THE DEVELOPMENT OF XYLEM WITHIN THE TOMATO AND ITS INFLUENCE ON THE MOVEMENT OF WATER AND CALCIUM INTO THE FRUIT

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The Development of Xylem within the Tomato and its Influence on the Movement of Water and Calcium into the Fruit.

BY

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Abstract

Functional associations of xylem vascularization, calcium transport, and water flux were studied in *Lycopersicon esculentum* Mill. "Tiny Tim". Xylem formation of the pedicel and fruit was traced from the emergence of the floral primordia through to the development of mature fruit. Berberine hemi-sulphate, an apoplastically mobile dye, was used to follow water movements through the xylem systems of intact stems and trusses. ^{45}Ca was used to identify areas of calcium localization within the plant and correlated with transpiration rates measured for the selected plant parts.

Water flow and calcium distribution in the plant were not uniform and the pattern was dependent on the extent of xylem vascularization. Leaves, sepals, and the smallest immature fruit (<30 mm^3), which were all well supplied with xylem, showed the greatest accumulations of calcium. Fruit in this class also showed the highest transpiration rates. In contrast, calcium concentration was lowest in mature fruit, which also had the lowest proportion of xylem vascularization and lowest transpiration rates.

These findings bear on the mechanism of induction of blossom end rot, a phytopathological condition regarded as a calcium-deficiency disease of tomatoes. The suggestion that the deficiency might first occur in very small fruit as a result of their early, rapid increase in volume is not supported by the present data which demonstrate that such fruit, due to high water flux and transpiration rate, accumulate large amounts of calcium. The distal portion of larger fruit may become calcium deficient because apoplastic water does not reach the far blossom end of these fruit.

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Chapter 1

Introduction

1.1. Floral Development

The inflorescence of the tomato (*Lycopersicon esculentum* Mill.) originates as an elongated vegetative apex. The apex is flattened, as is typical of inflorescence initiation, and the first flower is formed from this flattened dome. The individual parts of the flower are formed in series, beginning with the development of sepals at the outer margin and progressing to the center where carpels are formed. Usually five or occasionally six sepal primordia develop through meristematic activity, independent of each other, at the periphery of the flattened apex, and form a helix around the apex margin. The sepals arise in a clock-wise direction, resulting in a circle around the apex dome (Sawhney and Greyson 1972). Their growth is more rapid on their abaxial sides so that the tips incline towards each other as they elongate and partially enclose the cavity beneath them. After sepal differentiation, a circle of independent meristematic regions develop alternate to the sepals and grow into the petals, in a similar fashion to the sepals. The staminal whorl then develops opposite to the calyx and alternate with the corolla. Each primordium of the staminate whorl consists of homogeneous tissue, which as the tissue grows, becomes differentiated into the distinct parts of anther and filament. The fourth whorl of five or six primordia

develops into the fused carpels of the gynoecium. The placenta on the axillary wall of each carpel enlarges so that it, with the developing ovules and jelly-like mesocarp, fills the locules of the carpels. Ovules are attached by their funiculus directly to the placental tissue in a series of rows. The end result of this process is a pentamerous or hexamerous, hypogenous, actinomorphic, bisexual flower (Hayward 1938).

Subsequent flowers arise as lateral buds from the preceding flower and develop in a similar fashion, until a helicoid-cyme results, usually of five to seven flowers. The first flower to grow has a developmental advantage over the second, and the second over the third to the end of the truss. Ordinarily, no more than two flowers of an inflorescence are open simultaneously. Because of this progressive development, a single cyme may have small fruit, flowers, and buds at the same time (Cooper 1927).

1.2. Xylem Development

Xylem differentiation within the flower bud begins at the inner side of a trace procambium near the base of the bud. It advances acropetally towards the bud tip and basipetally through the bud base to connect to the existing xylem of the vegetative stem. The pattern is similar to that of a vegetative bud (Jacobs and Morrow 1957). Several vertical files of tracheary elements may be initiated at the isolated locus before one extends to connect with the xylem of the vegetative stem. The upward differentiating file may not directly connect with the downward differentiating file. The two strands of xylem are then joined laterally by the differentiation of the few intervening procambial cells to form a short chain of connecting xylem cells (Jacobs and Morrow 1957).

The first xylem tissue to form at the base of the flower bud is the protoxylem, which matures before the flower bud has elongated. As the cells adjacent the protoxylem extend during bud elongation, the non-living tracheary elements of the protoxylem are stretched or often destroyed. The metaxylem differentiates during bud elongation and matures after this process is finished. Therefore, the elements of the metaxylem are not destroyed by stretching and persist in the mature inflorescence. In the event of large secondary growth, the metaxylem usually becomes non-functional. However, in most flower buds and pedicels, it remains as the sole water-conducting tissue (Esau 1953).

The protoxylem usually contains relatively few tracheary elements, and a large proportion of parenchyma cells. The metaxylem is a more complex tissue whose tracheary elements are generally wider and are accompanied by fibres along with parenchyma cells. The high proportion of cells with secondary cell walls gives the metaxylem a more compact and sturdy appearance than the protoxylem (Esau 1953).

Once floral development is complete, a continuous cylinder of metaxylem occurs through the length of the pedicel, which is formed by the bundles of vessels and tracheids, and cells of fibres and xylem parenchyma. It encloses the internal phloem and pith and is bordered by the external phloem, all of which is surrounded by cortex. This xylem reaches the receptacle or point of attachment to the floral parts. Traces diverge into the sepals, petals, anthers, and gynoecium. Each sepal usually has as many traces as a leaf of the same plant. Traces then branch into the corolla, from the metaxylem cylinder, usually one to each petal.

A single xylem trace travels the length of the stamen. Several xylem bundles may feed into the carpels: the typical number is three traces to each carpel with small branches connecting the carpellary system to the ovules. There are also strands of xylem leading to the stigma (Esau 1953).

Mature xylem tissue of the tomato plant consists of vessel members, tracheids, fibres, and parenchyma cells. The tracheary elements (vessels and tracheids) are the water-conducting portions of this tissue. Vessels consist of series of individual cells, the vessel elements, whose end walls are partly or completely dissolved at later stages of cell maturation, thus forming the long open vessels (see Zimmermann 1983). The tracheids are usually much longer than vessel elements and connect across cell walls with bordered pits. Both types of cells have secondary wall thickenings that enable them to retain their shape when dead, despite the pressure of the surrounding cells (Aloni 1987).

1.3. Water Transport into and through the Xylem

The rate and direction of water flow through vessel elements and tracheids is dependent on water potential. Water potential is expressed as the combined effects of osmotic, turgor, and matrix potentials. Osmotic potential is a function of solute concentration and is measured against a reference potential of pure water at atmospheric pressure. This reference is taken to be zero, therefore the osmotic potential of any solution is always negative. The more concentrated a solution, the lower its osmotic potential. Pressure potential is the result of turgor pressures within cells, balancing the difference between the osmotic and total water potential, and transpirational pulls within vascular channels. The matrix

potential or adhesion of water to surfaces such as cell walls is typically negligible inside the plant that is not in a water stressed condition. Hence, water movement is directional and is defined by the magnitude of the gradient of water potential: water always moves toward the region of lowest potential (see Boyer 1985).

The rate of water flux across the root of the plant and in the xylem tracheary elements of the stem is determined by the root pressure during periods of darkness and low water stress and the rate of transpiration from the surface of the plant during the day (see Marschner 1986).

Soil solution, which contains dissolved ions, diffuses into the cortical cell region and travels apoplastically through the cell walls of the cortex. Symplastic movement through plasmodesmata into endodermal cells must also occur, as water passage through the apoplast is blocked by the suberin-impregnated Casparian band surrounding the endodermal cells (see Clarkson 1984, Peterson 1988). Once across the barrier, ions are released again into the apoplast of the stele. Secondary and tertiary wall building of the endodermal cells of mature roots may destroy the attachment of the plasmalemma and the Casparian band, opening small apoplastic channels in the plane of the endodermal cells to allow ion passage (Sanderson 1983). This flux of ions into the stele results in lowered ion concentration of the cortex relative to that of the stele and establishes an osmotic gradient across this region from cortex to stele. The water potential of the cortex is then higher than in the stele and causes water to diffuse into it. This diffusion is sufficient to establish a substantial hydrostatic pressure in the stele, resulting in water flow up the xylem elements of the stem (Barrs 1966). This gradient results

in the phenomenon of root pressure which alone is insufficient to cause the movement of water through the plant without the greater effects of transpiration from the plant surface.

Transpiration creates a negative pressure within the vessel elements and tracheids due to evaporation from leaves and causes water to be pulled from the lower stem to the evaporating surfaces such as leaves and sepals. Hence, an increase in the transpiration rate enhances both the uptake and the translocation of mineral elements in the xylem (see Marschner 1986). The rate of transpiration is dependent on several factors, such as time of day, relative humidity, age of the plant, and solute concentration of the absorbed soil solution. Leaves create the greatest transpirational pull and more than 90% of this transpiration is stomatal. Transpiration rates and movement of mineral elements are much higher during the day than during the dark period, due to the opening of stomata for gas exchange in photosynthesis and energy availability for the water phase change. Short term declines in the translocation rates of minerals at the onset of darkness reflect the change from transpiration to root pressure-mediated volume flow in the xylem (Crosset 1968). An increase in the relative humidity of the environment will decrease transpiration rates from the plant surface as the water potential difference between the plant tissue and the atmosphere decreases. Similarly, an increase in solute concentration of the soil solution decreases its osmotic potential. However, soil solutions are rarely so concentrated that they inhibit transpiration from the leaves (see Boyer 1985). In seedlings and very young plants, the effects of transpiration are small because leaf surface area is small: water uptake and

transport to the shoots is determined primarily by the root pressure (see Marschner 1986).

1.4. Transport of Calcium

Unlike the majority of mineral elements, calcium is translocated in the xylem rather than the phloem. The concentrations of all solutes except calcium are several times greater in the phloem exudate than in the xylem exudate. The rate of uptake and transport in the cytoplasm is therefore severely restricted and it is the only mineral nutrient other than boron that exists mainly outside the cytoplasm in the apoplast. Calcium transport has been examined in stems of *Nicotiana* L. (Hocking 1980) and in the peduncles of lupins, *Lupinus albus* L., (Pate *et al.* 1974) and calcium is considered a xylem-mobile mineral with trace amounts, if any, found in the phloem. Radiotracer studies have shown that calcium does move into phloem tissue but it is not translocated (Biddulph *et al.* 1959) and thus only negligible amounts are ever present in phloem exudates. Calcium phosphate is precipitated inside the sieve elements in the presence of high phosphate levels and a high phloem sap pH of 7.5 to 8.5. Most sieve elements have a filamentous proteinaceous content referred to as a p-protein, the appearance of which coincides with the onset of translocation within the phloem. It has been suggested that contraction and oscillation of these filaments propel the fluid of the sieve tubes (MacRobbie 1971). The p-proteins have been shown to be sensitive to calcium similar to actin-like structural proteins (Kleinig *et al.* 1971). Actin is known to be sensitive to calcium concentrations (Williamson 1975) which may be another reason for calcium exclusion from sieve elements.

Calcium is therefore limited to translocation in the xylem and its movement is a function of both mass flow of water and ion exchange reactions on the walls of tracheary elements. Bell and Biddulph (1963) postulated that calcium ions on entry to the xylem were adsorbed onto negatively- charged sites lining the inner walls of the vessels. Upward movement through the vessel would occur as a transfer of calcium from one exchange site to the other. The rate of calcium ascent would depend on the degree of transpirational tension inside the vessels. Increasing transpirational pull would result in faster water flow through the xylem and a faster translocation of calcium, presumably because the ions would have travelled farther before being readsorbed at another exchange site (Emmert 1969). The mobility of calcium is also promoted by other divalent cations that compete with calcium for adsorption on the exchange sites. The higher the concentration of these cations, the faster the movement of calcium through the xylem (Millikan and Hanger 1966). Also, neutralization of the calcium ionic charge by chelation with malic or citric acids allows freer movement of calcium through the plant (Millikan and Hanger 1965).

1.5. Function of Calcium

Calcium is a relatively large divalent cation with a hydrated ionic radius of 0.412 nm. It readily enters the apoplast and is bound in an exchangeable form to cell walls and the exterior surface of the plasma membrane (see Marschner 1986). Most of its activity is related to providing stable but reversible intermolecular linkages, predominantly in the cell walls and at the plasma membrane. Thus, a high proportion of total calcium in plant tissue is located in the cell walls in

contrast to other macronutrients. In the middle lamella, it is bound to carboxylic groups of polygalacturonic acid (pectin) where it may contain up to 50% of the total plant calcium (Armstrong and Kirkby 1979). In both the middle lamella and plasma membrane, calcium regulates membrane permeability and strengthens cell walls. In leaves receiving a high level of calcium during growth, a large proportion of pectic material exists as calcium pectate. This makes the tissue firm and highly resistant to degradation by polygalacturonase (Cassells and Boulass 1976). The proportion of calcium pectate in the cell walls is also of importance for the ripening of fruits. Rigney and Wills (1981) showed that during tomato fruit development, the calcium content of the cell walls increases up to the fully-grown green stage, and subsequently drops just before the onset of ripening and softening of the tissue. Simultaneously, a shift in the binding stage of calcium occurs in which water- soluble calcium is favored over wall-bound calcium. Comparisons between normally-ripening tomatoes and the nonripening *rin* mutant shows the importance of calcium for fruit firmness and its solubilization for fruit ripening. *rin* mutants show an increase of bound calcium during fruit maturation, whereas in other cultivars the content of bound calcium declines. This decline is associated with an increase in polygalacturonase activity (Poovaiah 1979).

Calcium stabilizes cell membranes by bridging phosphate and carboxylate groups of phospholipids and proteins at membrane surfaces (Legge *et al.* 1982). As a divalent cation, it reacts with negatively charged phosphate groups of the phospholipids in membranes and stabilizes them. Calcium enables membranes to

function as barriers against uncontrolled permeation processes. Selective ion uptake at the plasmalemma is mediated by calcium (Epstein 1961). The fundamental role of calcium in membrane stability was demonstrated by van Goor (1968), who induced an increased leakage of low-molecular-weight solutes, mainly potassium ions, from cells of calcium-deficient tomato fruits. Potassium ions are antagonistic to the function of calcium because of their potential to replace calcium on its binding site if calcium concentrations are low, which would increase cell permeability (see Bangerth 1979). Increased respiration rate of the calcium-deficient tissue also results from low calcium as a consequence of leakage of respiratory substrates from vacuoles to the respiratory enzymes in the cytoplasm (Bangerth *et al.* 1972). This leakage due to calcium deficiency is similar to the characteristics of tissue senescence. Cut carnation flowers experience a 70% decline in ATP-dependent uptake of calcium into microsomal vesicles during post-harvest development. Paliyath and Thompson (1988) suggest that the inhibition of ATP-dependent calcium uptake into vesicles could be the result of lipid membrane changes that would allow calcium ion leakage into the cytoplasm and thereby facilitate senescence.

