

Late-season temperature effects on the carbon economy and tree performance of 'Royal Gala' apple (*Malus domestica*) trees

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Abstract Potted 'Royal Gala' apple on M.9 rootstock (*Malus domestica*) trees were grown after fruit harvest at constant temperature conditions (18/8°C day/night) for 3, 6, and 9 weeks to manipulate their carbohydrate reserves. On several occasions, leaf gas exchange was measured and selected trees were destructively harvested before and after each treatment to measure the dry weight of the component parts. Samples were also taken for carbohydrate analysis. After treatment, the remaining trees were returned outdoors and, in spring, selected trees from each treatment were destructively harvested for dry weight and carbohydrate analysis. Budbreak was then measured and fruit weight and crop load determined next autumn and the trees destructively harvested for dry weight and carbohydrate analysis. There was no change in tree dry weight after each late-season temperature treatment. Carbohydrate concentrations, averaged over the whole tree, increased by 10–15% compared to the pre-treatment trees, consistent with

a net increase in carbon acquisition. Over winter, the total carbohydrate concentration had declined by 25–40%. In spring, time of budbreak differed significantly; trees exposed for 3 weeks to 18/8°C in late-season broke buds 9–19 days earlier than the other treatments in the following spring. The early budbreak was associated with not only the largest increase in carbohydrate concentration during treatment but also the greatest decrease thereafter. Although there is some support for the conclusion that high carbohydrate reserves confer a direct benefit on the budbreak process, it remains an open question if it was the increase in reserves, or their subsequent consumption that advanced budbreak.

Keywords budbreak; carbohydrates; fruit weight; crop load; photosynthesis; respiration; carbon balance

INTRODUCTION

There have been several recent attempts to assess why New Zealand apple (*Malus domestica* (Borkh.)) yields are relatively higher compared with other countries. The generally accepted hypothesis (Tustin et al. 1997; Wünsche et al. 2000; Greer et al. 2002), is that the relatively mild and long period after harvest in New Zealand enables more carbon to be acquired and stored as reserves in the period up to leaf fall. These reserves could then contribute to a higher carbon availability at budbreak than might occur in cooler Northern Hemisphere orchards. As a consequence, apple trees in New Zealand orchards may reach autotrophic growth sooner and hence have improved growth conditions in spring.

Several different approaches have been followed to address the question of whether extended periods of carbohydrate accumulation in autumn contribute to high crop yields in the subsequent growing season. Tustin et al. (1997) shortened the growing season by partial or full defoliation and these treatments delayed both time of budbreak and full bloom, and reduced yield through reduced fruit size.

This suggested that carbohydrate reserves influenced fruit development.

In another approach, Greer et al. (2002), manipulated the carbon economy in the period after harvest by exposing trees to a range of controlled temperature conditions. Marked differences in total carbon acquisition over a 5-week period occurred between trees in the different temperature regimes, with generally more carbon acquisition as temperature increased. However, differences in carbohydrate concentration were less clear-cut, with the highest concentrations occurring in trees treated at moderate temperatures and lower concentrations at the highest and lowest temperatures. These differences in carbohydrate concentration, nevertheless, had marked effects on budbreak and crop yield in the subsequent growing season, higher carbohydrate reserves being particularly correlated with earlier budbreak and higher apple yields. However, at high temperatures, the 'Braeburn' trees broke bud and flowered profusely during the treatment, thus apparently consuming much of the previously fixed carbon.

To fully assess the hypothesis that high carbohydrate reserves benefit budbreak and crop yield, there is a need to examine if carbon fixed in the period after harvest contributes to these outcomes without the confounding influence of new growth flushes during the treatment phase. The objective, therefore, of the present study was to assess the carbon economy of 'Royal Gala' apple trees during varying temperature exposure periods after harvest and tree performance over the subsequent growing season.

MATERIALS AND METHODS

This experiment was carried out using the facilities of the New Zealand Controlled Environment Laboratory in Palmerston North, New Zealand.

Plant material and growth conditions

Twenty potted trees, contained in 30-litre capacity planter bags, of 3-year-old 'Royal Gala' apple on M.9 rootstock (*Malus domestica* (Borkh.)) were grown outdoors under standard cultural conditions throughout the growing season until fruit were harvested in March. Six trees were transferred to each of three controlled environment rooms in mid April.

The trees were grown at a day/night temperature of $18/8 \pm 0.5^\circ\text{C}$ and at a photon flux density of 700

$\mu\text{mol m}^{-2} \text{s}^{-1}$ at mid-tree height for 10 h in each room for durations of 3, 6, or 9 weeks. The light was provided by a water-screened array of four high-pressure discharge and four tungsten iodide lamps, as described by Greer et al. (1995). The vapour pressure deficit was $0.4/0.3 \pm 0.05$ kPa (day/night). All plants were supplied with a modified Hoaglands nutrient solution (Brooking 1976) throughout the experiment.

At the completion of this stage, four trees from each treatment were returned to outdoors and monitored through the subsequent spring and summer and the other two trees were destructively harvested.

Dry matter measurements

At the start of the experiment, two trees were destructively harvested and separated into components of leaves, spurs (all ages), 1-year-old wood (current season's growth), older wood, trunk, fine roots, coarse roots (>2 mm diam.), and the main root. From each tissue component, a subsample of c. 1 g was randomly collected and stored at -80°C for later carbohydrate analysis. The remaining tissues were vacuum-dried at 40°C for up to a week until dry weights were constant. These are named hereafter as the "pre-treatment" trees.

