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Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear

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Summary In contrast to most temperate woody species, apple and pear and some other woody species of the Rosaceae family are insensitive to photoperiod, and no alternative environmental seasonal signal is known to control their dormancy. We studied growth and dormancy induction in micropropagated plants of four apple (*Malus pumila* Mill.) and one pear (*Pyrus communis* L.) commercial rootstock cultivars in controlled environments. The results confirm that growth cessation and dormancy induction in apple and pear are not influenced by photoperiod, and demonstrate that low temperature (< 12 °C) consistently induces both processes, regardless of photoperiodic conditions. Successive stages of the autumn syndrome (growth cessation, formation of bud scales and winter buds, leaf senescence and abscission, and dormancy induction) occurred in response to low temperature. Long days increased internode length at higher temperatures, but had no significant effect on leaf production in any of the cultivars. Chilling at 6 or 9 °C for at least 6 weeks (about 1000 h) was required for dormancy release and growth resumption, whereas treatment at 12 °C was marginally effective, even after 14 weeks of exposure. We are thus faced with the paradox that the same low temperature conditions that induce dormancy are also required for dormancy release in these species.

Keywords: chilling, elongation growth, internode length, leaf production, rootstocks.

Introduction

Winter dormancy is an important adaptive mechanism for plant survival in temperate and cold climates. It is essential that the dormant condition is established within the plant well in advance of the cold season. This requires the timely sensing and physiological processing of a regular and reliable environmental seasonal signal.

Following the pioneering discovery by Garner and Allard (1923), the important role of short photoperiods as the dormancy-inducing signal has been amply documented in a variety of temperate-zone woody plants (e.g., Kramer 1936, Downs and Borthwick 1956, Wareing 1956, Nitsch 1957, Heide 1974, Håbjørg 1978, Li et al. 2003). Temperatures within the normal growth range do not change the critical photoperiods for dormancy induction significantly, although warm conditions usually advance the progress to dormancy (Heide 1974, 2003, Junttila et al. 2003), whereas near-freezing nighttime conditions (21/4 °C, 14/10 h) may induce growth cessation even in continuous light (Heide 1974).

An important exception to the widely demonstrated short day (SD) control of dormancy in temperate trees and shrubs has been reported for apple (*Malus pumila* Mill.) and some other woody genera of the Rosaceae family, in which growth is unaffected by photoperiod (Garner and Allard 1923, Wareing 1956, Nitsch 1957). However, no alternative environmental signal is known to control dormancy induction in these species, and their dormancy is therefore considered to be under entirely endogenous control (Wareing 1956, Battey 2000). To test this assumption, we grew saplings of one pear (*Pyrus communis* L.) and four apple rootstock cultivars of contrasting growth vigor in controlled environments under diverse temperature and day-length regimes. Our results confirm that photoperiod has no dormancy-inducing effect in these plants, whereas low temperature consistently induced growth cessation and dormancy, regardless of the photoperiodic regimes.

Materials and methods

Plant material and cultivation

The experiments were done in the Ås phytotron (60° N, 11° E) in daylight compartments combined with adjacent growth rooms for photoperiodic manipulation. In vitro micropropagated plants of the commercial apple and pear rootstock cultivars listed in Table 1 were obtained from an authorized horticulture nursery. The plants had been established in soil and grown in 3-cm plug trays for 4 weeks to a height of 4 to 5 cm before delivery. When received, the plants were transplanted to 10-cm plastic pots containing a peat-based potting compost and kept at 21 °C in a 24-h photoperiod for one week for establishment before the experimental treatments began. The plants were fertilized twice weekly with a complete fertilizer solution and otherwise watered with tap water as required. All plants received natural daylight for 10 h per day (0800–1800 h), and day-length extension to 24-h photoperiod was provided by low-intensity light from 75 W incandescent lamps (about

Table 1. Origin and growth characteristics of the apple (*Malus pumila* Mill.) and pear (*Pyrus communis* L.) rootstock cultivars in the experiments. For further information on origin and characteristics of the rootstocks, see Ferree and Carlson (1987).

