POTASSIUM ACCUMULATION WITHIN THE MESOPHYLL APOPLAST OF PEA (*PISUM SATIVUM* CV. ARGENTEUM)

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A DISSERTATION

submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1990

ABSTRACT

POTASSIUM ACCUMULATION WITHIN THE MESOPHYLL APOPLAST OF PEA (*PISUM SATIVUM* CV. ARGENTEUM)

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A study was conducted to evaluate the influence of long/short transport processes on leaf mesophyll apoplastic K^+ content as related to the regulation of leaf K^+ status. Apoplastic K^+ content was measured in leaflet tissue from hydroponically cultured pea (*Pisum sativum* cv. Argenteum) plants utilizing an elution method.

Fusicoccin, an plasma membrane ATPase promoter, reduced leaf apoplastic K^+ content when injected into leaf pulvini at a concentration of 250-1000 μM. Vanadate had no effect on apoplastic K^+. Blockage of phloem transport, by either steam girdling or PCMBS injection (5×10^{-2} μmole) into the petiole significantly increased apoplastic K^+ content. Apoplastic K^+ content was lowered when xylem volume flux was altered by ABA injection or application of an antitranspirant to the leaf.

Mesophyll apoplastic K^+ and total leaflet K^+ content exhibited a diurnal fluctuation ranging from approximately 2 to 10 μeq/g fr wt and 50 to 85 μeq/g fr wt, respectively, with the highest levels occurring between 1300 and 2300 hr. During leaf ontogeny, apoplastic K^+ content declined only minimally. Transpirationally induced xylem import rate was thought to be the primary factor influencing apoplastic K^+ content within mesophyll tissue on the short term.

Since estimated apoplastic K^+ concentration (10-40 or 40-100 mM) during leaf

ontogeny were sufficiently high to promote continued influx and net accumulation of K^+ within mesophyll cells, ontogenetic declines in total leaflet K^+ were hypothesized to be due to phloem loading from the symplast or apoplast and net export from the leaf.

ACKNOWLEDGEMENT

I would like to thank the following people - Dr. Widders, Dr. Buckvac, Dr. Klampler, Dr. Ries and Dr. Safir - for their efforts in correcting and editing this dissertation. Especially, I want to thank Dr. Widders, my major advisor, for his help and patience in the past four years. I believe it would be extremely difficult for me to go through the entire doctoral program without his understanding and support. Dr. Widders first gave me confidence and time to overcome language problem. He also paid a lot of attention in my experiments. The friendship between us built during those days will last forever.

I would also want to thank my wonderful roommates, Sping Wang and Renee Polega, for their support in those hard days. I appreciate not only their help in my experiments but also their love in the daily life. Their friendship is my another achievement in the States. I want to say I love them very much.

Of course, I would never forget the love and care from my husband Jordan Wu. Without his encouragement I believe I am still a person who cannot speak English and a Ph.D degree will still a dream. He deserves half of this degree.

Dedicated to my parents and Jordan.

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INTRODUCTION

Potassium (K^+) is an important macronutrient for plant growth and development. Numerous research efforts have contributed to the elucidation of the mechanisms for its uptake, transport, distribution and other diverse roles in metabolism within the plant. The supply of K^+ to the root system can influence leaf morphology and subcellular structures (Beringer and Nothdurft, 1985), water stress resistance (Mengel and Arneke, 1982), disease resistance (Vierick, 1983), numerous physiological processes including assimilation rate, and ultimately crop yield and quality.

When K^+ is taken up and transported via the xylem to the shoot and ultimately into the leaves, it is first unloaded into the leaf free space, the apoplast, where K^+ can be absorbed by the mesophyll cells or be loaded into and retranslocated by the phloem. Potassium accumulation and distribution among different cell types and subcellular compartments within leaf lamina tissue varies with leaf age and depends on the equilibrium of solute fluxes between the symplast and apoplast (Cosgrove and Cleland, 1983a; Hancock, 1977; Meinzer and Moore, 1988). The symplast is known to be the major compartment for metabolism. The apoplast, however, is not as well characterized as the symplast. Also, most of the research concerning the apoplast has

concentrated on the root. An understanding of the regulation of the ionic status of the mesophyll apoplast in leaf tissue is essential for gaining a full understanding of the varied potential roles of the apoplast in plant metabolism and growth.

In contrast to the symplast, the apoplast provides a pathway for low resistance transport of water and solutes, including mineral ion movement and distribution from the xylem within leaf lamina tissue (Weatherly, 1970). The leaf apoplast is also an integral pathway for assimilate transport from mesophyll into the phloem tissue in certain plant species (Turgeon and Wimmers, 1988). It is assumed that ions such as K^+, which are readily accumulated and exported within the phloem may follow a similar pathway. Potassium accumulation within the apoplast, which significantly lowers the osmotic potential of the extracellular compartment, would also be expected to influence leaf water content and cell turgor (Cosgrove and Cleland, 1983a; Flowers and Yeo, 1988). In addition, the direction and rate of K^+ fluxes into leaf mesophyll cells is influenced by the magnitude of changes in apoplastic K^+ concentration (Pitman et al., 1974b) and thus electro-chemical potential gradients.

The ionic composition of the leaf apoplast was postulated (Pitman et al., 1974b) as being regulated by fluxes through the xylem and phloem and net uptake into the symplast (Jensen, 1980; Widders and Lorenz, 1983a). The relative activity of these transport processes and their ultimate effect on apoplastic K^+ are thought to be influenced by environmental factors. An ontogenetic decline in leaf K^+ level was suggested to be the result of net efflux of K^+ out of leaf cells induced by a reduction in the extracellular K^+ concentration below some threshold concentration at which

flux equilibrium occurs (Widders and Lorenz, 1983b).

The study of the apoplast, as a potentially important compartment for ions within leaf tissue, has been facilitated by the development of an elution method to measure ionic content in mesophyll of the pea (Long and Widders, 1990). This method has the advantage of preventing wounding and ageing effects caused by leaf slices and discs, and is the only known procedure which utilizes relatively intact leaf tissue.

The objectives of these studies were: 1) to gain a better understanding of the regulation of apoplastic K^+ content and concentration within leaf mesophyll tissue, and 2) to determine the potential role of the apoplast as an extracellular ionic compartment which influences ontogenetic changes in the K^+ content of leaf lamina tissue and as a potential pathway for ion distribution within leaf lamina tissue. These objectives were addressed by:

1. Investigating the effects of ATPase promoters and inhibitors on apoplastic and total K^+ in leaf mesophyll tissue.

2. Determining the effects of altered xylem and phloem transport rates on K^+ accumulation within the leaf apoplast.

3. Characterizing the diurnal and ontogenetic changes in leaflet apoplastic K^+ concentration in the Argenteum mutant of the pea.

4. Evaluating the effects of leaflet position on the stem axis on the total and apoplast K^+ within leaflet tissue as related to transpirational water loss.

LITERATURE REVIEW

1. Apoplast of Leaf Tissue

1.1 Definition and characteristic of apoplast ---

The apoplast is the entire volume of the tissue external to the plasma membrane including cell walls, middle lamella, intercellular spaces (typically air spaces), mature xylem vessel elements and tracheids (Esau, 1977). The apoplast can be further divided into water free space (WFS) and Donnan free space (DFS). The WFS is the space where ions can move freely in the water and sorbed by the cell wall, while the DFS consists of the fixed anion charges of the wall where cations cannot move freely and free anions are extremely limited (Briggs and Robertson, 1957; Briggs et al., 1961; Higinbotham et al., 1962; Pitman, 1965; Van Steveninck and Chenoweth, 1972). The functional apoplast is the volume of the extracellular compartment which is filled with solution under normal growing conditions including the cell wall but excluding intercellular air spaces.

The volume of the WFS is in the range of 0.023 to 0.25 ml/g fr wt depending on the degree of infiltration of bathing solution (Pitman et al., 1974a; Smith and Fox,

1975; Widders and Lorenz, 1983a). As stated by Pitman et al. (1974a) and Van Steveninck and Chenoweth (1972), the WFS in the transpiring leaf should occupy only a limited proportion of the cell wall volume. Transpiration can be expected to maintain water potential at a level that would not allow free water in the intercellular space except perhaps in interstices where capillary surfaces compensate for the difference in water potential between cells and free water. The DFS with a volume of 0.013 ml/g fr wt contains cation exchange sites of approximately 3.6 μeq/g fr wt (Pitman et al., 1974a) and 6.8 μeq/g fr wt (Widders and Lorenz, 1983a). However, Smith and Fox (1975) reported a much higher concentration of exchangeable cations, approximately 20-25 μeq/g fr wt. Pitman et al. (1974a) calculated the Donnan volume and concluded that the entire cell wall in leaves is a DFS. According to this view, the fixed negative charges in the DFS is proportional to the wall thickness and is variable between plant species, tissue, cell types and age. The pH value in the external solution could also affect the cation change capacity in the DFS. Low external pH of 4.5 could reduce 50% of the fixed negative charges in the DFS for beets (Pitman, 1965).

Virtually all of the DFS was found to exchange with the rate constant of the WFS. Bush and McColl (1987) suggested "Mass-action expressions" by assuming that ions are partitioned between two distinct phases: a solid-exchanger phase and an aqueous-solution phase. Cations in these two phases are free to exchange with one another. Ions associated with the wall can be treated as part of the exchanger phase where cation exchange activities are proportional to their mol fractions (Sposito,

1981). The affinity of the cell wall for each cation can be characterized without assumptions about solution-phase activity coefficients in the region of the wall or the volume of the wall involved in cation binding. According to this theory, the relative affinity of the wall for the cations was: $H^+ >> (K^+ \geq Ca^{2+}) > Mg^{2+}$. Small changes in the pH near the wall will cause large changes in the solution concentration of Ca^{2+}, Mg^{2+}, and K^+ (Bush and McColl, 1987).

The pH value of the leaf apoplast is in the range of 4.5 to 5.5 (Aloni et al., 1988; Giaquinta, 1977). In some plants, such as *Phaseolus vulgaris*, the pH can be as high as 6.8 (cf. Raven and Farquhar, 1981). Transport of amino acids, ions, sugars and other solutes across the plasmalemma is known to be by a H^+-cotransport mechanism, a process highly dependent on the pH gradient across the plasma membrane. The pH changes of the external medium can be due to changes in proton pumping activity (Aloni et al., 1988) and membrane leakage.

The capacity of the apoplast is limited by the volume of the cell wall and by the concentration of non-diffusible anions in the wall (Pitman et al., 1974a; Robinson, 1971). Since the apoplast is in communication with the symplast via the plasmalemma, its composition and content are determined in part by cell metabolism and seem to be maintained at a certain equilibrium with cell contents (Kursanov, 1984; Preston, 1979). Meinzer and Moore (1988) reported that about 13% of the osmolality of the apoplastic solution was attributable to reducing sugars and sucrose and another 18% to K^+. Cation concentration in the apoplast was figured at about 28 mM with K^+ concentration of 6.4 mM from *Venus flytrap* (Jacobson, 1971).

Bernstein (1971) calculated total leaf apoplastic solution concentration in the range of 2 to 10 mM. In *Suaeda maritima*, Harvey et al. (1981) estimated apoplastic K^+ concentration of 13 to 17 mM. Potassium concentration in the DFS was estimated at approximately 70 mM (Pitman et al., 1974a). More recently, Long and Widders (1990) reported leaf apoplastic K^+ concentration in Argenteum pea raged from 22-36 mM and 56-90 mM based on the assumption that the volume of the apoplast was 0.1 and 0.04 ml/g fr wt, respectively.

1.2 Role of apoplast ---

The apoplast is thought to be a site of numerous physiological processes (Preston, 1979). Since enzymes and solutes exist in the cell wall, some biochemical reactions such as sucrose hydrolysis and cell wall expansion could take place there (Giaquinta, 1980; Northcote, 1972). It was suggested that the apoplastic solutes contribute to the growth-induced water potential (Cosgrove and Cleland, 1983b). The apoplastic osmotic potential is lower in the elongation region of the leaf (Meinzer and Moore, 1988). The "velamen" (multiple epidermis) in the aerial roots of tropical Orchidaceae and epiphytic Araceae, as well as in some terrestrial monocotyledons consists of the thickened wall which is involved in water absorption and reduction in water loss. For example, sucking hairs of *Tillandsia* function as an apoplastic compartment for water storage (Dolzmann, 1964). The ingrowing cell wall of the transfer cells in xylem parenchyma may favor mass flow of solutes and water (Hill

and Hill, 1973) and facilitate transmembrane flux of solutes (Pate and Gunning, 1972).

It is possible that the apoplastic pathway is involved in transferring sugars from mesophyll to the phloem (Geiger, 1975; Giaquinta, 1983). Of course, one should not ignore that the apoplast participates in ion transport and transpirational water loss within a leaf (Tanton and Crowdy, 1972; Thompson et al., 1973). In plants with the diurnal leaf movement, the apoplast is the major route for the circadian ion movement (Hosokawa and Kiyosawa, 1983; Lee and Satter, 1987). Smith and Fox (1975) claimed that the DFS might act as a considerable extracellular reservoir or sink for cations which are not readily absorbed or may be extruded by the cells. The high capacity of the apoplast to accumulate ions especially in salt tolerant plants (Flowers and Yeo, 1988) indicates that the apoplast might play a physiological role beyond current knowledge such as participating in cell turgor maintenance. In the later stages of leaf ontogeny, if xylem import exceeds absorption by mesophyll cells, the apoplastic ionic concentration would be expected to rise and lead to a reduction in cell turgor (Clipson et al., 1985; Oertli, 1968).

1.3 Characterization of apoplast ---

The volume of the apoplast was declared relatively constant throughout fruit development except at the later stages of maturity when the apoplastic volume increased sharply (Damon et al., 1988). The volume of the apoplast can be estimated

in an ion uptake experiment from the amount of tracer labeled ionic solution which can be eluted out of a tissue by water within a relatively short period of time.

The content and concentration of the leaf apoplast is variable, determined by the exchange properties of the cell wall, supply of ions from the vascular system, and fluxes in the cells. For example, free space content of K^+ increased as the Ca^{2+} level was reduced (Pitman et al., 1974a). Much effort has been expended to determine the apoplastic solute content and concentration, but quantitative analysis especially in the leaf apoplast has been limited by the methods employed. Methods such as centrifugation (Jacobson, 1971), perfusion (Cosgrove and Cleland, 1983a), applied pressure (Cosgrove and Cleland, 1983a; Meinzer and Moore, 1988) and exudation (Long and Widders, 1990; Widders and Lorenz, 1982b) were introduced to extract the apoplastic solution. It was found that 25% of the apoplastic solutes in the growing stem were inorganic electrolytes (Cosgrove and Cleland, 1983a) which is ascribed to a high solute requirement for cell growth as well as a high rate of transpirational water loss from the cell wall.

By applying pressure to leaves, Jachetta et al. (1986) calculated the apoplastic solute concentration of the cell wall and minor vein fraction at approximately 8 Mol/Kg. Total cation concentration in the apoplast of *Venus flytrap* leaves, estimated by the centrifugation method, was about 28 mM, with a K^+ concentration of 6.4 mM (Jacobson, 1971). Bernstein (1971) applied vacuum perfusion to leaves and calculated total apoplastic solution concentration in the range of 2 to 10 mM. Recently, Long and Widders (1990) employed an "elution method" to elute the apoplastic solutes

from intact leaves of Argenteum pea in which the epidermis could be peeled off without damaging mesophyll cells. After removing the epidermis and attaching a glass cylinder to the mesophyll, a certain amount of $CaCl_2$ was placed in the cylinder to elute the apoplastic solutes. They reported a higher range of apoplastic K^+ concentration of 22-36 mM and 56-90 mM based on the assumption that the volume of the apoplast was 0.1 and 0.04 ml/g fr wt, respectively.

There are problems in the methods mentioned above. During exudation, solute composition and concentration change with time. Pressure application may somewhat damage tissue, resulting in contamination from damaged cells. Either centrifugation or perfusion is an indirect method to estimate apoplastic solution concentration. The use of leaf slices has the disadvantages of aging and wounding effects and the increase in the volume of the apoplast caused by damaged cells (Van Steveninck, 1976), even though Pitman et al. (1974b) concluded that cells in leaf slices retain the same capacity for ion uptake as cells in an intact leaf. The "elution method", which avoids many of the drawbacks of using leaf slices, is still restricted to estimating the bulk solutes of the minor vein/mesophyll cell wall. X-ray microanalysis seems to serve as a direct method to locate ions in different compartments and even in different parts of the apoplast. But the sample preparation, especially the leaf sample for water soluble ions, is extremely difficult.

2. Factors Influencing Apoplastic Ion/solute Composition

Potassium content and concentration within the leaf free space would be expected to be influenced by the following changes: a) net fluxes across mesophyll cell membrane, b) K^+ transported into the leaf apoplast via the xylem, and c) K^+ redistributed to other tissue via the phloem.

2.1 Ion fluxes across cell membrane ---

Potassium flux across the leaf cell membrane is either a passive or active process and is regulated by the availability of energy for active uptake (Jeschke, 1976), negative feedback from vacuolar concentrations on influx rates, the permeability and integrity of cellular membranes, and the K^+ concentration in the free space (Pitman, 1975; Pitman et al., 1974b). These factors might be expected to change during leaf ontogeny (van Steveninck, 1976). The passive K^+ transport was postulated through binding K^+ to membrane ionophores such as valinomycin, nonactin, enniatin B, and gramicidin or ionophore-like molecules to form a very mobile ionophore-K^+ complex and release K^+ to the other side of membrane (Dobler et al. 1969; Mertzler, 1977). The rate of passive K^+ flux relies on the electrochemical potential gradient for K^+ between the outer solution and the cytoplasm and on the permeability of the membrane for K^+ (Poole, 1978).

Active K^+ transport involves membrane-bound protein ATPases where one K^+

across the membrane coincides with one H^+ released to the other side (Cheeseman and Hanson, 1979). ATPase, a reversible enzyme complex containing nine different polypeptide chains with a molecular weight of about 50,000 daltons, is a transmembrane protein. Factors such as leaf age, energy supply and ATPase-sensitive chemicals definitely would affect the activity of ATPase. As the leaf matures and starts to senesce, a series of major changes in the molecular organization of membrane occur. A decrease in the phospholipid and an ensuing rise in the sterol phospholipid ratio (Thompson et al., 1982) result in a change in membrane fluidity, causing a reduction in ATPase activity (Paliyath and Thompson, 1988).

In the absence of K^+ ions in the incubation solution, the inhibition of media acidification may be due to a K^+ requirement of the H^+-ATPase for optimal activity (Travis and Booz, 1979) or the involvement of an ATPase-driven K^+/H^+ antitransport system across the plasmalemma of mesophyll cells (Wyse and Komor, 1984). The activity of the H^+-translocating ATPase could be modified by changing cellular turgor through exposing the tissue to solutions of different osmotic concentrations or applying pressure. Under pressure-induced dehydration, both the greatly increased pH and ABA content of the apoplastic solution may affect the activity of ATPases (Hartung et al., 1988). In *Escherichia coli*, changes in turgor pressure alter the expression of genes in the kdp operon which is involved in K^+ transport through the cell membranes (Epstein and Laimins, 1980). It is, thus, the apoplastic K^+ concentration which would probably be increased during water stress due to the inactivity of ATPases in pumping protons out of cells to exchange K^+.

Many reports showed that ATPase inhibitors or stimulators such as vanadate (Bowman et al., 1978; Cocucci et al., 1980), ABA (Lurie and Hendrix, 1979), and fusicoccin (De Michelis et al., 1989; Marre, 1979; Rasi-Caldogno et al., 1986) could modulate H^+/K^+ exchange system, and consequently modify the pH value and K^+ concentration in the incubation medium. Orthovanadate at a concentration between 5 to 20 μM was shown to inhibit ATPase activity and 200 μM markedly inhibited H^+ extrusion and K^+ influx (Cocucci et al., 1980). However, in higher plants such as beans, both vanadate-sensitive and insensitive ATPases exist. Only vanadate-sensitive ATPases are inhibited by the presence of vanadate (Rogers and Anderson, 1987). Environmental factors like relative humidity are able to alter the effects of the ATPase inhibitor such as DNP (2,4-dinitrophenol). Low relative humidity (50%) increases K^+ efflux which could be inhibited 30-40% by DNP; however, at 90% RH DNP has no significant inhibition effect (Jensen and Kylin, 1980).

Fusicoccin (FC) is known to promote extrusion of H^+ from cells and thus enhance K^+ influx by stimulating H^+-ATPase activity (Marre, 1979). The availability of K^+ in the WFS might regulate FC-promoted H^+ extrusion (Marre et al., 1974) by its depolarizing activity on membrane potential (Marre et al., 1988). In the presence of ferricyanide, the reduction of which depolarizes membrane potential, FC-induced H^+ extrusion no longer requires the presence of K^+ in the medium (Marre et al., 1988). Treating leaves with fusicoccin could decrease leaf exuded sap (apoplastic) K^+ concentration, greatly reduce the pH rise, and completely eliminate the increase in ABA within the apoplast upon dehydration (Hartung et al., 1988). It was reported

that when applied FC to isolated membrane, a saturation effect could be reached below 1000 nM (De Michelis et al., 1989; Rasi-Caldogno et al., 1986). The saturation effect was proposed due to the saturation of the FC binding receptors in the plasma membrane (De Michelis et al., 1989).

2.2 K^+ *fluxes* ---

The zero net K^+ flux in leaves, an equilibrium between influx and efflux rates, occurs at an extracellular K^+ concentration of 1-5 mM as measured in leaf lamina tissue without much change throughout leaf ontogeny (Pitman et al., 1974b; Widders and Lorenz, 1983b). The concentration of 5 mM was interpreted relative to free space properties since it was the concentration in solution external to the leaf and not that in the cell walls of the leaf (Pitman et al., 1974b). The rates of active K^+ influx and efflux across cellular membrane are differentially regulated during leaf expansion and senescence. Jacoby and Dagan (1969) reported that both net flux and influx to the vacuoles increased during leaf expansion and decreased rapidly in senescing leaves. However, Widders and Lorenz (1983b) found that in the tomato, leaf age has a relatively large effect on influx rates but a much less effect on efflux. In the mature leaf tissue and the onset of leaf senescence, the rate of decline in influx rate was considerably slower. Thus, they claimed that the decrease in K^+ concentration in the mature leaf cannot be adequately explicated by a dramatic increase in K^+ efflux and a loss in the capability of leaf cells to accumulate K^+.

The K^+ influx efficiency was highest in very young leaves and declined dramatically during leaf expansion (Jacoby et al., 1973; Widders and Lorenz, 1982a, 1983a, 1983b; Woodhouse et al., 1978). Young leaves with high K^+ uptake capacity might be still in the phase described by Steward and Mott (1970) as "the formation of new structure in the cytoplasm" which needs K^+ specifically. The rapid decrease in K^+ influx in growing leaves was suggested to be related to a diluted concentration of K^+ transporter sites resulting from an increase in cell volume and weight. In the tomato, the mature leaf and the onset of senescence exhibited only a slow loss in K^+ uptake capability (Widders and Lorenz, 1983a). However other researchers (Friedrich and Huffaker, 1980; van Steveninck, 1976) found that ion uptake might be dramatically altered during leaf senescence as a result of numerous metabolic and physiological changes occurring within leaf tissue.