At the conclusion of each treatment period in the controlled environment conditions, two trees were destructively harvested and separated into the same components. Subsamples were taken for carbohydrate analysis and the remaining tissues dried and weighed as above.

In spring, before budbreak, two trees from each treatment were destructively harvested as above with subsamples from each component again taken for carbohydrate analyses and dry matter determination. Budbreak was then monitored on the two remaining trees from each of the three treatments to determine percentage and time of budbreak. At harvest, all fruit were picked from each tree to determine yield and fruit numbers, after which time the trees were destructively harvested for dry matter and carbohydrate analyses as above.

Photosynthesis and respiration

Immediately after the treatments were imposed, and just before the different temperature durations were completed (3, 6, and 9 weeks), short-term leaf respiration rates were measured in the dark on each of four leaves of each tree, using a leaf chamber and Ciras (PP Systems, Hitchin, United Kingdom) gas exchange system. One hour after the lights came on,

short-term leaf photosynthetic rates were measured on four leaves per tree using the same gas exchange system and leaf chamber at a photon flux density (PFD) of 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Leaf temperature was controlled to match room temperature (18°C) in each instance.

Diurnal changes in photosynthesis and respiration were measured on individual leaves, using a leaf chamber similar to that described by Laing et al. (2002), at the growth temperature and PFD conditions. Between four and six individual leaves were measured for each treatment on each of three occasions throughout the treatment period.

Carbon acquisition was calculated from the daily integration of photosynthesis and respiration and the total leaf area of the trees at each measurement date and then summed over the duration of each treatment, according to the method of Greer & Jeffares (1998).

Non-structural carbohydrate determinations

For each tissue component, a subsample of c. 0.1 g was ground, lyophilised, and then extracted with 20 ml of 80% ethanol at 60°C. The insoluble residue was analysed for starch and the filtrate dried and sugars analysed by gas chromatography according to Greer (1998). For each tissue, the concentrations of fructose, sucrose, glucose, and the sugar alcohol inositol were pooled, since these were individually low in concentration, and are hereafter referred to as the soluble fraction.

Data analysis

All data were analysed using general linear models (SAS 1996) and least squares means and standard errors calculated. Each tree was used as an

experimental unit, hence there were two replications per treatment per sampling time.

RESULTS

Changes in dry matter

After harvest occurred on these apple trees and just before the study, the pre-treatment apple trees were averaging 137 g of leaf dry matter, 466 g of woody tissue, and 276 g of root tissue (Table 1), giving a total of $886 \pm 27 \text{ g tree}^{-1}$. Proportions of dry matter allocation between the various fractions were typically between 0.7% and 15%, whereas the trunk fraction averaged nearly 40% of the total tree dry weight. There was no change in this general pattern of dry matter allocation after the various postharvest treatments were applied (Table 1). However, trees treated for 3 and 6 weeks had markedly higher overall dry weights (1138 ± 21 and $1160 \pm 7 \text{ g tree}^{-1}$), whereas those treated for 9 weeks had less ($801 \pm 16 \text{ g tree}^{-1}$), than those harvested initially. However, these differences may have reflected high variability between the two replicate trees in each treatment. There was no discernible evidence of new growth in any of the trees.

Individual total tree dry weights at the spring harvest ranged between 826 ± 4 and $942 \pm 48 \text{ g tree}^{-1}$ and averaged $896 \pm 16 \text{ g}$, well within the range of the trees sampled before and after treatment, indicating no new growth occurred over winter. However, at the final harvest, the individual dry weights of the trees ranged from 1102 ± 5 to $1147 \pm 4 \text{ g tree}^{-1}$ and averaged $1119 \pm 6 \text{ g}$, again within the typical range for these trees, but also indicating that new growth had occurred.

Table 1 Dry matter of the tree components of the ‘Royal Gala’ apple (*Malus domestica*) trees (mean \pm SE, $N = 2-6$) before (initial) and after treatment at a constant temperature regime of 18/8°C for 3, 6, and 9 weeks after harvest. Also included are the dry matter for the trees in the subsequent spring just before budbreak and also at harvest in the subsequent autumn. Woody stems includes the 1-year-old wood, older wood, and the trunk while the root includes the main root and the fine and coarse roots. For the spring and autumn data, the treatments are pooled as there were no marked differences between them.

Tree component	Treatment					
	Control	3 weeks	6 weeks	9 weeks	Spring	Autumn
Leaves	137.2 \pm 12.1	157.8 \pm 9.3	153.7 \pm 2.7	112.6 \pm 13.5	–	69.9 \pm 8.8
Spurs	6.2 \pm 0.1	7.3 \pm 0.2	5.8 \pm 0.1	5.4 \pm 0.4	6.1 \pm 0.2	6.8 \pm 0.2
Woody stems	466 \pm 44	660 \pm 32	605 \pm 18	436 \pm 21	595 \pm 40	965 \pm 124
Roots	276 \pm 47	314 \pm 39	397 \pm 13	248 \pm 27	295 \pm 18	772 \pm 23
Total	886 \pm 27	1138 \pm 21	1160 \pm 7	801 \pm 16	896 \pm 16	1119 \pm 6