Species	Cultivar	Country	Growth vigor
Apple	'A2' (Alnarp 2)	Sweden	Vigorous
	'B9' (Budagovsky 9)	Russia	Semidwarf
	'M9' (Malling 9)	U.K.	Dwarf
	'MM106' (Malling-Merton 106)	U.K.	Semidwarf
Pear	'Brokmal'	USA	Semidwarf

8 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF)). Plants receiving SD treatment were kept in darkness from 1800 to 0800 h. Therefore, the plants received nearly the same daily light integral regardless of day-length conditions. Whenever the quantum flux in the daylight compartments was less than about 150 μmol m^{-2} s^{-1}, an additional 125 μmol m^{-2} s^{-1} PPF was automatically provided by high-pressure metal halide lamps (400 W; Philips HPI-T). Temperatures were controlled to ± 1.0 °C and a water vapor pressure deficit of 530 Pa was maintained at all temperatures above 6 °C.

Experimental designs

Four experiments were carried out during the years 2000 to 2003. Experiment 1 was designed to examine the time course of elongation growth of 'M9' and 'MM106' apple rootstock cultivars at four temperatures (9, 12, 15 and 21 °C) and photoperiods of 10 and 24 h. The experiment was started on May 30, 2000 and lasted for 8 weeks. The experiment was repeated in 2001 and expanded to include the pear rootstock cultivar 'Brookmal' (Experiment 2, started on May 22, 2001). Experiment 2 included an extra set of plants at 21 °C (long day (LD) and SD treatments) that was transferred to 9 °C after 5 weeks of treatment. All day lengths were as described for Experiment 1.

Experiment 3 was designed to determine the time course of extension growth of the apple rootstock cultivars 'A2' and 'B9' in SD and LD at constant (9, 12, 15 and 21 °C) and fluctuating (21/9 °C) day/night temperatures. Diurnal changes in light and temperature were synchronized to give 10 h of high temperature/high-intensity light and 14 h of low temperature/darkness (SD) or low-intensity light (LD). The experiment was started on September 23, 2002.

Experiment 4 was designed to investigate the induction and subsequent breaking of dormancy in the apple rootstock cultivars 'M9' and 'MM106'. All plants were initially grown at 21 °C for 24 days to establish high growth rates. Thirty plants of each cultivar were assigned to each of the temperatures 6, 9 and 12 °C and growth cessation (dormancy induction) monitored. After 6, 10 and 14 weeks at each of these temperatures, groups of 10 plants of each cultivar were transferred to 21 °C for forcing and monitoring of growth resumption (dormancy release). The plants were maintained under 10-h SD conditions throughout Experiment 4.

Measurements and statistical analyses

The time course of elongation growth was monitored by weekly measurements of plant heights. These observations provided accurate information of plant growth capacity and the time of growth cessation. Production of new leaves was determined by marking the last developed leaf (> 2 cm) of each plant at start of the experiments and counting additional leaves at various times during the experiment. All experiments were factorial of the split-plot design with temperatures as the main plots and photoperiods and cultivars as sub-plots. The analysis of variance (ANOVA) of the data was completed with the Systat Version 5.0 program package, with each plant (pot) considered as a replicate. All experiments had 10 plants of each cultivar per treatment.

Results

Cultivar 'MM106' grew more vigorously than cultivar 'M9', but otherwise the two cultivars responded similarly to the various treatments. Both shoot elongation growth and production

