J Plant Growth Regul (1989) 8:301–307

Journal of
Plant
Growth
Regulation



# Thidiazuron Substitution for Chilling Requirement in Three Apple Cultivars

G. L. Steffens and G. W. Stutte

US Department of Agriculture, ARS, Beltsville Agricultural Research Center, Beltsville, Maryland 20705, USA; and Department of Horticulture, University of Maryland, College Park, Maryland 20742, USA

Received January 18, 1989; accepted May 15, 1989

**Abstract.** Thidiazuron [(TDZ) *N*-phenyl-*N'*-1,2,3-thidiazol-5-ylurea] at 750 μM was applied to buds of apple trees to determine if it could substitute for the chilling requirement to induce bud break. Shoots of cv. 'Anna' (low chill), 'Delicious' cv. Redchief (medium chill), and 'Northern Spy' (high chill) were untreated, treated with TDZ prior to chilling (before-chill), and treated with TDZ at various intervals after the accumulation of specific amounts of chilling (after-chill). Shoots were stored in a cold room at 4°C. TDZ applied prior to chilling reduced the chill unit (CU) requirement (1 CU = 1 h at 4°C) for the promotion of bud break on 1-year-old shoots of 'Anna' and 'Northern Spy' and 1- and 2-year-old wood of 'Delicious.' TDZ applied after-chill promoted bud break only for 'Anna' and buds on 2-year-old wood of 'Delicious.' While accumulating CUs, untreated buds or buds treated with TDZ on 1-year 'Delicious' and 'Northern Spy' did not respond to the cold treatment even after 1848 h of CU accumulation. For all three cultivars, TDZ treatment was more effective in promoting bud break when applied before the initiation of chilling.

Shoots of many temperate-zone woody plants cease elongating in late summer or early fall and form terminal buds. These buds then require a period of time before growth can resume in the spring (Powell 1986). Axillary buds behave similarly. The amount of cool temperature required to overcome endodormancy (dormancy regulated by physiological factors within the affected structure) of the bud is referred to as the chilling requirement (Lang et al. 1985;

The use of a company or product name does not constitute an endorsement by USDA or the University of Maryland nor imply approval to the exclusion of other suitable products.

Saure 1985). The chilling requirement is considered to be satisfied in apple when 50% of the buds have opened after 20 to 25 days at ~20°C (Gianfagna and Mehlenbacher 1985; Young and Werner 1985). Various models, including the Utah model (Richardson et al. 1974), have been developed for calculating the amount of chilling required to release bud dormancy. The Utah model assigns one chill unit (CU) per hour to temperatures from 2.5–9.0°C, with reduced chilling effectiveness for temperatures higher or lower than these.

It is not clear how chilling releases buds from endodormancy; however, it is generally considered that endogenous plant hormones have a role in controlling this type of dormancy (Powell 1986; Westwood 1978). A number of chemicals, including cytokinins (Broome and Zimmerman 1976; Kender and Carpenter 1972; Pieniazek 1964) can substitute for part of the chilling requirement in apples. Thidiazuron [(TDZ) *N*-phenyl-*N'*-1,2,3-thidiazol-5-ylurea] has many cytokinin-like properties and is highly active at low dosages (Capelle et al. 1983; Isogai 1981; Wang et al. 1986; Yip and Yang 1986). This investigation was undertaken to determine if TDZ could substitute for the chilling requirement to stimulate bud break in three apple cultivars having widely differing chilling requirements.

