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Metabolic Changes Associated with Bud Break Induced by Thidiazuron

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Abstract. Bud break in apple (Golden Delicious, *Malus domestica* Borkh) was induced by thidiazuron (N-phenyl-N′-1,2,3-thidiazol-5-ylurea). In control and thidiazuron-treated shoots, higher amounts of soluble carbohydrates (sorbitol, fructose, glucose, sucrose) and galacturonic acid were found in the phloem, but higher amounts of starch and cell wall polysaccharides, including cellulose and xylose, were found in the xylem. A decrease in soluble carbohydrates and starch in both phloem and xylem was associated with induction of bud break by thidiazuron. However, little change in cell wall polysaccharides was found. Total carbohydrates were higher in the upper than in the lower portion of shoots. The breaking of dormancy by thidiazuron was also associated with an increase in organic acid content and respiration in buds. KCN inhibited bud respiration during all stages of development. Organic acid content was inversely related to carbohydrate content in developing buds. Axes contained more carbohydrates and organic acids than did scales.

Bud development is one of the major factors affecting productivity of apple trees. Apple buds are mostly dependent for their development on the transport of carbohydrates from the reserves of the tree (Priestley 1981). Carbohydrates in the shoots increased considerably between early autumn and midwinter and then decreased in early spring. The decrease was attributed to the translocation of reserves into new growth (Priestley 1970, 1981). We previously reported that the plant bioregulant [N-phenyl-N′-1,2,3-thidiazol-5-ylurea (thidiazuron;

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Dropp; SN49537; TDZ)] releases lateral buds from dormancy and that this is correlated with an increase in DNA, RNA, protein, 1-aminocyclopropane-1-carboxylic acid, 1-(malonylamino)cyclopropane-1-carboxylic acid, and S-adenosylmethionine levels as well as with greater polyamine formation (Wang et al. 1986a). The present study was initiated to determine the changes of carbohydrates, organic acids, and respiration in buds and the changes of carbohydrates in phloem and xylem of shoots during and after bud break induced by thidiazuron.

Materials and Methods

Plant Material and Treatments

Terminal shoots were collected from mature Golden Delicious (*Malus domestica* Borkh) apple trees at the Beltsville Agricultural Research Center orchard on January 6, 1986, and placed with their bases in jars of distilled water. The shoots were randomized so that 7 or 8 shoots (replications) per treatment were sampled. Thidiazuron (100 μM) was prepared in 2.5% DMSO plus 0.5% Tween 20 and applied directly to the buds with a brush until runoff. Treatments were applied only within nodes 1–10, counting from the apex. The treated area was divided into two regions: upper (nodes 1–5) and lower (nodes 6–10). Within each region, five buds were treated. Results obtained from each region were presented as a unit. Radiation sources consisted of natural day length and cool white fluorescent lamps which provided a PAR level of about 320–400 $\mu mols^{-1}$ m^{-2} for 10 h per day (0700–1700 h). Temperatures were approximately 25°C during the day and 20°C at night, and RH was 65%. Control and thidiazuron-treated shoots and buds were sampled at weekly intervals during a 4-week period. Phloem (bark) and xylem (wood) from the upper and lower portion of these shoots were separated and cut into thin slices, frozen, and used for carbohydrate analysis. Buds were analyzed as a whole or separated into scales and axes, for carbohydrate and organic acid.

Carbohydrate Analysis

The extraction, purification, and derivatization procedures for nonstructural and cell wall carbohydrates have been described previously (Wang et al. 1985, 1986b,c). A Hewlett-Packard 5880 gas chromatograph equipped with a flame ionization detector and a fused silica capillary column (methylsilicone fluid, 12.5 × 0.2 mm) was used for separation of sugars. Separated sugars were compared with derivatized sugar standards for qualitative and quantitative determinations.

