Spur Leaves Increase Calcium in Young Apples by Promoting Xylem Inflow and Outflow

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ABSTRACT. The effects of spur leaf removal on xylem sap flows and calcium accumulation in fruit of apple (*Malus × domestica* Borkh. 'Royal Gala') were determined 56 to 61 days after full bloom. Fruit calcium concentrations were reduced but fruit size was not influenced by partial spur defoliation at bloom. Apples exchanged xylem sap with the tree in daily cycles of flow reversal. The presence of local spur leaves promoted this exchange by accentuating the xylem sap drawn out of the fruit during the day, requiring more to flow back into the fruit at night to replace it. Calcium concentrations were lower in the xylem sap leaving the fruit than in that entering it. The reduced calcium accumulation in the fruit borne on defoliated spurs can therefore be attributed to the reduced volume of xylem sap exchanged between tree and fruit.

The close relationship between apple (*Malus × domestica*) calcium concentration at commercial harvest and fruit condition after storage is well known (Ferguson and Watkins, 1989). Fruit with low calcium concentrations are more susceptible to disease and physiological disorders such as bitter pit and lenticel blotch, and fruit senescence may be hastened compared with fruit of high calcium concentrations. Calcium accumulation in apples is influenced by a number of factors including cultivar, season and, to some extent, cultural practices such as crop loading and fruit thinning, summer and winter pruning. Many of these factors affect fruit calcium by changing fruit size; fruit calcium concentration and fruit size are inversely related (Perring and Jackson, 1975) and a reduction in fruit size is often associated with higher fruit calcium concentrations (Ferguson and Watkins, 1989).

Alteration of the number of spur leaves associated with the fruit is one canopy factor that might change fruit calcium independent of any influence of fruit size. It has been shown that fruit borne on spurs with large spur leaf areas have similar fruit size but higher calcium concentrations than fruit borne on spurs with lower leaf areas (Jones and Samuelson, 1983; Volz et al., 1994). The relationship has, moreover, been shown to be causative and not merely correlative. Experiments in which primary spur leaves and/or bourse shoots have been removed shortly before (Ferree and Palmer, 1982), during (Volz et al., 1996), or after bloom (Jones and Samuelson, 1983) have resulted in fruit with lower calcium contents. We must conclude that the presence of a large number of nearby leaves on a spur increases calcium transport into a fruit, although the mechanism by which this occurs is not known.

Calcium is known to move in the xylem but not in the phloem (Marschner, 1983). The mass flow of dissolved calcium in the xylem sap is therefore expected to be the main means for its entry to an apple (Ferguson and Watkins, 1989), although extravascular movement of calcium has been suggested (Bangerth et al., 1989). Ignoring other minor exchanges, the volume of water entering a fruit, i.e., the xylem

plus phloem inflow, will equal the volume transpired from its surface plus that stored in the fruit (Lang, 1990). The bagging of individual fruit thus can reduce their transpiration and fruit calcium input (Jones and Samuelson, 1983; Witney et al., 1991). However other studies have shown that for some seasons and periods of fruit growth there is little effect of bagging on fruit calcium (Cline and Hanson, 1992; Ford, 1979), suggesting that a simple fluid balance model is inadequate. Other factors would seem to be involved. The inadequacy of the simple model is further shown by the work of Jones et al. (1983), who found discrepancies between the measured and calculated rates of calcium accumulation in young apples based on net xylem inflows.

Several studies have shown that apples commonly shrink during the day and expand at night (Tromp, 1984; Tukey, 1964) and that this pattern is due in part to xylem cycling—a daily reversal in the direction of xylem sap flow to the fruit (Lang, 1990). Xylem sap generally flows from fruit to tree during the day but from tree to fruit during the night with greater outflows and subsequent inflows when the weather favors high rates of transpiration. Changes in apple water potential have been found to parallel similar changes in leaf water potential during the day (Goode et al., 1979), which supports the concept that xylem sap exchange between tree and fruit occurs in response to diurnal change in the water potential of the spur.