## Materials and Methods

Shoots (50–70 cm) were collected October 1, 1986 prior to accumulating 50 CUs from cvs. 'Anna,' 'Delicious' cv. Redchief, and 'Northern Spy' apple trees growing at the US Department of Agriculture, Beltsville, MD. The 'Anna' shoots were from wood that had been top-grafted onto self-rooted 'Northern Spy' trees. The 'Northern Spy' shoots were from the ungrafted trees. 'Delicious' shoots were from trees grafted onto MM.106 rootstock. These cultivars were chosen because of their widely differing chilling requirements for bud break: 'Anna' (low chill), 400–1000 CUs; 'Delicious' (medium chill), 1600–2400 CUs; and 'Northern Spy' (high chill), 2600–3600 CUs (Miller and Baker 1982; Powell 1986). Shoots of each cultivar were sorted into groups of four (4 shoots per lot × 8 or 10 sampling dates × 3 treatments). They were defoliated, treated, packed in moistened peat, wrapped in plastic, and stored in a cold room maintained at 4°C. The three treatments were as follows: (1) control, untreated; (2) before-chill, treated with TDZ prior to chilling; and (3) after-chill, treated with TDZ following CU accumulation. Shoots were dipped in a 750 μM aqueous solution of TDZ containing 3.75% DMSO and 0.75% Tween 20 surfactant. The before-chill TDZ treatment was applied just prior to placing the shoots in the 4°C cold room (0 CUs). The after-chill TDZ treatment was applied after shoots accumulated the number of CUs shown in Fig. 1, just prior to bud break conditions. A fourth treatment was included for 'Delicious' only, an aqueous solution containing 3.75% DMSO and 0.75% Tween 20 was used to treat the shoots at 0 time (before-chill). Information concerning this fourth treatment is not reported as shoots responded in a manner similar to those of the 'Delicious' untreated control group (treatment 1).

Shoots from each treatment group were periodically (see Fig. 1 for specific times) removed from the cold room, 2–3 cm cut from the stem base to facili-

tate water uptake, and then the cut ends placed in glass jars containing tap water. They were maintained in a room at 23–25°C and lighted by cool-white fluorescent tubes 24 h/day (25 μmol $m^{-2}$ $s^{-1}$, minimum). At weekly intervals, the basal ends of the shoots were recut, and the water in the containers changed. At the end of 21 days [9828 growing degree hours (Richardson et al. 1974)], the number of buds showing visible green leaves or flowers were noted for each of the four shoots. For 'Anna,' 18–20 mixed buds and for 'Northern Spy,' 18–20 vegetative buds (excluding terminal buds), from 1-year wood were evaluated. In the case of 'Delicious,' 15–18 vegetative buds on 1-year wood (excluding terminal buds) and 8–10 mixed buds on 2-year wood were evaluated. The percentage "bud break" was calculated for individual shoots and averaged over the four shoots per treatment for each sampling time. Shoots were removed from the cold room as follows: 'Anna,' at approximately 4-day intervals, 8 samplings from 0 through 29 days (0–696 CUs); and 'Delicious' and 'Northern Spy,' at approximately 8-day intervals, 10 samplings from 0 through 77 days (0–1848 CUs). For each cultivar-treatment combination, quadratic equations were derived for the bud break percentage over time (0–696 CUs or 0–1848 CUs), and coefficients of determination ($R^2$) were calculated for each. The formula for each quadratic curve thus fitted is also presented.

## Results

For low chill cv. 'Anna' (1-year wood) both after-chill and before-chill TDZ treatments promoted early bud break as compared to control. (Fig. 1A). Untreated buds began to break after receiving about 384 CUs. Approximately 50% of the buds broke for all three treatments by the time they received ≥600 CUs. However, TDZ-treated buds had a greater percentage of bud break as compared to untreated buds by the end of the 696 h chill period. The percentage bud break data from the control and both TDZ-treatment groups fitted quadratic curves equally well as shown by the coefficient of determination values ($R^2$, see Fig. 1).

TDZ applied before-chill to the medium-chill-requiring 'Delicious' resulted in bud break on both 1- and 2-year-old shoots that received 384 CUs (Fig. 1B, C). By the time 600 CUs had accumulated, buds on 1-year-old wood treated before-chill with TDZ reached over 50% bud break and thereafter increased to about 70% by the time 1800 CUs had accumulated. Control and after-chill TDZ-treated 1-year-old buds showed only low bud break percentages. For 'Delicious' buds on 2-year wood, the before-chill TDZ application resulted in nearly 100% bud break by the time 1000 CUs accumulated (Fig. 1C). After-chill TDZ treatment also promoted bud break on 2-year wood but not as consistently as the before-chill treatment.

The curve-fitting data for 1-year 'Delicious' buds from control and after-chill TDZ-treatment groups provided minimal information due to low bud break percentages over the 1848 h chilling time. However, the before-chill TDZ data fitted a quadratic curve quite well ($R^2 = 0.934$). Bud break data for 2-year-old 'Delicious' buds of control and before-chill TDZ treatments fitted a quadratic curve better ($R^2 = 0.90$) than the after-chill TDZ treatment ($R^2 = 0.71$).