Organic Acid Analysis

A Baker 10 extraction system was used for purification of organic acids in apple bud tissue. An aliquot of ethanol extract was adjusted to pH 7.0 and placed onto a 3-ml quaternary amine column. The columns were conditioned

with hexane and methanol. Organic acids were eluted with 3 ml 0.1 N HCl. The eluate was concentrated to dryness under vacuum. Derivatization and determination of organic acids were similar to those for nonstructural carbohydrate except that the initial oven temperature was reduced to 100°C and held isothermal for 3 min; temperature program rate was 4°C min^{-1}, and final oven temperature was 230°C.

Respiration Measurement

Oxygen uptake by apple buds was obtained using a Clark-type oxygen electrode (YSI 5331, Yellow Springs Instruments Co., Yellow Springs, OH, USA) in 3 ml 10 mM MES buffer (pH 6.0) at 25°C.

Results and Discussion

Bud Break and Growth

Thidiazuron stimulated bud break in the treated area of shoot (Fig. 1). Three days after treatment with 100 μM thidiazuron, buds started to swell. Promotion of bud break and growth was more evident in the upper portion of the shoots. In previous work, bud break and growth decreased in a basipetal direction in apple seedlings in response to thidiazuron treatment (Wang et al. 1986a) and suggested a gradient of increasing rest from shoot apex to base. Untreated buds on the thidiazuron-treated shoots and buds on the control shoots remained dormant, as previously reported in apple seedlings (Wang et al. 1986a). This suggests that thidiazuron was not translocated in apple shoots.

Nonstructural Carbohydrate Content

Carbohydrate content of shoots. Phloem contained a higher amount of soluble carbohydrates, including sorbitol, fructose, glucose, and sucrose, whereas xylem contained a higher amount of starch (Figs. 2 and 3). The upper portion of the shoots generally contained a higher amount of carbohydrates than the lower portion of the shoots (Figs. 2 and 3). Priestley (1960) reported that the amounts of carbohydrates per unit of dry weight may be related to the proportion of living to nonliving cells in the tissues. The higher carbohydrate content in bark (phloem) than in wood (xylem) may corrrespond to the proportion of living cells in these two tissues. Sorbitol (D-glucitol) is an alditol, distributed widely in plants. It is found mainly in the Rosaceae and plays a significant role in carbohydrate metabolism (Bieleski 1969, Hansen and Grauslund 1973, Webb and Burley 1962).

Sorbitol was the major sugar in the phloem (Fig. 2); it moves apoplastically in the free space or symplastically in the cytoplasm and is converted to glucose and fructose by compartmentalizing enzymes, while the unmetabolized fraction is pumped into the vacuole and accumulated (Yamaki and Ishikawa 1986). Probably much of the energy to sustain rapid bud growth and development is obtained via the conversion of storage sorbitol to sucrose, glucose, and fruc-

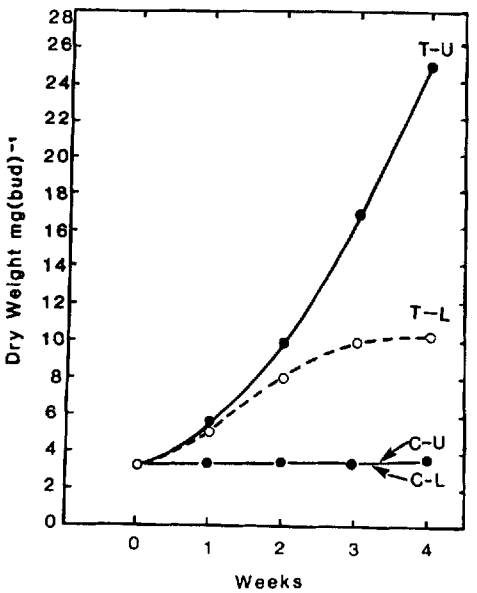

Fig. 1. Changes in bud dry weight in the upper (U) and lower (L) portions of shoots induced by 100 μM thidiazuron (T). C = Control. LSD (5%), 1.79.

