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ACCUMULATION OF CALCIUM IN APPLE FRUIT

By

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ABSTRACT

ACCUMULATION OF CALCIUM IN APPLE FRUIT

By

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Low calcium (Ca) concentrations in the apple can affect fruit quality and the incidence of Ca-disorders. Apple fruit often receive inadequate Ca even when levels in the soil and remainder of the tree are sufficient. How Ca is translocated to apple fruit is unclear. The xylem stream is thought to be the main source of fruit Ca, however indirect evidence suggests that a significant phloem supply may also be operational. Translocation of Ca to apple fruit was studied in three related field experiments during 1988 and 1989.

'Red Delicious' fruit were collected from orchards in Massachusetts, Michigan, Ontario, and Virginia to determine how the pattern of Ca accumulation might vary under different environments. Similar patterns of Ca accumulation were observed from these locations despite the wide variation in weather, soils. and possible management of the trees. Fruit Ca content increased rapidly early in the season, until the rate of uptake began to decline 10 to 14 weeks after bloom. In four of seven observations, an apparent decline in Ca content occurred just prior to harvest which did not appear weather related.

In a second experiment, the relative humidity surrounding apple fruit was altered during four periods of growth to determine the

importance of transpiration and the xylem system in supplying Ca to fruit. Fruit exposed to high humidities usually contained less Ca.

In a third experiment, the amount of Ca potentially supplied to fruit via the xylem was estimated, based on Ca concentrations in xylem exudate and water flow into fruit. Water flow was estimated to be the sum of water incorporated into fruit growth and that transpired. Calcium concentrations in the transpiration stream entering the fruit were assumed to be equal to levels in xylem sap extracted from shoots under vacuum. When compared to actual rates of Ca accumulation, this model markedly overestimated the rate of uptake late in the season. Seasonal changes in tree water relations, and diurnal shrinkage in fruit size provided indirect evidence that Ca was exported from the fruit.

The central observations from this research were that no further accumulation of Ca in 'Red Delicious' fruit is likely three weeks prior to harvest, the xylem system appears to be an important source of Ca throughout fruit growth, and a possible export of Ca may occur from the fruit under certain conditions.

To Michelle, my wife, for her love, inspiration,

and constant support.

To my mother and father, Barbara and Robert Cline,

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Guidance Committee:

The journal paper format was chosen for this thesis in accordance with departmental and university regulations. The thesis is divided into three chapters in which the first is intended for publication in *HortScience* and the last two in *The Journal of the American Society for Horticulture Science*.

TABLE OF CONTENTS

APPENDIX

LIST OF TABLES

List of Tables (cont.)

LIST OF FIGURES

INTRODUCTION

Certain physiological disorders in plants are correlated with insufficient levels of calcium (Ca) in specific organs or tissues. Bitter pit is a common Ca-related disorder of apple and pear which reduces fruit quality, storage life, and market value. Extensive research has been conducted on the Ca nutrition of apples (Bünemann,1972; Ferguson and Watkins, 1989). Various disorders have been correlated with low fruit Ca levels (Delong, 1936), although it is unclear whether Ca is entirely responsible. Consequently, most remedial measures to control Ca disorders (ie. by post- and pre- harvest applications of Ca salts) have had varying degrees of success.

Significant progress in understanding Ca's function has been made however. It is known that low cytosolic levels of Ca are crucial for maintaining vital cellular processes. Also, Ca associated disorders are often the result of inefficient distribution rather than limited soil supply or uptake, as leaves contain fifty-fold more Ca than fruit flesh, and Ca disorders, even on Ca-fertile soils, are not uncommon. Some cultural practices such as pruning and fertilization influence the occurrence of the disorders, yet Ca translocation and deposition in the fruit needs to be better understood in order to optimize quality.

How Ca is translocated to apple fruit is not clear. Ferguson and Watkins (1989) suggested that xylem is the major pathway of Ca supply to fruit, however indirect evidence suggested that phloem may also be

important. Differences in patterns of Ca accumulation between seasons, cultivars, and locations also exist. Ca-disorders might be alleviated if the supply of Ca to fruit could be increased, perhaps by redirecting Ca transport from alternate plant sources high in Ca, or by increasing the solubility of Ca in the xylem and/or phloem system. Clarification of the factors affecting the translocation and supply of Ca in fruit is needed in order to develop techniques to increase fruit Ca content.

REVIEW OF LITERATURE

CALCIUM DISORDERS, FUNCTION, DISTRIBUTION, AND TRANSLOCATION IN APPLE TREES

1.0 CALCIUM DISORDERS OF FRUIT

Many fruit and vegetables are prone to physiological disorders related to tissue Ca concentrations. Although the exact etiology of such disorders is unclear, the involvement of Ca is easily shown since increasing the Ca concentration of a tissue will usually decrease the occurrence and progression of the disorder. The history and description of Ca disorders (Shear, 1975; Simmon, 1978) and physiological changes occurring with them (Bangerth, 1973, 1979; Faust *et al.*, 1968) have been reviewed in detail. Vang-Peterson (1980) addressed the Ca nutrition of apple trees specifically and Ferguson and Watkins (1989) recently reviewed the factors leading to the development of bitter pit.

Apple disorders associated with inadequate fruit Ca levels include bitter pit, cork spot, fruit cracking, internal breakdown, Jonathan spot, lenticel blotch, lenticel breakdown, low temperature breakdown, senescent breakdown, and water core (Shear, 1975). Location, time of appearance, and environmental conditions associated with occurrence are important factors in distinguishing between disorders (Faust and Shear, 1968).

Bitter pit, a common corking disorder in apple, was reported as a production problem during the 19th Century (Jäger, 1869 -referenced by Bünemann,1972), and was later associated with low fruit Ca levels (Delong, 1936). It is characterized by slight indentations in the skin with small, brown, desiccated lesions below the peel which become corky and bitter in flavor. Pits are usually concentrated towards the calyx

end of the fruit. Although bitter pit is initiated in fruit on the tree, visual symptoms may appear before harvest or more often after fruit storage (Faust and Shear, 1968).

Calcium disorders are caused primarily by the limited capacity of the plant to uniformly distribute Ca to various plant tissues (Marschner, 1983). Resulting disorders are probably due to impaired membrane function and disruption in compartmentalization at the cellular level (Bangerth, 1979).

Species differ in the cation exchange capacity (CEC) of the cell wall which influences the affinity and concentration of apoplastic Ca at the cellular level. For example, the quantity of free carboxylic groups of pectins (polygalacturonic acid) and of Ca^{2+} bound to the middle lamella, partially explains cross-species discrepancies in Ca concentration (Marschner, 1983). Dicotyledons have a higher CEC and also much higher tissue Ca concentrations than monocotyledons (Marschner, 1983). Dicotyledonous plants also have a higher prevalence of Ca-related disorders than monocots (Hanson, 1983).

Susceptibility of fruit to Ca disorders is dependent partly on cultivar (Ferguson and Watkins, 1989; Faust *et al.*, 1971; Titus and Ghosheh, 1963; Perring and Pearson, 1986) and rootstock (Sistrunk and Campbell; 1966; Faust *et al.*, 1971; Bukovac *et al.*, 1958; Kennedy *et al.*, 1987). Cultural practices which aggravate Ca disorders include low fruit set, over thinning, low soil moisture, heavy pruning, high nitrogen application, and early harvest (Ferguson and Watkins, 1989).

Calcium disorders in many ways are associated with fruit ripening, senescence (Faust and Shear, 1968; Poovaiah and Leopold, 1973), and ethylene production (Sharples and Johnson, 1977). Maintenance of high

tissue Ca concentrations delays fruit ripening and senescence though low concentrations of Ca in the cytoplasm are required simultaneously for normal cell functions (Poovaiah, 1988). Motto and Lieberman (1977) found that the ethylene-synthesizing system is located in the cell wall-membrane matrix (outside the cytoplasm) and that Ca influences cell membrane permeability, ethylene production, and senescence. Reduction in fruit Ca concentration, perhaps a result of dilution caused by growth or a decline in physiologically active Ca (Himelrick and Walker, 1982), can be considered an important step in fruit ripening. The enzyme polygalacturonase removes bound Ca from the middle lamella, resulting in fruit softening, senescence, and potential onset of disorders (Bangerth, 1979; Poovaiah, 1979). Postharvest infiltration of Ca, a procedure widely used to control Ca disorders, has increased fruit firmness and decreased membrane permeability, respiratory CO_2 evolution (Bangerth *et al.*, 1972; Faust and Shear, 1972), and ethylene production (Ferguson and Watkins, 1989).

1.1 Predicting Calcium Related Disorders

Attempts have been made to predict how well fruit will store by measuring fruit Ca levels prior to storage. Predications are difficult because large variations in fruit Ca concentrations exist between fruit from the same orchard, tree, and even branch. Why fruit Ca concentrations vary so much is not clear, but factors such as fruit size (Perring & Jackson, 1975; Perring, 1979), content of other nutrients (Faust and Shear, 1973), cultural practices (Ferguson and Watkins, 1989), growing environment (Wiersum, 1979; Tromp, 1979b) and seed number (Bangerth, 1976; Tomala and Dilley, 1989; Bramlage *et al.* 1990) have

Table 1. Suggested threshold values for fruit calcium concentrations used to predict fruit susceptibility to Ca-disorders.[z]

Cultivar	Country	Type of sample[y]	Threshold at harvest mg Ca·100 g^{-1} fruit fresh wt.[x]	Reference
Cox's	U.K.	a	5.0	Perring, 1968
Orange		-		Sharples, 1980
Pippin		a		Perring & Jackson, 1975
		a	5.5	Perring & Sharples,1975
		a		Perring & Preston, 1974
		a	5.4 - 6.0	Johnson *et al.*, 1987
		b	5.4 - 5.7	van Goor,1971
	N.Z.	c	2.5 - 7.0	Wills *et al.*, 1976
		d	2.5 - 3.0	Ferguson & Reid, 1979
Egremont	U.K.	e	9 - 10[u]	Chiu & Bould, 1977
Russet		f	4.4 - 5.5	Chiu & Bould, 1977
R. Del.	N.Z.	d	2.5 - 3.0	Ferguson & Reid, 1979
G. Del.	N.Z.	d	2.5 - 3.0	Ferguson & Reid, 1979

[z] Adopted from Ferguson and Reid, 1979.
[y] Key to Sample Preparation. a - whole fruit minus seeds and pedicel; b - whole fruit slices; c - whole fruit minus core and pedicel; d - cortical plugs just beneath skin; e -peel sample; f=fruit juice; "-" method not provided
[x] to convert to ppm Ca, multiply by a factor of 10
[u] 20% dry matter in peel assumed

been shown to affect fruit Ca levels.

Due to the insidious manner in which disorders develop, fruit Ca concentration thresholds have been established above which little or no disorders are expected and below which fruit are more susceptible. (Table 1). Most work on Ca thresholds has been conducted on Cox's Orange Pippin, a highly bitter pit susceptible variety grown commercially in England and New Zealand. Ferguson and Reid (1979) report that Cox's Orange Pippin accumulates less Ca and is more susceptible when grown in New Zealand than in the United Kingdom. Susceptible cultivars grown commercially in North America include Northern Spy, Jonathan, and Red Delicious (Cline, 1983), however threshold levels for these are few or lacking.

If Ca concentrations in the fruit flesh drop below a critical threshold of approximately 5 mg Ca·100 g^{-1} or 50 ppm Ca (fresh weight, with seed and stems removed), physiological disorders such as bitter pit, cork spot, or internal breakdown are likely to develop (Himelrick and McDuffie, 1983). Inconsistencies in critical values in Table 1 may reflect the different fruit sampling and analysis methods used (Holland *et al*, 1975; Turner *et al*., 1977) since Ca is unevenly distributed throughout fruit (Perring and Wilkinson, 1965) and values will vary markedly according to which tissue is sampled (Ferguson and Reid, 1979).

Monitoring levels of other nutrients may also be important in avoiding Ca disorders. In the United Kingdom, critical values for leaf and fruit N, P, K, and Mg in addition to Ca have been established. Sharples (1980) suggested that a balance of leaf N (2.4-2.8 % D.M.), P (0.2-0.25), K (1.3-1.6), Mg (0.25-0.30) and fruit N (5-10 mg Ca·100 $f.w.^{-1}$, harvest), P (not less than 11), and K (130-160) be maintained to

assure good fruit keeping quality.

2.0 FUNCTION OF CALCIUM IN PLANTS

Several authors have recently reviewed the function of Ca in plants (Hanson, 1984; Marmé, 1985; Christiansen and Foy, 1979; Millaway & Wiersholm, 1979; Poovaiah and Reddy, 1987; Faust and Klein, 1974) and therefore this review will be limited to those reports which address the function of Ca with respect to Ca-disorders. The functions of Ca in plants can be classified into four general categories: a) cell walls; b) membrane permeability; c) enzymes; and d) Ca-phytohormone interactions (Bangerth, 1979).

2.1 Cell Wall

Water and solutes can move through plants in the apoplast, the intercellular spaces, including cell walls and dead vascular tissue, or the symplast, the intracellular regions joined by plasmodesmata (Münch, 1930). Much of the apoplastic Ca in plants is considered to be bound to cell walls or the middle lamella, the intercellular substance composed mostly of Ca-pectate compounds that cement the primary walls of contiguous cells. Cell-wall Ca functions principally to maintain cell integrity and offer structural support to plants (Marschner, 1986).

2.2 Cell Membrane

Calcium plays an important role in maintaining the structure and permeability of cell membranes (Rousseau *et al.*, 1972). Many Ca-related disorders, particularly in fruits, are probably due to impaired membrane function and the disruption of membrane compartmentalization (Bangerth,

1974). Although not a constituent of cell membranes, Ca is believed to influence the permeability of the plasmalemma by controlling ATPase dependent ion transport (Hanson, 1983). Under Ca deficient conditions, cell membranes become more pervious (Jones and Lunt, 1967) and cells are less able to maintain their integrity. For instance, the inefficient uptake of sorbitol into fruit cells, probably one of the reasons for watercore and internal breakdown in apple, can be improved by Ca applications which reduce sorbitol leakage (Bangerth *et al.*, 1972).

2.3 Calcium and Enzymes

Calcium appears to activate enzymes primarily associated with the plasma membrane where ATPases are common (Poovaiah, 1988). The low concentration of Ca^{2+} in the cytoplasm (<1 μM) would suggest it is of minimal importance in cytoplasmic enzyme activity, especially when a thousand-fold increase of the closely associated Mg^{2+} ion ($\approx$ 1000 μM) exists. The opposite is however true. With improved Ca^{2+} detection methods, Poovaiah (1988) suggested that cytoplasmic Ca^{2+} is pivotal in the activation of enzymes such as calmodulin (CaM).

2.4 Regulation of Cytoplasmic Calcium

The importance of the Ca ion (Ca^{2+}) as a macronutrient (an element required at relatively high concentrations for plant growth and development) is well accepted. Recent studies in plants and animals have concentrated on how Ca may function as a micronutrient (an element required at lower concentrations) and led to an understanding of its involvement in transducing extracellular responses (Poovaiah and Reddy, 1987; Poovaiah, 1988).

Measurement of cytoplasmic Ca^{2+} concentrations is complicated by the presence of cell walls, large vacuoles, chloroplasts, and high turgor pressures. Intercellular Ca in the cell wall and vacuole exceeds that in the cytoplasm by several orders of magnitude (Mengel and Kirkby, 1987). Cytoplasmic Ca^{2+} concentrations range from 0.01 to 1 μM (Macklon, 1975; Wiersum, 1979; Poovaiah and Reddy, 1987; Mengel and Kirkby, 1987) depending on the tissue measured. Such low levels of free Ca^{2+} must be maintained to prevent precipitation of inorganic phosphate, competition with Mg^{2+} binding sites, and control activation of certain enzymes (Marschner, 1986). Low cytoplasmic Ca^{2+} concentrations appear to be maintained by the plasmalemma barrier, Ca^{2+}-efflux pumps, and the Ca^{2+}-binding protein calmodulin (Marschner, 1986).

Intracellular Ca^{2+} ions are now considered a major regulator of several processes in plants (Hepler and Wayne, 1985). Poovaiah (1988) postulated that Ca may operate in the cell in the following ways: 1) the free cytoplasmic Ca concentration is less than 1 μM and is under metabolic control; 2) the cytoplasmic Ca concentration can be regulated by various extra- or intra-cellular signals such as light, gravity, and hormones; and 3) the cytoplasmic Ca binds to CaM, thereby activating it. Enzymes can bind to the activated Ca-CaM complex leading to a response.

Regulation of intracellular Ca distribution is vital, since excessive levels of free Ca^{2+} in the cytoplasm (> 1 μM) are toxic (Hepler and Wayne, 1985). To assist in maintaining a balance of Ca^{2+}, organelles including the endoplasmic reticulum, mitochondria, chloroplasts, and vacuoles are known to accumulate relatively large amounts of Ca^{2+}. ATPase Ca pumps shunt Ca^{2+} from the cytoplasm into the respective organelles. These Ca-transporting ATPases, in turn, are

believed to be controlled by the regulator protein calmodulin (Dieter and Marmé, 1980).

2.5 Calmodulin

The Ca-binding protein calmodulin (CaM) exists in both animals and plants (Poovaiah, 1985). Since its discovery in plants (Muto and Miyachi, 1977) there has been escalating interest in studying the role of Ca as a secondary messenger. The concentration of cytoplasmic Ca^{2+} is extremely low and is influenced by extracellular signals such as light, gravity, and hormones (Poovaiah, 1985). Poovaiah (1988) has reported several processes altered by changes in extracellular and intracellular Ca levels including cell elongation, abscission, senescence, tuberization, geotropism, stomatal control, secretion, hormone dependent changes, enzyme activation, and protein phosphorylation.

The mechanism through which CaM functions is not fully understood, but seems to involve a series of activation steps. In the first step a stimulus (ie. light, gravity, hormones) results in a cytoplasmic surge in Ca^{2+} above 1 μM (Poovaiah, 1985). This transient increase in Ca^{2+} can originate from the mitochondria, plasmalemma or vacuole. Four Ca^{2+} ions then couple with and activate one inactive CaM molecule via conformational changes at the molecular surface. Once activated, the Ca-CaM complex recognizes a receptor protein (enzyme) and binds to it. This CaM-Ca-enzyme complex then elicits the actual response (Poovaiah, 1985).

3.0 CALCIUM ABSORPTION AND TRANSPORT IN PLANTS

3.1 Soil Calcium and Root Uptake

The majority of soils seem to provide enough Ca to meet plant requirements (Vang-Petersen, 1980). The Ca concentration of soil may vary from less that 0.05% to greater than 25% (in calcareous soils). Soil Ca averages 3.6% for mineral soils (Hausenbuiller, 1978), and accounts for 65-85% of the total cation exchange capacity in limed arable soils (Chapman, 1966). The availability of Ca to plants is related to the proportion in exchangeable form.

Other ions in the rhizosphere may affect Ca uptake. For example, K^+, Mg^+, and NH_4^+ may compete (cation antagonism) with Ca for uptake (Kirkby, 1979; Geraldson,1971) whereas NO_3^-, HPO_4^{2-} or $H_2PO_4^-$ (Jakøbsen, 1979; Kirkby and Knight, 1977) may increase Ca uptake. A great number of other interactions may affect Ca uptake as well (Bangerth, 1979).

In soils high in Ca, the movement of Ca to plant roots occurs largely by mass flow rather than by diffusion (Marschner, 1986; Hausenbuiller, 1978). The Ca concentration in most soil solutions varies from 3.4 to 14 *mM*, whereas concentrations of 0.1 to 1 *mM* at the root surface appear adequate for most plants, provided concentrations of other ions are in balance (Fried and Shapiro, 1961; Loneragan and Snowball, 1969). Root uptake of Ca is influenced by the environment of the rhizosphere, total root volume, root density, periodicity of growth, transpirational demand for water, and availability of Ca^{2+} (Himelrick and McDuffie, 1983; Bangerth, 1979).

Calcium is translocated predominantly in the apoplast of the root, however some symplastic movement does occur. Apoplastic Ca must be absorbed in the immature region of the root, closest to the root tip,

where the casparian strip is not fully developed (Ferguson and Clarkson, 1976). Distal to the root tip, Ca^{2+} may also move in the apoplastic free space of the root cortex as far as the endodermis, where further movement is restricted by the suberized endodermal barrier. This Ca^{2+} is forced to pass symplastically to enter the stele. Conditions favoring root proliferation, such as adequate aeration, warm soil temperatures, and ample moisture are important in maximizing Ca uptake in roots (Russell & Clarkson, 1976).

3.2 Xylem Transport of Calcium

Translocation of Ca within the xylem occurs by a combination of mass flow and cation exchange, since its movement is not directly proportional, but nevertheless believed to be dominated by, the transpirational demand for water (Buchloh, 1974). Translocation of Ca within the xylem was shown to occur by a series of cation exchange reactions with lignin (Shear and Faust, 1970) or other negatively charged sites on cell walls of the xylem tissue (Bell and Biddulph, 1963; Biddulph *et al.*, 1959, Biddulph *et al.*, 1961; Faust and Shear, 1973; Ferguson and Bollard, 1976; van de Geijn *et al.*, 1979). Water and solutes are drawn acropetally by a slight negative water potential caused by the evaporation of water at the leaf or fruit surface. At night or during periods of low temperature, high humidity, and adequate to excessive soil moisture, xylem Ca may move to plant parts usually poorly supplied with Ca. This phenomenon, achieved by positive root pressure or by considerably reduced xylem tension, occurs in smaller plant species such as vegetables (Collier and Tibbitts, 1984). Although its importance in trees is unknown, a similar phenomenon may be

operating when high fruit/shoot ratios exists (Ferguson and Watkins, 1989), or perhaps when extreme diurnal fluctuations in temperature and/or humidity exist.

The transpiration rate of an organ is one factor determining the ratio of minerals imported by the xylem and phloem (Marschner, 1983). If Ca moves primarily in the xylem in response to transpirational losses of water, high relative humidity (RH) surrounding an organ would be expected to decrease transpiration and Ca supply. High RH has decreased Ca accumulation in tomato fruit (Armstrong and Kirkby, 1979; Bradfield and Guttridge, 1979; Banuelos *et al.*, 1987; Ehret and Ho, 1986), lettuce leaves (Collier and Tibbitts, 1984), cabbage leaves (Wiebe *et al.*, 1977), paprika and bean fruit (Mix and Marschner, 1976a, 1976b), strawberry leaves (Bradfield and Guttridge, 1979) and apple fruit (Ford, 1979b); however, the RH level imposed on apple fruit was not recorded. Low RH applied to entire apple trees increased leaf and fruit Ca concentrations but had no effect on their content since tree growth was reduced (Tromp, 1979b; Tromp and Oele, 1972).

Bollard (1953, 1957) devised a method to extract xylem exudate from woody plants. Translocation of organic compounds (Bollard, 1957) and Ca (Bradfield, 1976) has been measured throughout the season in the xylem of apple trees. Total Ca reaches a maximum of ≈180 ppm at full bloom, falls to ≈50 ppm five weeks later and remains at this level for the rest of the season. Roughly 50% of the Ca in xylem sap is complexed (Bradfield, 1976). Tromp (1979a) observed that xylem Ca concentrations peaked at ≈330 ppm three weeks prior to full bloom and suggested that the high concentrations of Ca and other nutrients near full bloom were primarily re-mobilized from branches, rather than transported from the

root, since the transpiration rate is low at this time. Mason and Whitfield (1960) observed similar seasonal patterns of nutrient contents in the xylem of apple trees.

3.3 Phloem Transport of Calcium

A difficulty in studying the phloem mobility of Ca in apple is the inability to obtain adequate volumes of phloem exudate. However, Ca concentrations in the phloem exudate of other plant species range from 21-63 ppm Ca (*Lupinus albus* and *Lupinus angustifolia)*, 83 (*Nicotiana glauca*), 49 (*Quercus rubra)*, 4-92 (*Ricinus communis*) (Hall *et al.*, 1971; Smith and Milburn, 1980; Wiersum, 1979), and 12 ppm Ca (*Yucca flaccida)* (Tammes and Van Die, 1964). It is difficult to predict what Ca concentrations in phloem of apple might be considering this wide range in other plant species and the potential for cross contamination from xylem exudate. A large discrepancy exists between the Ca levels measured in the phloem sap exudate and in the cytoplasm of plants (Poovaiah, 1988). A method to quantify Ca levels in the phloem of apple shoots and fruit is needed before its mobility can be reported with certainty.

The phloem mcbility of Ca in apple has been studied through indirect methods. Faust and Shear (1973) observed that ^{45}Ca administered to roots of apple trees appeared to be transported through the phloem. The phloem transport was slow, not responsive to transpirational stress, and was reportedly under hormonal control. However, when ^{45}Ca was applied to the basal end of cut stems, it was transported through the xylem. The xylem transport was rapid and responsive to concentration gradients and transpirational rates. Stebbins and Dewey (1972) observed evidence that Ca may move in the phloem by supplying ^{45}Ca to the roots of apple seedlings. Removing the

phloem tissue by girdling stems restricted ^{45}Ca movement to the leaves, suggesting that Ca is phloem mobile. However, it was unclear whether the girdling treatment altered root absorption of Ca. If the roots of girdled plants were starved of photosynthate, decreased root uptake may have occurred, hence the decline in xylem transport. Furthermore, lateral transfer of Ca between xylem and phloem (Ferguson and Watkins, 1989) and re-mobilization of Ca deposited in bark to the xylem may have occurred (Mason and Whitfield, 1960; Wieneke and Führ, 1975). The effect of girdling on Ca transport in apple trees needs to be further evaluated.

The relative importance of the xylem and phloem in transporting minerals to legume fruits has been studied in detail (Pate *et al*, 1974; Pate and Hocking, 1978) since phloem exudate can be collected from these species. The phloem was estimated to supply 80% of the C, N, and S; 70-80% of the P, K, Mg, and Zn; 65% of the Fe, Mn, and Cu, and only 30% of the Ca. These proportions may however fluctuate diurnally (Hocking and Pate, 1978). When Ca was added to phloem sap of *Yucca flaccida* and *Ricinus communis* at 20-40 ppm it caused precipitation of Ca-phosphate, whereas K and Mg, over the usual range of concentrations, did not cause precipitate formation (van Goor and Wiersma,1974). Only small additions of Ca exceeded the solubility limit.

4.0 CALCIUM DISTRIBUTION IN THE TREE AND FRUIT

4.1 Calcium Distribution in the Tree

The distribution of Ca in apple trees was reviewed by Himelrick and McDuffie (1983), Terblanche *et al*. (1979), and Mason and Whitfield (1960). Calcium disorders in plants are often related to localized Ca

deficiencies caused by poor distribution or re-mobilization in the tree rather than poor Ca uptake. The range in Ca concentrations in various components of an apple tree support this claim (Table 2). For example, Ca-deficiency disorders are common in the fruit, but deficiency symptoms are rare in leaves.

Interestingly, fruit comprise 18 % of the total tree dry weight, but only 1 % of total tree Ca. Calcium concentrations in fruit are one fiftieth the level in leaves. The Ca requirement of a mature tree is surprisingly high in comparison to other nutrient elements. In fact the Ca content is almost equal to that of N, K, P, and Mg combined (Himelrick and McDuffie, 1983). These figures stress the dissimilarity of Ca concentrations in trees.

4.2 Calcium Distribution in Fruit

Although Ca concentrations are similar in various parts of the fruit early in the growing season (Wieneke, 1974), concentration gradients develop as the season progresses (Perring and Clijsters, 1974; Ford, 1979a; Perring and Wilkinson, 1965; Wilkinson and Perring, 1964). By harvest, Ca concentrations are highest in the pedicels (5,600 ppm, dry wt.) and seeds (2,000), lowest in the flesh (300) and intermediate in the core (400) and peel (600) (Faust *et al.*, 1967; Kohl, 1966). In addition, flesh Ca concentrations are highest in the exposed side and the calyx end of the fruit (Perring and Wilkinson, 1965; Lewis & Martin, 1973; Lewis, 1980).

The Ca concentration is particularly low in the outer cortex of mature fruits where pitting occurs commonly. The pitted zones may actually contain higher concentrations of Ca than surrounding regions

(Perring and Plocharshi,1975; Hopfinger and Poovaiah, 1978; Chamel and Bossy, 1981; Askew *et al.*, 1958). Accumulation of Ca in pitted zones appears to occur simultaneously with the development of the disorder, after cortical cells begin to disorganize, but not prior to visual symptoms of bitter pit (Ford, 1979a; Faust *et al.*, 1968).

Table 2: Calcium composition and concentration of a mature apple tree at mid summer; fruit levels at harvest.

Tissue	Percent of Total Tree Weight[z]	Percent of Total Plant Ca[z]	Approximate Calcium Concentration ppm (dry wt.)[y]	Approximate Relative Amount of Ca in Tissue (fruit flesh=1)[x]
Leaves	13	29	14,000	50
Wood	40	9		
Bark	11	44	25,000	80
Branch	--	--	6,000	20
Stem	--	--	8,000	27
Trunk	--	--	15,000	50
Xylem sap	--	--	50[v]	0.16
Phloem sap[w]	--	--	.04[v]	1.3×10^{-4}
Root	18	17	1,200	4
Fruit	18	1		
Pedicel	--	--	5,600	20
Seed	--	--	2,000	7
Peel	--	--	600	2
Core	--	--	400	1.3
Flesh	--	--	300	1
Total	100	100		

[z] Adapted from Terblanche *et al.*, (1979)
[y] Adapted from Himelrick and McDuffie (1983)
[x] Tissues compared w.th fruit flesh (tissue most often Ca-deficient). Values based on ppm Ca fresh weight.
[w] estimated levels found in cytoplasm - levels unreported
[v] values expressed as ug Ca ml^{-1} xylem/phloem exudate

4.3 Factors Influencing Fruit Calcium Levels

It is evident that tissue Ca concentrations are influenced by several orchard factors which affect translocation within the xylem. Reports of treatment effects on Ca concentration and Ca-related disorders often conflict (Table 3). Treatments which alter fruit size will likely affect fruit Ca concentrations since an inverse relationship exists between these parameters (Perring & Jackson, 1975). It is imperative therefore to consider whether treatments affected fruit size when interpreting influences on fruit Ca levels.