Leaf position and the whole plant developmental stage have little influence on K^+ influx into leaf cells. The differences in K^+ uptake rates for various leaf positions at each plant developmental stage were considered due to differences in physiological age of the individual leaves and not to inherent differences in absorption capability (Widders and Lorenz, 1983a). But the steepness of the K^+ concentration gradient between leaves in different nodal positions within a plant could be affected by the supply of K^+ to the root (Wakhloo, 1980). Tomato plants grown at low levels of K^+ exhibited a steep gradient between the apical and the basal leaves; this gradient became smaller as K^+ concentrations in solution increased. Under an ample supply of K^+, the available K^+ for mesophyll cell uptake in lower nodal positions was

increased due to the luxury accumulation of K^+ in cells (Wakhloo, 1980). Leaf cells responding to the increased K^+ concentration in the free space might be shown on an increase in net transport into the vacuoles (Pitman et al., 1974b) by increasing K^+-stimulated ATPase activity (Falk and Stocking, 1976) or increasing permeability of cells to ions.

Potassium efflux was generally considered as a passive process not requiring metabolic energy (Van Steveninck, 1976). But the induction of K^+ efflux by UV light was inhibited by CCCP and anaerobiosis implied that metabolic energy is required (Murphy, 1988; Murphy and Wilson, 1982). The need for energy was suspected to reflect a need for gene expression. By applying inhibitors of translation, cycloheximide and emetine, Murphy (1988) observed a stimulated net K^+ efflux from cultured cells of *Rosa damascena*. This efflux was transient; continued incubation for 21 hours could allow cells to recover the K^+. This author hypothesized that in the short term, a reduction in protein synthesis activity, on which the accumulation of K^+ depends, might stimulate the opening of the K^+ channel in the plasma membrane (Kohler el al., 1986) for K^+ efflux. In the long term, it was suggested that the inhibition of K^+ efflux might be due to the lack of continued synthesis of the protein with short life which serves as a positive regulator of the K^+ channel (Murphy, 1988).

Leaf age appears to have an impact on K^+ efflux. Potassium efflux rate was observed to decrease as the leaf aged (Dhindsa et al., 1981; Widders and Lorenz, 1983b). The rate of Rb^+ efflux from preloaded discs of *Nicotiana tabacum* exhibited a linear decline after full leaf expansion (Dhindsa et al., 1981). In the later stage of

senescence, K^+ efflux from cotyledons increased significantly (Ferguson and Simon, 1973). It was reported that at the onset of senescence, the cell membrane looses its integrity (Thompson, 1988); the synthesis of membrane proteins decreases and membrane proteins breakdown (Brady, 1988; Peoples and Dalling, 1988); the electrochemical potential gradient across the membrane may also change; thus, resulting in dramatically increased K^+ efflux.

In general, factors which would be expected to influence K^+ efflux rates also include the rate of active K^+ uptake across both the plasmalemma and tonoplast, the electrochemical potential gradients, the rate of exchange diffusion across the tonoplast (Raven, 1976), and the integrity and permeability of cellular membranes (Mengel and Kirkby, 1978). Increased turgor pressure might change resistance of symplastic connections, thereby increasing efflux without changes in membrane permeability (Ehwald et al., 1984).

2.3 Xylem transport ---

The regulation of K^+ transport from the root to the shoot and ultimately into individual leaves is important for determining the K^+ status of the leaf (Pitman, 1975) and fruit during plant development. A sufficient, constant rate of upward transport for supporting shoot growth requires the transporting organ of the root, the endoplasmic reticulum, to function parallel to the root cells (Lazof and Cheeseman, 1986). Rapid exchange occurs across the endoplasmic reticulum membrane with the

external medium responsible for delivery of ions to the xylem (Lazof and Cheeseman, 1988). The rate of K^+ transport to the shoot is controlled by the growth rate of the shoot and its meristematic activities (Pitman, 1972; Widders and Lorenz, 1982a), and depends on the concentration of K^+ in the xylem stream and the volume flux (Sutcliffe, 1976).

Lower net rates of K^+ transport via the xylem were found into older leaves than into younger leaves (Greenway and Pitman, 1965). This difference can be attributed to the increased diffusive resistances for water vapor exchange in mature and senescing leaves (Christopher, 1987; Davies et al., 1977; Friedrich and Huffaker, 1980) which results in a lower transpiration rate, thus reducing the net rate of K^+ import into leaves via the transpirational stream (Sutcliffe, 1976). However, K^+ transport to the shoot was not with the xylem K^+ flux and transpiration rate (Cheeseman and Wickens, 1986; Pitman and Wellfare, 1978). In younger plants, the shoot regulated the transport of K^+ from the root to the shoot by controlling the availability and translocation of metabolic substrates to the root (Jensen, 1978). In mature plants the reduced root growth activity and increased proportion of non-metabolically active parenchyma cells in the root reduce active ion uptake and transport into the stele without concomitant change in resistance to water entry, resulting in a decrease in the xylem sap K^+ concentration (Bowling, 1968; Jensen, 1978; Widders and Lorenz, 1982b, 1983a).

With respect to long distance transport of mineral nutrients, both the rates and the mechanisms facilitating fluxes within the xylem have been reported to be a

function of the time of day (Van de Geijn and Smeulders, 1981). For example, an important driving force for xylem flow during the dark period, especially in young seedlings (Jensen, 1980), is a positive root pressure. Conversely, during the daytime, negative pressures within the xylem resulting from transpirationally induced water potential gradients within the plants drive mass flow through the xylem.

It was postulated by Zimmermann (1978) that osmoregulation in higher plants may be mediated by a delicate pressure-sensing mechanism. At night, the closure of stomates (Karmoker and van Steveninck, 1979) could result in a positive pressure of the xylem contents counteracting with the root pressure. Consequently a lower potassium transport might occur if such a pressure-sensing mechanism was operative in xylem vessels. Apart from this consideration, perhaps more significantly, a decrease in transpiration rate (Jensen, 1978) is expected to reduce removal of ions from xylem vessels, hence, a higher concentration of ions is likely to build up. Allaway (1976) reported a Substantial increase in the concentrations of K^+ and Cl^- in the xylem sap extracted by the scholander bomb method when stomatal transpiration in *Vicia faba* decreased from 42.6 $mg/m^2/s$ in the light to 6.9 $mg/m^2/s$ in the dark. It was also shown that the partition of the labeled phosphate and $^{86}Rb^+$ via the xylem into leaves was proportional to the transpiration streams (Mauk et al., 1985; Neumann and Nooden, 1984).

The decrease in stomatal conductance and transpiration rate can be achieved in the day time by treating leaves with either vanadate or ABA (De Silve et al., 1985; Saxe and Rajagopal, 1981). In *Phaseolus vulgaris*, application of vanadate (100 μM)

was observed to reduce the stomatal conductance and transpiration rate by 50% within 2 hours (Saxe and Rajagopal, 1981). ABA, at 10^{-6} to 10^{-5} mM strongly inhibited stomatal opening of *Commelina communis* (De Silve et al., 1985).

Besides the diurnal changes in transpiration rate, the changes in phloem sap sugar concentration is another factor influencing xylem K^+ transport within a 24 hr period. In tree tobacco and *Lupinus angustifolius*, diurnal fluctuations in K^+ concentration within tracheal sap were in phase with daily changes in sucrose concentration within the phloem (Hocking, 1980; Hocking et al., 1978). The mean daily amplitude of the fluctuations in concentration of K^+ was reported around $\pm$ 32.5%.

Low relative humidity within the leaf canopy resulted in enhanced efflux rates of K^+ ($^{86}Rb^+$) from root cells as compared to the efflux at higher relative humidities (Jensen and Kylin, 1980). It appears that low relative humidity might generate a 'drought' condition (Jensen and Kylin, 1980), decrease water potential (Erlandsson, 1979) in shoots and then increase the water potential gradient along the xylem, leading to an increase in transpiration rate. The increased transpiration rate could result in more water and ions released to the xylem, thus, causing more K^+ transported into leaves. This view point is supported by the observation of the diurnal variation of exudation rate as well as ion concentrations in the cucumber (Masuda and Gomi, 1982).

The uptake of water and potassium is closely related to solar radiation, maximum uptake rates occur during the brightest part of the day (Adams, 1980;

Gislerod and Adams, 1983; Winser et al., 1980). Arrival of K^+ via the xylem into leaves in the light is about 5 to 10 times that during the darkness (Conti and Geiger, 1982). The ratio of potassium uptake to water is generally higher in the evening than during the morning (Gislerod and Adams, 1983). In addition, the respiration rate of the roots (Hansen and Jensen, 1977; Huck et al., 1962) which determines the active uptake of K^+ into root cells is proportional to the prevailing light intensity and is greater at the end than at the beginning of a photoperiod. Light intensity of the preceding day may influence the uptake rate.

Increasing K^+ concentration in the mineral solution from 2 mM to 10 mM resulted in a twofold and threefold increase in the rate of K^+ transported to the leaves within 5 to 10 min after raising the K^+ concentration of the root bathing solution (Conti and Geiger, 1982). The increase in K^+ transport rate did not parallel the increase in K^+ concentration. This result was interpreted as being due to a sequestering by the roots of up to 99% of the absorbed K^+ and thus reducing the availability of K^+ for subsequent transport by the xylem to the shoot (Bowling and Wlahhrley, 1964).

The K^+ concentration in the xylem stream has been shown according to position along the stem axis. In guttating tomato plants, a gradient of salt content within the xylem sap was observed; the lower K^+ concentrations were measured in the apical and higher in the basal portions of the stem (Klepper and Kaufmann, 1966). The gradient in xylem ionic concentration within the shoot of a plant can be attributed to the capacity of stem and petiole tissues to remove and release solutes from the

ascending xylem stream (Pate, 1975). Van Bel (1978) reported a threefold increase in the number of xylem vessels between young and old tomato stem internodes. This anatomical change might influence the absorption of K^+ by neighboring parenchyma tissues within the stem as it is transported through the xylem to individual leaves.

This diversion of transported K^+ via the xylem into the leaf apoplast might be due to the differences among leaves in stomatal conductance (Neumann, 1987), hydraulic resistance (Black, 1979; Koide, 1985; Neumann and Stein, 1984), and transpiration rates (Mauk et al., 1985; Neumann and Stein, 1984). The extraction of ions from cells along the xylem as well as xylem to phloem and xylem to xylem transfers (Van Bel et al., 1981; Van de Geijn and Smeulders, 1981; Wolterbeek et al., 1985) also alter the amount of potassium transported into the leaf apoplast.

2.4 Phloem export ---

Potassium retranslocation within the plant is thought to explain, to a large extent, the ontogenetic drift of total potassium content and concentration with leaf age. Young leaves export only a limited amount of potassium but import quite a lot of potassium as a result of phloem translocation from other plant organs. About 50% of the potassium taken up by the rapidly developing young leaf tissue is derived from within the plant (Greenway and Pitman, 1965). As a leaf approaches maturity, phloem export rates increase (Greenway and Pitman, 1965; Thrower, 1962) and retranslocation becomes most pronounced.

Greenway and Pitman (1965) reported an ontogenetic increase in phloem export of K^+ out of leaves. In three-leaf-seedlings of *Hordeum vulgare*, the rate of K^+ export from the youngest leaf is approximately 1.1 μeq/day and from the oldest, 2.8 μeq/day (Greenway and Pitman, 1965). Potassium is commonly present in the phloem sap at relatively high concentrations, which contributes to the high mobility of this ion in plants. Redistribution via the phloem is considered sufficient to account for the cessation of net potassium uptake in the leaf. It was suggested that different degrees of retranslocation during leaf development can arise as a result of competition between cells of the leaf and the retranslocation system (Greenway and Pitman, 1965).

Potassium concentration in the phloem sap is in range of 30 to 100 mM (Hall and Baker, 1972; Ziegler, 1975). In young seedlings the concentration may be higher. Scherer (1982) found K^+ concentrations in wheat seedlings between 100-170 mM, with concentrations <100 mM K^+ resulting in significant growth depressions. This concentration is much higher than that within the leaf apoplast (Bernstein, 1971; Harvey et al., 1971; Jacobson, 1971; Long and Widders, 1990). Thus, K^+ is thought to be actively transported into phloem tissue. This process has been suggested to involve H^+/K^+ exchange ATPase (Giaquinta, 1983; Malek and Baker, 1978) to balance the electropotential gradient caused by the proton/sucrose system (Baker, 1985) for sucrose loading.

Accordingly, it seems reasonable to propose that K^+ loading to the phloem might parallel sucrose loading. In soybeans, 'Amsoy 71', there is a higher assimilate

export at night, whereas 'Wells II' has a higher day time export (Mullen and Koller, 1988). One would probably expect a higher K^+ export rate at night in 'Amsoy 71' and higher rate during the day time in 'Wells II'.

Conti and Geiger (1982) reported that the rate of K^+ export in the sugar beet was a function of the time of day, estimated to be 0.8 to 2.6 μeq/dm/h during the day and 0.5 to 1.5 μeq/dm/h at night. Total amounts of both daytime and nighttime export might rise with increasing photon flux density (Mullen and Koller, 1988). The external potassium supply to the root can also influence the K^+ movement in the phloem. With an abundant K^+ supply, the osmotic potential of the phloem sap would be low, the water potential in the phloem sap thus decreases and induces more water uptake to the sieve tube/companion cell complex, resulting in a stronger push which then increases the flow rate. The phloem sap flow rate can be nearly twice as high in the treatment with a higher K^+ supply (Mengel and Haeder, 1977). A net loss of 0.5 μeq/day was observed from leaves in potassium deficient wheat seedlings while 1.0 μeq/day, from well fertilized seedlings (Ward, 1958). Geiger and Conti (1983) found that with an ample K^+ supply, K^+ is rapidly translocated from older to younger leaves. The higher flow rate was not found to result in a dilution of the phloem sap solutes.

CHAPTER 1

Modifiers of Ion Transport Alter Apoplastic Potassium Content in Leaf Mesophyll Tissue of *Pisum sativum* cv. Argenteum

INTRODUCTION

The apoplast is an important extracellular compartment along with the symplast (intracellular space) within leaf tissue (Pitman, 1975; Robinson, 1971). A dynamic equilibrium is believed to exist between the apoplastic and symplastic solutes (Cosgrove and Cleland, 1983a; Hancock, 1977; Meinzer and Moore, 1988) as influenced by changing electro-chemical potentials across the plasma membrane. Pitman et al. (1974b) proposed that the K^+ status of leaf cells is regulated primarily by the "free space" potassium concentration. Potassium concentration within the apoplast of leaf tissue however has not been well characterized and probably varies depending upon the rate of import via the xylem and phloem, export in the phloem (Pitman et al., 1974b) and net uptake into the symplasm (Jensen, 1980; Widders and Lorenz, 1980).

ATPase inhibitors (Bowman et al., 1978; Cocucci et al., 1980) or stimulators (De Michelis et al., 1989; Marre, 1979; Rasi-Caldogno et al., 1986) such as vanadate and fusicoccin have been reported to modulate the H^+/K^+ exchange system across

the plasma membrane, modifying the pH and K^+ concentration in the extracellular medium. Much ion transport research has been conducted using isolated protoplasts or vesicles with relatively little work research evaluating transport kinetics in intact plant tissue. In intact leaves, the effects of import/export of ions via the xylem and phloem on the ionic composition of the extracellular environment surrounding leaf mesophyll cells needs to be investigated to fully understand the regulation of K^+ accumulation in leaf lamina tissue.

Potassium is involved in phloem loading through the H^+/K^+ exchange system (Giaquinta, 1983; Malek and Baker, 1978) which maintains a high K^+ content/concentration in the phloem to balance the charge resulting from the proton/sucrose cotransport system (Dolros and Bonnemain, 1985). Phloem loading is inhibited by PCMBS and ABA (Baker, 1985; Turgeon and Wimmers, 1988) and promoted by Fusicoccin (Malek and Baker, 1978). Ion transport via the xylem includes ion uptake by the root, transport from the root to the stele (Karmoker and Van Steveninck, 1979) and the xylem flow rate. Both ABA and vanadate may affect the stomatal conductance which regulates xylem flux rate by affecting ATPase activity (Saxe and Rajagopal, 1981; Snaith and Mansfield, 1982). The potential influence of altered phloem and xylem transport rates on the ionic composition of the apoplast still remains unclear.

The objectives of this study are: a) to determine with transport inhibitors and stimulators how the various ion transport processes influence apoplastic K^+ content and concentration, and b) to gain a better understanding of the role of the apoplast

as a pathway for K^+ distribution and as a compartment influencing K^+ uptake and accumulation by leaf mesophyll cells.

MATERIAL AND METHODS

Plant Material

Argenteum (*Pisum sativum*) peas were cultured in a hydroponic system containing half-strength modified Hoagland solution (Hoagland and Arnon, 1950; Johnson et al., 1957) in a growth chamber or greenhouse. The growth conditions in the controlled environment chambers were characterized by day/night temperatures of 25/23 C, 75% relative humidity, an irradiance 380 level of E m^{-2} s^{-1} at the top of the canopy, and a 16 hr photoperiod. The growing conditions in the greenhouse were 21-23 C temperatures, 50-80% relative humidity, and an approximately 16 hr photoperiod (May, 1989, in East Lansing Michigan). Recently expanded mature leaves from nodes 7 to 10 counting acropetally from the plant base at vegetative and early flowering stages were selected for treatment and apoplastic K^+ measurement.

A randomized complete block experimental design was used. Means were compared using a LSD test when the F value for treatments was significant.

Phloem Blocking -- Steam Girdling

Leaf petioles to be steam girdled were supported by a split wooden toothpick. Care was taken not to mechanically damage petiole and leaf lamina tissue. Steam was then directed at a 0.5 cm exposed region of the petiole using a transfer pipette nozzle for about 5 sec. A cardboard shield was used to avoid damage to other parts of the leaf or plant. Petioles of control plants were treated with water at room temperature.

Antitranspirant Treatment

To avoid the difficulty of removing the epidermis under the sticky, heavy mantle of antitranspirant, a 10% suspended solution was applied to achieve only a thin layer of antitranspirant covering the leaf lamina surface. Only one leaflet from a petiole was briefly dipped into the antitranspirant solution and allowed to air dry. The control leaflet on the opposite side of the petiole was bathed in water only. Under growth chamber conditions, treated leaves dried out in about 30 min.

Treatment with Promoters and Inhibitors

p-chloromercuribenzenesulfonic acid (PCMBS), ABA, and fusicoccin (FC) were each dissolved in water. Vanadate was dissolved either in water (solution pH was

around 11) or in a 1 mM Na-phosphate buffer adjusted to pH 6.6. A 10 μl micro syringe with a 0.11 mm diameter needle was used to inject 10 μl of various solutions into the pulvinus. Deionized water was injected in control plants. Apoplastic K^+ content was shown to remain relatively constant in control leaves during the period following injection of treated leaves. Preliminary experiments using RbCl as a label have demonstrated that injected solutes are transported via the xylem into the leaflet lamina tissue within 5 min. The injection did not cause any rapid wounding effect (Table I).

The response of the inhibitor and promoter injection was evaluated utilizing the following equation: ((d - c) / c) - ((b - a) / a),
where a and b equal the experimentally determined apoplastic K^+ content of the control measured before and after injection, respectively. c and d equal the experimentally determined apoplastic K^+ content of the specific treatments sampled before and after injection, respectively.

Estimation of Apoplastic K^+

The quantity of apoplastic K^+ from leaf lamina tissue was estimated by an elution method (Long and Widders, 1990). Leaflets were detached from petioles at predetermined time intervals after treatment and before elution analysis. Immediately after detachment of leaflets from plants, the leaflets were placed between moist, ice-cold filter papers in a plastic weighing boat floating on slushy ice. After leaflets were

detached from the plants, remaining procedures were conducted in the weighing boats on slushy ice in order to maintain tissue temperature near 1 C. A glass cylinder, 0.75 cm I.D. x 0.8 cm height, was then gently attached to the mesophyll using a silicon sealant. A 300 μl aliquot of 5 mM $CaCl_2$ solution (1°C)was pipetted into each cylinder. After predetermined periods (2, 5, 10, 20, 30, 40, 50 and 60 min), a 200 μl sample was withdrawn from each cylinder and replaced with 200 μl of fresh $CaCl_2$.

Each sample was placed into a 4 ml plastic vial containing 400 μl of 1000 ppm CsCl in 1% HCl. Samples were analyzed for K^+ by atomic emission spectrophotometry. The leaf apoplastic K^+ content was estimated from the time course curve of eluted K^+ as described by Long and Widders (Long and Widders, 1990). Estimation of the apoplastic K^+ concentration was based on the assumption that the apoplastic volume was approximately 0.1 ml/g fr wt.

Transpiration Rate and Stomatal Conductance

Transpiration rate and stomatal conductance were measured using a LI-1600 steady state porometer (LI-COR. INC/LI-COR, Ltd.). For leaflets treated with antitranspirant, measurements were taken after the leaf surface was visibly dry.

RESULTS

Steam Girdling

Steam girdling is a method used to thermally destroy petiole cells including sieve tube and companion cells. Phloem transport is thought to be temporarily blocked while xylem transport is not significantly limited. Wilting of girdled leaves was observed within approximately 2 min after treatment. A reduction of stomatal conductance and transpiration was noted within the same time period (Fig. 1). The wilted leaves regained turgidity within approximately 2 hr.

K^+ accumulated rapidly within the apoplast of petiole-steam-girdled leaves (SGL) (Table II). At 20 min through 24 hr after girdling, apoplastic K^+ content for the SGL ranged from 6.19 to 8.09 μeq/g fr wt as compared to 2.84 to 4.61 μeq/g fr wt for the controls. There was no detectable difference in total leaf K^+ between SGL and the control. The younger leaves, one or two nodes above the treated leaves, were found not to be affected by the steam girdling treatment within 24 hr in both total leaf K^+ and apoplastic K^+ content (Table III).

