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Respiratory Oxygen Response and Respiratory Quotient of Apple Stem Sections during Chilling

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Abstract. **During natural leaf abscission, 2-year-old potted apple trees *(Malus domestica* Borkh. cv. MM.111 EMLA) were placed in a room at 6C for chilling [0,600, 900, or 1400 chilling units (CU)]. After each chilling treatment, respiration of shoot segments was measured as CO_2 evolved and O_2 consumed at 22C in several O_2 concentrations. Respiration increased with oxygen concentration after all CU treatments. Carbon dioxide evolved at the several O_2 levels did not show a pattern related to CU, but O_2 consumed decreased at a decreasing rate with additional CU. Respiratory quotient was <1 at 0 and 600 CU and equal to 1 at 900 and 1400 CU, indicating a possible shift in respiratory substrate with chilling.**

Deciduous fruit trees require a minimum amount of chilling (4-10C) for release from dormancy (Perry, I97 1; Samish, 1954) and initiation of growth. This transition from the beginning of dormancy to active growth is accompanied by many metabolic changes, some of which are temperature-regulated. Changes in DNA and RNA levels (Barskaya and Oknina, 1959). protein content (Titus and Kang, 1982; Wang et al., 1985), hormone levels (Wood, 1983), and polyamincs (Wang et al., 1985) have been reported. Carbohydrate levels also change as reserves arc broken down and mobilized for new shoot and root growth (Hansen, 1971: Quinlan, 1969).

Compared to herbaceous plants, woody perennials have a high concentration of degradable carbohydrate reserves (Priestly, 1962), which are unlikely to be exhausted during dormancy under normal circumstances. Nevertheless, many trees' roots and shoots demonstrate a marked seasonal fluctuation in respiration rate when measured at constant temperature (Cox, 1975; Shiroya et al.. 1966; Strain, 1969). Wang et al. (1991) found a significant increase in respiration of apple buds induced to break dormancy by thidiazuron compared to untreated buds: also, respiration of apple root and shoot tissue increased during forcing at 23C only after trees had been fully chilled (Young et al., 1987). Studies also have shown that the Q_{10} and energy of activation (EA) for shoot respiration, measured after forcing at 20C, decreased with increased chilling given before forcing (Young, 1990). One possible explanation for the observed decrease in respiratory EA after chilling is a shift in the metabolic source of the CO_2 evolved. The EA measured was an average for all pathways that evolved CO_2, rather than mitochondrial respiration per se. These results indicate a possible change in C metabolism associated with dormancy release, which may also mean a change in respiratory substrate. The ratio of CO_2 evolved to O_2 consumed in respiration (RQ) is an indication of the original substrate used in respiration. If glucose only is respired, RQ = 1, organic acids yield an RQ > 1, and fatty acids yield an RQ < 1; e.g., stearic acid respiration results in RQ = 0.7, and with palmitic acid, RQ = 0.36 (Noggle and Fritz, 1976).

The RQ in dormant pea (*Pisum sativum* L.) seeds increases from 0.4 to between 0.7 and 0.8 as seeds develop toward germination (Obrucheva and Kovadlo, 1985), which is thought to be due to oxidation of storage lipids early in the dormancy period. A similar situation occurs in dormant apple seeds (Dawidowicz-Grzegorzcwska, 1989). where degradation of lipid bodiesdepends on chilling, which is necessary for germination. The RQ in apple buds during dormancy has not been reported, but Lui et al. (1991) found that lipase activity in apple buds increased greatly as chilling progressed.

A recent study of low-oxygen storage of fully chilled, quiescent apple trees (Young and Blankenship, 1991) showed that O_2 concentrations <5%, combined with 6.0C, inhibited budbreak for up to 35 weeks with no detectable effects on subsequent shoot growth. It is not known how O_2 level affects respiration during dormancy and whether or not any effects would change with changes in respiratory substrate.

The purposes of this study were 1) to measure respiration of apple shoot tissue during dormancy as both CO_2 evolution and O_2 consumption to calculate RQ values. and 2) to determine the effects of atmospheric O_2 concentration on these respiration measures.