Environmental and cultural practices which affect the translocation of Ca to fruit can be separated into those eliciting a "below-" or "above-" ground influence, and again into those affecting tree growth or Ca translocation. Below-ground factors, known to reduce fruit Ca levels by directly affecting root growth and/or Ca uptake, include low soil temperatures (Tromp, 1979b), high K and Mg fertilization (Bangerth, 1979), and low soil moisture (Tromp, 1979b). In contrast, Ca and B fertigation has reduced the frequency of cork spots (Smith *et al.*, 1987), and root pruning during dormancy or full bloom has increased fruit Ca levels (Schupp and Ferree, 1987).

Above-ground treatments such as increased light intensity (Tromp, 1979b) increased Ca uptake. Also, elevating tree air temperatures from 19° to 24°C increased fruit Ca, N, Mg, and P content. For these examples, treatment effects on fruit Ca levels were invariably co-related to larger sized fruit.

Some treatments interact in a more complex way. Nitrogen, for example, influences Ca-disorders and the storage quality of fruit (Faust and Shear, 1968). Fertilization with N is important with respect

Table 3. Selected treatments known to alter the Ca concentration and other parameters in apple trees and fruit.

Treatment	Effect on Fruit Calcium[z]	Summary	Reference
BELOW GROUND TREATMENTS			
↑ Soil Moisture	+	Irrigation increased soil uptake of Ca	Perring, 1979
	+	Low soil moisture decreased tree dry matter, Ca, and K uptake.	Tromp, 1979b
	Ø	Irrigation had no effect on BP.[y]	Goode & Hyrycz 1964; Goode & Ingram, 1971
	-	Irrigation increased BP by increasing fruit growth.	Faust & Shear, 1968
	NA	BP increased with high available soil water.	Lötter *et al.*, 1985
↑ Soil Temperature	-	Higher soil temp increased total tree dry matter and water uptake. At lower soil temps. Ca uptake increased while K uptake decreased. More roots observed at increased soil temp.	Tromp, 1979b
↑ Fertilization	Ø	Fertilization of N,P,K had no effect on fruit Ca except that caused by increased fruit size.	Perring, 1979
↑ Nitrogen	Ø	N application had no effect on BP and fruit color.	Preston & Perring, 1974
Ca & B	NA	Ca + B applied in trickle irrigation reduced the number of cork spots per fruit and increased the number of sound fruit. No effect on fruit size.	Smith *et al.*, 1987
Root Pruning	+	Root pruning during dormancy increased fruit Ca levels.	Schupp & Ferree, 1987.
ABOVE GROUND TREATMENTS			
↑ Light	+	Higher light intensity increased dry matter, Ca & K and water uptake.	Tromp, 1979b
↓ Light	Ø	Tree shading reduced BP, fruit size and number but N,P,K,Ca, or Mg conc. did not differ in similar sized fruit.	Jackson *et al.* 1971, 1977
Summer vs.	+	Summer pruning increased fruit Ca concentration irrespective of fruit size.	Perring, 1979

Table 3 (cont.)

	NA	Summer pruning reduced BP development, advanced blossom formation, and increased fruit color, but had no effect on yield. Fruit size was not reported.	Preston & Perring, 1974
	Ø	Summer pruning decreased BP and fruit yield but also reduced fruit size. Fruit Ca levels did not differ for similar sized fruit.	van der Boon, 1980
↑ Fruit Thinning	Ø	Reduced yield but no effect of BP and fruit Ca levels for similar sized fruit	van der Boon, 1980
↑ Air Temp.	Ø	Higher air temperature increased fruit Ca, N, Mg, and P content, but this was mostly attributed to increased fruit size at higher temperatures.	Tromp, 1975
↓ Leaf Transpiration	+	The incidence of BP was reduced with antitranspirants.	Schumacher *et al.*, 1976

[z] '+'=increase; '-'= decrease; 'Ø'= no response; 'NA' - Ca not measured
[y] BP- denotes bitter pit

to the exchange of Ca in xylem tissue (Faust and Shear, 1973). Nitrate nitrogen (NO_3^--N) ascends freely in the exchange column of the xylem, whereas NH_4^+-N can block Ca exchange and movement (Faust and Shear, 1973). Nitrogen also stimulated tree growth (Goode *et al.*, 1978), delayed fruit maturity, increased the leaf:fruit ratio, reduced fruit firmness, and increased fruit size (Preston and Perring, 1974; Perring, 1979). Other effects of N fertilization are complex since N is known to alter the fruit:shoot and shoot:root ratios of trees (Faust and Shear, 1968).

Factors other than mass flow and exchange may influence Ca transport. The endogenous plant growth substance auxin may influence Ca transport (Bangerth, 1976; Banuelos *et al.*, 1987, 1988; Fuente and Leopold, 1973). When the auxin transport inhibitor 2,3,5-triiodobenzoic acid (TIBA) is sprayed on apple trees shortly after bloom Ca accumulation in the fruit is reduced and bitter pit is enhanced (Bangerth and Firuzeh, 1971; Oberly, 1973; Stahly and Benson, 1970, 1972, 1976, 1982; Stahly, 1986). Himelrick and Ingle (1981), however, found no consistent influence of TIBA sprays on Ca concentrations in the leaf, fruit flesh or peel tissues. TIBA reduced ^{45}Ca movement into fruit and leaves of tomato (Banuelos *et al.*, 1987), cucumber (Beyer and Quebedeaux, 1974), and lettuce (Banuelos *et al.*, 1988). Bangerth (1976) attributed the lower Ca contents of parthenocarpic apple and pear fruit to reduced seed number, since seeds are known to be rich in auxin. Seed number was positively correlated with Ca concentration in apple fruit of the cultivars 'McIntosh', and 'Spartan' (Tomala and Dilley, 1989) and 'Red Delicious' (Bramlage *et al.*, 1990), but not 'Empire' (Tomala and Dilley, 1989).

Basipetal transport of auxin from fruit may exert control over the delivery of Ca to fruit. Environmental and cultural factors which alter the basipetal transport of auxin, such as poor pollination and seed set or removal of shoot tips by summer pruning, may affect the Ca supply to fruit (Banuelos *et al.*, 1987; Ferguson and Watkins, 1989).

5.0 CALCIUM TRANSLOCATION AND ACCUMULATION IN APPLE FRUIT

5.1 Seasonal Accumulation of Ca in Apple Fruit

Wilkinson (1968) proposed that Ca accumulates in apple fruit in two stages. During stage I (pollination to 4-5 weeks thereafter), cell division, fruit growth and Ca uptake are very rapid. During stage II (end of stage I to harvest), fruit growth continues at a rapid rate, but Ca accumulation either continues at a slower rate, ceases altogether, or Ca is exported back into the tree. Empirically, Ca accumulation may increase rapidly then level off (Tromp 1972,1975; Jones *et al.* 1983, Jones and Samuelson, 1983; Himelrick and Walker, 1982; Quinlin, 1969; Wilkinson, 1968) or accumulate linearly up until harvest (Rogers and Batjer, 1954; Oberly, 1973; Tromp, 1975, 1979b; Tomala *et al.*, 1989). Calcium content has also been observed to decline towards the end of some seasons (Tromp and Oele, 1972, Tromp, 1979b; Wilkinson, 1968; Hanson, unpublished data).

The three seasonal Ca accumulation patterns are depicted in Figure 1. The magnitude and duration of accumulation can vary considerably between fruit cultivars and different growth rates (Table 4). Although Wilkinson (1968) proposed that Ca uptake rates decline 4-6 weeks after full bloom (end of stage I), a compilation of reported patterns of Ca accumulation indicates that the transition between stage I and II often

occurs 12-16 weeks after full bloom (Jones *et al*, 1983; Jones and Samuelson, 1983). This shift may be due to differences in environment (Ferguson and Watkins, 1989) or cultivars. Weather conditions which reduced the accumulation of Ca late in the season, such as low temperatures (Tromp, 1975; Ford, 1979b), would shift this transition earlier. Since most observations are from non-controlled conditions, the confounding effects of climate on fruit growth make it difficult to directly relate one specific environmental parameter to Ca accumulation.

Moisture supply (Goode *et al.*, 1978, 1979; Irving and Drost, 1987), relative humidity (Tromp, 1979b), air and soil temperature (Tromp, 1979b; Ford, 1979b), and fruit cultivar (Tromp, 1975) affect the quantity and duration of Ca accumulation. The effects of soil moisture,

Figure 1. Typical patterns of seasonal calcium accumulation in apple fruit. Adapted from Faust, 1989.

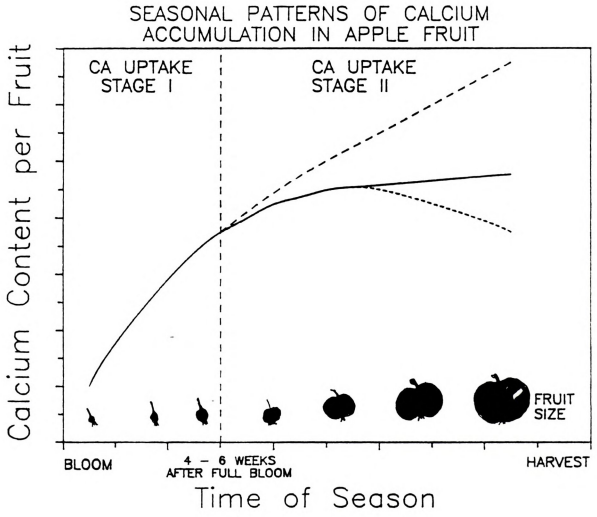

SEASONAL PATTERNS OF CALCIUM
ACCUMULATION IN APPLE FRUIT
CA UPTAKE
STAGE I
CA UPTAKE
STAGE II
Calcium Content per Fruit
FRUIT
SIZE
BLOOM
4 – 6 WEEKS
AFTER FULL BLOOM
HARVEST
Time of Season

Table 4: Seasonal patterns of calcium accumulation in selected apple cultivars from various locations.

Cultivar	Location	Peak Ca content (mg/fruit)	Period separating stage I & II (weeks AFB)[z]	Reference
Linear uptake of Ca in stage I, Ca content levels off stage II				
Bramley	U.K.	9	12	Jones *et al.*, 1983
Cox's Orange Pippin	U.K	5	16	Jones *et al.*, 1983
		5	8	Wilkinson, 1968
Delicious[y]	U.S.	9	6	Himelrick and Walker, 1982
Egremont	U.K.	7	12	Jones *et al.*, 1983
Golden Delicious	Neth.	6	`8	Tromp, 1975
		6	10	Tromp and Oele, 1972
	U.K.	8	12	Jones and Samuelson, 1983
	U.S.	8	16	Stahly & Benson, 1982
Laxton's Fortune	U.K.	6	8	Quinlan, 1969
Linear uptake of Ca in stage I and II (no stage II)				
Cox's Orange Pippin	Neth.	7	-	Tromp, 1975
		4	-	Tromp, 1979b
		9	-	Tromp, 1979b
Delicious[y]	U.S.	13	-	Rogers and Batjer, 1954
Golden Delicious	Neth.	6	-	Tromp, 1975
McIntosh	Poland	8	-	Tomala *et al.*,1989
Northern Spy	U.S.	11	-	Oberly, 1973
Spartan	Poland	10	-	Tomala *et al.*, 1989
Linear uptake of Ca in stage I, Ca content levels and declines during stage II				
Cox's Orange	Neth.	4	6	Tromp, 1979b
	U.K.	5	8	Wilkinson, 1968
G. Del.	Neth..	6	10	Tromp and Oele, 1972.
R. Del.	U.S.	12	14	Hanson, 1987 (unpublished data)

[z] AFB indicates after full bloom
[y] Strain not indicated

soil temperature, and air humidity (Tromp and Oele, 1972; Tromp 1975, 1979b; Ford, 1979b -temperature only) on the seasonal pattern of Ca accumulation show that trees subject to low temperatures or high humidities early in the growing season maintain an essentially linear seasonal pattern of Ca uptake, but fruit Ca content is reduced. In general, Ca uptake is often limited if trees are under stress during stage II.

Soil moisture has affected the pattern and level of Ca accumulation in apple fruit inconsistently. Soil moisture has had no measurable effect on fruit Ca levels (Goode *et al.*, 1978; Irving and Drost,1987; Tromp, 1979b), although both high and low soil moisture treatments have reduced (Goode *et al.*, 1978; Irving and Drost, 1987) and increased (Lötter *et al.*,1985; Wilkson, 1968) the incidence of bitter pit. Wilkinson (1968) suggested that periods of drought cause late-season export of Ca from fruit. Movement of Ca out of fruit was confirmed by studies in which ^{45}Ca was applied to the fruit surface and later found in leaves and shoots (Millikan, 1971)

Despite these differences, early season uptake of Ca by roots appears vital in maintaining the rate of Ca supply to the fruit, since it affects both the pattern of uptake and final Ca content (Tromp, 1979b). In contrast, fruit Ca content later in the season seems more limited by above-ground environmental factors, since relative humidity changed the pattern of Ca accumulation in stage II (Tromp, 1979b). Soil moisture and temperature may influence Ca uptake by altering root growth.

5.2 Importance of Xylem and Phloem in Translocation of Calcium to Fruit

The literature on Ca transport to apple fruit can be classified into two categories. There are those who seem to support 1) the exclusive xylem mobility of Ca (Tromp & Oele,1972; Vang-Peterson, 1980; Redmond, 1975) or 2) a combination of both xylem and phloem mobility of Ca (Faust and Klein, 1974; Jones *et al.*, 1983; Stebbins and Dewey, 1972; Faust and Shear, 1973).

Our understanding of the translocation of Ca into apple fruit is incomplete (Ferguson and Watkins, 1989; Ferguson, 1979; Hanger, 1979). A theory put forward by Wiersum (1966) and developed by others (Wilkinson, 1968; Ferguson, 1979; Hanger, 1979; Ferguson *et al.*, 1987; Ferguson and Watkins, 1989) suggested that the xylem is the primary route of Ca supply early in the season whereas the phloem may predominate later. Early in the season, Ca absorbed by plant roots is distributed principally with water throughout the plant. Leaves and other organs which receive large amounts of water also accumulate higher Ca levels than fruit or other plant parts with low transpiration rates. Young fruit have a relatively large surface area and a high surface permeability to water with active stomata (Blanke and Lenz, 1988) which allow a high rate of transpiration. Young fruit also photosynthesize actively (Blanke, 1989) and therefore have a high water requirement and low external photosynthetic need. The water supply to young fruit (stage I) likely originates from the xylem in which Ca moves comparatively freely.

Wiersum (1966) further postulated that as fruit increase in size (stage II), the surface area:volume ratio decreases, the fruit surface

becomes more waxy, stomatal density decreases, stomata become less functional, and the leaf:fruit ratio increases. These changes decrease fruit transpiration and xylem supply, disfavoring xylem movement of Ca into the fruit. The net rate of Ca uptake decreases through the season while the supply of phloem-mobile nutrients (K, Mg, P & N) and photosynthate increase or remain the same.

Despite the numerous times this conjecture has been put forward, and in spite of its logic (Ferguson and Watkins, 1989) it needs to be demonstrated that appreciable amounts of Ca are phloem mobile and that actual phloem flow of water changes with fruit development. Calcium concentrations in the phloem of apple are expected to be low, based on levels in phloem sap of other plant species (Hall *et al.*, 1971; Wiersum, 1979; Tammes and Van Die, 1964) and lack of re-mobilization of Ca from apple leaves (Himelrick and McDuffie, 1983). Since fruit typically accumulate little Ca later in the season, there is no need for a specific role of phloem to explain a late season reduction in fruit Ca uptake, since xylem alone could be responsible.

Ferguson *et al.* (1987) suggested that fruit growth may dilute fruit Ca and thereby decrease concentrations at the fruit wall. Cortical tissue is diluted to the greatest extent since it undergoes greater cell expansion and has fewer vascular connections. The continued supply of other nutrients throughout the season is due to the ability of the plant to transport these nutrients in both xylem and phloem tissue. Since the phloem is comprised of living cells which maintain micromolar cytoplasmic Ca levels, negligible transport in the phloem is expected to occur (Ferguson and Watkins, 1989). As a result, Ca accumulates where it is first deposited by the xylem (mainly leaves)

and is not markedly re-mobilized thereafter. Other nutrients in contrast can move in the phloem out of leaves into developing fruit, along with sugar needed for fruit development. The flow of Ca into developing fruit declines as the water supply from the xylem is reduced, whereas K, Mg, P, and N increase due to increased phloem supply (Wiersum, 1966).

Jones *et al.* (1983) estimated the xylem supply of Ca to apple fruit to be the product of xylem sap Ca concentration and water flow to the fruit. Xylem Ca supply was estimated for the cultivars Egremont Russet, Bramley, Cox's Orange Pippin, and Golden Delicious, and compared with actual accumulation rates to determine if the xylem system alone may account for Ca content of fruit. Xylem sap was collected from shoots under suction (Bollard, 1957) and water flow was estimated to be the sum of the net water uptake for fruit growth and that lost from the fruit by evaporation. Two methods were used to estimate water loss (Jones and Higgs, 1982): 1) weight loss of water from detached fruit hanging in the tree, and; 2) determination of surface conductance of attached fruit to water loss (permeability) with adjustment for daily water vapor pressure deficits. Their findings consistently underestimated Ca uptake rates by developing fruits early in the season and markedly overestimated observed rates late in the season. Calcium transport is greater than predicted during initial stages of fruit development, while less significant later on. This is consistent with other findings which suggest a decline in xylem transport 4 to 6 weeks after petal-fall.

Possible reasons for the discrepancies between actual and predicted values are important to consider and include: 1) erroneous

measurement of actual Ca uptake; 2) incorrect estimate of net mass flow of water into fruit; 3) erroneous estimation of Ca concentrations in the xylem sap entering the fruit; and/or 4) a back-flow of Ca out of fruit occurred.

It is most likely that the measurement of net water movement to fruit is in error since transpiration of attached fruit is difficult to measure; although transpiration rates are easier to measure earlier in fruit development when fruit are small. The assumption that attached and detached fruit maintain similar transpiration rates is suspect. Furthermore, *in situ* measurements of fruit transpiration rates, made with a modified steady state porometer, required lengthy (> 60 minutes) equilibrium times which may be a source of error.

Another reason why xylem may not account for the total amount of Ca in fruit at harvest is that movement of Ca from the fruit to the tree may be occurring. During seasons of low soil moisture, fruit compete with leaves for water and generally lose. Under these conditions Ca migration from fruit has been reported to occur (Wilkinson, 1968). Cultural practices which reduce leaf transpiration and vegetative competition, such as summer pruning (Perring, 1979; Preston and Perring, 1974; van der Boon, 1980), anti-transpirants (Schumacher *et al.*, 1976), or over-head irrigation (Goode *et al.*, 1979), have sometimes decreased Ca disorders or increased fruit Ca levels.

Diurnal fluctuations in water relations of the tree and fruit may cause movement of Ca from the fruit. Fruit growth is greatest at night (Tukey, 1964, 1974; Tromp, 1979b) and diurnal changes in fruit diameter (Tukey, 1964, 1974; Jones, 1985) and water potential gradients from fruit to leaves in apple (Goode *et al*, 1979) suggest that water may flow

out of the fruit. When trees are stressed, the xylem column would be under tension and a potential backflow of water could occur, taking with it Ca. There is also evidence that xylem movement from fruit occurs in grape berries (Lang and Thorpe, 1989), cowpea fruit (Pate *et al.*, 1985), and pea fruit (Hamilton, 1988). Further work is needed to quantify actual diurnal water movement from fruit since Jones *et al.* (1983) found that evaporation at the fruit surface may be sufficient to cause the magnitude of diurnal shrinkage observed which would suggest that there was no displacement of xylem sap. The 'heat-pulse' method would perhaps be a non-destructive, more definitive way to measure xylem flow of water through fruit pedicels (Cermák *et al.*, 1973; Valancogne and Nasr, 1989). Nonetheless, the importance of the above assumptions needs to be verified to comment further on the accuracy of their predictions.

LITERATURE CITED

Armstrong, M.J. and E.A. Kirkby. 1979. The influence of humidity on the mineral composition of tomato plants with special reference to calcium distribution. Plant and Soil 52:427-435.

Askew, H.O., E.T. Chittendon, R.J. Monk and J. Watson. 1958. Chemical investigation of bitter pit of apples. III. Chemical composition of affected and neighboring healthy tissues. N.Z. J. Agric. Res. 3:169-178.

Bangerth, F. 1973. Investigations upon Ca related physiological disorders. Phytopathol. Z. 77:20-37.

Bangerth, F. 1974. Untersuchungen und Uberlegungen zur Vorausschatzung des Stippenbefalls. Erwerbsobstbau 16:169-172.

Bangerth, F. 1976. A role for auxin and auxin transport inhibitors on the Ca content of artificially induced parthenocarpic fruits. Physiol. Plant. 37:191-194.

Bangerth, F. 1979. Calcium-related physiological disorders of plants. Ann. Rev. Phytopathol. 17:97-122.

Bangerth, F., D.R. Dilley, and D. H. Dewey. 1972. Effect of postharvest calcium treatments on internal breakdown and respiration of apple fruits. J. Amer. Soc. Hort. Sci. 97:679-682.

Bangerth, F., and P. Firuzeh. 1971. Der Einfluss von 2,3,5-Trijodobenzoesäure (TIBA) auf den Mineralstoffgehalt und die Stippigkeit von 'Boskoop' Früchten. Z. Pflanzenkrankheiten u. Pflanzenschutz 78:93-97.

Banuelos, G.S., F. Bangerth, H. Marschner. 1987. Relationship between polar basipetal auxin transport and acropetal Ca^{2+} transport into tomato fruits. Physiol. Plant. 71:321-327.

Banuelos, G.S., F. Bangerth, H. Marschner. 1988. Basipetal auxin transport in lettuce and its possible involvement in acropetal calcium transport and incidence of tipburn. J. Plant Nutrit. 11:525-533.

Bell, C.W., and O. Biddulph. 1963. Translocation of calcium. Exchange versus mass flow. Plant Physiol. 38:610-614.

Beyer, E.M., Jr., B. Quebedeaux. 1974. Parthenocarpy in cucumber: mechanism of action of auxin transport inhibitors. J. Amer. Soc. Hort. Sci. 99:385-390.

Biddulph, O., R. Cory, and S. Biddulph. 1959. Translocation of calcium in the bean plant. Plant Physiol. 34:512-519.

Biddulph, O., F.S. Nakayama, and R. Cory. 1961. Transpiration stream and ascension of calcium. Plant Physiol. 36:429-436.

Blanke, M.M and F. Lenz. 1988. Kann die Apfelfrucht selbst zur Stoffbildung beitragen ? (Does the apple fruit itself contribute to matter production?) Erwerbsobsbau 30:44-47.

Blanke, M.M and F. Lenz. 1989. Fruit photosynthesis. Plant Cell and Environ. 12:31-46.

Bollard, E.G. 1953. The use of tracheal sap in the study of apple-tree nutrition. J. Exp. Bot. 4:363-368.

Bollard, E.G. 1957. Translocation of organic nitrogen in the xylem. Aust. J. Biol. Sci. 10:292-301.

Bradfield, E.G. 1976. Calcium complexes in the xylem sap of apple shoots. Plant & Soil 44:495-499.

Bradfield, E.G. and C.G. Guttridge. 1979. The dependence of calcium transport and leaf tipburn in strawberry on relative humidity and nutrient solution concentration. Ann. Bot. 43:363-372.

Bramalage, W.J. S.A. Weis, and D.W. Greene. 1990. Observations on the relationships among seed number, fruit calcium, and senescent breakdown in apple. HortScience 25:351-352

Buchloh, G. 1974. Uptake and translocation of calcium. Acta Hort. 45:53-75.

Bukovac, M.J., S.W. Wittwer, and H.B. Tukey. 1958. Effect of stock-scion interrelationships on the transport of ^{32}P and ^{45}Ca in the apple. J. Hort. Sci. 33:145-152.

Bünemann, G. 1972. Bibliographische Reihe der Technischen Universitatät Berlin. Annotated bibliography on bitter pit of apples. Vol. 2.

Cermák, J., M. Deml, and M. Penka. 1973. A new method of sap flow rate determination in trees. Biol. Plant. 15:171-178.

Chamel, A.R. and J.P. Bossy. 1981. Electron-microprobe analysis of apple fruit tissues affected with bitter pit. Scientia. Hortic. 15:155-163.

Chapman, H.D. 1966. Calcium, p. 65-92 In: H.D. Chapman (ed.) Diagnostic Criteria for plants and soils. Univ. Cal., Div. Agr. Sci., Riverside.

Christiansen, M.N. and C.D. Foy. 1979. Fate and function of calcium in tissue. Commun. Soil Sci. Plant Anal. 10:427-442.

Chiu, T.F., and C. Bould. 1977. Sand-culture studies on the calcium nutrition of young apple trees with particular reference to bitter pit. J. Hort. Sci. 52:19-28.

Cline, R.A. 1983. Bitter pit control in apples. Ontario Ministry of Agriculture and Food. Bulletin Agdex 211/690.

Collier, C.F., and T.W. Tibbitts. 1984. Effects of relative humidity and root temperature on calcium concentration and tipburn development in lettuce. Amer. Soc. Hort. Sci. 109:128-131.

Delong, W.A. 1936. Variations in the chief ash constituents of apples affected with blotchy cork. Plant Physiol. 11:453-456.

Dieter, P. and D. Marmé. 1980. Ca^{++} transport in mitochondrial and microsomal fractions from higher plants. Planta 150:1-8.

Ehret, D.L. and Lim C. Ho. 1986. Translocation of calcium in relation to tomato fruit growth. Ann. Bot. 58:679-688.

Faust, M. 1989. Physiology of fruit trees. Academic Press, New York

Faust, M., and J.D. Klein. 1974. Levels and sites of metabolically active calcium in apple fruit. Proc. Amer. Soc. Hort. Sci. 99:93-94.

Faust, M., and C.B. Shear. 1968. Corking disorders of apples. A physiological and biochemical review. Bot. Rev. 34:441-469.

Faust, M., and C.B. Shear. 1972. The effect of calcium on respiration of apples. J. Amer. Soc. Hort. Sci. 97:437-439.

Faust, M., and C.B. Shear. 1973. Calcium translocation patterns in apples. Proc. Res. Inst. Pomology, Skierniewice, Poland, Ser. E. 3:423-436.

Faust, M., C.B. Shear, G.B. Oberle, and G.T. Carpenter. 1971. Calcium accumulation in fruit of certain apple crosses. HortScience 6:542-543.

Faust, M., C.B. Shear, and C.B. Smith. 1967. Investigations of corking disorders of apples. I. Mineral element gradients in 'York Imperial' apples. Proc. Amer. Soc. Hort. Sci. 91:69-72.

Faust, M., C.B. Shear, and C.B. Smith. 1968. Investigation of corking disorders of apples II. Chemical composition of affected tissues. Proc. Amer. Soc. Hort. Sci. 92:82-88.

Ferguson, I.B. 1979. The uptake and transport of calcium in the fruit tree, p.183-192 In: Mineral Nutrition of Fruit Trees. Atkinson, D., J.E. Jackson, and R.O. Sharples (eds.). Buttersworths Press, London, Toronto.

Ferguson, I.B. and E.G. Bollard. 1976. The movement of calcium in woody stems. Ann. Bot. 40:1057-1065.

Ferguson, I.B. and D.T. Clarkson. 1976. Simultaneous uptake and translocation of magnesium and calcium in barley (*Hordeum vulgare* l.) roots. Planta 28:267-269.

Ferguson, I.B., F.R. Harker, and B.K. Drøbak. 1987. Calcium and apple fruit. The Orchardist of New Zealand. May:119-121.

Ferguson, I.B. and M.S. Reid. 1979. Calcium analysis and the prediction of bitter pit in apple fruit. N.Z. J. Agric. Res. 22:485-490.

Ferguson, I.B., and C.B. Watkins. 1989. Bitter pit in apple fruit, p. 289-355 In: Hort Reviews. J. Janick (ed.). Timber Press, New York

Ford, M. 1979a. The distribution of calcium in mature apple fruits having bitter pit disorders. J. Hort. Sci. 54:91-92.

Ford, M. 1979b. Effect of post-blossom environmental conditions on fruit composition and quality of apple. Commun. Soil Sci. Plant Anal., 10:337-348.

Fried, M. and R.E. Shapiro. 1961. Soil-plant relationships in ion uptake. Ann. Rev. plant Physiol. 12:91-112.

Fuente, R.K. and A.C. Leopold. 1973. A role for calcium in auxin transport. Plant Physiol. 12:91-112.

Geraldson, C.M. 1971. Intensity and balance concept as an approach to optimal production, p. 352-354 In: R.M. Samish (ed.) Recent advances in plant nutrition. Gordon and Breach Sci. Pub., New York.