PCMBS, evaluated as a potential phloem transport inhibitor, was injected into the pulvinus to avoid mechanical damage to the petiole tissue and thus contamination of the xylem with K^+. PCMBS applied at 1 mM (net 1 x 10^{-2} μmole injected) did not affect total leaf K^+ and apoplastic K^+ within 2 hr of treatment (Table IV). When PCMBS was increased to 5 mM (equals to 5 x 10^{-2} μmole) a significant increase in

Figure 1. **Short term effect of steam girdling on transpiration rate and stomatal conductance. Steam girdling was applied at time zero. Measurement were collected from three leaves from different plants.**

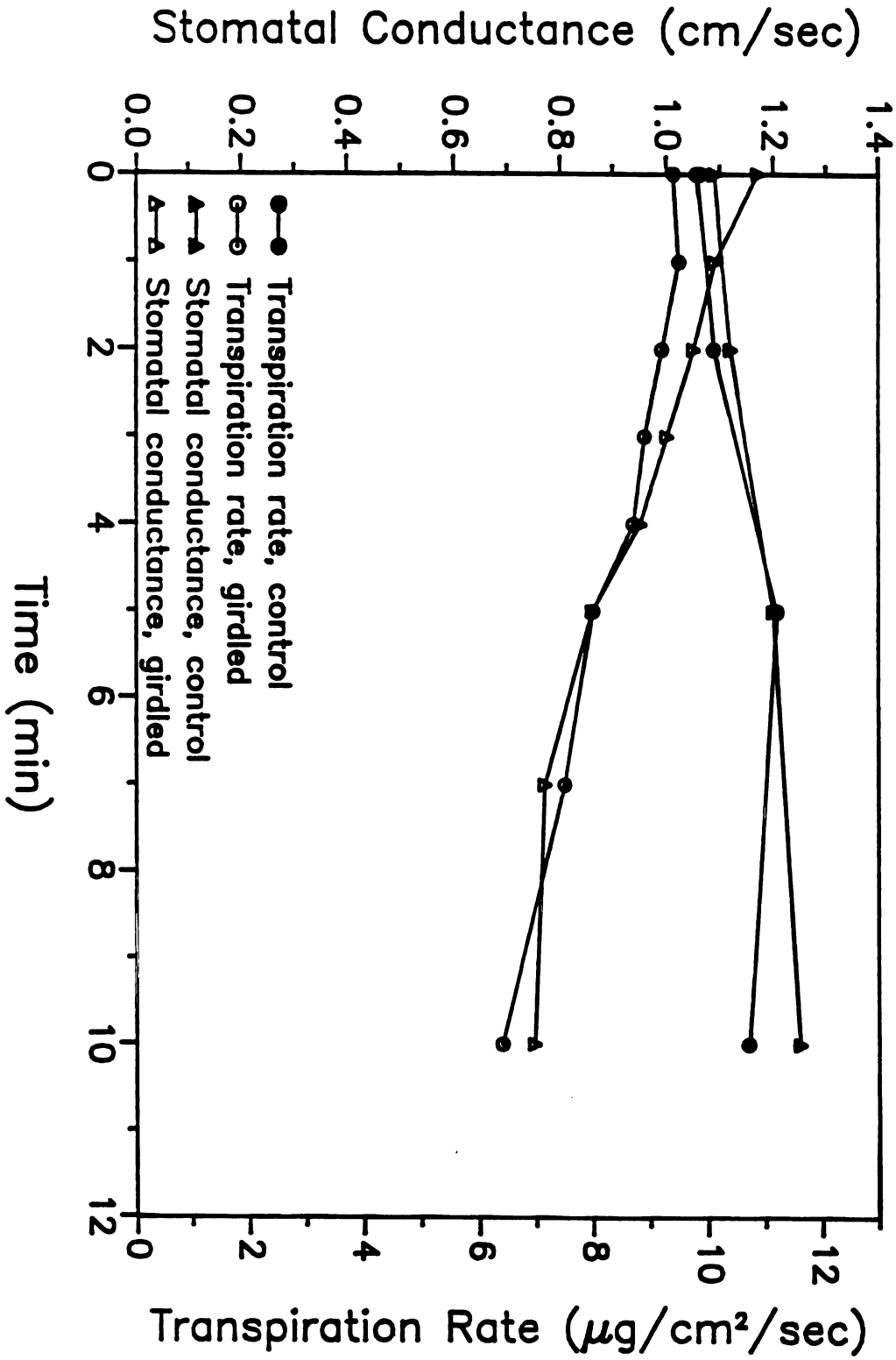
Stomatal Conductance (cm/sec)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Time (min)
0
2
4
6
8
10
12
Transpiration Rate (μg/cm²/sec)
0
2
4
6
8
10
12
Transpiration rate, control
Transpiration rate, girdled
Stomatal conductance, control
Stomatal conductance, girdled

Table I. *The effect of PCMBS as compared to water (control) injection into the pulvinus on total leaf and apoplastic K^+ contents after 20 min.*

Treatment		Total Leaflet K^+	Apoplastic K^+ Content	Apoplastic K^+
Chemical	Time[a]			
	min	*μeq/g fr wt*	*μeq/g fr wt*	*% of total*
Control	0	143.91	6.19	4.30
	20	144.19	6.17	4.28
F test		NS	NS	NS
PCMBS[b]	0	155.24	8.86	5.71
	20	157.52	8.82	5.60
F test		NS	NS	NS

[a] The measurement at 0 min was taken just before injection. The measurement at 20 min was made 20 min after injection.

[b] The concentration of PCMBS was 1 mM.

Table II. *Effect of steam girdling on apoplastic and total K^+ content in mesophyll tissue of Argenteum pea leaves in relation to time after steam girdling.*

Control petioles were treated with water at room temperature. Petioles of leaves at nodal position 5 or 7 were steam girdled. Each value is the mean of 6 leaves.

Time	Treatment Girdling	Total Leaflet K^+	Apoplastic K^+ Content	Apoplastic K^+
		μeq/g fr wt	*μeq/g fr wt*	*% of total*
0.3	0[a]	60.77	2.84	4.67
	+	62.66	7.30	11.65
3.0	0	56.14	3.80	6.77
	+	61.41	7.42	12.08
6.0	0	51.79	4.61	8.90
	+	57.77	8.09	14.00
24.0	0	54.63	3.60	6.59
	+	52.69	6.19	11.75
Average	0	55.83	3.72	6.59
	+	58.63	7.25	12.37
Time		NS	NS	NS
Treatment		NS	*[b]	*

[a] "0" and "+" indicate with and without steam girdling treatment.

[b] F value for treatment (steam girdling) effect significant at 5% level.

Table III. *Effects of steam girdling on apoplastic and total K^+ content in mesophyll tissue in Argenteum pea leaves one or two nodes above steam girdle treated leaf.*
Control petioles were treated with water at room temperature. Petioles of leaves at nodal position 5 or 7 were steam girdled. Each value is the mean of 6 leaves.

Girdling	Total Leaflet K^+	Apoplastic K^+ Content	Apoplastic K^+
	μeq/g fr wt	*μeq/g fr wt*	*% of total*
0[a]	55.24	2.47	4.47
+	55.68	2.10	3.77
F test	NS	NS	NS

[a] "0" and "+" indicate with and without steam girdling treatment.

Table IV. *Relative effects of PCMBS and ABA injections into the pulvinus of pea leaves on the total leaf and apoplastic K^+ content in mesophyll tissue.*

ABA injection immediately following PCMBS injection. Both chemicals were injected at the same volume. Chemical injected leaves were sampled 150 min after injection.

Chemical	Conc	Total Leaflet K^+	Apoplastic K^+ Content	Apoplastic K^+ (% of total)
	mM			
PCMBS	0	0.00	0.00	0.00
	1	0.23	0.81	0.41
	5	0.09	1.05	0.96
	10	1.10	-1.88	-2.11
	20	0.02	-1.88	-1.94
PCMBS +	10	0.08	0.07	-0.07
ABA (0.1)	20	-0.04	-0.01	0.06
LSD 5%		NS	0.87	0.69

apoplastic K^+ was observed. At higher concentrations of PCMBS (10 and 20 mM), however, apoplastic K^+ content was depleted within a 2 hr period, even though total leaflet K^+ remained unaltered. Both 10 and 20 mM concentrations caused mosaic-water-soaked spots in leaves on one or both sides of the main vein, sometimes towards the acropetal portion soon after injection. Within 24 hr these water-soaked spots became dark green or yellow. The elution procedure was conducted in the area without visible mosaic-water-soaked spots. Both the decline in apoplastic K^+ content and mosaic-water-soaked spots were avoided if ABA 0.1 mM (equals to 10^{-3} μmole) was injected immediately following the injection of 10 or 20 mM PCMBS.

Transpiration Reduction

The antitranspirant application reduced stomatal conductance and leaf transpiration by nearly 40% (6.22 $\mu g/cm^2/sec$: 10.27 $\mu g/cm^2/sec$) and 50% (0.61 : 1.20 cm/sec), respectively (Table V). Since only a 10% suspended solution was applied, the leaf surface was evenly but not fully covered by antitranspirant after the leaf dried. The apoplastic K^+ content declined to 65% of the control within 20 min (4.13 μeq/g fr wt : 6.31 μeq/g fr wt) and decreased to 33% within 3 hr (1.38 μeq/g fr wt : 4.17 μeq/g fr wt). Total leaflet K^+ did not change within the 3 hr period. With time after antitranspirant application, the apoplastic K^+ fraction, % of total K content in lamina tissue, became increasingly smaller.

Table V. *Effect of antitranspirant on total and apoplastic K^+ content in mesophyll tissue of Argenteum pea leaflets at specific time after application.*

Antitranspirant was applied by submersing leaflets into solution for a few sec. Each value is the mean of 6 values. "*" means significant at 5% level.

Time	Treatment Antitrans.	Total Leaflet K^+	Apoplastic K^+ Content	Apoplastic K^+
min		*μeq/g fr wt*	*μeq/g fr wt*	*% of total*
5	0[a]	67.57	4.56	6.75
	+	64.27	4.91	7.64
20	0	75.12	6.31	8.40
	+	71.28	4.13	5.79
180	0	72.46	4.17	5.75
	+	70.46	1.38	1.96
Average	0	71.72	5.01	6.97
	+	68.67	3.37	5.13
F test				
Time		NS	*	*
Treatment		NS	*	*
Time x Treatment		NS	**[b]	**

[a] "0" and "+" indicate with and without antitranspirant application.

[b] F value for interaction of treatment x time significant at 1% level.

Abscisic Acid

The response to ABA injection was concentration dependent. ABA at 0.1 to 1 mM (net injected, 1 x 10^{-3} - 10^{-2} μmole) clearly reduced apoplastic K^+ levels relative to controls (Table VI) without altering total leaf K^+. No significant effect was observed when 0.05 mM (net injected, 5 x 10^{-4} μmole) ABA was injected. Injection of 1mM ABA also reduced transpiration by approximately 30% (9.22 $\mu g/cm^2/sec$ vs. 13.09 $\mu g/cm^2/sec$) and stomatal conductance by 33% (0.89 cm/sec vs. 1.33 cm/sec) as compared to controls (data not presented).

Fusicoccin and Vanadate

Vanadate treatment in both Argenteum and the normal pea did not affect transpiration rate or stomatal conductance (Table VII), as reported in wheat (45), regardless of the concentration at which it was applied or the solvent (Na-phosphate buffer or water only) in which vanadate was dissolved. In addition, total leaf K^+ and apoplastic K^+ did not differ from controls (Table VIII, IX).

Fusicoccin, on the other hand, significantly reduced apoplastic K^+ content without a change in total leaf K^+ level (Table X). Similar responses to fusicoccin were observed within the concentration range of 250 μM to 1000 μM.

Table VI. *Total and apoplastic K^+ content in mesophyll tissue as a function of ABA concentration in solution injected into pulvinus of Argenteum pea leaves.*

ABA injected leaves were sampled 150 min after treatment. Each value is the mean of 12 values.

ABA	Total Leaflet K^+	Apoplastic K^+	% of Apoplastic K^+ in 1 Leaf
mM			
0.00	0.00	0.00	0.00
0.05	-0.05	-0.18	-0.04
0.10	-0.03	-0.82	-0.79
1.00	-0.10	-0.81	-0.66
LSD 5%	NS	-0.77	NS

Table VII. *Transpiration rate and stomatal conductance of fully expanded Argenteum pea leaflets as influenced by vanadate concentration.*

Vanadate	Phosphate	Time	Stomatal Conductance[a]	Transpiration[a]
mM	*mM*	*hr*	*cm/sec*	*μg/cm²/sec*
		Argenteum		
0		0	1.00	1.00
0	10	2	1.05	1.16
0	10	5	1.08	1.08
0	10	8	0.99	1.05
0.15	10	3	0.97	1.00
0.15	10	6	0.97	0.98
0.15	100	2	1.06	1.10
0.15	100	5	1.04	1.07
0.15	100	8	1.01	1.01
F test			NS	NS
		Normal pea		
1	100	1.5	0.87	0.90
10	100	1.5	0.80	0.86
100	100	1.5	0.92	0.90
1	100	2.5	0.91	0.92
10	100	2.5	0.89	0.90
100	100	2.5	0.97	0.92
F test			NS	NS

[a] Calculation of presented values: (d/c) / (b/a). a, water control at time zero; b, water control at time X; c, treatment at time zero; d, treatment at time X. Control, injected deionized water. Each treatment (in terms of time course or concentrations) had its own 'water control'.

Table VIII. *Effect of vanadate (VAN) injection on total and apoplastic K^+ content in mesophyll tissue of Argenteum pea leaflets as a function of time.*

Vanadate 10 μl (10 mM) was injected into leaf pulvinus. Each value is the mean of 4 values.

Time	Treatment vanadate	Total Leaflet K^+	Apoplastic K^+	% of Apoplastic K^+ in 1 Leaf
hr				
2.5	0[a]	0.00	0.00	0.00
	+	-0.01	-0.54	-0.53
5.0	0	0.00	0.00	0.00
	+	0.22	0.05	-0.23
30.0	0	0.00	0.00	0.00
	+	0.15	0.95	0.65
Time		NS	NS	NS
Treatment		NS	NS	NS

[a] "0" and "+" indicate with and without vanadate injection.

Table IX. *Total and apoplastic K^+ content in mesophyll tissue as a function of vanadate (VAN) concentration in solution injected into the pulvinus of Argenteum pea leaves.*

Vanadate injected leaves were sampled 120 min after treatment. Each value is the mean of 3 values.

Vanadate	Total Leaflet K^+	Apoplastic K^+	% of Apoplastic K^+ in 1 Leaf
mM			
0	0.00	0.00	0.00
1	-0.10	0.10	0.23
10	-0.07	0.97	0.82
100	-0.12	0.40	0.59
LSD 5%	NS	NS	NS

Table X. *Total and apoplastic K^+ content in mesophyll tissue as influenced by concentration of fusicoccin (FC) injected into the pulvinus of Argenteum pea leaflets.* Fusicoccin injected leaves were sampled 180 min after treatment. Each value is the mean of 6 values.

Fusicoccin	Total Leaflet K^+	Apoplastic K^+	% of Apoplastic K^+ in 1 Leaf
μM			
0	0.00	0.00	0.00
250	-0.04	-1.29	-0.92
500	0.02	-1.55	-1.53
1000	-0.04	-1.47	-1.25
LSD 5%	NS	-1.11	-1.25

DISCUSSION

According to Pitman's model for ion compartmentation and transport in leaf tissue, the K^+ content and concentration within the functional apoplast should be altered if 1) phloem loading and export K^+ from the leaf were blocked (Baker, 1985), 2) stomatal conductance and thus the import rate of K^+ via the transpirational stream through the xylem were reduced (De Silva et al., 1985; Snaith and Mansfield, 1982), or 3) if flux rates across the plasmalemma of mesophyll cells were altered through the use of an ATPase inhibitors or promoters (Baker, 1985; Rijven and Gifford, 1983; Schmitt et al., 1984).

Although steam girdling has been used to temporarily block phloem transport (Hall et al., 1971; Touraine et al., 1988), the observed accumulation of K^+ within the leaf apoplast within 20 min after steam girdling (Table I) probably does not indicate a cessation in phloem export and thus a reduction in ion loading from the apoplast. The net increase in apoplastic K^+ within 20 min after steam girdling is extremely high, 4.45 μeq/g fr wt (higher than what would be anticipated if phloem loading were simply inhibited).

Relatively few estimates of ion (e.g. K^+) transport rates via the phloem have been reported in the literature. Moreover the phloem export rate to a large extent depends on the nutrient supply (Geiger and Conti, 1983; Mengel and Haeder, 1977; Ward, 1958), leaf age (Greenway and Pitman, 1965), the time of day (Conti and Geiger, 1982) and other environmental factors. If one assumes that the calculated

transport rates of Conti and Geiger (1982) (2.6 μeq/dm/hr) and of Greenway and Pitman (1965) (2.8 μeq/day) are reasonable and representative of K^+ movement in the pea, such a large increase in apoplastic K^+ should theoretically not occur. This is supported by the observation that PCMBS did not increase apoplastic K^+ within 2 hr (Table II).

In addition, the short term increase in apoplastic K^+ after steam girdling cannot be attributed to an enhancement in xylem flow rates. In fact, wilting of the leaves was observed within 2 min after steam girdling which suggests that the resistance to xylem flow had been significantly increased. The anticipated result would be a reduction in the xylem flow rate and thus a reduction in the import rate of ions via the xylem rather than an increase if xylem solution ionic concentration were to remain constant.

The short term increase in K^+ within the leaf apoplast might be the result of K^+ which had leaked out and subsequently been redistributed via extracellular water from the heat destroyed petiole tissue. Additionally, the membrane permeability of the leaf mesophyll cells might have been temporarily altered in response to the wounding (steam girdling) of the petiole tissue (Gronewald and Hanson, 1980; Zimmermann and Steudle, 1980; Zocchi and Hanson, 1982), giving rise to enhanced K^+ efflux from the cells.

PCMBS, which has been shown to be an inhibitor of sucrose loading and phloem transport in the pea (Estruch et al., 1989; Turgeon and Wimmers, 1988) and other plant species (Giaquinta, 1976), was found to enhance apoplastic K^+. PCMBS (5 mM) injected into the pulvinus resulted in a significant increase in apoplastic K^+

(Table IV). Since K^+ influx into sieve tubes driven by H^+-ATPase is recognized to balance the proton charge as a result of sucrose/proton cotransport, phloem loading of K^+ from the apoplastic pathway can not be neglected.

The recognition site of PCMBS on the plasma membrane is still unequivocal. It was suggested that PCMBS might have two sites of inhibitory action: one at the sucrose carrier and the other at the ATPase-mediated proton pump (Giaquinta, 1983). The lack of effect of PCMBS on the movement of Lucifer Yellow in *Ipomea tricolor* (Madore et al., 1986), being transported through the symplastic pathway regulated by ATPase activity (Erwee and Goodwin, 1985; Madore et al., 1986), supports the suggestion that the action of PCMBS is predominantly on the sucrose carrier (sucrose/proton exchange system). Lichtner and Spanswick (Lichtner and Spanswick, 1981) reported that sucrose-induced depolarization of the membrane potential could not be induced by the addition of sucrose to the PCMBS-treated cotyledons indicating that PCMBS inhibits the sucrose carrier. However, PCMBS was also shown to inhibit K^+-stimulated ATPase activity of cell membranes isolated from corn roots (Giaquinta, 1980) and K^+ uptake into root cells (Lin, 1980). Thus the accumulated K^+ within the apoplast after injecting PCMBS might be ascribed to: a) the direct inhibition on ATPase activity, or b) the indirect inhibition on activity of H^+/K^+ exchange ATPase resulting from the inhibition of the sucrose/proton exchange system. Moreover, regardless of the mechanism of PCMBS inhibition in phloem loading, the reduction in the ion volume flow through the phloem will result in an accumulation of intercellular K^+ within sieve tube and companion cells, thus

limiting the continued uptake of K^+ from the apoplast.

The opposite effect of PCMBS, which depletes apoplastic K^+ at higher concentrations (10-20 mM), is believed to be related to the toxic effects which may or may not produce mosaic-water-soaked spots. ABA injection (10^{-3} μmole) immediately following PCMBS injection was found to totally prevent this effect (Table IV). The leaves did not show any toxic symptoms. It is difficult to propose a satisfactory interpretation for the relationship between PCMBS and ABA. It might be possible that ABA binds to a specific membrane receptor, which consequently alters the membrane potential (Hartung et al., 1980) or the three dimensional conformation of the membrane, in a manner affecting the action of PCMBS.

Fusicoccin, a catalyzer of H^+-ATPase activity, is known to stimulate K^+ uptake and thus deplete the extracellular K^+ content (Hartung et al., 1988; Marre, 1979). It also has been reported to be a phloem loading promoter (Malek and Baker, 1978). The observed decline in apoplastic K^+ in FC treated leaves as compared to controls (Table X) reveals that K^+ within the apoplast is either taken up into mesophyll cells or loaded into the phloem for export. FC-induced activation of the plasma membrane H^+-ATPase is through the binding of FC to its plasma membrane receptor (De Michelis et al., 1989) and saturates at a concentration below 100 μM when FC is applied to isolated membrane systems (De Michelis et al., 1989; Rasi-Caldogno et al., 1986). The data presented showed that the saturation effects were achieved when a finite amount, 2.5 μmoles, was injected into the leaves (Table X). FC is thought not to interact with the sucrose/proton exchange system in the promotion of phloem

loading. FC is believed to directly stimulate H^+-ATPase.

Both the application of the antitranspirant (Table V) and of ABA (Table VI) had similar effects on K^+ accumulation within the leaf apoplast. Volume flux through the xylem into the leaf was thought to have been reduced due to a reduction in stomatal conductance and ultimately in net transpirational water loss from the leaf (Karmoker and Van Steveninck, 1979). The observation that stomatal conductance and transpiration rate had only been reduced by 40-50% within three hr by the application of antitranspirant suggests full coverage of the leaf surface may not have been achieved. The 67% decrease in apoplastic K^+ (Table V) indicates the significant importance of xylem flow in supplying ions to the mesophyll apoplast. The effect of antitranspirant probably does not affect K^+ export via the phloem and uptake into mesophyll cells. In other words, antitranspirant would not be expected to specifically affect ATPase activity in leaf lamina tissue. ABA-induced stomatal closure, on the other hand, is known to affect ATPase by binding to its receptor on the plasma membrane (Hornberg and Weiler, 1984).

It was reported that there is a considerable transient K^+ efflux out of guard cells induced by ABA-related stomatal closure (MacRobbie, 1981; Weyers and Hillman, 1980). It seems unlikely that the mesophyll apoplastic K^+ level would be influenced significantly by the K^+ efflux out of guard cells due to the air barrier between the epidermis and the underlying mesophyll tissue in the Argenteum mutant of pea (Hock et al., 1980; Mark, 1982).

It is believed that the effects of antitranspirant and ABA injection on stomatal

conductance and transpiration rate were not necessarily the result of an altered leaf water potential. The decrease in stomatal conductance could occur without a change in leaf water status (Bates and Hall, 1981; Gollan et al., 1986). Therefore, a recent report that lower turgor triggered higher phloem loading of sucrose (Estruch et al., 1989), which would result in a decrease in apoplastic K^+, is thought not to contribute to the observed decrease in K^+ within the apoplast.

Vanadate is one of the inhibitors modulating ATPase activity. It was reported that in barley leaves vanadate could reduce stomatal conductance and transpiration rate by 50% within 2 hr at a concentration of 100 μM and 40 μM, respectively (Saxe and Rajagopal, 1981). In this study, no such results were obtained either by increasing the reaction time course or by raising the concentration up to 100 mM (Table VIII, IX). Since vanadate did not even produce a stomatal response in the normal pea, Argenteum and the normal pea probably lack a vanadate-sensitive ATPase in the plasma membrane. It has been reported that a vanadate-insensitive ATPase was present in leguminous species such as bean (Rogers and Anderson, 1987).

This study shows that apoplastic K^+ content is highly responsive to changes in K^+ flux across the plasmamembrane, and in the rates of xylem import and phloem import/export. The apoplast is suggested to function as a temporary ion (e.g. K^+) pool from which ions can be uptaken into mesophyll cells or redistributed extracellularly to surrounding lamina tissue. However, further studies are necessary to confirm such changes in apoplastic K^+ content, such as which might occur

diurnally or during plant ontogeny in an intact plant system.

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CHAPTER 2

Diurnal Changes in the Leaf Potassium Status of the Argenteum Mutant of Pea

INTRODUCTION

It is well established that numerous plant physiological processes exhibit diurnal changes in activities such as growth rate (Schnyder and Nelson, 1988; Schnyder et al., 1988), mineral transport (Hocking et al., 1978; Van de Geijn and Smeulders, 1981), photosynthesis (Kalt-Torres and Huber, 1987; Kalt-Torres et al., 1987; Usuda et al., 1987), assimilate export (Bernadette and Geiger, 1982), enzyme activities (Sieciechowicz et al., 1985; Vassey, 1989), and leaf movement (Lee and Satter, 1987). Long distance transport of mineral nutrients from the root via the xylem, both the volume flux rates and ionic concentrations within the xylem have been reported to vary depending on time of day (Hocking, 1980; Hocking et al., 1978; Hosokawa and Kiyosawa, 1983; Teng, 1990a). The mechanisms facilitating xylem flow are also different during a diurnal period of time (Van de Geijn and Smeulders, 1981). For example, an important driving force for xylem flow during the dark period, especially in young seedlings (Jensen, 1980), is a positive root pressure. Conversely, negative pressure in the xylem during the light period, which results from transpirationally induced water potential gradients, drives mass flow through the xylem. The rates of

mineral and assimilate export via the phloem are a function of the time of day and vary depending on the plant species (Kalt-Torres and Huber, 1987; Mullen and Koller, 1988).