A particular disease condition of tomato, blossom end rot (BER), illustrates the importance of calcium movement and the consequence of its deficiency in the plant. As the name implies, the condition is characterized by the appearance of extensive, brownish-black lesions on the distal end of the developing fruit. Spurr (1959) described the cells of affected fruits as appearing to be in "an advanced state of disorganization": cells of the necrotic tissue collapse, the cytoplasm

coagulates, and the nuclei are of abnormal shape. Van Goor (1968) suggested that BER is a symptom of local calcium deficiency, which would increase ion permeability of cell membranes, and could account for tissue necrosis. BER is induced under conditions that affect the movement of calcium and/or water to the fruit (Armstrong and Kirkby 1979, Shaykewich *et al.* 1971, Ward 1973, Wiersum 1966). For example, application of nitrogen fertilizer may or may not cause BER, depending on the chemical form used. Application of ammonium during fruiting induces BER (Taylor and Smith 1957), because of induced resistance to water flux that reduces the calcium content of the fruit (Pill *et al.* 1978). Conversely, application of nitrate results in a higher organic acid content of the plant. Chelation of calcium ions by these acids increases both mobility and concentration of calcium in the xylem sap (see Hanger 1979).

Environmental effects and physiological processes also affect distribution and deficiency of calcium in the tomato. Gerard and Hipp (1968) reported that an increase in relative humidity reduced the incidence of BER in tomatoes, and that a reduction in leaf transpiration enhanced calcium movement into the fruit. Bradfield and Guttridge (1984) found that calcium intake into the tomato fruit was greater when nights were humid rather than dry and nutrient solution dilute rather than concentrated. Positive root pressure at night apparently promotes transport of calcium into tissues and organs that have restricted transpiration.

This study was motivated by the observation that the extent of xylem concentration varied within and among plant organs. In particular, xylem is reduced in the abscission zone of the pedicel. Because calcium, an essential plant

mineral, can be supplied to various plant parts only through the apoplast, the hypothesis of a potential restriction to apoplastic water flow could be tested by tracing the development of xylem within the flower and fruit, observing water flow patterns through intact plant segments and correlating this with the areas of calcium accumulation. Deficiencies of calcium classically arise in the fruit, in the form of BER. Previous work suggests that much of the water supplied to the fruit arrives via the phloem, especially during the early fast growth phase (Wolterbeek *et al.* 1987, Ho *et al.* 1987). Presumably, deficiency of calcium should arise at this time. The following study attempts to correlate the extent to which xylem pattern and transpiration rates influence calcium concentration, specifically within fruit ranging in age from time of pollination to maturity.

Chapter 2

Materials and Methods

Tomato plants, *Lycopersicon esculentum* Mill. "Tiny Tim" were grown in a 3:1 mixture of peat based potting soil and vermiculite at pH 6. The plants were maintained at an average of 65% relative humidity under fluorescent lamps (Sylvania, Incandescent fluorescent 30W, 80 μE s^{-1} m^{-2}) with daily 16 h light 8 h dark periods. They were watered daily as required and fertilized with Hoagland's solution (Appendix A) once every four days.

2.1. Establishment of Xylem Pattern

Floral primordia and inflorescences of various ages were excised from tomato plants. These were boiled in 95% ethanol to remove photosynthetic pigments, rehydrated, then cleared by autoclaving (15 min. liquid cycle) in 10% KOH. Lignin and cell wall material were stained by autoclaving (15 min. liquid cycle) in a 1:1:1 solution of glycerol, 85% lactic acid, and 0.1% chlorazol black E (Allied Chemical Co. New York, N.Y., C.I. No. 30235, Lot 140). This preparation is a modification of Brundrett *et al.* (1984). Pedicels were longitudinally bisected to discern the xylem pattern and whole fruits were sliced longitudinally into 3 mm thick disks. Flower buds were stained without dissection. The plant parts were mounted in a 1:1 ratio of glycerol and water and observed with light microscopy.

Morphometric analysis was used to estimate xylem surface area relative to total surface area (Toth 1982). Measurements were made from photographs of cleared and stained (as mentioned) sepals and fruit disks.

The size at which each floral primordium first exhibited xylem vessels was noted. Flower size was estimated by a measurement of length from pedicel attachment point to the sepal tips, and combined with a diameter measurement of the flower across the middle of the ovary. Measurements were taken from enlarged photographs of flower buds and fruit. The pattern and distribution of xylem were studied from the primordial stage through to the development of mature fruit. The presence or absence of a pedicel abscission zone was correlated with the size of the flower. Leaf abscission zones were also cleared and stained in the above manner for use as a comparison with pedicel abscission zones. Structural differences of abscission zones of the leaf and the fruit would suggest possible functional differences between the two. Vessel member lengths and diameters within the abscission zone and the proximal and distal portions of the pedicel were measured from photographs of the tissue.

Pedicels for estimation of the xylem tissue cross-sectional area were harvested on a weekly basis from two days following anthesis to maturity at eight weeks. A single pedicel was sectioned for each week. Tissues were dehydrated in an ethanol-butanol series and embedded in paraffin wax for sectioning (Jensen 1962).

Serial microtome cross-sections of 40 μm thickness were made from the

proximal to the distal end of each pedicel. These were stained with phloroglucinol and HCl, mounted in 1% CMC10, (BDH Chemicals Ltd. Poole, England) a non-resinous aqueous mountant with a refractive index of 1.38, prior to photomicroscopy. The photographed sections were projected onto a digitizing apparatus and cross-sectional areas of total pedicel and xylem were measured in μm^2.

2.2. Distribution of Fluorescent Tracer

The alkaloid berberine is an apoplastically mobile dye (Strugger 1938) that does not flow through the symplast if the tissues are left intact. The dye used in these experiments was berberine hemi-sulphate (Sigma, No. B-3376, Lot 15F-0259) which fluoresces bright yellow under UV epifluorescence. The dye is carried along the transpiration stream and was used to show the proportion of xylem that functioned as water-conducting tissue (Dixon and Peterson 1989).

Plant stem portions with attached trusses were cut and their ends immersed into 0.03% berberine hemi-sulphate in 0.05 M phosphate buffer at pH 6. They were then left under lamps (Sylvania, Incandescent fluorescent 30W, 80 $\mu E\ s^{-1}\ m^{-2}$) in an environment of 20.5 °C and 65% relative humidity for 3 h respectively. Diameter of the fruit was measured and fruit color was noted before immersion of stems in dye to judge its ripeness and developmental stage. Hand cross-sections were made of the proximal, middle, and distal portion of the fruit and immediately photographed to observe the position of the dye and extent of impregnation along the fruit xylem vasculature. Photographs were also taken of the pedicel and sepal hand sections. The peduncles of fruit with their sepals

removed were also immersed in dye to determine the effect of sepal removal on the transpiration stream and dye pathway. The cut ends remaining after sepal excision were coated with petroleum jelly to prevent water loss through these ends. A fluorescence standard was made using a haemocytometer with a dye concentration of 0.03% and solution depth of 0.1 mm. The measured fluorescence at 6.3x was 20.6. All relative fluorescence values were measured at the same magnification and dye concentration as the standard. Observations were made with a Zeiss Photoscope III using a Zeiss filter 487718 with maximum transmission between 395-425 nm. The photoscope was equipped with UV epifluorescence and an attached Zeiss PM1 photometer head for the quantitative fluorescence comparisons. Fluorescence values of the stained, wet mounted tissue are represented as "relative fluorescence units".

2.3. Distribution of ^{45}Ca

Tomato plant stems with intact inflorescences and leaves were excised and the stem ends immersed into Hoagland's Nutrient Solution containing 0.59 MBq of ^{45}Ca per 50 mls of nutrient solution. The form of calcium used was $CaCl_2$ and references to calcium are to the ion. These plant portions were maintained for four days at 21 °C and 65% relative humidity under fluorescent lamps (Sylvania, Incandescent fluorescent 30W, 80 $\mu E\ s^{-1}\ m^{-2}$) with a diurnal 16 h light and 8 h dark period. Leaves, petioles, pedicels, sepals, fruits, flowers, and flower buds were then excised and weighed separately. The fruit was cut into proximal and distal halves and each weighed. Fruit with a fresh weight of less than 0.01 g were left whole. These fruit are referred to as "whole" in further comparisons. The

leaf used for organ comparison on each stem was a mature, fully-expanded leaf closest to the fruiting truss. In each case the petiole was that of the leaf used in the analysis. Flowers and flower buds were left intact and included sepals, petals, anthers, and ovary. Each portion was oven-dried at 60 °C for 4-5 days until no change in weight could be measured. The dried samples were then ashed at 650 °C for 36 h. An acid extract of the ash in 50% HCl was then oven-dried at 60 °C. The remaining residue was redissolved in 200 μl of water. Ten mls of liquid scintillation fluid was added to this solution and then scintillation counted between 200 and 750 keV. Plant portions prepared in the above manner but without ^{45}Ca labeling were used as control samples. Scintillation counts were converted to disintegrations per minute and analysis was based on these values. Conversions from dpm to μmoles calcium were as follows:

- 1 dpm = 60 dps
- 1 dps = 1 Bq
- 1480 Bq = 1 μg Ca
- 1 μg Ca/40.08 = 1 μmol Ca

The resulting μmol Ca represents the amount of labeled calcium added to the nutrient solution. The nutrient solution contained 125 μmol unlabeled calcium. The total amount of calcium in the beginning of the experiment represented the sum of the labeled and unlabeled calcium. Calcium was assumed to be absorbed from the solution at a constant ratio of labeled to unlabeled, so the total amount of calcium in each plant part is the product of μmol labeled Ca times the ratio of labeled to total calcium at the beginning of the experiment.

Two measurements of the largest fruit diameter were made at right angles and the volume of each fruit was estimated on the assumption that the fruit is essentially a sphere (Ehret and Ho 1986a). Volumes were estimated before and after the four day experimental period and the increases of volume were calculated as the differences between their volumes during this time. Relative increase of volume is the difference in volume over the four day period divided by the original volume.

Fruit of various ages were harvested, weighed, and their diameters measured. They were maintained at 20°C and 65% relative humidity for an hour and weighed at the end of that time. The difference in weight was equal to the transpiration rate of each fruit. Water intake by the fruit is equal to growth of the fruit plus the amount of water transpired. To estimate total water intake, the units of growth measured over four days, and the units of transpiration measured in hours were standardized by multiplying transpiration per hour by 96. The amount of transpiration in 96 hours by a fruit of a particular volume was added to the amount of growth in four days of a fruit of the same volume. The epidermal surface of the fruit, sepals, and leaves was examined for the presence of stomata.

An analysis of variance of the calcium content of the plant part groups was used to confirm the hypothesis of unequal means among leaves, sepals and fruit of different ages. Significant differences in the calcium content of the remaining plant part groups were shown with a Newman-Keuls multiple range test. Linear regression illustrated the relationships among calcium content, relative growth,

transpiration, and fruit volume. Tests were done using SpssX and Minitab and graphs were drawn with SpssGraphics.

Chapter 3

Results

3.1. Establishment of Xylem Pattern

Floral buds of various ages were examined to follow the stages of xylem development. Inflorescences, at an early stage of development (with a total length of 0.4 mm), showed no trace of xylem vessels but the young leaves (1 mm in length) surrounding the inflorescences were supplied by continuous and connected strands of xylem (Figure 1, A). The elements developed first in the proximal portion of the pedicel and advanced as single strands acropetally toward the sepal tips of the bud (Figure 1, B). Tracheary elements appeared in sepals of buds that were 0.3 mm long and were often present singly, some were non-continuous (Figure 2, A). Continuous strands of vessels running through the pedicel, sepals, and petals and which were connected to the main floral axis, were first seen in buds 0.6 mm long (Figure 2, B). The abscission zone was evident in buds 0.8 mm long. The vessel elements that first developed within the pedicel were short relative to other vessel elements of mature vegetative tissue and were twisted or crooked in shape (Figure 3, A). At bud maturity, longer vessel members, tracheids, and fibres developed alongside the crooked vessel members. At flower maturity and during fruit development, only two areas remained composed solely of short, crooked tracheary elements: the pedicel abscission zone and the xylem

of the fruit core leading to the ovules. Stubby vessel members in the pedicel, bordered on the proximal and distal sides by elongated vessel members, were used to identify the pedicel abscission zone. The mean length of these stubby vessel members at the abscission zone was 0.029 mm with a range from 0.009 mm to 0.06 mm compared with the vessel members of the proximal and distal portions that averaged 0.4 mm. The mean width of the stubby vessel members was 0.012 mm and did not differ in width from the vessel members proximal and distal to the abscission zone. The short vessel members were present for the duration of fruit development, forming an irregular ring of strands separated by parenchyma cells. Hence, the abscission zone had less xylem relative to the portions of the pedicel proximal and distal to it (Figure 3, B). This reduction in xylem at the abscission zone was a consistent feature of all pedicels sectioned and stained with phloroglucinol and HCl from two days after anthesis to fruit maturity (Figure 4). The pedicel sectioned two days after anthesis was designated as week 0 in Figure 4. The values for the proportion of xylem to total pedicel cross-sectional areas for the proximal and distal portions of each pedicel were chosen from sections approximately 0.5 mm from the abscission zone. The proportion of xylem was greatest in the proximal segments with a mean of 50% for all pedicels, and somewhat less for the distal segments with a mean of 40%. Corresponding values for xylem at the abscission zone for the same pedicels ranged from 4% to 13%.

Xylem tissues of the leaf abscission zone and the zone of attachment of the pedicel with the fruit were composed primarily of fibres and tracheids. Xylem of the pedicel extended to the point of attachment of the sepals and fruit where the

Figure 1: A. Floral primordia. No xylem vessels apparent. B. Entire inflorescence. Tracheary elements advancing acropetally toward flower bud tips. Stained with 0.1% chlorazol black E.