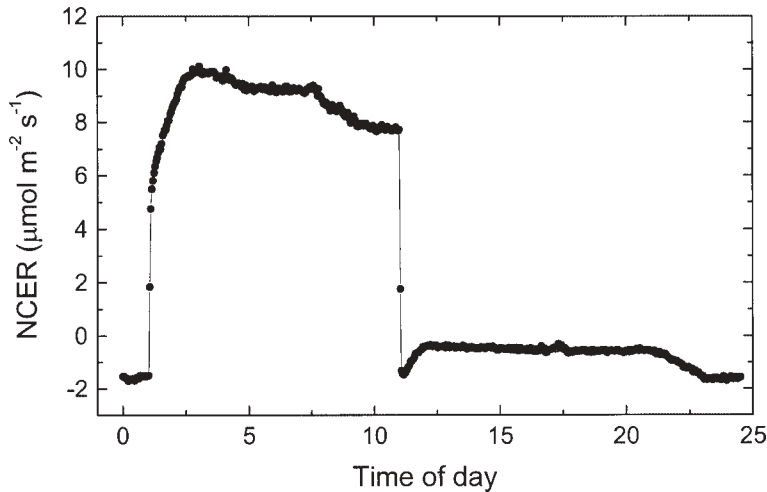


Fig. 1 Changes in gas exchange (photosynthesis and respiration) of 'Royal Gala' apple (*Malus domestica*) leaves in response to diurnal changes in photon flux density and temperature. (NCER, net carbon dioxide exchange rate.)

Table 2 Rates of photosynthesis and respiration (mean \pm SE, $N = 8$) measured on leaves of 'Royal Gala' apple (*Malus domestica*) trees throughout the treatment. Trees were transferred to the controlled environment conditions in mid April.

Treatment	Date of measurement			
	17 Apr	8 May	29 May	19 Jun
Photosynthesis				
3 weeks	6.7 \pm 1.1	6.6 \pm 0.7	–	–
6 weeks	6.8 \pm 1.1	4.2 \pm 0.6	6.9 \pm 0.5	–
9 weeks	5.8 \pm 0.9	6.2 \pm 0.8	3.1 \pm 0.4	6.3 \pm 0.5
Respiration				
3 weeks	-1.1 \pm 0.8	-1.7 \pm 0.04	–	–
6 weeks	-1.0 \pm 0.2	-1.4 \pm 0.2	-1.4 \pm 0.1	–
9 weeks	-1.2 \pm 0.1	-1.1 \pm 0.2	-1.3 \pm 0.2	-1.5 \pm 0.1

Photosynthesis and respiration

Short-term rates

In general, short-term rates of photosynthesis remained reasonably constant between the trees and over time and averaged 5.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 2). However, there were some differences, notably between the 6- and 9-week treatments at the 29 May measurements, with the rate of photosynthesis on trees in the 9-week treatment averaging <45% of the rates on the trees in the 6-week treatment. Nevertheless, by the end of the 9-week treatment, the photosynthetic rates had fully recovered to the levels occurring 40 days earlier.

Rates of respiration (Table 2) tended to increase from the time of application of the treatment conditions, averaging -1.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the start and -1.7, -1.4, and -1.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ after 3, 6, and 9 weeks of treatment, respectively.

Diurnal rates

Photosynthetic rates increased rapidly with the diurnal increase in PFD but only reached a daily maximum rate of c. 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at c. 150 min after the light was turned on (Fig. 1). Thereafter the rates of photosynthesis declined such that by the end of the photoperiod they were c. 8 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Respiration rates at night were generally low but did show slight changes in response to the diurnal changes in temperature.

In general, daily maximum rates of photosynthesis were highest (Table 3) on trees treated to the temperature conditions for 3 weeks. The rates then declined progressively, from c. 9 to 7 $\mu\text{mol m}^{-2} \text{s}^{-1}$, as the treatment duration increased up to 9 weeks. Furthermore, the extent of the diurnal decline in photosynthetic rates also significantly ($P < 0.01$) decreased between all treatments, from 35% to 18%, as the duration increased.

Estimated carbon acquisition (photosynthesis plus respiration) for the trees treated for 3 weeks averaged 46.3 ± 11.8 g tree⁻¹ and this increased on average to 48.8 ± 4.7 g tree⁻¹ over the next 3 weeks and then declined over the final 3 weeks to an average of 31.1 ± 5.7 g tree⁻¹. By the conclusion of the treatment, the apple trees had accumulated an estimated 126.3 g (carbon) tree⁻¹.

Table 3 Daily maximum rates of leaf photosynthesis (P_{\max}), the rates of leaf photosynthesis at the end of the daily photoperiod (P_{end}), and the percentage change in rates of photosynthesis through the photoperiod (mean \pm SE, $N = 4-7$) for ‘Royal Gala’ apple (*Malus domestica*) trees treated for different durations at 18/8°C.