It has been hypothesized (Pitman et al., 1974b) that the ionic composition of the free space within leaf mesophyll tissue is regulated in part by the rates of import and export to and from the leaf via the xylem and phloem and by ion fluxes across the plasmamembrane into the symplast (Jensen, 1980; Teng, 1990a; Widders and Lorenz, 1983a). Thus, diurnal fluctuations in ionic concentration would be expected to occur within the apoplastic compartment. Leaf mesophyll apoplastic ionic concentrations, as influenced by the time of day, have not been investigated previously. It cannot be assumed that these concentrations fluctuate in parallel to changes in xylem sap ionic concentrations.

The objectives of the present study, therefore, are: 1) to evaluate potential diurnal changes in K^+ concentration and content within the mesophyll apoplast of pea leaves, 2) to gain a better understanding of the role of the apoplast as a compartment modified by changes in xylem flux rate and thus influencing K^+ uptake and accumulation into leaf mesophyll cells.

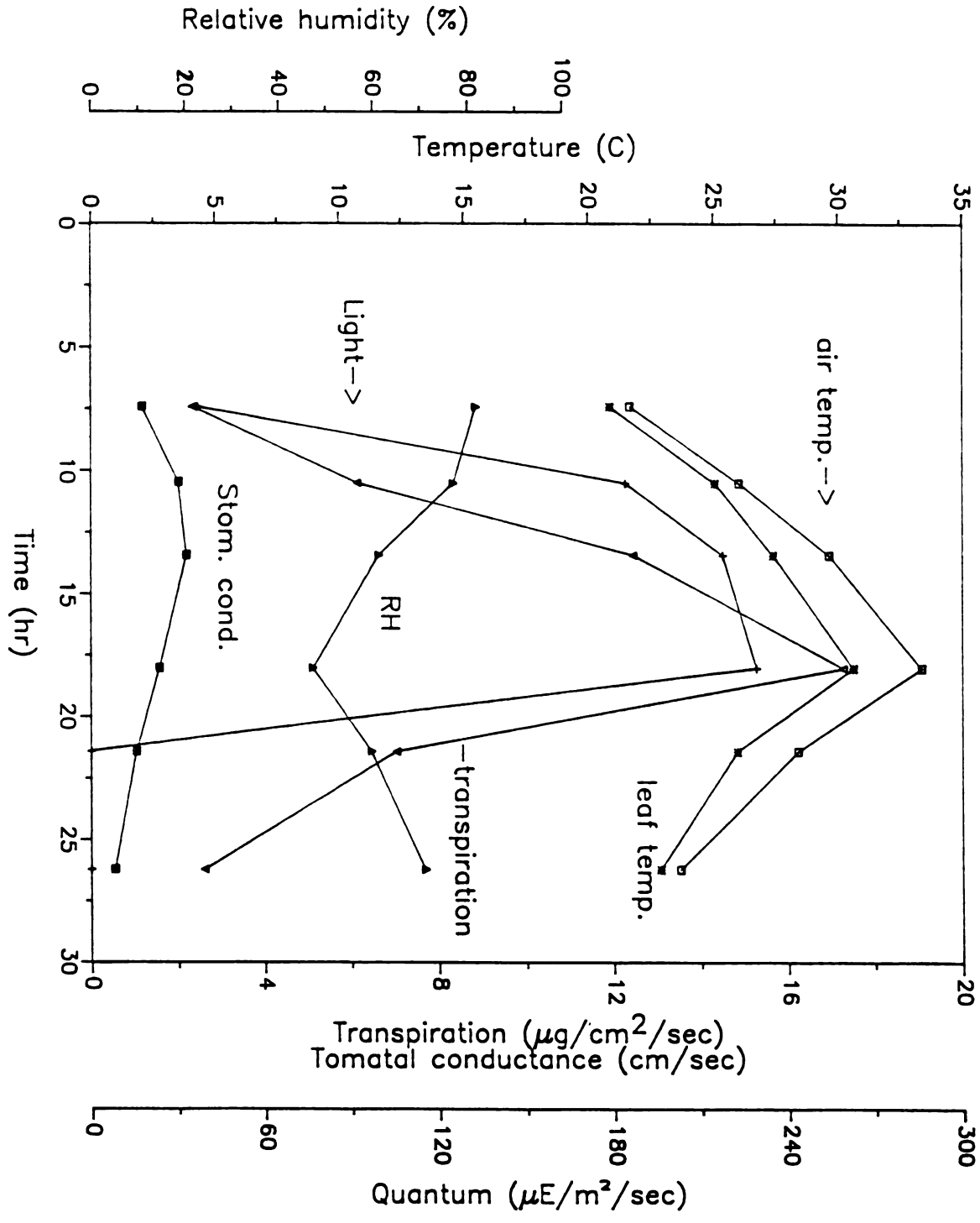
MATERIAL AND METHODS

Plant Culture

Pea (*Pisum sativum* cv. Argenteum) seeds, presoaked for one day in water, were planted into Turface (Blue Mountain Co.) in a growth chamber or a research greenhouse at Michigan State University. Seedlings were transferred to containers with nutrient solution (8 plants/30 l) prior to the unfolding of the second or third leaf. The nutrient solution (a half strength modified Hoagland solution (Hoagland and Arnon, 1950; Johnson et al., 1957) containing 3 mM K^+ was replaced weekly. All experiments were performed at least twice. Growth conditions in the controlled environment chamber were a 16-hr photoperiod, 25/23 C day/night temperature, 75% humidity and 350-400 μmole m^{-2} s^{-1} irradiance level.

Greenhouse experiments were conducted during August 1987 and May 1989. Although the results were similar in both experiments, the magnitude of the diurnal fluctuations of the measured parameters were significantly greater in August when larger diurnal variation occurred within the greenhouse environment. Only summer data is presented. Typical environmental conditions over a 24-hr period in the greenhouse is presented (Fig. 1). In August, the light period extended from 06:00 to 21:30 hr with maximum quantum on sunny days above 1000 μmole m^{-2} s^{-1}. Since maximum expansion of leaflets was achieved at lower irradiance levels, a shading screen was provided to lower the quantum to nearly 400 μE m^{-2} s^{-1}.

Figure 1. **Diurnal changes in greenhouse environment (temperature, irradiance and relative humidity) and leaf temperature, transpiration and stomatal conductance in Argenteum pea.**


Relative humidity (%)
0
20
40
60
80
100
Temperature (C)
0
5
10
15
20
25
30
35
Time (hr)
0
5
10
15
20
25
30
Light—>
air temp.—>
Stom. cond.
RH
—transpiration
leaf temp.
0
4
8
12
16
20
Transpiration (μg/cm2/sec)
Tomatal conductance (cm/sec)
0
60
120
180
240
300
Quantum (μE/m2/sec)

Irradiance levels at the surface of the leaf canopy increased rapidly after dawn, followed by a slower increase after 10:00 hr and reached a maximum level (240 μmole m^{-2} s^{-1}) at 19:00 hr (Fig. 1). Air temperature ranged from 21.5 C at 07:00 hr, to a high of 33.5 C at 19:00 hr and then declined rapidly. The leaf temperature was consistently lower than the air temperature, ranging from 0.5 C at dawn and 3.5 C lower at 19:00 hr. Relative humidity was 85% in the early morning, decreasing to 50% in the afternoon hours and increasing again at night.

Leaves 7 or 8 counting acropetally from the base were selected for elution experiments from both locations. Elution analysis was initiated one hour after the beginning of the light period and continued at approximately three hour intervals during a diurnal period. Six randomly selected fully expanded leaflets from separate plants were measured each time. Leaf temperature, stomatal conductance and transpiration rate were measured from the same six plants before leaf sampling. Xylem sap was collected from growth chamber plants.

Elution Procedure

Apoplastic K^+ content from leaf lamina tissue was estimated by an elution method (Long and Widders, 1990) which minimizes mechanical damage and ageing effects (Van Steveninck, 1976) to the mesophyll tissue. Cell damage during the experimental procedure was almost undetectable (Long and Widders, 1990). Immediately after detaching, leaflets were placed between, moist, ice-cold (1 C) filter

papers in a plastic weighing boat floating on slush ice. The following procedures were conducted in the weighing boat. The leaflets were prerinsed with deionized water and the abaxial epidermis was detached with care. A glass cylinder (75 mm I.D. x 80 mm height) was then gently attached to the mesophyll tissue using a silicon sealant. A 300 μl aliquot of 5 mM ice-cold $CaCl_2$ solution was pipetted into each cylinder from which a 200 μl sample was withdrawn and replaced with the same amount of fresh $CaCl_2$ after predetermined periods (2, 5, 10, 20, 30, 40, 50 and 60 min). Each sample collected was transferred to a 4 ml plastic vial containing 400 μl of 1000 ppm CsCl in 1% HCl. Samples were analyzed for K^+ by atomic emission spectrophotometry.

Leaf apoplastic K^+ content was estimated from the time course curve of eluted K^+ as described by Long and Widders (Long and Widders, 1990), Estimation of K^+ concentration in the apoplastic solution was based on the assumptions that the apoplastic volume was 0.1 ml/g fr wt.

Collection and Analysis of Xylem Sap

A Scholander pressure chamber (Soilmoisture Equipment Corp.) was used to collect xylem sap. Plants from which leaves were selected for elution experiments were used for collection. The stem was cut at the third or fourth internode and then placed into the pressure chamber along with moist towels to maintain a high internal relative humidity. After the cut stem surface was rinsed with deionized water, a pressure sufficient to induce exudation was applied gradually. The first 3-5 μl of sap

were discarded. The subsequent 10 to 20 μl of xylem sap collected were diluted with 1000 ppm CsCl in 1 % HCl for K^+ analysis by atomic absorption spectrophotometry.

Measurement of Transpiration and Stomata Conductance

Transpiration rate and stomatal conductance were measured with a LI-1600 steady state porometer (LI-COR. INC/LI-COR, Ltd.) from the same plants in which leaflets were harvested for elution analysis.

Calculation of Estimated Potassium Transported into Leaflets

The rate of K^+ import into a leaf via the xylem was estimated using the equation:

$J_s = J_v (1-@) * C * A$ where

J_s - total and active solute fluxes (μeq/g fr wt/min)

C - xylem sap concentration (solute concentration, mM)

@ - reflection coefficient of the solute (assumed to be 0 in xylem)

J_v - total volume flux (transpiration rate, $cm^3/cm^2/sec$ or ml/sec)

A - leaf area (cm^2/g fr wt)

RESULTS

Greenhouse Experiments

Transpiration rate exhibited a large daytime increase in response to high temperatures and low relative humidities at midday (Fig. 1). Minimum diurnal transpiration rates (approximately 2 $\mu g/cm^2/sec$) were observed in the early morning and during the night period. Maximum transpiration rates (approximately 17 $\mu g/cm^2/sec$), measured between 18:00-20:00 hr, coincided with the time when the irradiance, the temperature and the air-leaf temperature differential were at their highest levels. Maximum stomatal conductance (2.5 cm/sec) was measured at midday, with partial stomatal closure occurring during the afternoon and evening hours.

Total K^+ content in leaf tissue changed significantly during the diurnal period for leaves at the three stages of leaf maturity evaluated (Table I). The largest increases in total leaf K^+ content (up to 25 $\mu g/g$ fr wt) were observed between approximately 10:00-17:00 hr, in parallel with increases in transpiration rate, irradiance and air/leaf temperature (Fig. 1). In rapidly expanding young leaves, the enhanced K^+ content was somewhat maintained during the subsequent night period. However, as the leaflets matured, a larger nocturnal reduction in total leaflet K^+ content was observed.

The diurnal fluctuations in apoplastic K^+ content ranged from 2.7 to 6.6, 0.4 to 8.4 and 2.2 to 10.6 $\mu eq/g$ fr wt for the three leaf developmental stages (rapidly

Table I. *Diurnal changes in total K^+, apoplastic K^+ from mesophyll tissue at three stages of leaflet ontogeny from greenhouse cultured pea plants.*

Time	Total K^+	Apoplastic K^+	
		content	Concentration[a]
hr	*μeq/g fr wt*	*μeq/g fr wt*	*mM*
	Rapidly expanding leaf		
7:30	50.6	2.66	26.6
10:45	54.7	4.78	47.8
13:45	66.7	4.50	45.0
16:50	64.0	6.60	66.0
20:15	65.3	2.69	26.9
23:05	73.0	4.88	48.8
2:30	72.9	3.84	38.4
LSD 5%	8.5	3.13	31.3
	One day after full leaf expansion		
7:30	53.8	2.05	20.5
10:45	52.6	4.71	47.1
13:45	73.3	7.98	79.8
16:50	85.1	8.41	84.1
20:15	60.1	5.24	52.4
23:05	81.3	4.73	47.3
2:30	70.5	0.43	4.3
LSD 5%	10.5	4.60	46.0
	Ten days after full leaf expansion		
7:30	54.4	5.01	50.1
10:45	57.3	4.65	46.5
13:45	67.6	6.06	60.6
16:50	77.0	10.59	105.9
20:15	66.1	7.31	73.1
23:05	74.1	4.71	47.1
2:30	61.3	2.23	22.3
LSD 5%	11.8	5.22	52.2

[a] Assume the volume of apoplastic is 0.1 ml/g fr wt

expanding leaves, recent fully expanded leaves and leaves ten days after fully expansion) (Table I). Assuming that the volume of the apoplast is approximately 0.1 ml/g fr wt (Long and Widders, 1990), the range of apoplastic K^+ concentration for these three leaf developmental stages would be 27-66, 5-84 and 22-106 mM, respectively. Both total leaflet K^+ and apoplastic K^+ exhibited concurrent diurnal fluctuations. The percent of apoplastic K^+ relative to the total K^+ content only varied from 3.6% to 13.8%. The amount of durnal change in total leaflet K^+ content consistently exceeded the change in apoplastic K^+ content.

Growth Chamber Experiment

A midday increase in transpiration rate, stomatal conductance (Table II), and apoplastic K^+ content and concentration (Table III) in plants cultured in the controlled environment chamber occurred in spite of the fact that leaf and air temperatures were maintained constant (Table II). The magnitude of the diurnal change (increase) in apoplastic K^+ was not as high as that observed in greenhouse cultured plants although apoplastic K^+ levels were significantly higher in the growth chamber plants. The apoplastic K^+ reached a diurnal maximum level of 12.06 μeq/g fr wt at approximately 9 hr after the light period began (Table III) which coincided with the time of maximum transpiration rates (19 μg/cm/sec) and the highest stomatal conductance (1.6 cm/sec) were measured (Table II). A positive correlation between the transpiration rate and apoplastic K^+ was found ($r = 0.77$) (Fig. 2).

Compared to apoplastic K^+ content, the highest total leaflet K^+ occurred 3 to 6 hr later (Table III). The percentage of apoplastic K^+ relative to the total was in the range of 3.05 to 6.85%.

Xylem sap K^+ concentrations, in contrast, started increasing one to two hr before the night period began and reached a maximum of about 22 mM within the first hr during the night period (Table IV). Similarly, the osmotic potential of the xylem sap began increasing later in the light period and remained high at approximately 67 m mole/Kg (0.17 MPa) throughout the dark period. The estimated K^+ imported into the leaf apoplast via the xylem as a function of xylem sap K^+ concentration and transpiration rate gradually increased to a maximum of 0.77 μeq/g fr wt/min later in the light period. It then decreased to a minimum of 0.17 μeq/g fr wt in the mid dark period (Table IV) when the highest xylem sap K^+ concentration (Table IV) and the lowest transpiration rates were measured (Table II).

Table II. *Diurnal changes in leaf/air temperature, stomatal conductance and transpiration from leaves in growth chamber cultured plants.*

Light period was from 07:00 to 23:00 hr. Each value is the mean of 8 plants.

Time	Temperature		Stomatal Conductance	Transpiration
	Air	Leaf		
hr	*°C*		*cm/sec*	*μg/cm²/sec*
07:45	23.18	21.76	0.99	7.14
10:23	23.18	21.76	1.00	10.95
13:16	24.40	22.41	1.45	16.22
16:23	24.60	22.28	1.61	18.78
19:09	24.73	22.63	1.58	19.05
22:03	24.88	23.10	1.07	13.91
23:40	22.95	21.38	1.02	8.12
04:58	23.50	22.51	0.60	4.95
06:07	23.55	22.45	0.58	6.95
LSD 5%	NS	NS	0.12	1.28

Table III. *Diurnal changes in total K^+, apoplastic K^+ and intracellular K^+ from mesophyll tissue in growth chamber cultured pea plants.*

Light period was from 07:00 to 23:00 hr. Each value is the mean of eight data. The apoplastic volume is assumed to be 0.1 ml/g fr wt.

Time	Total	Apoplastic K^+	
	Leaflet K^+	content	conc
hr	*μeq/g fr wt*	*μeq/g fr wt*	*mM*
07:45	155.37	6.08	60.75
10:23	143.32	6.10	60.95
13:16	157.98	6.15	61.53
16:23	169.59	12.06	120.57
19:09	171.20	9.49	94.88
22:03	183.45	9.97	99.65
23:40	159.92	4.74	47.36
04:58	178.21	5.47	54.68
06:07	177.42	5.06	50.59
LSD 5%	9.31	5.73	57.29

Figure 2. Correlation analysis between transpiration rate and leaf apoplastic K content. Data is from one of the diurnal experiments in greenhouse. Different symbols represent different position on a leaflet. ($r = 0.77$).

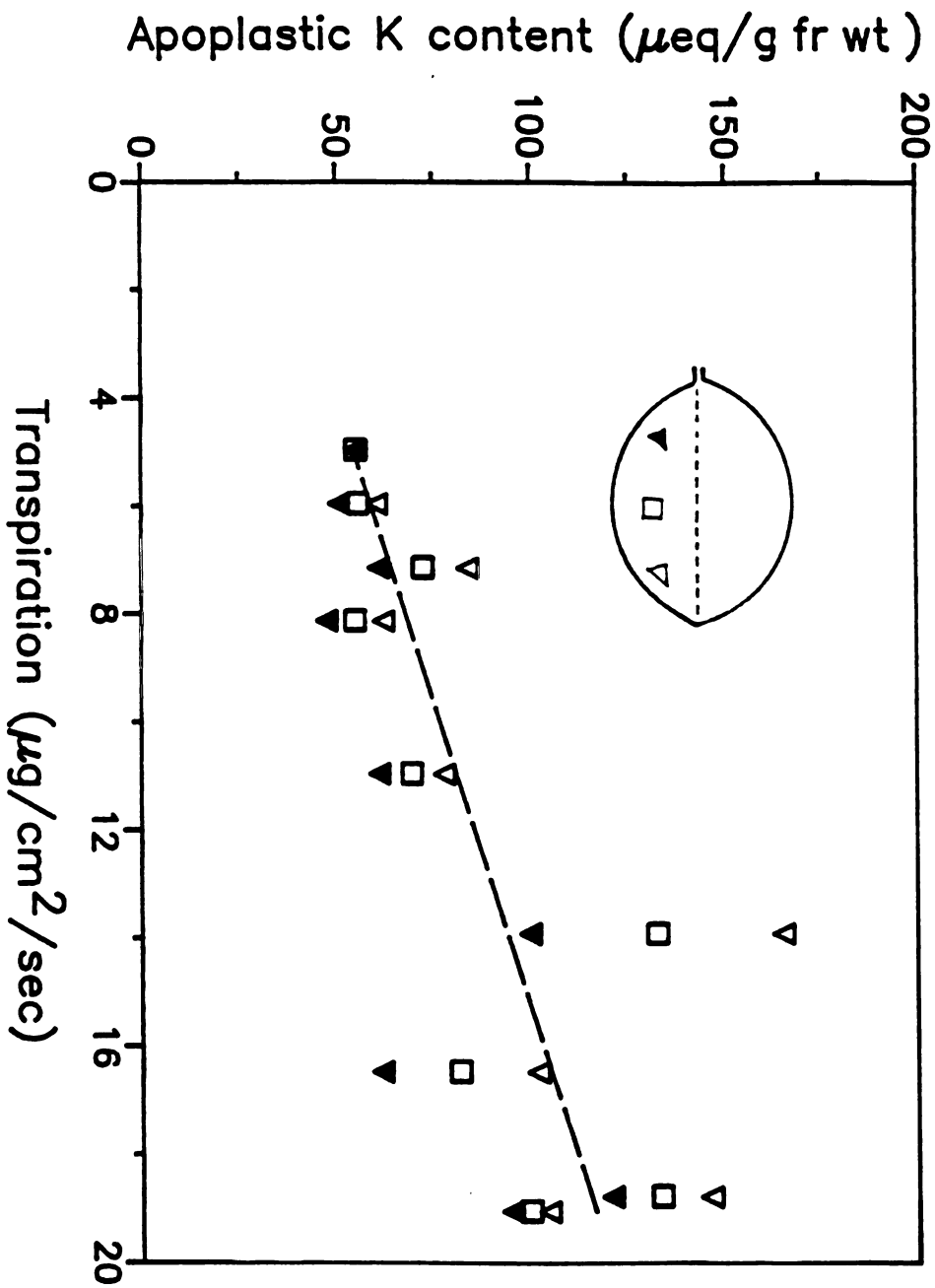

Apoplastic K content (μeq/g fr wt)
0
50
100
150
200
Transpiration (μg/cm²/sec)
0
4
8
12
16
20

Table IV. *Diurnal changes in osmotic potential, xylem sap K^+ and estimated K^+ transported via the xylem into the leaf apoplast from growth chamber cultured plants.* Light period was from 07:00 to 23:00 hr. Each value is the mean of eight values.

Time	Osmalalitity	Xylem Sap K^+	Estimated K^+ Import via the Xylem
hr	*MPa*	*mM*	*μeq/g fr wt /min*
07:45	0.0829	8.24	0.33
10:23	0.0896	9.86	0.40
13:16	0.0804	9.05	0.54
16:23	0.0768	9.63	0.67
19:09	0.0731	8.82	0.60
22:03	0.1151	14.62	0.77
23:40	0.1383	21.66	0.58
04:58	0.0975	10.50	0.17
06:07	0.1633	14.04	0.29
LSD 5%	0.0311	5.18	0.35

DISCUSSION

The increase in apoplastic K^+ content and concentration during the day time is thought to be largely determined by a high rate of xylem K^+ import into the leaf resulting from high midday transpirational water loss from the leaves. Apoplastic K^+ content and concentration were observed to fluctuate diurnally (Table I, III) in parallel to estimated fluxes of xylem sap K^+ into the leaf (Table IV). Evidence is consistent with the hypothesis that ions within the xylem vessels (within the vascular bundles) in leaf lamina tissue can freely diffuse into the leaf apoplast. This suggests that the apoplast may constitute an important compartment with low resistance facilitating K^+ distribution to mesophyll cells within the interveinal region of the leaf lamina. It must be recognized, however, that gradients in ionic concentration may exist within the apoplast (cell wall) even though they could not be detected by the elution method.

Potassium import into a leaf should be considered as being a function of the volume flow rate of the xylem and the K^+ concentration of the xylem sap. However, the actual amount of K^+ being ultimately transported into the target leaf is also affected by additional factors: the degree of K^+ binding to fixed negatively charged sites in the xylem vessels, the concentrations of other cations within the xylem solution, the extraction of ions from cells along the xylem as well as xylem to phloem and xylem to xylem transfers (Pitman, 1965; Sieciechowicz et al., 1985; Van Bel et al., 1981; Wolterbeek et al., 1985) under different transpiration rates.

