4. Environmental, physiological and biological factors affecting plant response to foliar fertilization

4.1. Introduction

The response of plants to the foliar application of nutrients varies not only between species and cultivars but also depends upon the plant's phenology, the physiological status and the environment in which the plant is growing. Understanding these responses is key to optimizing the efficacy and reproducibility in performance of foliar fertilizers (Kannan, 2010; Marschner, 2012; Weinbaum, 1988).

The physical and physiological characteristics of a plant can alter the efficacy of foliar fertilization in two ways; differences in canopy surface area and the characteristics of the plant surface have a quantitative impact on the amount of applied nutrient that penetrates the surface barriers; while differences in physiological processes (uptake, storage and retranslocation) alter both the immediate and long-term biological efficacy of the nutrient once it has entered the plant.

The environment also influences the efficacy of foliar fertilizers through direct effects on the physico-chemical properties of the spray on the leaf surface (Chapter 3), and by affecting biological processes in the plant over both the immediate and long-term. The immediate conditions of light, temperature and humidity at the time of foliar application affect plant metabolic status and hence may directly influence absorption processes across the leaf surface and from within the internal leaf spaces. Environmental conditions following application can determine the persistence of the treatments on leaf surfaces and affect nutrient redistribution within the plant following their absorption. Over a longer time frame, the environment in which a plant is growing can alter the efficacy of foliar fertilisers through its effect on leaf surface characteristics, the size and composition of the canopy, and its effect on plant nutrient status, morphology and physiology. These interactions are summarized in Table 4.1.

The complexity of the possible interactions between the environment and the biology of the plant on the efficacy of foliar applications greatly complicates the conduct and interpretation of field research and hence their agronomic efficacy. Historically, there have been relatively few studies of nutrient foliar applications that have directly characterized the environmental determinants of uptake integrated with the physiological basis of the observed plant response and consequently this has limited the development of broadly applicable, biologically-based guidelines for the use of foliar fertilizers in diverse crops.

Table 4.1. The physical structure and physiology of the leaf and the plant canopy interact with the local environment affecting retention, absorption and utilization of foliar-applied fertilizers.

Leaf age, leaf surface, leaf ontogeny, leaf homogeneity and canopy development	Physical structure of the leaf affects spray retention	hairs, trichomes, surface architecture
		stomatal distribution and density
		presence of discontinuities (lenticels, cracks…)
	Chemical composition of the leaf affects penetration, distribution, absorption and 'availability' of foliar-applied nutrients	cuticle thickness, cuticle composition
		apoplast binding and complexation
	The physiological state of the leaf at the time of spraying affects nutrient assimilation and mobilization	leaf expansion and source/sink status
		leaf senescence and remobilization
Plant architecture and metabolic status	Canopy architecture and phenology have a quantitative effect on spray retention and penetration	canopy size and leaf age distribution
		new growth, presence of leaf or floral buds, presence of reproductive structures
	Plant metabolic activity and crop phenology affect uptake and remobilizations	shoot and root growth activity alters demand and source:sink dynamics
		metabolic status of plant affects availability of substrate and energy for absorption and assimilation
Short- and long-term environmental interactions	Temperature, light, humidity	immediate effects on energy and metabolites required for nutrient absorption, metabolism and transport
		long-term effects on physical and chemical properties of leaf and plant
	Plant nutrient status alters leaf structure and physiology and may alter leaf assimilation of foliar applied nutrients	
	Biotic and abiotic stress (pests, temperature, water)	

The purpose of this chapter is to review the existing literature on the role of the environment and biology in foliar fertilization efficacy and to use this information to identify common principles and knowledge gaps.

4.2. Leaf age, leaf surface, leaf ontogeny, leaf homogeneity and canopy development

There is considerable evidence from field and laboratory studies that leaf and plant age can have a significant impact on the efficacy of foliar application of nutrients. These effects may reflect differences in ultrastructure, chemical and physical properties and metabolic state of the leaf as described earlier, but may also be a result of differences in the physiological status of the plant which acts to alter the availability of energy and substrate for absorption and assimilation as well as the rate at which absorbed nutrients are translocated out of the leaf (Weinbaum, 1988). When interpreting field studies that show an effect of leaf age on efficacy of foliar fertilization it is critical to consider possible confounding effects of environment (temperature, light, humidity) that generally vary coincident with plant and canopy development and consequently act to reduce the surface area available for spray retention.