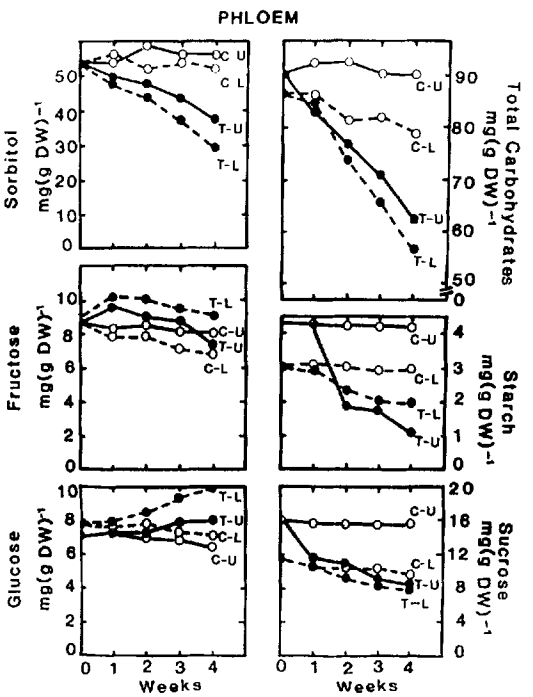

Fig. 2. Changes in carbohydrate content in phloem tissue of the upper (U) and lower (L) portions of the shoots following treatment of buds with 100 μM thidiazuron (T). C = Control. LSD (5%) for sorbitol, 4.61; fructose, 1.45; glucose, 1.05; sucrose, 1.59; starch, 0.41; total carbohydrates, 3.57.

tose. Xylem contained 20-fold more starch than phloem. Sucrose concentrations were 3–4 times lower than sorbitol in phloem and xylem tissue. Fructose and glucose were also relatively low in both phloem and xylem (Figs. 2 and 3).

Sorbitol, sucrose, and total carbohydrate content decreased in thidiazuron-

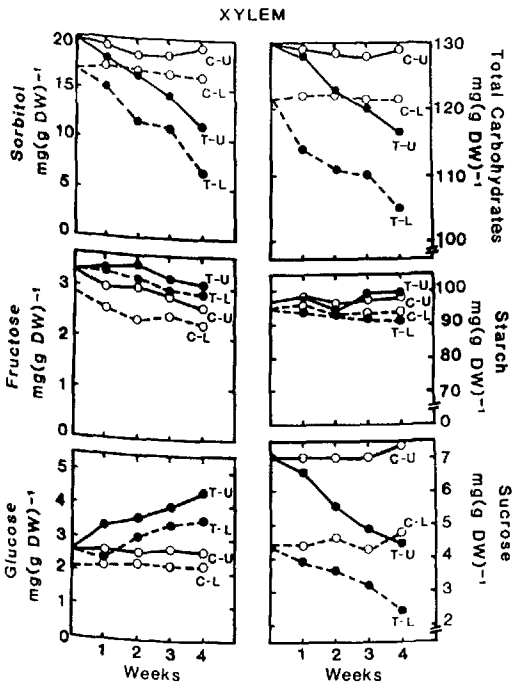

Fig. 3. Changes in carbohydrate content in xylem tissue of the upper (U) and lower (L) portions of the shoots following treatment of buds with 100 μM thidiazuron (T). C = Control. LSD (5%) for sorbitol, 1.20; fructose, 0.33; glucose, 0.80; sucrose, 0.68; starch, 1.17; total carbohydrates, 3.13.

treated shoots during bud development (Figs. 2 and 3). These reductions indicate that sugars might have been mobilized to support bud growth and development. Priestley (1960) found that a lower level of sugar in the shoots was associated with a higher rate of new growth.

Skene (1971) stated that starch dissolution is predominantly a consequence of the increased metabolic requirement of an active growth. The rapid starch depletion in shoots of apple phloem tissue occurred between weeks 1 and 2 after thidiazuron treatment, indicating that the buds used the starch in the phloem for their development (Fig. 2). Priestley (1970) found that toward the end of winter, starch was partly converted into glucose for supporting bud growth. Starch in xylem remained at a relatively constant level during a 4-week experimental period (Figs. 2 and 3). Decreases in sorbitol, sucrose, and total carbohydrate content in xylem were perhaps due to lateral translocation to phloem to support bud growth. The carbohydrate content in the control shoot remained constant (Figs. 2 and 3).