Xylem K^+ concentrations do not predict K^+ content and concentration within the leaf apoplast as being evidenced by xylem sap K^+ concentrations in the dark period being higher than in the day time (Table IV). In contrast, day time transpiration rates were 2 to 4 times higher than night transpiration rates (Fig. 1, Table II). The partitioning of labeled phosphate and ^{86}Rb via the xylem into leaves was reported to be in proportion to transpirational water loss (Mauk et al., 1985; Neumann and Nooden, 1984). The previous study (Teng, 1990a) showed that apoplastic K^+ level was significantly reduced when stomatal conductance and transpiration rate were inhibited by an antitranspirant. A positive relationship between transpiration and apoplastic K^+ was also obtained after correlation analysis (Fig. 2). It appears that the volume flow rate of the xylem is the primary factor influencing K^+ supply to the leaf apoplast during a diurnal period.

During the period of high transpiration, which typically occurs at midday, K^+ fluxes into mesophyll cells from the apoplast might not keep pace with the rate of xylem import, thus resulting in an increase in apoplastic K^+ content. Assuming no net change in the apoplastic volume, K^+ concentration would be expected to increase temporarily within the apoplast. Potassium concentration within the apoplastic compartment therefore appears to be highly sensitive to fluctuations in xylem volume flow rates into the leaf rather than K^+ influx into mesophyll cells.

The apoplastic K^+ concentrations measured during the diurnal period were higher than the extracellular concentrations reported to be necessary to achieve a zero net flux in leaf lamina tissue (Widders and Lorenz, 1983b). This indicates that

apoplastic K^+ concentrations would drive net K^+ uptake into mesophyll cells even at night. The observation of the decline in total leaf K^+ content in fully expanded mature leaves which occurs during the night when apoplastic K^+ concentrations were still relatively high suggests that phloem export is actively occurring (Table I). Therefore, phloem loading might not only involve uptake from an apoplastic pool of K^+ in the mesophyll tissue, although the previous study (Teng, 1990a) did show blockage of phloem export resulted in an increase in apoplastic K^+ concentration. Whether the symplastic or apoplastic pathway is the dominant pathway for phloem loading of K^+ from mesophyll cells to companion cells and sieve tubes during a diurnal course needs to be further investigated.

Leaf developmental stage seems to have an impact on the maximum levels of apoplastic K^+ content and concentration during a diurnal course. The decrease in K^+ influx into mesophyll cells with leaf age (Widders and Lorenz, 1983a) would be expected to contribute to the higher daytime maximum levels of apoplastic K^+ in mature leaves. Potassium uptake into mesophyll cells may be in response to an efflux of H^+ from the symplasm which is necessary for cell wall loosening (Cleland, 1987) during expansive growth. This may explain the decrease of apoplastic K^+ in expanding leaves.

It was hypothesized (Clipson et al., 1985; Flowers and Yeo, 1988) that the control of osmotic pressure of the free space solution within certain limits would act as a mechanism to buffer the cells from changes in the external water conditions. The increased apoplastic K^+ content and concentration in the afternoon (Table I, Table

III) would significantly reduce the extracellular solution osmotic potential by approximately -0.65 to -1 bar which supports this hypothesis. This decreased osmotic potential might give rise to a net efflux of water from the mesophyll cells in response to a water potential gradient, diluting the apoplastic solution and thus preventing further reduction of cell turgor. The decrease in osmotic potential might also increase the water potential gradient along the pathway of water movement in a transpiring plant, thus facilitating water transport through the xylem.

In conclusion, the leaf apoplast acts as an ion pool within leaf mesophyll tissue. Potassium concentrations within this compartment fluctuate in response to changes in the environmental conditions especially those factors influencing transpiration rate, and leaf growth. Xylem K^+ import rate is considered to be the most significant factor affecting the diurnal changes in K^+ contents within the leaf apoplast. Since apoplastic K^+ concentrations are much higher than the theoretical threshold concentration needed for a zero net K^+ flux across the plasma membrane, this favors net K^+ influx into mesophyll cells. It is suggested that phloem loading of K^+ may involve more than the apoplast.

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CHAPTER 3

Ontogenetic Changes in Potassium Content within Leaf Tissue of Argenteum Mutant Pea

INTRODUCTION

Potassium concentration in leaf lamina tissue is influenced by leaf age (Jacoby et al., 1973; Waughman and Bellamy, 1981; Widders and Lorenz, 1983b), the position of the leaf on the shoot axis (Wakhloo, 1980; Widders and Lorenz, 1983a), the developmental stage of the plant (Widders and Lorenz, 1982a), as well as environmental factors (Erlandsson, 1979; Glass, 1989; Jensen and Kylin, 1980). In many plant species, leaf K^+ concentration declines during leaf ontogeny (Waughman and Bellamy, 1981; Widders and Lorenz, 1982b). The reduction is ascribed primarily to K^+ export to other growing tissues where K^+ is needed to facilitate numerous biophysical and metabolic processes associated with growth (Hocking and Pate, 1977; Nooden, 1984; Widders and Lorenz, 1982a).

Studies on ontogenetic changes in K^+ flux in leaf tissues showed that an extracellular K^+ concentration of 1.0 to 3.5 mM (Widders and Lorenz, 1983b) is required to achieve zero net K^+ flux. Jacoby and Dagan (1969) reported that net Na^+ flux in bean significantly increased during leaf expansion and decreased rapidly in

senescing leaves. A possible deduction from these studies would be that an ontogenetic decline of leaf K^+ level results from a reduction in extracellular K^+ concentration below the threshold level which might ultimately result in a net efflux out of leaf cells (Widders and Lorenz, 1983b).

Although such a hypothesis appears reasonable, recent research (Teng, 1990b) examining diurnal changes in apoplastic K^+ content in recently expanded mature leaves suggests that apoplastic K^+ concentrations are higher than originally reported, <20 mM (Bernstein, 1971; Jacobson, 1971; Harvey et al., 1981). At midday, apoplastic K^+ concentrations of 100-120 mM (Teng, 1990b) were estimated assuming conservative estimate of apoplastic volume, 0.1 ml/g fr wt. K^+ flux kinetics within such a high K^+ concentration apoplastic environment suggested a net influx into the mesophyll cells. Flowers and Yeo (1988) proposed that leaf apoplastic ionic concentrations were significantly higher than that within the xylem. Xylem sap K^+ concentrations are usually higher than the concentration needed for zero net flux (Widders and Lorenz, 1983b).

An ontogenetic decrease in extracellular K^+ concentration in leaf tissue does not appears to be conducive to a depletion in K^+ content within the leaf mesophyll due to a K^+ concentration driven net efflux. Resolution of this controversy necessitates the analysis of apoplastic K^+ concentrations and contents within leaf lamina tissue in relation to ontogenetic changes in leaflet K^+. This study was conducted to examine the ontogenetic changes in leaf apoplastic K^+ concentration, utilizing an elution method (Long and Widders, 1990). The results are discussed with

respect to the regulation of K^+ transport and accumulation in leaf tissue of the pea as well as the possible role of apoplastic K^+.

MATERIAL AND METHODS

Plant culture

Pea (*Pisum sativum* cv. Argenteum) seeds were presoaked in water for two days and then sown in Turface (product of Blue Mountain Co.) in a controlled environmental room. Seedlings were transferred to half strength modified Hoagland solution (Hoagland, 1950; Johnson et al., 1957) containing 3 mM K^+ nutrient solution (8 plants/30 l) prior to the unfolding of the second leaf. The nutrient solution was replaced every week. The growing conditions were: day/night temperature of 25/23 °C, 75% humidity, irradiance levels of 320-370 μmole m^{-2} s^{-1} and a 16hr photoperiod.

Elution procedure

Apoplastic K^+ content and K^+ efflux rates from leaf lamina mesophyll tissue were estimated by an elution method (Long and Widders, 1990). In the elution method, intact leaves rather than narrow leaf slices or discs were used. This elution method is considered superior because surgical and ageing effects (Van Steveninck,

1976) are minimized, cell integrity is maintained and cell damage during the experimental procedure is almost undetectable (Long and Widders, 1990).

Leaflets from leaves 6 and 7 counting acropetally were sampled at one or two day intervals prior to full leaf expansion. Subsequent sampling of leaflets during maturation and the onset of senescence was conducted every four to six days. Immediately after detachment, the leaflets were placed between, moist, ice-cold filter papers in a plastic weighing boat floating on slush ice. Procedures conducted after detaching leaflets from plants were conducted on weighing boats at 1 C.

The leaflets were rinsed briefly with deionized water and the abaxial epidermis was carefully detached. A glass cylinder of 7.5 mm I.D. with 8.0 mm height was then gently attached with silicon sealant to mesophyll at both basipetal and acropetal positions of the leaflet. A 300 μl aliquot of 5 mM ice-cold $CaCl_2$ solution was pipetted into each cylinder. Following predetermined time periods (2, 5, 10, 20, 30, 40, 50 and 60 min) a 200 μl sample was withdrawn from each cylinder and replaced with 200ul of fresh $CaCl_2$.

Each sample was transferred into a 4 ml plastic vial containing 400 μl of 1000 ppm CsCl in 1% HCl. Samples were analyzed for K^+ by atomic emission spectrophotometry.

Equations for determination of apoplastic potassium (K_t), Donnan Phase potassium (K_d) and potassium concentration in apoplastic solution (K_s)

$K_s = (K_t - K_d) / v$

$K_d = e * (1 - (S_{max}\, e\, (-(X_1 * K_s + X_2 * K_s^2))$

K_t - total estimated eluted potassium from apoplast (μeq/g fr wt)

K_d - estimated amount of potassium bound to Donnan phase (μeq/g fr wt)

K_s - concentration of potassium in solution within apoplast (μeq/ml)

V - estimated volume of the apoplast (0.1 or 0.04 ml/g fr wt)

S_{max} - adsorption capacity of the Donnan phase

X_1, X_2 - constants

Leaf apoplastic K^+ content was estimated from the time course curve of eluted K^+ as described by Long and Widders (1990). Estimation of K^+ concentration in the apoplastic solution was based on assumptions of the apoplastic volume (0.04 to 0.1 ml/g fr wt) and sorption characteristics of the Donnan phase. Simultaneous equations were used to calculate K^+ concentration in the apoplastic solution (K_s).

Measurement of transpiration and stomata conductance

Transpiration rate and stomatal conductance were measured with a LI-1600 steady state porometer (LI-COR. INC/LI-COR, Ltd.). Six plants were randomly

selected for each sampling. Data were usually collected in the middle of the light period.

Collection and analysis of xylem sap

A Scholander pressure bomb (Soilmoisture Equipment Corp.) was used to collect xylem sap from the stem of pea plants. Collections were made from the same plants used for transpiration measurement. The plant stem was cut at the third or fourth internode in vegetative plants and at the sixth or seventh internode in mature plants. The cut shoot was then placed in a pressure chamber along with moist towels to maintain high humidity. Following rinsing of the cut stem surface with deionized water, a pressure of approximately 7-9 bars was applied gradually to induce exudation. The first 3-5 μl of sap were discarded. The subsequent 10 to 20 μl sample of xylem sap collected was diluted with 1000 ppm CsCl in 1 % HCl for K^+ analysis.

Calculation of estimated potassium transported into leaflets

The rate of K^+ import into a leaf via the xylem was estimated using the following equation:

$J_s = J_v (1-@) * C * A$

J_s - total and active solute fluxes (μmole/g fr wt/min)

C - xylem sap concentration (solute concentration, μM)

@ - reflection coefficient of the solute (usually close to 0 in xylem)

J_v - total volume flux (transpiration rate, $cm^3/cm^2/sec$ or ml/sec)

A - leaf area (cm^2/g fr wt)

RESULTS

Leaflet expansion ceased approximately 6 days following unfolding from the stipules (Fig. 1). Concomitantly, leaf area per unit fresh weight declined rapidly during leaf expansion and continued to decline slowly as the leaflets matured. The first flower was observed when plants were about 26 days old. Rapid seed filling occurred between 48 to 54 days.

Total K^+ concentration in leaflet tissue was relatively high in expanding tissue, approximately 90 to 128 μeq/g fr wt, but declined slowly as leaves matured (Fig. 2). The basipetal portion of the leaflet consistently contained more K^+ than the acropetal portion.

Total apoplastic K^+ content in leaflet mesophyll tissue was highly variable especially in acropetally positioned tissue, ranging from 1 to 4 μeq/g fr wt (Fig. 3). When K^+ partitioning between the fixed and the solution phase within the apoplast was evaluated, assuming a total cation exchange capacity of 2.627 μeq/g fr wt for the DFS (Long and Widders, 1990), the Donnan phase was found to be nearly fully saturated throughout the entire period of leaf growth except during the final stages

Figure 1. Ontogenetic change in leaf area (per leaflet) and leaf area relative to lamina fresh weight of leaf 6 counting acropetally in Argenteum pea plants. Mean values of 6 plants. Plant age was counted from the day of seedling emergence.

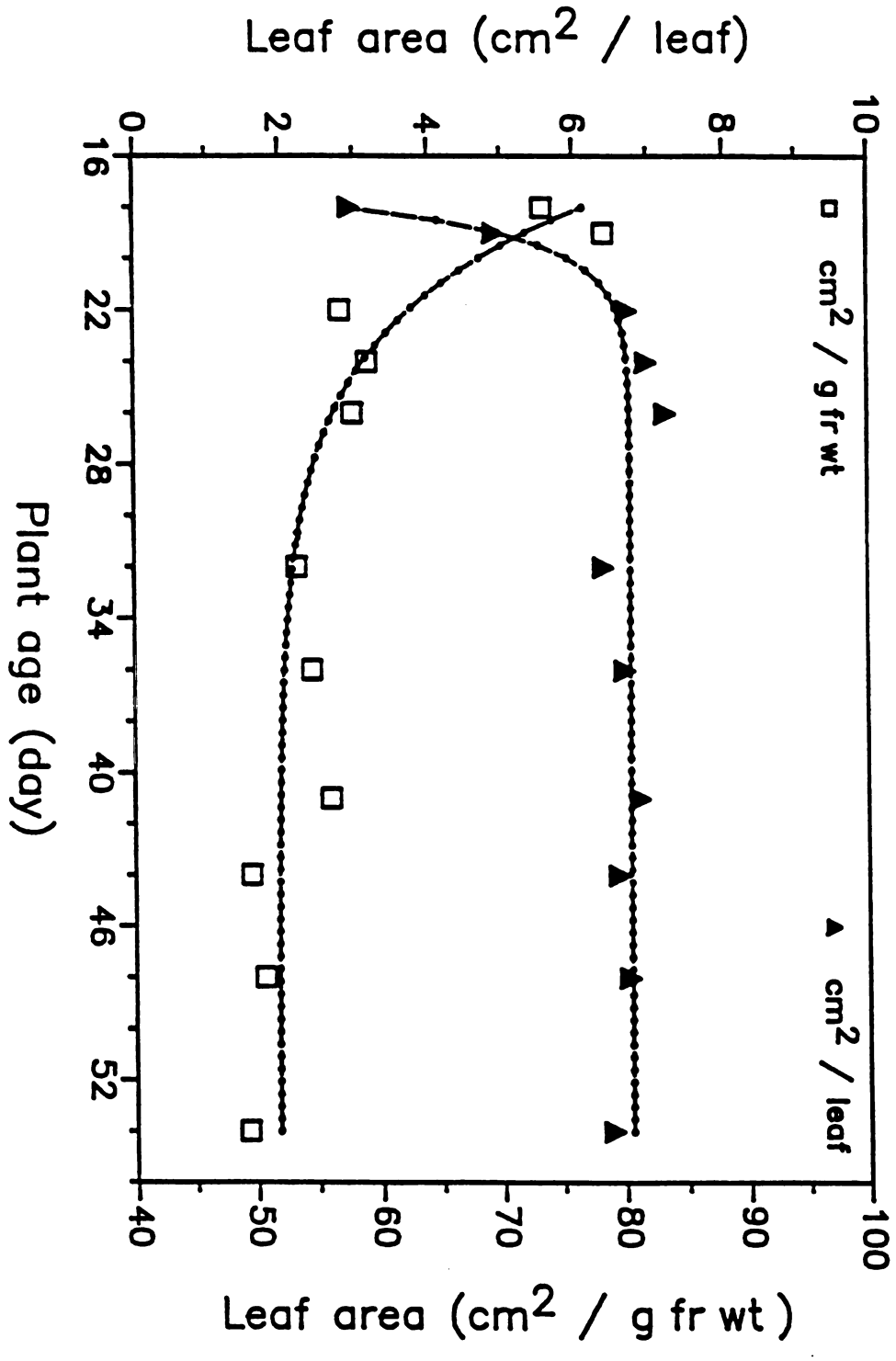

Leaf area (cm2 / leaf)
0
2
4
6
8
10
16
22
28
34
40
46
52
Plant age (day)
cm2 / g fr wt
cm2 / leaf
40
50
60
70
80
90
100
Leaf area (cm2 / g fr wt)

Figure 2. Changes in total K^+ concentration (ueg/g fr wt) in lamina tissue from the acro- and basipetal positions of leaf 6 counting acropetally as a function of age of Argenteum pea plants. Mean values of 6 plants. Plant age is counted from the day of seedling emergence.

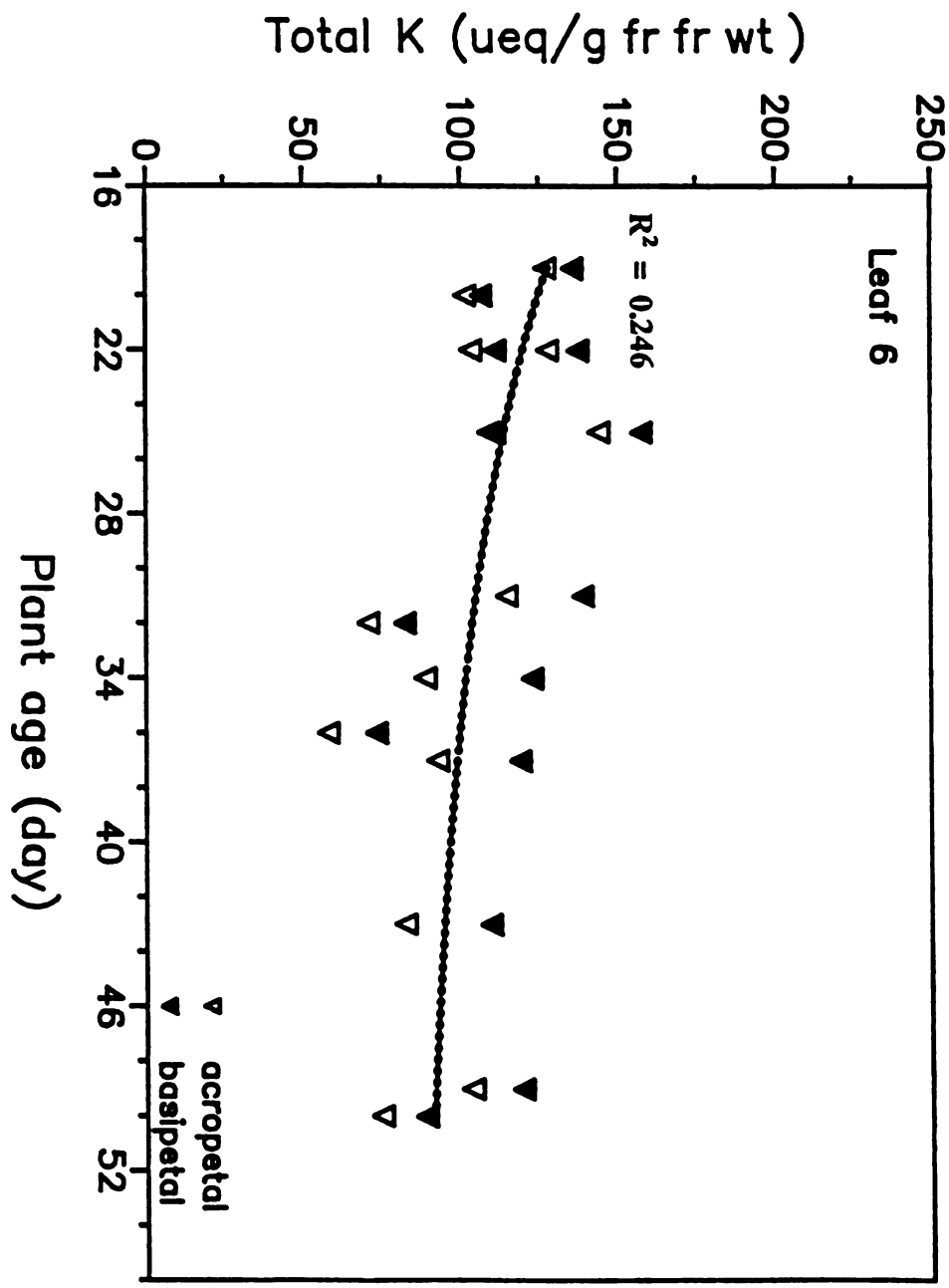
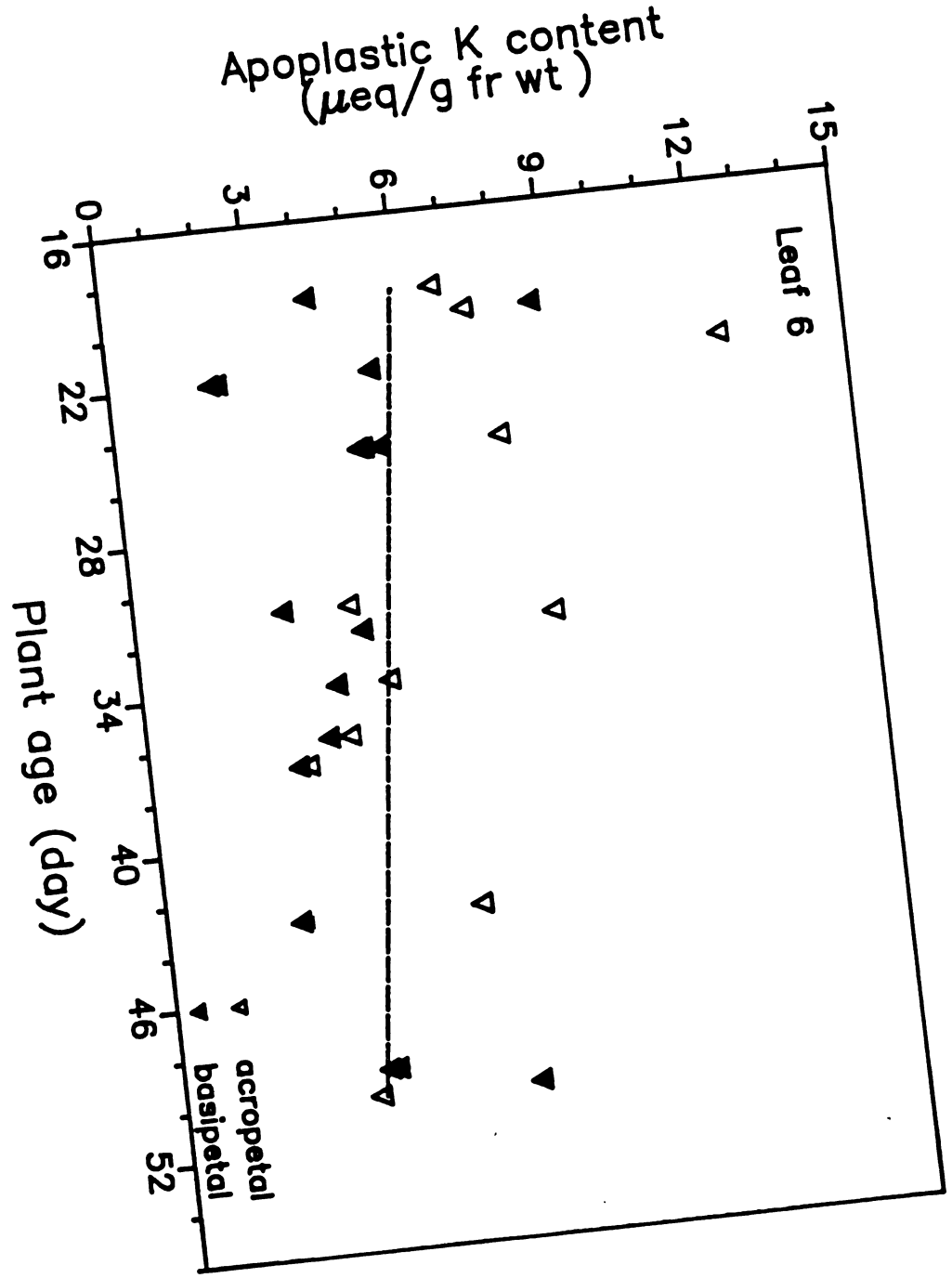

Total K (ueq/g fr fr wt)
0
50
100
150
200
250
16
22
28
34
40
46
52
Plant age (day)
Leaf 6
R² = 0.246
acropetal
basipetal

Figure 3. Apoplastic K^+ content in lamina mesophyll tissue from acro- and basipetal positions of leaf 6 counting acropetally in relation to plant age of Argenteum pea. Mean values of 6 plants. Plant age is counted from the day of seedling emergence.