Material and Methods

Forty 1 -year-old rooted layers of Malling Merton 111 (MM. 111) apple [chilling requirement 1450 chill units (CU) (Hauagge and Cummins, 1991; Young and Werner, 1985)] were lifted from a commercial stoolbed (Treco, Woodburn, Ore.). All trees were pruned to 60 cm above the root collar, and all lateral branches were removed. Trees were potted in 3.5-liter containers in damp calcined clay (Turface) and placed in a room at 5 ± 2C. After each chilling treatment (0, 600, 900, or 1400 CU), 10 trees were removed and terminal shoots cut into four 10-cm sections, excluding the top and bottom 10 cm. One of each of these sections was placed in each of forty 100-ml test tubes containing a piece of moist filter paper to prevent desiccation. Shoot sections were placed randomly into the various oxygen concentrations to randomize any variation due to acrotony and basitony. Groups of four test lubes were each thoroughly blushed with one of 10 gas mixtures containing O_2 at 0.5%, 2%, 4%, 6%, 8%, 11%, 13%, 15%, 18%, or 21% ± 0.5%. Gas mixtures were made by metering compressed air (0.03% CO_2, 21% O_2, 78% N_2) and compressed nitrogen (99.5% N_2, 0.5% O_2) together in various ratios. The final mixtures were monitored each time the tubes were flushed. Tubes were then sealed with a rubber serum stopper and inverted into a pan of 22C water to prevent gas leaks and maintain a constant temperature. Shoot sections were equilibrated in these atmospheres for4 h, then tubes were reflushed with identical gas mixtures and sealed as before. A 1-ml gas sample was withdrawn through the serum

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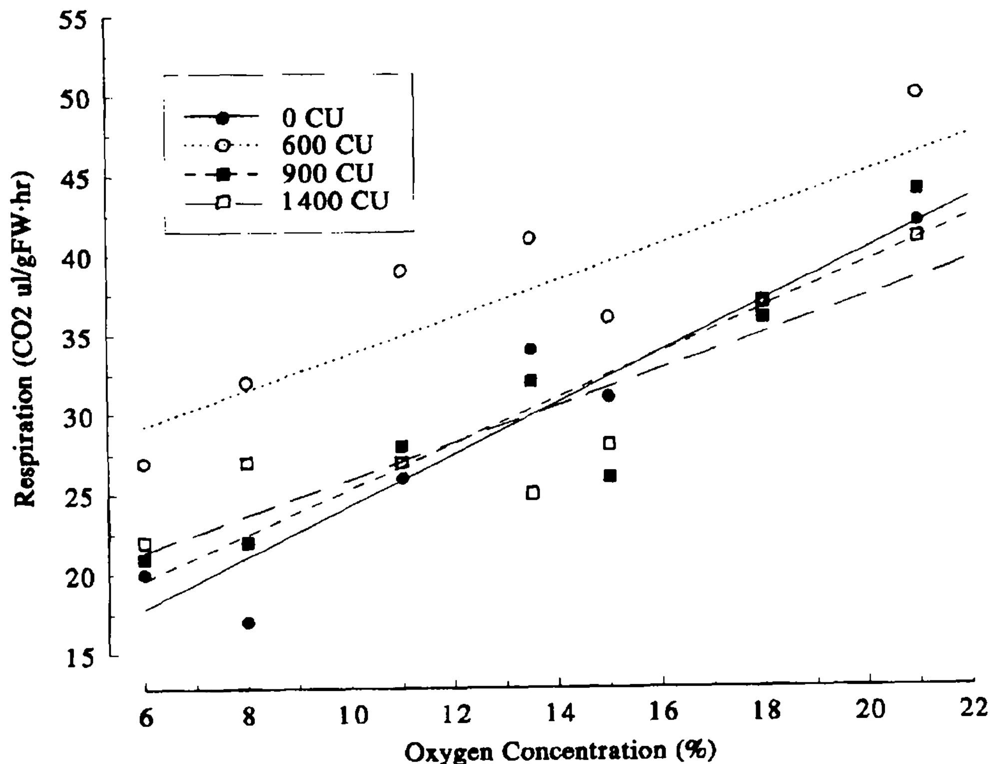

Fig. 1. Respiration at various atmospheric O_2 concentrations as measured by CO_2 evolution of 10 cm-long apple shoot segments that had previously received chillin of 0, 600, 900, or 1400 CU. Data shown are means of four replications. Linear regression equations are as follows: 0 CU, $Y = 1.6X + 8.5$, $r^2 = 0.92$**; 600 CU, $Y = 1.1X + 23.0$, $r^2 = 0.70$*; 900 CU, $Y = 1.4X + 10.5$, $r^2 = 0.85$**; 1400 CU, $Y = 0.9X + 16.5$, $r^2 = 0.79$*.

stopper from each test tube immediately after resealing and after an additional 2 h. Carbon dioxide and O_2 levels were measured on a gas chromatograph using a molecular sieve (13×) column at 50C with a thermal conductivity detector, with helium as a carrier gas. Fresh weight of each shoot section was determined after obtaining respiration measurements. Respiration was calculated as microliters of CO_2 evolved and O_2 consumed per gram fresh weight. The RQ was calculated as the ratio of CO_2 evolved to O_2 consumed per gram fresh weight. Polynomial regression analysis was applied to the rates of CO_2 evolved and O_2 consumed, and logarithmic regression analysis was used for the RQ data.

