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# Freezing temperature treatment induces bud dormancy in 'Granny Smith' apple shoots

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**Abstract**

One-year-old, 'Granny Smith' apple (*Malus* × *domestica* Borkh.) shoots were selected randomly from commercial orchards in 1999 and 2003, cold stored at temperatures between 1 and 13 °C for varied periods, following a 12/12 h freezing temperature pre-treatment of −1/13 °C (supposedly non-chilling temperatures) for 1 or 2 weeks. The rate of budburst, determined after forcing at 25 °C, was used to follow the progression of bud dormancy. The freezing pre-treatment clearly enhanced (deepened) bud dormancy in all experiments. Clearly definable influences due to the presence of leaves during the pre-treatment were not observed on shoots cold stored at chilling temperatures. Trees were defoliated before leaf drop in late summer/autumn under field conditions by hand (2001) and using a spray containing 3% urea and 1.8% zinc sulphate in 2003. In 2001 the first two hand defoliation dates significantly enhanced dormancy, while in 2003 chemical defoliation had no effect. These data indicate that the leaves are not clearly involved in the perception of factors that are responsible for dormancy induction. As with dormancy release it is possible that the perception of induction factors, i.e. low temperatures, and as shown in these data, freezing temperatures, occurs within the buds themselves.
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*Keywords:* Apple bud dormancy; Temperature; Defoliation; *Malus* × *domestica* Borkh

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## 1. Introduction

In temperate climates terminal buds of apple shoots rapidly enter dormancy in autumn and then start to exit dormancy initially slowly but more rapidly in late winter before spring budburst (Cook et al., 1998). Buds have the ability to enter into and exit from dormancy even at warm temperatures (>15 °C) (Crabbé, 1994; Mauget, 1983; Mauget and Rageau, 1988). Cold temperatures, however, are generally considered as the environmental cue responsible for the initial induction (in autumn) and subsequent alleviation (in winter) of the chilling requirement during bud dormancy (Crabbé, 1994; Crabbé and Barnola, 1996). The role of freezing temperatures in dormancy induction and release is less clear. Rinne et al. (1997) showed how freezing temperatures enhance dormancy release in *Betula* plants that had already entered dormancy.

At warmer temperatures apple (Cook and Jacobs, 2000; Heide and Prestrud, 2004) and walnut buds (Mauget, 1983) take longer to enter into and exit from dormancy. Cook and Jacobs (2000) found that 'Granny Smith' shoots reached maximum dormancy after only 100 Utah Chill Units (CU) in a cold area while those from a warmer area reached maximum dormancy after 600 CU. It was speculated that temperatures other than those used to calculate the chill units, possibly freezing temperatures, may enhance the progression of dormancy. In subsequent work a pre-dormancy freezing temperature treatment (−1/13 °C) was tested (Jacobs et al., 2002). This pre-treatment killed autumn harvested 'Granny Smith' apple shoots but not winter harvested shoots. When it was applied to winter harvested shoots it had small, but significant effect on the progression of bud dormancy. In these trials winter harvested shoots were treated for 2 weeks and autumn harvested shoots for only 1 week. These findings prompted the re-evaluation of this work. In this study the possible role of freezing temperatures in the induction of dormancy was investigated. The role of leaves in the perception of the inductive stimulus that triggers dormancy was questioned.

## 2. Materials and methods

In 1999 shoots were harvested from mature bearing 'Granny Smith' apple trees near Somerset West (34°S, 80 m). Shoots were harvested in winter (7 July), defoliated and cut to a length of 50 cm. Following a 0 or 2 week pre-treatment of a 12/12 h freeze treatment at −1 °C night and 13 °C day temperatures the shoots were wrapped in moist paper and placed in plastic bags, and stored upright during cold storage at 1, 4, 7, or 10 °C for 0, 4, 8 or 12 weeks. Thirty shoots per treatment were bundled in three replicate bundles of 10 shoots. The experimental design was a completely randomised design with three factors (freeze treatment, cold storage temperature and cold storage time).

The bundles were then placed at random in 5 L buckets with their bases in ≈1 L of water containing 5 mL $L^{-1}$ household bleach (5% sodium hypochlorite), and forced at 25 °C with continuous illumination (ca. 200 μmol $m^{-2}$ $s^{-1}$ photosynthetically active radiation). The water in the buckets was replaced three times a week, removing the bottom 5 mm of each shoot weekly. During forcing the shoots were checked three times a week for budburst, which was considered at the first visible signs of green expanding leaves, i.e., ''green tip''.