Apoplastic K content
(μeq/g fr wt)
0
3
6
9
12
15
Leaf 6
Plant age (day)
16
22
28
34
40
46
52
acropetal
basipetal

of leaf maturity (data not presented). Assuming the volume of the solution phase within the apoplast to be 0.04-0.1 ml/g fr wt (Long and Widders, 1990), K^+ concentration within the apoplastic solution was estimated to be in the range of 40-100 and 10-40 μeq/ml, respectively (Fig. 4). It should be recognized that the apoplastic volume is not constant for a specific tissue but is thought to depend on mesophyll cell size, cell wall deposition, and the extent of tissue hydration. From Figure 4, it is apparent that ontogenetic changes in K^+ concentration within the apoplastic solution would be relatively small if the volume of the Donnan free space were 0.1 ml/g fr wt. A lower Donnan free space volume of 0.04 ml/g fr wt would cause K^+ concentration within the apoplastic solution to decline more significantly with leaf age and perhaps slightly increase again when leaflets senesce (Fig. 4). The acropetal portion contained a higher apoplastic K^+ content (Fig. 3) compared to the basipetal lamina.

Transpiration and stomata conductance

Midday stomatal conductance was relatively high, 1.8 cm/sec, in young unfolding leaflets but decreased with leaf maturity to 0.9 cm/sec. During fruit setting, stomatal conductance again increased to approximately 1.7 cm/sec over time (Fig. 5). The transpiration rate varied during leaf ontogeny (Fig. 5), however a slow downward trend between 9.0 to 6.5 $\mu g/cm^2/sec$ was apparent.

Figure 4. Ontogenetic changes in apoplastic solution K^+ concentration within lamina mesophyll tissue on basipetal position of leaf 6 in Argenteum pea plants. Mean values of 6 plants. Plant age is counted from the day of seedling emergence.

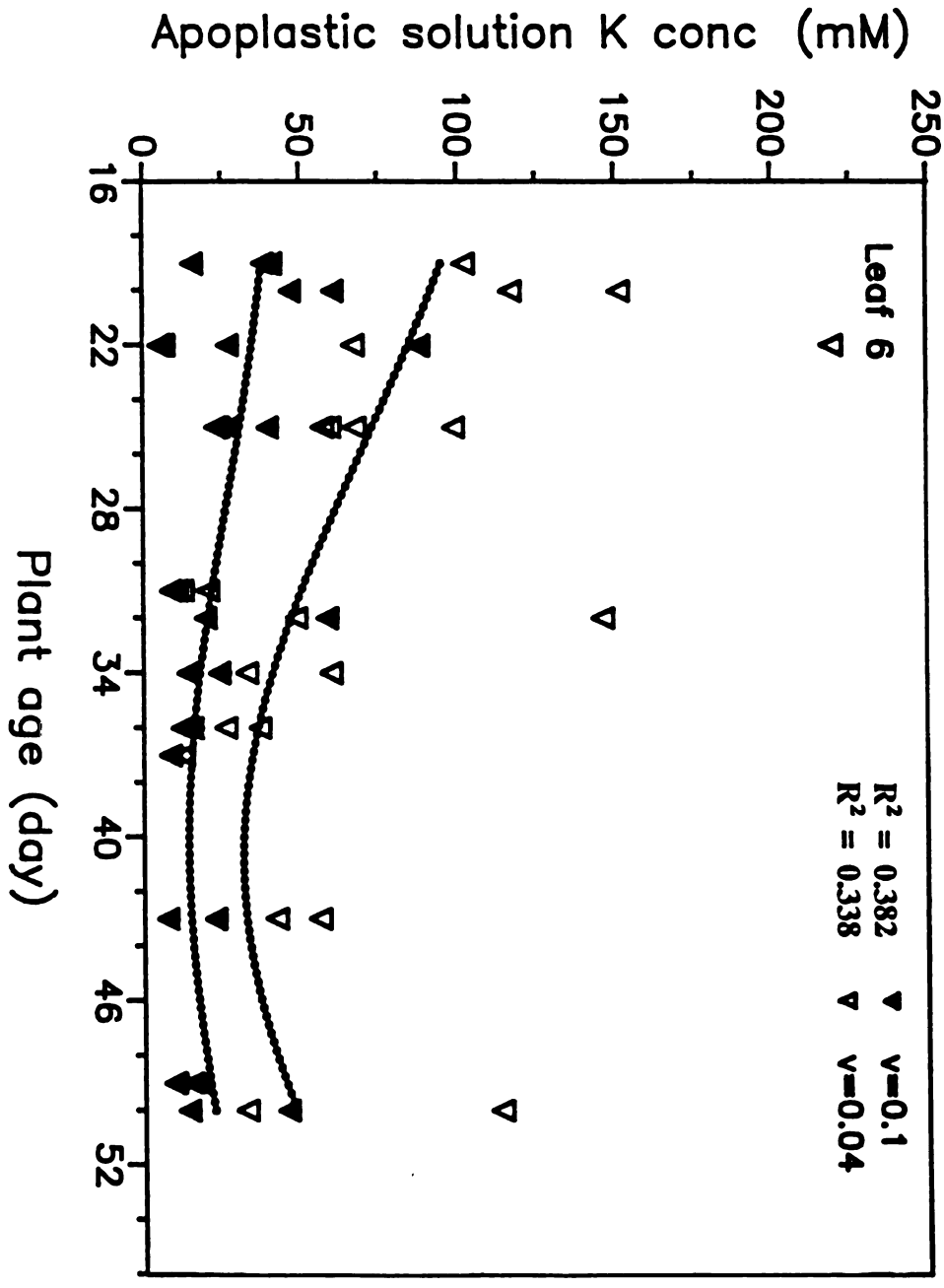
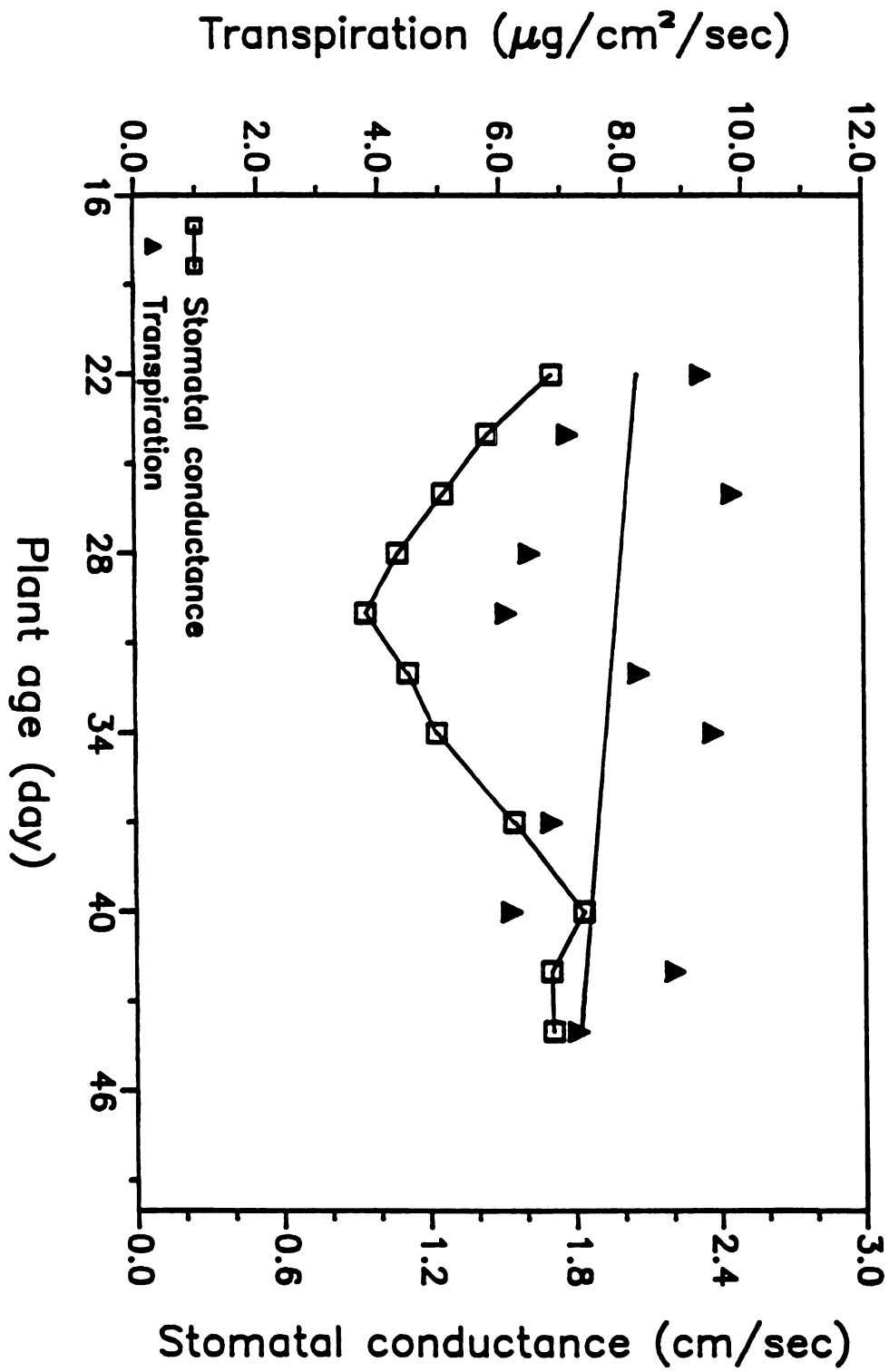

Apoplastic solution K conc (mM)
250
200
150
100
50
0
16
22
28
34
40
46
52
Plant age (day)
Leaf 6
R² = 0.382
R² = 0.338
v=0.1
v=0.04

Figure 5. Ontogenetic changes in midday stomatal conductance and transpiration rate in Argenteum pea plants. Mean values of 6 plants. Plant age is counted from the day of seedling emergence.


Transpiration (μg/cm²/sec)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
Stomatal conductance
Transpiration
Plant age (day)
16
22
28
34
40
46
Stomatal conductance (cm/sec)
0.0
0.6
1.2
1.8
2.4
3.0

Xylem sap K^+ and estimated K^+ transport into leaflets

A relatively minimal change in xylem sap K^+ concentration was observed during plant ontogeny (Fig. 6). K^+ concentration within the xylem sap fluctuated within the range of 10-15 mM. Xylem import K^+ exhibited a trend similar to that of transpiration rate throughout leaf ontogeny (Fig. 7).

Figure 6. Changes in xylem sap K^+ concentration as a function of plant age in Argenteum pea. Mean values of 6 plants. Plant age is counted from the day of seedling emergence.

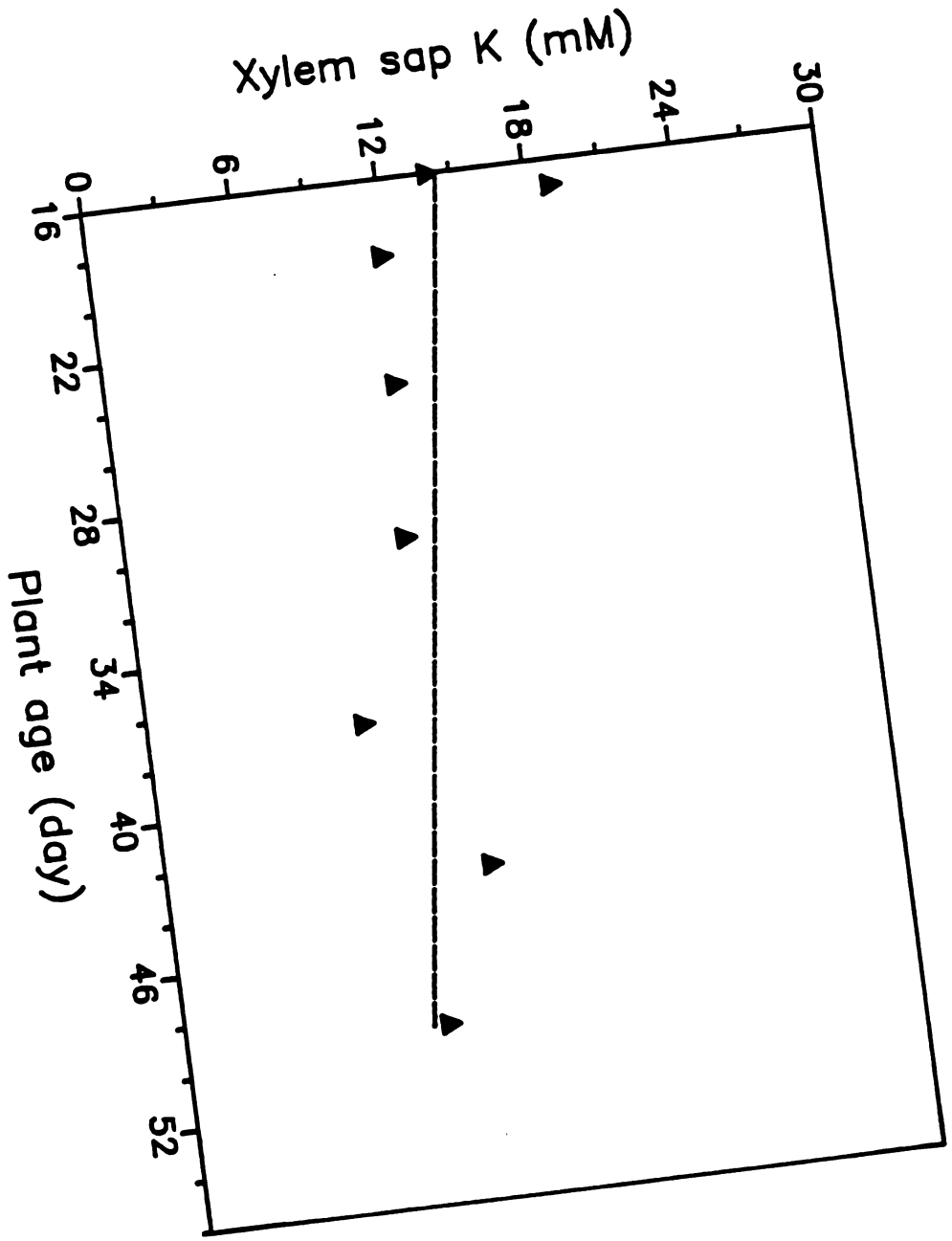
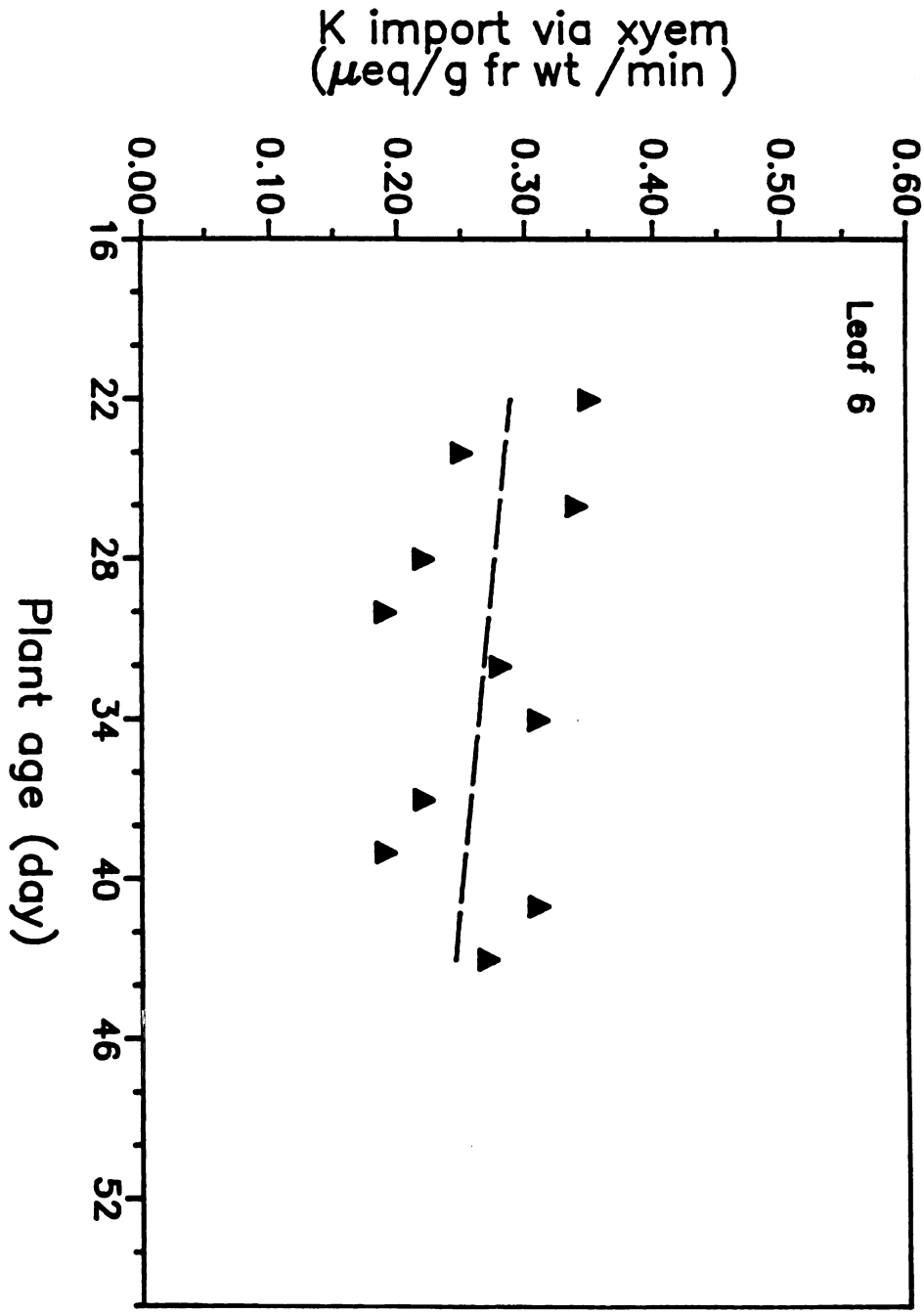

Xylem sap K (mM)
0
6
12
18
24
30
Plant age (day)
16
22
28
34
40
46
52

Figure 7. Estimated rate of K^+ import into leaf 6 via the xylem as a function of plant age in Argenteum pea. Mean values of 6 plants. Plant age is counted from the day of seedling emergence.


K import via xyem
(μeq/g fr wt /min)
0.00
0.10
0.20
0.30
0.40
0.50
0.60
Leaf 6
16
22
28
34
40
46
52
Plant age (day)

DISCUSSION

Ontogenetic declines in the content of certain mineral elements (i.e. K^+) within leaf tissue are common in higher plants. This is attributed largely to dilutionary effects during rapid tissue expansion, decreased uptake and accumulation by leaf tissue (Widders and Lorenz, 1983a) or high rates of phloem export (Greenway and Pitman, 1965; Thrower, 1962). Potassium content and concentration within the leaf extracellular compartment, the apoplast, is thought to be regulated by the rate of ion uptake into the symplast, transpiration-induced xylem import, phloem import, and phloem export to other growing tissue (Greenway and Pitman, 1965; Pitman et al., 1974b; Teng, 1990a). The integration of transport processes and its ultimate net effect on K^+ accumulation within leaf tissue might change during ontogeny. Data presented in this study indicates an ontogenetic decline in apoplastic K^+ content but with a high degree of variability (Fig. 3). In contrast, the decrease in K^+ concentration within the apoplastic solution was estimated to change to a greater extent during leaf growth, maturation and senescence. (Fig. 4).

Changes in K^+ flux kinetics would be expected to influence apoplastic K^+ content throughout leaf ontogeny. Jacoby and Dagan (1969) reported that net flux increased during leaf expansion and decreased in senescing leaves. However, Widders and Lorenz (1983a, 1983b) found relative little effect of leaf age on net flux since both influx and efflux rates at specific extracellular K^+ concentrations did not change dramatically as the mesophyll tissue matured and approached senescence.

It is not suggested that the rate of K^+ supply to the mesophyll apoplast via the xylem during leaf ontogeny parallels the net rate of K^+ influx into mesophyll cells although the magnitude of fluctuation in the xylem transported K^+ (Fig. 7) was similar to that of apoplastic K^+ content (Fig. 4), It was suggested in the diurnal study (Teng, 1990b) that the increase in apoplastic K^+ concentration in the afternoon hours especially in tissue from mature leaves was partly ascribed to a higher xylem K^+ import rate than to a lower rate of K^+ influx into mesophyll cells.

Net K^+ import into the apoplast of mature leaves is considered to be a function of transpiration rate and xylem sap K^+ concentration. The import of solutes via the phloem in mature leaves is considered minimal. The relationship between transpiration and ion transport depends on plant species, age, the specific ion and cultural conditions (Pitman, 1977, 1982; Salim and Pitman, 1984a, 1984b). Increased diffusive resistance for water vapor exchange in mature leaves (Christopher, 1987) reduces transpirational water loss. Neumann and Nooden (1984) and Mauk et al. (1985) reported that the xylem fluxes of ^{86}Rb into leaves was in proportion to the transpiration rate. In the diurnal study (Teng, 1990b), transpiration rate was found to be the primary factor influencing apoplastic K^+ level. During leaf ontogeny, the fluctuation of xylem K^+ import was observed to be concomitant with the ontogenetic trend observed in leaf transpiration rate (Fig. 5, 7) rather than with xylem sap K^+ concentration (Fig. 6). Therefore, it is not unreasonable to assume transpiration rate might have a more significant influence on leaf apoplastic K^+ content during leaf ontogeny than does K^+ concentration within the xylem.

The apoplastic volume determines the estimated K^+ concentration within the apoplast. In this study, apoplastic K^+ concentration would not be expected to change much during leaf ontogeny if apoplastic volume was maintained at 0.1 ml/g fr wt; however, a significant ontogenetic change would occur if the volume were 0.04 ml/g fr wt (Fig. 4). Apparently, with a larger apoplastic volume, a slight change in apoplastic K^+ content will not alter apoplastic K^+ concentration as markedly as when the volume is relatively small. In the latter case, a similar change of apoplastic K^+ content would result in a dramatic difference in concentration.