	P_{\max}	P_{end}	% change
3 weeks	9.2 ± 0.20	5.9 ± 0.23	36.5 ± 2.1
6 weeks	8.5 ± 0.16	6.4 ± 0.17	24.9 ± 1.1
9 weeks	7.3 ± 0.22	6.1 ± 0.22	18.0 ± 1.1

Changes in carbohydrate concentration

Pre-treatment trees

Before treatment, these trees had very high concentrations of non-structural carbohydrates in both the fine and coarse root fractions (Fig. 2), predominantly as starch, averaging 250–300 mg g⁻¹ compared with all other tree fractions. Leaves had similar concentrations of sorbitol and starch (80 mg g⁻¹) and the highest concentration of soluble sugars (45 mg g⁻¹). In all other fractions, sorbitol was <50 mg g⁻¹ and soluble sugars were <30 mg g⁻¹. Thus, initially, total carbohydrate concentration of the apple trees averaged 206.7 ± 20 mg g⁻¹.

Treatments at 3, 6, and 9 weeks

After each of the three treatments, the distribution of carbohydrate concentrations (Fig. 2) remained generally similar with the initial pattern, in that of all fractions, roots had the highest concentrations of starch and leaves had high concentrations of sorbitol. In both instances, these generally increased with time

Table 4 Carbohydrate content (mean \pm SE) for ‘Royal Gala’ apple (*Malus domestica*) trees before (control), after postharvest 18/8°C exposure for 3, 6, and 9 weeks, and also in the following spring and at the subsequent harvest.

Treatment	Carbohydrate content (g)			
	Before temperature treatment (Apr)	After temperature treatment	Before budbreak (Sep)	Harvest (May)
Control	173 ± 5.8	–	–	–
3 weeks		242 ± 8.1	101.6 ± 5.7	233.5 ± 10.7
6 weeks		244 ± 8.6	128.5 ± 6.3	230.8 ± 10.2
9 weeks		168 ± 6.5	135.2 ± 6.4	225.9 ± 8.1

Table 5 Percentage and time of 50% budbreak during spring and fruit number and mean fruit weight per tree at harvest for ‘Royal Gala’ apple (*Malus domestica*) trees treated at 18/8°C for 3, 6, and 9 weeks in the preceding postharvest period.

Treatment	Budbreak (%)	Budbreak (Days from 1 Oct)	Fruit number (No./tree)	Fruit weight (kg/tree)
3 weeks	81.7 ± 2.3	8.8 ± 0.5	17 ± 4	2.29 ± 0.21
6 weeks	72.2 ± 9.7	27.3 ± 3.5	37 ± 10	3.17 ± 0.29
9 weeks	76.2 ± 11.9	17.5 ± 1.4	29 ± 6	2.76 ± 0.25