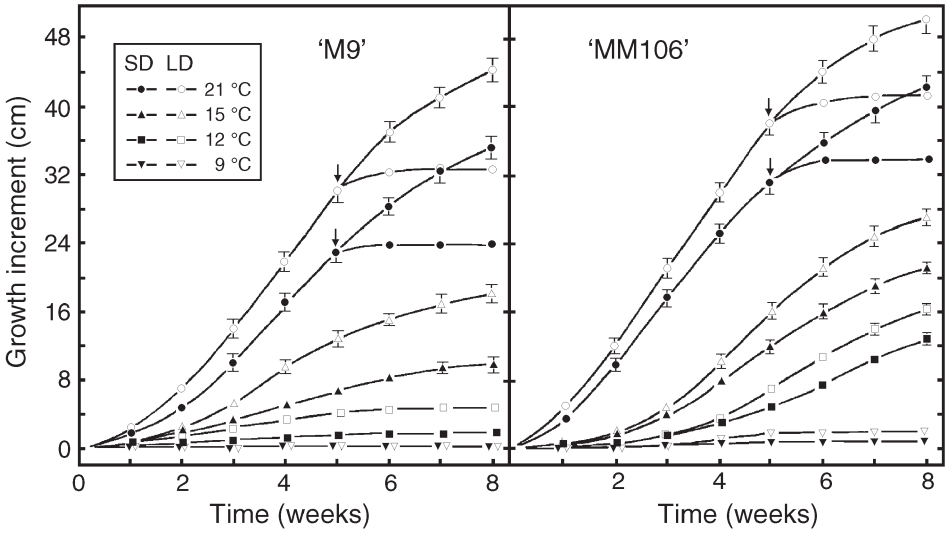

Figure 1. Time courses of elongation growth of the apple rootstock cultivars 'M9' and 'MM106' grown under different temperature and day-length regimes. Arrows indicate time of transfer from 21 to 9 °C. Data are weighted means ± SE for two experiments over two years, except for the temperature transfer results, which are based on one experiment only. Abbreviations: SD = short day treatment; and LD = long day treatment.

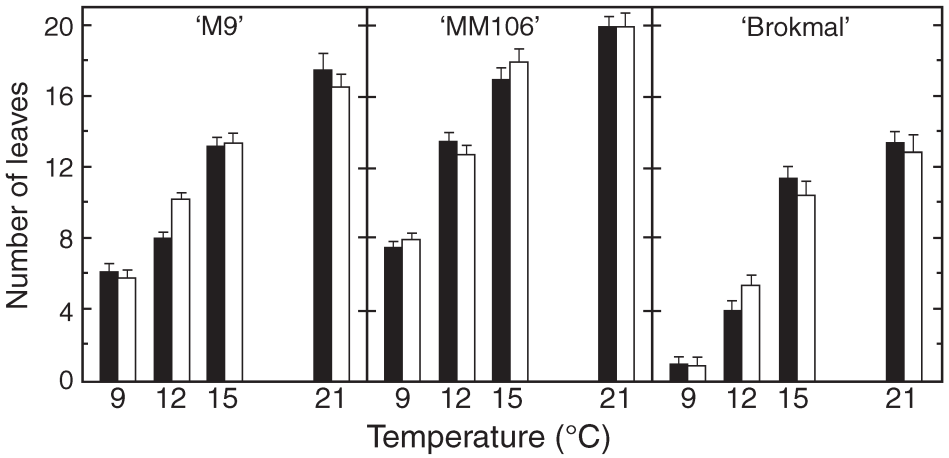

Figure 2. Effects of temperature and day length on the production of new leaves in the apple rootstock cultivars 'M9' and 'MM106' and the pear rootstock cultivar 'Brokmal' after eight weeks of growth under various conditions. Filled columns represent the 10-h short day (SD) treatment and open columns represent the 24-h long day (LD) treatments. Data are weighted means ± SE for two experiments over two years for apple cultivars, and for a single experiment with 'Brokmal'.

of new leaves increased with increasing temperature (Figures 1 and 2). At temperatures of 21 and 15 °C, continuous growth was maintained in all plants in both day lengths throughout the experimental period, although the growth rate levelled off somewhat after about 6 weeks, possibly because of root restriction in the pots after prolonged growth. At 12 °C, 'M9' ceased growing after about 5 weeks, whereas 'MM106' maintained a relatively low growth rate throughout the experimental period in both photoperiods. However, at 9 °C, complete growth cessation took place in all plants of both cultivars in both SD and LD. Also, when plants were transferred to 9 °C after 5 weeks of active growth at 21 °C, elongation growth ceased after one week in both cultivars at both day lengths (Figure 1). Growth cessation at low temperature was associated with a sequential reduction in leaf lamina size and the formation of bud scales and winter buds (cf. Abbott 1970).

At all temperatures above 9 °C, elongation growth was significantly stimulated by long photoperiods ($P < 0.001$). This was attributed to increased internode length in LD, because the production of new leaves was not significantly affected by photoperiod (Figure 2). Thus, there was a highly significant effect of temperature in both cultivars ($P < 0.001$), whereas the effect of photoperiod was not significant for either cultivar ($P > 0.05$). However, there was a marginally significant ($P = 0.045$) temperature × photoperiod interaction for 'M9', but not for 'MM106'.