Additionally, compared to the effect of the symplastic volume to symplastic K^+ concentration, the relatively small volume of the apoplast (0.04 to 0.1 ml/g fr wt) would have more effect on K^+ concentration within the apoplast in the K^+ partition between these two compartments. It was reported that if more than 1% of the Na^+ transported to the leaf remained in the apoplast, the apoplastic ionic concentration would be expected to rise faster than that in the protoplast (Flowers and Yeo, 1988). The volume of the apoplast would be expected to increase with leaf growth in part due to the expansive growth of individual cells resulting in larger intercellular spaces and the laying down of the secondary cell wall. An increase in the concentration of fixed negatively charged sites for cation binding, the Donnan phase, accompany the formation of new cell wall. The ontogenetic changes in apoplastic K^+ would be even more significant if the apoplastic volume increased from 0.04 to 0.1 ml/g fr wt instead of remaining constant at a fixed volume (Fig. 4). The water free space was reported to only occupy a limited proportion of the cell wall volume in transpiring

leaves (Pitman et al., 1974b; Van Steveninck and Chenoweth, 1972). Therefore, when the same amount of K^+ remained within the apoplast, the concentration could probably change depending upon transpiration rate and the ultimate hydration status of the leaf.

Estimated K^+ concentrations within the apoplastic solution (10-40 and 40-100 mM based on the assumed apoplastic volumes of 0.1 and 0.04 ml/g fr wt, respectively) in Argenteum pea (Fig. 4) (Long and Widders, 1990) during leaf ontogeny are quite high compared to earlier reports (<20 mM) (Bernstein, 1971; Harvey et al. 1981; Jacobson, 1971). Optimum K^+ concentration in the cytoplasm was generally thought to be in the range of 100-200 mM (Gibson et al., 1984; Hsiao and Lauchli, 1986; Wyn Jones and Pollard, 1983; Wyn Jones et al., 1979). Subtracting apoplastic K^+ from the total tissue content and assuming the volume of the intracellular space to be approximately 0.9 ml/g fr wt (Long and Widders, 1990), estimated intracellular K^+ concentrations for pea lamina tissue would be within the range of 95-130 mM. The average percentage of apoplastic K^+ relative to the total content is about 2 to 6%. Although there is limited information available on K^+ partitioning between the extra and intracellular compartments in leaf tissue of other plant species, these ranges do not seem unrealistic.

The high apoplastic K^+ content observed in this study might also be attributed to the hydroponic system which minimized the diurnal fluctuations of water and nutrient availability to the root systems. However, the concentration of K^+ measured within the xylem (10-15 mM) was within the range of that reported in other plant

species. This suggests the maintenance of a relatively constant level of K^+ within the mesophyll tissue during leaf development.

It was hypothesized (Widders and Lorenz, 1983b) that the ontogenetic decline of leaf K^+ was due to a net K^+ efflux out of mesophyll cells resulting from a long term ontogenetic reduction in extracellular K^+ concentration below 2 to 3 mM, which was required to maintain a zero net flux into the symplast. The high apoplastic K^+ concentrations observed in this study, either including or excluding the Donnan phase K^+, were much higher than these threshold concentrations. Certainly the ontogenetic decline of the leaf K^+ level in Argenteum pea cannot be satisfactory explained by such hypothesis only (Widders and Lorenz, 1983b). In fact, K^+ concentration within the apoplast of pea is considered sufficiently high to sustain continued net uptake and accumulation of K^+ within mesophyll cells throughout ontogeny. Therefore, the symplastic pathway for phloem loading of K^+ might play an important role in K^+ retranslocation to other tissue. This is not to suggest, however, that the apoplastic pathway does not directly provide a pool of K^+ for loading into the phloem (Teng, 1990a).

The high K^+ concentration within the apoplast may suggest an important osmotic function in the regulation of cellular water potential. The osmotic consequences of high ionic concentrations within the leaf apoplast were first outlined by Oertli (1968). It was suggested that osmotic potential of the free space solution might serve as a buffer to changes in cellular water status within certain limits (Clipson et al., 1985; Cosgrove and Cleland, 1983a). An ionic concentration of 150

mM would constitute a considerable osmotic component, a reduction of -2.44 bar (Leigh and Tomos 1983; Nobel, 1970). A reduction in leaf apoplastic osmotic potential might contribute to the development of low water potentials which serve to facilitate xylem solution import and distribution throughout leaf lamina tissue. The decrease in apoplastic K^+ concentration during leaf expansive growth (Fig. 4) (Meinzer and Moore, 1988) suggests that the requirement of ion uptake for intracellular osmotic maintenance and continued water uptake for cell expansion could diminish apoplastic ionic concentrations. In addition, apoplastic ionic concentrations are also important in determining net uptake rates in leaf mesophyll cells since ion flux kinetics are dependent upon electro-chemical potentials across the plasma membrane of the cell. Whether or not K^+ accumulation in the cell wall is of widespread significance and its role in the osmotic relations of plant cells during leaf ontogeny awaits further investigation.

In conclusion, the ontogenetic changes (decrease) in apoplastic K^+ concentration are more apparent than changes in apoplastic K^+ content. The high apoplastic K^+ concentrations indicate that during K^+ retranslocation, the apoplastic pathway might not be the primary pathway for phloem loading of K^+. K^+ content within the apoplast is suggested to play a role in regulating cell water potential.

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CHAPTER 4

Whole Plant Leaf Nodal Position Effects on Apoplastic K Content in Mesophyll Tissue of Pea (*Pisum sativum* cv. Argenteum)

INTRODUCTION

An ontogenetic decline in leaf tissue K^+ concentration during reproductive growth was hypothesized (Widders and Lorenz, 1983a, 1983b) to be partially due to an ontogenetic decline in apoplastic K^+ concentration. During reproductive growth, assimilates are partitioned into developing fruits at the expense of continued root growth and maintenance of the energy status of the root for supporting such processes as mineral nutrient uptake. Ionic concentrations, K^+ etc, within the xylem of a variety of plant species decline during plant development (Hocking, 1980; Widders and Lorenz, 1982). However, diurnal and ontogenetic changes in xylem sap K^+ concentrations have been shown to have less influence than transpiration rate upon the rate of K^+ import into a leaf and consequently upon the extracellular K^+ concentrations within leaf lamina tissue (Teng, 1990a, 1990b).

Transpiration rate was suggested to be the primary factor influencing xylem K^+ fluxes into leaf mesophyll (Teng, 1990a, 1990b). In addition, variations in transpiration rate among leaves within a shoot canopy should influence the

partitioning of xylem transported K^+ moving from the root. Differences in transpiration-driven xylem import of K^+ into different leaves would be expected to alter apoplastic K^+ concentrations and might be responsible for the apparent gradients in leaf K^+ content within the shoot.

Developmental events such as flowering and fruit growth might also have an impact on total leaf K^+ and apoplastic K^+ levels. The relationship between plant development and apoplastic K^+ content/concentration has not been examined. This study evaluates total and apoplastic K^+ levels in lamina tissue at various nodal positions along the stem at several different developmental stages of the pea in order to gain a better understanding of the impacts of transport processes within plants on leaf K^+ concentration throughout leaf and plant ontogeny.

MATERIAL AND METHODS

Pea plants (*Pisum sativum* cv. Argenteum) were hydroponically cultured in a modified Hoagland solution containing 3 mM K^+ (Hoagland, 1950; Johnson et al., 1957) in a controlled environment room. Environmental conditions were 25/23 °C day/night temperature, 75% relative humidity, 16 hr photoperiod (08:00-24:00) and irradiance levels 320-370 μmole m^{-2} s^{-1}. Leaves beyond the fifth node counting acropetally were evaluated at three different developmental stages: vegetative, flowering and seed filling. At each sample time, leaflets from all nodal positions on

the primary stem axis between the fifth node and the unfolding leaflets in the top canopy were sampled for K^+ analysis. Elution analysis described by Long and Widders (1990) was used to determine apoplastic K^+ content in mesophyll tissue. Leaflets were sampled from 10:00-18:00 hr since preliminary experiments indicated minimal fluctuation of leaf potassium during this period. Six leaflets from six different plants were analyzed on consecutive days. Calculations of apoplastic K^+, Donnan phase K^+ and estimated transported K^+ into the leaf apoplast via the xylem were described in detail by Teng (1990b).

Stomatal conductance and transpiration rate for each leaflet were measured every two days at midday with a LI-1610 steady state porometer (LI-COR.). Eight preselected plants were used throughout plant ontogeny. Variability in air/leaf temperature within the plant canopy did not exceed 2 C. The rate of xylem import of K^+ into individual leaves was estimated based on the assumption that it was a function of the concentration of K^+ within the xylem sap and volume flux rate through the xylem as driven by transpiration rate.

RESULTS

Argenteum plants became determinate during seed filling after producing 16-18 leaves. Leaflet size of apical leaves were less than lower leaves at a similar stage of leaf maturity. The profile in leaf width from apex to base paralleled the ontogenetic

trend in width of an individual leaflet (Fig. 1) (Teng, 1990b). Leaf length, however, was more variable than width among leaves from the various nodal positions. Maximum leaflet length was consistently between nodes 3-5 counting basipetally (Fig. 1). Compared to leaflets developed during the vegetative phase, the size of leaves developing after flowering were smaller at full leaflet expansion.

Young expanding leaflets at the stem apex commonly contained the highest total K^+ levels except before rapid seed filling. During seed filling, K^+ accumulated to quite high concentrations in leaflets from intermediate nodes (10 to 14), greater than 125 μeq/g fr wt. The lowest total leaflet K^+ concentrations, approximately 90 μeq/g fr wt, were found in the basal leaves (node 5 and 6) throughout plant growth and development (Fig. 2).

At vegetative and flowering stages of plant development, relatively high apoplastic K^+ contents were measured in young expanding leaflets. As the plants started seed filling, the apoplastic K^+ content in the growing leaflets declined markedly from > 5.5 to approximately 3 μeq/g fr wt. The lowest apoplastic K^+ contents were consistently observed in recently fully expanded leaves, 4 or 5 nodes counting from the apex. In reproductive plants, it is at these nodes where fruit trusses are positioned.

Estimations of K^+ concentration within the apoplastic solution of leaf mesophyll tissue, taking into account K^+ sorption to a fixed Donnan phase, were influenced both by nodal position of leaf in the plant and stage of plant development in a manner similar to apoplastic K^+ content (Fig. 3). The volume of solution within the

Figure 1. Leaflet length and width from nodal positions at three developmental stages of Argenteum pea plants: a) vegetative, b) flowering and fruit set, and c) seed filling stage. Leaf nodal positions were determined in an acropetal direction within the plant. Plant ages at vegetative, flowering and fruit setting, and seed filling stages were approximately 20, 30 and 50 days, respectively. Mean values of 6 plants. Plant age is counted from seedling emergence.

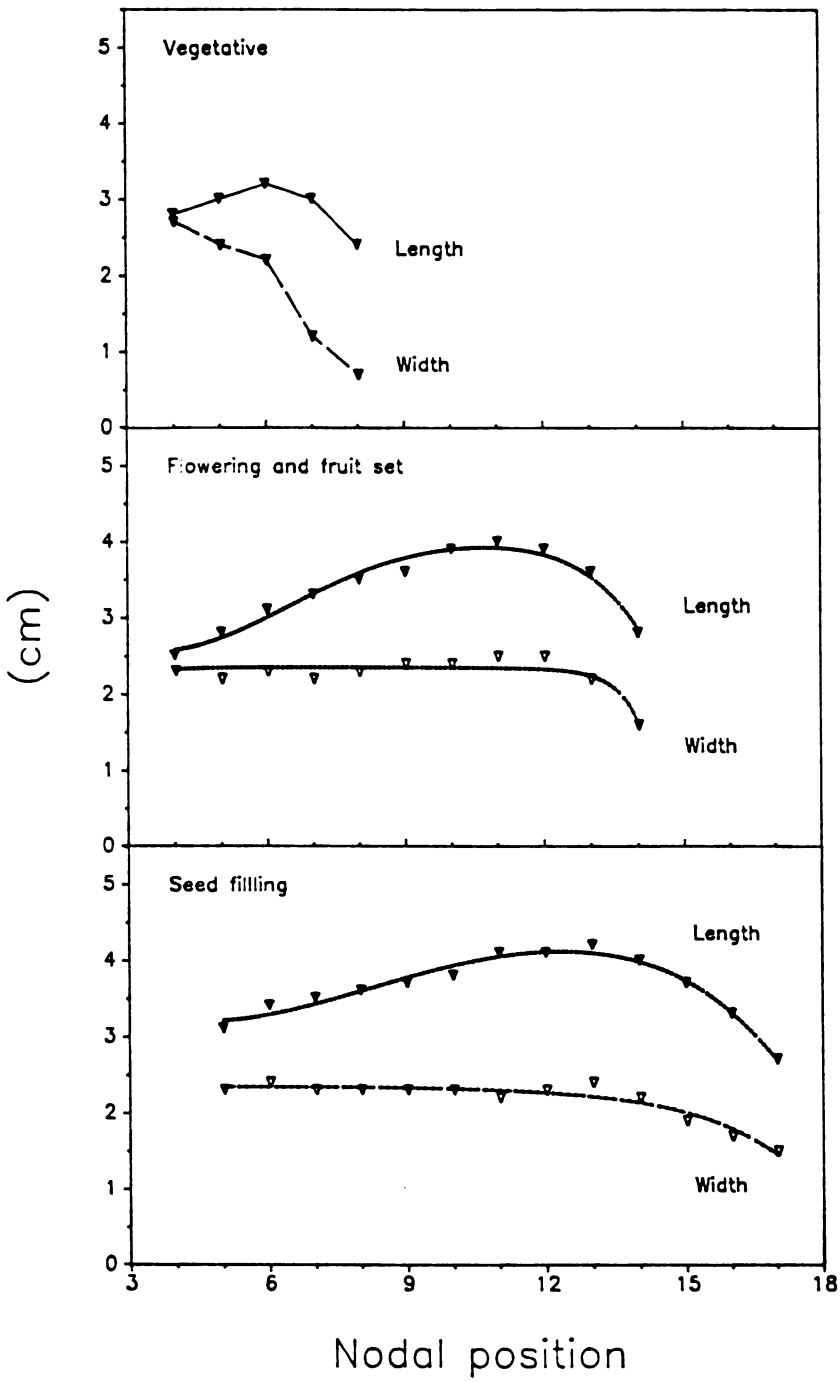

Vegetative
Length
Width
Flowering and fruit set
Length
Width
Seed fillling
Length
Width
(cm)
0
1
2
3
4
5
3
6
9
12
15
18
Nodal position

Figure 2. Total leaf and apoplastic K^+ content in lamina tissue as influenced by leaf nodal positions in Argenteum pea plants at three stages of growth and development of Argenteum pea plants. Stages of plant growth and development are described in Fig. 1. Leaf nodal positions were determined in an acropetal direction within the plant. Open and solid symbols represent acro- and basipetal positions on the leaflet, respectively. Mean values of 6 plants.

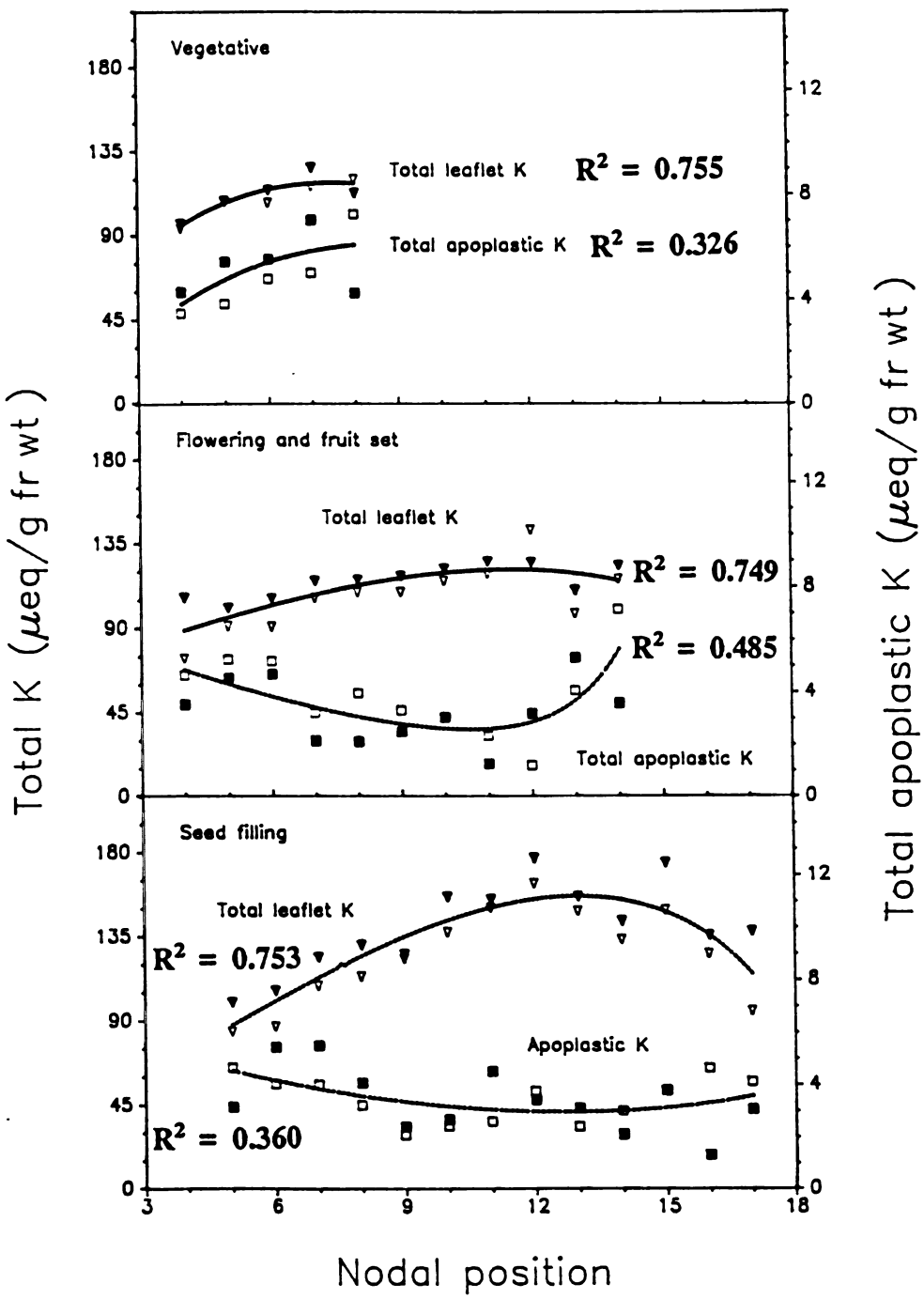
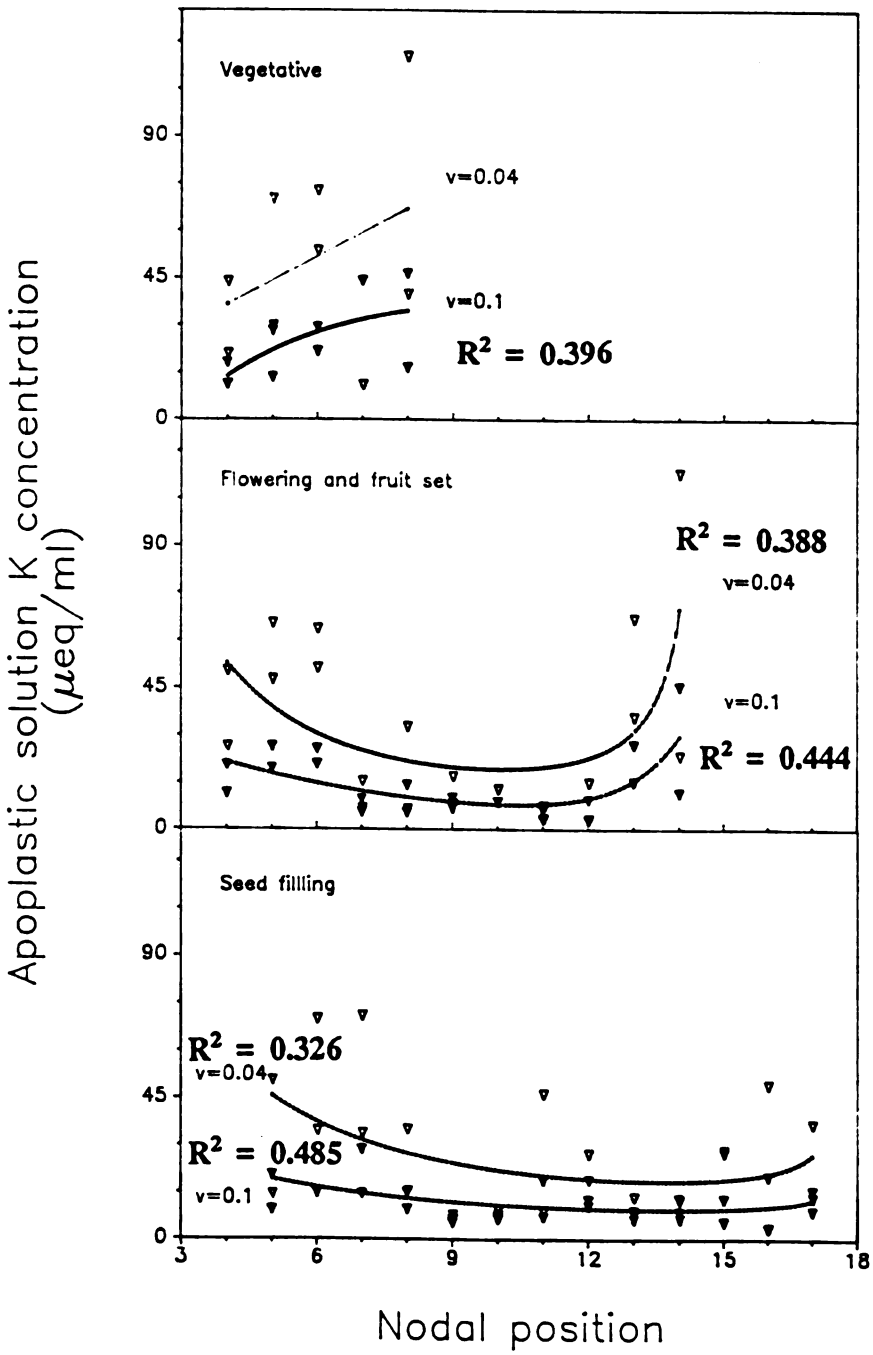

Vegetative
Total leaflet K
R^2 = 0.755
Total apoplastic K
R^2 = 0.326
Flowering and fruit set
Total leaflet K
R^2 = 0.749
R^2 = 0.485
Total apoplastic K
Seed filling
Total leaflet K
R^2 = 0.753
Apoplastic K
R^2 = 0.360
Total K (μeq/g fr wt)
Total apoplastic K (μeq/g fr wt)
Nodal position
0
45
90
135
180
4
8
12
3
6
9
12
15
18

Figure 3. Estimated K^+ concentration within the mesophyll apoplast as influenced by nodal positions of the leaf at three developmental stages of Argenteum pea plants. Volume of the apoplast was estimated to be 0.04 and 0.1 ml/g fr wt. Leaf nodal positions were determined in an acropetal direction within the plant. Stages of plant growth and development are described in Fig. 1. Mean values of 6 plants. Plant age is counted from the day of seedling emergence.