The rate of budburst was determined from the inverse of the time (*d*) for budburst to occur on five shoots per bundle, i.e., 1/(days to 50% budburst) (Cannell, 1989). The shoots were maintained in the buckets until buds no longer burst.

In 2003 a similar trial was repeated on shoots harvested from another commercial 'Granny Smith' orchard in Elgin (34°S, 300 m). These shoots were harvested in early autumn (9 April) before the accumulation of any winter chilling. In this trial only half the shoots were defoliated before a 0 or 1 week pre-treatment of a 12/12 h freeze treatment at −1 °C night and 13 °C day temperature. Thereafter, the non-defoliated shoots were defoliated. The shoots were then cold stored at 1, 5, 9, or 13 °C for 0, 3, 6, 9 or 12 weeks. Throughout the pre-treatment and cold storage the shoots were held in water in plastic buckets (as above). All shoots were bundled, 20 shoots per treatment in two replicate bundles of 10 shoots, and forced as above. Data for each cold storage temperature were analysed as four separate experiments, each with a completely randomised design and three factors (freeze treatment, defoliation, and cold storage time), and two replications.

In three separate experiments 'Granny Smith' apple trees in commercial orchards were defoliated in late summer. The subsequent progression of bud dormancy was followed by harvesting and forcing shoots (as above) during winter. In 2001 'Granny Smith' apple trees in Stellenbosch (34°S, 115 m) were defoliated by hand on three dates (30 May, 20 June and 11 July). In 2003 'Granny Smith' apple trees were defoliated using a spray containing 3% urea and 1.8% zinc sulphate on three dates in Elgin (23 April, 13 May and 3 June) and in the Koue Bokkeveld, Ceres (33°S, 945 m) on 24 April, 13 May and 3 June. In the three trials non-defoliated controls were included. The experimental design was a randomised complete block design with four treatments (defoliation), seven forcing dates (time), and two (2001) or three (2003) replications.

For each experiment the analysis of variance was conducted using the General Linear Models (GLM) procedure of SAS® (SAS Institute, Cary, NC).

## 3. Results

In 1999, no difference in chilling efficiency was observed between chilling temperatures of 1, 4, 7, and 10 °C, however, a −1/13 °C freeze pre-treatment significantly lowered the bud growth potential and enhanced dormancy (Fig. 1). In 2003 chilling at 1 °C significantly increased the rate of bud burst with time more so than chilling at 5, 9, and 13 °C (Fig. 2). As in 1999, the freeze pre-treatment significantly enhanced dormancy in all the 2003 experiments (1, 5, 9, and 13 °C; Fig. 2). No clear effect of defoliation on the progression of bud dormancy was observed in shoots cold stored at 1, 5, and 9 °C (Fig. 2). In shoots stored at 13 °C an interaction between defoliation and the freeze pre-treatment was observed (Fig. 2).

Hand defoliation in the field on the first two dates significantly affected the progression of dormancy; a deeper dormancy was observed. Dormancy progressed similarly in the control and the final defoliation date (Stellenbosch 2001; Fig. 3). Chemical defoliation had no effect on the progression of dormancy in both areas (Ceres 2003 and Elgin 2003; Fig. 3).

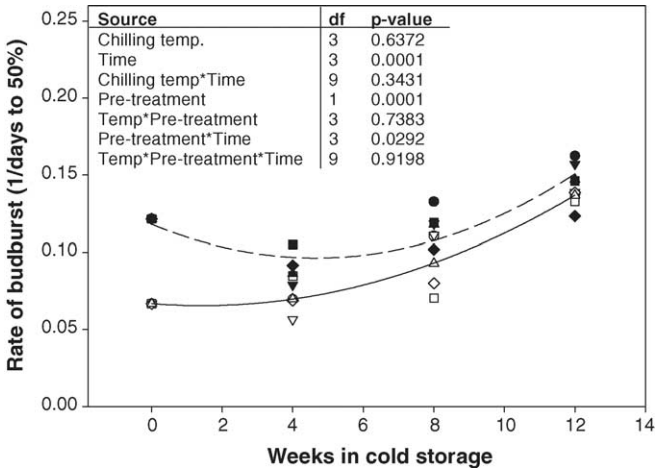


Fig. 1. The change in rate of budburst in 1999 after 0, 4, 8, or 12 weeks of cold storage at 1 °C (circles), 4 °C (triangles), 7 °C (squares) and 10 °C (diamonds) following a 12/12 h pre-treatment of −1/13 °C for 0 (closed symbols, broken line), or 2 (open symbols, solid line) weeks of 'Granny Smith' apple shoots. Analysis of variance results and significant trendlines are shown.