A simple net xylem flow model for predicting fruit calcium nutrition assumes the calcium concentrations of the inflowing and outflowing saps to be the same. If they are not, then measurement of the actual inflows and outflows, and their calcium concentrations, is required for predicting fruit calcium nutrition and the pattern of xylem sap cycling becomes much more important. Jones et al. (1983) indeed present evidence that the outflowing sap contains much less calcium than the inflowing sap. Accepting that xylem sap cycles in and out of the fruit daily and that the sap leaving the fruit contains substantially less calcium than that entering it, it is to be expected that any factor which increases the amplitude of xylem inflow will also increase the accumulation of calcium in the fruit. We can anticipate also that transpiration of spur leaves will emphasize the cycle of water potential change in the spur, so increasing the volume of xylem sap exchanged with the fruit. Combining these ideas, we can hypothesize that the net import of calcium to a fruit may be promoted by an increased xylem sap exchange associated with a large area of subtending spur leaves.

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In this study we examined the above hypothesis. We measured the effect of subtending spur leaf area on fruit calcium nutrition and on the volumes of xylem sap cycling between tree and fruit, and we measured the calcium concentrations in the xylem sap entering and leaving the fruit.

Materials and Methods

TREES. Twenty 'Royal Gala' apple trees in a single row at the Massey University Orchard in Palmerston North, New Zealand were chosen for the leaf area experiment. The orchard is situated on a sandy-silty loam with excellent drainage characteristics. Trees had been topworked 5 years previously onto 'Captain Kidd' trees planted in 1978 on MM 106 rootstock. In a separate experiment the fruit and spur wood from which xylem sap was extracted were obtained from four 'Royal Gala' trees at the HortResearch Orchard in Hawkes Bay, New Zealand. These trees were planted in 1985 on MM 106 rootstock and grew in a deep, fertile, recently deposited alluvium. All trees at both orchards were irrigated and managed according to normal commercial practice except for the trees at Massey University to which calcium and chemical thinning sprays were not applied. All clusters on the trees were hand thinned to one fruit 21 d after full bloom (DAFB) (30 Oct.).

LEAF AREA MANIPULATION. Four spur clusters (2- to 3-year) were chosen on each of the 20 trees located at Massey University at king full bloom (9 Oct.) and all lateral flowers were removed to leave a single flower on each. Two of these spurs, selected at random, were left as intact controls and, from the other two, most of the primary spur leaves and all of the developing shoot (bourse) leaves were removed. A minimum of one to two primary spur leaves was left per spur to encourage fruit set. Any regrowth of bourse shoots on the treated spurs was removed at 7- to 14-d intervals during the study to maintain the treatment. Fruit drop was similar for both treatments with a total of 26 and 22 fruit from intact and defoliated spurs respectively, available for analyses.

XYLEM FLOW MEASUREMENTS. From the 48 spurs that bore fruit, 10 similar-sized fruit on seven adjacent trees were selected for xylem sap flow measurement starting 56 DAFB (4 Dec.) and continuing for the following 4 d. During the first 3 d these included a comparison of four fruit borne on partially defoliated/deboursed spurs and six on control spurs. The remaining spur leaves on the four partially defoliated/deboursed spurs were removed at 56 DAFB to maximize differences between them and the six control spurs. After 3 d of measurement, primary spur leaves and bourse shoot(s) were removed from the six control spurs so that for the final 2 d of measurement none of the instrumented fruit had any associated primary spur or bourse leaves.

