and measuring gas exchange processes only one time during the day would have provided misleading data in some cases. If one-time-of-day measurements are to be used to separate plant response to environmental treatments, the lack of treatment effect on daily fluctuations in these processes must be documented.

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Changes in Superoxide Dismutase Activity during Thidiazuron-induced Lateral Budbreak of Apple

Shiow Y. Wang, Hong J. Jiao¹, and Miklos Faust

Fruit Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705

Additional index words. *Malus domestica*, free radicals, growth substance

Abstract. The effects of thidiazuron on changes in superoxide dismutase (SOD) activity in apple (*Malus domestica* Borkh cv. York Imperial) buds during dormancy break were determined. Dormant buds had low SOD activity. Increased SOD activity was correlated with budbreak induced by thidiazuron. Buds contained three distinct forms of SOD: Cu, Zn-SOD; Mn-SOD; and Fe-SOD. Most of the SOD activity in apple buds was attributed to Cu and Zn-SOD, followed by Mn-SOD. Iron-SOD was not present in dormant buds, and only a trace amount was found as buds started rapid expansion. Chemical name used: *N*-phenyl-*N'*-1,2,3-thidiazol-5-ylurea (thidiazuron).

Free radicals are formed during normal cell metabolism. The superoxide radical O_2^- , generated in aerobic biological systems during enzymatic and nonenzymatic oxidation, is very toxic to all forms of life (Fridovich, 1986a). Mechanisms probably exist in plant cells to detoxify such free radicals. Superoxide dismutase (SOD) (EC 1.15.1.1) is a class of metal-containing proteins scavenging superoxide radicals. The mechanism involves an alternating reduction and oxidation of the metal associated with the enzyme. Superoxide dismutase occurs in three molecular forms, with Cu-Zn, Mn, or Fe as prosthetic metals, and has been detected in a wide range of living organisms (Fridovich, 1986b). Superoxide dismutase catalyzes the breakdown of O_2^- to O_2 and H_2O_2 , removes singlet oxy-

gen as well as O_2^- , prevents formation of OH^- (Fridovich, 1973; Paschen and Wester, 1973) and has been implicated as an essential defense against the potential toxicity of oxygen (McCord, 1979).

The plant bioregulator thidiazuron (Dropp; SN49537; TDZ) was 20 times more effective than cytokinin in breaking dormancy in lateral buds of apple (Wang et al., 1986). Our preliminary findings from electron spin resonance spectrometry (ESR) signals indicated that free radicals are formed in dormant buds (Wang and Faust, 1988). Thidiazuron induced budbreak and diminished free radical formation (Wang and Faust, 1988). No information is available on the relationship between dormancy, budbreak, and activity of SOD. This paper describes the isolation of SOD and the changes in its activity in apple buds during dormancy, and during budbreak and bud growth induced by thidiazuron.

The growing conditions of 'York Imperial' apple seedlings and treatment methodology used in this study have been described (Wang et al., 1986). The seedlings were planted in 3.5-liter pots and grown in a greenhouse and had never received any chill-

ing. Plants were generally 1 m high with a single stem. The buds were in paradormancy, and removal of apical dominance could induce lateral budbreak. A total of 1200 seedlings with completely randomized design were used for the experiment. One-half of the plants were used as control, and the others were treated with thidiazuron. Thidiazuron (100 μ M) was prepared in 2.5% (v/v) dimethyl sulfoxide (DMSO) plus 0.5% (v/v) Tween-20, and applied to the upper five buds with a brush until runoff. Control plants were treated with only DMSO and Tween 20. Natural daylight was supplemental with 400-W high-pressure sodium lamps (Energy Technics, York, Pa.) that provided a PAR level of ≈ 400 to $500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for 12 h-day⁻¹ (0700 to 1900 HR). Temperatures were $\approx 25/20^\circ\text{C}$ (day/night), and relative humidity was 65%. Buds from control and thidiazuron-treated plants were sampled at 2-day intervals for 12 days. Triplicate bud samples of 0.2 g fresh weight per sample were collected at each date. Depending on their size, the number of buds required ranged from approximately nine to 142. Excised bud

Fig. 1. Inhibition of the photoreduction of nitro blue tetrazolium by SOD in apple bud tissue extract. Since inhibition is not linearly correlated with SOD concentration, a V/v transformation was used to obtain linearity (V = basic reaction rate without apple bud extract, v = reaction rate with extract). When 50% inhibition of basic reaction rate was used as one unit of SOD activity, the equation for the line was as illustrated. Correlation coefficient for this line was 0.987.

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¹On leave from the Dept. of Horticulture, Guangxi Agricultural College, Nanning, Guangxi, Peoples' Republic of China.

Fig. 2. Effects of thidiazuron (T) (100 μ M) on fresh and dry weights of apple buds. C, control. Small letters indicate stage of bud development as follows: a, dormant bud; b, first swell; c, full swell; d, side green; e, green tip; f, bud expansion; g, bud open. Data are the means of three replicate samples. Vertical bars denote LSD at $P = 0.05$.