The pear rootstock 'Brokmal' responded to the treatments in much the same way as the apple cultivars 'MM106' and 'M9' (Figures 2 and 3). Shoot elongation growth was significantly stimulated by both increasing temperature ($P < 0.001$) and long photoperiods ($P = 0.015$). Because growth cessation occurred at low temperature regardless of photoperiodic conditions, there was also a highly significant temperature × photoperiod interaction ($P < 0.003$) on elongation growth (growth increment data after 8 weeks of cultivation). Transfer of actively growing 'Brokmal' plants from 21 to 9 °C after 5 weeks resulted in an almost instantaneous cessation of growth in both day lengths (Figure 3). Production of new leaves increased significantly with increasing temperature ($P < 0.001$), with no significant effect of photoperiod or the interaction of temperature and photoperiod (Figure 2). Thus, the increased elongation growth in LD at 21 and 15 °C was entirely due to increased internode length in LD.

The relatively vigorous-growing apple cultivars 'B9' and 'A2' had high growth rates at high temperature, but growth was more sensitive to low temperatures than growth of 'M9' and 'MM106' (Figure 4). This was particularly marked for 'A2' which produced little or no growth at 15 °C and lower temperatures. Low night temperature (21/9 °C) also strongly reduced elongation growth, and more so in 'A2' than in 'B9', but did not cause growth cessation in any of the cultivars (Figure 4). The main effect of temperature was highly significant for both cultivars ($P < 0.001$), whereas photoperiod had no significant effect on elongation growth in either cultivar ($P > 0.05$). There was a significant temperature × photoperiod interaction on elongation growth in cultivar 'B9' ($P < 0.05$), because elonga-

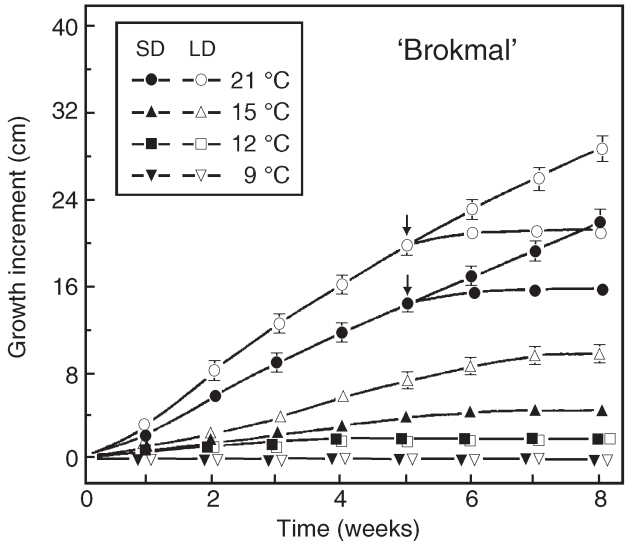

Figure 3. Time courses of elongation growth of the pear rootstock cultivar 'Brokmal' grown under different temperature and day-length regimes as indicated. The arrow indicates time of transfer from 21 to 9 °C. Data are means ± SE for one experiment with 10 plants per treatment. Abbreviations: SD = short day treatment; and LD = long day treatment.