A = 116x B = 116x

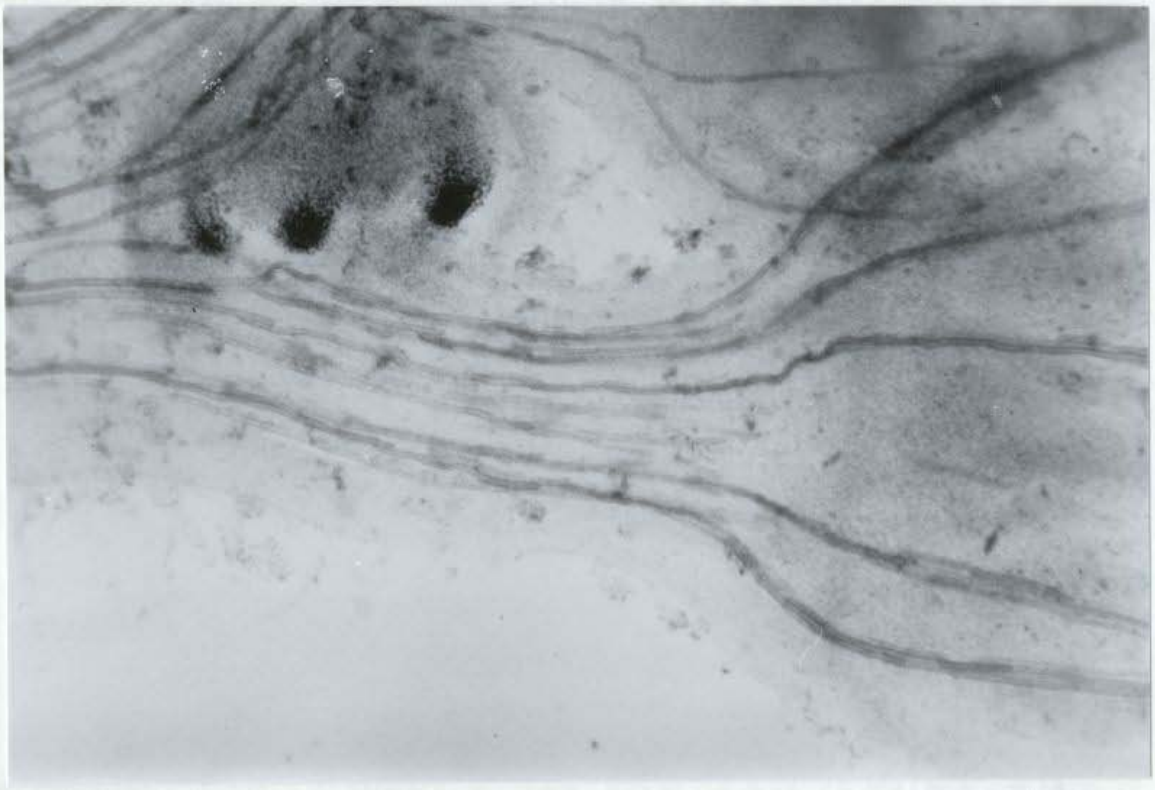
A

B

Figure 2: A. Entire inflorescence with tracheary elements reaching the tip of the largest bud. B. Xylem tissue of the pedicel and sepals of a bud. No abscission zone is evident. Stained with 0.1% chlorazol black E.

A = 116x B = 116x

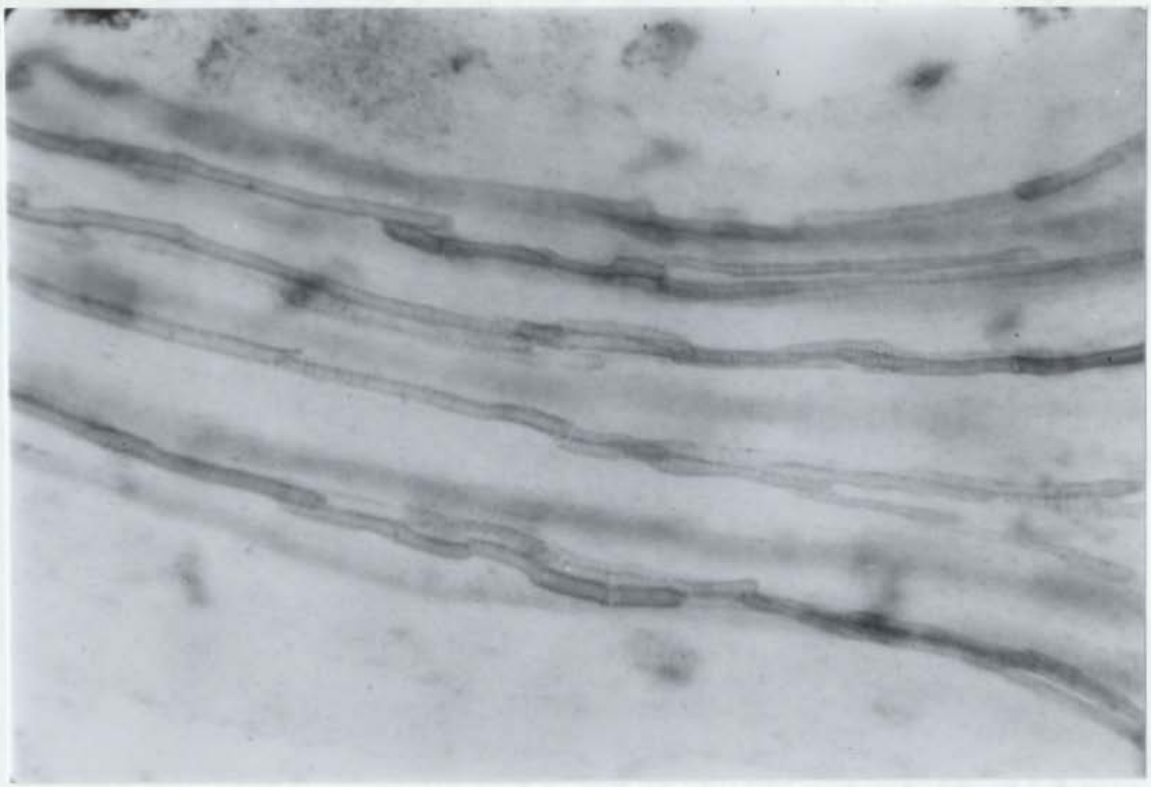
A



B

Figure 3: A. Enlargement of tracheary elements through the pedicel of a bud. Elements are relatively short, but no abscission zone is evident. B. Whole pedicel illustrating decreased xylem at the abscission zone. Stained with 0.1% chlorazol black E.

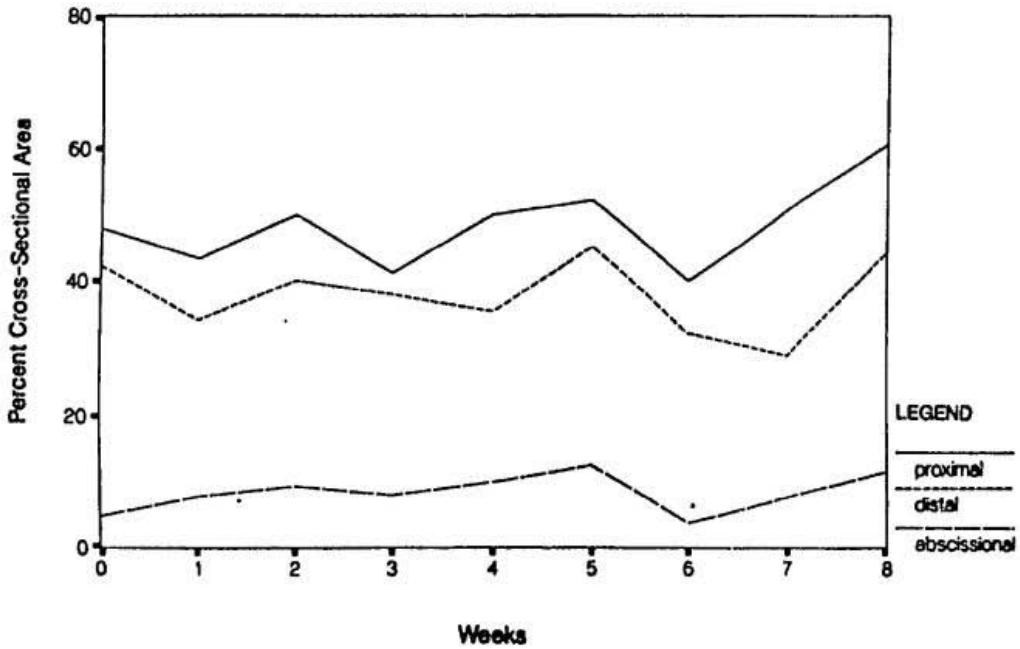
A = 290x B = 18.75x



A

B

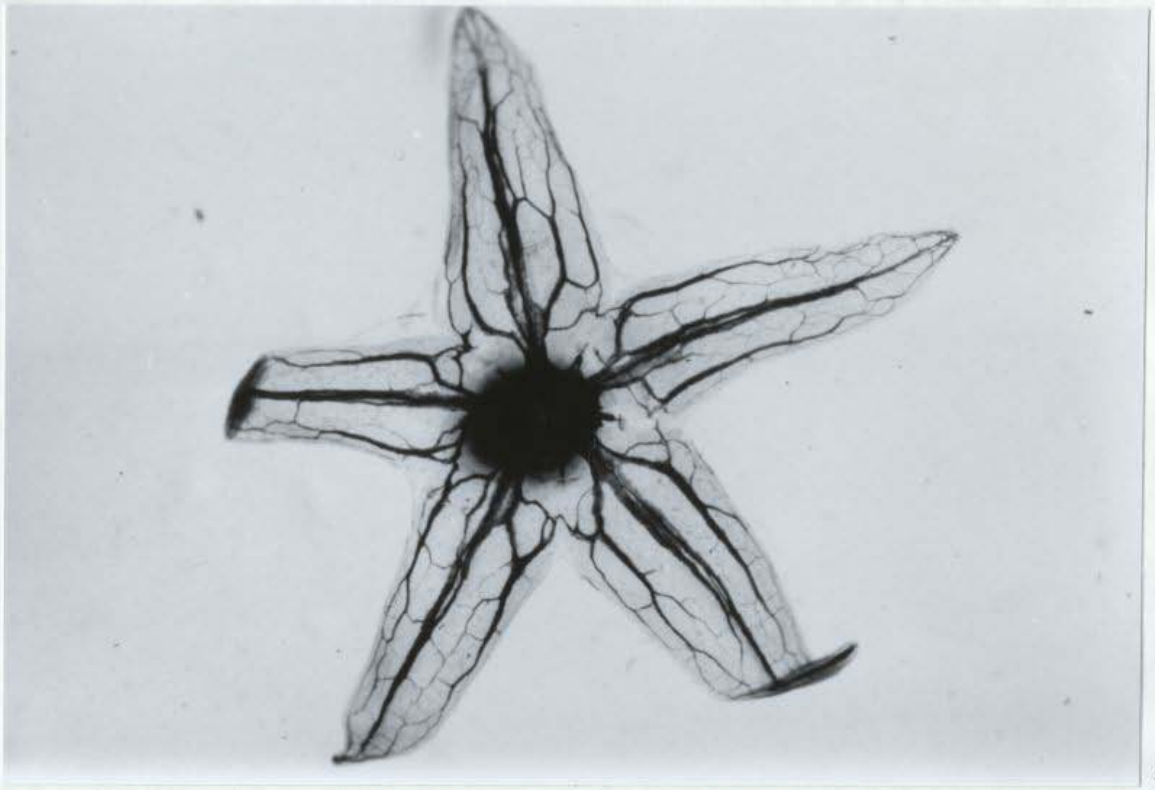
Figure 4: Percent of xylem relative to total cross-sectional area in the proximal, distal, and abscissional segments of pedicels aged two days after anthesis (0 weeks) to eight weeks.


Percent Cross-Sectional Area
80
60
40
20
0
0
1
2
3
4
5
6
7
8
Weeks
LEGEND
proximal
distal
abscissional

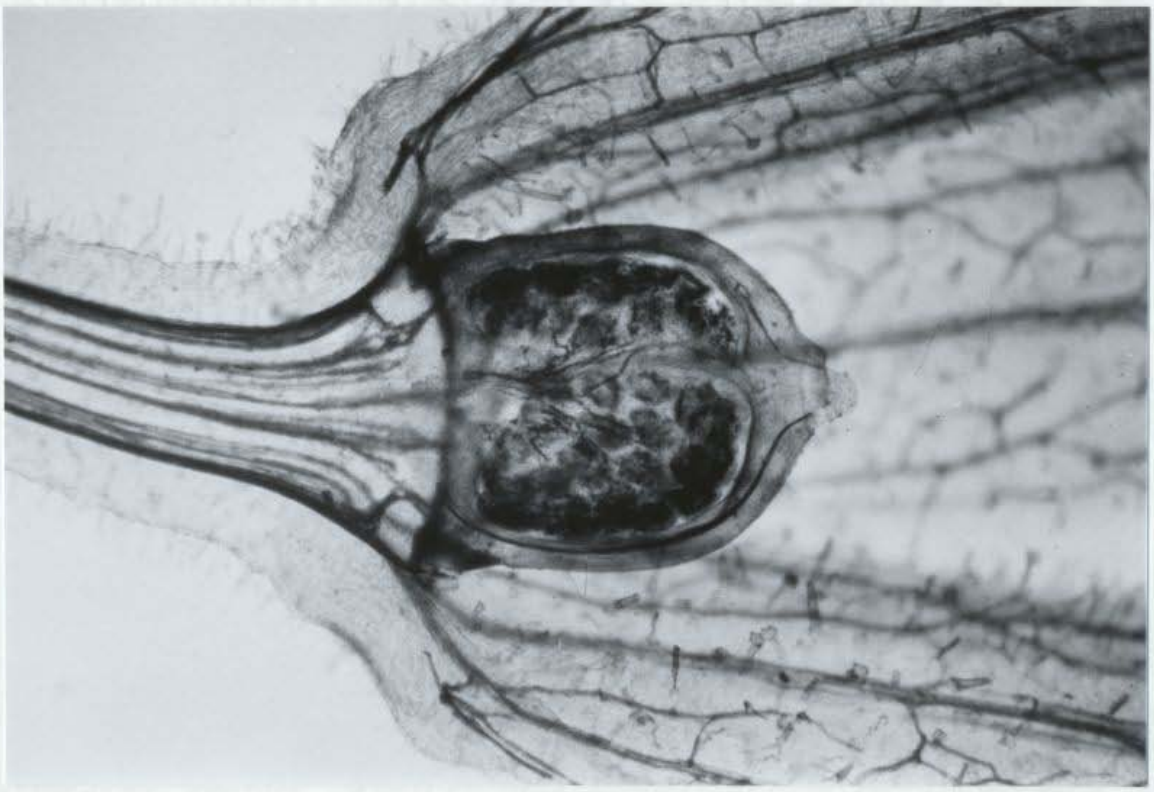
majority of it extended into the sepals to form a continuous net pattern with venation similar to that of a leaf (Figure 5, A). A main mid-vein formed and vessel elements branched laterally to the sepal edge to connect with strands of xylem running along the sepal periphery. The venation was complete, with no blind ends. Approximately 42% of the surface area of the cleared and stained sepals was xylem tissue. The xylem vasculature of the ovary, and later the fruit, was less extensive relative to organ size. Small fruit (< 5 mm diameter) contained approximately 26% xylem tissue per fruit disk surface area. Xylem tissue comprised approximately 14% of the surface area of cleared and stained disks of larger fruit (> 10 mm diameter). Tracheary elements extended from the circle of xylem in the receptacle tissue at the fruit's proximal point of attachment. The majority of it passed through pedicel tissue directly to the ovules. Remaining branches of the xylem spread along the fruit wall in the proximal fruit half and then branched further into the distal half. Typically, the fruit wall contained three to five arms of xylem that reached from the proximal to the distal end of the fruit within the fruit wall. Here they converged as two bundles into the style. After fertilization, the ovary wall began to swell and the style excised, leaving broken ends of xylem at the distal end of the fruit (Figure 5, B). Generally, the xylem of the fruit wall was continuous with no blind ends except for these two broken bundles. The distal portion of the fruit appeared to be poorly supplied with xylem relative to the proximal half primarily because the majority of the xylem vessels of the fruit lay within the placental tissue where they branched directly to the ovules (Figure 6, A). The xylem of the placenta was composed solely of short and crooked vessel members (Figure 6, B).