Goode, J.E., K.H. Higgs, and K.J. Hyrycz. 1978. Nitrogen and water effects on the nutrition, growth, crop yield and fruit quality of orchard-grown Cox's Orange Pippin apple trees. J. Hort Sci. 53:295-306.

Goode, J.E., K.H. Higgs, and K.J. Hyrycz. 1979. Effects of water stress control in apple trees by misting. J. Hort. Sci. 54:1-11.

Goode, J.E., and K.J. Hyrycz. 1964. The response of Laxton's Superb apple trees to different soil moisture conditions. J. Hort. Sci. 39:254-276.

Goode, J.E. and J. Ingram. 1971. The effects of irrigation on the growth, cropping and nutrition on Cox's Orange Pippin trees. J. Hort. Sci. 46:195-208.

Hall, E.G., D.A. Baker, and J.A. Milburn. 1971. Phloem transport of ^{14}C-labelled assimilates in *Ricinus* phloem exudate. Planta 100:200-207.

Hamilton, D.A., and P.J. Davies. 1988. Mechanism of export of organic material from the developing pea. Plant Physiol. 86:956-959.

Hanger, B.C. 1979. The movement of calcium in plants. Commun. Soil Sci. Plant Anal. 10:171-193.

Hanson, J.B. 1983. The roles of calcium in plant growth, p. 1-24 In: Current papers in plant biochemistry and physiology. D.D. Ransall, G.G. Bierins, R. Larson (eds.). Univ. of Missouri, Columbia.

Hanson, J.B. 1984. The functions of calcium in plant nutrition, p. 149-208 In: Advances in plant nutrition. Volume 1. P.B. Tinker and A. Lauchli (eds.). Praeger Pub., N.Y.

Hausenbuiller, R.L. 1978. Soil Science - principles and practices. 2nd Edition. W.C. Brown Co., Iowa.

Helper, P.K. and R.O. Wayne. 1985. Calcium and plant development. Ann. Rev. Plant Physiol. 36:397-439.

Himelrick, D.G. and M. Ingle. 1981. Calcium levels of apple leaves and fruit following tree sprays with EDTA, oxalic acid, TIBA, and calcium chloride. HortScience 16:167-168.

Himelrick, D.G. and R.F. McDuffie. 1983. The calcium cycle:uptake and distribution in apple trees. HortScience 118:147-150.

Himelrick, D.G. and C.E. Walker. 1982. Seasonal trends of calcium, magnesium, and potassium fractions in apple leaf and fruit tissues. J. Amer. Soc. Hor.. Sci. 107:1078-1080.

Hocking, P.J., and J.S. Pate. 1978. Accumulation and distribution of mineral elements in annual lupins Lupinus albus and *Lupinus angustifolius* L. Aust. J. Agric. Res. 29:267-280.

Holland, D.A., M.A. Perring, R. Rowe, D. J. Fricker. 1975. Discrepancies in the chemical composition of apple fruits as analyses by different laboratories. J. Hort. Sci. 50:301-310.

Hopfinger, J.A. and B.W. Poovaiah. 1978. Calcium and magnesium gradients in apples with bitter pit. Commun. Soil Sci. Plant Anal. 9:237.

Irving, D.E., and J.H. Drost. 1987. Effects of water deficit on vegetative growth, fruit growth and quality in Cox's Orange Pippin apples. J. Hort. Sci. 62:427-432.

Jackson, J.E., J.W. Palmer, M.A. Perring, and R.O. Sharples. 1977. Effects of shade on the growth and cropping of apple trees. III.Effects on fruit growth, chemical composition, and quality at harvest and after storage. J. Hort. Sci. 52:267-282.

Jackson, J.E., R.O. Sharples, and J.W. Palmer. 1971. The influence of shade and within-tree position on apple fruit size, colour and storage quality. J. Hort. Sci. 42:277-287.

Jäger, G. 1869. Uber das Pelzig oder Stippigwerden der Kernobstfrucht. Illustr. Monatsh. f. Obst-u.Weinbau, 318-319.

Jakøbsen, S.T. 1979. Interaction between phosphate and calcium in nutrient uptake by plant roots. Commun. Soil Sci. Plant Anal. 10:141-152.

Johnson, D.S., M.J. Marks, and K. Pearson 1987. Storage quality of Cox's Orange Pippin apples in relation to fruit mineral composition during development. J. Hort. Sci. 62:17-25.

Jones, H.G. and K.H. Higgs. 1982. Surface conductance and water balance of developing apple (*Malus pumila* Mill.) fruits. J. Exp. Bot. 33:67-77.

Jones, R.G.W., and O.R. Lunt. 1967. The function of calcium in plant roots. Commun. Bot. Rev. 33:407-426.

Jones, H.G., K.H. Higgs, and T.J. Samuelson. 1983. Calcium uptake by developing apple fruits. I. Seasonal changes in calcium content of fruits. J. Hort Sci. 58:173-182.

Jones, H.G. and T.J. Samuelson. 1983. Calcium uptake by developing apple fruits. II. The role of spur leaves. J. Hort. Sci. 58:183-190.

Kennedy, A.J., R. Watkins, J.M. Werts. 1987. Variation in leaf calcium in a range of apple rootstocks. Fruit Varieties J. 41:13-16.

Kirkby, E.A. 1979. Maximizing calcium uptake by plants. Commun. Soil Sci. Plant Anal. 10:89-114.

Kirkby, E.A. and A.H. Knight. 1977. Influence of the level of nitrate nutrition on uptake and assimilation, organic acid accumulation, and cation-anion balance in whole tomato plants. Plant Physiol. 60:349-353.

Kohl, W. 1966. Die Calciumverteilung in Apfeln und ihre Veranderung wahrend des Wachstums. Gartenbauwissenschaft 31:513-547.

Lang, A., and M.R. Thorpe. 1989. Xylem, phloem and transpiration flows in a grape: Application of a technique for measuring the volume of attached fruits to high resolution using Archimedes' Principle.

Lewis, T.L. 1980. The rate of uptake and longitudinal distribution of potassium, calcium, and magnesium in the flesh of developing apple fruits of nine cultivars. J. Hort. Sci. 55:57-63.

Lewis, T.L., and D. Martin. 1973. Longitudinal distribution of applied calcium and naturally occurring calcium, magnesium, and potassium in Merton apple fruits. Aust. J. Agr. Res. 24:363-371.

Loneragan, J.F. and K. Snowball. 1969. Calcium requirements of plants. Aust. J. Agr. Res. 20:465-478.

Lötter, J. De V., D.J. Beukes, and H.W. Weber. 1985. Growth and quality of apples as affected by different irrigation treatments. J. Hort. Sci. 60:181-192.

Macklon, A.E.S. 1975. Cortical cell fluxes and transport to the stele in excised root segments of *Allium cepa* L. II. Calcium. Planta 122:131-141.

Marmé, D. 1985. The role of calcium in the cellular regulation of plant metabolism. Physiol Veg. 23:945-953.

Marschner, H. 1983. General introduction to the mineral nutrition of plants. In: Encyclopedia of Plant Physiology. New Series Vol 15A. Inorganic Plant Nutrition. Lauchli (ed.).

Marschner, H. 1986. Functions of mineral nutrients: macronutrients, In: Mineral Nutrition of Higher Plants. Academic Press, London.

Mason, A.C. and A.B. Whitfield. 1960. Seasonal changes in the uptake and distribution of mineral elements in apple trees. J. Hort. Sci. 35:34-55.

Mengel, K. and E.A. Kirkby. 1987. In: Principles of Plant Nutrition. International Potash Institute, Switzerland.

Millaway, R.M. and L. Wiersholm. 1979. Calcium and metabolic disorders. Commun. Soil Sci. Plant Anal. 10:1-28.

Millikan, C.R. 1971. Mid-season movement of ^{45}Ca in apple trees. Aust. J. Agric. Res. 22:923-930.

Mix, G.P. and H. Marschner. 1976a. Calciumgehalte in Früchten von Paprika, Bohne, Quitte und Hagebutte im Verlauf des Fruchtwachstums (Calcium content in fruits of paprika, bean, quince, and hip during fruit growth). Z. Pflanzenernaehr. Bodenkd. 5:537-549.

Mix, G.P. and H. Marschner. 1976b. EinfluB exogener und endogener Faktoren auf den Calciumgehalt von Paprika- und Cohnenfrüchten. (Effect of external and internal factors on the calcium content of paprika and bean fruits). Z. Pflanzenernaehr. Bodenkd. 5:551-563.

Motto, A.K., and M. Lieberman. 1977. Localization of the ethylene-synthesizing system in apple tissue. Plant Physiol. 60:794-799.

Münch, E. 1930. Die Stoffbewegungen in der Pflanze. (Translocation in Plants.) Fischer, Jena.

Muto, S. and S. Miyachi. 1977. Properties of a protein activator of NAD kinase from plants. Plant Physiol. 59:55-60.

Oberly, G.H. 1973. Effect of 2,3,5-triiodobenzoic acid on bitter pit and calcium accumulation in 'Northern Spy' apples. J. Amer. Soc. Hort. Sci. 98:269-271.

Pate, J.S., and P.J. Hocking. 1978. Phloem and xylem transport in the supply of minerals to a developing legume (*Lupinus albus* L.) fruit. Annal. Bot. 42:911-921.

Pate, J.S. , P.J. Sharkey, O.A.M. Lewis. 1974. Xylem to phloem transfer of solutes in fruiting shoots of legumes, studies by a phloem bleeding technique. Planta 122:11-26.

Pate, J.S., M.B. Peoples, A.J.E. van Bel, J. Kuo, and C.A. Atkins. 1985 Diurnal water balance of the cowpea fruit. Plant Physiol. 77:148-156.

Perring, M.A. 1968. Mineral composition of apples. VII. The relationship between fruit composition and some storage disorders. J. Sci. Food Agric. 19:186-192.

Perring, M.A. 1979. The effects of environment and cultural practices on calcium concentration in the apple fruit. Commun. Soil Sci. Plant Anal. 10:279-293.

Perring, M.A. and H. Clijsters. 1974. The chemical composition and storage characteristics of apples grown in black cloth bags. Qualitas Plantarum 23:379-393.

Perring, M.A. and C.H. Jackson. 1975. The mineral composition of apples. Calcium concentrations and bitter pit in relation to mean mass per apple. J. Sci. Food Agric. 26:1493-1502

Perring, M.A. and K. Pearson. 1986. Redistribution of minerals in apple fruit during storage. Effects of storage temperatures, varietal difference, and orchard management. J. Sci. Fd. Agric. 37:607-617.

Perring, M.A. and W. Plocharski 1975. Differences in the mineral composition of sound and disordered apple fruits and of sound and pitted tissues. J. Sci. Food Agric. 26:1819-1823.

Perring, M.A. and A.P. Preston. 1974. The effects of orchard factors on the chemical composition of apples. III. Some effects of pruning and nitrogen application on Cox's Orange Pippin fruit. J. Hort. Sci. 49:85-93.

Perring, M.A. and R.O. Sharples. 1975. The mineral composition of apples. Composition in relation to disorders of fruit imported from the Southern Hemisphere. J. Sci. Food Agric. 26:681-689.

Perring, M.A. and B.G. Wilkinson. 1965. The mineral composition of apples. IV. The radial distribution of chemical constituents in apples, and its significance in sampling for analysis. J. Sci. Food Agr. 16:535-541.

Poovaiah, B.W. 1979. Role of calcium in ripening and senescence. Commun. Soil Sci. Plant Anal. 10:83-88.

Poovaiah, B.W. 1985. Role of calcium and calmodulin in plant growth and development. HortScience 20:347-352.

Poovaiah, B.W. 1988. Molecular and cellular aspects of calcium action in plants. Calcium in Horticulture Symposium. HortScience 23:267-271.

Poovaiah, B.W., and A.C. Leopold. 1973. Deferral of leaf senescence with calcium. Plant Physiol. 52:236-239.

Poovaiah, B.W., and A.S.N. Reddy. 1987. Calcium messenger in plants, p. 47-103 In: CRC Critical reviews in plant sciences. Vol. 6. CRC Press., New York.

Preston, A.P. and M.A. Perring. 1974. The effect of summer pruning and nitrogen on growth, cropping and storage quality of Cox's Orange Pippin apple. J. Hort. Sci. 49:77-83.

Quinlin, J.D. 1969. Chemical composition of developing and shed fruits of Laxton's Fortune apple. J. Hort. Sci. 44:97-106.

Redmond, W.J. 1975. Transport of Ca in apple trees and its penetration into the fruit. Ccmmun. Soil Sci. Plant Anal. 6:261-272.

Rogers, B.L. and L.P. Batjer. 1954. Seasonal trends of six nutrient elements in the flesh of Winesap and Delicious apple fruits. Proc. Amer. Soc. Hort. Sci. 63:67-73.

Rousseau, G.G., F.J. Haasbroek, and C.J. Visser. 1972. Bitter pit in apples:the effect of calcium on permeability changes in apple fruit tissue. Agroplantae 4:73-80.

Russell, R.S. and D.T. Clarkson. 1976. Ion transport in root systems, p. 401-411 In: Perspectives in experimental Biology. Vol. 2. Botany. N. Sunderland (ed). Pergamon Press, Oxford and New York.

Schumacher, R., F. Fankhauser, and W. Stadler. 1976. Versuche mit Calciumchlorid, antitranspiranten, und Borsäure zur verminderune der Stippebildung. Schweiz Z. Obst. u. Weinb. 112:300-304.

Schupp, J.R. and D.C. Ferree. 1987. Effect of root pruning at different growth stages on growth and fruiting if apple trees. HortScience 22:387-390.

Sharples, R.O. 1980. The influence of orchard nutrition on the storage quality of apples and pears grown in the United Kingdom, p. 17-28 In: Mineral Nutrition of Fruit Trees. Atkinson, D., J.E. Jackson, and R.O. Sharples (eds.). Buttersworths Press, London, Toronto.

Sharples, R.O., and D.S. Johnson. 1977. The influence on senescence changes in apple. Ann. Appl. Bio. 85:450-453.

Shear, C.B. 1975. Calcium-related disorders of fruits and vegetables. HortScience 10:361-365.

Shear, C.B. and M.B. Faust. 1970. Calcium transport in apple trees. Plant Physiol. 45:670-674.

Simon, E.W. 1978. The symptoms of calcium deficiency in plants. New Phytol. 80:1-15.

Sistrunk, J.W. and R.W. Campbell. 1966. Calcium content difference in various apple cultivars as affected by rootstock. Proc. Am. Soc. Hort. Sci. 88:38-40.

Smith, C.B., C.T. Morrow, G.M. Greene. 1987. Corking of 'Delicious' apple (*Malus domestica* Borkh.) on four rootstocks as affected by calcium and boron supplied through trickle irrigation. J. Plant Nut. 10:1917-1924.

Smith, J.A.C., and J.A. Milburn. 1980. Osmoregulation and the regulation of phloem-sap composition in *Ricinus communis* L. Planta 148:28-34.

Stahly, E.A. 1986. Time of application of calcium sprays to increase fruit calcium and reduce fruit pitting of apples sprayed with TIBA. HortScience 21:95-96.

Stahly, E.A., and N.R. Benson. 1970. Calcium levels of 'Golden Delicious' apples sprayed with 2,3,5-triiodobenzoic acid. J. Amer. Soc. Hort. Sci. 95:726-272.

Stahly, E.A., and N.R. Benson. 1972. Restriction of ^{45}Calcium translocation into apple fruit by 2,3,5-triiodobenzoic acid. HortScience 7:172-173.

Stahly, E.A., and N.R. Benson. 1976. Calcium levels of 'Golden Delicious' apples as influenced by calcium sprays, 2,3,5-triiodobenzoic acid, and other plant growth regulator sprays. J. Amer. Soc. Hort. Soc. 101:120-122.

Stahly, E.A., and N.R. Benson. 1982. Seasonal accumulation of calcium and potassium in the cortex of 'Golden Delicious' apple fruit sprayed with 2,3,5-triiodobenzoic acid. HortScience 17:781-783.

Stebbins, R.L. and D.H. Dewey. 1972. Role of transpiration and phloem transport in accumulation of $_{45}$calcium in leaves of young apple trees. J. Amer. Soc. Hort. Sci. 94:471-474.

Tammes, P.M.L., and J. van Die. 1964. Studies on phloem exudation from *Yucca flaccida* Haw. I. Some observations on the phenomenon of bleeding and the composition of the exudate. Acta Bot. Neerl. 13:76-83.

Terblanche, J.H., L.G. Wooldridge, I. Hesebeck, and M. Joubert. 1979. The redistribution and immobilization of calcium in apple trees with special reference to bitter pit. Commun. Soil Sci. Plant Anal. 10:195-215.

Titus, J.S., and N.S. Ghosheh. 1963. Some varietal and stock-scion effects on the cation distribution in Jonared and Golden Delicious apple trees. J. Amer. Soc. Hort. Sci. 82:35-45.

Tomala, K., M. Araucz, and B. Zaczek. 1989. Growth dynamics and calcium content in McIntosh and Spartan apples. Commun. Soil Sci. Plant Anal. 20:529-537.

Tomala, K., and D.R. Dilley. 1989. Calcium content of "McIntosh" and "Spartan" apples is influenced by the number of seeds per fruit. Int. Sym. on Diagnosis of Nutritional Status of Deciduous Fruit Orchards. Int. Soc. Hort. Sci., Warsaw, Poland. Abstr. pg. 66.

Tromp, J. 1975. The effect of temperature on growth and mineral nutrition of fruits of apple with special reference to calcium. Physiol. Plant. 33:87-93.

Tromp, J. 1979a. Seasonal variations in the composition of xylem sap of apple with respect to K, Ca, Mg, and N. Z. Pflanzenphysiol. Bd. 94:189-194.

Tromp, J. 1979b. Mineral absorption and distribution in young apple trees under various environmental conditions, p. 173-182. In: Mineral Nutrition of Fruit Trees. Atkinson, D., J.E. Jackson, and R.O. Sharples (eds.). Buttersworths Press, London, Toronto.

Tromp, J., and J. Oele. 1972. Shoot growth and mineral composition of leaves and fruits of apple as affected by relative are humidity. Physiol. Plant 27:253-258.

Tukey, L.D. 1964. A linear electronic device for continuous measurement and recording of fruit enlargement and contraction. Proc. Am. Soc. Hort. Sci. 84:653-660.

Tukey, L.D. 1974. Some relationships in the growth and development of apple fruits. Proc 19th Intern. Hort. Congr. 3:35-45.

Turner, N.A., I.B. Ferguson, and R.O. Sharples. 1977. Sampling and analysis for determining relationship of calcium concentration to bitter pit in apple fruit. N.Z. J. Agr. Res. 20:525-532.

Valancogne, C., and Z. Nasr. 1989. Measuring sap flow in the stem of small trees bya heat balance method. HortScience 24:383-385.

van de Geijn, S.C., C.M. Petit, H. Roelofsen. 1979. Measurement of the cation exchange capacity of the transport system in intact plant stems. Methodology and preliminary results. Commun. Soil Sci. Plant Anal. 10:225-236.

van der Boon, J. 1980. Prediction and control of bitter pit in apples. II. Control by summer pruning, fruit thinning, delayed harvesting and soil calcium dressings. J. Hort. Sci. 55:313-321.

Vang-Petersen, O. 1980. Calcium nutrition of apple trees: a review. Sci. Hort. 12:1-9

van Goor, B.J. 1971. The effect of frequent spraying with calcium nitrate solutions and occurrence of bitter pit of apple Cox's Orange Pippin. J. Hort. Sci. 46:347-364.

van Goor, B.J. and D. Wiersma. 1974. Redistribution of potassium, calcium, magnesium, and manganese in the plant. Physiol. Plant. 31:163-168.

Wiebe,H.J., H.P. Schätzler, and W. Kühn. 1977. On the movement and distribution of calcium in white cabbage in dependence of the water status. Plant and Soil 48:409-416.

Wiersum, L.K. 1966. Calcium content of fruits and storage tissue in relation to the mode of water supply. Acta. Bot. Neerl. 15:406-418.

Wiersum, L.K. 1979. Effects of environment and cultural practices on calcium nutrition. Commun. Soil Sci. Plant Anal. 10:259-278.

Wieneke, J. 1974. Untersuchungen zur Translokation von ^{45}Ca im Apfelbaum. III. Ca-Auswaschung im Verlaufe der Vegetation-Speriode. Gartenbauwissenschaft 39:161-171.

Wieneke, J. and F. Führ. 1975. Untersuchungen zur Translokation von ^{45}Ca in Apfelbaum. IV. Sekundäre Ca-Verlagerung nach der Ruheperiode. Gartenbauwissenschaft 40:105-112.

Wilkinson, B.G. 1968. Mineral composition of apples. IX. Uptake of calcium by the fruit. J. Sci. Food Agric. 19:646-64.

Wilkinson, B.G. and M.A. Perring. 1964. Changes in the chemical composition of apples during development, and near picking. J. Sci. Food Agric. 15:146-152.

Wills, R.B.H., K.J. Scott, P.B. Lyford, and P.E. Smale. 1976. Prediction of bitter pit with calcium content of apple fruit. N.Z. J. Agric. Res. 19:519-519.

CHAPTER 1

Seasonal Accumulation of Calcium in 'Red Delicious' Apple Fruit

INTRODUCTION

Several disorders of apple fruit are associated with low fruit calcium (Ca) concentrations (Faust *et al.*, 1968; Himelrick and McDuffie, 1983; Ferguson and Watkins, 1989). Fruit Ca levels and the severity of the disorder varies from year to year, between orchards, and even between fruit of the same branch in the same production year. The unpredictable nature of Ca accumulation has been attributed, in part, to yearly differences in the weather (Tromp, 1979a; 1975) and in crop load (van der Boon, 1980).

The climate of the orchard may influence the supply of Ca to fruit since small differences in environment are suggested to have large effects on fruit growth, mineral composition, and the storage quality of apples (Ford, 1979; Cline, 1983). Factors such as root temperatures (Carlson, 1965; Tromp, 1975, 1978; 1979a, 1979b), air temperatures (Ford, 1979), light intensity (Tromp, 1975; 1979b), soil moisture (Goode *et al.*, 1978; Tromp, 1979a, 1979b) and relative humidity (Tromp, 1972, 1979a, 1979b) have inconsistently altered fruit Ca levels when studied independently. However, whether these factors interact in the orchard to influence the seasonal accumulation of fruit Ca is not well documented.

Wilkinson (1968) proposed that apple fruit accumulate Ca in two stages. Stage I is the 4-6 week period beginning at bloom when cell division and fruit growth are rapid and Ca uptake is linear and rapid. During stage II (end of stage I to harvest), the uptake of Ca usually is more gradual or ceases (Tromp 1972, 1975; Jones and Samuelson, 1983, Jones *et al.*, 1983; Himelrick and Walker, 1982; Quinlin, 1969; Wilkinson, 1968). A net export of Ca from the fruit (Tromp, 1972; Wilkinson, 1968; Hanson, unpublished data; Perring, 1979) may also

occur. Calcium may also accumulate linearly throughout the season (Rogers and Batjer, 1954; Tromp, 1975, 1979; Tomala *et al.*, 1989). If two stages can be distinguished, the transition between stage I and II can occur as late as 12-16 weeks after full bloom (Jones *et al*, 1983, Jones and Samuelson, 1983). This shift is probably a result of the environmental influences on fruit growth and nutrient transport, however cultivar differences are also possible (Ferguson and Watkins, 1989).

We have observed that 'Red Delicious' apple fruit in some years, seem more prone to the Ca-related disorder, 'bitter pit', than other cultivars, however the reports of Ca accumulation patterns in 'Red Delicious' fruit are limited to two (Himelrick *et al.*,1982; Roger and Batjer, 1954). The objective of this study was to describe the seasonal pattern of Ca accumulation in 'Red Delicious' fruit under varying environmental conditions.

MATERIALS AND METHODS

Apple fruit were sampled throughout the 1988 and 1989 growing seasons from 'Red Delicious' (*Malus domestica* Borkh.) orchards in Belchertown, Massachsetts (MA), East Lansing, Michigan (MI), Vineland Station, Ontario (ONT), and Blacksberg, Virginia (VA) (1988 only). Strain/rootstock combinations included 'Starking'/M.7 (MA and MI), 'Starking'/MM.111 (Va), and 'Red Spur'/MM.106 (ONT). Soil texture ranged from sandy loam (MA and MI), to loam (ONT), and silt loam (VA). Tree age in 1988 was 25 (MA), 32 (MI), 12 (ONT), and 24 (VA) years. All trees received standard management practices for the region without irrigation or application of Ca sprays. Daily maximum and minimum temperatures and precipitation data were collected at each site.

Experimental units (plots) consisted of two trees in MI and ONT, and single trees in MA and VA, and were replicated six times at each location. Samples consisting of 20 fruit early in the season, and 10 fruit thereafter, were collected from each plot approximately twice monthly. Fruit were selected which best represented the tree's average fruit size on each date. Fruit were weighed immediately, placed in plastic bags, refrigerated, and shipped to Michigan for Ca analysis. Virginia samples were weighed and freeze-dried prior to transport. Upon arrival, all samples were processed immediately. Fruit, with pedicels removed, were homogenized with a food processor and tissue moisture content was determined by weight loss following oven-drying at 65°C (1988 samples) or freeze drying (1989 samples - to improve drying efficiency) to a constant weight. Representative samples were ashed in a muffle furnace at 550°C for 6 hours. Ash was dissolved in 20 ml of 10% (v/v) nitric acid and filtered through low-ash Whatman #41 paper into scintillation vials. A subsequent aliquot was appropriately diluted, prepared in 1000 ppm lanthanum and 2% (v/v) nitric acid, and analyzed for Ca by atomic absorption spectrophotometry.

RESULTS

Orchard precipitation and temperatures differed markedly between the years (Table 1). The 1988 season was characterized by low and sporadic rainfall (total rainfall 64% less than 1989), and high average temperatures (7% greater than 1989) and accumulated heat units (10% greater than 1989). Total rainfall in 1989 was adequate and excessive in MI and MA, respectively, whereas the total rainfall by August in ONT was 55% and 25% less than in MA and MI, respectively. Temperatures

varied more between years than locations. Bloom dates in 1988 were 29 April, 9 May, 15 May, and 15 May in VA, MI, MA and ONT, and in 1989 were 15 May, 18 May, 26 May in MA, MI, and ONT, respectively.

Calcium accumulation, plotted against time, increased in a quadratic fashion with most uptake occurring in the first two-thirds of the season (Fig. 1). Calcium content, plotted against fruit weight to correct for environmental influence on fruit growth, followed a similar trend as in Figure 1 (appendix 3). Generally, Ca uptake into fruit continued almost linearly 10-14 weeks after bloom and then leveled off. Final Ca content in the 1988 fruit at commercial harvest ranged from approximately 8 to 10 mg Ca $fruit^{-1}$ over all locations. Fruit Ca concentrations were similar regardless of the location or year (Fig 2.).

Fruit growth increased sigmoidally both years at all locations (Fig. 3), although final fruit weight in 1989 was 20 to 30% less than in 1988. Final weight of the Va fruit was 20% less than their counterparts.

DISCUSSION

Similar patterns of Ca accumulation were observed over a wide range of seasonal temperatures, rainfall, and potential cropping factors. These observations indicate that 'Red Delicious' fruit are unlikely to accumulate additional Ca during the three weeks prior to harvest. Furthermore, in four of the seven observations fruit appeared to lose Ca prior to harvest.

Calcium uptake appeared to occur in two stages. Uptake was rapid and linear for the first 10 to 14 weeks of fruit development (stage I), then declined thereafter (stage II). Himelrick *et al.* (1982) observed that Ca uptake in 'Red Delicious' fruit increased linearly for 6 weeks

following bloom and then leveled off to 9 mg Ca·fruit^{-1} at harvest. The above data are consistent with seasonal patterns described in other cultivars as well (Tromp, 1972,1975; Jones *et al.*, 1983a; Wilkinson, 1968; Quinlan, 1969).

Variations in the transition period where Ca uptake shifts from predominantly linear (stage I) to curvilinear (stage II), appeared to be more related to fruit weight than Ca content, since fruit Ca concentrations were similar at any given time (Fig. 2) while fruit weight differed markedly between years (Fig. 3).

It is unclear why fruit weight was generally greater in 1988 than in 1989. Temperatures were higher in 1988, which may have increased fruit growth (Tromp, 1979b), while low soil moisture levels may not have caused adequate stress to decrease fruit size. Crop load may also affect fruit size (van der Boon, 1980), though cropping levels were not measured in this study.

A late-season export of Ca from fruit appeared to occur in 1988 in MA, MI, and in 1989 in MA and ONT (Fig. 1). Similar late season declines in Ca have been observed when trees are under moisture stress (Tromp and Oele, 1972; Tromp, 1979a; Wilkinson, 1968; Perring, 1979). However, Ca losses did not appear to result from moisture stress in this study. For example, the MA site received the greatest amount of rain each year, but was the only location where fruit appeared to lose Ca both years. We suggest that the movement of Ca in apple fruit may be controlled by factors in addition to soil moisture.

Table 1. Average monthly temperature, precipitation, and accumulated heat units for Amherst, Massachusetts; East Lansing, Michigan; Vineland Station, Ontario; and Blacksburg, Virginia in 1988, 1989.