A number of studies have shown that rates of uptake of applied chemicals by leaves declines with leaf age from initiation to full expansion (Sargent and Blackman, 1962; Zhang and Brown, 1999b). This may also be followed by a period of increasing permeability as mature leaves begin to senesce. For example, uptake of N from ^{15}N labelled urea and KNO_3 on a per unit leaf area of *Citrus paradisi* L. cv. Redblush was 1.6 to 6 fold greater for two month old leaves than for six month old leaves. In studies using isolated cuticles of Marsh grapefruit (*Citrus paradisi* Macfad), transcuticular movement of urea decreased as leaf age increased from three to seven weeks, but permeability increased in cuticles from leaves older than nine weeks (Orbovic *et al.*, 2001). In these studies, cuticle thickness, weight per area and the contact angle of urea solution droplets increased as leaves aged.

Though most researchers have focused on N in these studies, the effect of leaf age on foliar absorption has been observed for other elements. Walker (1955) reported a higher P absorption by young apple leaves than old ones; and immature pistachio and walnut leaves absorbed 55 and 25% more Zn than fully expanded leaves (Zhang and Brown, 1999b). Olive plants sprayed with three KCl concentrations (0.2 and 4%) showed a positive linear response to increasing leaf K applications and that foliar K uptake was higher in young than in mature leaves of olive and French prune (Restrepo-Diaz *et al.*, 2009; Southwick *et al.*, 1996).

Many researchers in diverse crops and environments have made the observation that the gross quantity and composition of the cuticle and the epicuticular waxes varies with leaf development, and have hypothesized that this variation with age influences foliar uptake (Hull *et al.*, 1975; Leece, 1976; Rhee *et al.*, 1998; Riederer and Friedmann, 2006; Swietlik and Faust, 1984; Zhang and Brown, 1999b). While correlations between absorption and gross changes in cuticles clearly occur, this is not sufficiently mechanistic to be considered causal. The difficulty in interpreting the role of gross cuticular characteristics on foliar absorption is illustrated by the contrast in foliar absorption by diverse species whose leaves frequently exhibit gross differences in cuticle structure, wax percentages and composition but these differences are poorly predictive of foliar absorption capacity.

To better understand the relationship between cuticle composition and foliar absorption as affected by leaf age, Riederer (1995) analyzed the change in specific waxes in *Fagus sylvatica*. In this species, the distribution of aliphatic[10] wax constituents, within the maximum range of C_{28} and C_{52} (number of carbon atoms), shifted within 20 days after bud expansion to a large single maximum of C_{28} waxes when the leaf reached final size (Riederer and Friedmann, 2006). Unfortunately, the physiological relevance of these changes was not determined. During leaf expansion of *Prunus laurocerasus*, the average chain length of alcohols and fatty acids of epicuticular waxes increased from C_{24} to approximately C_{32} (Bringe *et al.*, 2006; Jetter and Schaffer, 2001) which altered the wettability of cuticles (Chapter 2). In apples, this coincided with the decrease in height of cuticular ridges or 'wrinkles' (about 0.8–1.0 µm in height in youngest leaves), especially above the lumen of epidermal cells (Bringe *et al.*, 2006). In leaves and fruits of citrus, the same shift in wax composition during leaf expansion was observed and was coincident with a corresponding decline in wax concentration per unit of leaf area (Freeman *et al.*, 1979). During ontogenesis of peach leaves, the individual wax mass as well as the composition of major components (triterpenes and alkanes), and the average chain lengths of alcohols increased with leaf ontogenesis while the absolute amounts of alcohols largely remained constant or slightly increased (Bukovac *et al.*, 1979).