Figure 5: A. Xylem pattern of the sepals. B. Longitudinal median section of pedicel, sepals, and fruit illustrating xylem at the distal portion of the fruit. Age- one week after fertilization. Stained with 0.1% chlorazol black E.

A = 10x B = 75x



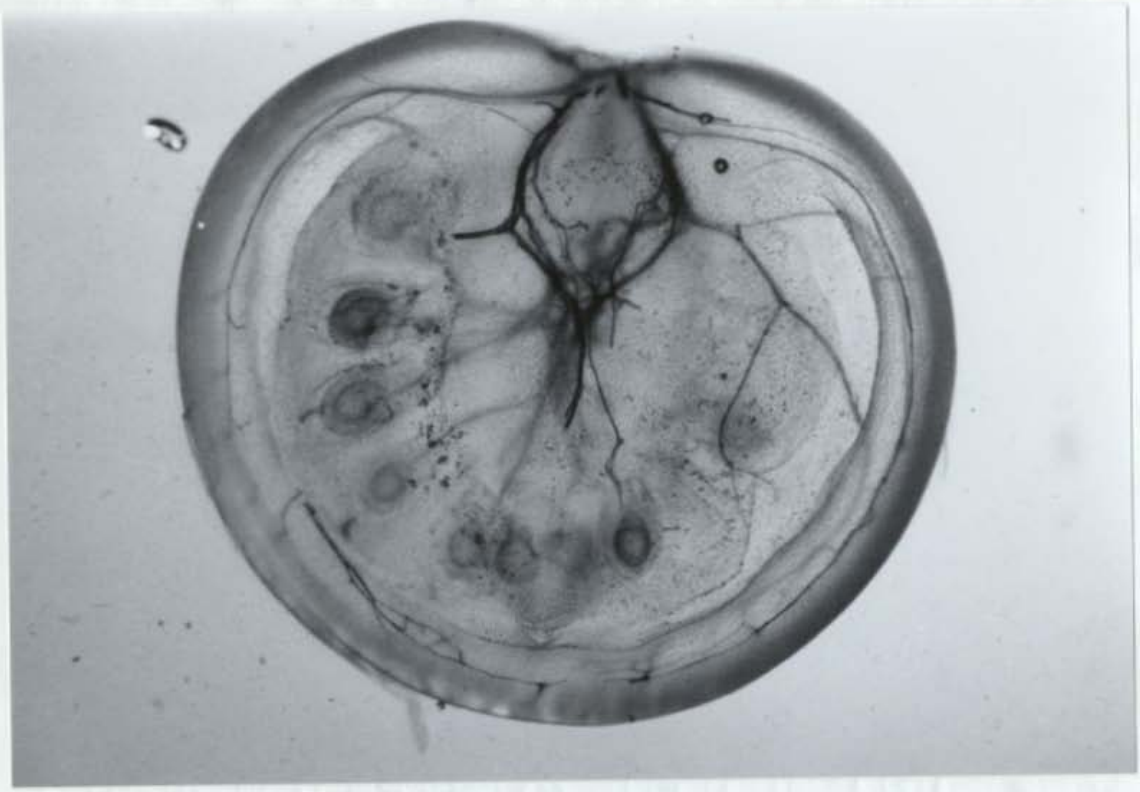
A



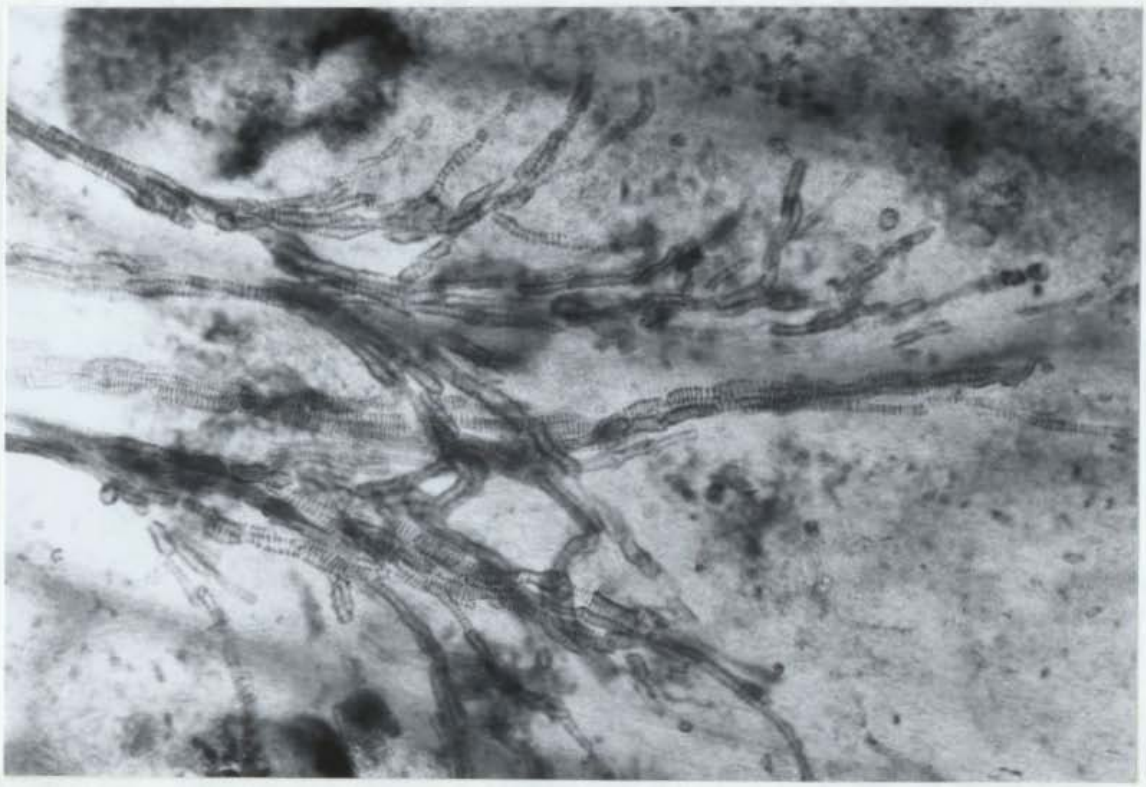
B

Figure 6: A. Median section of fruit illustrating the concentration of xylem in the placental tissue. B. Tracheary elements of the placenta leading to the ovules. Stained with 0.1% chlorazol black E.

A = 5.6x B = 116x



A



B

3.2. Distribution of Fluorescent Tracer

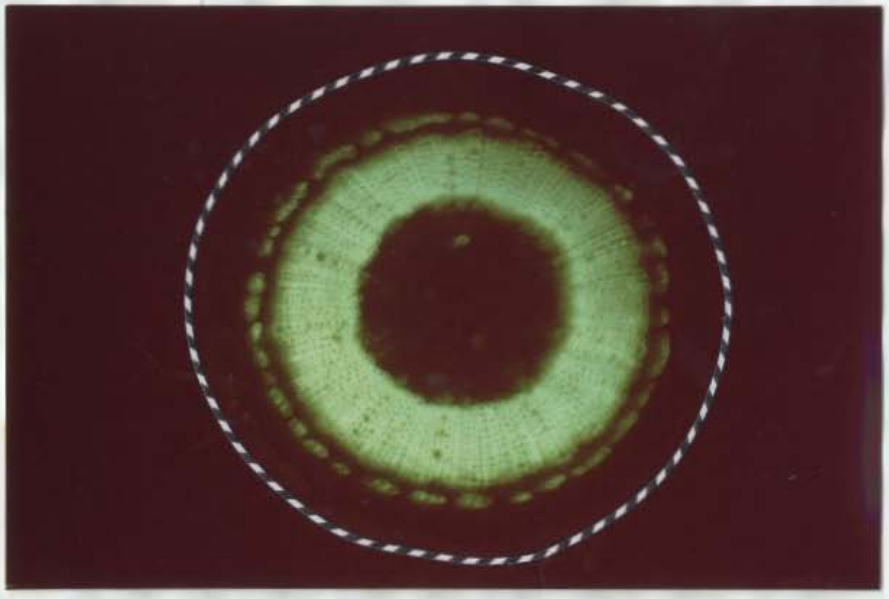
The pedicel xylem was heavily stained with berberine hemi-sulphate and lateral leakage of the dye resulted in all cells containing lignin to be stained. Figure 7 illustrates the extent of dye passage along the pedicel and the reduction of xylem at the abscission zone. At the point where the sepals attach to the pedicel, the dye travelled into both the sepals and the fruit (Figure 8). The proportion of berberine hemi-sulphate that entered the fruit concentrated in the placental tissue of the proximal half of the fruit (Figure 8, B). The mean fluorescence per unit surface area of the proximal fruit half was 28.5, whereas the mean of the distal fruit half was 13.9 (relative fluorescence units). The distal fruit half contained less relative fluorescence per unit surface area than the proximal fruit half. Spots of dye were rarely seen in the fruit wall. No dye was observed in the far blossom end of any fruit. This pattern of berberine distribution was consistent in all small and large green fruit, but mature ripe fruit showed no dye uptake. Removal of the sepals from around the fruit caused a significantly greater amount of dye to travel into the fruit (mean relative fluorescence of 63.6) compared with fruit with their sepals intact (mean relative fluorescence of 42.6), ($F=16.61$, $p=0.015$).

3.3. Distribution of ^{45}Ca

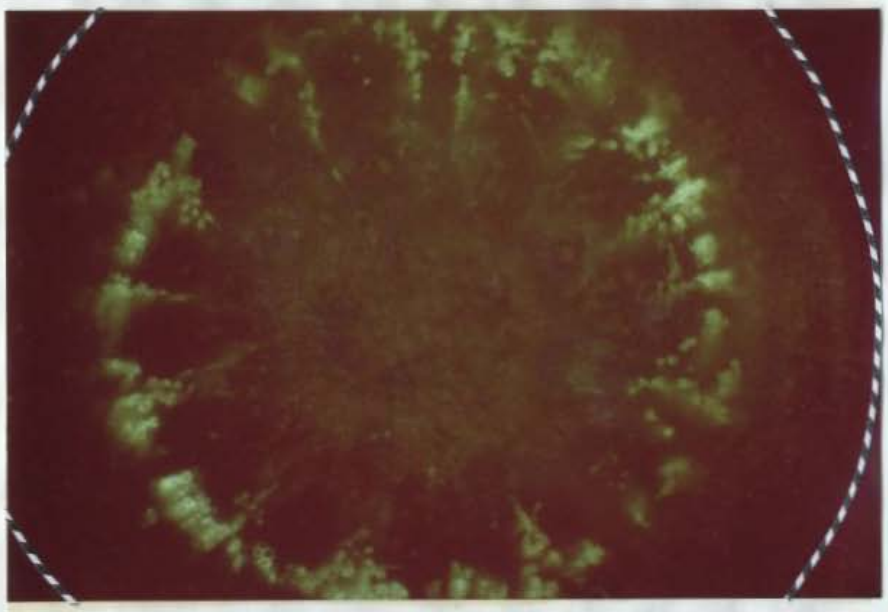
Fruit used in the following analyses were assigned to one of two classes: those that weighed 0.05 g (30 mm^3) or less (hereinafter referred to as "very small fruit") and those that weighed more than 0.05 g.

Figure 7: Proximal (A) abscissional (B) and distal (C) pedicel cross-sections illustrating decreased xylem at the abscission zone. Stained with 0.03% berberine hemi-sulphate.