Fig. 3. SOD activity in dormant control (C) buds and buds treated with thidiazuron (T) (100 μ M). Data are the means of three replicate samples. Vertical bars denote LSD at $P = 0.05$.

tissue was immediately pulverized with a cold mortar and pestle with 0.1 M Na-phosphate buffer (pH 7.8) containing 0.1 mM EDTA-Na, and 5% (w/v) insoluble polyvinyl pyrrolidone. The ratio of the bud tissue to the homogenizing medium was 1:1 (w/v). The homogenate was strained through four layers of miracloth and centrifuged at $15,000 \times g$ for 20 min at 0°C. The supernatant was purified by gel filtration using Sephadex G-25 (Pharmacia, Piscataway, N.J.) that had been equilibrated with the extraction buffer. Naturally occurring reductants or antioxidants with low molecular weight (Halliwell, 1984; Lee et al., 1986) were depleted by passing the extract through a Centricon 10 molecular filter (Amicon, Danvers, Mass.) before assay of SOD enzyme activity.

Total SOD activity was assayed photochemically (Giannopolitis and Ries, 1977; Monk et al., 1987), based on the photoreduction of nitro blue tetrazolium (NBT) by light in the presence of riboflavin and methionine. Nitro blue tetrazolium is reduced

to blue diformazan, which has a strong absorbance at a wavelength of 560 nm. Under aerobic assay conditions, SOD inhibits the formation of blue diformazan. Nitro blue tetrazolium can be reduced by either pyridine nucleotide or O_2^- (Thayer, 1990). Dicoumarol was included in the reaction mixture to inhibit reduction by pyridine nucleotide (Thayer, 1990) and to obtain a completely O_2^- -dependent reduction of NBT. The reaction mixture (1 ml) contained 100 μ M dicoumarol, 1.3 μ M riboflavin, 13 mM methionine 0.05 M Na_2CO_3 , 0.01 M Na-phosphate buffer (pH 7.8), and 0.1 ml of the enzyme extract; after 3 min, 68 μ M NBT was added. The mixtures were illuminated by a fluorescent lamp (light intensity was $170 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) for 3 min. Identical solutions kept in darkness served as blanks. One unit of SOD was defined as the amount of enzyme that produced a 50% inhibition of NBT reduction under assay conditions (Beauchamp and Fridovich, 1973). Since inhibition is not linearly correlated with SOD concentration, a V/v transformation was used to obtain linearity (V = basic reaction rate without apple bud extract, v = reaction rate with extract). Linear correlation gave the equation: $\text{SOD units/ml} = (0.532 V/v - 0.197) \times \text{dilution factor}$. The correlation coefficient for this line was 0.987 (Fig. 1).

Copper, Zn-SOD activity is inhibited by cyanide, but Fe- and Mn-SOD activities are not. Iron-SOD is inactivated by H_2O_2 while Mn-SOD is not (Asada et al., 1975). Thus, Cu, Zn-SOD was estimated as total SOD activity in the absence of KCN minus activity in the presence of 1 mM KCN. The Mn-SOD was estimated as total Fe- and Mn-SOD activity minus activity following incubation of the apple bud extract with 2 mM H_2O_2 and 1 mM KCN. Finally, Fe-SOD activity was calculated as the difference between the CN-resistant and the Mn-SOD activities. Pre-incubations were carried out at 25°C for 10 min. Protein was determined according to Bradford (1976), using bovine serum albumin as a standard.

The buds started to swell within 2 days of thidiazuron (100 μ M) application (Fig. 2).

Increases in fresh and dry weights were associated with budbreak and bud growth induced by thidiazuron, but untreated buds from the same plant and buds from control plants remained dormant, as previously reported (Wang et al., 1986, 1990).

Apple buds contained three distinct forms of SOD: Cu, Zn-SOD; Mn-SOD; and Fe-SOD (Fig. 3). Most of the SOD activity was attributed to the Cu, Zn-SOD, followed by Mn-SOD. In higher plants, Cu, Zn-SOD occurs mainly within chloroplasts, cytoplasm, mitochondria, and peroxisomes (del Rio et al., 1978; Henry et al., 1980; Matters and Scandalios, 1987; Sandalio and del Rio, 1987). Mn-SOD is localized in mitochondria and peroxisomes (Sandalio et al., 1987). Iron-SOD occurs predominantly in prokaryotes but is also found in the chloroplasts and peroxisomes of eukaryotes (Duke and Salin, 1985; Salin and Lyon, 1983; Sandalio and del Rio, 1987). Since the dormant bud is nonphotosynthetic, Fe-SOD is not present. Trace amounts were found as buds started rapid expansion (Fig. 3). Dormant buds had low SOD activity. The activities of total and Cu, Zn-SOD increased 2.9- and 3.7-fold, respectively, whereas bud weight showed no significant difference between control (dormant bud) and thidiazuron-treated buds at day 2. This relationship indicates that SOD may play a protective role in apple buds by detoxifying free radicals and inducing budbreak. Total and Cu, Zn-SOD activity reached their peaks when buds were in the early stage of swelling, 4 days after thidiazuron treatment. During this period, bud weight increased only slightly. Manganese-SOD activity reached its peak 8 days after thidiazuron treatment and declined thereafter, when buds were in the rapid expansion stage. This decline in activity could be the result of a reduction in O_2^- production in the bud.