Location	Average monthly temperature(°C)		Average monthly precipitation (mm)		Accumulated heat units (base 5°C)	
	1988	1989	1988	1989	1988	1989
MASSACHUSETTS						
May	14.6	14.6	83	244	298	297
June	18.0	18.8	63	169	390	414
July	24.2	21.2	231	120	595	504
Aug.	22.9	20.3	100	172	555	473
Sept.	18.0	17.4	54	188	350	347
AVG(TOTAL)	19.5	18.5	(531)	(893)	(2188)	(2035)
MICHIGAN						
May	16.8	12.7	15	125	330	219
June	20.1	19.1	4	85	473	440
July	23.4	22.0	61	46	573	527
Aug.	23.2	20.1	90	175	560	468
Sept.	16.5	15.1	94	150	321	301
AVG(TOTAL)	19.5	17.8	(265)	(582)	(2257)	(1955)
ONTARIO						
May	13.2	12.4	33	122	253	230
June	19.1	18.2	11	94	422	397
July	23.3	21.9	134	77	565	522
Aug.	21.7	20.3	75	30	518	474
Sept.	16.3	16.8	61	103	341	355
AVG(Total)	18.7	17.9	(314)	(426)	(2099)	(1978)
VIRGINIA						
May[z]	---	---	---	---	---	---
June	19.9	---	72	---	438	---
July	22.3	---	137	---	530	---
Aug.	22.4	---	142	---	530	---
Sept.	14.1	---	105	---	331	---
AVG(Total)	19.7	---	(456)	---	(1829)	---

zData unavailable

Fig. 1. Seasonal changes in calcium content of 'Red Delicious' fruit from several orchards in 1988 and 1989 plotted against time. Calcium content = $ax^2 + bx + c$, where

Location	a	b	c	r2
Massachusetts				
1988	-1.1×10^{-3}	0.29	-8.13	0.72
1989	-9.1×10^{-4}	0.21	-3.71	0.73
Michigan				
1988	-1.0×10^{-3}	0.24	-3.77	0.63
1989	-6.3×10^{-4}	0.16	-1.76	0.85
Ontario				
1988	-7.7×10^{-4}	0.20	-2.84	0.63
1989	-9.9×10^{-4}	0.20	-1.57	0.45
Virginia				
1988	-4.1×10^{-4}	0.13	-2.52	0.70

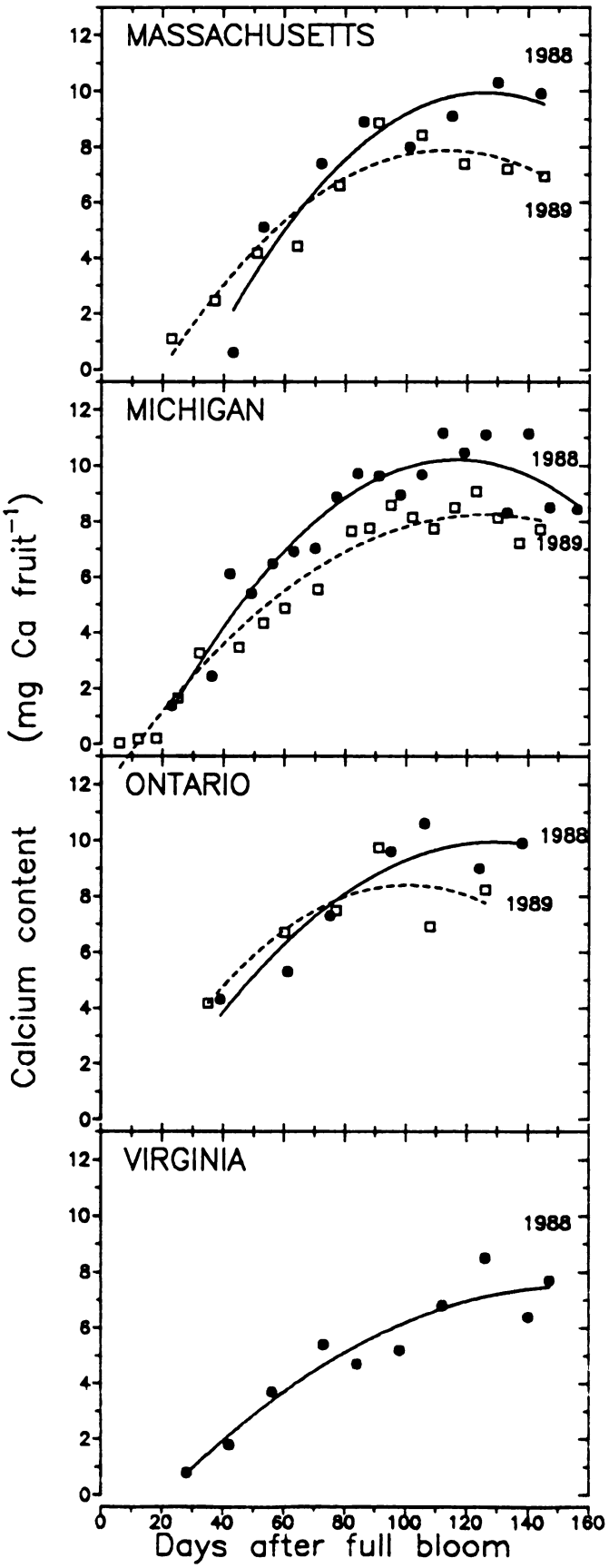

MASSACHUSETTS
1988
1989
MICHIGAN
1988
1989
ONTARIO
1988
1989
VIRGINIA
1988
Calcium content (mg Ca fruit^{-1})
Days after full bloom
0
20
40
60
80
100
120
140
160
2
4
6
8
10
12

Fig. 2. Seasonal changes in calcium concentrations (dry weight) of 'Red Delicious' fruit from several orchards in 1988 and 1989.

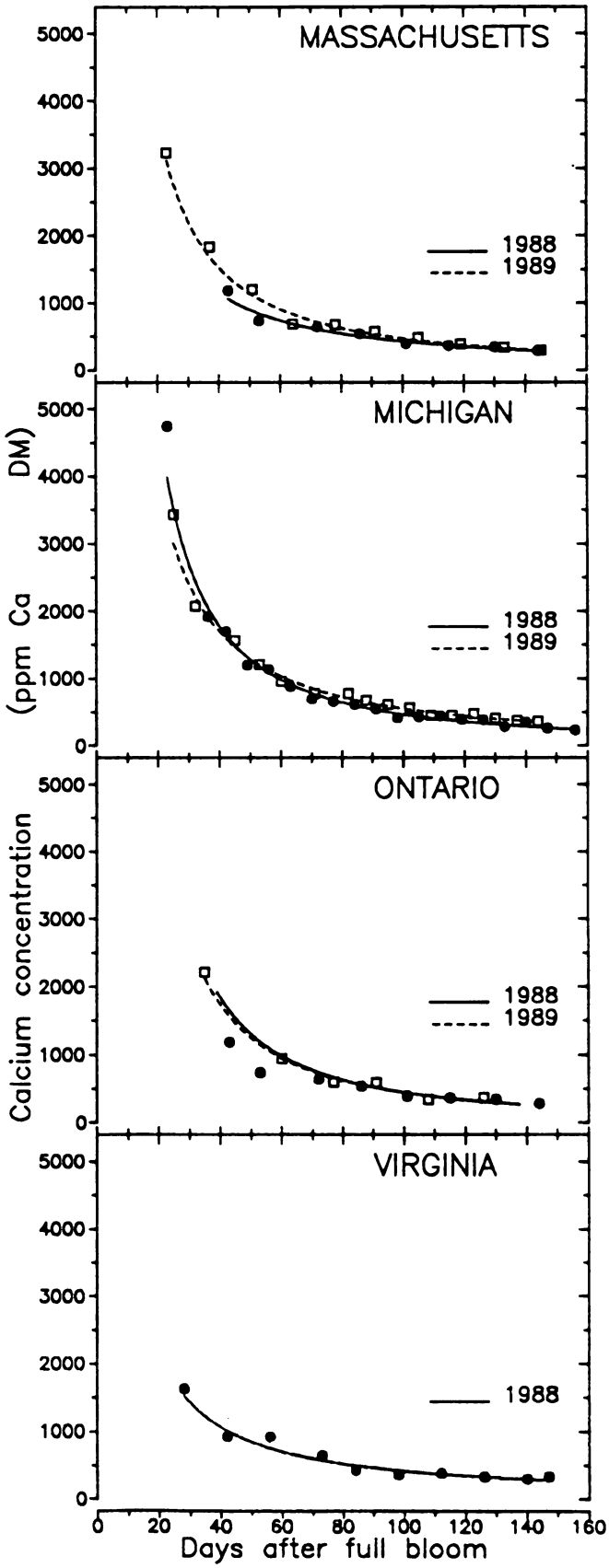
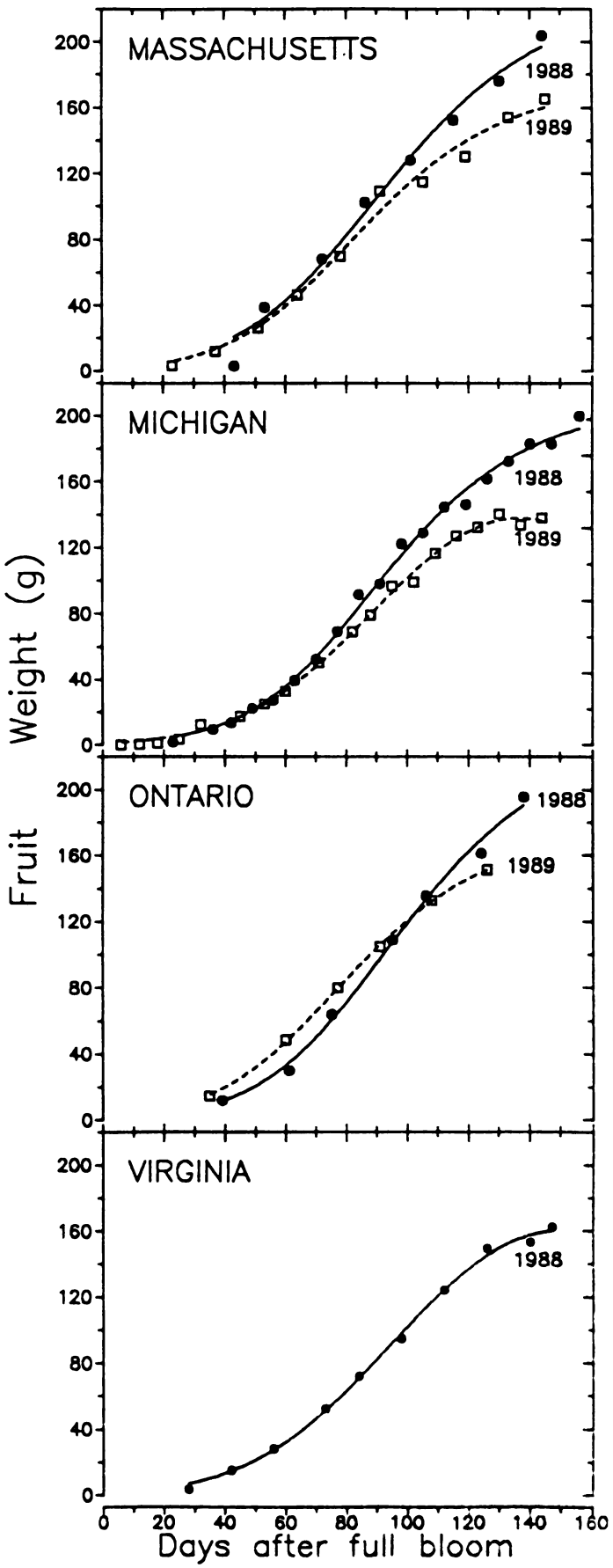

MASSACHUSETTS
MICHIGAN
ONTARIO
VIRGINIA
1988
1989
Calcium concentration (ppm Ca DM)
Days after full bloom
0
1000
2000
3000
4000
5000
0
20
40
60
80
100
120
140
160

Figure 1. Relative humidity and temperatures surrounding low RH treated fruit (bagged with desiccant), high RH treated fruit (bagged without desiccant) and control fruit (untreated, no bag). Relative humidity levels are a mean of two replications. Ambient temperature is a mean of all three treatments.


MASSACHUSETTS
1988
1989
MICHIGAN
1988
1989
ONTARIO
1988
1989
VIRGINIA
1988
Fruit Weight (g)
0
40
80
120
160
200
0
20
40
60
80
100
120
140
160
Days after full bloom

LITERATURE CITED

Carlson, R.F. 1965. Responses of Maller Merton clones and Delicious seedlings to different root temperatures. Proc. Amer. Soc. Hort. Sci. 86:41-45.

Cline, R.A. 1983. Bitter pit control in apples. Ontario Ministry of Agriculture and Food. Bulletin Agdex 211/690.

Faust, M., C.B. Shear, and C.B. Smith. 1968. Investigation of corking disorders of apples II. Chemical composition of affected tissues. Proc. Amer. Soc. Hort. Sci. 92:82-88.

Ferguson, I.B., and C.B. Watkins. 1989. Bitter pit in apple fruit, p. 289-355 In: Hort Reviews. J. Janick (ed.). Timber Press, New York

Ford, M. 1979. Effect of post-blossom environmental conditions on fruit composition and quality of apple. Commun. Soil Sci. Plant Anal., 10:337-348.

Goode, J.E., K.H. Higgs, and K.J. Hyrycz. 1979. Effects of water stress control in apple trees by misting. J. Hort. Sci. 54:1-11.

Goode, J.E., K.H. Higgs, and K.J. Hyrycz. 1978. Nitrogen and water effects on the nutrition, growth, crop yield and fruit quality of orchard-grown Cox's Orange Pippin apple trees. J. Hort Sci. 53:295-306.

Himelrick, D.G. and R.F. McDuffie. 1983. The calcium cycle:uptake and distribution in apple trees. HortScience 118:147-150.

Himelrick, D.G. and C.E. Walker. 1982. Seasonal trends of calcium, magnesium, and potassium fractions in apple leaf and fruit tissues. J. Amer. Soc. Hort. Sci. 107:1078-1080.

Irving, D.E., and J.H. Drost. 1987. Effects of water deficit on vegetative growth, fruit growth, and fruit quality in Cox's Orange Pippin apples. J. Hort. Sci. 62:427-432.

Jones, H.G., K.H. Higgs, and T.J. Samuelson. 1983. Calcium uptake by developing apple fruits. I. Seasonal changes in calcium content of fruits. J. Hort Sci. 58:173-182.

Jones, H.G. and T.J. Samuelson. 1983b. Calcium uptake by developing apple fruits. II. The role of spur leaves. J. Hort. Sci. 58:183-190.

Perring, M.A. 1979. The effects of environment and cultural practices on calcium concentration in the apple fruit. Commun. Soil Sci. Plant Anal. 10:279-293.

Rogers, B.L. and L.P. Batjer. 1954. Seasonal trends of six nutrient elements in the flesh of Winesap and Delicious apple fruits. Proc. Amer. Soc. Hort. Sci. 63:67-73.

Shear, C.B. 1979. Interaction of nutrition and environment on mineral composition of fruits. p. 41-50 In: Mineral Nutrition of Fruit Trees. Atkinson, D., J.E. Jackson, and R.O. Sharples (eds.). Buttersworths Press, London, Toronto.

Slowik, K. 1979. Effects of environmental and cultural practices on calcium nutrition of fruit trees. Commun. Soil Sci. Plant Anal. 10:295-302.

Tomala, K., M. Araucz, and B. Zaczek. 1989. Growth dynamics and calcium content in McIntosh and Spartan apples. Commun. Soil Sci. Plant Anal. 20:529-537.

Tromp, J., and J. Oele. 1972. Shoot growth and mineral composition of leaves and fruits of apple as affected by relative humidity. Physiol. Plant 27:253-258.

Tromp, J. 1975. The effect of temperature on growth and mineral nutrition of fruits of apple with special reference to calcium. Physiol. Plant. 33:87-93.

Tromp, J. 1978. The effect of root temperature on the absorption and distribution of K, Ca, and Mg in three rootstock clones of apple budded with Cox's orange pippin. Gartenbauwissenschaft 43:49-54.

Tromp, J. 1979a. The intake curve for calcium into apple fruits under various environmental conditions. Commun. Soil Sci. Plant anal. 10:325-335.

Tromp, J. 1979b. Mineral absorption and distribution in young apple trees under various environmental conditions, p. 173-182. In: Mineral Nutrition of Fruit Trees. Atkinson, D., J.E. Jackson, and R.O. Sharples (eds.). Buttersworths Press, London, Toronto.

van der Boon, J. 1980. Prediction and control of bitter pit in apples. II. Control by summer pruning, fruit thinning, delayed harvesting and soil calcium dressings. J. Hort. Sci. 55:313-321.

Wilkinson, B.G. 1968. Mineral composition of apples. IX. Uptake of calcium by the fruit. J. Sci. Food Agric. 19:646-64.

CHAPTER TWO

THE EFFECT OF RELATIVE HUMIDITY ON CALCIUM TRANSLOCATION INTO APPLE FRUIT

INTRODUCTION

Certain physiological disorders of plants are correlated with insufficient calcium (Ca) in specific organs or tissues. In apple fruit specifically, the development of Ca-related disorders reduces fruit quality and storage ability, and is closely associated with low Ca concentration in the fruit cortex (Faust and Shear, 1968; Ferguson and Watkins, 1989). Calcium related disorders are primarily the result of poor Ca distribution to the fruit (Himelrick and McDuffie, 1983) rather than limited root uptake (Bangerth, 1979; Hanger, 1979).

Disagreement exists regarding the phloem mobility of Ca in apple trees and how Ca is translocated to fruit (Ferguson and Bollard, 1976; Redmond, 1975; Shear and Faust, 1970; Vang-Petersen, 1980). A hypothesis initiated by Wiersum (1966) and developed by others (Wilkinson, 1968; Ferguson, 1979; Hanger, 1979; Ferguson *et al.*, 1987; Ferguson and Watkins, 1989) suggested that the xylem is the primary route of Ca supply early in the season, whereas the phloem predominates later. Early in the season Ca is distributed principally by the water demanded by rapidly transpiring organs such as leaves and young fruit. Young fruit are relatively strong sinks for water since they have a high surface area:volume ratio and permeability to water (Chapter 3; Blanke and Lenz, 1989; Blanke and Lenz, 1985). Young fruit are also photosynthetically competent (Blanke and Lenz, 1989) and require little external photosynthate. Water supply to young fruit is likely to be provided by the xylem where Ca moves comparatively freely.

During fruit growth the surface area:volume ratio decreases, the fruit cuticle becomes more lipophilic (Blanke and Lenz, 1989; Ferguson and Watkins, 1989), stomata are less dense and functional (Blanke and

Lenz, 1989), and the ratio of leaf:fruit number and surface area increases. These changes reduce fruit transpiration (Jones *et al*, 1983; Blanke and Lenz, 1985) and xylem water flow, ultimately disfavoring movement of Ca into the fruit. The net rate of Ca uptake decreases through the season while the supply of phloem-mobile nutrients (K, Mg, P, N), and photosynthate increase or remain the same (Tromp, 1975).

Although this theory seems logical, it is questionable whether the phloem can supply significant quantities of Ca to fruit. Although Ca concentrations in the phloem of apple trees have not been reported, due to the difficulty in collecting adequate volumes of sap, Ca levels in the phloem sap of other plant species are generally low (Hall *et al.*, 1971; Wiersum, 1979; Tammes and Van Die, 1964; Marschner, 1986). Indirect techniques modifying the phloem transport system and tracing ^{45}Ca indicate that Ca is phloem mobile (Stebbins and Dewey, 1972; Faust and Shear, 1973), however, lack of Ca re-mobilization from leaves (Himelrick and McDuffie, 1983) is evidence against the existence of a significant phloem supply. Since the rate of Ca accumulation in fruit typically decreases later in the season (Tromp and Oele, 1972, Tromp, 1975; Jones and Samuelson, 1983, Jones *et al.*, 1983; Himelrick and Walker, 1982; Quinlin, 1969; Wilkinson, 1968; Bangerth, 1979), only a limited phloem supply of Ca may be needed to explain this pattern of accumulation.

The importance of the xylem system in supplying Ca to plant organs has been demonstrated by imposing different relative humidity (RH) treatments which alters the transpiration rates of fruit (Armstrong and Kirkby, 1979; Banuelos *et al.*, 1987; Ehret and Ho, 1986; Mix and Marschner, 1976a, 1976b; Bradfield and Guttridge, 1979) and leaves

(Collier and Tibbitts, 1984; Wiebe *et al.*, 1977). When entire apple trees are subjected to low RH environments, leaf and fruit Ca contents were increased (Tromp, 1979; Tromp & Oele, 1972). However, these effects are difficult to interpret because dry matter accumulation and growth were also reduced. No reports of controlled studies on individual fruit are available. Ford (1979) observed that fruit covered with plastic bags on the tree had a greater incidence of bitter pit after storage, but Ca concentrations were not different.

If the xylem is the primary route of Ca flow to apple fruit, changing fruit transpiration rates would likely alter fruit Ca levels since xylem flow is controlled, in part, by transpiration . If the fruit were supplied with Ca predominantly from the phloem, changing fruit transpiration rates would have little effect on fruit Ca content. Also, if xylem transport of Ca is significantly reduced by the end of the season, changes in the RH surrounding fruit at this time would have little influence on the import of Ca to the fruit. The objective of this study was to determine the importance of the xylem in supplying Ca to fruit during different stages of development.

MATERIALS AND METHODS

Fruit on two adjacent 32 year old trees of *Malus domestica* (Borhk.) cv. 'Starking Red Delicious'/M.27 in East Lansing, Michigan were used for this study. Trees received standard pruning, and fertilization, herbicide, and pesticide practices, without irrigation or Ca sprays. The influence of changes in fruit transpiration rates on Ca supply was studied by altering the relative humidity around individual fruit during different periods of fruit development (Table 1).

Table 1. Periods during which humidity treatments were imposed on 'Red Delicious' fruit in 1988 and 1989.

Period	1988	1989
1	1 Jul. - 15 Aug.	8 Jun. - 4 Jul.
2	15 Aug. - 1 Oct.	7 Jul. - 5 Aug.
3	---	12 Aug. - 12 Sept.
4	---	12 Sept.- 12 Oct.

During 1988, the following 4 treatments were replicated 15 times on single, uniformly sized fruit selected randomly amongst the two trees: 1) untreated control fruit; 2) control fruit; 3) high RH; and 4) low RH. Humidity treatments were imposed by enclosing fruit in 2 mil, 1 litre-sized low density *Zip-Lock*™ polyethylene bags (Dow Chemical Company, USA) with or without a calcium chloride desiccant. Bags were secured around fruit with the help of a modified plastic container with a 12.5 cm diameter screw-top lid. An 8 mm hole was made in each lid and a slit was cut between the edge of the lid and the hole. The pedicel of the fruit ran through the hole and was surrounded by polyethylene foam to cushion it from the lid. The bottom was cut from the container leaving a 3-5 cm long threaded cylinder. Bags were placed inside each cylinder and the bag top was folded over the lip. The cylinder was screwed to the lid to provide an enclosed chamber. The apparatus was secured to the nearest stable branch using fibrous, weather-resistant tape. A rubber septum (10.5 mm diameter, 25 mm height) was placed in the top of the lid for gas sampling (using a syringe) or for insertion of a humidity/temperature sensor into the sealed plastic chamber. Fruit not enclosed in bags and fitted with lids served as 'control fruit', while fruit not treated at all served as 'untreated control fruit'.

The low RH treatment was imposed by placing 50 g of calcium chloride desiccant in the bags. The desiccant was enclosed in spunbound olefin (*Tyvek*™, Dupont Chemical Company) pouches (Shirazi and Cameron, 1989) which prevented the fruit from contacting the desiccant. The high RH treatment was imposed by enclosing fruit in the bags without the desiccant.

During 1989, two additional treatments were imposed as alternative methods of altering fruit transpiration. Entire fruit were dipped in either melted paraffin wax or anti-transpirant (10% v/v, *Wilt-Pruf*, Wilt-Pruf Products Inc, Connecticut). The number of replications for all treatments were also increased from 15 in 1988 to 30 in 1989.

Fruit weight was estimated at the initiation of each period in 1989 so that the treatment effect on weight gain could be calculated. This was achieved by measuring fruit diameter (d) and length (l), using calipers, at the initiation and termination of each treatment period, to estimate the surface area of the detached fruit [fruit surface area = [d x $(\frac{1}{2}d)^2$ + $(\frac{1}{2}l)^2$] (Long, 1980)]. Surface area was then used to estimate the weight of the fruit when attached to the tree, using an equation which correlated fruit weight taken at the termination of each treatment period, with fruit surface area.

Relative humidity and temperature were measured simultaneously during the last treatment period in 1988 and 1989 in the low and high RH treatment bags, and in the external environment. Analog temperature/humidity sensors (model 850-242, General Eastern, Massachusetts) connected to a *Polycorder*™ data logger (model 516C-64, Omni Data International, Utah) provided unattended continuous recording of the conditions for three days. Carbon dioxide and oxygen

concentrations from five high RH, low RH and control (atmospheric levels) fruit were monitored once each treatment period by analyzing 1 cm^3 of air using a standard infrared gas analyzer.

At the end of each treatment period, individual fruit, minus pedicels, were removed, weighed, and homogenized using a food processor. Tissue moisture content was determined by weight loss following oven-drying at 65°C (1988 samples) or freeze drying (1989 samples). Representative samples were ashed in a muffle furnace at 550°C for 6 hours, and then dissolved in 20 ml of 10% (v/v) nitric acid and filtered with low-ash Whatman #41 paper into scintillation vials. A subsequent aliquot was prepared in 1000 ppm lanthanum and 2% (v/v) nitric acid, diluted appropriately, and analyzed for Ca by atomic absorption spectrophotometry.

RESULTS

Relative humidity levels within the bags were distinctly different from ambient levels. Temperature and RH levels for high RH, low RH, and control fruit, over a three day period in 1988 (Figure 1), are representative of measurements on several other dates. High RH treatments (fruit bagged without desiccant) ranged from 75 % RH at mid-day to 100% at night. Low RH treatments (fruit bagged with $CaCl_2$ desiccant) ranged from 20-40% RH, while the RH surrounding untreated fruit (ambient levels in the tree canopy) remained between the levels in the high and low RH treatments. Air temperatures surrounding the bagged fruit only are presented (Figure 1) as these were similar to non-bagged fruit during each measurement period.

Treatments imposed during two different periods in 1988 had little

effect on fruit Ca content or concentration. Low RH, during period one (50 to 90 days after bloom), reduced fruit weight by 12 % and increased dry matter and fruit Ca concentration (ppm fresh wt.) compared to untreated fruit (Table 2). Treatments imposed later in the 1988 season, 103-153 days after bloom, had no effect on fruit Ca levels, however, low and high RH fruit had significantly lower dry matter content than untreated control fruit.

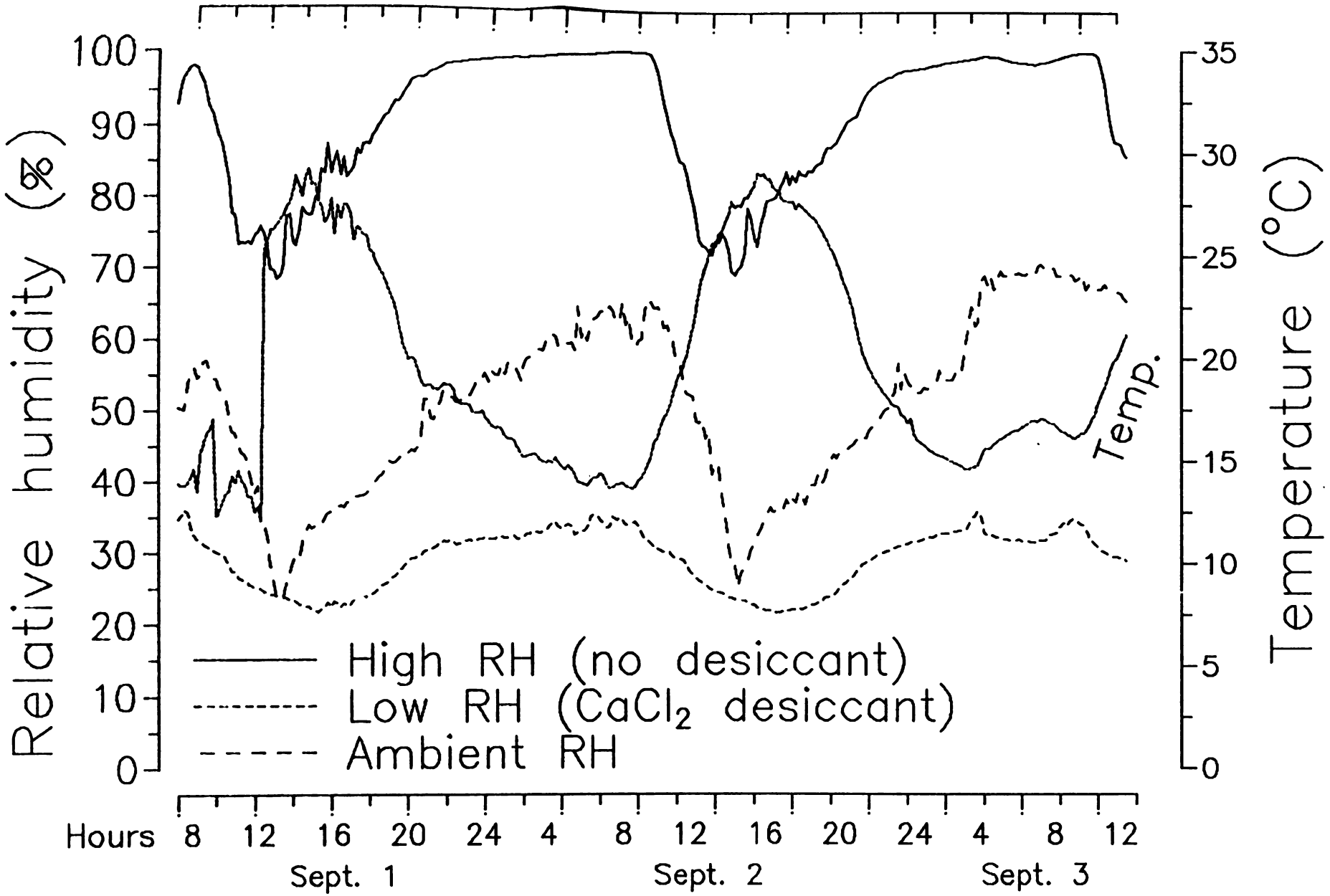
Relative humidity treatments did affect the Ca content of fruit in 1989 (Table 3). During periods 1,3, and 4, high RH decreased fruit Ca contents by 18, 16, and 11%, while low RH treatments increased Ca content by 20, 15, and 11%, respectively, compared with untreated fruit. During period 2 no significant effect of RH on Ca content was detected, however, high RH treatments tended to increase and low RH treatments tended to decrease Ca content. This was a reverse of what was observed for the three other treatment periods. High and low RH had no effect on final fruit weight during any time period when compared with each other or with untreated fruit. The change in fruit weight during each treatment period was similar for the untreated, high, and low RH fruit treatments with the exception that the low RH treatment reduced growth compared to untreated fruit during period 4. The specific effect of RH on fruit Ca concentration followed a noticeable and at times significant trend. Relative humidity had no effect on fruit Ca concentration (dry wt.) except during the last period when high RH decreased Ca concentration by 20% compared to low RH fruit. In general high RH treatments reduced fruit Ca concentrations while low RH treatments increased them, compared to untreated fruit. This trend was accentuated towards the end of the season.