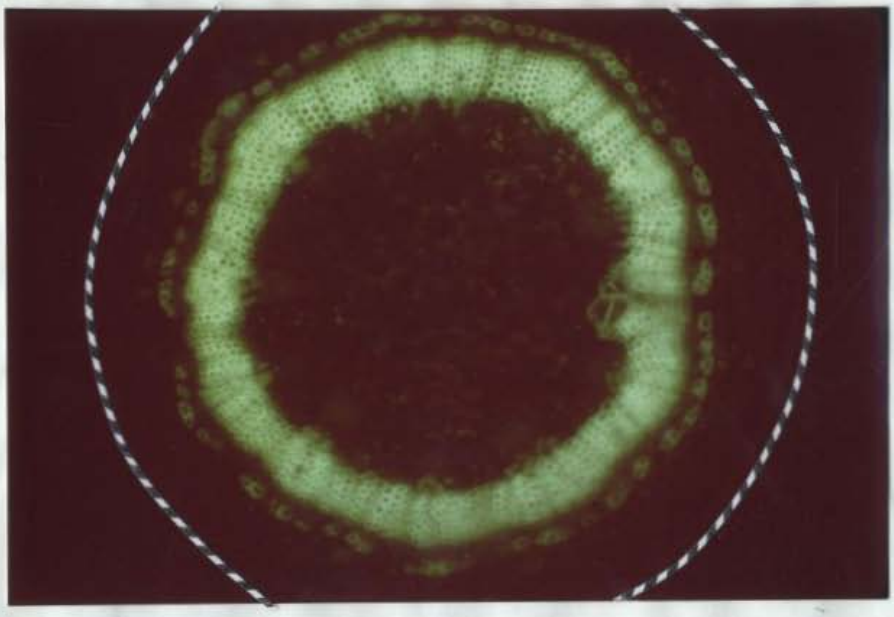
A = 73x B = 73x C = 73x



A



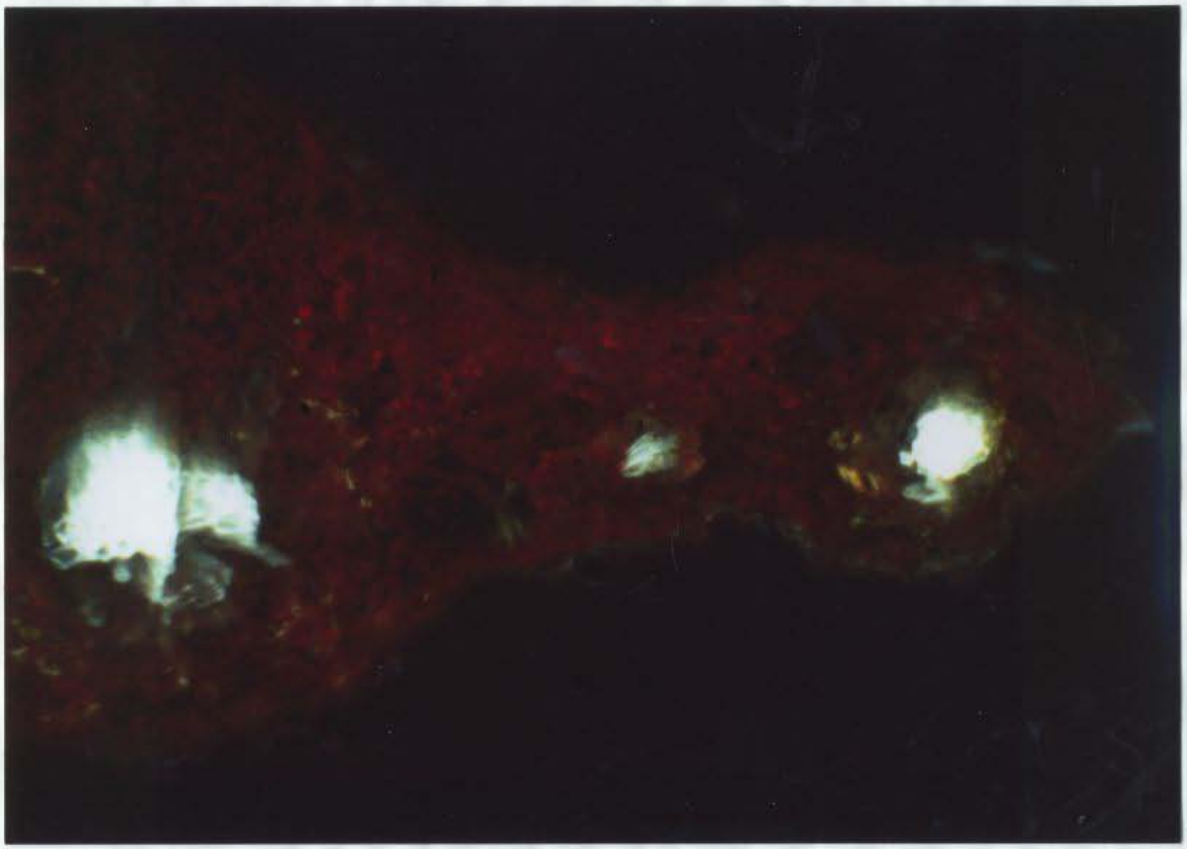
B



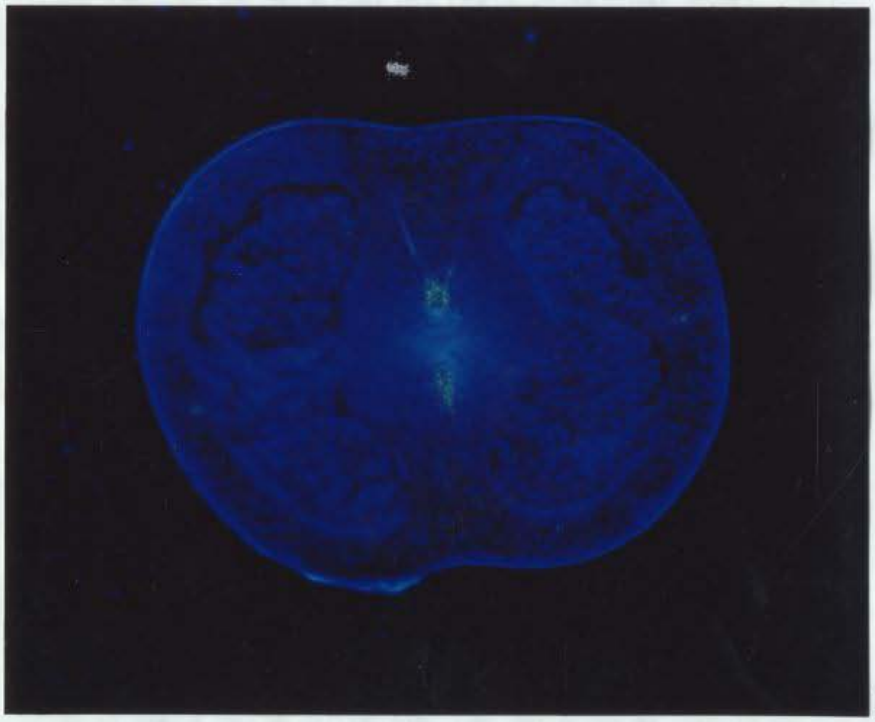
C

Figure 8: A. Cross-section of sepal illustrating xylem vessels.
B. Cross-section of the proximal end of the fruit. Yellow spots indicate berberine-stained xylem. Stained with 0.03% berberine hemi-sulphate.

A = 100x B = 5.6x



A



B

The leaves (n=13), inflorescences (n=15), and fruit (n=54) on tomato plant stems differentially accumulated calcium (Table 1). The radioactivity counted in the leaves, (the greatest accumulators), was 508 times greater than the distal half of fruit weighing more than 0.05 g fresh weight. Whole fruit weighing less than 0.01 g (n=8), accumulated significantly more calcium per fresh weight ($4.2x10^6$) than did medium and larger fruit ranging from 0.5 g-7.5 g ($2.3x10^5$, n=46), ($F=68.58$, $p<0.001$).

Analysis of variance of the nine plant part groups showed a significant difference in calcium accumulation between leaves and all other tissues ($F=7.15$, $p<0.001$). Fruit greater than 0.5 g accumulated the least calcium ($F=37.19$, $p<0.001$); other maximum nonsignificant ranges are indicated in Table 1. Proximal halves of fruit always contained more calcium than distal halves, but the difference was not significant ($F=4.05$, $p=0.06$).

Calcium uptake into the fruit was dependant on the total water intake by the fruit (increase of volume plus transpirational loss) over the four day experimental period (Figure 9). Regression analysis on n=30 fruit gave the following equation:

$$Y = -4.74x10^3 + 3.29x10^2(X)$$

Y = radioactivity of calcium and X = total water intake

The regression coefficient is r = 0.89

Table 1: Mean ^{45}Ca dpm values of plant parts.

Plant Parts	Mean Calcium(dpm) ($X \pm 1$ s.e.m.(n))	Maximum Nonsignificant Ranges
Leaf	$3.2x10^7 \pm 1.7x10^7$ (13)	A
Sepal	$7.5x10^6 \pm 6.7x10^5$ (54)	B
Flower Bud	$7.4x10^6 \pm 1.8x10^6$ (11)	B
Flower	$7.4x10^6 \pm 1.3x10^6$ (4)	B
WholeFruit<0.01g	$4.2x10^6 \pm 1.1x10^6$ (8)	C
Pedicel	$3.1x10^6 \pm 1.5x10^5$ (54)	C
Petiole	$3.1x10^6 \pm 3.6x10^5$ (4)	C
Proximal Fruit Half	$1.6x10^5 \pm 5.8x10^4$ (46)	D
Distal Fruit Half	$6.3x10^4 \pm 2.5x10^4$ (46)	D

Fruit with a large volume increase accumulated significantly more calcium over the experimental period ($F=113.0$, $p<0.001$).

Calcium accumulation compared to the fruit's increase in volume was constant for all fruit greater than 30 mm^3 in volume and 0.05 g in fresh weight. However, fruit smaller than 30 mm^3 or 0.05 g accumulated, on average, 7.5 times the calcium per increase in volume than did fruit larger than 30 mm^3. The mean calcium accumulation relative to growth for a very small fruit was $1.1x10^4$ dpm/volume increase compared to the constant value of $1.3x10^3$ dpm/volume change for fruit between 0.5 g and 7.5 g or 325 mm^3 and 6600 mm^3 respectively. Logarithmic transformation of the X axis variable allowed any variability of calcium content among very small fruit to be visually obvious. Figure 10 illustrates the difference between calcium accumulation relative to volume increase for fruit less than 30 mm^3 compared with fruit larger than 30 mm^3.

Transpirational losses per unit fruit surface area were also greatest for very small fruit (<30 mm^3) where cuticular conductance was 0.18 mg/h compared to the mean cuticular conductance of 0.002 mg/h for fruit ranging from 325 mm^3 to 6600 mm^3 (Figure 11). Fruit less than 30 mm^3 showed an exponential water loss per unit area that averaged 90 times more than larger fruit from 325 mm^3 to 6600 mm^3. Fruit between 30 mm^3 and 325 mm^3 showed an intermediate rate of water loss. The mean transpiration rate of 0.002 mg/h for the larger fruit represents a constant rate of water loss per unit surface area for all these fruit.

Over the four day experimental period, the increase of fruit volume relative

Figure 9: Calium (dpm) relative to total fruit water intake over the four day experimental period.

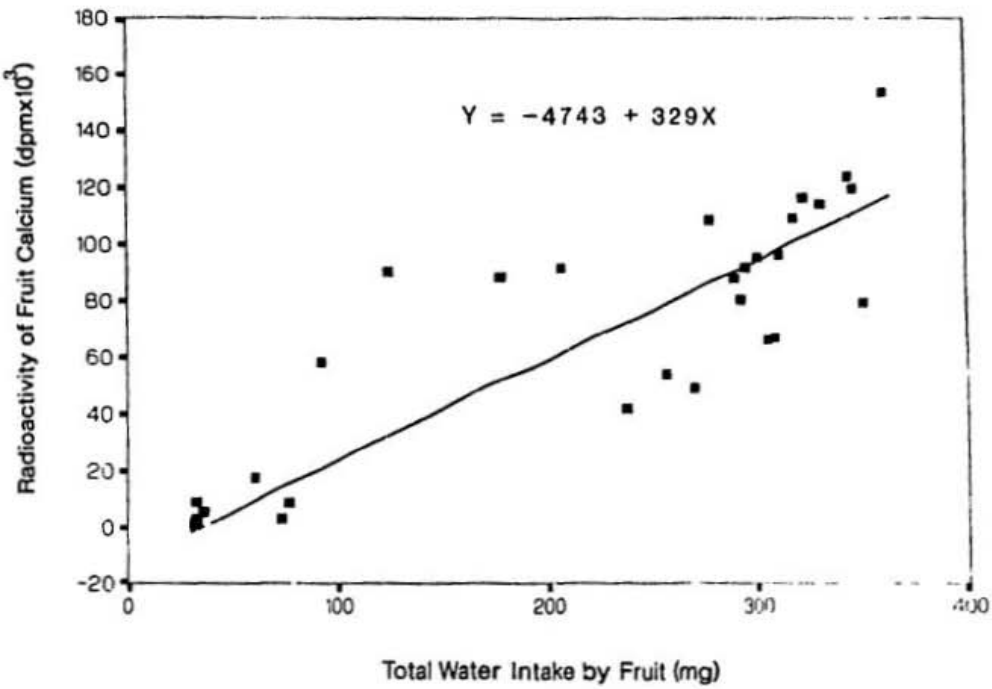

Y = −4743 + 329X
Radioactivity of Fruit Calcium (dpmx10³)
Total Water Intake by Fruit (mg)
180
160
140
120
100
80
60
40
20
0
-20
0
100
200
300
400

Figure 10: Calcium (dpm) relative to growth over the four day experimental period for all fruits tested.

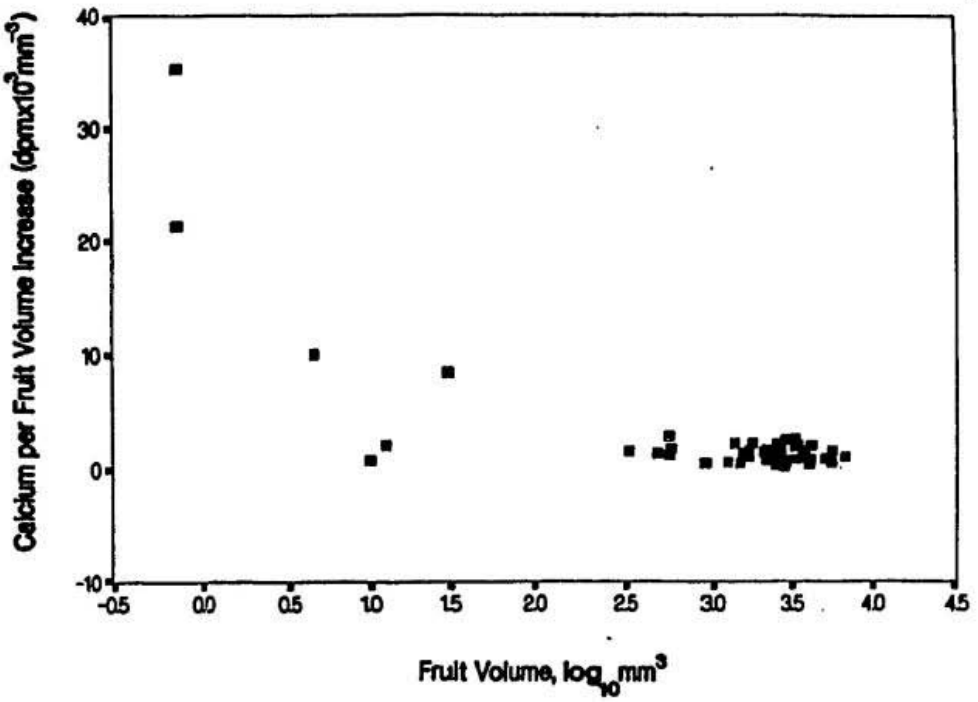
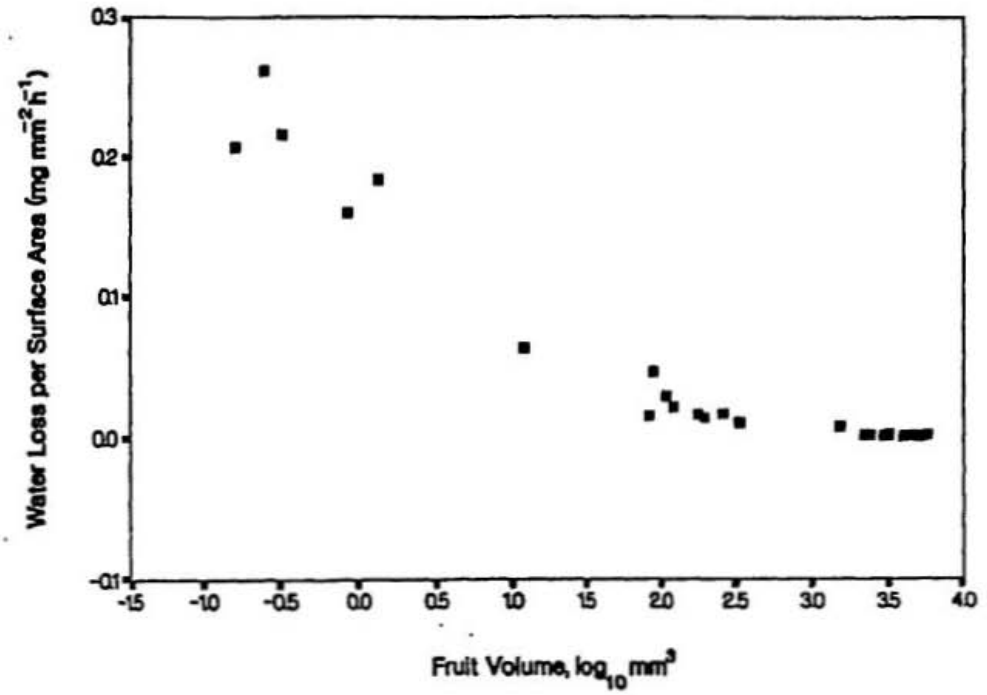