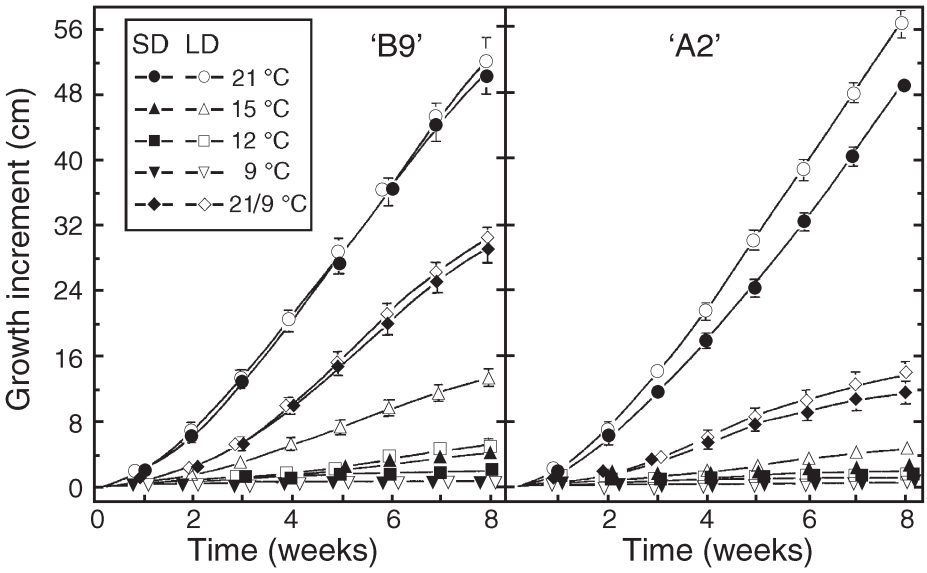

Figure 4. Time courses of elongation growth of the apple rootstock cultivars 'B9' and 'A2' grown under different temperature and day-length regimes as indicated. Data are means ± SE for one experiment with 10 plants per treatment. Abbreviations: SD = short day treatment; and LD = long day treatment.

tion growth increased in LD at 15 and 12 °C, whereas day length had no effect at either higher or lower temperatures. No such interaction was found for 'A2'. Temperature also had a highly significant effect ($P < 0.001$) on leaf production in both cultivars, whereas the effect of photoperiod and the interaction with temperature was not significant ($P > 0.05$) (Figure 5).

To test if growth cessation at low temperature is directly associated with true bud dormancy, actively growing plants of 'M9' and 'MM106' were exposed to temperatures of 6, 9 and 12 °C for 6, 10 and 14 weeks, and then exposed to 21 °C for forcing and monitoring of growth resumption (dormancy release) (Figure 6). Plants of cultivar 'M9' ceased growing completely at all three temperatures after 1 to 2 weeks of exposure, formed winter buds, shed their leaves and apparently went dormant. Increasing duration of chilling at 6 and 9 °C progressively increased the ability of buds to resume growth at high temperature, 6 °C being the most effective temperature. Time to growth resumption decreased and bud growth potential increased with increasing time of chilling at 6 and 9 °C, whereas plants at 12 °C were unable to resume normal growth even after 14 weeks of exposure (Figure 6). The 'MM106' plants ceased growing after 1–2 weeks of exposure to 6 and 9 °C, but growth cessation did not occur until after about 4 weeks of exposure to 12 °C. These plants did not shed their leaves completely as did those at the lower temperatures. An immediate growth resumption after transfer to 21 °C following 6 weeks at 12 °C indicated that the 'MM106' plants were not fully dormant at 12 °C. A slower and less vigorous growth resumption after 10 weeks at 12 °C suggested a deeper state of dormancy at this stage, whereas continued exposure to 12 °C for 14 weeks reestablished a somewhat more vigorous growth at high temperature. However, plants exposed to 6 and 9 °C ex-