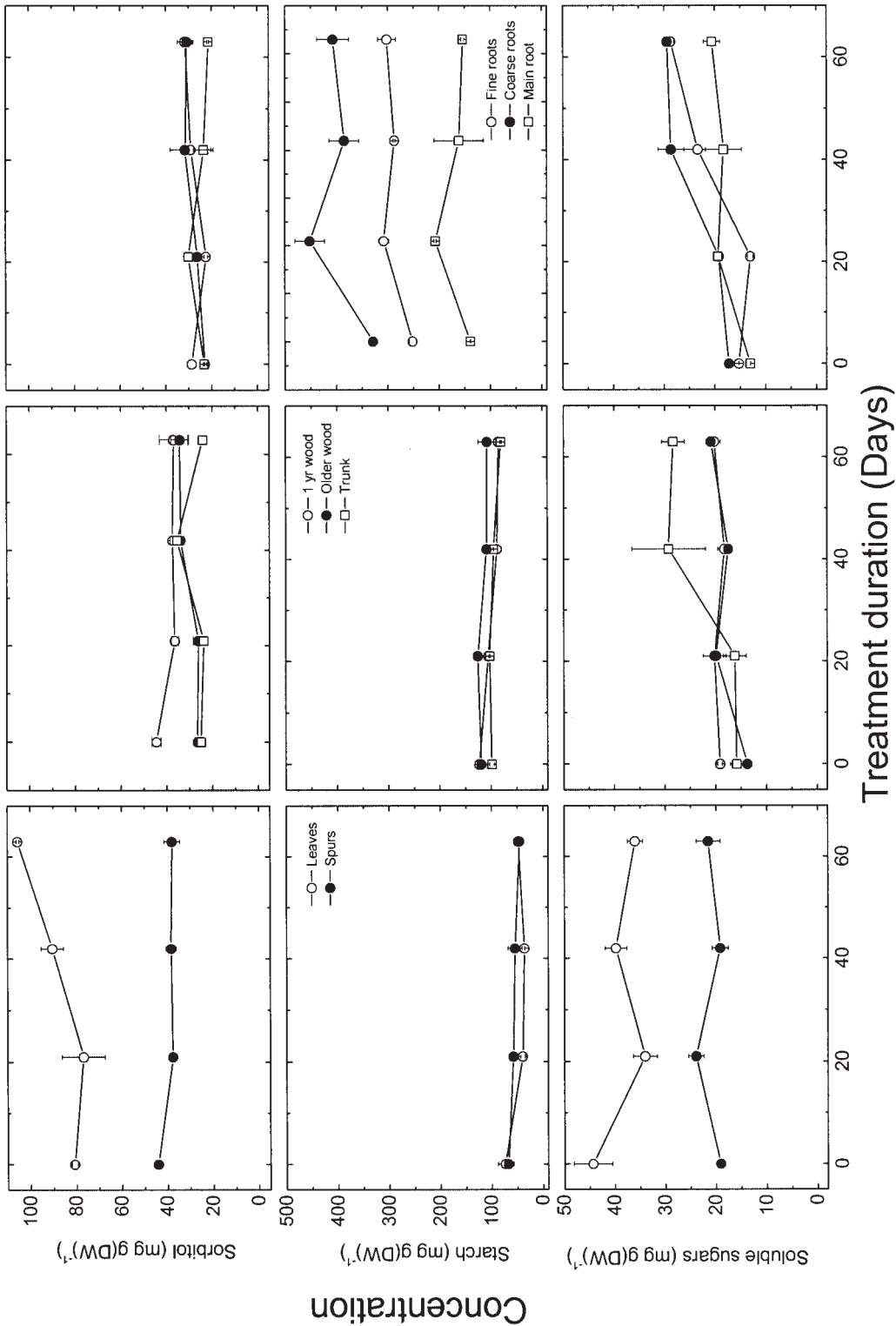


Fig. 2 Carbohydrate concentrations (mean \pm SE, $N = 2$) in the different tree components; leaves, spurs, current season's shoots (1-year-old wood), older wood, tree trunk, fine and, coarse roots, and the main root of the 'Royal Gala' apple (*Malus domestica*) trees both before and after 3, 6, and 9 weeks of treatment at 18/8°C. (DW, dry weight.)

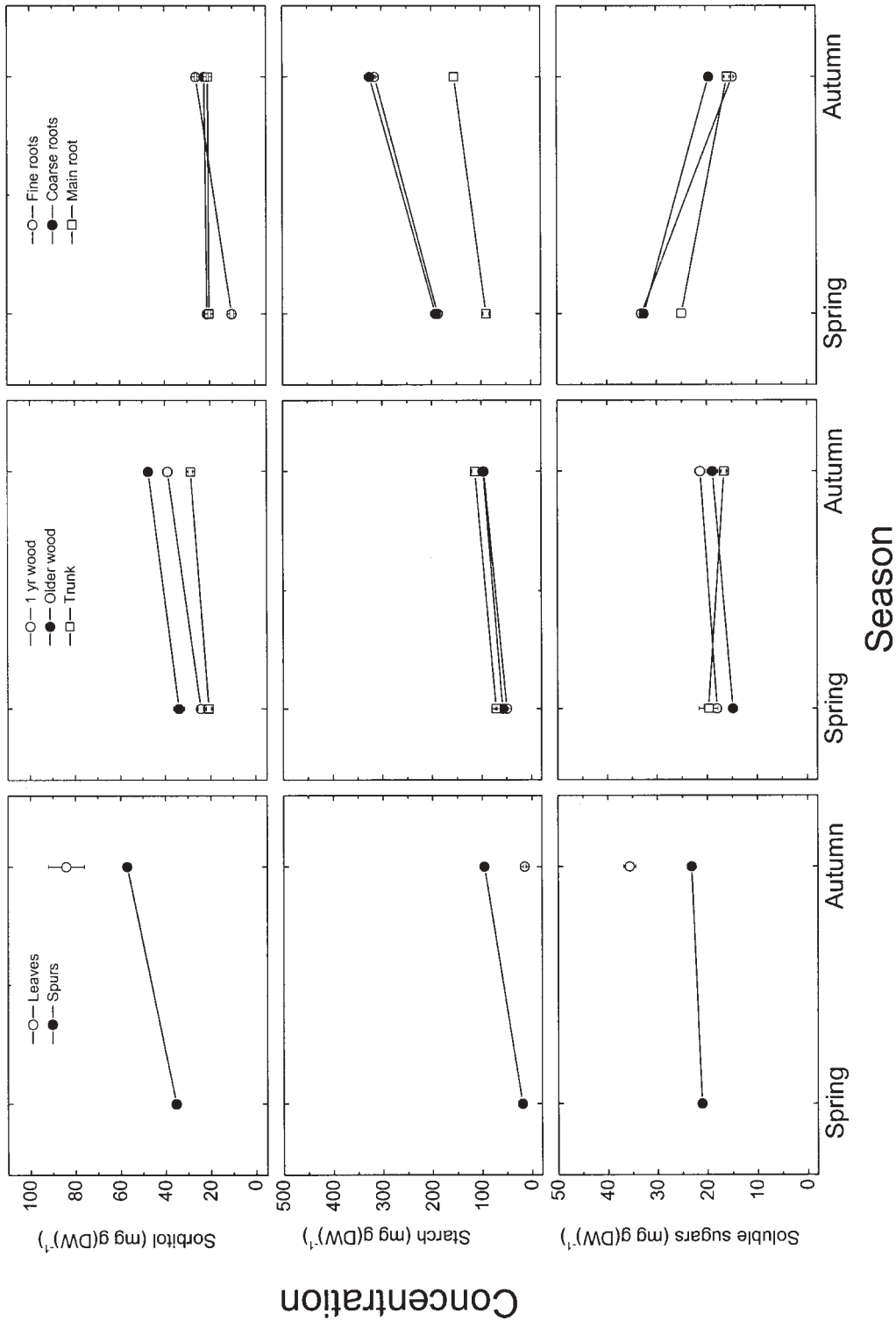


Fig. 3 Carbohydrate concentrations (mean \pm SE, $N = 2-6$) in the different tree components; leaves, spurs, current season's shoots (1-year-old wood), older wood, tree trunk, fine and coarse roots, and the main root of the 'Royal Gala' apple (*Malus domestica*) trees treated with different postharvest treatments and measured in the subsequent spring and at harvest. Since there were no differences in carbohydrate concentrations between the treatments at these times, data have been pooled. (DW, dry weight.)

of treatment. Compared to the initial concentration, starch in both fine and coarse roots increased typically to between 300 and 400 mg g⁻¹. The total carbohydrate concentration for each treatment averaged 211.8 ± 31.7, 202.7 ± 27.1, and 201.2 ± 30.2 mg g⁻¹ for the 3-, 6-, and 9-week treatments, respectively, thus no different from at the start of the treatments.