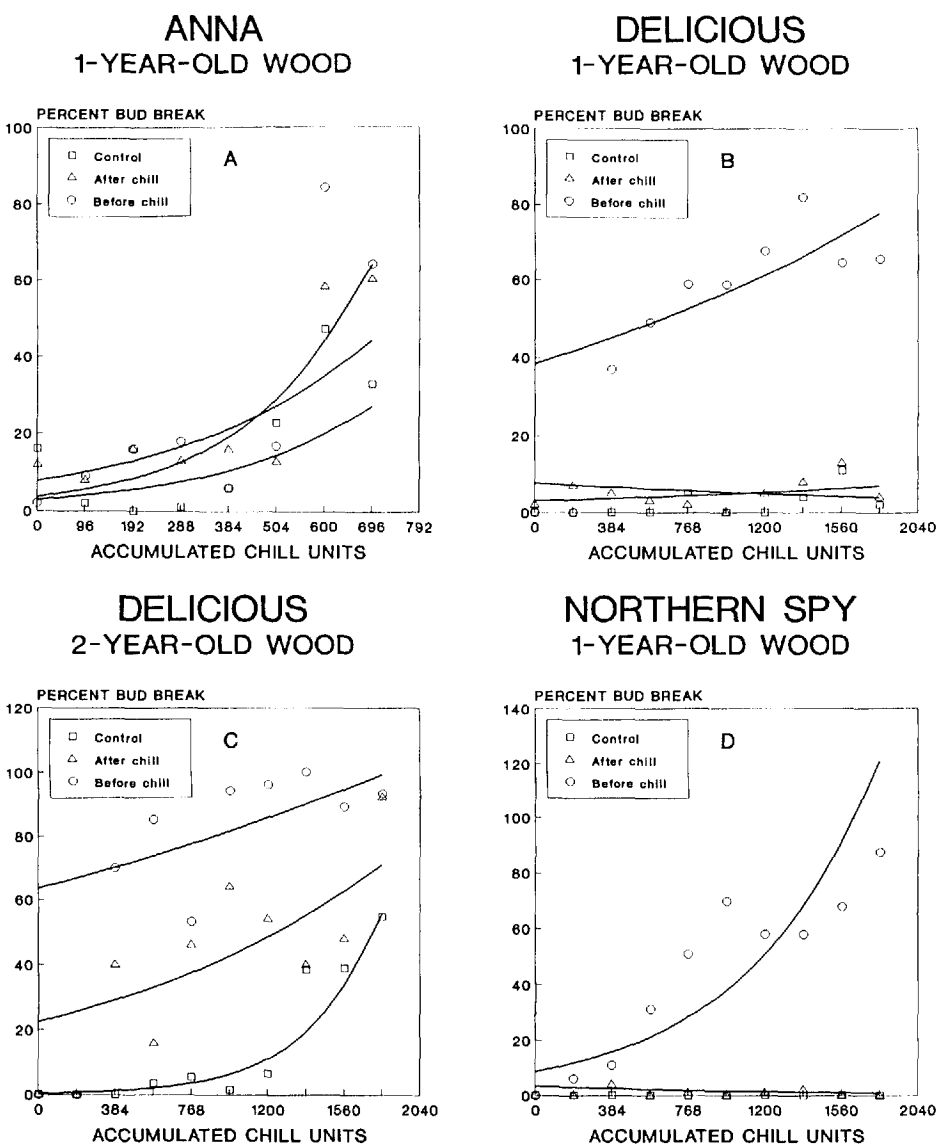


**Fig. 1.** Percent bud break for control, untreated; before-chill TDZ, 750 μM TDZ applied prior to chilling; and after-chill TDZ, 750 μM TDZ applied after specific amount of CU accumulation. Quadratic equations and coefficient of determination ($R^2$) derived from bud break percentages vs. CU accumulation. **A** Low-chill-requiring cv. 'Anna' (1-year-old wood stored at 4°C over 696 h): control [10.51 − 1.62 (CU) + 0.10 $(CU)^2$], $R^2$ = 0.752; before-chill TDZ [7.99 − 1.08 (CU) + 0.12 $(CU)^2$], $R^2$ = 0.716; after-chill TDZ [14.62 − 1.71 (CU) + 0.12 $(CU)^2$], $R^2$ = 0.829. **B** Medium-chill-requiring cv. 'Delicious' (1-year-old wood stored at 4°C over 1848 h): control (minimal response, no calculation); before-chill TDZ [−6.12 + 2.66 (CU) − 0.02 $(CU)^2$], $R^2$ = 0.934; after-chill TDZ (minimal response, no calculation). **C** Medium-chill-requiring cv. 'Delicious' (2-year-old wood stored at 4°C over 1848 h): control [−1.73 − 0.49 (CU) + 0.02 $(CU)^2$], $R^2$ = 0.905; before-chill TDZ [2.78 + 3.49 (CU) − 0.03 $(CU)^2$], $R^2$ = 0.911; after-chill TDZ [2.26 + 1.16 (CU) − 2.82 $(CU)^2$], $R^2$ = 0.714. **D** High-chill-requiring cv. 'Northern Spy' (stored at 4°C over 1848 h): control (no response); before-chill TDZ [−5.41 + 1.84 (CU) − 9.32 $(CU)^2$], $R^2$ = 0.889; after-chill TDZ (minimal response, no calculation).

