauagge and Cummins, 1991a).

^y $\Delta\text{Seedlings}/\Delta\text{parents}$ index was calculated by: $[\text{m}(\text{GDH})_{\text{sa}} - \text{m}(\text{GDH})_{\text{sb}}]/[(\text{GDH})_{\text{pa}} - (\text{GDH})_{\text{pb}}]$, where $\text{m}(\text{GDH})_{\text{s}}$ is equal to the population mean GDH accumulated between leaf fall and budbreak; and $(\text{GDH})_{\text{s}}$ is equal to the mean GDH accumulated between leaf fall and budbreak of the respective grafted seed parent. Index was calculated from the mean of at least 70 seedlings and eight of each grafted female parent growing in similar conditions.

cultivars (Oppenheimer, 1962; Oppenheimer and Slor, 1968). Although terminal bud formation, leaf senescence, abscission, and even winter hardiness can be induced in young apple seedlings (C. Stushnoff, personal communication), their state of dormancy is not a good indicator of their genetic constitution (Tables 2 and 3; Fig. 1 A–H). The length of time to budbreak after overwintering of seedling populations agef either 70 to 150 days at the time of dormancy induction is related to the bud CR of their parents (Tables 2 and 3; Fig. 1 A and B). However, there was an almost complete overlapping of CR classes between seedling populations originated from standard ('Golden Delicious') and low-CR ('Anna') female parents, which disappeared at a later seedling age (Fig. 1). Unique CR classes in 'Anna' OP in relation to 'Golden Delicious' OP began to appear when seedlings were 200 days old at the time of dormancy induction, and these classes remained relatively stable afterwards (Fig. 1 A–H). The relationship between the length of the dormancy period in OP seedling populations and their female parents, expressed by the $\Delta\text{seedlings}/\Delta\text{parents}$ index, also showed certain stabilization around 200 days (Table 4). The impossibility to discriminate between 'Anna' OP and 'Golden Delicious' OP CR classes in populations younger than 200 days indicates that some high-CR seedlings have shallow dormancy, which makes them behave like low-CR seedlings. Therefore, comparison between mature clones and seedlings would be safer if done in

terms of standard units. Shallow seedling dormancy has also been observed in *Abies fraseri* (Hinesley, 1982) and possibly in other species, such as *Picea* spp. (Dormling et al., 1968; Young and Hanover, 1977) and *Pseudotsuga* spp. (Helmers, 1959; Worral, 1971). It seems that seedlings of some species cannot complete the growth cycle during the normal season. Somehow they are laggard in responding to environmental cues. A significant proportion of terminals will not form buds on 1- or 2-year-old apple seedlings under cold conditions.

Effects of ethephon and paclobutrazol on the length of bud dormancy in apple seedlings. Ethephon or ethephon plus paclobutrazol made the dormancy period of seedling populations resemble those of older populations of similar genetic constitution (Fig. 1 A, B, I–L). Effects on the frequency distribution of classes were more pronounced in 'Golden Delicious' OP populations, where classes were skewed toward the seed parent. In both populations, means were similarly affected. (Table 5). Population variability expressed by the coefficient of variation (cv) of their means was increased in 'Anna' OP and decreased in 'Golden Delicious' OP by the action of both compounds (Table 5). Indeed, 'Anna' has extremely low bud CR, and considerably higher variability can be expected (Hauagge and Cummins, 1991b, 1991c). Crosses involving high-CR x high-CR parents, as in the case of 'Golden Delicious' OP populations, do not produce progeny with short dormancy periods (Miller and Sherman, 1980). The use of ethephon and/or paclobutrazol may be a useful tool to help discriminate among dormancy classes in segregating populations of young apple seedlings. There is, however, a need for further studies concerning their action in even younger populations. The fact that these chemicals stimulated different responses in the two populations signifies that some part of the dormancy induction process is missing in the lower-CR seedlings and that it could not be supplied by either chemical treatment. New internode growth was drastically reduced in both populations by the action of both chemicals. Although leaf size was much reduced in all 'Golden Delicious' seedlings, in the 'Anna' OP population, leaf size was reduced only in those seedlings in which budbreak occurred very late (data not shown). If confirmed with other populations, it could constitute yet another differential response reflecting the length of the dormancy period in apple seedlings.