Fruit volume is known to change continuously as a result of various fluid exchanges taking place between the fruit and the tree (via the pedicel) and between the fruit and the atmosphere (via the epidermis). Under the conditions of our experiment, fruit volume changes are expected to comprise a xylem component, a phloem component and a fruit transpiration component—the fruit were not wet so that a component of osmotic water uptake through the epidermis did not occur. Xylem sap flows were calculated from the fruit volume profiles by subtracting estimates of the contributions made by the phloem and by fruit transpiration based on separate measurements in which fruit stalks had been either steamed (to stop phloem transport) or detached (to stop phloem and xylem transport) (Lang, 1990). A total of 50 profiles of cumulative xylem flow were produced, 18 of these were for fruit with foliage (6 fruit $\times$ 3 d) and 32 without foliage (4 fruit $\times$ 3 d and 10 fruit $\times$ 2 d). From each profile we were thus able to extract values for xylem efflux, xylem influx and net xylem influx. The experiment was organized as a split-plot design so

that tree, day number and leaf area effects on xylem flows could be determined separately.

Volume changes in fruit water flows were estimated using a method similar to that described in Lang (1990), employing linear-variable-displacement transducer (LVDT) equipment. The LVDT equipment provided an accurate ($\pm 1 \mu\text{m}$) measurement of the diameter of each fruit every 15 min. Temperature recordings made at the same time allowed corrections to be made for the thermal expansion of the fruit, of the measuring apparatus and for any temperature sensitivity of the LVDTs and associated electronic equipment. We made the assumptions that a significant change in fruit shape did not occur diurnally with water gains/losses or as a result of the small amount of growth during the 5 d measurement period. This allowed LVDT measurements of diameter change to be expressed as diurnal profiles of volume change where, for a small fractional diameter change f , the corresponding fractional volume change is $3f$. Sphericity was assumed, in using diameter to convert fractional volume changes to mL and small changes in fruit volume were assumed to represent equal changes in water content of the fruit.

FRUIT CALCIUM AND LEAF AREA MEASUREMENTS. When sap flow measurements were completed 61 DAFB (9 Dec.), all spurs bearing fruit were harvested. For each spur, fruit fresh mass was recorded and the area of primary spur and bourse leaves was measured using a leaf area meter (LI-3100; LI-COR Inc., Lincoln, Neb.). Each fruit was then completely digested fresh in nitric/perchloric acids and analyzed for total calcium by atomic absorption spectrophotometry (Turner et al., 1977).

XYLEM SAP EXTRACTION. To assess the importance of xylem cycling to fruit calcium nutrition we determined calcium concentration of the xylem sap as it flowed out of the fruit to the tree and back. Samples considered to be representative of the outflowing xylem sap were obtained from fruit and of the inflowing xylem sap from subtending shoots from the 'Royal Gala' trees in Hawkes Bay. At 20 to 40 d intervals during the season, xylem sap was extracted from fruit taken at random from within the tree canopies. On each occasion fruit were collected, just predawn, and with the spur still attached, placed in polythene bags pending sap extraction during the morning of the same day. For sap extraction, the fruit pedicel was cut with a sharp blade just distal to the point of attachment to the spur. The fruit was then placed in a pressure bomb with the cut surface of the pedicel connecting with the outside via a silicone rubber adapter and a length of fine stainless steel tube (Lang and Ryan, 1994). A steady pressure of 1.38 MPa was then applied, which is similar to negative values of fruit water potential measured in young developing apples (Mills et al., 1996). Under these conditions xylem sap was expressed steadily into a small vial at a rate of $\approx 0.15 \text{ mL}\cdot\text{h}^{-1}$. Where sap could be extracted from the pedicel, volumes of $\approx 0.1 \text{ mL}$ per fruit were collected and calcium concentrations determined using atomic absorption spectrophotometry.

Wood xylem sap was extracted from 2-year wood bearing a terminal fruiting cluster on 20 Oct., 1 Dec., and 27 Jan. of the following year, which spans the time when most calcium is moving into 'Royal Gala' fruit (Volz et al., 1996). This material was also collected early in the day. Fruit and leaves were removed and the lengths of stem were placed in polythene bags. Sap extraction later the same day was by vacuum with air displacement (Bollard, 1953). For this, a straight length of 2-year wood, 100 mm long, was cut with a sharp blade and vacuum was applied to the proximal end. This arrangement yielded $\approx 0.1 \text{ mL}$ of xylem sap in 5 min, which was collected into a small vial. Calcium analysis of sap samples from wood and fruit was carried out by atomic absorption spectrophotometry.