Following spring

Four weeks before budbreak, concentrations of carbohydrates were very low (Fig. 3). This was caused by a marked decrease in the concentration of starch in the coarse and fine roots to below 200 mg g⁻¹, but also the trunk starch concentration declined c. 30% (cf. Fig. 2 and Fig. 3), whereas soluble sugars in all root fractions increased over the winter. For other tree components, carbohydrate concentrations were less than c. 50 mg g⁻¹. Generally, all trees showed a similar response, with the total carbohydrate concentrations for the 3-week treatment averaging 130.4 ± 14.9 mg g⁻¹ and for the 6- and 9-week treatments, 146.4 ± 18.4 and 150.3 ± 19 mg g⁻¹, respectively. Overall, total carbohydrate concentration declined by 25–38%, relative to the concentration determined after each treatment in the preceding autumn. This suggested carbohydrates were consumed by metabolism during winter and early spring.

Following autumn

Over the subsequent growing season, the trees restored carbohydrate concentrations to levels (Fig. 3) that were comparable to those at the end of the previous growing season. Distribution patterns of carbohydrates across all tree components were also consistent with these occurring previously. There were no differences between the trees from the three treatments at this stage. Starch concentrations of the two main root fractions averaged over 300 mg g⁻¹ and there were also higher concentrations in other tree components. It was notable that leaf starch concentration was again very low and sorbitol the predominant carbohydrate. The concentrations in the various tree components were, however, comparable with those determined before treatment in the previous autumn. Overall, total carbohydrate concentrations averaged 203.0 ± 24.4, 219.4 ± 23.2, and 214.8 ± 20.7 mg g⁻¹ for the 3-, 6-, and 9-week treatments, respectively, thus comparable with those concentrations measured after treatment in the previous autumn.

Changes in carbohydrate content

Consistent with the increase in carbohydrate concentration for the treatment trees was also a marked increase in carbohydrate content (Table 4), at least for up to 6 weeks. Because of the low dry weight of the trees sampled at 9 weeks of treatment and a slight decrease in carbohydrate concentration, these trees showed an apparent decline in carbohydrate content over the course of the treatment. This decline is, nevertheless, consistent with lower rates of photosynthesis and lower net carbon fixation at 9 compared with 6 weeks of treatment.

In spring (Table 4), there was a considerably lower carbohydrate content for the trees from all treatments, typically between 100 and 135 g, again indicative of marked consumption of carbohydrate over the winter. By autumn, however, carbohydrate contents had increased again, to be about comparable with the contents of those trees at the end of the treatment period.

Budbreak and final fruit number and weight

The date of budbreak (time for 50% of buds to break) across treatments covered a 19-day period (Table 5), with trees from the 3-week treatment significantly ($P < 0.05$) earlier on the 8 October and the 6- and 9-week treatment trees delayed until 27 and 17 October, respectively. The percentage of total buds breaking averaged 77% and there were no significant treatment effects.

Fruit numbers (Table 5) ranged between 17 ± 4 and 37 ± 10 but were not significantly different, probably because of the high tree-to-tree variability. Total fruit fresh weight averaged between 2.3 ± 0.2 and 3.2 ± 0.3 kg, but it was statistically different ($P < 0.05$) between the 3-week treatment and the 6- and 9-week treatments. The lower yield of the 3-week treatment is consistent with the lower crop load of these trees, even though average fruit weights were higher (130 cf. 86 and 95 g for the 3-, 6-, and 9-week treatments, respectively).

DISCUSSION

For the 'Royal Gala' apple trees exposed to a postharvest growth temperature of 18/8°C, there were no new growth flushes over the 9 weeks of treatment. High tree-to-tree variability may have obscured some growth responses, especially in the root component, but no buds began to break during the treatment. In the earlier study of Greer et al.

(2002), 'Braeburn' apple trees treated during the postharvest period at temperatures of 24/19°C and 19/14°C had new growth flushes of leaves, flowers, and roots. However, in their 14/9°C temperature treatment, there were no obvious growth flushes and some leaf abscission occurred. This indicated, at least for 'Braeburn' trees in the postharvest period, that the 14/9°C temperature regime appeared to be below the threshold for active growth. Results for the 'Royal Gala' trees in the present study were consistent with this result, possibly reflecting mean temperatures in each study being similar (11.5°C for the earlier and 13°C for the present study).