Calcium per Fruit Volume Increase (dpmx10³mm⁻³)
40
30
20
10
0
-10
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Fruit Volume, log10 mm³

Figure 11: Cuticular conductance of fruit relative to their surface area.


Water Loss per Surface Area (mg mm^{-2} h^{-1})
0.3
0.2
0.1
0.0
-0.1
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Fruit Volume, log$_{10}$ mm^3

to initial volume was greatest for fruit less than 30 mm^3 and was 7 times greater than medium and large sized fruit ranging from 325 mm^3 to 6600 mm^3. The mean relative growth increase of very small fruit (<30 mm^3) was $3.6x10^{-1}$ g over the experimental time, whereas the mean growth increase of fruit from 325 mm^3 to 6600 mm^3 was $5.0x10^{-2}$ g over the same period (Table 2). Logarithmic transformation of fruit volume illustrates that a constant slope exists for the relationship between relative increase of volume and fruit volume (n=54), (Figure 12):

$$Y = 3.96x10^{-1} - 1.0x10^{-1}\log_{10}X$$

Y = relative increase of volume and X = fruit volume

The regression coefficient is r = 0.98

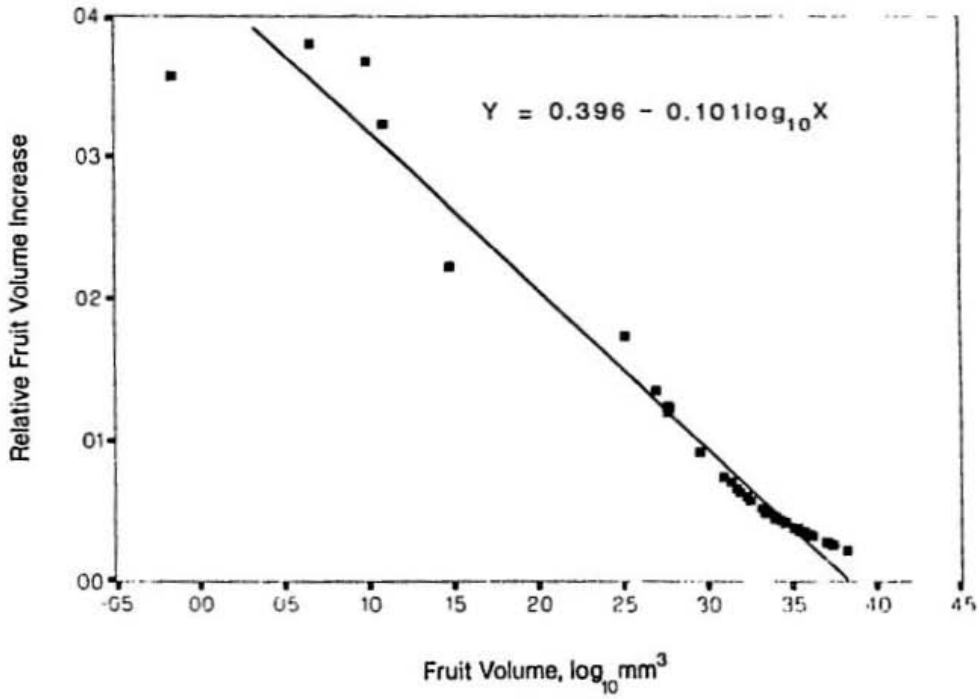
The total amount of water entering an expanding fruit equals the increase in volume plus transpirational losses. The amount of calcium in each fruit relative to its total water accumulation over the four day experimental period was independent of fruit volume. The ratio of calcium to water intake was consistent for all fruit sizes. Very small fruit, less than 30 mm^3, did accumulate less calcium per total water influx than larger fruit but this was not a significant difference (Table 2).

The growth and amount of calcium accumulated by a fruit was also dependent on its position on a truss. The first (most proximal or closest to the main plant stem) fruit on a truss, accumulated significantly more calcium than

Table 2: Size Classes of Tomato Fruit with Their Mean Transpiration Rate, Calcium Accumulation, and Relative Growth ($X \pm 1$ s.e.m. (n))

Fruit Size mm^3	Water Loss $mg\ mm^{-2}\ h^{-1}$	^{45}Ca/Volume Change $dpm\ mm^{-3}$	^{45}Ca/Water Intake $dpm\ mg^{-1}$	Volume Change mm^3
0.7-30	$1.8x10^{-1} \pm 2.7x10^{-2}(6)$	$1.1x10^{4} \pm 3.7x10^{3}(9)$	$1.8x10^{2} \pm 5.0x10^{1}(9)$	$3.6x10^{-1} \pm 5.0x10^{-3}(9)$
30-325	$2.1x10^{-2} \pm 3.2x10^{-3}(8)$	$5.0x10^{3} \pm 3.5x10^{3}(2)$	$4.9x10^{2} \pm 1.8x10^{2}(2)$	$2.0x10^{-1} \pm 2.5x10^{-2}(2)$
325-6600	$2.0x10^{-3} \pm 3.8x10^{-4}(16)$	$1.3x10^{3} \pm 1.0x10^{2}(43)$	$4.9x10^{2} \pm 1.5x10^{1}(43)$	$5.0x10^{-2} \pm 4.1x10^{-3}(43)$

Figure 12: Relative volume increase compared to initial fruit volume.


Y = 0.396 - 0.101log10X
Relative Fruit Volume Increase
Fruit Volume, log10mm3
04
03
02
01
00
-05
00
05
10
15
20
25
30
35
40
45

the most distal fruit of the same truss ($F=13.92$ $p=0.01$). The fruit in between these two extremes accumulated less calcium with each position away from the first fruit. The amount of calcium accumulated is related to the fruit position (Figure 13):

$$Y = 2.15 \times 10^5 - 2.70 \times 10^4 X$$

Y = radioactivity of calcium and X = truss position

The regression coefficient is $r = 0.81$

The above equation was for a single truss with eight fruit. Variability in dpm counts between trusses changed the value of the y-intercept, however, the linear relationship remained the same.

A similar relationship existed for the increase in volume over the experimental period and fruit position for the same truss of eight fruit (Figure 14):

$$Y = 1.61 \times 10^2 - 1.47 \times 10^1 X$$

Y = increase in volume and X = truss position

The regression coefficient is $r = 0.82$

The change in volume of the first fruit was significantly greater than all others of the truss and declined to the most distal fruit ($F=15.34$, $p=0.008$). However, as a proportion of growth relative to size at the beginning of the

Figure 13: Calcium content relative to fruit position along the truss. Fruit #1 is the most proximal.

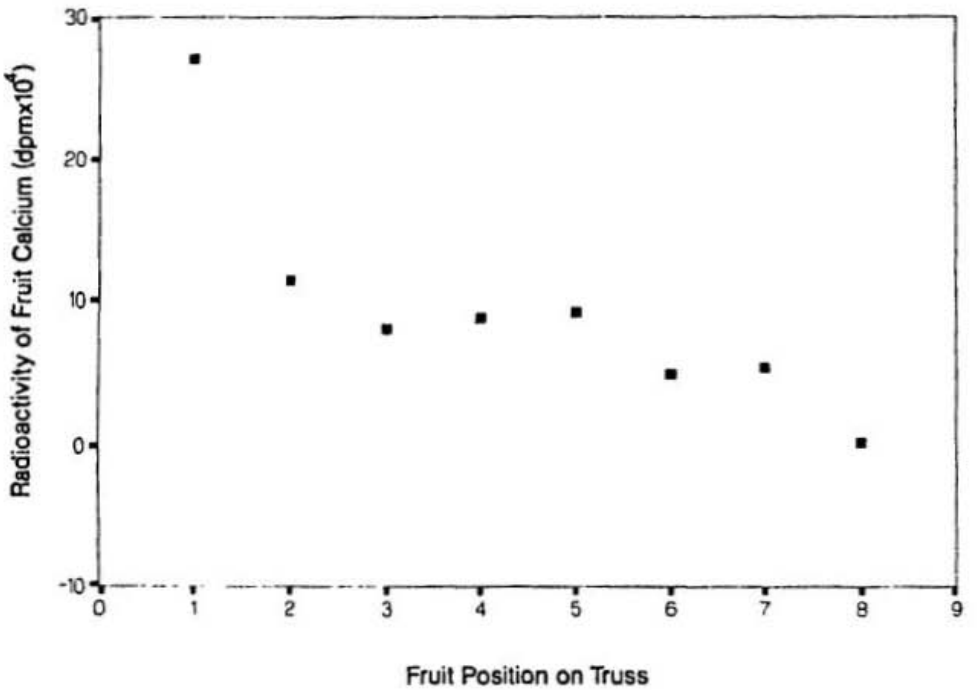
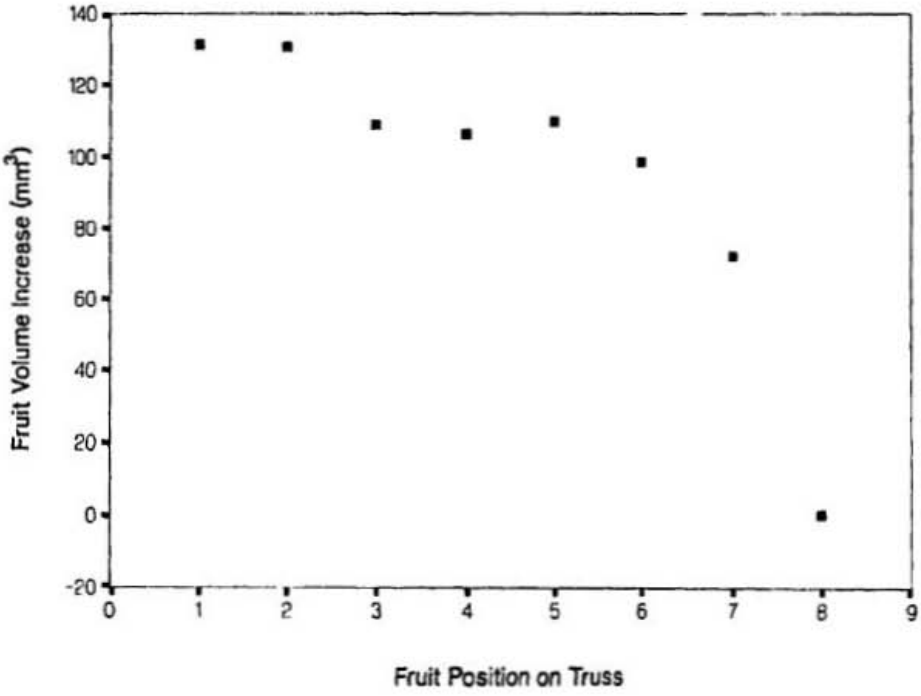

Radioactivity of Fruit Calcium (dpmx10⁴)
30
20
10
0
-10
0
1
2
3
4
5
6
7
8
9
Fruit Position on Truss

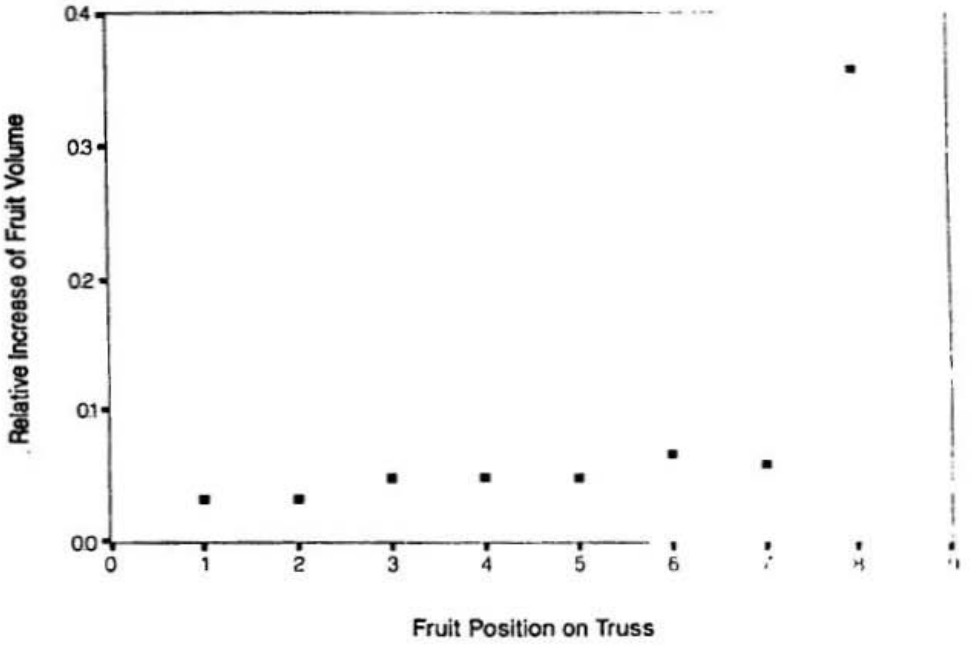
Figure 14: Increase of volume relative to fruit position along the truss. Fruit #1 is the most proximal.