Vegetative
v=0.04
v=0.1
R^2 = 0.396
Flowering and fruit set
R^2 = 0.388
v=0.04
v=0.1
R^2 = 0.444
Seed fillling
R^2 = 0.326
v=0.04
R^2 = 0.485
v=0.1
90
45
0
3
6
9
12
15
18
Apoplastic solution K concentration (μeq/ml)
Nodal position

apoplast, however, would be a major determinant of concentration of K^+ and the variation within the plant. Assuming a volume of 0.04 to 0.1 ml/g fr wt, apoplastic K^+ concentrations ranged from approximately 5 to nearly 120 μeq/ml. During the vegetative growth and flowering stages, the highest apoplastic K^+ concentrations were estimated from young expanding and mature leaves which are beginning to senesce. At seed filling, relatively small differences in K^+ concentration within the apoplast were observed between leaves from the various nodal positions, especially if the volume of apoplastic solution was high (0.1 ml/g fr wt).

Midday transpiration rates in young leaves were relatively high and tended to decline as leaves matured, especially in leaves at lower nodal positions (Fig. 4). The stage of plant development had some effect on transpiration rate. In vegetative plants, a linear, basipetally decline in leaf transpiration rate was measured. During reproductive development, however, similar transpiration rates in the range of approximately 7.0 to 7.5 $\mu g/cm^2/sec$ were observed at the intermediate nodal positions on the plant. Again, the highest rates were observed in expanding leaves. The highest transpiration within a plant were quite constant throughout plant ontogeny, about 8.5 to 5.0 $\mu g/cm^2/sec$, respectively.

The relative differences in K^+ transported via the xylem into the mesophyll apoplast among leaves along the stem counting basipetally in vegetative plants (Fig. 5) is similar to that of a single leaf ontogeny (Teng, 1990c.). Import rates of K^+ declined from the young to the older leaves. The profile of xylem import rates observed in reproductive plants, however, was somewhat different. During seed

Figure 4. Transpiration rate of leaflet from different nodal positions at three developmental stages of Argenteum pea plants. Leaf nodal positions were determined in an acropetal direction within the plant. Stages of plant growth and development are described in Fig. 1. Mean values of 8 plants. Plant age is counted from the day of seedling emergence.

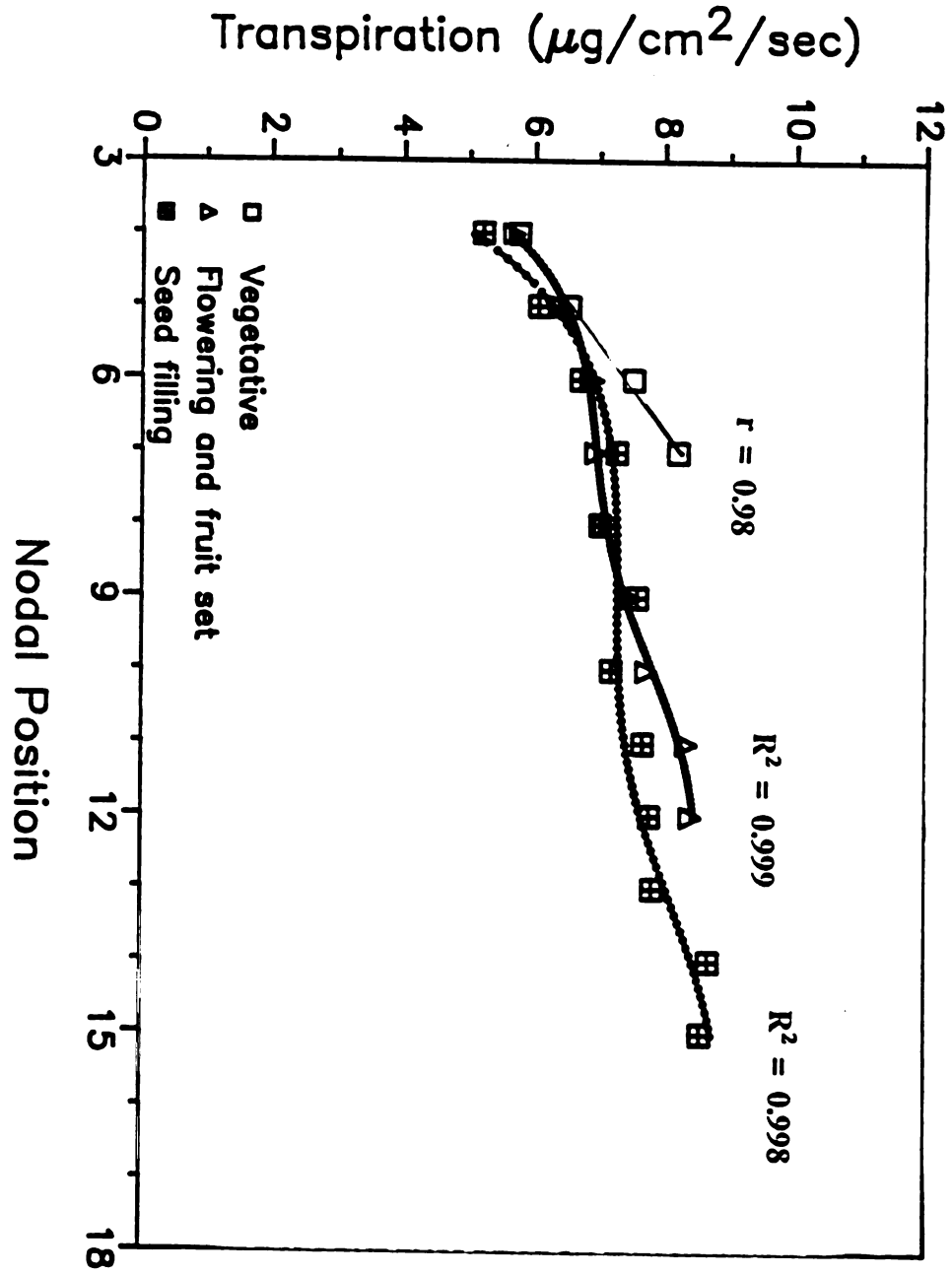
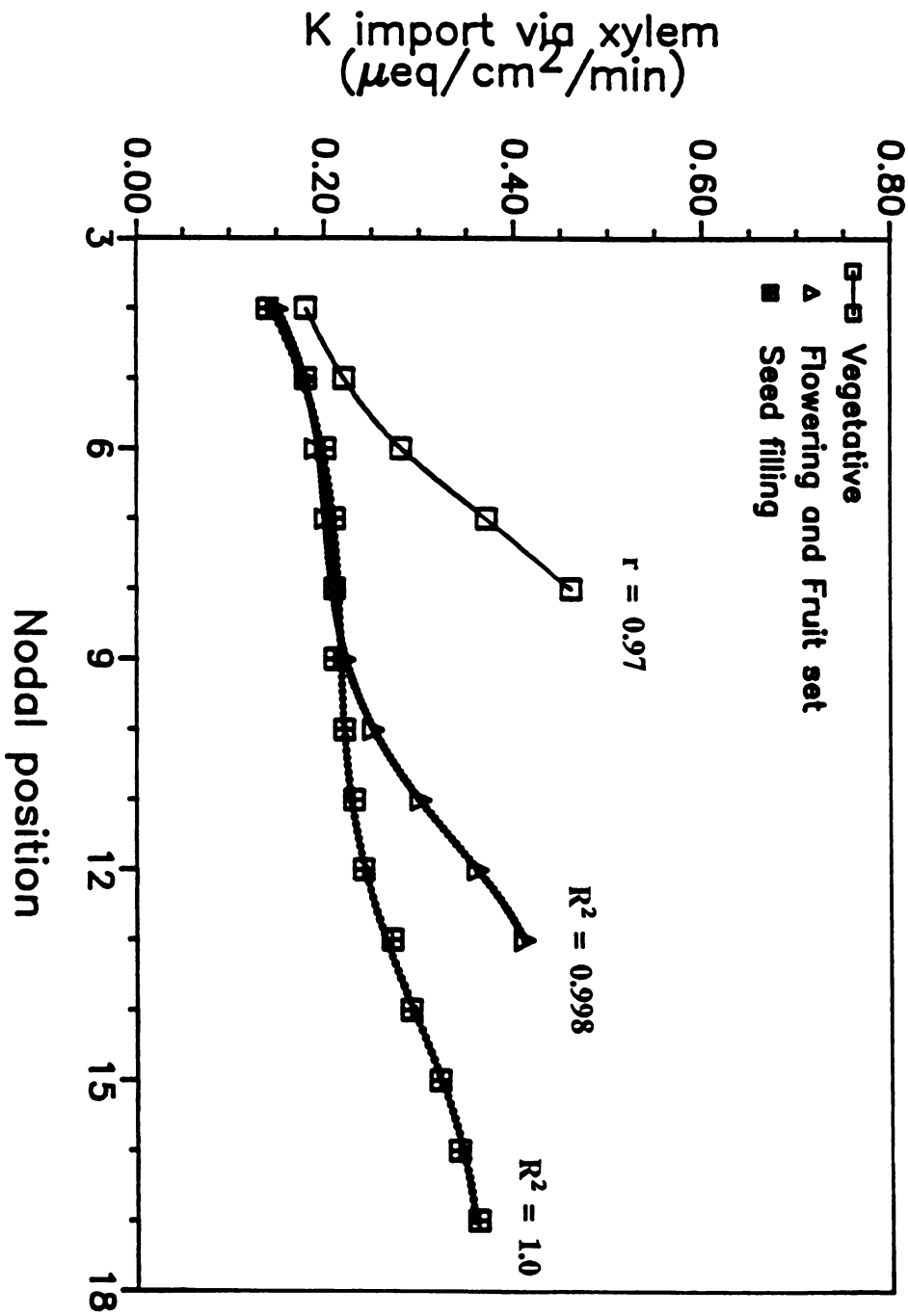

Transpiration (μg/cm²/sec)
0
2
4
6
8
10
12
3
6
9
12
15
18
Nodal Position
Vegetative
Flowering and fruit set
Seed filling
r = 0.98
R² = 0.999
R² = 0.998

Figure 5. Influence of leaflet nodal position on estimated K^+ import rate via the xylem into leaf apoplast at three developmental stages of Argenteum pea plants. Leaf nodal positions were determined in an acropetal direction within the plant. Stages of plant growth and development are described in Fig. 1. Mean values of 6 plants.


K import via xylem
(μeq/cm²/min)
0.00
0.20
0.40
0.60
0.80
3
6
9
12
15
18
Nodal position
Vegetative
Flowering and Fruit set
Seed filling
r = 0.97
R² = 0.998
R² = 1.0

filling, the rates of xylem import became increasingly lower as it proceeded from the most apical leaf basipetally down the plant. K^+ transport rate via the xylem into leaves positioned at nodes 6 through 9 (counting acropetally) exhibited little variance. The rates decreased slightly in the most basal leaves.

DISCUSSION

Stages in plant development such as reproductive growth and plant senescence, at which the changes in transport processes occur, are expected to influence mineral element levels within individual leaves. Thus, ontogenetic changes in both short and long distance transport processes within a whole plant would contribute to differences in K^+ accumulation within leaf lamina tissue at different nodal positions.

Leaves at different nodal positions on the stem appear to differ significantly in the uptake and accumulation of K^+ during plant growth and development. In vegetative and flowering plants, apical leaves contained higher levels of K^+ than leaves in more basal positions (Fig. 2). During seed filling, however, fully expanded apical leaves (Fig. 1) contained less K^+ than leaves more closely associated with flower/fruit trusses (node 10-14) (Fig. 2). Argenteum pea became determinate after producing 16-18 leaves. The apical leaves developing after flowering and fruit set were smaller than leaves between nodes 10 and 14. Leaves developing after flowering and fruit set are thought unable to compete as effectively for K^+ as developing seeds which are recognized as strong sinks for phloem transported K^+.

Basal leaves did not change significantly in total K^+ level throughout plant growth and development. This may be due to the steady supply of abundant K^+ in the hydroponic system which provided luxury absorption. Little ontogenetic decline in xylem imported K^+ into basal leaves was observed. Thus, the balance between the import and export of K^+ might be responsible for the fact that no decline in basal

leaf K^+ content was observed.

It is hard to provide a satisfactory explanation for the high K^+ accumulation in leaves from nodes 10-14 during seed filling. It is possible that the anatomy of the mesophyll may influence tissue K^+ content. In mature plants (without new developing leaves) leaves along the stem could be divided into three general groups based on size (Fig. 1). The largest leaves were found in the flowering region. In comparison, apical leaves were relatively narrow while basal leaves were significantly wider but shorter in length. Larger leaves generally contain larger cells which have larger vacuole. The vacuole, which constitute >90% of the symplastic volume in mature cells, accumulates ions, therefore, the high K^+ content observed in leaves in the flowering region (node 10-14) during seed filling may be ascribed to the accumulation of K^+ in the larger vacuole.

Differences in K^+ accumulation within leaf lamina tissue between leaves from different nodal positions at various stages of plant development cannot be attributed directly to differences in extracellular K^+ concentrations. During seed filling, the highest levels of leaf K^+ were at nodes 10-14 where apoplastic K^+ levels were comparatively low (Fig. 2). Conversely, during reproductive development, high apoplastic K^+ contents were found in the basal leaves which did not accumulate high levels of K^+. The lack of correlation between apoplastic K^+ concentration and tissue K^+ content suggests that some unknown factors are preventing high accumulation of K^+ within the mesophyll tissue.

Apoplastic K^+ content among leaves at different nodal positions appears to be

affected more by the age of each individual leaf rather than degree of plant developmental. K^+ content and concentration within the apoplast rapidly decreased during leaf expansion (Fig. 2, 3). Mature leaves in the flowering region contained the lowest apoplastic K^+ levels (Fig. 2, 3). Rates of xylem imported K^+ (Fig. 5), organic nitrogen (Simpson et al., 1983), calcium (Van de Geijn and Smeulders, 1981) and phosphates (Stryker et al., 1974) into basal leaves were lower than into apical leaves. The more apical leaves appear to be more competitive than basal leaves for nutrient supplies transported via the xylem. This might be due to a comparatively high amount of water (Neumann and Stein, 1984; Zimmermann, 1983) and mineral nutrients (Mauk et al., 1985; Neumann and Nooden, 1984) being transported into apical leaves as compared to the basal leaves.

In apical leaves, the high apoplastic K^+ concentrations may be a function of the high xylem K^+ import rates driven by transpiration (Fig. 4, 5) (Teng, 1990a, 1990b). Similarly, the lower apoplastic K^+ concentrations in basal leaves might be related to the lower xylem K^+ flux rate in the xylem into the leaves. Stomatal conductance (Neumann, 1987), hydraulic resistance (Black, 1979; Koide, 1985; Neumann and Stein, 1984) and transpiration rate (Mauk et al., 1985; Neumann and Stein, 1984) have been shown to vary among leaves at nodal position. K^+ uptake by cells along the xylem transport pathway within the stem as well as absorption by phloem tissue (Van Bel et al., 1981; Van de Geijn and Smeulders, 1981; Wolterbeek et al., 1985) would be also expected to influence the amount of K^+ transported into individual leaves.

High levels of apoplastic K^+ are present in the most basal leaves (below node 9) (Fig. 2, 3), in which the rates of xylem import of K^+ are not significantly different from leaves between nodes 9 and 12 (Fig. 5). This suggests that at a later stage of leaf maturation the mesophyll tissue looses the ability to take up K^+. It might also be due to an accelerated rate of phloem export. At the onset of leaf senescence, an increase in apoplastic K^+ content might be ascribed to the inability of the mesophyll cells to maintain intracellular K^+ concentrations. Associated with the onset of senescence is: a) the loss of cell membrane integrity (Thompson, 1988), b) a decrease in the synthesis relative to breakdown of membrane carrier proteins (Brady, 1988; Peoples and Dalling, 1988), and c) changes in electrochemical potential gradients across cellular membranes due to ontogenetic changes in activity of electrogenic pumps (e.g. ATPase).

During the period of leaf senescence, tissue around the vascular bundle (Butler and Simon, 1971; Peoples et al., 1980; Shaw and Manocha, 1965) and sieve tubes remains functional until an advanced stage of mesophyll senescence, even after the onset of chlorosis (Benner and Nooden, 1984; Matile and Winkenbach, 1971). Therefore, K^+ retranslocated from leaves to developing fruits would be expected to continue in senescing leaves. Yet the pathway for K^+ movement and the site of ultimate loading into the phloem may change. It is possible that as leaves mature, an increasingly higher percentage of the K^+ loaded into the phloem might originate directly from the apoplastic pathway rather than the symplastic pathway.

This study indicates that plant growth and development affect leaf K^+ content

at different nodal positions. Apoplastic K^+ content, however, was confirmed to be less influenced by plant development. Although most variations in mesophyll apoplastic K^+ content among leaves from the various nodal positions is thought to be attributable to difference in leaf age, apoplastic K^+ concentration is not considered to be major factor in the regulation of leaf K^+ content.

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SUMMARY

The elution method developed by Long and Widders (1990) and used in these experiments has the advantage of facilitating quantitative estimation of K^+ fluxes across the plasma membrane of intact leaf mesophyll tissue with minimal aging and wounding effects. The mesophyll tissue is thought to be not wounded as a result of removal of the epidermis. Although Pitman et al (1974b) claimed that the absorption kinetics of barley leaf slices remained unchanged during the subsequent experimental period, one cannot ignore the potential alterations in plasma membrane permeability and thus in ion fluxes after slicing.

The elution method is valuable in the estimation of ion compartmentation within mesophyll tissue. Not only can apoplastic content be estimated, but also the mean content within the symplast. Subcellular compartmentation, however, can not be effectively evaluated by elution analysis. Energy dispersive X-ray microanalysis of cryosectioned frozen hydrated tissue, in theory, would be the best method for quantitative and qualitative analysis of subcellular ion localization. Unfortunately such technique has not been successfully developed to date. The presence of cell wall and large intercellular air space make it extremely difficult to thin section frozen hydrated tissue, which is essential for high spacial resolution X-ray analysis. In

addition, ice crystal formation during rapid freezing disrupts the integrity of the membranes and thus of the subcellular compartments.

The elution method can also represent an useful approach in the investigation of leaf elemental nutrition if mesophyll apoplastic ionic levels are found responsive to environmental and developmental changes. To date, little research has focused on the potential changes which might occur within the leaf mesophyll apoplast in response to environmental changes, such as irradiance level, temperature, relative humidity, and the availability of elemental nutrients and water within the root environment. The evaluation of diurnal periodicity in xylem solution ionic levels and in the mineral content of leaves have been reported (Hocking, 1980; Hocking et al., 1978). The amplitudes of daily fluctuation of most minerals were within 20 to 50 and 4 to 10 per cent of the mean daily concentration for xylem sap and leaflet, respectively. Leaf mesophyll apoplastic ionic levels in response to environmental changes, however, was not available due to the lack of a suitable evaluation method available to determine mineral composition within the mesophyll apoplast.

This dissertation is probably the first one to present the diurnal fluctuation of ionic levels within mesophyll apoplast, in xylem sap and in whole leaf in an integrated manner. I report that diurnal changes in apoplastic K^+ contents and concentrations fluctuate synchronously within diurnal changes in quantum, relative humidity and temperature. The significance of this synchronous relation is that the elution method provides a valuable tool for evaluating short term responses by plants to water stresses or to changes in elemental nutrient availability to the root system.

This will open another interesting field for future study.

Given the low functional volume of the mesophyll apoplast and the relatively high estimated K^+ content within the apoplast especially during midday, one would anticipate relatively high apoplastic concentrations within leaf mesophyll tissue, up to 10 mM, higher than are typically evaluated for ion flux studies of leaf tissue. In this study, apoplastic K^+ concentrations were estimated to range from 4.3-105.9mM, assuming an apoplastic volume of 0.1 ml/g fr wt, and depending upon the time of day and the stage of leaf ontogeny. This suggests that the apoplast is a temporary pool for K^+ accumulation within the leaf prior the absorption into the symplast or retranslocation to other growing tissue. This is consistent with what claimed by Smith and Fox (1975) that the apoplast might act as a considerable extracellular reservoir or sink for ion which are not readily absorbed by the cells.

The rates of ion transport from the roots through the xylem and into the mesophyll apoplast can be calculated through measurement of xylem sap ionic concentration, volume flow rate through the xylem and apoplastic ionic content. Rate of phloem loading and export from leaf mesophyll apoplast can also be calculated. It is apparent from this study, however, that net flux rates of K^+ into mesophyll cells, the rates of phloem loading and export from leaves might be higher than estimated from studies with leaf slices of discs or with isolated protoplasts (Conti and Geiger 1982; Greenway and Pitman, 1965; Jacoby et al., 1973; Mullen and Koller, 1988; Pitman et al., 1974b).

The conclusion is based upon the observation that net import rates of K^+ via

the xylem are quite high, of approximately 0.3-0.8 μeq/g fr wt/min during periods of rapid transpiration at midday or in the afternoon. At such high import rates, the net uptake of K^+ into mesophyll cells and/or the retranslocation to other tissue via the phloem would also be expected to be relatively high considering the consequent extracellular K^+ concentrations. In addition, considering that apoplastic K^+ contents and concentrations in pea leaf tissue do not decline significantly during leaf maturation and increase again while leaf senesce, a period when total tissue K^+ content is being depleted in leaves, the levels of K^+ within the apoplastic environment surrounding phloem tissue should be conducive to rapid rates of influx and thus subsequent export.

Analysis of specific ion transport processes or compartments which are involved in influencing elemental nutrient levels within leaf tissue can be only achieved through modeling. Modeling enables mathematical integration of all transport functions and pools within a system and thus the evaluation of the regulation of specific components of that system. Although a complete model for leaves was not developed in this dissertation, such a model could be readily formulated and tested with the data presented here and in the literature.

An understanding of the regulation of K^+ transport within leaf tissue is critical to more effective management of agronomic crops so as to optimize their K^+ nutritional status during plant ontogeny. From this study we have learned that both the leaf apoplastic K^+ levels and total leaflet K^+ could be probably regulated not only by fertilization but by modifying ambient environmental factors such irradiance

levels, temperature, relative humidity and water/nutrient availability to the root system. A strategy of managing a field crop to maintain higher K^+ concentration in leaf tissue during ontogeny, however, might not be achieved unless more about the relationship between the apoplastic K^+ content/concentration and total leaflet K^+ level is understood. Besides, before modifying environment to regulate K^+ level within leaflet, it is necessary to examine the effect of each single factor within the modeled system and its effect on apoplastic K^+ concentration.

In conclusion, the question of whether the symplastic or apoplastic pathway is more important for K^+ loading into the phloem and retranslocated to other tissue still merits additional investigation. It was hypothesized (Widders and Lorenz, 1983b) that ontogenetic decline in leaflet K^+ is due to the apoplastic K^+ concentration below the threshold of 3-5 mM, resulting in a net K^+ efflux out of mesophyll cells. The result from my study, however, indicates a continuous K^+ uptake into mesophyll cells throughout leaf ontogeny. It is known that leaflet K^+ levels decline during leaf maturation and senescence. If the above mentioned hypothesis correct, the apoplastic K^+ concentration should decline to below the threshold during leaf maturation in order to sustain an efflux of the symplastic K^+ and ultimately the export and depletion in K^+ content of the tissue.

Information from the literature (Widders and Lorenz, 1983a) and my experiments indicate that K^+ concentration is higher than threshold concentration within the apoplast during leaf growth. It is, therefore, necessary to propose another hypothesis about the route of ontogenetic decline of leaflet K^+. A possible hypothesis

is that K^+ loaded into the phloem is largely via a symplastic pathway. Further research is needed to test this hypothesis. Additional benefit from testing this hypothesis is that it may also provide an evidence of the pathway for sucrose loading into the sieve tubes, which is still a controversial topic, since K^+ has been shown to play an role in phloem loading of assimilates (Giaquinta, 1983).

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