Analysis of variance and regression analyses of data in each experiment were carried out using the GLM procedure of SAS (SAS Institute, Cary, N.C.).

Fig. 1. A typical fine-day profile of cumulative xylem flow from and into a 'Royal Gala' apple from a defoliated spur over a 24-h period from 0800 HR on 4 Dec. to 0800 HR on 5 Dec. 1991. The xylem sap flow measurements used in the subsequent analyses are (a) the xylem efflux, (b) the xylem influx, and (c) the net xylem influx.

Results

LEAF AREA AND XYLEM CYCLING. Fruit xylem sap exhibited diurnal cycles of outflow (during the day) and inflow (during the night) with significant variation from day to day. The average daily net inflow of xylem sap was $0.26 \pm 0.04 \text{ mL} \cdot \text{d}^{-1}$, and a diurnal change in direction always occurred (Fig. 1). Rates of fruit transpiration ($0.32 \pm 0.04 \text{ mL} \cdot \text{d}^{-1}$) and phloem sap inflow ($1.23 \pm 0.04 \text{ mL} \cdot \text{d}^{-1}$) also fluctuated over any 24-h period but their direction of flow did not change.

About half of the xylem sap volume entering the fruit at night was for replacing water drawn out to the tree during the early part of the day. The other half contributing (along with the water entering in the phloem sap) to the daily increment in fruit volume growth and to offset transpiration losses through the epidermis (Fig. 1, Table 1). There was a strong positive linear relationship between efflux and influx for individual days and the influx was about twice the amount of the efflux (Fig. 2).

Tree and day number both had considerable effects ($P < 0.001$) on xylem flows, consequently, xylem flows were adjusted for tree and day effects to clarify relationships with leaf area. Defoliation reduced ($P = 0.01$) volumes of xylem sap exchanged between tree and fruit (Table 1). For each 100 cm^2 increase in spur leaf area, xylem efflux and influx increased linearly by 0.029 and $0.076 \text{ mL} \cdot \text{d}^{-1}$ respectively (Fig. 3). Fruit with no leaves present had significant efflux and influx. Defoliation did not change the general pattern of diurnal reversal of direction.

FRUIT SIZE AND CALCIUM. Partial defoliation of spur leaves at bloom reduced calcium accumulation in the fruit but not fruit mass (Table 2). For individual spurs, fruit calcium content increased linearly with increasing total leaf area (Fig. 4).

XYLEM SAP CALCIUM CONCENTRATION. It became progressively more difficult to obtain xylem sap samples from apples using the pressure bomb technique as the season progressed (data not shown) and is reflective of a changing property of the fruit rather than a weakness of the technique (Lang and Ryan, 1994). As a result, the majority of the 85 fruit sap samples were obtained during the 70 d period from bloom. Fruit xylem sap calcium concentrations ($0.17 \pm 0.04 \text{ mmol} \cdot \text{L}^{-1}$) did not change significantly with time. Xylem sap samples obtained from spur wood by vacuum extraction were available throughout the season. Again there was no evidence of change in calcium concentration during the season (data not shown) but the average values ($1.27 \pm 0.32 \text{ mmol} \cdot \text{L}^{-1}$) were nearly seven times higher than in the saps obtained from the fruit.

Discussion

Partial defoliation of spur leaves at bloom reduced calcium accumulation in 'Royal Gala' fruit, confirming reports for this (Volz et al., 1994, 1996) and other cultivars (Ferree and Palmer, 1982; Jones and Samuelson, 1983). In an earlier study on 'Royal Gala' it was suggested that decreases in fruit calcium uptake in response to leaf area reduction may be less for those fruit borne on spurs with larger as opposed to smaller leaf areas (Volz et al., 1994). In contrast, our data in the present work indicate that the fruit calcium response to leaf area reduction was similar across a wide range of total leaf areas (Fig. 4). While it is difficult to ascertain if the different patterns of the calcium/leaf area relationship are real, the timing of harvest may explain them. In previous studies, fruit were picked at maturity (commercial harvest) while in our experiment, fruit were harvested much earlier, 9 weeks after bloom.