Bud break was evident only for the before-chill TDZ treatment on 1-year-old wood of the high-chill-requiring cv. 'Northern Spy' (Fig. 1D). Bud break for the before-chill TDZ treatment was about 40% after 600 CUs accumulated and reached 60% or greater after 768 CUs. No bud break occurred on control or after-chill TDZ-treated shoots by the end of the experiment (after 1848 CUs). However, bud break data from the before-chill TDZ treatment fitted a quadratic curve well ($R^2 = 0.889$).

## Discussion

TDZ applied before chilling substituted and/or reduced the number of CUs required for bud break on 1-year-old 'Anna,' 'Delicious,' and 'Northern Spy' and on 2-year-old wood of 'Delicious.' For 'Anna,' TDZ application after chilling did not differ from the before-chilling treatment, and both were effective in breaking dormancy. Thus, in this case it appears that TDZ substituted for chilling in breaking bud dormancy. In the case of 'Delicious' and 'Northern Spy,' treatment of 1-year-old wood before chilling was much more effective than treatment after chilling, with the after-chilling treatment being essentially ineffective on both cultivars. It is of interest to note that 'Delicious' and 'Northern Spy' were as responsive, or more responsive, than 'Anna' to TDZ treatment within the first 600 h of chilling, although 'Delicious' and 'Northern Spy' have a much higher chilling requirement than 'Anna.' 'Delicious' buds on 2-year-old wood were more responsive than those on 1-year-old wood, particularly for the TDZ treatment following CU accumulation. In this case TDZ reduced or substituted for part of the chilling requirement. Buds on 2-year-old wood also appeared to have a lower CU requirement than those on 1-year-old wood. Positional effects on the chill unit requirement for bud break have been reported in excised shoots (Chandler 1960) and single node cuttings (Crabbe 1984).

Our experiments may be interpreted to suggest that there are multiple components of bud dormancy that respond differently to TDZ treatment. If one assumes that chilling enhances dormancy, then TDZ treatment before dormancy (before chilling) would counter the development of endodormancy. Treatment following the onset of chilling would become less effective as endodormancy developed. However, if this were the only component of bud dormancy, a gradual decline in TDZ effectiveness would be expected to correlate with CU accumulation instead of the observed minimal response of the after-chilling TDZ treatment.

It could also be argued that a paradormant (dormancy regulated by physiological factors outside the affected structure) response related to apical dominance is inhibiting bud break in apple. Two-year-old wood was more responsive to TDZ both before and after CU accumulation. This may be related to the age of the wood or to a lack of apical control. Chandler (1960) reported that rest was satisfied sooner in 2-year-old wood than in 1-year-old wood. He also reported a positional effect on bud break in 1-year-old wood which correlated with apical dominance. These results were essentially confirmed and expanded upon by Crabbe (1984) using single node cuttings. However, when buds are

excised and isolated in culture, no such positional effect was detected (Borkowska and Powell 1979). Before- and after-chilling treatments were both effective in overcoming dormancy in 'Anna' suggesting a weak apical influence on the chilling requirement.

There are several reports of cytokinin applications promoting bud break in apple (Broome and Zimmermann 1976; Kender and Carpenter 1972; Pieniazek 1964; Wang et al. 1986), although some chilling was required before these treatments were effective. Benzylaminopurine injected directly into isolated buds of sour cherry was effective for promoting bud break early in dormancy (Arias and Crabbé 1975).