Literature Cited

Anderson, J. L., E.A. Richardson, and C.D. Kesner. 1986. Validation of chill unit and flower bud phenology models for 'Montmorency'

Table 5. Effect of ethephon (500 ppm a.i.) and paclobutrazol (300 ppm a.i.) on the population means for GDH from leaf fall until budbreak and the population mean coefficient of variation (cv) for GDH in 'Anna' and 'Golden Delicious' OP seedling populations. Ethephon or paclobutrazol was sprayed to runoff 110 and 80 days after seedling emergence, respectively. Dormancy induction began when seedlings were 150 days old. Seedlings were overwintered under alternating-temperature regime.

Chemical		GDH (thousands)		cv	
Ethephon	Paclobutrazol	Anna OP	Golden Delicious OP	Anna OP	Golden Delicious OP
No	No	8.4 a ^z	16.4 a	18 a	24 a
Yes	No	12.2 b	22.3 b	20 a	16 b
Yes	Yes	12.0 b	25.8 c	23 b	13 c

^zMeans separation in columns by Duncan's multiple range test, $P = 0.05$.

- sour cherry. *Acts Hort.* 184:71-78.
- Coston, D. C., T.E. Elkner, and J.G. Williamson. 1986. Mode of action of chemical treatments for peach bloom delay. *Acts Hort.* 179:157-162.
- Dormling, I., A. Gustafsson, and D. von Wettstein. 1968. The experimental control of life cycle in *Picea abies* (L.) Karst. I. Some basic experiments on the vegetative cycle. *Silvae Genet.* 17:44-64.
- Funt, R. C. and D. C. Ferree. 1986. Ethephon induced bloom delay on peach trees, Ohio, USA. *Acts Hort.* 179:163-169.
- Hauagge, R. and J.N. Cummins. 1991a. Phenotypic variation of length of bud dormancy in apple cultivars and related *Malus* species. *J. Amer. Soc. Hort. Sci.* 116:100-106.
- Hauagge, R. and J.N. Cummins. 1991b. Seasonal variation in intensity of bud dormancy in apple cultivars and related *Malus* species. *J. Amer. Soc. Hort. Sci.* 116:107-115.
- Hauagge, R. and J.N. Cummins. 1991c. Genetics of length of dormancy period in *Malus* vegetative buds. *J. Amer. Soc. Hort. Sci.* 116:121-126.
- Hedden, P. and J. E. Graebe. 1985. Inhibition of gibberellin biosynthesis by paclobutrazol in cell-free homogenates of *Cucurbita maxima* endosperm and *Malus pumila* embryos. *J. Plant Growth Regulat.* 4:111-122.
- Helmers, H. 1959. Photoperiodic control of bud development in Coulter pine and bigcone Douglas fir. *For. Sci.* 5:138-141.
- Hinesley, L.E. 1982. Dormancy in *Abies fraseri* seedlings at the end of the first growth cycle. *Cair. J. For. Res.* 12:374-383.
- Jonkers, H. 1979. Bud dormancy of apple and pear in relation to the temperature during the growth period. *Scientia Hort.* 10:149-154.
- Kobayashi, K. D., L.H. Fuchigami, and M.J. English. 1982. Modeling temperature requirements for rest development in *Comus sericea*. *J. Amer. Soc. Hort. Sci.* 107:914-918.
- Lever, B.G. 1986. 'Cultar'—A technical overview. *Acts Hort.* 179:459-466.
- Maage, F. 1986. The effect of growth regulators on bud dormancy and winter injury in red raspberry. *Acts Hort.* 179:149-156.
- Miller, E.P. and W.B. Sherman. 1980. Origin and description of 'Dorsett Golden' apple. *Proc. Fla. State Hort. Soc.* 93:108-109.
- Oppenheimer, C. 1962. Breeding of apples for a subtropical climate. *Proc. 16th Intl. Hort. Congr., Brussels.* 3:18-24.
- Oppenheimer, C.H. and E. Slor. 1968. Breeding apples for subtropical climate II. Analysis of two F2 and nine backcross populations. *Theor. Applied Genet.* 38:97-102.
- Proebsting, E. L., Jr., and H.H. Mills. 1976. Ethephon increases cold hardiness of sweet cherry. *J. Amer. Soc. Hort. Sci.* 101:31-33.
- Shaltout, A.D. and C.R. Unrath. 1983. Rest completion prediction model for 'Starkrimson Delicious' apples. *J. Amer. Soc. Hort. Sci.* 108:957-961.
- Worral, J. 1971. Absence of "rest" in the cambium of Douglas fir. *Can. J. For. Res.* 1:84-89.
- Young, E. and J.W. Hanover. 1977. Development of the shoot apex on blue spruce. *Can. J. For. Res.* 7:614-620.