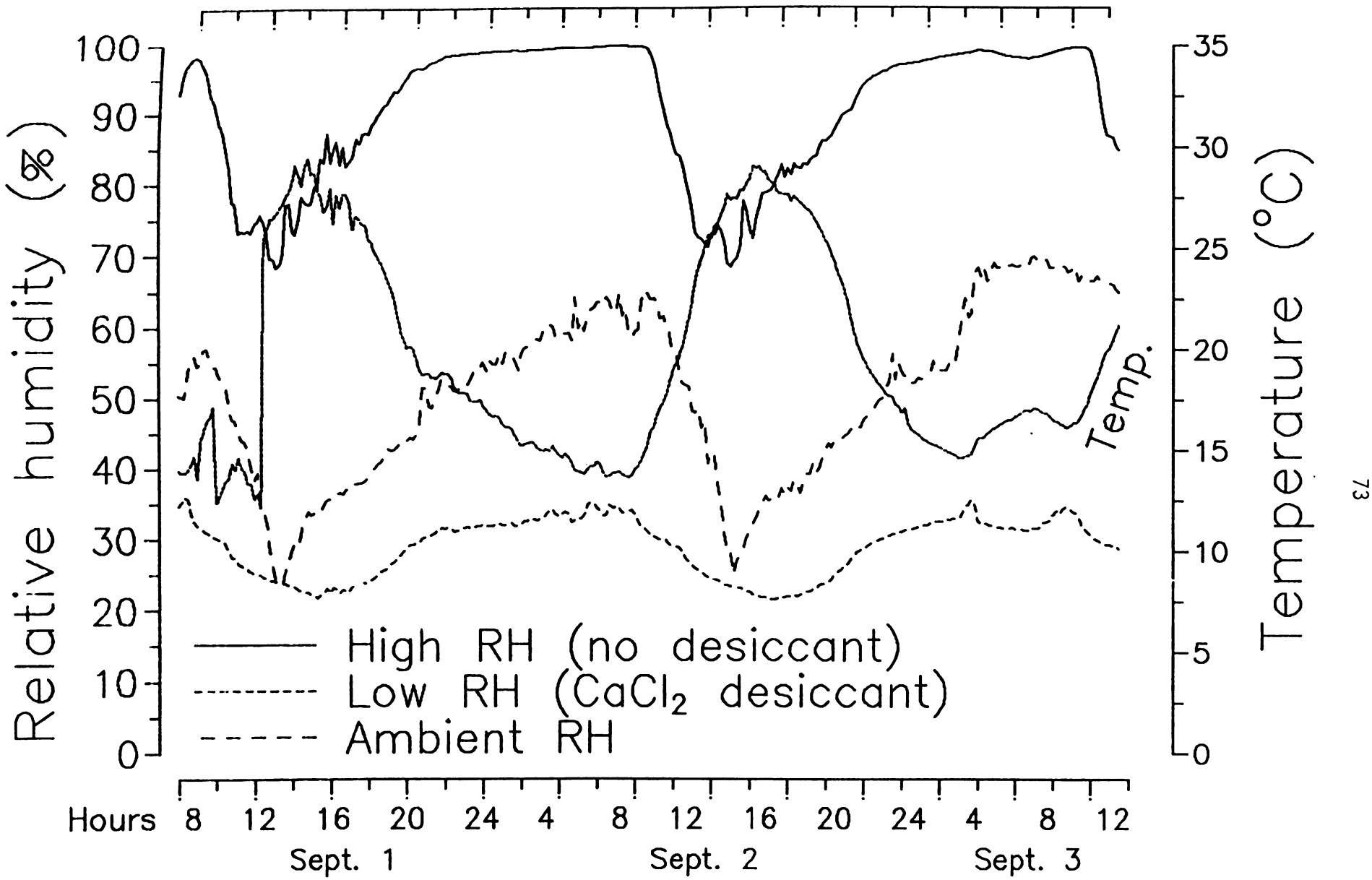
Wax treated fruit had significantly less Ca than untreated control fruit during periods 2 and 3, however, on the average, fruit growth was reduced by 30% during all the treatment periods. Anti-transpirants appeared to have little influence on Ca content other than that related to differences in fruit growth.

Seasonal rates of fruit growth during the treatment periods were greatest during period 2 and 3, at which time fruit were increasing more in diameter than in length.

Fruit abscission was greatest early in the season when treatments were applied prior to post-bloom drop (June drop) and for the low RH treatment, for which, both years combined, 6 fruit abscised during each period. Three fruit in total abscised for the other treatments.

Carbon dioxide and oxygen levels within the bagged treatments were not significantly different from atmospheric levels at each time measured (data not presented).


Relative humidity (%)
100
90
80
70
60
50
40
30
20
10
0
Temperature (°C)
35
30
25
20
15
10
5
0
Temp.
High RH (no desiccant)
Low RH ($CaCl_2$ desiccant)
Ambient RH
Hours
8 12 16 20 24 4 8 12 16 20 24 4 8 12
Sept. 1
Sept. 2
Sept. 3


Relative humidity (%)
100
90
80
70
60
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40
30
20
10
0
Temperature (°C)
35
30
25
20
15
10
5
0
Temp.
High RH (no desiccant)
Low RH ($CaCl_2$ desiccant)
Ambient RH
Hours
8 12 16 20 24 4 8 12 16 20 24 4 8 12
Sept. 1
Sept. 2
Sept. 3

Table 2. The effect of relative humidity on apple (cv. 'Red Delicious') fruit growth, percent dry matter, calcium concentration and calcium content for 2 treatment periods. 1988.[z]

Treatment	Gain in fruit diameter (mm)	Gain in fruit length (mm)	Predicted initial fruit weight (g)	Actual final fruit weight (g)	Predicted change in fruit weight (g)	Percent Moisture (%)	Calcium concentration		Calcium content mg·fruit^{-1}
							g Ca· g dm^{-1}	µg Ca· g fw	
PERIOD 1: 1 JULY - 15 AUGUST									
Untreated control[y]	---[x]	---	---	128 a	---	83.7	429	70 b	8.8
Control[w]	---	---	---	125 ab	---	84.4	503	79 b	9.7
Low RH	---	---	---	113 b	---	78.6	495	104 a	11.8
High RH	---	---	---	132 a	---	84.3	577	90 ab	11.6
PERIOD 2: 23 AUGUST - 14 OCTOBER									
Untreated control	---	---	---	200	---	82.6 b	300	52	10.4
Control	---	---	---	205	---	83.0 ab	371	62	13.2
Low RH	---	---	---	220	---	83.4 a	322	53	11.7
High RH	---	---	---	203	---	83.6 a	440	72	14.8

[z]Mean separation within columns by Least Significant Difference; numbers followed by similar letters not significantly different at $P = 0.05$.
[y]Untreated fruit
[x]Data not available (collected in 1989 only)
[w]Fruit treated with lid apparatus only

Table 3. The effect of relative humidity, paraffin wax, and anti-transpirant on apple (cv. 'Red Delicious') fruit growth, percent dry matter, and calcium levels during four treatment periods in 1989.[z]

Treatment	Gain in fruit diameter (mm)	Gain in fruit length (mm)	Predicted initial fruit weight (g)	Actual final fruit weight (g)	Predicted change in fruit weight (g)	Percent Moisture (%)	Calcium concentration		Calcium content mg·fruit^{-1}
							µg Ca· g dm^{-1}	µg Ca· g fw^{-1}	
PERIOD ONE: 8 JUNE - 5 JULY									
Untreated control[y]	19.4 a	12.1 abc	5.3	20.4 ab	15.0	86.4	1520	202	4.13 ab
Control[x]	20.0 a	13.1 a	5.4	21.6 a	16.2	87.6	1859	233	4.83 a
Low RH	20.6 a	12.6 ab	5.2	21.7 a	16.4	86.3	1668	229	4.93 a
High RH	20.1 a	12.5 ab	5.6	22.1 a	16.5	87.0	1199	156	3.39 b
Anti-transpirant[w]	19.2 a	11.7 bc	5.1	18.8 bc	13.6	87.0	1293	169	3.15 b
Paraffin wax	17.4 b	11.3 c	5.1	17.0 c	11.9	87.0	1666	200	3.33 b
PERIOD TWO: 7 JULY - 10 AUGUST									
Untreated control	18.0 a	12.3 a	31.2	78.7 a	47.4	85.9 ab	791	110 b	8.67 ab
Control	18.4 a	12.5 a	29.4	73.7 a	44.3	85.4 b	733	106 b	7.83 b
Low RH	18.0 a	12.7 a	29.8	75.9 a	46.0	85.5 b	747	108 b	8.28 ab
High RH	18.6 a	10.9 b	30.5	73.5 a	43.0	85.2 b	903	132 a	9.65 a
Anti-transpirant	18.0 a	12.2 a	29.1	75.0 a	45.8	85.5 b	758	110 b	8.14 b
Paraffin wax	13.0 b	9.0 c	30.3	58.3 b	28.0	86.6 a	761	101 b	5.96 c
PERIOD THREE: 12 AUGUST - 12 SEPTEMBER									
Untreated control	9.1 bc	9.5 a	79.6 bc	129.4 bc	50.1 ab	83.9 b	507	82 b	10.68 ab
Control	10.3 ab	7.8 b	89.6 a	140.4 a	51.1 a	86.5 a	495	67 c	9.40 bc
Low RH	7.9 c	6.3 c	84.2 ab	127.5 bc	43.7 bc	83.0 c	574	96 a	12.30 a
High RH	10.6 a	8.1 b	84.6 ab	136.0 ab	51.8 a	86.6 a	495	66 c	9.02 bc
Anti-transpirant	8.8 c	9.7 a	77.4 c	124.7 cd	47.8 ab	86.2 a	509	69 bc	8.73 c
Paraffin wax	7.7 c	7.0 bc	79.5 bc	117.2 d	38.1 c	86.6 a	553	73 bc	8.57 c
PERIOD FOUR: 12 SEPTEMBER - 12 OCTOBER									
Untreated control	3.9	3.4	128.6	156.8	28.2 a	85.3 cd	419 b	61 bc	9.69 ab
Control	2.9	3.1	128.2	152.4	24.2 ab	85.8 bc	496 a	70 a	10.77 a
Low RH	2.9	1.3	132.1	148.1	16.0 c	85.2 d	491 a	73 a	10.73 a
High RH	3.2	2.7	130.4	152.4	22.0 abc	85.8 ab	397 b	56 c	8.58 b
Anti-transpirant	2.4	2.6	135.2	154.5	19.4 bc	85.5 bcd	488 a	70 a	10.94 a
Paraffin wax	1.9	2.9	125.9	143.1	17.3 bc	86.3 a	487 a	66 ab	9.53 ab

[z]Mean separation within columns by Least Significant Difference; numbers followed by similar letters not significantly different at $P = 0.05$.
[y]Untreated fruit
[x]Fruit treated with lid apparatus only
[w]10% (v/v) 'Wilt-Pruf' anti-transpirant.

DISCUSSION

This study suggests that the xylem system may be an important source of Ca throughout fruit development, though its contribution diminishes towards harvest. Exposing fruit to high RH in 1989 reduced fruit Ca content. These results are similar to those observed when tomato (Armstrong and Kirkby, 1979; Banuelos *et al.*, 1987; Ehret and Ho, 1986), paprika and bean fruit (Mix and Marschner, 1976a, 1976b) and leaves of lettuce (Collier and Tibbitts, 1984), cabbage (Wiebe *et al.*, 1977), and strawberry (Bradfield and Guttridge, 1979) were subjected to high RH.

It is unclear why treatments had little effect on fruit Ca levels in 1988. The 1988 season was characterized by higher temperatures and less precipitation than in 1989 (Table 4). These trees were not irrigated and may have been under some moisture stress in 1988. How this stress might have influenced treatment effects specifically is not clear.

Table 4. Average monthly temperature, precipitation, and accumulated heat units during 1988 and 1989. East Lansing, Michigan.

Month	Average monthly temperature(°C)		Average monthly precipitation (mm)	
	1988	1989	1988	1989
May	16.8	12.7	15	125
June	20.1	19.1	4	85
July	23.4	22.0	61	46
Aug.	23.2	20.1	90	175
Sept.	16.5	15.1	94	150
AVG(TOTAL)	19.5	17.8	(264)	(582)

The variability of fruit Ca levels did not appear to limit our ability to detect treatment differences in 1988 and 1989 since coefficients of variation were similar both years. Therefore increasing the number of replications in 1989 from 15 to 30 did not appear to be beneficial. During period 2 in 1989, it is unclear why fruit Ca levels were higher for the high RH treatments while lower for the low RH treated fruit, since this was opposite the effect observed during other treatment periods.

Wax treatments generally reduced Ca levels and increased the moisture content of the fruit compared to controls, but these data were confounded by a treatment effect on fruit size, since a physical and possibly physiological impedance on fruit growth was observed. The moisture content of waxed fruit was most often higher than untreated fruit indicative of reduced transpiration rate. Anti-transpirant did not have a significant influence on fruit Ca levels. A repeated application may have been beneficial considering the possible 'rinsing' effect of rain by the end of the treatment interval. Antitranspirants applied to whole trees have reduced the incidence of bitter pit (Schumacher, 1976).

This study did not address the possible relationship between RH and phloem transport specifically, however one would be expected if a difference in dry matter accumulation was detected between low and high RH treated fruit. Low RH treated fruit tended to accumulate more dry matter or less water compared with high RH treated, but it is difficult to know on what basis RH may have influenced phloem transport.

The plastic bags appeared to be adequately sealed to maintain different RH levels around the fruit, however CO_2 and O_2 levels in the

bags were similar to ambient conditions. This suggests that bags were not fully sealed and that small spaces, likely around the pedicels, allowed some gas exchange.

A slight decrease in fruit transpiration rates as they progress through the season has been observed (chapter 3; Blanke and Lenz, 1985). Although this would indicate that the effect of the RH treatments should be less towards period 4, there was still an influence on Ca accumulation towards the end of the season.

Late in the 1989 season, just prior to harvest, RH treatments appeared to affect the water content and Ca content of the fruit, even though no apparent accumulation of Ca in untreated fruit during period 4 was occurring. It is possible that rather than primarily altering fruit transpiration and Ca import, RH was affecting the export of Ca from the fruit by its effect on fruit water potential (Goode *et al*, 1979). For example, high RH treatments had higher moisture contents and appeared to be enhancing Ca export from the fruit. Low RH treatments, as a result of their possible lower water content and perhaps more negative water potential, may have prevented Ca export from the fruit. In grape berries (Lang and Thorpe, 1989), cowpea (Pate *et al.*, 1985), and pea fruit (Hamilton and Davies, , 1988) evidence has been established for a reverse flow of water from the fruit to the plant later in fruit development.

LITERATURE CITED

Armstrong, M.J. and E.A. Kirkby. 1979. The influence of humidity on the mineral composition of tomato plants with special reference to calcium distribution. Plant and Soil 52:427-435.

Bangerth, F. 1979. Calcium-related physiological disorders of plants. Ann. Rev. Phytopathol. 17:97-122.

Banuelos, G.S., F. Bangerth, H. Marschner. 1987. Relationship between polar basipetal auxin transport and acropetal Ca^{2+} transport into tomato fruits. Physiol. Plant. 71:321-327.

Blanke, M.M and F. Lenz. 1985. Spaltöffnungen, Fruchtoberfläche und Transpiration wachsender Apfelfrüchte der Sorte 'Golden Delicious'. Erwerbsobstbau 27:139-143.

Blanke, M.M and F. Lenz. 1989. Fruit photosynthesis. Plant Cell and Environ. 12:31-46.

Bradfield, E.G. and C.G. Guttridge. 1979. The dependence of calcium transport and leaf tipburn in strawberry on relative humidity and nutrient solution concentration. Ann. Bot. 43:363-372.

Collier, C.F., and T.W. Tibbitts. 1984. Effects of relative humidity and root temperature on calcium concentration and tipburn development in lettuce. Amer. Soc. Hort. Sci. 109:128-131.

Ehret, D.L. and Lim C. Ho. 1986. Translocation of calcium in relation to tomato fruit growth. Ann. Bot. 58:679-688.

Faust, M., C.B. Shear, and C.B. Smith. 1968. Investigation of corking disorders of apples II. Chemical composition of affected tissues. Proc. Amer. Soc. Hort. Sci. 92:82-88.

Faust, M., and C.B. Shear. 1973. Calcium translocation patterns in apples. Proc. Res. Inst. Pomology, Skierniewice, Poland, Ser. E. 3:423-436.

Ferguson, I.B. 1979. The uptake and transport of calcium in the fruit tree, p.183-192 In: Mineral Nutrition of Fruit Trees. Atkinson, D., J.E. Jackson, and R.O. Sharples (eds.). Buttersworths Press, London, Toronto.

Ferguson, I.B. and E.G. Bollard. 1976. The movement of calcium in woody stems. Ann. Bot. 40:1057-1065.

Ferguson, I.B., F.R. Harker, and B.K. Drøbak. 1987. Calcium and apple fruit. The Orchardist of New Zealand. May:119-121.

Ferguson, I.B., and C.B. Watkins. 1989. Bitter pit in apple fruit, p. 289-355 In: Hort Reviews. J. Janick (ed.). Timber Press, New York

Ford, M. 1979. Effect of post-blossom environmental conditions on fruit composition and quality of apple. Commun. Soil Sci. Plant Anal., 10:337-348.

Goode, J.E., K.H. Higgs, and K.J. Hyrycz. 1979. Effects of water stress control in apple trees by misting. J. Hort. Sci. 54:1-11.

Hall, E.G., D.A. Baker, and J.A. Milburn. 1971. Phloem transport of ^{14}C-labelled assimilates in *Ricinus* phloem exudate. Planta 100:200-207.

Hamilton, D.A., and P.J. Davies. 1988. Mechanism of export of organic material from the developing pea. Plant Physiol. 86:956-959.

Hanger, B.C. 1979. The movement of calcium in plants. Commun. Soil Sci. Plant Anal. 10:171-193.

Himelrick, D.G. and C.E. Walker. 1982. Seasonal trends of calcium, magnesium, and potassium fractions in apple leaf and fruit tissues. J. Amer. Soc. Hort. Sci. 107:1078-1080.

Himelrick, D.G. and R.F. McDuffie. 1983. The calcium cycle:uptake and distribution in apple trees. HortScience 118:147-150.

Jones, H.G. and T.J. Samuelson. 1983. Calcium uptake by developing apple fruits. II. The role of spur leaves. J. Hort. Sci. 58:183-190.

Jones, H.G., K.H. Higgs, and T.J. Samuelson. 1983. Calcium uptake by developing apple fruits. I. Seasonal changes in calcium content of fruits. J. Hort Sci. 58:173-182.

Lang, A., and M.R. Thorpe. 1989. Xylem, phloem and transpiration flows in a grape: Application of a technique for measuring the volume of attached fruits to high resolution using Archimedes' Principle.

Long, M.S. 1980. Cuticle development and incidence of russet on 'Golden Delicious' apple as influenced by subclone susceptibility and shelters. Michigan State University. MS Thesis.

Marschner, H. 1986. Functions of mineral nutrients: macronutrients, In: Mineral Nutrition of Higher Plants. Academic Press, London.

Mix, G.P. and H. Marschner. 1976a. Calciumgehalte in Früchten von Paprika, Bohne, Quitte und Hagebutte im Verlauf des Fruchtwachstums (Calcium content in fruits of paprika, bean, quince, and hip during fruit growth). Z. Pflanzenernaehr. Bodenkd. 5:537-549.

Mix, G.P. and H. Marschner. 1976b. EinfluB exogener und endogener Faktoren auf den Calciumgehalt von Paprika- und Cohnenfrüchten. (Effect of external and internal factors on the calcium content of paprika and bean fruits). Z. Pflanzenernaehr. Bodenkd. 5:551-563.

Pate, J.S., M.B. Peoples, A.J.E. van Bel, J. Kuo, and C.A. Atkins. 1985 Diurnal water balance of the cowpea fruit. Plant Physiol. 77:148-156.

Quinlin, J.D. 1969. Chemical composition of developing and shed fruits of Laxton's Fortune apple. J. Hort. Sci. 44:97-106.

Redmond, W.J. 1975. Transport of Ca in apple trees and its penetration into the fruit. Commun. Soil Sci. Plant Anal. 6:261-272.

Schumacher, R., F. Fankhauser, and W. Stadler. 1976. Versuche mit Calciumchlorid, antitranspiranten, und Borsäure zur verminderune der Stippebildung. Schweiz Z. Obst. u. Weinb. 112:300-304.

Sharazi, A., and A.C. Cameron. 1989. Modified packaging: a new concept for extending the shelf life of fresh produce. Submitted to J. Fd. Sci. for publication.

Shear, C.B. and M.B. Faust. 1970. Calcium transport in apple trees. Plant Physiol. 45:670-674.

Stebbins, R.L. and D.H. Dewey. 1972. Role of transpiration and phloem transport in accumulation of ^{45}calcium in leaves of young apple trees. J. Amer. Soc. Hort. Sci. 94:471-474.

Tammes, P.M.L., and J. van Die. 1964. Studies on phloem exudation from *Yucca flaccida* Haw. I. Some observations on the phenomenon of bleeding and the composition of the exudate. Acta Bot. Neerl. 13:76-83.

Tromp, J. 1975. The effect of temperature on growth and mineral nutrition of fruits of apple with special reference to calcium. Physiol. Plant. 33:87-93.

Tromp, J., and J. Oele. 1972. Shoot growth and mineral composition of leaves and fruits of apple as affected by relative are humidity. Physiol. Plant 27:253-258.

Tromp, J. 1979. Mineral absorption and distribution in young apple trees under various environmental conditions, p. 173-182. In: Mineral Nutrition of Fruit Trees. Atkinson, D., J.E. Jackson, and R.O. Sharples (eds.). Buttersworths Press, London, Toronto.

Vang-Petersen, O. 1980. Calcium nutrition of apple trees: a review. Sci. Hort. 12:1-9

Wiebe,H.J., H.P. Schätzler, and W. Kühn. 1977. On the movement and distribution of calcium in white cabbage in dependence of the water status. Plant and Soil 48:409-416.

Wiersum, L.K. 1966. Calcium content of fruits and storage tissue in relation to the mode of water supply. Acta. Bot. Neerl. 15:406-418.

Wiersum, L.K. 1979. Effects of environment and cultural practices on calcium nutrition. Commun. Soil Sci. Plant Anal. 10:259-278.

Wilkinson, B.G. 1968. Mineral composition of apples. IX. Uptake of calcium by the fruit. J. Sci. Food Agric. 19:646-64.

CHAPTER 3

Estimated Importance of the Xylem Supply of Calcium to Developing Apple Fruit

INTRODUCTION

Poor distribution of Ca in apple trees is the primary reason that fruit are predisposed to Ca-disorders such as 'bitter pit' (Ferguson and Watkins, 1989), even when levels are adequate in the remainder of the tree (Himelrick and McDuffie, 1983) and soil (Bangerth, 1979; Hanger, 1979; Mason, 1979). Knowledge of the factors affecting the translocation of Ca to fruit would be useful in developing techniques which would enhance the transport of Ca to fruit.

Various seasonal patterns of Ca accumulation have been observed in apple fruit. Calcium may accumulate linearly in the fruit throughout the season (Rogers and Batjer, 1954; Oberly, 1973; Tromp, 1975, 1979b,; Tomala *et al.*, 1989), increase linearly early in the season, then slow and cease accumulation 6-16 weeks after bloom (Tromp 1972,1975; Jones *et al.*, 1983, Jones and Samuelson, 1983; Himelrick and Walker, 1982; Quinlin, 1969; Wilkinson, 1968) or increase linearly early then decline prior to harvest (Tromp, 1972, 1979b; Wilkinson, 1968; Hanson, unpublished data) (Chapter 1).

The two potential pathways of fruit Ca supply are the xylem and the phloem. Tromp & Oele (1972) and Vang-Peterson (1980) suggested that the xylem supplies the majority of fruit Ca, however, indirect evidence of phloem transport in apple has also been reported (Stebbins *et al.*, 1972; Faust and Shear, 1973). A hypothesis initiated by Wiersum (1966) and developed by others (Wilkinson, 1968; Ferguson, 1979; Hanger, 1979; Ferguson *et al.*, 1987; Ferguson and Watkins, 1989; Faust *et al*, 1974; Jones *et al.*, 1983) suggests that the xylem is the primary route of Ca supply early in the season when fruit accumulate Ca rapidly, whereas the phloem predominates later in the season when fruit Ca content increases

less rapidly (Ferguson and Watkins, 1989). A difficulty with this hypothesis is that Ca concentrations in phloem of other plant species are generally low (Hall *et al.*, 1971; Wiersum, 1979; Tammes and Van Die, 1964; Komor *et al.*, 1989). Furthermore, the remobilization of Ca in phloem from leaves to fruit is limited compared with phloem-mobile nutrients such as N, P, or K (Himelrick and McDuffie, 1983). Consequently, it is unlikely that the phloem can supply significant quantities of Ca to fruit. Also, since the Ca content of fruit usually increases only gradually, if at all, late in the season, a phloem supply of Ca may not be needed to explain the accumulation patterns typically observed.

The importance of the xylem system in supplying Ca to fruit was estimated for the apple cultivars 'Bramley', 'Egremont Rusett', 'Cox's Orange Pippin' and 'Golden Delicious' by measuring the Ca concentrations in the xylem sap and the water flow into the fruit (Jones *et al.*, 1983). Sap was collected from shoots under pressure (Bollard, 1957) and water flow was estimated to be the sum required for fruit growth and transpiration. Fruit transpiration was estimated by measuring weight loss from detached fruit hanging in the tree, and actual transpiration rates of attached fruit (Jones and Higgs, 1982). This theoretical level was compared with the observed patterns of Ca accumulation. Xylem supply consistently underestimated Ca uptake rates of fruit early in the season and markedly overestimated observed rates late in the season.

One explanation for the discrepancy between predicted and actual rates of Ca accumulation might be erroneous estimates of fruit transpiration rates. *In situ* measurement of fruit transpiration rates later in the season, with a modified steady state porometer, required up

to 60 minutes to reach equilibrium (Jones *et al.*, 1983). The importance of this potential error needs to be verified to comment further on the accuracy of their predictions.

The objective of this study was to clarify the role of the xylem system in supplying Ca to 'Red Delicious' fruit using a more precise method to measure fruit transpiration.

MATERIALS AND METHODS

Calcium accumulation in (*Malus domestica*, Borhk.) apple fruit was studied for two years (1988-1989) in a 32 year-old orchard of 'Starking cv. Red Delicious'/M.7 and for one year (1989) in a 10 year-old 'Viking'/M.27 orchard, both located in East Lansing, Michigan. Trees were managed by standard practices and did not receive irrigation or Ca applications. Experimental units (plots) consisted of two adjacent trees and were replicated six times. Trees were selected each year which carried a uniform crop load. The potential contribution of the xylem system to fruit Ca was estimated based on xylem Ca concentrations and water flow into the fruit, over the course of the season, using the following methods.

Fruit Sampling

Fruit samples were collected weekly for Ca analysis and consisted of 20 fruit each early in the season and 10 fruit thereafter. Fruit were chosen which best represented the average fruit size on the tree. Fruit fresh weight, length, and width were measured. The surface area of 20 fruit was estimated using Table 1 Eq.[4] (Long, 1980). Pedicels were removed and whole fruit were homogenized in a food processor. Approximately 5 g of tissue was weighed into porcelain crucibles and

dried at 65°C to a constant weight. Samples were weighed again and ashed in a muffle furnace at 550°C for 6 hours. Samples in 1989 were homogenized, then freeze-dried, rather than oven-dried. The ash was dissolved in 20 ml of 10% (v/v) nitric acid and filtered with low-ash Whatman #41 paper into scintillation vials. A subsequent aliquot was diluted appropriately and prepared in a final concentration of 1000 ppm lanthanum and 2% (v/v) nitric acid, and analyzed for Ca by atomic absorption spectrophotometry.