The results described in this report indicate that SOD appears to play a role in dormancy and budbreak. The increased activities of Cu, Zn-SOD; Mn-SOD; and Fe-SOD in apple buds were correlated to budbreak. These enzymes may remove the possible toxic effects of radicals and may be necessary for inducing budbreak in apple.

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Influence of Rootstock, Scion, and Water Deficits on Growth of 'Colt' and 'Meteor' Cherry Trees

Thomas G. Ranney¹, Nina L. Bassuk, and Thomas H. Whitlow
Department of Floriculture and Ornamental Horticulture, Cornell University, Ithaca, NY 14853

Additional index words. *Prunus avium* × *pseudocerasus*, *Prunus cerasus*, drought, growth analysis, net assimilation rate, relative growth rate, water stress

Abstract. Growth and physiological characteristics were evaluated in autografted and reciprocally grafted plants of *Prunus avium* L. × *pseudocerasus* Lindl. 'Colt' and *Prunus cerasus* L. 'Meteor'. Containerized plants were grown for 150 days in a greenhouse under either well-watered or water-stressed conditions. Both the scion and rootstock influenced growth (relative growth rate, $\bar{R}$), morphological [leaf area : root surface area (LARSA) and specific leaf area (SLA)], and physiological (mean net assimilation rate, $\bar{E}$) characteristics of grafted plants. Regardless of the watering regime, plants with 'Meteor' scions and 'Colt' rootstocks maintained higher $\bar{R}$ than plants with 'Colt' scions and 'Meteor' rootstocks. This enhanced growth occurred as a result of higher $\bar{E}$. Measurements on water-stressed plants also showed that the graft combination of 'Meteor' on 'Colt' had the lowest LARSA, while the reciprocal combination of 'Colt' on 'Meteor' had the highest. Differences in LARSA among water-stressed plants primarily reflected changes in SLA, as influenced by both rootstock and scion, and not in partitioning of dry weight between these organs.

A wide range of morphological and physiological characteristics has been found to be affected by rootstocks, scions, and their resulting interactions (Lockard and Schneider, 1981; Syvertsen, 1985; Tubbs, 1973). Many of these characteristics have the potential for improving plant water relations and growth during water stress. For example, rootstocks have been found to influence transpiration rate and crop water-use efficiency in peach (*Prunus* spp.) (Natali et al., 1983), leaf conductance in apple (*Malus* spp.) (Giulivo et al., 1985), leaf osmotic potential at full turgor and calculated hydraulic conductivity in apple (Olien and Lakso, 1986), root distribution in citrus (*Citrus* spp.) (Castle and Krezdorn, 1975, 1977), and midday leaf water potentials in citrus (Castle and Krezdorn, 1977), peach (Natali et al., 1983), and apple (Giulivo et al., 1985; Olien and Lakso, 1986). Few of these experiments, however, have examined how the influence of different rootstocks and scions can contribute to whole-plant growth and maintenance of growth under water stress.

Classical plant growth analysis provides a comprehensive method for evaluating whole-plant growth that integrates growth over time and permits the partitioning of growth into

morphological and physiological components (Hunt, 1982). Plant growth analysis has been used extensively as a method for evaluating and comparing growth of various species and progenies (Cain and Ormrod, 1984; Grime and Hunt, 1975; Ledig and Perry, 1969; Pollard and Wareing, 1968; Sweet and Wareing, 1968), the effects of water stress on growth processes (Ashenden et al., 1975; Jarvis and Jarvis, 1963), and the influence of rootstocks on plant growth and structure (Dudney, 1974; Vyvyan, 1955).

In this study, we combined the reciprocal grafting protocol of Vyvyan (1955) and plant growth analysis to examine the relationships between scion, rootstock, and water stress in two cherry cultivars with varying growth habits: *Prunus cerasus* 'Meteor', a strongly determinate grower, and *P. avium* × *pseudocerasus* 'Colt', a strongly indeterminate grower. The objective of this study was to evaluate the relative influence of rootstock and scion on plant growth and partitioning patterns in graft combinations of 'Meteor' and 'Colt' cherry trees under well-watered and water-stressed conditions.

All plant material was clonal and ranged in stem caliper from 1 to 2 cm. Stems were pruned to a uniform length of 20 cm from the stem-root junction. Root systems were pruned to a uniform overall dimension of 21 cm in diameter and 30 cm in length to conform to container dimensions. Cleft grafts, consisting of two scions with three buds each, were made between 2 and 23 Feb. 1985. Plants were then placed in the dark at 5°C with roots packed in moist peat before being planted.

Plants were potted on 14 Mar. 1985 in 11.4-liter black plastic containers with 8 liters of medium consisting of 2 sand : 1

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¹To whom reprint requests should be addressed. Current address: Dept. of Horticultural Science, North Carolina State Univ., Mountain Horticultural Crops Research and Extension Center, 2016 Fanning Bridge Rd., Fletcher, NC 28732.