Our experiment involved estimating fruit water volumes and subsequent xylem water fluxes from fruit growth measurements, and several assumptions were made in these determinations (Lang, 1990). While errors in these estimates due to our assumptions being incorrect are possible, we believe that they would not have been substantial enough to alter our interpretation of the data. First, we have calculated a likely error introduced by our assumptions, and found it to be similar to that introduced by natural differences in growth among individual fruit (10% to 15%). Second, whatever the assumptions made underlying the calculation of the sap flows, errors arising from any of these would have been the same across our treatments.

The typical pattern of xylem daytime outflow and nighttime inflow found for 'Royal Gala' fruit is also consistent with an earlier study (Lang, 1990). Xylem sap cycling is thought to be driven by cycles of change in spur water potential, the daily excursions of spur water being more pronounced when transpiration is greatest (Lang, 1990; Tromp, 1984). This raises the question of the relative importance to spur water potential (and thus to the cycling of xylem sap between tree and fruit) of transpiration from the various parts of the tree (i.e. transpiration through the epidermis of the fruit, the associated spur leaves, or the canopy as a whole). A study by Jones and

Table 1. Mean daily xylem sap efflux and influx for young 'Royal Gala' apples 56 to 61 d after full bloom, as affected by spur leaf defoliation. Individual fruit values were adjusted for tree and day effects before ANOVA and means are averages of these values.

Treatment	Leaf area/spur (cm^2)	Efflux ($\text{mL} \cdot \text{d}^{-1}$)	Influx ($\text{mL} \cdot \text{d}^{-1}$)
Intact	195	0.29 a ^z	0.61 a
Defoliated	0	0.23 b	0.46 b

^zValues within the same column followed by different letters are significantly different by pairwise *t* test ($P = 0.01$).

Fig. 2. Relationship between xylem efflux and influx for 'Royal Gala' apples. Each point represents one measurement within a 24-h period for a single fruit. The slope is significant ($P < 0.001$) and the intercept is not significantly different from zero. Influx = $-0.0381 + 2.20$ efflux; $r^2 = 0.74$.

Higgs (1985) supported the idea that whole canopy transpiration was more important than transpiration from the fruit itself. Our finding of a significant effect of spur leaf removal on xylem sap flow agrees with this but suggests that it is the transpiration from the foliage most closely associated with the fruit (i.e. of the spur leaves) that dominates the daily shrinking and swelling of the fruit. Our observation, however, that spur leaf removal reduced, but did not stop, xylem outflows indicates that spur water potential is also subject to the effects of transpiration from foliage further away in the canopy.

Jones and Higgs (1985) found that defoliating individual spurs had no significant effect on fruit shrinkage—a result contrary to ours—but then it is likely that evapotranspirative demand (in UK) was less than in our New Zealand study so that the local effect would be less pronounced. In another study with 'Golden Delicious' (Jones and Higgs, 1982), it was found that diurnal shrinkage was positively related to solar radiation. However, in contrast to our results here and to some previous studies (Lang, 1990; Tromp, 1984), it was concluded that fruit rather than leaf transpiration, had the major effect on fruit shrinkage. This interpretation is considered unlikely for apple for several reasons. First, where on a number of occasions we have measured rates of xylem backflow in apple, we have found them to be between three and ten times greater than rates of fruit transpiration (Lang, 1990). Xylem backflow is considered therefore the more likely to dominate a fruit's water balance. Second, if shrinkage was due to a fruit's own transpiration, the fruit would shrink fastest when the evapotranspirative demand is greatest—in the early afternoon. Instead we find that apples generally shrink fastest during the morning, the rate of shrinking slowing to zero by the early afternoon

(Fig. 1). Shrinking ceases altogether at the stage that evapotranspiration rates reach a maximum. This behavior is consistent with a model in which fruit water balance is dominated by the reasonably free exchange of xylem water with the tree such that the fruit's own water status (and therefore volume) remains more-or-less in phase with that of the tree. Third, the positive linear relationship between daytime efflux and subsequent nighttime influx also lends strong support to the sap replacement concept.