Carbohydrate content of buds. Sorbitol, fructose, glucose, sucrose, and starch were detected in vegetative buds (Fig. 4). The axes contained higher carbohydrate levels than the scales during all stages of bud development (Table 1). The dormant buds contained substantial amounts of sorbitol and sucrose. As metabolic activity within the bud increased, sucrose, sorbitol, and starch content declined. Marked reduction of carbohydrates in shoots has been observed during bud break (Priestley 1981, Wang et al. 1986b). This may be attributed to translocation of reserves into new growth or to respiration loss (Priestley 1970, 1981). Sorbitol is the major soluble carbohydrate in leaves, wood, roots, and fruits of many species in the Rosaceae (Bieleski 1969, 1977,

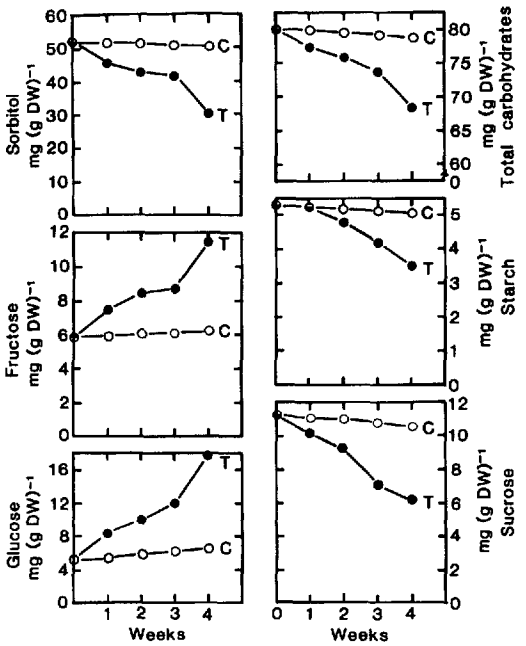

Fig. 4. Changes in carbohydrate content during the development of apple vegetative buds induced by 100 μM thidiazuron (T). C = Control. LSD (5%) for sorbitol, 4.27; fructose, 2.31; glucose, 2.02; sucrose, 1.18; starch, 0.85; total carbohydrates, 3.14.

Grant and apRees 1981, Priestley 1981). It also plays an important role in translocation of photosynthate. The translocated sorbitol in each organ seems to be converted into other sugars, such as glucose, fructose, sucrose, or starch (Bieleski 1969, Webb and Burley 1962). Sorbitol in bud scales decreased only slightly during the first 3 weeks after thidiazuron treatment (Table 1). Sorbitol in bud scales may not be involved in interconversion with other sugars or may be steadily replenished from the shoots. However, the sorbitol in bud axes decreased dramatically following thidiazuron-induced bud break (Table 1). There was a sharp decline in sucrose content accompanied by a large increase in glucose in the buds, scales, and axes (Fig. 4; Table 1). Fructose was present in all components of the bud and increased in axes and buds during bud break. Buds, scales, and axes also contained a low amount of starch which decreased only 3 weeks after treatment (Fig. 4; Table 1).

Cell Wall Carbohydrate Content

The noncellulosic neutral sugars, including rhamnose, arabinose, xylose, mannose, glucose, and galactose, occurred in the cell wall of both phloem and xylem. Xylose was the predominant noncellulosic neutral sugar. Xylem contained a higher amount of xylose and cellulose, whereas phloem contained a higher amount of galactose and galacturonic acid (Table 2). Differences in cell wall composition of xylem and phloem have been reported in other woody trees (Thornber and Northcote 1961). The content of cell wall carbohydrate in shoots did not change significantly in phloem and xylem when bud break and bud growth were induced by thidiazuron (data not shown). This indicates that cell wall carbohydrates may not be associated with the various phases of bud

Table 1. Carbohydrate content of axes and scales of vegetative buds following treatment of buds with 100 μM thidiazuron (TDZ).