Results and Discussion

RQ values increased to between 3.0 and 5.5 for trees subjected to O_2 concentrations 14% (data not shown), indicating that respiration had become anaerobic (Wills et al., 1981): therefore, only data for O_2 between 6% and 21% are shown. Respiration, as measured by CO_2 evolved, generally increased as atmospheric O_2 increased across the range of concentrations used for all chilling treatments (Fig. 1). The rate of decrease in respiration appeared to show a slight parabolic curve between 18% and 8% O_2; however, only the linear portion of the polynomial regression analysis was significant for each curve (Fig. 1). There were no significant differences due to chilling treatments in the response of CO_2 evolution to O_2 concentration (i.e., regression slopes were not significantly different). Trees receiving 600 CU had somewhat higher respiration rates over the whole range than the rest (Fig. 1), although the regression intercept was not significantly different from that of the other chilling treatments.

Respiration as measured by O_2 consumption decreased as O_2 concentration decreased across the range of concentrations (Fig. 2). As in Fig. I, the data appeared to be curvilinear, particularly at 0 CU, but only the linear portion of a polynomial regression was significant. The apparent increase in respiration between 21% and 18% O_2 for the 0 CU trees (Fig. 2) may have been due to unusually low respiration rates at 21% O_2 rather than an actual increase as O_2 decreased. The change in O_2 consumption with O_2 concentration, as measured by the slope of the regression lines (Fig. 2), decreased significantly as the trees were chilled from 0 to 600 CU and 600 to 900 CU. No significant difference in O_2 consumption was found between trees chilled for 900 and 1400 CU. These data indicate that apple shoot sections consume less O_2 as they progress through chilling, and that the response curve of respiration to O_2 concentration changes with chilling.

The RQ values did not change significantly at O_2 concentrations between 21% and 15%, but increased somewhat from 15% to 6% (Fig. 3). RQ in 21% to 15% O_2 was ≈ 0.25 for trees that had received 0 CU, 0.50 for trees chilled 600 CU, and 1.00 for trees chilled 900 or 1400 CU (Fig. 3). The RQ values indicate that these trees were using lipids as a significant portion of the substrate for respiration early in the dormancy period. Since the RQ values had increased to 1.0 after 900 CU. the primary respiratory substrate had probably changed to carbohydrates, such as sucrose, by that point in dormancy development.

The results presented here provide further indications that the change in respiratory energy of activation with chilling, reported previously (Young, 1990). is likely to be due to a change in the dominant respiratory pathway during chilling. This pattern could account for changes in energy of activation and RQ values for respiration of apple shoots during and after chilling.