Fruit Volume Increase (mm³)
140
120
100
80
60
40
20
0
-20
0
1
2
3
4
5
6
7
8
9
Fruit Position on Truss

experiment, the change in volume was relatively constant for all fruit of the truss larger than 30 mm^3 or 0.05 g. These very small fruit grew the fastest compared to others on the truss even though they were the most distal from the plant stem (Figure 15). The proportional growth of the remaining fruit was approximately equal, regardless of their truss position. Figure 15 illustrates the general trend of increase in volume relative to initial size for the different fruit along the truss. Data of the calcium content and volume increase of the remaining fruit of trusses are listed in Appendix E.

The fruit epidermal surface lacked stomata, but stomata were present on both surfaces of leaves and sepals. The abaxial surface of the leaves contained the highest mean stomatal density of $45/mm^2$ and an adaxial mean of $9/mm^2$. The mean number of stomata on the abaxial side of the sepals was $24/mm^2$ and $9/mm^2$ on the adaxial side. Pedicels contained a mean of $3/mm^2$ on their epidermal surfaces.

Figure 15: Relative growth of fruit compared to their position along the truss. Fruit #1 is the most proximal.


Relative Increase of Fruit Volume
04
03
02
01
00
0
1
2
3
4
5
6
Fruit Position on Truss

Chapter 4

Discussion

The development of xylem tissue within the primordia of the tomato inflorescence through to fruit maturity affects the mode of water and calcium supply to the pedicel, sepals, and fruit. The extent of vascularization within these organs plus effects of transpiration determine the sites which accumulate calcium.

The inflorescence of the tomato differentiates from the vegetative apex and develops in the manner detailed by Sawhney and Greyson (1972). At flower maturity, a complete ring of xylem vasculature extends through the pedicel to the receptacle, where it branches to the sepals, petals, anthers, and the ovary tissues. An abscission zone in the middle of the pedicel is indicated by a dramatic decline in xylem tissue cross-sectional area. The data presented here show that the abscission zone xylem is composed of short, irregular vessel members that persist for the duration of fruit development. Within the abscission zone, vessel members, tracheids, and fibers of the metaxylem and secondary xylem fail to develop as they do in the proximal and distal portions on either side of the abscission zone. Jensen and Valdovinos (1967) described the anatomy of the abscission zone of the tomato flower at anthesis and noted that vascular tissue is continuous through the zone, but did not describe the xylem tissue or its relative

amounts within the pedicel. Hudak (1987) noted that the xylem in the abscission zone was significantly less than the proximal and distal pedicel portions. The xylem pattern was comparable to that of the pedicel of the mango (Barnell 1939) in which the amount of xylem was decreased and the cylinder of vascular tissue was broken into an irregular ring of xylem strands at the abscission zone. Baird and Webster (1979) noted that xylem vessels are unusually small or absent from the abscission zones of most fruit and considered the zone as a region of abrupt structural transition. Aloni (1987) studied the irregular vascular attachment site between the xylem of a leaf and stem of the palm *Rhapis excelsa*. At the node between the leaf and plant stem, the vessel system of the leaf connects to the vessels of the stem via narrow tracheids. The tracheids in the node region presumably protect the vessels of the stem from cavitation when the leaf drops off. Lee (1989) proposed that the abscission zone in the tomato pedicel resists water flow through the xylem and transfers the demand for water by the fruit to the phloem, thus linking water and dry matter influx to the fruit.

Generally, the presence of a fluorescent dye such as berberine hemi-sulphate, can be detected in smaller quantities than can non-fluorescent dyes (O'Brien and McCully 1981), making any water passage through the xylem obvious. If transpirational tensions are high or suction is applied to the end of the stem, berberine hemi-sulphate flows with the water pathways and therefore will indicate functional water-conducting vessels and tracheids (Dixon and Peterson 1989). Generally, berberine stains lignin and suberin in plant tissues (Brundrett *et al.* 1988). However, the dye tends to bleed laterally through vessel elements and

stain cells in which the dye was not carried, making quantification of water-conducting vessels and tracheids impossible. In every section, xylem vessels and tracheids were stained plus any cells containing lignin (Figure 7). The technique of staining functional xylem is presently being refined (Peterson, pers. comm.). Berberine hemi-sulphate is preferable to a phloroglucinol and HCl stain because the latter stains for a variety of polysaccharides besides lignin, whereas the presence of berberine illustrates areas of water flow- the dye stains lignin wherever it travels with the water. Therefore, berberine staining is useful to show apoplastic continuity through the length of the pedicel.

The xylem tissue of the fruit concentrated within the placental region. These tracheary elements were stained with berberine because of water movement to the ovules. Even though xylem existed in the distal fruit portions, dye did not pass to the blossom-end of the fruit. The vessels either did not function as water-conducting passageways or, more probably, transpirational water losses from the fruit were insufficient to draw dye to the distal end. As the fruit surface lacks stomata, transpiration must be cuticular. A significantly greater amount of dye travelled into the fruit when sepals were removed before the stem ends were immersed in dye. This supports the idea that transpirational pull and amount of water loss from the sepals diverts water and dye into the sepals rather than allowing it to continue from the pedicel into the fruit. Wiersum (1966) immersed tomato trusses into a solution containing the dye, Light Green. The dye travelled apoplastically, staining the vasculature of the pedicel and calyx but abruptly ended within the tissue of the receptacle. Dye was rarely detected in veins of the

fruit wall. Wiersum did not confirm whether or not the majority of dye flowed through the placental tissue to the ovules. The results of this thesis support the view that little apoplastic water flow appears to enter the fruit. In these experiments, the presence of berberine hemi-sulphate correlated with the observation of greatest xylem concentration within the placental tissue.

Ehret and Ho (1986a) have shown that the hydraulic conductance of xylem sap differs among the pedicel, pedicel-fruit junction, and fruit tissues. Pressurized water forced through the xylem tracheary elements showed a restriction to xylem flow that increased in the sequence of pedicel $<$ pedicel-fruit junction $<$ fruit. A low hydraulic conductance implies either a low amount of xylem water transport, some form of restriction to flow within the vessels, or few vessels to transport water. The fruit contains less xylem per cross-sectional area than does the pedicel, and has a low transpiration rate, therefore it may be speculated that water within the pedicel is primarily attracted to the sepals that exhibit a high transpiration rate. Measurements of water potential gradients within the tomato plant showed that a resistance to water flow exists between the pedicel and tomato fruit (Lee *et al.* 1989). This observation may partly be the consequence of the short, crooked vessel members that characterize the xylem vasculature of the pedicel-fruit junction. However, it is not expected that vessel members with simple perforation plates would greatly impede water flow.

The organs of the tomato plant accumulate radioactive calcium to different extents. Leaves contained more labeled calcium per wet weight than all other plant parts examined. Developing and mature leaves contained a greater

proportion of xylem vessel strands than did flowers or fruit and were well supplied with stomata on both sides. These characteristics would contribute to a high transpirational capacity, thereby drawing calcium into the leaf in the transpiration stream. Armstrong and Kirkby (1979) noted that tomato plants grown in a high humidity environment (95% R.H.), showed a marked difference in calcium distribution relative to plants in a lower humidity (50% R.H.). In high humidity, the level of calcium in the young leaves was very low and calcium accumulated in the stems. Therefore, during periods of high humidity and hence low transpiration, calcium tended to accumulate in stems instead of leaves.

Whole flowers and buds, both of which have intact sepals, and sepals taken separately, all accumulated relatively large amounts of calcium. This was expected, since sepals have stomata on both surfaces and are well supplied with xylem vessels.

The pedicels and petioles accumulated less calcium than did leaves, sepals, buds, and flowers. These organs represent vascular passageways and the occurance of calcium in the pedicels and petioles is dependent on transpirational pull of xylem water plus calcium exchange on negatively-charged sites of the xylem vessel surface (see Hanson 1984). Because of lateral leakage from xylem vessels (see Hanger 1979), some calcium was absorbed by the pedicels and petioles. Considerably more calcium was expected to accumulate in organs which maintained a greater transpirational pull on the xylem water.

The tomato fruit contained the smallest amount of calcium per wet weight

relative to all other plant parts sampled. This pattern was consistent for all replicate trusses and was correlated to the relative amounts of xylem vasculature. As previously described, fruit are poorly supplied with xylem relative to pedicel, sepal, and leaf. The proximal fruit half consistently contained more calcium per unit wet weight than the distal half (Table 1). In a study involving calcium distribution within plants grown at various salinities, Ehret and Ho (1986b) also observed a lower calcium concentration in the distal half of the fruit relative to the proximal half. They noted that removal of sepals produced an increased incidence of BER and suggested that the sepals draw water toward the fruit because of their high transpirational capacities. However, the present results show that sepal removal results in an increased flow of dye into the fruit. Sepal transpirational pull enhances water flow across the pedicel, and water flow would be diverted into the sepals rather than into the fruit.

Developing flower buds, flowers, and fruit receive significantly less calcium than mature leaves of the same plant. It has previously been recognized that calcium is not significantly translocated from older tissues to younger plant parts even under calcium stress conditions (see Hanger 1979). When calcium is abundant in the sap, the distribution of the ion will be closely related to the rate of transpiration and calcium will move primarily into transpiring leaves (see Clarkson 1984). Once it enters the leaf, much of the calcium absorbed by leaf cells will be bound by oxalic acid, generated during nitrate reduction. Application of calcium to the soil and to the foliage of plants does not increase calcium concentration in the fruits, because it becomes bound as calcium oxalate due to

the high oxalic acid content of leaves and stems (Evans and Troxler 1953). Growth in organs such as fruits and transpiring leaves influence calcium distribution. When large quantities of calcium are absorbed through the xylem of the root, the concentration differential between leaves and fruit is minimal, although the ratio changes diurnally. Calcium flows to transpiring leaves because of negative transpirational pull during the day, and to fruits and meristem tissues at night when the pull of transpiration decreases and root pressure increases. Organs with low transpiration rates, such as meristems and fruit, accumulate needed calcium from xylem sap delivered by root pressure during the night (Bradfield and Guttridge 1984). However, an alternative exists to acquiring calcium. Growing points, such as meristems and fruit, undergo new wall synthesis and thereby create apoplastic binding sites (mainly cell wall carboxyl groups) that can release the calcium bound to exchange sites on the xylem vessel walls (see Clarkson 1984). This may explain why preferential calcium transport in the dark period into meristems and fruit is not limited to intact plants with root pressure but is also observed in isolated shoots although at a lower level of calcium influx. The distribution of calcium usually favors leaves but in low transpiration environments, the differential between leaves and fruit is less (van de Geijn and Smeulders 1981).

Fruit generally contain less calcium than leaves. Tomato fruit contain a higher percentage of citric acid in their cell sap than stems and leaves. Organic acids generated in the respiration of young shoots would be utilized in the synthesis of proteins, whereas fruit have a low protein synthesis and therefore low

utilization of organic acids. The chelation capacity of citric acid can interfere with calcium assimilation (Evans and Troxler 1953). Fruit must also maintain low calcium level during rapid cell expansion. Calcium would otherwise be deposited as calcium pectate, which would make cell walls rigid and would interfere with membrane permeability and influx of assimilates and minerals into the expanding cell.

The growth of a tomato fruit, following the first four days after anthesis, is essentially the accumulation of water in existing cells. Most of the cell division in the pericarp takes place during the first week after anthesis (Davies and Cocking 1965). In this study, calcium was proportional to the increase in fruit volume over the four day period. Fruit that grew the fastest during the experimental period, that is those in which water intake was greatest, accumulated the most calcium. The increase of calcium as a proportion of growth was constant for all fruit regardless of developmental stage, except for the very small fruit. For fruit larger than 30 mm^3, calcium was transported into the fruit as a constant proportion of volume increase or water flow into the fruit. Very small fruit, however, accumulated a disproportionately large amount of calcium relative to their increase in volume during the experimental period. This trend can be explained by considering transpirational rates for these fruit. The surface area per volume ratio of small fruit was greater than that of large fruit, but water loss per unit surface area of fruit was constant except for fruit less than 30 mm^3 in size. Very small fruit lost a disproportionate amount of water from their surface. This excessive water loss accounted for the larger proportion of calcium that entered

these fruit. Gerard and Hipp (1968) reported a similar relationship between transpiration rate and fruit weight. Transpiration of small fruit was several times that of larger fruit in a high air temperature. low relative humidity environment-conditions conducive to high transpiration. In a study involving transpiration rates of tomato fruit grown in different nutrient salinities, Ehret and Ho (1986a) showed that fruit less than 5 g fresh weight had a disproportionately large rate of water loss when compared to larger fruit grown in the same salinity. However, the increased transpiration rate of small fruit relative to larger fruit per unit surface area was consistent.

Rapid water loss from very small fruit might be explained by the structure of the cuticle and fruit epidermis. The fruit surface lacks stomata, but on the very young fruit, trichomes extend from the epidermis (Wilson and Sterling 1975) that are later dehisced from the fruit surface. These trichomes would increase the surface area of very young fruit, and would not have been accounted for in surface area calculations. If the trichomes lose water to the air, then the increased surface area relative to volume would serve to increase the transpirational surface of the fruit and result in greater water loss for very small fruit. Generally, the cuticle of the tomato fruit is a homogenous covering of wax and cutin over the epidermal cells of the fruit which prevent water loss and ion leakage from the fruit surface (Martin and Juniper 1970). Wilson and Sterling (1975) showed that the cuticle of full size, green fruit is much less homogenous than the cuticle of ripe fruit and is partly composed of irregular, globular masses. It may be speculated that the cuticle of very young fruit is similarly irregular

and/ or incompletely differentiated, and may contribute to excessive water loss. Also, the cuticle of immature fruit is significantly thinner than that of mature, ripe fruit. A steady increase in cuticle weight per unit surface area occurs during fruit development that results in a several fold increase in cuticle thickness between green and ripe fruit (Baker *et al.* 1982) thus decreasing the rate of water loss as the fruit matures.