Tissue	Treatment	Duration (weeks)	Carbohydrate (mg · g^{-1} DW)					
			Sorbitol[a]	Fructose	Glucose	Sucrose	Starch	Total
Axes	Control	0	102.3c	11.6b	7.8c	19.6c	8.7c	150.0d
	Control	3	95.7c	13.0b	6.9c	17.4c	9.3c	142.3d
	TDZ	3	68.0b	14.4c	23.4d	9.7b	5.4b	120.9c
Scales	Control	0	43.9a	4.5a	1.8a	9.4b	4.3b	63.9b
	Control	3	40.8a	6.0a	1.5a	8.0b	5.0b	61.3b
	TDZ	3	39.3a	5.5a	4.4b	4.8a	1.6a	55.6a

[a] Means in a column separated by Duncan's multiple-range test, 5% level.

Table 2. Cell wall polysaccharide composition in phloem and xylem of upper and lower portions of shoots. Samples were collected on January 6, 1986.

		Polysaccharide concentration[a] (mg · g^{-1} DW)							
Tissue	Portion	Rha[b]	Ara	Xyl	Mann	Glu	Gal	Cellu	Gaua
Phloem	Upper	3.5a	7.7a	36.5a	3.2a	10.0a	9.7b	168.7a	140.0b
	Lower	3.4a	8.1a	36.1a	2.4a	9.7a	9.2b	166.0a	154.0b
Xylem	Upper	1.8a	5.3a	46.7b	2.9a	10.1a	3.6a	253.3b	91.0a
	Lower	1.8a	6.1a	46.8b	3.1a	10.2a	3.5a	244.3b	97.3a

[a] Data represent the mean of nine analyses. Means in a column separated by Duncan's multiple-range test, 5%.

[b] Sugar abbreviations: Rha, rhamnose; Ara, arabinose; Xyl, xylose; Mann, mannose; Glu, noncellulosic glucose; Gal, galactose; Cellu, cellulose; Gaua, galacturonic acid.

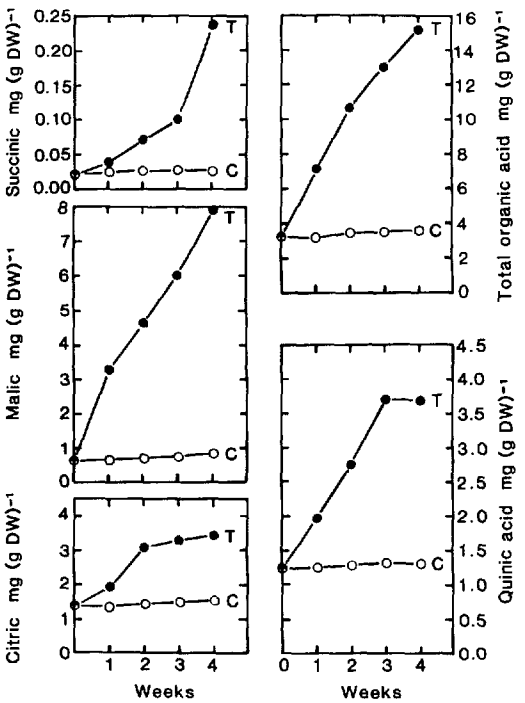

Fig. 5. Changes in organic acid content during the development of apple vegetative buds induced by 100 μM thidiazuron (T). C = Control. LSD (5%) for succinic, 0.03; malic, 1.23; citric, 0.56; quinic, 0.65; total organic acid, 2.17. C, control; T, thidiazuron.

growth and bud development during the 4-week experimental period. Cell wall carbohydrate content in nontreated shoots also remained constant (data not shown).

Organic Acids

Most studies on organic acids have been carried out in apple fruit tissue, and relatively little information is available in apple buds. Buds, scales, and axes all contained malic, quinic, and citric acids and trace amounts of succinic acid

Table 3. Organic acid content of axes and scales of vegetative buds following treatment of buds with 100 μM thidiazuron (TDZ).