Xylem Sap Ca Concentrations

Xylem sap was collected biweekly by vacuum extraction (Bollard, 1953). On each date, four 2-year-old, 10 to 15 mm diameter shoots were collected per plot. The basal 3 cm of bark and cambium tissue was stripped from each shoot, so that phloem exudate was not collected. Exudate was collected from all four shoots and combined (approximately 5 ml total). Samples were frozen and later diluted and analyzed for Ca by atomic absorption spectrophotometry.

Estimation of Fruit Transpiration

Hanging Fruit Method

The transpiration rates of fruit were estimated by weight loss from detached fruit (Jones *et al.*, 1983). Samples of 5 fruit were collected from each plot at weekly intervals and weighed. Fruit were placed in plastic mesh (vexar) bags which were hung in the trees exposed to conditions similar to unpicked fruit. Fruit were removed and re-weighed one week later. The weekly transpiration rates of fruit were estimated to be equal to the weight loss of fruit hanging in the trees.

Fruit Conductance Method

A second estimate of fruit transpiration utilized the measurement

of fruit surface conductance to water loss under controlled conditions (Shirazi and Cameron, 1989, 1990; Table 1 [Eq.1]). At 3-day intervals early in the season and weekly intervals thereafter, fruit were collected in the morning, as soon as the fruit surface was dry, placed in plastic bags and transported to the laboratory (this required 15-20 minutes). The tips of the pedicels were dipped in melted paraffin wax and the basal ends of fruit were sealed with petroleum jelly so that transpiration occurred from the fruit surface only. Fruit were placed on a 0.1 mg sensitivity balance (model AE163, Mettler Instruments, Switzerland). As the number of fruit per weighing was limited to the capacity of the balance (<163 g), 10 fruit early in the season and only one fruit by the end of the season were selected. Three replications were conducted for each cultivar per week, weather permitting. The weighing area of the balance was enclosed in a small chamber (760 cm^3) supplied with a steady flow ($\approx$ 400 $ml \cdot min^{-1}$) of compressed air in which the relative humidity (RH) was adjusted to 70% (model WG600, Analytical Development Co., England). The balance was connected to a desktop computer programmed (Shirazi, 1989) to record fruit weights at 5 minute intervals for 1 hour. A sensor (model 850-242, General Eastern, USA) positioned in the chamber and linked to a *Polycorder*™ data logger (Omni Data International, Utah, USA) recorded the chamber temperature and RH concomitantly with fruit weight. Transpiration was indicated by a slight decrease in fruit weight (Table 1 Eq.[2]), assuming that the decrease in weight equaled the water transpired. Fruit conductance was calculated using the RH and temperature in the balance chamber [Eq.3] and the fruit surface area (Eq.[4]). Conductance of fruit was then used to predict the transpiration rate of fruit under orchard conditions

(Eq.[5]).

Evaporation of water from an open pan served as a measure of the average vapor pressure deficit within the orchard. Two plastic pans, one in each orchard, with inside dimensions of 37 x 45 cm (1582 cm^2) and 10 litre capacity, were placed in the center of the tree canopy 1.5 m from the ground. A wooden cover was placed 60 cm above the evaporative surface to keep rain water out of the pan. The pan was filled weekly with water and the volume remaining one week later was measured.

Diurnal Measurements

Diurnal measurements of shoot xylem Ca concentration and fruit and leaf water potentials were made from 'Red Delicious' trees for 24-hour cycles (4 hour intervals) each 3-4 weeks throughout the 1989 growing season. Diurnal changes in fruit size were also measured periodically throughout the season for a 3 to 4 day period. Measurements from 'Viking' trees were taken only once.

Leaf and fruit water potentials were measured in a pressure chamber (Soil Moisture Equipment Corp., USA) to determine if a relationship between water potential and water movement in and out of fruit existed. Measurements were made on ten fruit and leaf samples at each sampling. The liquid exuding from fruit pedicels was also collected when fruit water potential measurements were made. Approximately 50 - 500 μl of sap was collected from the 10 fruit using a syringe and #28 hypodermic needle. Four replications of shoot xylem exudates were also collected under vacuum as described previously.

Table 1: Equations used for calculating fruit transpiration rates based on fruit conductance to water loss (see appendix 2 for sample calculation).

Conductance (C) of a fruit surface to water loss was calculated using the relationships (Cameron, 1982):

Water flux per unit surface area ($cm \cdot sec^{-1}\ KPa^{-1}$)

$$C_{SA} = \frac{J_v}{SA_{fruit} \times VPD}, \quad [1.1]$$

Water flux per unit fruit weight (mmol H_2O g $fruit^{-1}\ KPa^{-1}$)

$$C_{FRUIT} = \frac{J_v}{\text{fruit wt.(g)} \times VPD}, \quad [1.2]$$

where,

J_v [mmol $H_2O \cdot sec^{-1}$] = water flux

$$= \frac{(\text{Fruit weight})_{t0} - (\text{Fruit weight})_{t1}}{t1 - t0\ (\text{sec})} \quad [2]$$

where t_0 = starting time and t_1 = ending time

VPD (KPa) = the difference in vapor pressure of the fruit and the atmosphere surrounding the fruit within the laboratory.

$$= \frac{\text{Saturation Vapor Pressure} \times [1\text{-RH}(\%)]}{100\ \%} \quad [3]$$

$SA_{FRUIT}(cm^2)$ = estimated surface area of the fruit [4]
= [w x $(\frac{1}{2}w)^2 + (\frac{1}{2}l)^2$], where 'w' and 'l' represent fruit width and length, respectively (Long, 1980)

To convert transpiration rates from the laboratory to the orchard:

Fruit Transpiration in the Orchard ($ml\ week^{-1}$)

$$= \frac{\text{Pan Evaporation}}{SA_{PAN}} \times \frac{C_{SA}}{C_{WATER}} \times SA_{FRUIT} \quad [5]$$

Where,

Pan Evaporation = ml H_2O evaporated per week

SA_{PAN} = surface area of water evaporating from the pan (1554 cm^2)

C_{WATER} = 3.0×10^{-2} $cm \cdot sec^{-1}\ KPa^{-1}$ = conductance of water from an open surface of water measured empirically using [Eq.1.1] under controlled conditions

To address the potential diurnal movement of water from the fruit, the change in fruit diameter was measured on the 24-hour, 4-hour cycle seasonal schedule for a 4 day period using linear voltage displacement transducers (model 350-000, Trans-tek Inc., USA) linked to a model 570 *Polycorder* data logger (Omni Data International, Utah, U.S.A.). Transducers were secured to a stable ladder and set tangentially against individual, stabilized fruit. A single transducer was secured to each of two fruit. Since our methods were principally the same as Tukey (1964) and Higgs and Jones (1984), with exception to the data logging methods, further detail may be obtained by writing the authors.

RESULTS

Weather and Pan Evaporation

Extremes in temperature and rainfall were experienced during the two years of this study (Chapter 1, Table 1). Low rainfall and high temperatures prevailed in 1988, whereas rainfall was adequate to excessive and temperatures much cooler in 1989. Pan evaporation was generally higher in 1988 than 1989, especially early in the season (Fig. 2). Evaporation in the 'Viking' orchard was slightly greater than in the 'Red Delicious' orchard in 1989. Pan evaporation data correlated well with standard EPAN data collected in the area.

Fruit Growth

Both cultivars followed sigmoidal growth patterns (Fig. 1). 'Red Delicious' fruit at harvest weighed 30% more in 1988 than in 1989. 'Viking' fruit matured 8 weeks earlier than 'Red Delicious', but the final fruit size was similar for both. Bloom dates were 9 May for 'Red

Delicious' in 1988 and 15 May in 1989 for 'Viking' and 'Red Delicious'.

Calcium Concentrations in Xylem Exudate

Calcium concentrations in xylem exudate from 'Red Delicious' shoots in 1988 were highest at full bloom (150 ppm Ca) and leveled off to 75 ppm Ca seven weeks later (Fig. 3). In 1989 xylem Ca levels for 'Viking' and 'Red Delicious' were consistently higher and more variable than those in 1988; Ca levels tended to decrease linearly from 175 ppm Ca at bloom to 100 ppm Ca by the end of the season. No measurable differences were apparent in the diurnal Ca concentration of shoots (Appendix 4) or fruit pedicel extracts. Calcium concentration of 'Red Delicious' fruit pedicel extracts did not appear to follow a seasonal trend; concentrations ranged from 50 to 80 μg Ca ml^{-1}, which were half those measured in the shoot xylem (Table 2).

Table 2. Calcium concentrations of fruit pedicel exudate extracted in a pressure chamber. cv. Red Delicious, 1989.

Days after bloom (May 15)	Number of[z] samples	Ca conc. ppm	Std. Dev.
33	2	50	± 0
36	3	57	± 9.4
37	2	65	± 5
60	1	60	
67	3	80	± 20
68	2	65	± 50

[z] samples consisted of extracts combined from 10 fruit to provide sufficient volumes for measurement

Fruit Transpiration and Evapo-transpiration

Fruit transpiration, estimated by measuring the weight loss of hanging fruit, varied considerably during the season and between cultivars (Fig. 5). 'Red Delicious' fruit transpired from 0.5 to 3.5 ml of H_2O $week^{-1}$ $fruit^{-1}$, corresponding closely with the pan evaporation data. 'Viking' fruit transpired 0.5 ml H_2O (l) $week^{-1}$ $fruit^{-1}$ early in the season, and up to 4.5 ml H_2O (l) $week^{-1}$ $fruit^{-1}$ at harvest. These data were not closely related to the pan evaporation data.

The conductance of the fruit surface to water, expressed per unit fruit weight, decreased during fruit development (Fig. 4). Fruit conductance was 50 times greater early in the season and decreased exponentially towards harvest as fruit size increased. Fruit conductance, expressed per unit surface area (Appendix 5), decreased from 0.16 to 0.08 mmol H_2O m^{-2} sec^{-1} KPa^{-1} for 'Viking' early and late in the season, respectively, and from 0.08 to 0.01 mmol H_2O m^{-2} sec^{-1} KPa^{-1} for 'Red Delicious' early and late in the season, respectively. The fruit conductance method of estimating evapo-transpiration followed the same seasonal trend as that measured by the hanging fruit, but the magnitude and sampling variability were greater at times, especially for the 'Red Delicious' fruit. Evapo-transpiration rates varied from 1.0 to 5.5 ml H_2O (l) $week^{-1}$ $fruit^{-1}$ in 1988, but were always less that 1 ml H_2O (l) $week^{-1}$ $fruit^{-1}$ in 1989. 'Viking' fruit ranged from 1.0 to 5 ml H_2O (l) $week^{-1}$ $fruit^{-1}$ which corresponded well with the hanging fruit estimates.

Actual (Measured) versus Predicted Ca Content

Actual and estimated fruit Ca contents differed between 1988 and 1989 (Fig. 6). The actual Ca content of 'Red Delicious' fruit increased

quadratically in both years, with the greatest intake occurring the first 10 weeks after bloom. In 1988 an apparent decrease in Ca content occurred just prior to harvest. Actual Ca content of 'Viking' fruit increased linearly throughout the season.

The predicted Ca content of 'Red Delicious' fruit increased sigmoidally both seasons. During 1988 both methods of estimating the xylem flow of water and Ca levels underestimated Ca content until 15 weeks after bloom and then overestimated it by as much as 40%. The predicted final Ca content at the end of the 1989 season was twice that of the measured value for both cultivars at harvest.

Figure 1. Fruit growth of 'Red Delicious' in 1988 and 1989 and 'Viking' in 1989, East Lansing, Michigan. Sigmoidal equation describing fruit weight where, x = days after full bloom:

$$\text{Fruit weight (g)} = \frac{A}{[\,1 + B \cdot e^{-(Cx + Dxx)}\,]}$$

Year	Cultivar	A	B	C	D	R^2
1988	Red Del.	234	230	7.31×10^{-2}	-1.83×10^{-4}	0.97
1989	Red Del.	699	650	7.43×10^{-2}	-2.72×10^{-4}	0.99
1989	Viking	215	410	1.11×10^{-1}	-4.59×10^{-4}	0.99

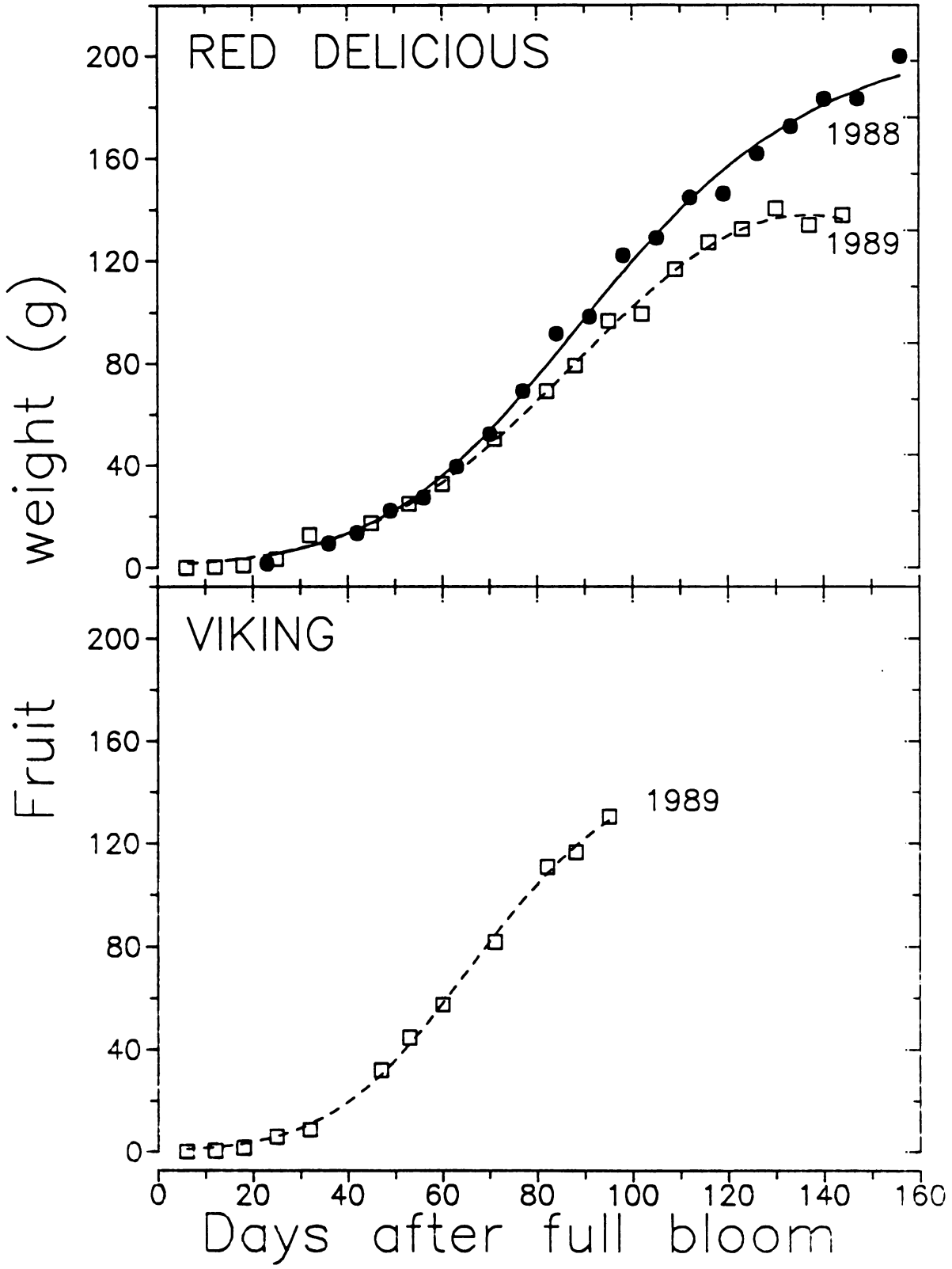

RED DELICIOUS
1988
1989
VIKING
1989
Fruit weight (g)
0
40
80
120
160
200
0
20
40
60
80
100
120
140
160
Days after full bloom

Figure 2. Evaporation of water from a pan placed within the 'Red Delicious' and 'Viking' tree canopy. East Lansing, Michigan, 1988, 1989. (Not a standard EPAN)

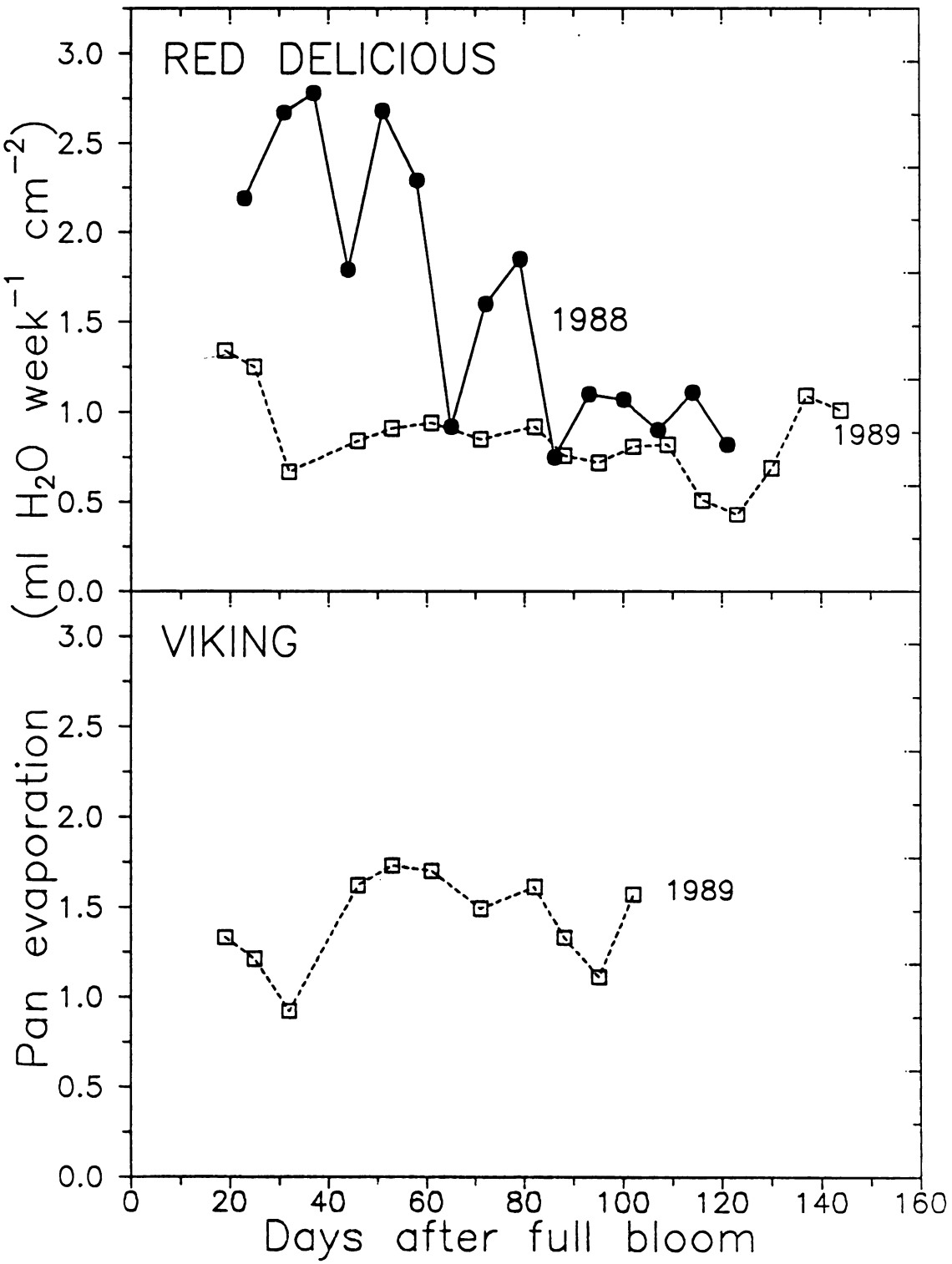

RED DELICIOUS
1988
1989
VIKING
1989
Pan evaporation (ml H_2O $week^{-1}$ cm^{-2})
Days after full bloom
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
20
40
60
80
100
120
140
160

Figure 3. Seasonal changes in calcium concentration in the xylem exudate from 'Red Delicious' apple shoots. Regression equations are as follows, where DAFB indicates 'days after full bloom': 1988 Red Del.= -112 x $(1-e^{(-0.051 \times DAFB)})$, r^2=0.75; 1989 Red Del.= -0.75(DAFB)+186.4, r^2=0.26; 1989 Viking = -0.78(DAFB)+173.2, r^2=0.21. Values represent the mean $\pm$ SE of six observations.

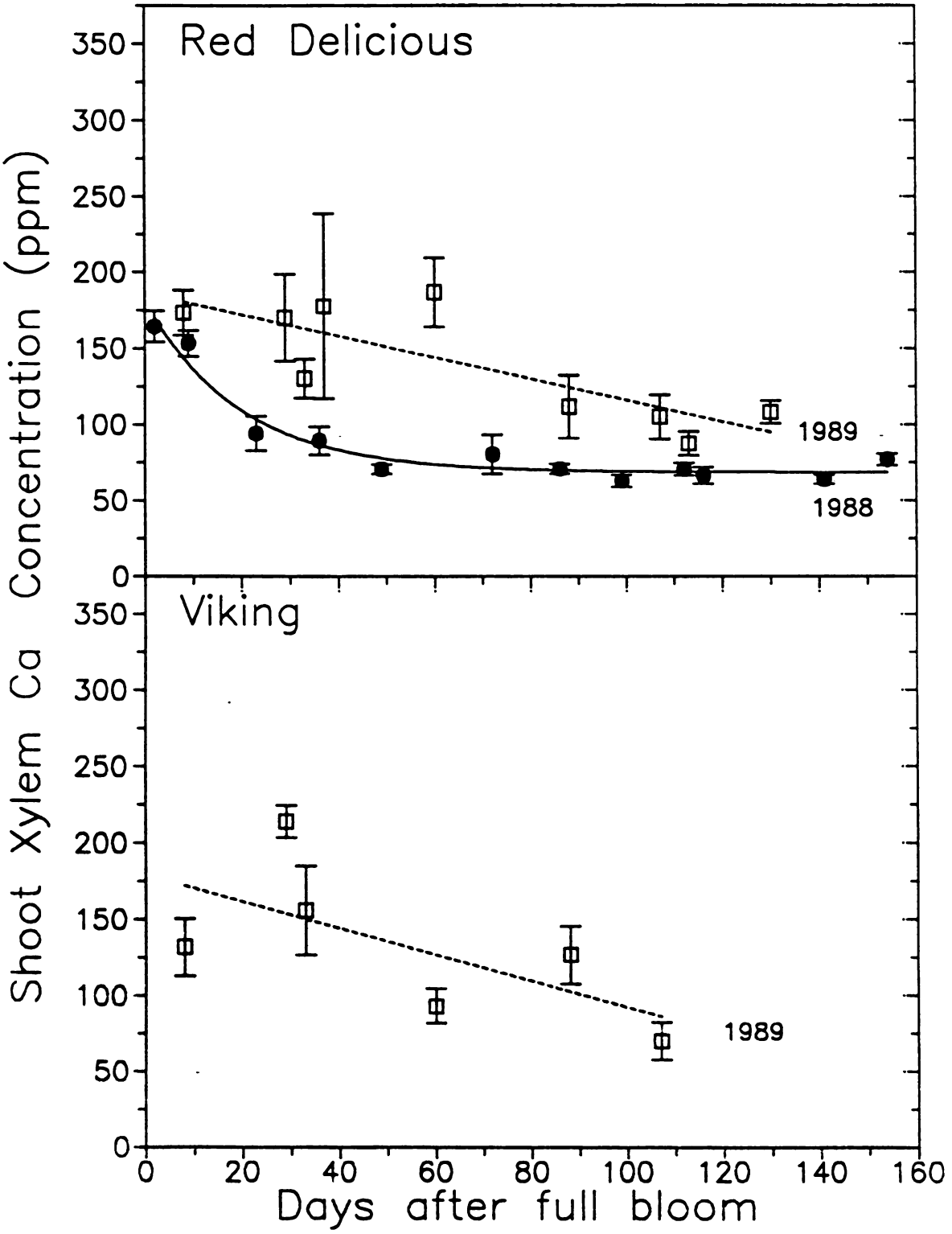

Red Delicious
Viking
1989
1988
1989
Shoot Xylem Ca Concentration (ppm)
Days after full bloom
0
50
100
150
200
250
300
350
0
20
40
60
80
100
120
140
160

Figure 4. Seasonal pattern of fruit conductance to water loss in 'Red Delicious' and 'Viking' trees, 1988 and 1989. Best Fit Equation: $C_{fruit} = (DAFB^{-A})xB$. For 1988 R. Del.: A=-1.41; B=2.83 (r^2=0.64; n=41), 1989 R. Del.: A=-2.18; B=69.3 (r^2=0.99; n=15), and 1989 Viking: A=-2.01; B=70.84 (r^2=0.96; n=18). Fruit surface area (SA) increased linearly with fruit growth [SA (cm^2) = mX + b, where 'X' = fruit weight (g)]. For 1988 R. Del.: m=0.47; b=5.4 (0.91; n=52), 1989 R. Del: m=0.45; b=1.8 (0.91; n=334) and 1989 Viking: m=0.52; b=1.7 (0.99; n=202), where n = the number of observations.

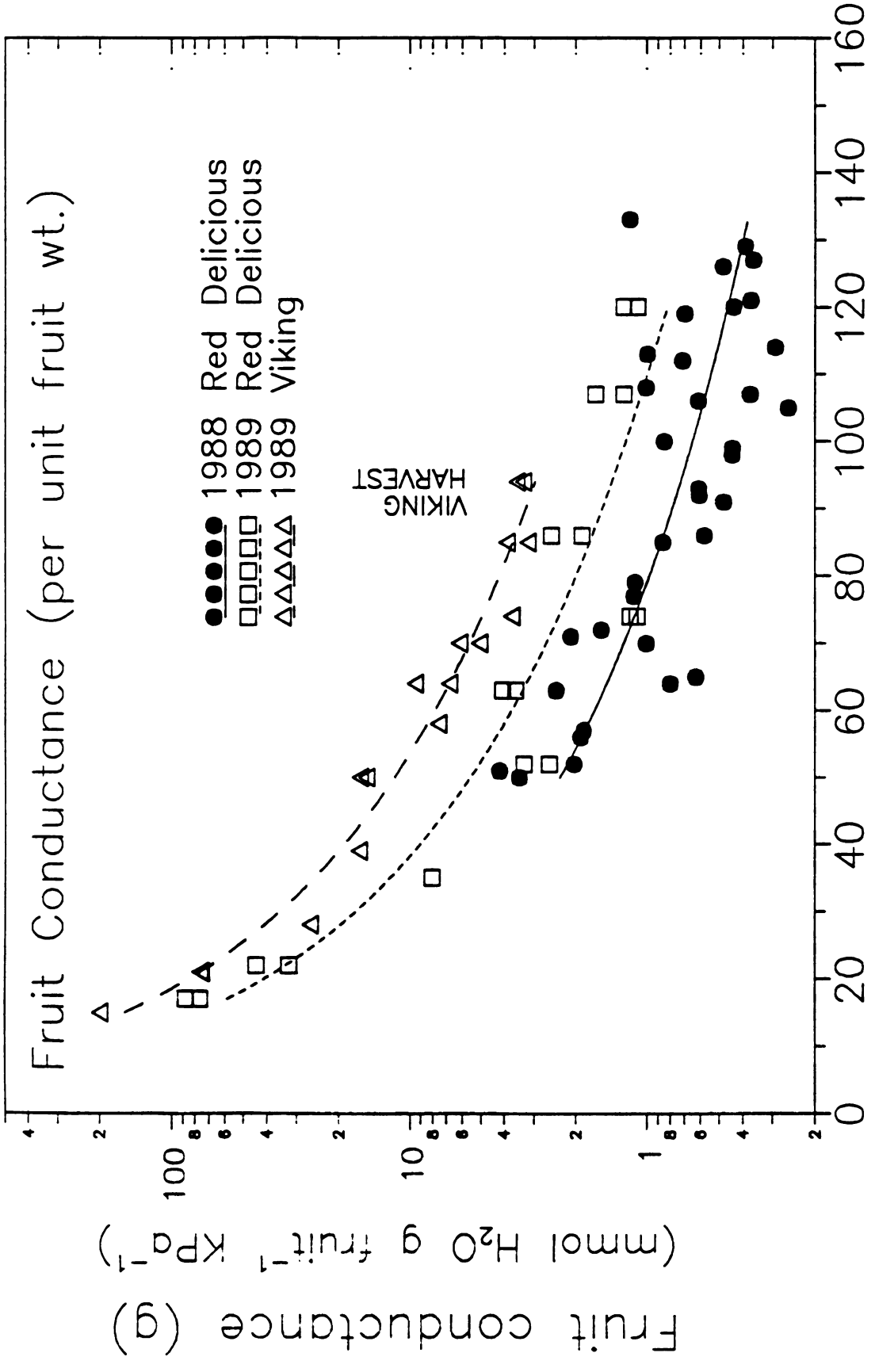

Fruit Conductance (per unit fruit wt.)
1988 Red Delicious
1989 Red Delicious
1989 Viking
VIKING HARVEST
Days after full bloom
0
20
40
60
80
100
120
140
160
100
10
1
Fruit conductance (g)
(mmol H2O g fruit-1 KPa-1)

Figure 5. Transpiration rates of 'Red Delicious' and 'Viking' fruit detached from the tree and measured by two methods: 1) weekly weight loss of fruit hanging in the tree, and; 2) Fruit conductance to water loss measured in the laboratory under controlled conditions. Values represent the mean ± SE of six observations (method 1).