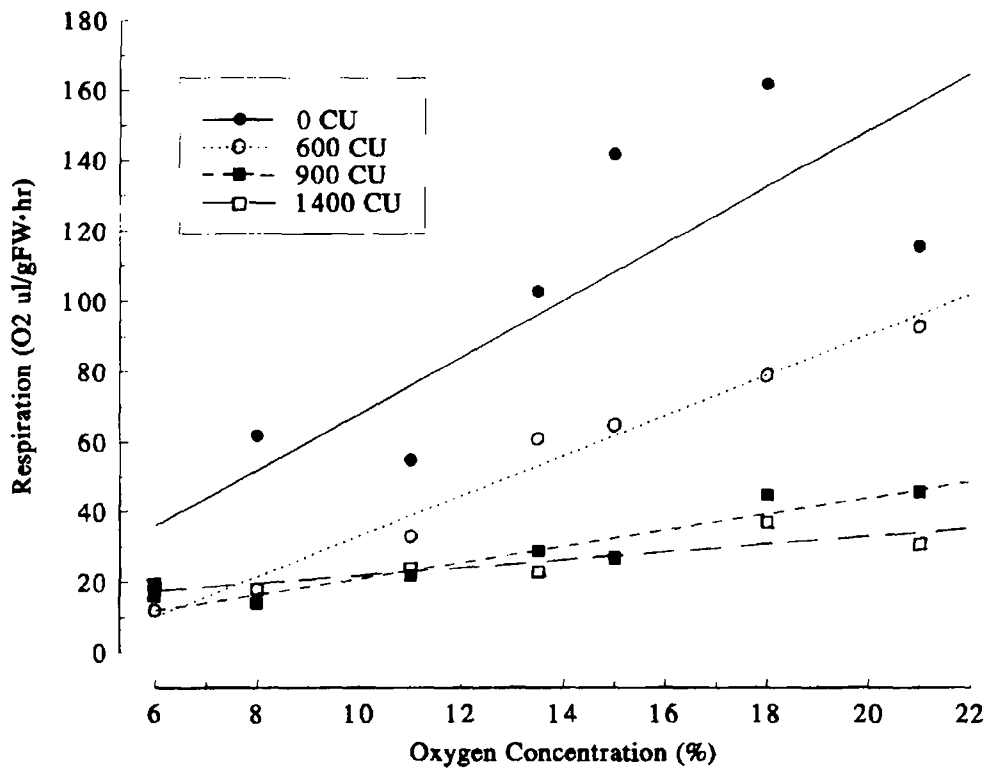

Fig. 2. Respiration at various atmospheric O_2 concentrations as measured by O_2 consumption of 10-cm-long apple shoot segments that had previously received chilling of 0, 600, 900, or 1400 CU. Data shown are means of four replications. Linear regression equations are as follows: 0 CU, Y = 8.1X – 12.5, r^2 = 0.71*; 600 CU, Y = 5.7X – 24.4, r^2 = 0.97**; 900 CU, Y = 2.3X – 1.9, r^2 = 0.91**; 1400 CU, Y = 1.1X + 10.9, r^2 = 0.77*.

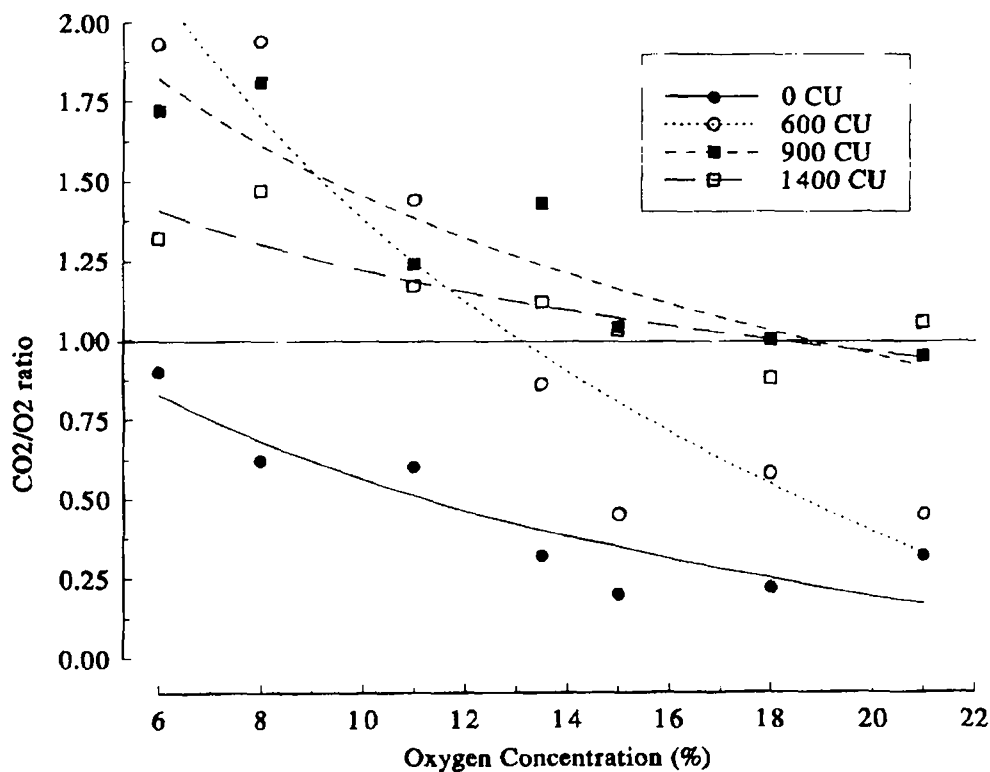

Fig. 3. Respiratory quotient at different atmospheric O_2 concentrations of 10 cm-long apple shoot segments that had previously received chilling of 0, 600, 900, or 1400 CU. Data shown are means of four replications. Logarithmic regression equations are as follows: 0 CU, Y = 1.8 – 0.5lnX, r^2 = 0.83**; 600 CU, Y = 4.7 – 1.4lnX, r^2 = 0.90**; 900 CU, Y = 3.1 – 0.7lnX, r^2 = 0.83**, 1400 CU, Y = 2.1 – 0.4, r^2 = 0.71*.

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