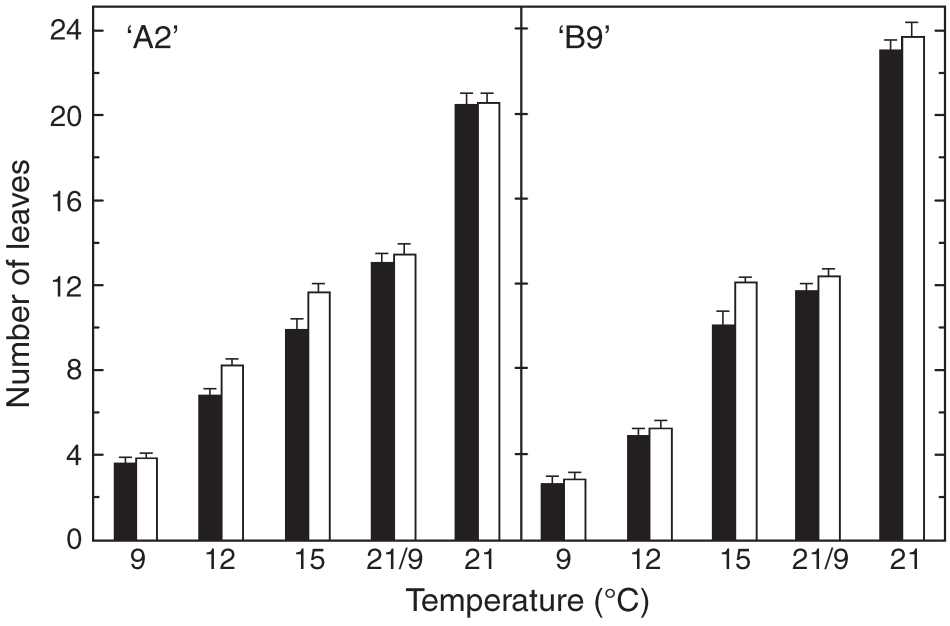

Figure 5. Effects of temperature and day length on the production of new leaves in the apple rootstock cultivars 'A2' and 'B9' after 8 weeks of growth under the various conditions. Filled columns represent the 10-h short day (SD) treatment and the open columns represent the 24-h long day (LD) treatment. Data are means ± SE for one experiment with 10 plants per treatment.

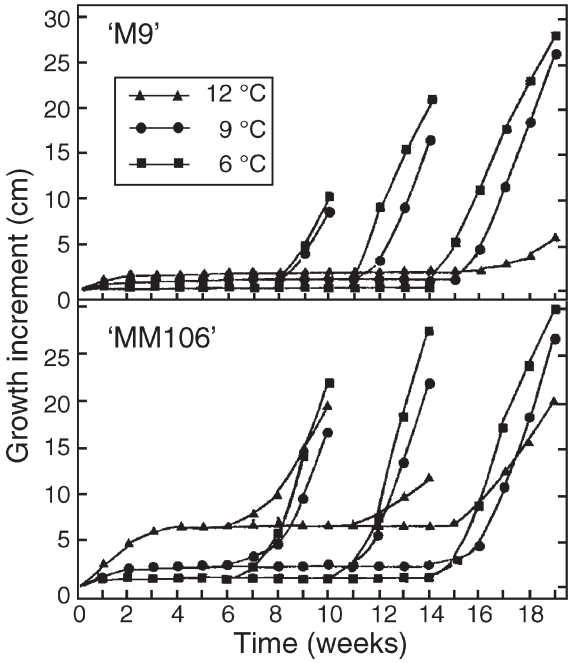

Figure 6. Effects of low temperatures on dormancy induction and its release in the apple rootstock cultivars 'M9' and 'MM106'. Groups of 10 plants were transferred from each of the indicated temperatures to 21 °C after 6, 10 or 14 weeks, and their capacity for growth at 21 °C during the following four weeks determined. Data are for one experiment with 10 plants per treatment.

hibited a greater growth potential than those at 12 °C with all chilling times, and as for 'M9', 6 °C was the most effective temperature in restoring bud growth potential (Figure 6). An ANOVA of shoot growth data after 4 weeks at high temperature (combined for the two cultivars) revealed highly significant main effects of temperature ($P < 0.001$), chilling time ($P < 0.001$) and cultivar ($P = 0.003$), as well as a significant three-factor interaction of the three variables.

Discussion

We confirmed that photoperiod has no effect on growth cessation and dormancy induction of apple and pear plants at normal growth temperatures (Nitsch 1957), although shoot elongation was significantly increased by long photoperiods in most cultivars as a result of increased internode length in LD (Figures 1–5). The occurrence of increased internode length in LD has long been recognized in a number of tree species (Wareing 1956), but the response is independent of, and should not be confounded with, dormancy (Thomas and Vince-Prue 1997). However, both processes are photoperiodically controlled and respond to night interruption in a red–far-red reversible manner (Nitsch 1957), indicating phytochrome mediation. The apple cultivar 'B9' that did not respond to LD with increased internode length (Figure 4) has dark red leaves, and we speculate that this difference in response may be associated with modified red-light-absorbing properties of the red leaves.

Previous studies have indicated an absence of photoperiodic control of growth cessation and dormancy induction in apple and pear, leading to the conclusion that these processes are endogenously controlled (Wareing 1969, Battey 2000). However, we found that temperatures below 12 °C consistently induced growth cessation and formation of winter buds in all tested cultivars regardless of photoperiodic conditions. Particularly convincing was the abrupt growth cessation of plants transferred from high to low temperature during active growth (Figures 1, 3 and 4). The plants went through successive stages of the autumn syndrome including apical growth cessation, gradual reduction of lamina size of successive leaves and formation of bud scales and winter buds as described by Abbott (1970), and finally, acropetal leaf senescence and abscission. The results in Figure 6 further demonstrate that the buds entered a true state of dormancy under these conditions and that chilling at the same temperatures was required for breaking dormancy and restoration of bud growth potential.