When total water intake is measured as the increase in volume plus transpirational losses, these very small fruit did not accumulate significantly less calcium per total water than fruit larger than 30 mm^3. This calcium accumulation was directly related to total water intake and was independent of fruit size or developmental stage. An increase in total water intake to the fruit resulted in an increased accumulation of labeled calcium in the fruit. Calcium as a proportion of total water, including the transpirational losses, was a constant percent of volume, regardless of fruit size. Because calcium travels along the transpirational stream in the xylem, and its rate of translocation increases with increased levels of transpiration, it was expected that the greater the influx of water into a fruit, the greater the amount of calcium carried with it. Calcium transport is not completely dependent on water flux, however, if water movement into the fruit is at a constant rate, then translocation of calcium into the fruit would also follow at a constant rate, and remain so for the duration of fruit development. Bangerth (1979) concluded that calcium travelling to above ground organs is translocated primarily as a function of water flow and transpiration rate rather than as a consequence of physiological requirement for the mineral.

There is considerable evidence that during the early fast growth phase of tomato fruit, most of the water, minerals, and all assimilates travel mainly via the phloem rather than the xylem. Wolterbeek *et al.* (1987) have calculated that the phloem contributes 84% of the water and 98% of the total dry matter. Ho *et al.* (1987) calculated that 90% of the water which enters the tomato fruit arrives via the phloem. It has therefore been argued that high phloem import induces BER in young fruit, and that a high growth rate of small fruit is correlated with a low calcium content (see Hanger 1979, Wiersum 1966). This is not supported by the present data, which show that very small fruit experienced the fastest volumetric growth. The growth of all the fruit over the four day period appeared as a typical sigmoidal growth curve without the initial lag phase. Very small fruit grew exponentially compared to larger ones whose growth tapered once the fruit reached more than 325 mm^3 in size. The great increase in volume of fruit less than 30 mm^3 represents the greater amount of water intake by these fruit. These very small fruit also accumulated more calcium relative to their increase in volume than fruits of a larger size. The calcium content relative to volume increase for larger fruit declined sharply and then remained constant. Therefore apoplastic water delivery was expected to be constant. The fruit continued to grow, but the decreased calcium accumulation suggests that much of the water entering the fruit may be contributed by the phloem. The high calcium content of these very small fruit may be partially explained by the observation that these fruit have a greater proportion of xylem vasculature than older and larger fruit. An increased supply of xylem elements and a high transpiration rate would increase both water flux and calcium uptake. This would indicate that at very

early stages of development the apoplastic pathway significantly contributes to the delivery of water to the fruit. Hardham (1976) noted that, in peas, the pattern of calcium distribution is directly related to the pattern of vascular supply. Organs with low transpiration such as fruit may obtain more water via the phloem than the xylem, however this need not necessarily induce BER as suggested in a review by Bangerth (1979). Sufficient apoplastic water enters the tomato fruit to avoid BER. The fruit has two modes of water supply, the xylem and the phloem, and the relative contributions of each are dependent on transpiration rate, xylem content, and developmental stage. A probable explanation for the discrepancy in results shown here compared to published observations on calcium uptake is that the published results only included fruit that had passed the early post-anthesis period when transpiration per unit area of the fruit surface was still very high.

Growth over the experimental period was influenced by fruit position along the truss. In many cases, the most proximal fruit develops first, with each fruit following in sequence as a lateral primordial bud from the preceeding developing flower. Hence, the most proximal fruit is often the largest, with a gradual decline in fruit size for the remaining fruit along the truss (Bangerth and Ho 1984). Increase in size would therefore be greatest in the first fruit with gradual decline in size to the fruit most distal along the truss. However, growth relative to initial volume at the beginning of the experimental time was independent of fruit position. The percent growth of each fruit was either constant or not significantly different for all fruit of a truss with some variability between trusses. Fruit less

than 30 mm^3 did not follow this trend but grew disproportionately compared to others of the same truss even though these fruit were often the most distal on the truss.

Position of the fruit along the truss influenced the total amount of calcium uptake into the fruit. The most proximal fruit on the truss accumulated more calcium than the last fruit (most distal on the truss). This is explained by the movement of calcium in the transpirational stream. Calcium is transported through the peduncle of the truss along the water flow transferring from one xylem vessel exchange site to another. The first pedicel that the water encounters will be that of the most proximal fruit. Growing fruit, with their expanding cell wall surfaces, provide new exchange sites that act as a sink for calcium. Therefore, calcium will first bind to these sites and when these sites are saturated, it will be carried through the xylem stream to the next binding site. This process would result in the most proximal fruit constantly being the first to receive incoming calcium. Russell and Morris (1982) noted that the preferred pathway for movement of solutions is along the most direct vascular connection. The most proximal fruit is in a more direct position to acquire calcium transported through the vasculature than are the more distal fruit. Differences among fruits of the same truss was noted by Bangerth and Ho (1984) who concluded that the induction sequence of the flowers and therefore fruit on the truss, affected the sink activity of the fruit with the first-induced fruit having a greater sink activity than the later-induced fruit. They suggested that this trend may be the result of the increased cell number in proximal fruit or the presence of an increased

amount of the hormone indolacetic acid to attract assimilates. It is understandable how an increased cell number of the proximal fruits could contribute to a greater number of cell wall receptor sites for calcium during cell expansion.

The incidence of the calcium deficiency disease, BER, is primarily influenced by the water status of the plant. The xylem is its sole passageway for long distance transport and the pattern of xylem concentration of the leaves, sepals, and fruit influences calcium distribution within these organs. The deficiency disease arises at the blossom end of the fruit, where the supply of vasculature is less relative to the proximal portion and because of the generally low transpiration rate of fruit relative to other organs. The majority of xylem vasculature passes to the ovules rather than to the distal end of the fruit. Very small fruit (30 mm^3) that exhibited a high growth rate did not represent the initial stage of BER induction, contrary to suggestions in the literature. The very small fruit accumulated significantly more calcium per relative increase in volume than larger fruit. This increased amount of calcium reflected the mode of transport of this mineral. Fast growing fruit may create increased numbers of cell wall receptor sites for calcium as cell walls extend to accommodate the expanding cells. Therefore, BER should not be induced during the fast growth phase but following it, when the creation of sites on new cell walls declines and when the transpiration rate per unit area drops to a constant value.

Chapter 5

Conclusion

The first trace of xylem tissue within the floral bud primordia exists in buds 0.3 mm long. The abscission zone of the pedicel is first present in buds 0.8 mm long and is composed of stubby vessel elements without tracheids or xylem fibres. The abscission zone xylem does not undergo further development, but is maintained to fruit maturity as an area with weak vascular connections and potential for xylem water flow restriction.

Much of the xylem water flowing through the pedicel of an intact truss is diverted into the sepals rather than entering the fruit. The extensive vascular system and presence of stomata result in higher transpirational pull of water by the sepals relative to the fruit.

The distribution of calcium effectively followed the areas of xylem concentration and high transpiration rate. Fruit accumulated the least calcium of all organs examined and this trend parallels their relatively poor xylem vasculature and low transpiration rate. Very small fruit did not exhibit a tendency for calcium deficiency as may have been expected from their rapid increase of volume. These fruit showed a high transpiration rate and greater

amount of xylem vasculature relative to larger fruit. Calcium deficiencies should be induced following the fast growth phase of the fruit, when transpiration and growth rate decline. High rates of transpiration and associated high levels of calcium accumulation suggest that apoplastic water may play a major role in the very early stages of fruit development.

Chapter 6

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Appendix A

Hoagland's Nutrient Solution

A.1. Macronutrients

10 ml	1 M $Ca(NO_3)_2$
10 ml	1 M KNO_3
2 ml	1 M $MgSO_4$
4 ml	1 M KH_2PO_4
2 ml	Fe EDTA 0.5 g/100 ml

A.2. Micronutrients

2.86 g	Boric Acid
1.81 g	$MnCl_2$
0.11 g	$ZnCl_2$
0.05 g	$CuCl_2$
0.02 g	Na_2MoO_4

- Dissolve micronutrients in 1 l water.
- Add the 28 ml of the macronutrient solution to 2 ml of the micronutrient solution.
- Dilute these 30 ml in 2 l water.

Appendix B

Percent Cross-sectional Area of Xylem of the Proximal, Abscissional and Distal Portions of Pedicels Aged Two Days after Anthesis 0 Weeks to Eight Weeks, Stained with Phloroglucinol and HCl

Age	Proximal	Abscissional	Distal
0	47.6	4.9	42.2
1	43.5	7.9	34.9
2	50.0	9.5	41.7
3	41.2	8.1	38.0
4	50.4	10.1	35.5
5	52.3	12.6	45.3
6	40.9	3.9	32.2
7	50.1	7.8	28.9
8	59.8	11.7	44.6

Appendix C

Data of Fruit Volume, Increase of Volume, Relative Increase of Volume and ^{45}Ca per Increase of Volume

Volume (mm^3)	Volume Increase (mm^3)	Volume/Volume	^{45}Ca/Volume (dpm mm^{-3})
900.0	82.5	0.0917	507.9
0.7	0.3	0.4286	6855.2
12.4	4.0	0.3226	2164.8
10.0	3.7	0.3680	795.7
4.6	1.8	0.3804	10004.6
2525.0	111.3	0.0441	2227.1
30.9	6.9	0.2223	8465.2
0.7	0.3	0.4286	20637.8
325.0	56.3	0.1731	1568.6
585.0	72.5	0.1239	1243.9
500.0	67.5	0.1350	1358.0
0.7	0.3	0.4286	2824.0
0.7	0.4	0.5714	9343.2
2900.0	120.0	0.0414	800.9
2500.0	113.8	0.0455	838.5
1719.0	103.8	0.0603	1043.9
3250.0	122.5	0.0377	1965.3
3670.0	130.0	0.0354	1587.8
1562.0	98.8	0.0632	1367.1
600.0	73.8	0.1229	1782.4
0.7	0.4	0.5714	3623.5
4090.0	131.3	0.0321	2060.9
4050.0	130.6	0.0322	872.8
2275.0	109.0	0.0479	736.0
2187.0	106.3	0.0486	826.7
2290.0	110.0	0.0480	831.0
1500.0	98.6	0.0658	496.3

Appendix C continued

Volume (mm^3)	Volume (mm^3)	Volume/Volume	^{45}Ca/ Volume (dpm/mm^3)
1250.0	72.5	0.0580	712.9
0.7	0.3	0.3571	8580.0
6600.0	145.0	0.0220	1058.7
4950.0	137.5	0.0278	869.6
5450.0	140.0	0.0257	1602.1
2500.0	111.3	0.0445	344.1
2825.0	118.8	0.0420	201.1
2900.0	119.4	0.0412	556.6
4000.0	131.3	0.0328	405.1
1715.0	102.5	0.0598	1628.9
2517.0	115.0	0.0457	1530.1
3265.0	122.5	0.0375	2624.8
5100.0	137.5	0.0270	900.4
5400.0	140.0	0.0259	564.5
3750.0	130.0	0.0347	1313.6
3500.0	125.0	0.0357	930.0
2870.0	118.8	0.0414	2565.4
2700.0	117.5	0.0435	1724.8
2200.0	108.8	0.0494	1696.6
1800.0	102.5	0.0569	2242.6
2550.0	113.8	0.0446	1940.5
2800.0	117.5	0.0420	560.7
2100.0	107.5	0.0512	1529.8
1380.0	97.5	0.0706	2271.3
3270.0	122.5	0.0375	889.6
3400.0	130.0	0.0382	2115.1
585.0	70.0	0.1197	2850.5

Appendix D

Data of Water Loss per Hour, Surface Area, and Volume of Fruit

Water loss ($mg\ h^{-1}$)	Surface area (mm^2)	Volume (mm^3)	Water loss/Surface area ($mg\ min^{-2}\ h^{-1}$)
3.3	1804.66	5766.85	0.0018
1.7	1469.14	5293.69	0.0012
2.2	1434.42	5107.19	0.0015
1.7	1469.82	5297.36	0.0012
1.6	1436.00	5115.79	0.0011
1.8	1236.61	4088.04	0.0015
2.5	1052.09	3208.07	0.0024
2.4	1372.28	4778.91	0.0017
2.0	1356.57	4698.87	0.0015
1.3	1249.11	4037.77	0.0010
1.4	1049.79	3197.56	0.0013
1.6	1025.24	3083.50	0.0016
1.4	998.74	2972.18	0.0014
1.5	864.65	2394.50	0.0017
1.7	819.40	2209.09	0.0021
4.8	644.22	1537.16	0.0075
2.4	235.06	338.01	0.0102
3.3	201.06	268.02	0.0164
2.3	166.96	201.97	0.0138
2.5	156.26	183.42	0.0160
2.6	119.60	122.95	0.0217
4.5	97.12	89.97	0.0463
3.2	110.85	109.71	0.0289
1.4	92.29	83.35	0.0152
1.6	25.34	12.00	0.0631
0.7	4.37	0.86	0.1602

Appendix D continued

Water loss (mg/h)	Surface area (mm^2)	Volume (mm^3)	Water loss/Surface area ($mg/mm^2/h$)
1.1	5.98	1.38	0.1839
0.5	2.32	0.33	0.2155
0.5	1.91	0.25	0.2618
0.3	1.45	0.16	0.2069

Appendix E

Data of Truss Number, Corresponding Fruit Position, Calcium Accumulation and Increase of Volume.

Truss #	Position	^{45}Ca (dpm)	Volume Increase (mm^3)
1	2	41899	82.5
1	3	17508	1.8
1	4	8659	4.0
1	5	2928	3.7
1	6	2934	0.3
2	1	247760	111.3
2	2	58156	6.9
2	3	88231	56.3
2	4	90184	72.5
2	5	91664	67.5
2	6	8833	0.3
2	7	5335	0.3
2	8	706	0.4
3	1	96105	120.0
3	2	95385	113.8
3	3	108308	103.8
4	1	240754	122.5
4	2	206420	130.0
4	3	134997	98.8
4	4	131454	73.8
4	5	2069	0.4

Appendix E continued

Truss #	Position	^{45}Ca (dpm)	Volume Increase (mm^3)
5	1	270499	131.3
5	2	114009	130.6
5	3	80224	109.0
5	4	87839	106.3
5	5	91742	110.0
5	6	48958	98.6
5	7	53827	72.5
5	8	2145	0.3
6	1	153515	145.0
6	2	224298	140.0
6	3	119566	137.5
7	1	53168	131.2
7	2	66444	119.4
7	3	23885	118.8
7	4	38277	111.2
8	1	321543	122.5
8	2	175960	115.0
8	3	166961	102.5
9	1	123810	137.5
9	2	79023	140.0
9	3	170763	130.0
9	4	116255	125.0
9	5	304638	118.8
9	6	202666	117.5
9	7	184508	108.8
10	1	229866	102.5
10	2	220727	113.8
11	1	274967	130.0
11	2	108971	112.5
11	3	65883	117.5
12	1	164453	107.5
12	2	221448	97.5
12	3	199532	70.0