We infer from our results that transpiration from the subtending spur leaves, amplifies the water potential excursion in the spur over and above that due to transpiration in the canopy as a whole. Spur leaves are anatomically closest to the fruit, but for the effect to be as pronounced as it is, it would seem that the spur would have to be somewhat isolated from the rest of the tree hydraulically. That is, the hydraulic resistance between the spur and the limb should be higher than that between the fruit and its associated leaves. This arrangement would force the fruit to act as a local water reservoir within the spur, buffering the water deficits arising from transpiration from the associated leaves. Zimmerman (1978) detected increased hydraulic resistance at the junction plane between twigs and branches for several tree species and correlated this with a reduction in vessel size. Preliminary studies of our own have indicated a high hydraulic resistance in the xylem of current year's growth.

The much higher calcium concentrations of sap extracted from detached shoots compared with fruit for 'Royal Gala' in Hawkes Bay agree closely with those reported by Jones et al., (1983) for a range

Fig. 3. Relationship between spur leaf area and xylem efflux or influx for 'Royal Gala' apples. Each point represents one measurement within a 24-h period for a single fruit. Influx and efflux values have been adjusted for tree and day effects and regression analyses have been carried out using these adjusted values. For efflux and influx, the slopes are significant ($P < 0.001$) and the intercepts are significantly different from zero ($P < 0.001$). Efflux = $0.230 + 2.94 \times 10^{-4}$ area; $r^2 = 0.49$. Influx = $0.461 + 7.55 \times 10^{-4}$ area; $r^2 = 0.49$.

Table 2. Fresh fruit mass and calcium content for young 'Royal Gala' apples 61 d after full bloom as affected by spur leaf defoliation at bloom.

Treatment	Leaf area (cm ²)	Fresh fruit mass (g)	Fruit Ca content (mg/fruit)
Intact	286	14.6 a ²	3.7 a
Defoliated	18	17.4 a	2.2 b

²Values within the same column followed by different letters are significantly different by pairwise *t* test ($P = 0.05$).

of cultivars in the United Kingdom and we can assume that these values are likely to represent concentrations for 'Royal Gala' trees at Massey University also. That much lower calcium concentrations were found from fruit pedicel sap compared with shoot exudate in our experiment is not surprising. Fruit in general (and apples in particular) are well known to be low in calcium in comparison with other plant organs (Marschner, 1983). Also, the apoplast is the most likely source for the xylem sap flowing out of the fruit and calcium concentrations here are expected to be particularly low because the greater proportion of fruit calcium is either vacuolar or is tightly bound to the pectin polymers in the cell walls (Harker and Venis, 1991).

On the assumption that our fruit and shoot sap measurements reflect the calcium concentrations of the incoming and outgoing xylem fluid, their difference takes on a special importance, in the light of the observed patterns of xylem cycling. The calcium concentration difference between inflowing and outflowing xylem saps and the higher inflow than outflow of xylem sap mean that much more calcium will be gained by the fruit when xylem sap is drawn out

during days of high transpiration rates and greater inflow of xylem sap into the fruit. Leaf area manipulation in the vicinity of a fruit seems to have its primary effect on the amplitude of the water potential excursion. The results suggest that other factors, which promote a deepening of the daily water potential excursions of the tree, and in particular the foliage surrounding a fruit, will also tend to improve fruit calcium nutrition.

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Fig. 4. Relationship between fruit calcium content and total leaf area for individual 'Royal Gala' apple spurs. The slope is significant ($P < 0.001$) and the intercept is significantly different from zero ($P < 0.001$). $\text{Calcium} = 2.33 + 5.55 \times 10^{-3} \text{ area}$; $r^2 = 0.64$.