Tissue	Treatment	Duration (weeks)	Organic acid (mg · g^{-1} DW)				
			Succinic[a]	Malic	Citric	Quinic	Total
Axes	Control	0	0.07a	2.80b	2.43b	2.41a	7.71b
	Control	3	0.20a	3.40b	2.67b	2.65a	8.92b
	TDZ	3	0.17a	7.81c	3.34c	3.77b	15.09c
Scales	Control	0	0.00a	1.08a	1.78a	2.11a	4.97a
	Control	3	0.12a	1.80a	1.52a	2.08a	5.52a
	TDZ	3	0.00a	3.61b	2.79b	2.69a	9.09b

[a] Means in a column separated by Duncan's multiple-range test, 5% level.

(Fig. 5; Table 3). Axes contained higher amounts of organic acids compared to scales (Table 3). Malic, quinic, citric, and total acids increased in axes and buds during bud break and bud development. The rate of increase was the greatest in malic acid, followed by quinic and citric. In the active growing buds, scales, and axes, malic acid was the predominant acid. The amounts of citric and quinic acid were comparable (Fig. 5; Table 3). The increase of organic acids in growing buds, scales, and axes was accompanied by a decrease in carbohydrate content (Tables 1 and 3; Figs. 4 and 5). Therefore, organic acid and sugar content were inversely related.

Respiration

The respiration rate increased during thidiazuron-induced bud break, and the difference between control and treated buds became significant after 2 weeks of treatment (Fig. 6). This increase in bud respiration was associated with a decrease in carbohydrate content. The respiration rate of untreated buds remained low (Fig. 6). An increase in O_2 consumption from the dormant stage in the winter to resumption of growth in the spring has also been reported in pear flower buds (Cole et al. 1982). Cyanide (0.1 mM) inhibited bud respiration by 20% in the dormant stage, and the inhibitory effect increased with time in thidiazuron-treated buds (Fig. 6). Similar increases in cyanide sensitivity occurred in pear flower buds as dormancy was broken (Cole et al. 1982). This may be attributed to the increased demand for energy during bud development.

Azcon-Bieto et al. (1983) found that in leaves of wheat and spinach, the alternative pathway (cyanide insensitive respiration pathway) was not engaged in the morning, when carbohydrate levels were low, but became engaged after several hours of photosynthesis. Carbohydrate levels in developing buds may not exceed the capacity of buds to utilize incoming carbon, as reported by Lambers (1980), and consequently the alternative pathway has not been initiated.

Conclusion

The carbohydrate changes occurring in the thidiazuron-treated shoots appeared to parallel the general pattern of carbohydrate metabolism during nat-

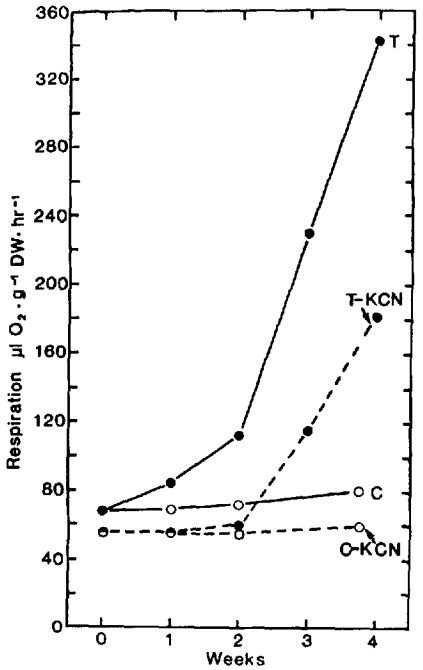

Fig. 6. Changes in respiration during the development of apple vegetative buds induced by 100 μM thidiazuron. Control (C) or thidiazuron-treated (T) buds were incubated with 3 ml 10 mM MES buffer (pH 6.0) with or without 0.1 mM KCN. LSD (5%), 18.17.

ural bud growth in the spring. Thidiazuron may initiate a regulatory process and lead to bud break and metabolic changes. This process may either convert reserved carbohydrates to a more readily usable form or promote efficient transport for bud break.

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