Although the cultivars responded in much the same way, some cultivar differences in threshold temperature for growth cessation were evident. Thus, the pear rootstock cultivar 'Brokmal' and the apple cultivars 'M9' and 'A2' ceased growing completely at both 9 and 12 °C, whereas the apple cultivars 'MM106' and 'B9' maintained some growth over several weeks at 12 °C (Figures 1 and 4). Cultivar 'A2' produced little growth even at 15 °C (Figure 4) and thus appeared to have a high critical temperature for growth cessation, a feature of possible relevance to its reputed winter hardiness (Ferree and Carlson 1987). Low night temperature (21/9 °C and 10-h photoperiod) reduced growth rate but did not induce growth cessation in the apple cultivars 'B9' and 'A2' (Figure 4). The fluctuating temperature regime, which provided an average daily mean temperature of 14 °C, produced significantly more growth than did a constant 15 °C in both cultivars, demonstrating that the effect of fluctuating temperature is not a simple mean temperature response.

Low temperature (chilling) is the main environmental factor for bud dormancy release in temperate trees and shrubs (Vegis 1964), such as apple and pear. However, it is a paradox that the same low temperature regime that induces dormancy in these species also controls its release. With some minor variations, temperatures below 12 °C were increasingly effective in inducing growth cessation and dormancy in all cultivars (Figures 1–6), and with parallel effectiveness on dormancy release (Figure 6). Both earliness of growth resumption and the magnitude of growth potential increased with decreasing chilling temperature, 6 °C being most effective and 12 °C only marginally effective for dormancy release (Figure 6). The same critical chilling temperature was found for *Betula* species by Myking and Heide (1995) and for *Picea abies* and *Pinus sylvestris* by Hänninen (1990). Chilling at 6 °C for 6 weeks (1008 h) allowed growth resumption of all plants of 'M9' and 'MM106', but was clearly not optimal because additional chilling to 10 or even 14 weeks markedly increased both rate and earliness of growth (Figure 6). These results are in agreement with the chilling requirements of 1200 to 1500 h (50–60 days) at 5–7 °C reported for apple and pear cultivars (Ryugo 1988).

The finding that temperatures below 12 °C control both dormancy induction and its release, demonstrates that plants in different states of development can respond to the same treat-

ment in opposite ways, possibly by utilization of different signal transduction pathways. The plants may even start accumulating chill hours as soon as growth has stopped and before dormancy is fully established. Jonkers (1979) grew young apple trees during spring and summer at temperatures ranging from 9 to 25 °C and found that, after 6 weeks of chilling at 2 °C, bud break and growth was earlier and more vigorous the lower the temperature had been during the preceding summer. Similar results are reported for other woody species (Westergaard and Nymann Eriksen 1997, Heide 2003, Junttila et al. 2003), demonstrating the complexity of temperature regulation of bud dormancy.

It is often argued that dormancy behavior of seedlings is not representative of the situation in mature trees. Thus, Hauagge and Cummins (1991) were unable to induce dormancy in 70-day-old apple seedlings even after 370 days of exposure to 8 °C and concluded that apple seedlings do not reflect the species' inherent dormancy responses before they are at least 200 days old. Micropropagated plants, as used in the present experiments, are usually maintained in a juvenile state and micropropagation is even used for rejuvenation of mature trees (Hackett 1985). However, several studies have shown that, although micropropagated plants may have all the morphological traits of juvenile plants, they do not always respond physiologically as truly juvenile plants. Such plants may for example be able to flower in response to photoperiodic induction and may also exhibit mature developmental behavior (examples in Hackett 1985). The contrasting dormancy induction responses of apple seedlings (Hauagge and Cummins 1991) and micropropagated apple plants, strongly suggest that our plants were not truly juvenile in this respect, but responded more like non-juvenile trees. Also, vegetative shoots of actively growing mature apple and pear trees usually keep growing into late autumn, indicating temperature control of growth cessation and dormancy also in mature trees. Because several members of the *Pomoide* subfamily of the *Rosaceae* are known to be insensitive to photoperiod (Wareing 1956, Nitsch 1957), it would be of interest to know whether the low temperature control of dormancy induction is of general occurrence within the subfamily.