Short-term rates of photosynthesis of the 'Royal Gala' leaves during the treatments, typically c. 6 $\mu\text{mol m}^{-2} \text{s}^{-1}$, were relatively low compared to other measured rates of photosynthesis in apple leaves (Flore & Lakso 1989; Wünsche et al. 1996; Palmer et al. 1997). However, the rates measured in the present study compared favourably with rates for apple trees without crop or taken a considerable time after harvest (cf. Terhoeven-Urselmans & Blanke 1999; Wünsche et al. 2000). When measured over the entire photoperiod, maximum observed rates of photosynthesis were higher than the short-term rates (cf. Tables 2 and 3), but these diurnal trends also revealed a marked depression in photosynthesis occurring each day. This is consistent with feedback inhibition of photosynthesis (Foyer 1988) and conforms with the removal of the fruit sink at harvest (Wünsche et al. 2000). In further support, this depression of photosynthesis was most marked in the first treatment after harvest (35%).

Feedback inhibition of photosynthesis is often associated with accumulation of starch in the leaves (Greer 1998; Wünsche et al. 2000), especially with leaf starch retained overnight. In this study, there was a marked decline in starch concentration in the leaves compared to the control trees. This lack of starch accumulation in leaves does not appear to conform with end-product inhibition of photosynthesis. However, as samples for carbohydrate analysis were collected only once, diurnal variation in starch concentration could not be assessed. However, apple leaves are known to accumulate starch during the light period in controlled growth conditions (Wang et al. 1997), thus it was possible that the diurnal depression in photosynthesis was caused by daily patterns of leaf starch accumulation.

Over the whole tree, there was an accumulation of starch, notably in the roots, indicative of an active sink for carbohydrates. Young (1989) has also shown for 'Starkspur Supreme Delicious' apple seedlings

that starch accumulated in the roots to a greater extent than in the shoots, at the end of the growing season (see also Chong 1971 and Kandiah 1979). The 'Royal Gala' trees may have subsequently acclimated to the treatment condition, as the extent of feedback inhibition of photosynthesis subsided between 6 and 9 weeks to <20%. However, between 6 and 9 weeks of postharvest 18/8°C treatment, the decline in both maximum photosynthesis and carbohydrate concentration was symptomatic of leaf senescence occurring over this time in the constant growth conditions. Senescence would also have occurred in orchard-grown trees over this time (mid May–early June).

The estimated net carbon balance of the trees remained high in each of the first two treatment periods, with nearly 50 g tree⁻¹ of carbon accumulated in each period. In the subsequent period (6–9 weeks), the net carbon balance declined to c. 30 g tree⁻¹, again probably caused by leaf senescence and the associated decline in photosynthesis. Over the whole 9 weeks, the apple trees fixed a net total of 126 g (carbon) tree⁻¹. This compares favourably with the net carbon fixation rates determined by Greer et al. (2002) for 'Braeburn' trees at similar temperatures. The overall increase in carbon supply throughout the treatment period is consistent with the increase in carbohydrate concentrations over the same time. Given that this increase, primarily of starch, was most evident in both the coarse and fine roots, marked export of carbon from the source leaves and transport to the roots for storage as reserves must have occurred. Consistent with this, Teng et al. (1999) showed that after leaf abscission, 60% of recoverable ¹³C from a pulse experiment was found in roots of pear.

In spring, the trees had markedly reduced concentrations of carbohydrates compared with the concentrations determined both before and after the postharvest treatments, with the 3-week treatment decreasing by the greatest amount. Nevertheless, the general pattern of concentration differences between the tree components was maintained, that is, highest concentrations occurred in coarse and fine roots. Thus, between winter and just before budbreak, these trees apparently consumed up to 25–38% of the total carbohydrate concentration. It is not clear what plant process consumed the carbon but presumably respiration by roots and stems would be primary contenders (Wibbe et al. 1994; Teng et al. 1999). Similar results have been shown in pear (Teng et al. 1999), apple (Brown et al. 1985), and blueberry (Darnell & Birkhold 1996).

The percentage of buds breaking in spring was generally similar across the three treatments, although time of budbreak was significantly earlier in the trees treated for 3 weeks compared to those treated for 6 and 9 weeks. Concomitant with this was also the greatest decline carbohydrate concentration, from 212 to 130 mg g⁻¹ in the trees treated at 3 weeks. It is, therefore, tempting to conclude that this consumption of carbohydrate contributed to the advanced budbreak (Worley 1979; Keller & Loescher 1989). However, it is not clear whether it was the high concentration of starch especially in the roots after the 3-week treatment, or the consumption of carbohydrate over winter, that conferred the change for budbreak to occur in these apple trees. Further work is thus needed to clarify the underlying relationships between carbohydrate accumulation, consumption, and budbreak.

Throughout the growing season, all trees fully recharged their carbohydrate pools, with the concentrations at harvest comparable with those at the previous harvest. Although there were some differences in fruit numbers and yield at harvest, there were no persistent effects of the treatments from the previous autumn. This contrasts with our earlier study (Greer et al. 2002), where crop load was strongly affected by the postharvest treatments in the preceding season, although this study focused on the influence of different temperatures.

In summary, maintaining the 'Royal Gala' trees at a moderate temperature after harvest for extended periods of time resulted in a general increase in carbohydrate concentration, consistent with increased carbon fixation. However, this extra carbohydrate and also a proportion of that stored as reserves had apparently been consumed in metabolic processes before budbreak occurred. Although this decline in carbohydrate concentration was consistent with other studies, the underlying relationship between winter metabolic activity and budbreak needs to be addressed. Thus, there still remains an open question about the relationship between carbohydrate accumulated in the postharvest period and the benefit conferred on the trees in the following spring.