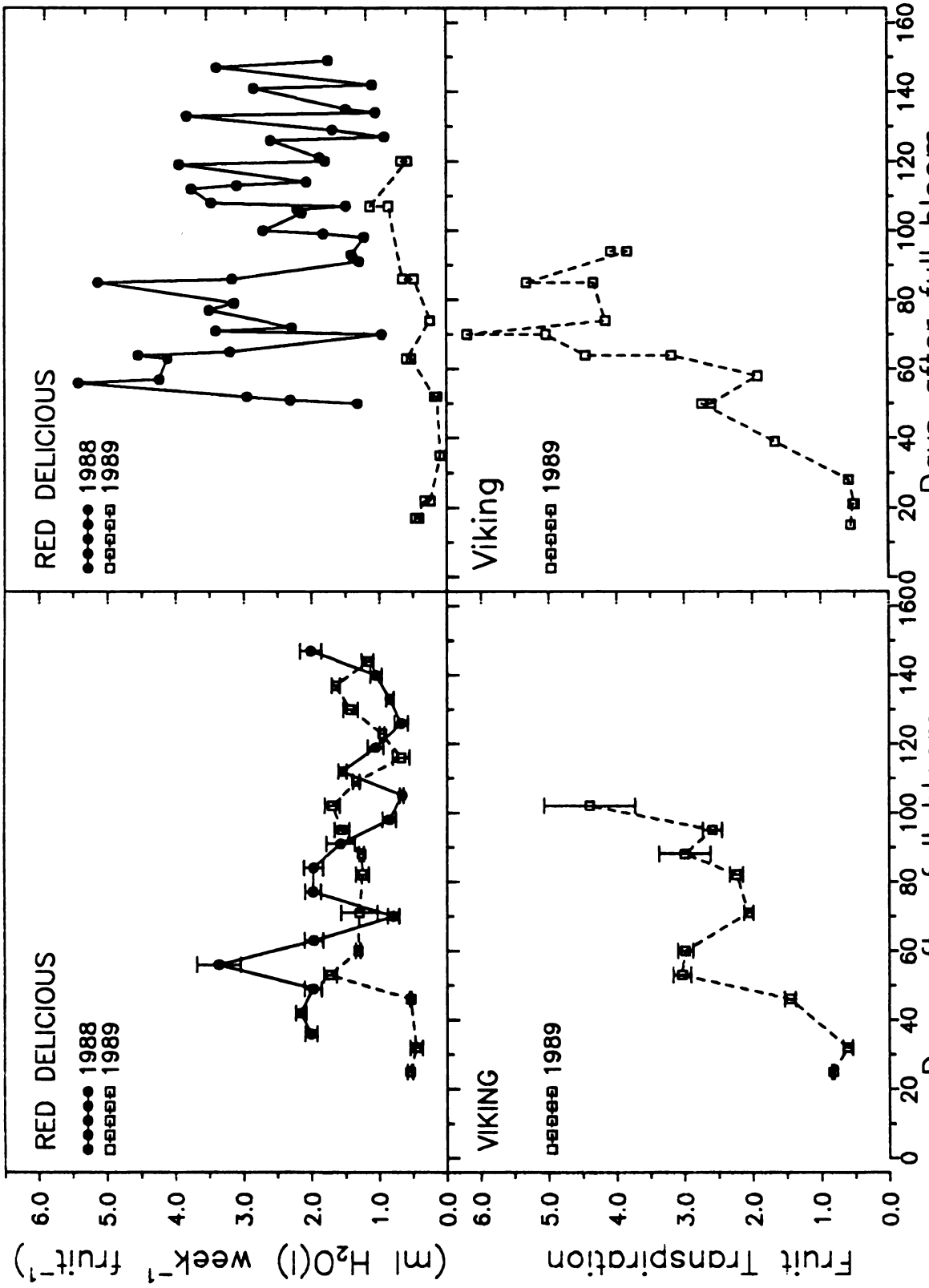

Method 1: Hanging Fruit in Tree
Method 2: Fruit Conductance
RED DELICIOUS
1988
1989
VIKING
Viking
Fruit Transpiration
(ml H2O(l) week-1 fruit-1)
Days after full bloom
0.0
1.0
2.0
3.0
4.0
5.0
6.0
0
20
40
60
80
100
120
140
160

Figure 6. Actual and predicted patterns of calcium accumulation in 'Red Delicious' and 'Viking' fruit. Predicted values are estimates of the xylem supply of calcium to fruit which were determined by the concentration of calcium in the xylem, and flow of water into the fruit. The quantity of xylem water flowing into the fruit was estimated to be the sum of the water transpired from the surface of the fruit (two methods used to estimate; Fig. 5) and that incorporated into fruit growth (85% of the gain in fruit weight was attributed to xylem water; Fig. 1)

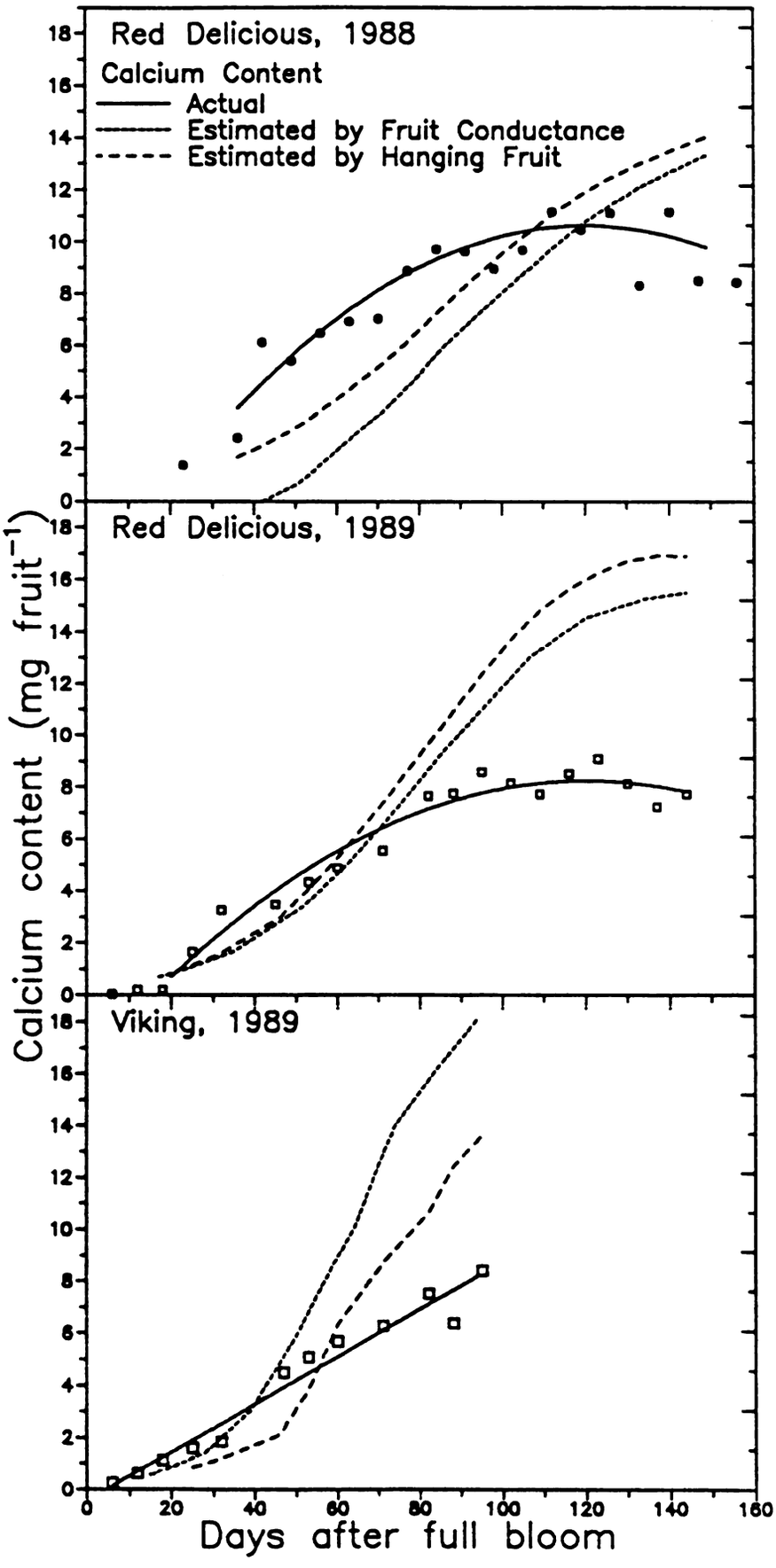
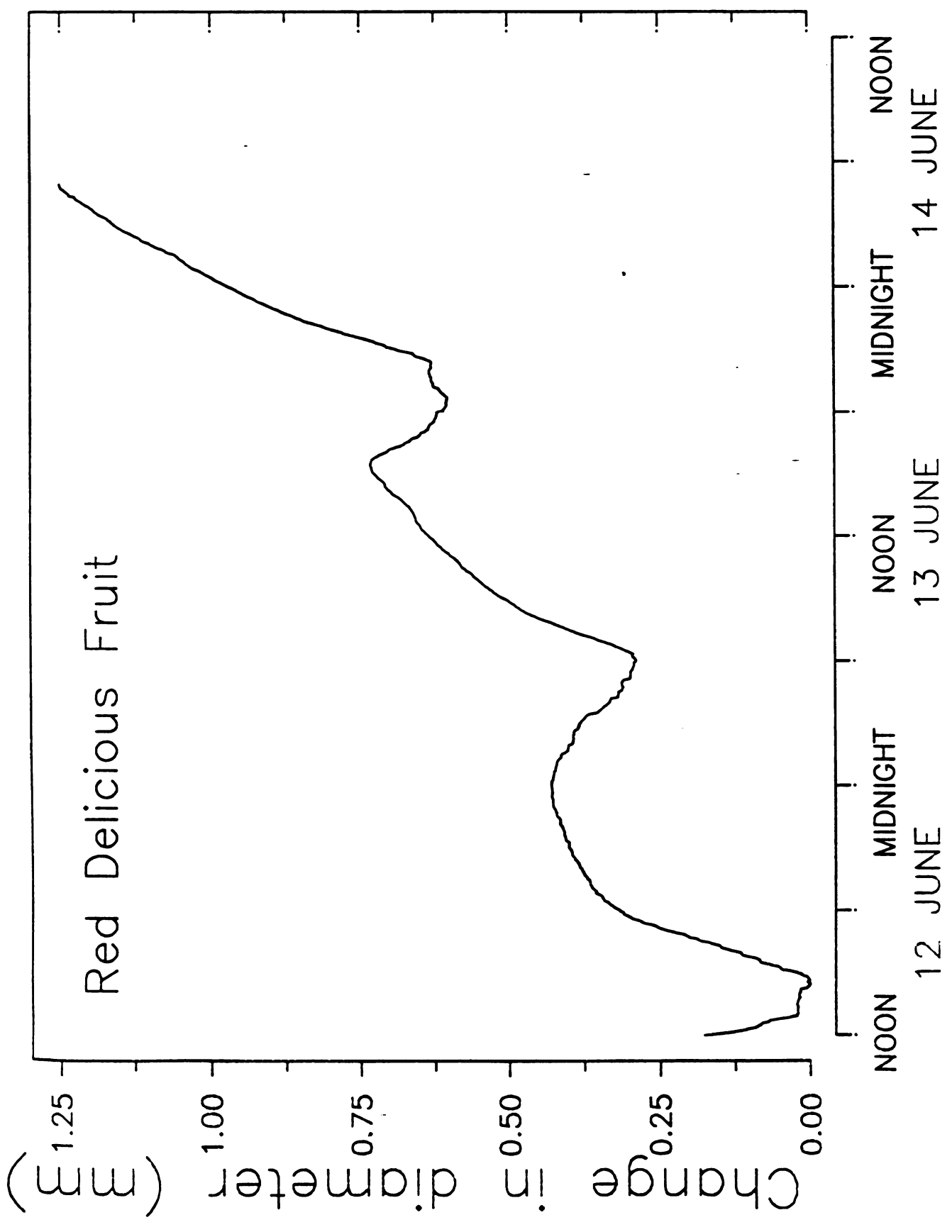

Red Delicious, 1988
Calcium Content
Actual
Estimated by Fruit Conductance
Estimated by Hanging Fruit
Red Delicious, 1989
Viking, 1989
Calcium content (mg fruit^{-1})
Days after full bloom
0
2
4
6
8
10
12
14
16
18
20
40
60
80
100
120
140
160

Figure 7. Diurnal leaf and fruit water potentials cv. 'Red Delicious' on two selected dates (20 June and 27 Sept., 1989). Values represent the mean ± SE of six observations.

Water potential (MPa)
0.00
−0.25
−0.50
−0.75
−1.00
−1.25
−1.50
Time (hours)
0
4
8
12
16
20
24
Red Delicious, 1989
Leaf
Fruit
20 June
27 Sept.

Figure 8. Diurnal change in 'Red Delicious' fruit diameter on 12-14 June, 1989. Values represent mean of two fruit.


Red Delicious Fruit
Change in diameter (mm)
1.25
1.00
0.75
0.50
0.25
0.00
NOON
MIDNIGHT
NOON
MIDNIGHT
NOON
12 JUNE
13 JUNE
14 JUNE

DISCUSSION

The xylem supply of Ca to apple fruit estimated by two different methods, generally underestimated Ca uptake during the first five to seven weeks of the season, and greatly overestimated uptake later in the season. Jones *et al.* (1983) used similar methods and also underestimated Ca content early in the season and overestimated it late in the season for four different cultivars. There are several possible explanations for the discrepancies between the predicted and actual patterns of Ca accumulation which occurred.

It is unlikely that the actual accumulation rates of Ca in the fruit differed greatly from those measured. The pattern of the Ca uptake curve was similar, but higher in magnitude, than that observed by Himelrick and Walker (1982). Rogers and Batjer (1954) observed a linear uptake of Ca in 'Red Delicious' fruit reaching 13 mg Ca per fruit by harvest. Nevertheless, the patterns observed in this study have been reported in other cultivars (Tromp 1972, 1975; Jones and Samuelson, 1983, 1983; Himelrick and Walker, 1982; Quinlin, 1969; Wilkinson, 1968; Chapter 1). The apparent decrease in Ca content in 'Red Delicious' fruit late in the season has also been reported in other cultivars (Tromp, 1972; Hanson, unpublished data; Perring, 1979) and may be related to the water status of the tree (Wilkinson, 1968; Tromp, 1979b). The cultivar 'Viking' was included in this study for comparison purposes because it matures earlier than 'Red Delicious'. Viking fruit accumulated Ca linearly throughout the season and attained a final Ca content equal to that of 'Red Delicious'fruit maturing 8 weeks later. The pattern of Ca accumulation in 'Viking' fruit has not been reported previously, although a similar pattern of Ca uptake has been observed in

other cultivars (Rogers and Batjer, 1954; Tromp, 1975, 1979; Tomala *et al.*, 1989).

The differences in the pattern of Ca uptake between the 'Red Delicious' and 'Viking' cultivars may be less related to fruit growth and more to late-season restrictions and limitations on fruit intake since Ca accumulation in 'Red Delicious' fruit ceased about the time 'Viking' were harvested. It is unclear whether this limitation is internally or externally related to the tree (ie. environmental or physiological).

We assumed that the Ca concentration in the exudate from shoots was representative of the levels in the xylem stream entering the fruit. These data are consistent with Bradfield (1986) and Jones *et al.* (1983) who generally found that Ca concentrations reached a maximum of 200 ppm Ca at bloom and then declined to approximately 50 ppm Ca by the end of the season. The fact that Ca levels in shoot exudates did not appear to vary diurnally (Appendix 4) indicates that samples collected in the morning would be representative of average levels throughout the day. It is possible that measured Ca levels in shoot exudates may be much higher than those actually delivered to the fruit. Since Ca^{2+} moves in the xylem by a series of exchange reactions and mass flow (Biddulph, 1959,1961), the extraction process could have released Ca from exchange sites leading to erroneously high levels. It is difficult to estimate the degree of this error.

For comparison, sap was also extracted from pedicels by placing the fruit under pressure. Exudate from pedicels contained lower Ca levels (Table 2) than shoot xylem exudates (Fig. 3). Pedicel extracts may contain a mixture of both xylem and phloem sap as the xylem could

not be sampled separately.

Our estimate of the xylem flow rate is a potentially large source of error in predicting the xylem supply of Ca to fruit. The quantity of xylem water flowing into fruit was estimated as the sum of the water transpired and that incorporated into growth. We estimated that a 150g fruit of either cultivar transpired approximately 20 to 30 ml of water during its growth. Jones *et al.* (1983) found that fruit of four cultivars transpired between 20 and 40 ml during the course of the season.

The amount of water encorporated into fruit growth can be measured directly, but the proportion of water supplied by the xylem must be estimated and could also be a large source of error. In order to determine the amount of water needed for growth, Jones *et al.* (1983) assumed that 85% of the increase in fruit fresh weight was contributed by water from the xylem (ie. all the water remaining in the fruit was supplied by the xylem). Since mature fruit are approximately 85% water, 128 g of water is retained in the fruit at harvest. This leaves 30 ml or roughly 20% of the total water (158 ml) to be contributed by the phloem. We have chosen the same percentage of water supplied by the xylem (85%), but if the phloem contribution is greater, a significant over prediction of Ca intake by the xylem may be occurring. If phloem supply of water is estimated as 4.6-6.7 times fruit dry matter increase (Jones *et al*, 1983), our hypothetical fruit would have received anywhere from 65% (103 ml) to 95% (150 ml) of its total water (158 ml) from the phloem. In order to achieve the observed content of 10 mg Ca per fruit at harvest, a phloem Ca concentration ranging from 22 μg Ca ml^{-1} to 64 μg ml^{-1} would be necessary, assuming a typical xylem Ca concentration of 50 μg

Ca ml^{-1}. Phloem Ca levels in other plant species have reportedly ranged from 10 μg Ca ml^{-1} in *Arenga saccharifera* (Tammes, 1958) to 4-92 μg Ca ml^{-1} in *Ricinus communis* (Hall *et al.*, 1971; Smith and Milburn, 1980; Wiersum, 1979; Komer *et al.*, 1989). Since phloem sieve tube sap is essentially a continuum of the cytoplasm (symplast system) where Ca concentrations are maintained at micromolar concentrations (1×10^{-3} μg Ca ml^{-1}) (Poovaiah 1985,1988) it is unlikely that such high levels exist in the phloem of intact plants. Considering the relatively small proportion of water transpired from the fruit surface in comparison to the quantity of water incorporated into fruit growth, fruit transpiration does not appear to be the driving force for Ca uptake into fruit. From the above calculations, it appears that if the phloem is supplying a significant percentage of water to the fruit, this would explain the over-prediction in fruit Ca contents.

An export of Ca from fruit could also explain why our calculations over-estimate Ca accumulation rates since we assumed a one-way flow of Ca into fruit. Given the flattening of the Ca accumulation curve observed in 'Red Delicious' fruit in both years (Fig. 6), and in other cultivars (Tromp 1972,1975; Jones *et al.*, 1983, Jones and Samuelson, 1983; Himelrick and Walker, 1982; Quinlin, 1969; Wilkinson, 1968), and the apparent decrease in Ca content in 1988, there is a possibility that an export of Ca from the fruit may be occurring (Wilkinson, 1968; Tromp, 1979b; Millikan, 1971). This phenomenon has been reported previously (Tromp, 1972, 1979b; Wilkinson, 1968) and appears to be caused by tree moisture stress (Wilkinson, 1968; Ferguson and Watkins, 1989). There is evidence that xylem movement from fruit occurs in grape berries (Lang and Thorpe, 1989), cowpea fruit (Pate *et al.*, 1985), and

pea fruit (Hamilton, 1988).

Changes in the water potential of 'Red Delicious' leaves and fruit (Fig. 7) and diurnal contraction and expansion of fruit (Fig. 8), during the 1989 season, appeared to support the possibility of a backflow of water and Ca out of the fruit during the day when the water potential of leaves is more negative than that of the fruit. Late in the season, the magnitude of this difference in water potentials was greater, possibly exerting a greater effect on Ca translocation out of the fruit (Appendix 6). In the fruit, as the season progressed, the magnitude of diurnal change decreased as the fruit osmotic potentials presumably increased. At night in August and September, when leaf water potentials increased to nearly zero (MPa), fruit water potentials were often 10 times more negative (-0.5 MPa) indicating that water movement would be from the tree to the fruit. In the day, leaf water potentials were nearly twice more negative than fruit suggesting a reversal of water flow from the fruit to the tree. This diurnal fluctuation which corresponded with a shrinkage and expansion of the fruit (Fig. 8) may have led to a net export of Ca from the fruit. A similar diurnal study in *Pissium sativum* L. revealed that water potential gradients between fruit and leaves were related to an apoplastic reverse-flow of water in the peduncle (Hamilton and Davies, 1988).

The magnitude of this potential loss of Ca can be estimated by assuming that for any given decrease in fruit size an equal volume of xylem sap is displaced from the fruit. Considering that a fruit approximates the volume of a sphere ($4/3\ \pi r^3$), the diurnal decrease in diameter measured during the season for 30 (early) and 70 (late) mm diameter fruit was approximately 0.10 and 1.0 mm, respectively. This

estimates that a displacement of 0.14 cm^3 early and 7.8 cm^3 late in the season would be occurring. Since the daily transpiration rates of fruit may range from four early in the season, to 0.20 ml H_2O (liquid water) late in the season (Fig. 4), it is unlikely that fruit transpiration alone can account for fruit shrinkage late in the season. Assuming that xylem Ca concentration is 50 μg Ca ml^{-3}, the potential loss of Ca could range from 7 μg to 390 μg per 12 hour period. This shrinkage has been confirmed in grape berries (Long and Thorpe, 1989). Calcium concentrations of xylem sap, measured diurnally at monthly intervals from bloom through harvest, indicate that no diurnal fluctuation in xylem Ca occurs (Appendix 4).

The main conclusion to be drawn from this study is that the xylem supply of Ca overestimates the actual Ca content of the fruit. Possible reasons for this overprediction are that Ca concentrations entering the fruit are less than those measured in shoot xylem exudates, that a significant amount of fruit water is derived by the phloem, or that a net export of Ca from the apple fruit may be occurring. The amount of water incorporated into fruit growth appears to be relatively more important in the delivery of Ca than fruit transpiration since fruit retained more water than they transpired. Further information on the xylem:phloem flow of water to apple fruit and the respective Ca concentrations of each, is necessary.

LITERATURE CITED

Bangerth, F. 1979. Calcium-related physiological disorders of plants. Ann. Rev. Phytopath. 17:97-122.

Biddulph, O., R. Cory, and S. Biddulph. 1959. Translocation of calcium in the bean plant. Plant Physiol. 34:512-519.

Biddulph, O., F.S. Nakayama, and R. Cory. 1961. Transpiration stream and ascension of calcium. Plant Physiol. 36:429-436.

Bollard, E.G. 1953. The use of tracheal sap in the study of apple-tree nutrition. J. Exp. Bot. 4:363-368.

Bollard, E.G. 1957. Translocation of organic nitrogen in the xylem. Aust. J. Biol. Sci. 10:292-301.

Cameron, A.C. 1982. Gas diffusion in bulky plant organs. PhD. Diss. University of California, Davis 126p. (Diss. Abstr. 43/04B p. 929)

Ehret, D.L., and L.C. Ho. 1986. Translocation of calcium in relation to tomato fruit growth. Ann. of Bot. 58:679-658.

Faust, M., and C.B. Shear. 1973. Calcium translocation patterns in apples. Proc. Res. Inst. Pomology, Skierniewice, Poland, Ser. E. 3:423-436.

Ferguson, I.B. 1979. The uptake and transport of calcium in the fruit tree, p.183-192 In: Mineral Nutrition of Fruit Trees. Atkinson, D., J.E. Jackson, and R.O. Sharples (eds.). Buttersworths Press, London, Toronto.

Ferguson, I.B., and C.B. Watkins. 1989. Bitter pit in apple fruit, p. 289-355 In: Hort Reviews. J. Janick (ed.). Timber Press, New York

Ferguson, I.B., F.R. Harker, and B.K. Drøbak. 1987. Calcium and apple fruit. The Orchardist of New Zealand. May:119-121.

Hall, E.G., D.A. Baker, and J.A. Milburn. 1971. Phloem transport of ^{14}C-labelled assimilates in *Ricinus* phloem exudate. Planta 100:200-207.

Hamilton, D.A., and P.J. Davies. 1988. Mechanism of export of organic material from the developing pea. Plant Physiol. 86:956-959.

Hanger, B.C. 1979. The movement of calcium in plants. Commun. Soil Sci. Plant Anal. 10:171-193.

Higgs, K.H., and H.G. Jones. 1984. A microcomputer-based system for continuous measurement and recording fruit diameter in relation to environmental factors. J. Exp. Bot. 35:1646-1655.

Himelrick, D.G. and R.F. McDuffie. 1983. The calcium cycle:uptake and distribution in apple trees. HortScience 118:147-150.

Himelrick, D.G. and C.E. Walker. 1982. Seasonal trends of calcium, magnesium, and potassium fractions in apple leaf and fruit tissues. J. Amer. Soc. Hort. Sci. 107:1078-1080.

Hocking, P.J., and J.S. Pate. 1978. Accumulation and distribution of mineral elements in annual lupins Lupinus albus and *Lupinus angustifolius* L. Aust. J. Agric. Res. 29:267-280.

Jones, H.G. and K.H. Higgs. 1982. Surface conductance and water balance of developing apple (*Malus pumila* Mill.) fruits. J. Exp. Bot. 33:67-77.

Jones, H.G., K.H. Higgs and T.J. Samuelson. 1983. Calcium uptake by developing apple fruits. I. Seasonal changes in calcium content of fruits. J. Hort Sci. 58:173-182.

Jones, H.G., and T.J. Samuelson. 1983. Calcium uptake by developing apple fruits. II. The role of spur leaves. J. Hort. Sci. 58:183-190.

Jones, H.G., and K.H. Higgs. 1985. Water movement into and out of apple fruits. Acta Hort. 171:353-358.

Komor, E., J. Kallarackal, C. Schobert, and G. Orlich. 1989. Comparison of solute transport in the phloem of the *Ricinus communis* seedling and the adult plant. Plant Physiol. Biochem. 27:545-550.

Lang, A., and M.R. Thorpe. 1989. Xylem, phloem and transpiration flows in a grape: Application of a technique for measuring the volume of attached fruits to high resolution using Archimedes' Principle.

Long, M.S. 1980. Cuticle development and incidence of russet on 'Golden Delicious' apple as influenced by subclone susceptibility and shelters. Michigan State University. MS Thesis.

Mason, A.C. and A.B. Whitfield. 1960. Seasonal changes in the uptake and distribution of mineral elements in apple trees. J. Hort. Sci. 35:34-55.

Millikan, C.R. 1971. Mid-season movement of ^{45}Ca in apple trees. Aust. J. Agric. Res. 22:923-930.

Oberly, G.H. 1973. Effect of 2,3,5-triiodobenzoic acid on bitter pit and calcium accumulation in 'Northern Spy' apples. J. Amer. Soc. Hort. Sci. 98:269-271.

Pate, J.S., and P.J. Hocking. 1978. Phloem and xylem transport in the supply of minerals to a developing legume (*Lupinus albus* L.) fruit. Annal. Bot. 42:911-921.

Pate, J.S., M.B. Peoples, A.J.E. van Bel, J. Kuo, and C.A. Atkins. 1985 Diurnal water balance of the cowpea fruit. Plant Physiol. 77:148-156.

Poovaiah, B.W. 1985. Role of calcium and calmodulin in plant growth and development. HortScience 20:347-352.

Poovaiah, B.W. 1988. Molecular and cellular aspects of calcium action in plants. Calcium in Horticulture Symposium. HortScience 23:267-271.

Perring, M.A. 1979. The effects of environment and cultural practices on calcium concentration in the apple fruit. Commun. Soil Sci. Plant Anal. 10:279-293.

Quinlin, J.D. 1969. Chemical composition of developing and shed fruits of Laxton's Fortune apple. J. Hort. Sci. 44:97-106.

Rogers, B.L. and L.P. Batjer. 1954. Seasonal trends of six nutrient elements in the flesh of Winesap and Delicious apple fruits. Proc. Amer. Soc. Hort. Sci. 63:67-73.

Shirazi, A. 1989. Modified humidity packaging of fresh produce. PhD Diss., Michigan State Univ., E. Lansing 123p. (Diss. Abstr. 50/07B p. 3125)

Shirazi, A., and A.C. Cameron. 1989. Modified packaging: a new concept for extending the shelf life of fresh produce. Submitted to J. Fd. Sci. for publication.

Sharazi, A., and A.C. Cameron. 1990. A rapid method for measuring transpiration rates of small fruits and vegetables. (in preparation)

Smith, J.A.C., and J.A. Milburn. 1980. Osmoregulation and the regulation of phloem-sap composition in *Ricinus communis* L. Planta 148:28-34.