Borkowska (1980) suggested that benzylaminopurine enhances the CU effectiveness at low temperatures, but does not alter dormancy per se. However, it is difficult to experimentally differentiate between decreased CU requirement for breaking dormancy and increased effectiveness of each CU. TDZ is relatively immobile and stable in plant tissue thus allowing it to remain in the bud throughout CU accumulation. This may explain why TDZ treatment before chilling was more effective than treatments after chilling.

TDZ, a highly active cytokinin-like compound, is effective in reducing the CU requirement of low, medium, and high chilling varieties when applied before CU accumulation. In the low-chill-requiring variety 'Anna' and the 2-year-old wood of medium-chill-requiring 'Delicious,' TDZ treatment was also effective after CU accumulation. It is suggested that both para- and endodormancy occur in apple buds, and that TDZ is more effective for overcoming the factors resulting in endodormancy than those that result in the paradormant response.

*Acknowledgments.* The authors thank Nor-Am Chemical Co., Wilmington, Delaware for providing thidiazuron.

## References

Arias O, Crabbé J (1975) Les gradients morphogénétiques du rameau d'un an des végétaux ligneaux, en repos apparent. Physiol Veg 13:69–81

Borkowska B (1980) Effect of chilling period, benzylaminopurine (BA) and abscisic acid (ABA) on physiological and biochemical changes in non-growing and growing apple buds. Fruit Sci Rep 7:101–115

Borkowska B, Powell LE (1979) The dormancy status of apple buds as determined by an *in vitro* culture system. J Am Soc Hortic Sci 104:796–799

Broome OC, Zimmerman RH (1976) Breaking bud dormancy in tea crabapple [*Malus hupehensis* (Pamp.) Rehd.] with cytokinins. J Am Soc Hortic Sci 101:28–30

Capelle SC, Mok DWS, Kirchner SC, Mok MC (1983) Effects of thidiazuron on cytokinin autonomy and the metabolism of $N^6$-($4^2$-isopentenyl)-8-$^{14}$C-adenine in callus tissues of *Phaseolus lunatus* L. Plant Physiol 73:796–802

Chandler WH (1960) Some studies of rest in apple trees. Proc Am Soc Hortic Sci 76:1–10

Crabbe JJ (1984) Correlative effects modifying the course of bud dormancy in woody plants. Z Pflanzenphysiol 113:465–469

Gianfagna TJ, Mehlenbacher SA (1985) Importance of leaf requirement for bud break and time of flowering in apple. HortScience 20:909–911

Isogai Y (1981) Cytokinin activities of *N*-phenyl-*N'*-(4-pyridyl) ureas. In: Guern J, Péaud-Lenoël C (eds) Metabolism and molecular activities of cytokinins. Springer-Verlag, Berlin, pp 115–128

Kender WJ, Carpenter S (1972) Stimulation of lateral bud growth of apple trees by 6-benzylamino purine. J Am Soc Hortic Sci 97:337–380

Lang G, Early J, Arryove N, Darnell R, Martin G, Stutte G (1985) Dormancy: Toward a reduced, universal terminology. HortScience 20:809–812

Miller EP, Baker LH (1982) An evaluation of apple cultivars for central and northern Florida. Proc Fl State Hortic Soc 95:88–90

Pieniazek J (1964) Kinetin induced breaking of dormancy in 8-month-old apple seedlings of 'Antonovka' variety. Acta Agrobot 16:157–169

Powell LE (1986) The chilling requirement of apple and its role in regulating time of flowering in spring in cold-winter climates. Acta Hortic 179:129–183

Richardson EA, Seeley SD, Walker DR (1974) A model for estimating the completion of rest for 'Redhaven' and 'Elberta' peach trees. HortScience 9:331–332

Saure MC (1985) Dormancy release in deciduous fruit trees. Hortic Rev 7:239–300

Wang SY, Steffens GL, Faust M (1986) Breaking bud dormancy in apple with a plant bioregulator, thidiazuron. Phytochemistry 25:311–317

Westwood M (1978) Temperate-zone pomology. WH Freeman, San Francisco

Yip WK, Yang SF (1986) Effect of thidiazuron, a cytokinin-active urea derivative, in cytokinin-dependent ethylene production systems. Plant Physiol 80:515–519

Young E, Werner DJ (1985) Chill unit and growing degree hour requirements for vegetative bud break in six apple rootstocks. J Am Soc Hortic Sci 110:411–413