## 4. Discussion

The freeze pre-treatment significantly enhanced the entrance into bud dormancy in all the experiments. According to the widely used Utah chill unit model the temperatures −1 and 13 °C should have little effect on dormancy progression (Richardson et al., 1974). This is confirmed in the 13 °C experiment (Fig. 2) where dormancy progressed poorly. Clearly, freezing temperatures (−1 °C) have an effect on the progression of apple bud dormancy, especially in the induction phase. This confirms other recent findings (Jacobs et al., 2002; Naor et al., 2003). For increased accuracy chilling models should take this into account.

In our previously reported trials the freeze pre-treatment had a significant but only small effect (Jacobs et al., 2002). The buds, treated in winter, were already well dormant (rate of budburst <0.1). In these trials the freeze treatment was applied earlier. The control shoots all had a rate of budburst >0.1 and the shoots responded to the freezing temperatures by rapidly (within 1 week) entering deeper into dormancy. These findings add further support for the role of freezing temperatures in the induction of dormancy.

In a broader context, the entrance into dormancy is a continuous process that involves growth cessation, terminal bud formation and leaf senescence (Abbott, 1970). The role of leaves and the process of leaf abscission in the entrance to dormancy have been debated. Under the warm local conditions leaf abscission is often delayed. In the warmer apple production regions abscission commonly only occurs in late winter. Leaf abscission is promoted by low temperatures; 2 weeks exposure to 9° day/4 °C night temperatures (Lakso et al., 1999); 1–2 weeks at 6, 9, or 12 °C after active growth at 21 °C (Heide and Prestrud, 2004). Photoperiod is not involved (Heide and Prestrud, 2004). However, under local conditions as observed from years of forcing experiments (personal experience), and as is