Stebbins, R.L. and D.H. Dewey. 1972. Role of transpiration and phloem transport in accumulation of $_{45}$calcium in leaves of young apple trees. J. Amer. Soc. Hort. Sci. 94:471-474.

Tammes, P.M.L. 1958. Micro- and macro-nutrients in sieve-tube sap of palms. Acta Bot. Neer. 7:233-234.

Tammes, P.M.L., and J. van Die. 1964. Studies on phloem exudation from *Yucca flaccida* Haw. I. Some observations on the phenomenon of bleeding and the composition of the exudate. Acta Bot. Neer. 13:76-83.

Tomala, K., M. Araucz, and B. Zaczek. 1989. Growth dynamics and calcium content in McIntosh and Spartan apples. Commun. Soil Sci. Plant Anal. 20:529-537.

Tromp, J., and J. Oele. 1972. Shoot growth and mineral composition of leaves and fruits of apple as affected by relative are humidity. Physiol. Plant. 27:253-258.

Tromp, J. 1975. The effect of temperature on growth and mineral nutrition of fruits of apple with special reference to calcium. Physiol. Plant. 33:87-93.

Tromp, J. 1979a. Seasonal variations in the composition of xylem sap of apple with respect to K, Ca, Mg, and N. Z. Pflanzenphysiol. Bd. 94:189-194.

Tromp, J. 1979b. Mineral absorption and distribution in young apple trees under various environmental conditions, p. 173-182. In: Mineral Nutrition of Fruit Trees. Atkinson, D., J.E. Jackson, and R.O. Sharples (eds.). Buttersworths Press, London, Toronto.

Tukey, L.D. 1964. A linear electronic device for continuous measurement and recording of fruit enlargement and contraction. Proc. Amer. Soc. Hort. Sci. 84:653-660.

Tukey, L.D. 1974. Some relationships in the growth and development of apple fruits. Proc 19th Intern. Hort. Congr. 3:35-45.

Vang-Petersen, O. 1980. Calcium nutrition of apple trees: a review. Sci. Hort. 12:1-9

Wiersum, L.K. 1966. Calcium content of fruits and storage tissue in relation to the mode of water supply. Acta. Bot. Neer. 15:406-418.

Wiersum, L.K. 1979. Effects of environment and cultural practices on calcium nutrition. Commun. Soil Sci. Plant Anal. 10:259-278.

Wilkinson, B.G. 1968. Mineral composition of apples. IX. Uptake of calcium by the fruit. J. Sci. Food Agric. 19:646-64.

SUMMARY AND CONCLUSIONS

Poor distribution of Ca in apple trees is the primary reason that fruit are predisposed to Ca-disorders such as 'bitter pit' (Ferguson and Watkins, 1989) even when Ca levels are adequate in the remainder of the tree (Himelrick and McDuffie, 1983) and in the soil (Bangerth, 1979; Hanger, 1979; Mason, 1979). Knowledge of the factors affecting the translocation of Ca to fruit would be useful in developing techniques to enhance its transport to fruit. By redirecting Ca transport from alternate plant sources high in Ca, or by increasing the solubility of Ca in the xylem and/or phloem systems, perhaps Ca-disorders might be prevented. Three experiments were conducted to describe the seasonal patterns of Ca accumulation in fruit and to estimate the importance of the xylem contribution of Ca to fruit.

The objective of the first experiment was to describe the seasonal pattern of Ca accumulation in 'Red Delicious' fruit and to determine how the pattern of accumulation might change under varying environmental conditions. Similar patterns of Ca accumulation were observed over a wide range of seasonal temperatures, rainfalls, and potential cropping levels. Calcium uptake was essentially linear and rapid for the first 10 to 14 weeks of fruit development, but began to decline in a quadratic manner thereafter. These data indicate that no further accumulation in the fruit is likely in the three weeks prior to harvest.

The earlier ripening Viking fruit were included in this study for comparison purposes. The pattern of Ca accumulation in 'Red Delicious' fruit, when compared to that of 'Viking', appeared to be more related to late-season limitations on fruit intake which may have been associated

environmental influences on or physiological changes within the fruit. The exact nature of this restriction was not studied.

The objective of the second experiment was to determine the importance of the xylem in supplying Ca to fruit during different stages of fruit development. Exposing fruit to high humidities reduced fruit Ca content and often increased fruit growth, although these effects were not consistently observed.

The objective of the third experiment was to clarify the role of the xylem system in supplying Ca to 'Red Delicious' and 'Viking' fruit. The xylem supply to the fruit, estimated by two methods, generally underestimated Ca uptake early in the season, and greatly overestimated uptake later in the season. These data provide evidence that a net export of Ca from the fruit may be occurring. It also could indicate that phloem is more important than xylem for Ca import later in the season.

This experiment also revealed that fruit retained more water than they transpired. This suggests that water incorporated into fruit growth is relatively more important in the delivery of Ca than fruit transpiration. Further information on the xylem:phloem flow of water to apple fruit and the respective Ca concentrations of each, would be valuable in understanding the regulation of Ca supply to the fruit.

The central observations from this research are that Ca accumulation in 'Red Delicious' apple fruit is not continuous throughout fruit development, the xylem system appears to be an important source of Ca throughout fruit growth, and that an export of Ca may occur from the fruit under certain conditions.

The xylem flow of water could be estimated more directly using the

'heat-balance' method (Cermak *et al.*, 1973; Valancogne and Nasr, 1989 - review of literature). In principle, the 'heat-balance' works by applying a predetermined quantity of heat to a plant stem (or pedicel) and estimates the amount of heat absorbed by the xylem sap, which is proportional to the xylem flow rate.

If phloem Ca concentrations are much greater than cytoplasmic levels and if appreciable quantities of water originate from the phloem, the phloem could be a significant source for fruit Ca. Although estimates of phloem sap Ca concentrations, from tissue of other plant species, range as high as 100 μg Ca ml^{-1} (Hall *et al.*, 1971; Tammes and Van Die, 1964), levels in intact plants approximate those found in the cytoplasm (< 0.04 μg Ca ml^{-1}) (Poovaiah, 1988). Techniques to collect adequate and representative phloem sap samples are needed to determine with certainty its contribution of Ca to apple fruit.

Evidence for Ca export from the fruit was indicated by the decrease in fruit Ca content at harvest and by the possible backflow of water from the fruit to the tree in response to seasonal changes in tree water relations. Diurnal shrinkage in fruit size also supports this claim. In four of seven measurements of Ca content in experiment one, the fruit appeared to lose Ca prior to harvest. This potential export appeared to be induced by factors unrelated to moisture stress.

The specific tissue(s) in the fruit where Ca is exported during periods of Ca backflow is(are) not clear. If Ca were being effluxed from areas, resulting in a reduction in the physiological competance of cells, this backflow could result in the development of Ca-disorders. However, if Ca were being redistributed from tissue relatively high in Ca, such as the seed or core, such losses may be less important. In

addition, the form of Ca exported, whether it is complexed or physiologically active, may be useful in determing how a specific tissue may become predisposed to a Ca-disorder. Further knowledge of the seasonal distribution of Ca within the different fruit components would be useful.

APPENDIX ONE: ADDITIONAL EXPERIMENTS

A. THE EFFECT OF MOISTURE STRESS ON CALCIUM UPTAKE IN APPLE FRUIT

INTRODUCTION

The objective of this study was to measure the effect of moisture deficit on calcium accumulation in apple fruit, primarily late in the growing season when Ca contents have previously been observed to decline (see chapter 1). It is hypothesized that a late season export of Ca from fruit will occur under periods of soil moisture stress.

MATERIALS AND METHODS

Two methods in 1988 were used in attempting to impose adequate moisture stress, one in a commercial orchard in Traverse City, Michigan, and the other at the Clarksville Research Station, Clarksville, Michigan.

Traverse City:

Soil underneath 7-8 year old 'Red Delicious' cv. Starking/M.26 was covered with 1.5 m widths of 4 mil black polyethylene plastic mulch to prevent rainfall from penetrating the soil. The experiment was initially designed as a 2 factor factorial (+/- mulch, +/- trickle irrigation) with 4 replications and 2 trees serving as experimental units with 2 border trees. However, as the irrigation was inoperative, the experiment was analyzed as a randomized complete block with two treatments and 12 replications. Gravimetric soil moisture levels at two depths (0-30, 30-45 cm) and fruit Ca levels were measured six times throughout the season. Noon-day and pre-dawn leaf water potentials (ψ_w) were measured 8/6 and 9/8, respectively, to estimate the degree of

water stress imposed.

Clarksville:

With the co-operation of Dr. Ron Perry, apple fruit were sampled at commercial harvest from two irrigation regimes (+/-) and two soil bed heights (level, 1.2 m). Two subsamples of five uniformly sized fruit per treatment, replicated 3 times, were analyzed for Ca, soluble solids, and fruit firmness.

RESULTS AND DISCUSSION

Traverse City Results

Mulch treatments reduced soil moisture at the 0-30 and 30-45 cm depths by 23 and 9% respectively (Table 1), but had no effect on leaf water potentials (Table 2), stomatal conductance and transpiration (data not included).

The orchard had received overhead irrigation just prior to initiation of treatments. It appeared that soil moisture was not depleted enough during the experiment to significantly affect the moisture status of the trees, even though the year was characterized as extremely 'dry' and 'hot'. Fruit Ca accumulation was not influenced by the changes in soil moisture (data not presented).

Clarksville Raised-Bed Experiment Results

Irrigation had a significant affect on fruit firmness and soluble solids, but neither treatment altered the calcium status of the fruit (Table 3). Fruit of similar size were selected for calcium determination, therefore treatment effects on fruit size were not determined.

Table 1. Gravimetric soil water content at two depths for mulch and control treatments. Traverse City, 1988.

	Gravimetric soil water content (%)	
Treatment/ Depth (cm)	Pretreatment 1 July	23 July
0-30		
Mulch	4.4	5.7
Control	4.1	7.4
	NS	**
30-45		
Mulch	-	6.5
Control	-	8.0
		*

NS,*,** Not significant, significant at $P = 0.05$ and $P = 0.01$, respectively.

Table 2. Water potential (ψ_w) of 'Red Delicious' apple trees treated with and without plastic mulch. Traverse City, 1988.

	Leaf water potential (ψ_w, Mpa)	
Treatment	Noon 6 August	Pre-dawn 8 Sept.
Mulch	4.71	4.14
No Mulch	4.77	4.10
	NS	NS

NS,*,** Not significant, significant at $P = 0.05$ and $P = 0.01$, respectively.

Table 3. The effect of elevated soil beds and irrigation on 'Red Delicious' fruit weight, firmness, soluble solids, and calcium levels. Clarksville Experiment Station, Michigan. Sept. 20, 1988[z]

Soil bed type	Irrigation (+/-)	Fruit weight (g)	Fruit[y] firmness (lbs)	Soluble solids (%)	Dry matter: whole fruit (%)	Dry matter: fruit[x] flesh (%)	Calcium: whole fruit µg Ca	Calcium: fruit[x] flesh fw	Calcium: whole fruit mg Ca
Flat	-	178	16.0 a	10.8 a	17.4	22.2	40	54	7.0
Flat	+	196	15.3 ab	10.4 b	17.0	22.9	38	56	7.2
Raised	-	179	15.8 ab	10.8 a	17.2	22.6	43	54	7.6
Raised	+	193	15.1 b	10.1 b	16.7	21.4	35	52	6.7

[z] means followed by same letters within column are not significantly different at P=0.05. Least significant Difference.
[y] average of 15 fruit
[x] tissue 1-3 mm beneath peel

B. ASSESSMENT OF LATE-SEASON PHLOEM EXPORT OF CALCIUM BY GIRDLING EXPERIMENTS

INTRODUCTION

The objective of this study was to measure the effect of disrupting phloem transport into apple fruit on Ca accumulation late in the growing season. It was hypothesized that late season phloem transport of calcium to the fruit is negligible.

MATERIALS AND METHODS

Pedicels from 20 Red Delicious fruit of similar size were girdled 9/5/88 using pressurized steam. Another 20 fruit served as controls. Fruit were harvested 15 days later on 9/20, weighed and Ca, Mg,and K determined for whole fruit tissue, as well as cortex tissue 1-2 mm below the peel. Girdling effectiveness was indicated by the browning of phloem tissue.

RESULTS AND DISCUSSION

Calcium levels of whole fruit tissue were unaffected by girdling treatment, but Ca levels of sub-peel samples (fresh weight) were 60% higher for girdled fruit. Also girdled fruit had a greater percent dry weight (less water), but fruit weight was reduced resulting in less total dry matter. Furthermore, girdled fruit had a slight increase in Mg concentrations (fresh weight) and total content (data not presented), though the latter is a fruit size effect. The effect of girdling on K accumulation, other than that caused by changes in fruit size, was negligible.

APPENDIX 2

SAMPLE CALCULATION OF FRUIT SURFACE CONDUCTANCE TO WATER LOSS

Sample Problem

Problem: What is the evapo-transpiration rate (ml H_2O per week) from an individual 20 g apple fruit early in the growing season given the following.

Abbreviations Used : VPD = vapor pressure deficit ; SVP = saturation vapor pressure ; J = flux rate of H_2O loss; SA = surface area; RH = relative humidity;

Parameter	Units	Value
Δ fruit wt (on balance)	mg hr^{-1}	6.9
RH surrounding fruit	%	70.0
Temp surrounding fruit	°C	21.0
Surface area of fruit(SA)	cm^2	5
Evaporation of water from an open pan of H_2O	mmol H_2O sec^{-1} KPa^{-1} cm^{-2}	3×10^{-2}
Typical evaporation from an open pan of water in the orchard	ml H_2O $week^{-1}cm^{-2}$	1.5

Solution

Conductance (C) of a fruit surface to water loss was calculated using the relationships (Cameron, 1982):

Water flux per unit surface area ($cm \cdot sec^{-1}$ KPa^{-1})

$$C_{SA} = \frac{J_v}{SA_{fruit} \times VPD},$$

Water flux per unit fruit weight (mmol H_2O g $fruit^{-1}$ KPa^{-1})

$$C_{FRUIT} = \frac{J_v}{\text{fruit wt.(g)} \times VPD}$$

where,

J_v [mmol $H_2O \cdot sec^{-1}$] = water flux

$$= \frac{(\text{Fruit weight})_{t0} - (\text{Fruit weight})_{t1}}{t1 - t0}$$

where t_0 = start time and t_1 = end time

$$= \frac{6.9 \text{ mg } H_2O}{1 \text{ hour}} \times \frac{1 \text{ mmol } H_2O}{18 \text{ mg } H_2O} \times \frac{1 \text{ hour}}{3600 \text{ sec}}$$

$$= \frac{1.06 \times 10^{-4} \text{ mmol } H_2O}{\text{sec}}$$

VPD (KPa) - the difference in vapor pressure of the fruit and the atmosphere surrounding the fruit within the laboratory.

$$= \frac{\text{Saturation Vapor Pressure} \times [1\text{-RH}(\%)]}{100\ \%}$$

$$\text{VPD} = \frac{2.52\ \text{KPa (@21°C)} \times (100\%\ \text{RH} - 70.0\ \%\ \text{RH})}{100\ \%}$$

$$= 0.755\ \text{KPa}$$

SA_{FRUIT} - estimated surface area of the fruit
- $[w \times (\frac{1}{2}w)^2 + (\frac{1}{2}l)^2]$, where 'w' and 'l' represent fruit width and length, respectively (Long, 1980)

$$= 5.0 \times 10^{-3}\ m^2$$

$$C_{SA} = \frac{1.06 \times 10^{-4}\ \text{mmol}\ H_2O\ \text{sec}^{-1}}{5.0 \times 10^{-3}\ m^2 \times 0.755\ \text{KPA}}$$

$$= 2.81 \times 10^{-2}\ \text{mmol}\ H_2O\ \text{sec}^{-1}\ \text{KPa}^{-1}\ m^{-2}$$

$$C_{FRUIT} = \frac{1.06 \times 10^{-4}\ \text{mmol}\ H_2O\ \text{sec}^{-1}}{5.0 \times 10^{-3}\ m^2 \times 20\ \text{g fruit}}$$

$$= 1.06 \times 10^{-3}\ \text{mmol}\ H_2O\ \text{sec}^{-1}\ \text{g fruit}^{-1}$$

Field Determination (Correction/Adjustment):

The conductance of water in the laboratory was used to predict the amount of water transpired from the fruit within the orchard. Since the environment of the orchard can be quite different from the controlled conditions in the laboratory, conductance values must be corrected for the different vapor pressures in the field. Evaporation from a free-standing open pan of water serves this purpose well.

To convert fruit conductance values from the laboratory to evapo-transpiration rates in the orchard:

$$\text{Fruit Evapo-transpiration in the Orchard (ml week}^{-1}) = \frac{\text{Pan Evaporation}}{SA_{PAN}} \times \frac{C_{SA}}{C_{WATER}} \times SA_{FRUIT}$$

Where,

Pan Evaporation = ml H_2O evaporated per week

SA_{PAN} = surface area of water evaporating from the pan

C_{WATER} = 1.3 mmol H_2O m^2 $\cdot sec^{-1}$ KPa^{-1} = conductance of water

from an open surface of water measured empirically using fruit conductance values under controlled conditions

$$= \frac{4000 \text{ ml week}^{-1}}{1.584 \times 10^{-1} \text{ m}^2} \times \frac{2.81 \times 10^{-2} \text{ mmol } H_2O \text{ m}^2 \text{ sec}^{-1} \text{ KPa}^{-1}}{1.3 \text{ mmol } H_2O \text{ m}^2 \cdot \text{sec}^{-1} \text{ KPa}^{-1}}$$

$$= 545 \text{ ml week}^{-1} \text{ m}^{-2}$$

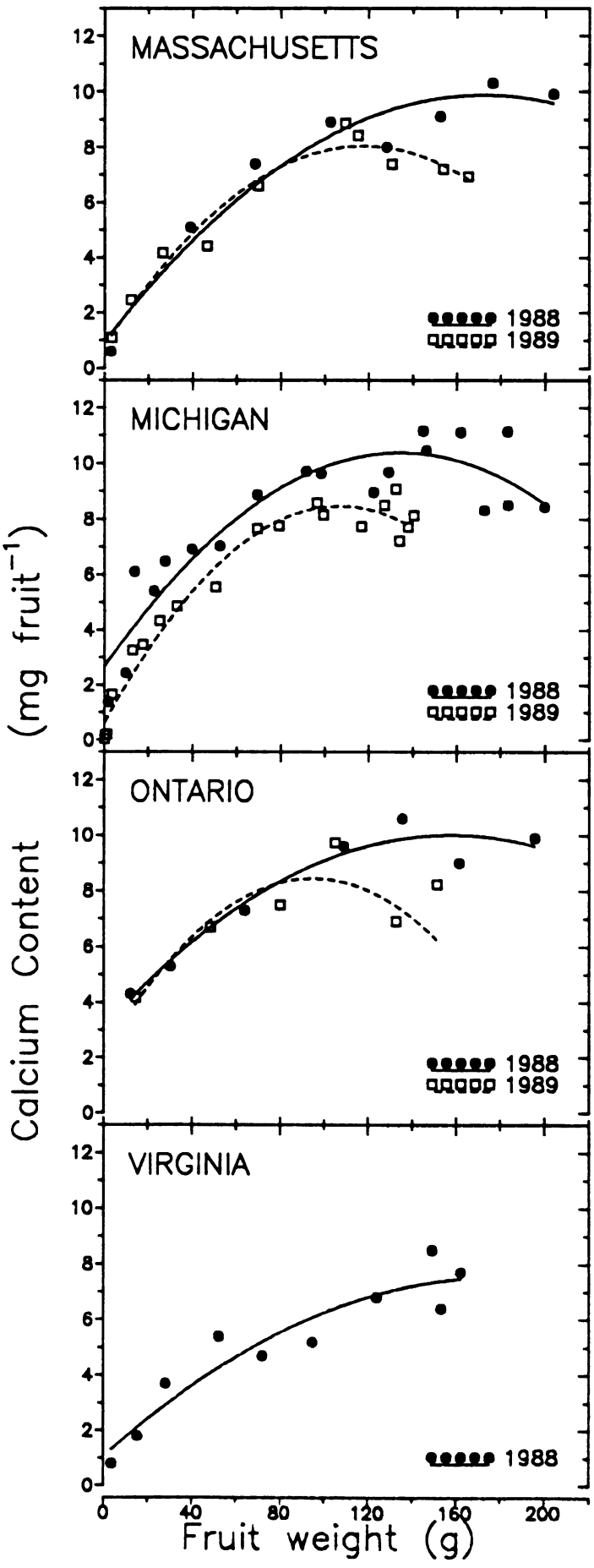
Given a fruit with a surface area of 50 cm^2, the total amount of water evapo-transpiring from its surface would be:

$$= 545 \text{ ml week}^{-1} \text{ m}^{-2} \times 5 \times 10^{-3} \text{ m}^2 \text{ fruit}^{-1}$$

$$= 2.72 \text{ ml fruit}^{-1} \text{ week}^{-1}$$

Appendix 3: Seasonal changes in calcium content of 'Red Delicious' fruit from several orchards in 1988 and 1989 plotted against fruit weight. Calcium content = $ax^2 + bx + c$, where a,b,c =

Location	a	b	c	r2
Massachusettes				
1988	-3.01×10^{-4}	0.104	0.958	0.94
1989	-5.23×10^{-4}	0.123	0.802	0.96
Michigan				
1988	-4.27×10^{-4}	0.115	2.68	0.86
1989	-6.84×10^{-4}	0.145	0.636	0.97
Ontario				
1988	-2.78×10^{-4}	0.0876	3.10	0.95
1989	-6.91×10^{-4}	0.132	2.20	0.82
Virginia				
1989	-1.98×10^{-4}	0.0713	1.06	0.90


MASSACHUSETTS
MICHIGAN
ONTARIO
VIRGINIA
1988
1989
Calcium Content (mg fruit^{-1})
Fruit weight (g)
0
2
4
6
8
10
12
40
80
120
160
200

Appendix 4. 'Red Delicious' and 'Viking' diurnal calcium concentrations in shoot xylem exudates collected under suction during the 1988 and 1989 growing seasons.[z]

Date	Days after full bloom	Time (hr)	Calcium concn. (ppm)	Std. Dev.
Red Delicious, 1988				
June 27	48	9 am	96.50	± 19.43
"	48	13 pm	87.58	± 7.87
"	48	17	92.42	± 37.55
"	48	21	103.50	± 32.10
June 28	49	1 am	115.75	± 11.98
"	49	4	84.83	± 12.33
"	49	8	106.67	± 21.34
Red Delicious, 1989				
June 21	36	15 pm	140.0	± 53.9
"	36	19	112.5	± 16.4
June 22	37	22	125.0	± 38.4
"	37	1 am	116.7	± 26.2
"	37	6	132.5	± 27.7
"	37	9	145.0	± 47.7
July 22	67	17 pm	107.5	± 23.8
"	67	21	72.5	± 49.2
July 23	68	1 am	80.0	± 48.5
"	68	5	100.0	± 21.2
"	68	9	110.0	± 40.6
"	68	13 pm	105.0	± 43.3
August 20	95	17 pm	137.5	± 79.5
"	95	21	115.0	± 32.0
August 21	96	9 am	60.0	± 52.0
"	96	13	92.5	± 41.5
"	96	17	102.5	± 80.4
Sept. 1	135	6 am	100.0	± 32.4
"	135	9	127.5	± 47.1
"	135	13 pm	132.5	± 48.2
"	135	17	122.5	± 50.7
"	135	21	127.5	± 39.6

Appendix 4. (cont.)

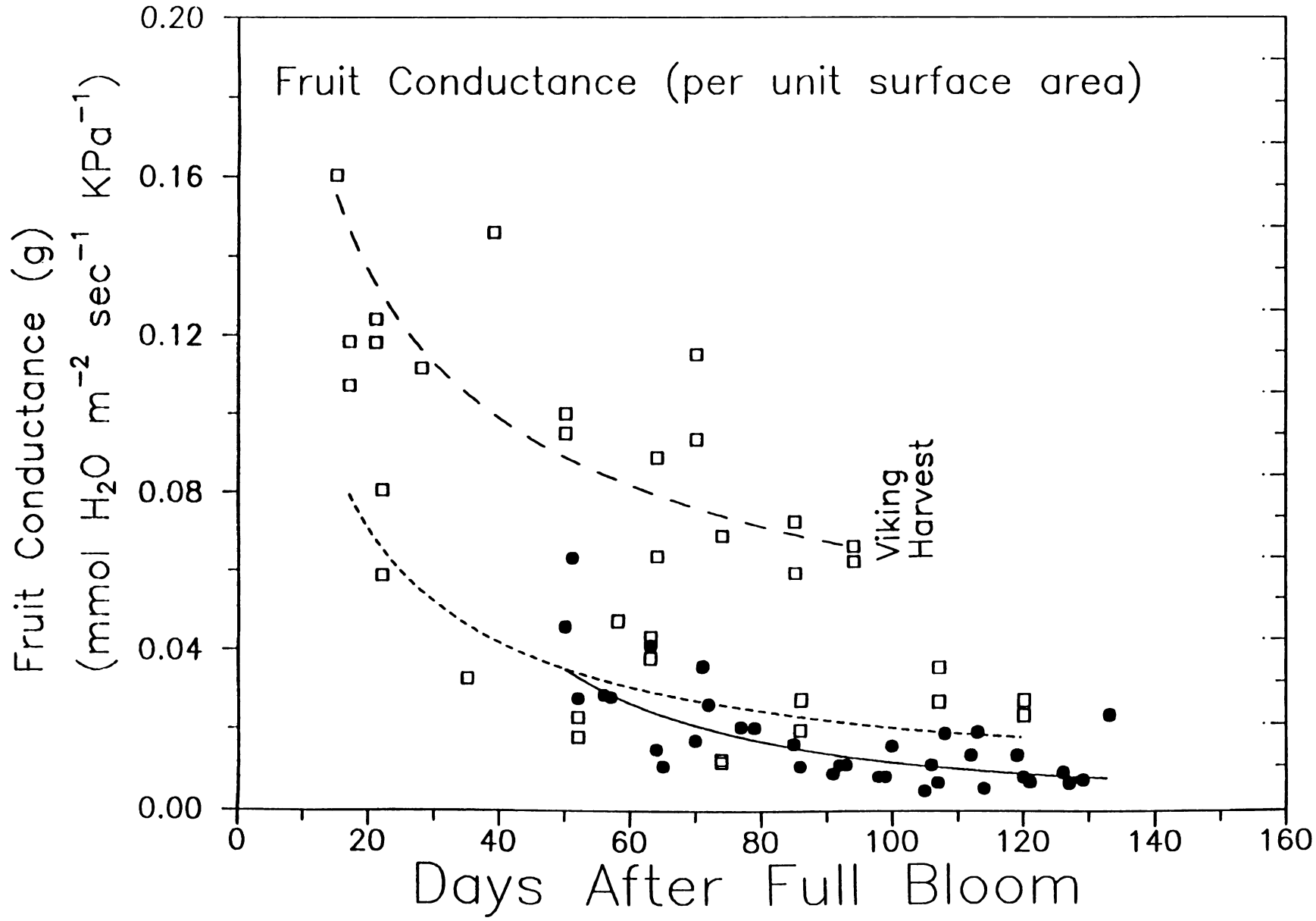
Viking, 1989[y]

July 14	60	18 pm	256.7	± 30.9
"	60	21	216.7	± 54.4
July 15	61	1 am	92.5	± 54.5
"	61	5	225.0	± 41.5
"	61	9	223.3	± 75.9
"	61	13 pm	267.5	± 10.9

[z] Values are the mean of four observations per sampling date

[y] samples not collected in 1988.

Appendix 5. Seasonal pattern of fruit conductance to water loss per unit surface area in 'Red Delicious' and 'Viking' trees, 1988 and 1989. Best fit equations: $C_{SA} = (DAFB^{-A})xB$, where A and B are: 1988 R. Del. A= -1.51; B = 12.92, 1989 R. Del A=-0.751; B=0.666, 1989 Viking A=-0.465; B=0.549.


Fruit Conductance (per unit surface area)
Fruit Conductance (g)
(mmol H_2O m^{-2} sec^{-1} KPa^{-1})
0.20
0.16
0.12
0.08
0.04
0.00
0
20
40
60
80
100
120
140
160
Days After Full Bloom
Viking
Harvest

Appendix 6. Diurnal water potential of 'Red Delicious' leaves and fruit measured throughout the 1989 growing season. East Lansing, Michigan.

Date	Days after full bloom	Time (hr)	Water Potential (PSI) Leaf	Std. Dev.	Fruit	Std. Dev.
June 20	36	14	100	± 19	94	± 10
"	36	18	88	± 22	89	± 14
"	36	22	28	± 12	40	± 7
June 21	37	2	10	± 0	27	± 7
July 21	67	18	40	± 8	98	± 8
	67	21	27	± 9	68	± 4
July 22	68	1	18	± 3	45	± 6
	68	5	8	± 3	40	± 9
	68	9	85	± 47	81	± 12
	68	13	143	± 33	86	± 11
August 18	95	17	111	± 32	76	± 12
	95	21	20	± 0	74	± 10
August 19	96	9	23	± 5	53	± 6
	96	13	149	± 18	66	± 6
	96	17	122	± 23	74	± 8
September 5	113	13	161	± 66	83	± 29
	113	17	152	± 54	68	± 2
	113	19	32	± 5	50	± 0
September 27	135	6	9	± 2	87	± 29
	135	9	16	± 5	44	± 10
	135	13	148	± 67	66	± 12
	135	17	144	± 29	80	± 17
	135	21	56	± 14	66	± 12