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REFERENCES

- Brooking, I. R. 1976: Soilless potting media for controlled environment facilities. *New Zealand Journal of Experimental Agriculture* 4: 203–208.
- Brown, C. S.; Young, E.; Pharr, D. M. 1985: Rootstock and scion effects on the seasonal distribution of dry weight and carbohydrates in young apple trees. *Journal of the American Society for Horticultural Science* 110: 696–701.
- Chong, C. 1971: Study of the seasonal and daily distribution of sorbitol and related carbohydrates within apple seedlings by analysis of selected tissues and organs. *Canadian Journal of Plant Science* 51: 519–525.
- Darnell, R. L.; Birkhold, K. B. 1996: Carbohydrate contribution to fruit development in two phenologically distinct rabbiteye blueberry cultivars. *Journal of the American Society for Horticultural Science* 121: 1132–1136.
- Flore, J. A.; Lakso, A. N. 1989: Environmental and physiological regulation of photosynthesis in fruit crops. *Journal of the American Society for Horticultural Science* 119: 596–603.
- Foyer, C. H. 1988: Feedback inhibition of photosynthesis through source-sink regulation in leaves. *Plant Physiology and Biochemistry* 26: 483–492.
- Greer, D. H. 1998: Photoinhibition of photosynthesis in dwarf bean (*Phaseolus vulgaris* L.) leaves: effect of sink-limitations induced by changes in daily photon receipt. *Planta* 205: 189–196.
- Greer, D. H.; Jeffares, D. 1998: Temperature-dependence of carbon acquisition and demand in relation to shoot growth of kiwifruit (*Actinidia deliciosa*) vines grown in controlled environments. *Australian Journal of Plant Physiology* 25: 843–850.
- Greer, D. H.; Laing, W. A.; Campbell, B. D. 1995: Photosynthetic responses of thirteen pasture species to elevated CO₂ and temperature. *Australian Journal of Plant Physiology* 22: 713–722.
- Greer, D. H.; Wünsche, J. N.; Halligan, E. A. 2002: Influence of postharvest temperatures on leaf gas exchange, carbohydrate reserves and allocations, subsequent budbreak, and fruit yield of 'Braeburn' apple (*Malus domestica*) trees. *New Zealand Journal of Crop and Horticultural Science* 30: 175–185.

- Kandiah, S. 1979: Turnover of carbohydrates in relation to growth in apple trees. II. Distribution of ^{14}C assimilates labelled in autumn, spring and summer. *Annals of Botany* 44: 185–195.
- Keller, J. D.; Loeschner, W. H. 1989: Non-structural carbohydrate partitioning in perennial parts of sweet cherry. *Journal of the American Society for Horticultural Science* 114: 969–975.
- Laing, W. A.; Greer, D. H.; Campbell, B. D. 2002: Strong responses of growth and photosynthesis of five C_3 pasture species to elevated CO_2 at low temperatures. *Functional Plant Biology* 29: 1089–1096.
- Palmer, J. W.; Giuliani, R.; Adams, H. H. 1997: Effect of crop load on fruiting and leaf photosynthesis of 'Braeburn'/M.26 apple trees. *Tree Physiology* 17: 741–746.
- SAS Institute Inc. 1996: SAS/STAT Software: Changes and enhancements through release 6.11. Cary, North Carolina.
- Teng, Y. W.; Tanabe, K.; Tamura, F.; Itai, A. 1999: Translocation of ^{13}C -assimilates in the spring following fall assimilation of $^{13}\text{CO}_2$ by 'Nijisseiki' pear (*Pyrus pyrifolia* Nakai). *Journal of the Japanese Society for Horticultural Science* 68: 248–255.
- Terhoeven-Urselmans, A.; Blanke, M. M. 1999: Post-harvest photosynthesis of apple leaves. *Gartenbauwissenschaft* 64: 233–238.
- Tustin, D. S.; Stanley, C. J.; Adams, H. H. 1997: Physiological and phenological responses of apple trees to artificial reduction of the growth period from harvest to leaf fall. *Acta Horticulturae* 451: 383–392.
- Wang, Z.; Yuan, Z.; Quebedeaux, B. 1997: Photoperiod alters diurnal carbon partitioning into sorbitol and other carbohydrates in apple. *Australian Journal of Plant Physiology* 24: 587–595.
- Wibbe, M. L.; Blanke, M. M.; Lenz, F. 1994: Respiration of apple trees between leaf fall and leaf emergence. *Environmental and Experimental Botany* 34: 25–30.
- Worley, R. E. 1979: Fall defoliation and seasonal carbohydrate concentration of pecan wood tissue. *Journal of the American Society for Horticultural Science* 194: 195–199.
- Wünsche, J. N.; Lakso, A. N.; Robinson, T. L.; Lenz, F.; Denning, S. S. 1996: The bases of productivity in apple production systems: the role of light interception by different shoot types. *Journal of the American Society for Horticultural Science* 121: 886–893.
- Wünsche, J. N.; Palmer, J. W.; Greer, D. H. 2000: Effects of crop load on fruiting and gas-exchange characteristics of 'Braeburn'/M.26 apple trees at full canopy. *Journal of the American Society for Horticultural Science* 125: 93–99.
- Young, E. 1989: First-year shoot development and carbohydrate distribution in fall- and spring-planted apple trees. *HortScience* 24: 234–236.