In conclusion, our results confirm that growth cessation and dormancy induction in apple and pear are not influenced by photoperiod (Nitsch 1957), and demonstrate that low temperature (< 12 °C) is highly effective in bringing about both dormancy induction and its release in these species. However, the results do not exclude the possible involvement of an additional endogenous circannual life cycle control mechanism in these trees.

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References

Abbott, D.L. The role of budscales in the morphogenesis and dormancy of the apple fruit bud. *In* Physiology of Tree Crops. Eds. L.C. Luckwill and C.V. Cutting. Academic Press, London, pp 65–80.

Battey, N.H. 2000. Aspects of seasonality. J. Exp. Bot. 51:1769–1780.

Downs, R.J. and H.A. Borthwick. 1956. Effect of photoperiod on growth of trees. Bot. Gaz. 117:310–326.

Ferree, D.C. and R.F. Carlson. 1987. Apple rootstocks. *In* Rootstocks for Fruit Crops. Eds. R.C. Rom and R.F. Carlson. John Wiley and Sons, New York, pp 107–143.

Garner, W.W. and H.A. Allard. 1923. Further studies in photoperiodism, the response of the plant to relative length of day and night. J. Agric. Res. 23:871–920.

Håbjørg, A. 1978. Photoperiodic ecotypes in Scandinavian trees and shrubs. Meld. Nor. Landbrukshøgsk. 57:1–20.

Hackett, W.P. 1985. Juvenility, maturation, and rejuvenation in woody plants. Hortic. Rev. 7:109–155.

Hänninen, H. 1990. Modelling bud dormancy release in trees from cool and temperate regions. Acta For. Fenn. 213:1–47.

Hauagge, R. and J.N. Cummins. 1991. Age, growing temperatures, and growth retardants influence induction and length of dormancy in *Malus*. J. Am. Soc. Hortic. Sci. 116:116–120.

Heide, O.M. 1974. Growth and dormancy in Norway spruce (*Picea abies*). I. Interaction of photoperiod and temperature. Physiol. Plant. 30:1–12.

Heide, O.M. 2003. High autumn temperature delays spring bud burst in boreal trees, counterbalancing the effect of climatic warming. Tree Physiol. 23:931–936.

Jonkers, H. 1979. Bud dormancy of apple and pear in relation to the temperature during the growth period. Sci. Hortic. 10:149–154.

Junttila, O., J. Nilsen and B. Igeland. 2003. Effect of temperature on the induction of bud dormancy in ecotypes of *Betula pubescens* and *Betula pendula*. Scand. J. For. Res. 18:208–217.

Kramer, P.J. 1936. Effect of variation in length of day on growth and dormancy of trees. Plant Physiol. 11:127–137.

Li, C., O. Junttila, A. Ernstsen, P. Heino and E.T. Palva. 2003. Photoperiodic control of growth and dormancy development in silver birch (*Betula pendula*) ecotypes. Physiol. Plant. 117:206–212.

Myking, T. and O.M. Heide. 1995. Dormancy release and chilling requirement of buds of latitudinal ecotypes of *Betula pendula* and *B. pubescens*. Tree Physiol. 15:697–704.

Nitsch, J.P. 1957. Photoperiodism in woody plants. Proc. Am. Soc. Hortic. Sci. 70:526–544.

Ryugo, K. 1988. Fruit culture: its science and art. John Wiley and Sons, New York, 344 p.

Thomas, B. and D. Vince-Prue. 1997. Photoperiodism in plants. 2nd Edn. Academic Press, London, 428 p.

Vegis, A. 1964. Dormancy in higher plants. Annu. Rev. Plant Physiol. 15:185–224.

Wareing, P.J. 1956. Photoperiodism in woody plants. Annu. Rev. Plant Physiol. 7:191–214.

Westergaard, L. and E. Nymann Eriksen. 1997. Autumn temperature affects the induction of dormancy in first-year seedlings of *Acer platanoides* L. Scand. J. For. Res. 12:11–16.