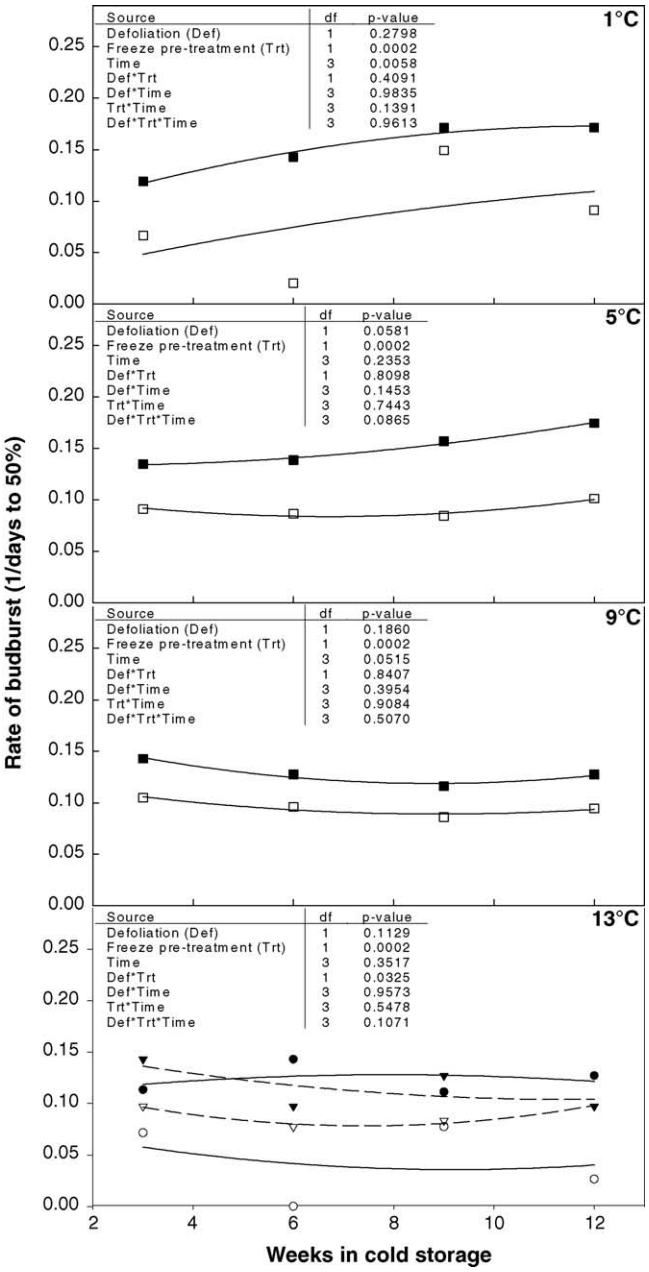


Fig. 2. The change in rate of budburst in 2003 after cold storage at 1, 5, 9, and 13 °C for 3, 6, 9, or 12 weeks following a 12/12 h pre-treatment of −1/13 °C for 0 or 1 week of 'Granny Smith' apple shoots. Freeze pre-treatment (open squares), no pre-treatment (closed squares), defoliation plus pre-treatment (open triangles, broken line), defoliation without pre-treatment (closed triangles, broken line), pre-treatment without defoliation (open circles), no pre-treatment without defoliation (closed circles). Analysis of variance results and significant trendlines are shown for each experiment.

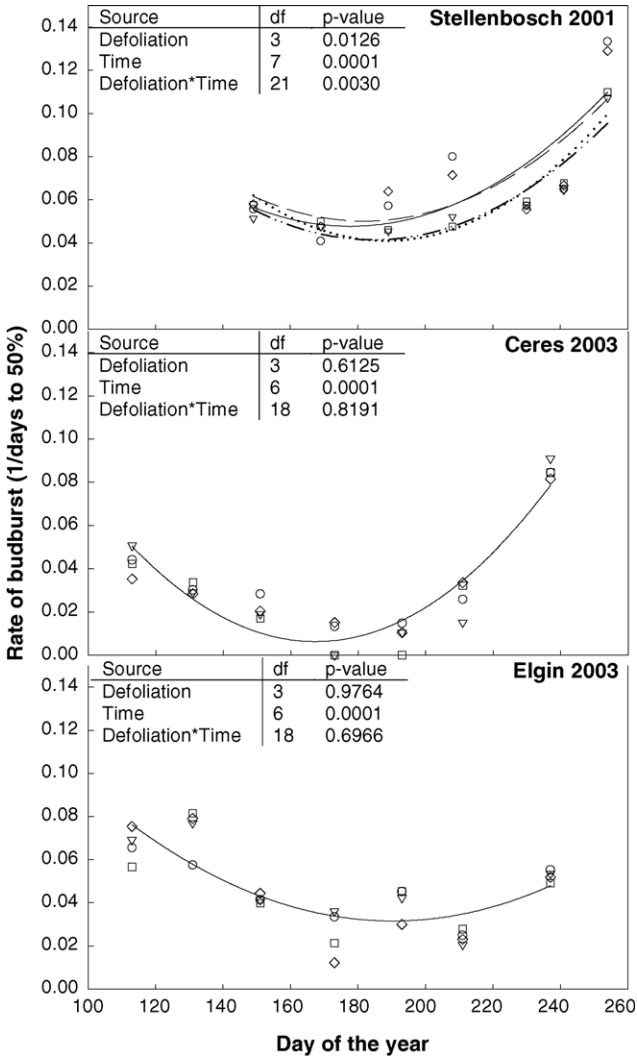


Fig. 3. The change in rate of budburst of 'Granny Smith' apple shoots harvested from trees defoliated in autumn by hand in 2001 and with 3% urea and 1.8% zinc sulphate in 2003. Treatments included a first (triangles), second (squares), and third (diamonds) defoliation date, and a control (natural defoliation, circles). Analysis of variance results and significant trendlines are shown for each experiment.

again confirmed in these data, buds enter into dormancy before leaf abscission in the field. It would appear that leaf abscission occurs independently from the process of dormancy induction.

Defoliating trees under field conditions had a small, but significant, effect in 2001 and no effect in 2003. The absence or presence of leaves during the freeze pre-treatment had no effect of shoots stored at normal chilling temperatures (1, 5, or 9 °C). An interaction between defoliation and the freeze pre-treatment was observed only at the non-chilling

temperature of 13 °C. It remains doubtful if the leaves have any primary role in the induction or progression of bud dormancy.

These data imply that the leaves are not directly involved in the perception of factors that are responsible for dormancy induction. As with dormancy release it is possible that the perception of induction factors, i.e. low and/or freezing temperatures, occurs within the buds themselves.

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