The cuticular wax of the adaxial surface of apple leaves was analyzed under controlled growth conditions for their chemical composition, micro-morphology and hydrophobicity just as the leaf unfolded (Bringe *et al.*, 2006). With increasing leaf age, the hydrophobicity of the adaxial leaf surface decreased significantly. The contact angles of solutions with the leaf surface also decreased with age, facilitating the absorption of solutes. The amount of apolar cuticular wax per unit area was lower in older than in young leaves. A similar effect was detected for the ester fraction: the C_{40}:C_{52} ratio was approximately 1:1.1 for the youngest leaf, and changed to 1:5 for the oldest one. These changes decreased the hydrophobicity of adaxial leaf surfaces and were associated with a decrease in the total amount of extractable surface waxes as well as modifications in the composition of wax compounds. The accumulation of OH-functional groups also seems to play an important role in increasing leaf wettability with leaf age. This effect may be explained by the increased polarity of the mature surface due to the accumulation of hydroxyl groups (Fernandez *et al.*, 2011). In agreement with Bringe *et al.* (2006), Hellmann and Stosser (1992) observed no consistent effect of leaf age or cultivar on total wax mass in apple (*Malus domestica* Borkh), while the proportion of alkanes and esters decreased during leaf ontogenesis and primary alcohols increased.

The conflicting results on the effect of leaf age on foliar absorption that are reported by different researchers are certainly a consequence of the species and the environment in which the experiments were conducted. The difference in response between field and laboratory research is striking. Bringe *et al.* (2006) observed that, in all stages of leaf development for apples grown in the laboratory, the wax mass of adaxial cuticles of the leaves remained low (10–15 µg cm^{-2}) as compared to about 280 µg cm^{-2} (total wax mass) and 76 µg cm^{-2} (epicuticular waxes) for apple leaves grown in the field (Hellmann and Stosser, 1992). The conclusions derived from research focused on one leaf surface or

[10]Organic compounds with an open chain structure, for instance, n-alkanes

species must also be considered with caution; in *P. laurocerasus* total cuticular waxes on the adaxial (280 μg cm^{-2}) were much less than on the abaxial (830 μg cm^{-2}) leaf surface (Jetter and Schaffer, 2001), and significantly greater than amounts detected for field grown *Malus domestica* (Hellmann and Stosser, 1992) which are 30 times greater than total cuticular waxes in laboratory grown *Malus domestica*. The differences in cuticular wax between these field and laboratory grown plants is a result of integrated differences in many factors including temperature, humidity, UV light, dust, mechanical strain, leaf phenology and other biotic and abiotic stresses.

- Leaf cuticular composition changes with leaf age and varies with species and the environment.
- Changes in leaf cuticular composition corresponds with changes in efficacy of foliar-applied fertilizers.
- It is not currently possible to predict how changes in leaf cuticular composition will alter the efficacy of foliar-applied fertilizers.
- With these uncertainties, empirical testing of foliar fertilizers is essential to ensure efficacy and safety.

It is well documented that the abaxial leaf surface takes up mineral nutrients more rapidly than the adaxial surface. According to Hull (1970), the greater nutrient absorption by the abaxial leaf surface results from the presence of a thinner cuticular membrane and a large number of stomata. Fernández *et al.* (2008) observed that adaxial cuticles isolated from pear leaves were thicker in contrast to abaxial ones, though the significance of abaxial cuticle characteristics in the uptake of foliar-applied fertilizers is currently unclear and requires further investigation. The theory that abaxial cuticles are thinner and therefore uptake will be more rapid has been inadequately validated as the majority of studies have been conducted with adaxial cuticles to avoid the complexities associated with the presence of stomata. Schlegel and Schönherr (2002) examined four plant species and observed that, during the first 24 hours, the absorption of Ca^{2+} by the abaxial leaf surface was much higher than that of the adaxial. In contrast, Boynton *et al.* (1954) concluded that both leaf surfaces differ only in the rate of nutrient absorption and not in their total absorption capacity. This conclusion was based on the observation that urea absorption by the abaxial leaf surface was rapid within the first 24 hours and then decreased rapidly. The adaxial leaf surface absorbed urea steadily for seven days after which the rate of this absorption was similar to the abaxial leaf surface (Boynton, 1954). Leaf surface influenced Zn adsorption but not Zn absorption in pistachio and had no effect on either Zn adsorption or absorption in walnut (Zhang and Brown, 1999a).

In addition to changes in leaf cuticle composition, the number of trichomes and the composition of the exudates also change with leaf development (Valkama *et al.*, 2004). Density of both glandular and non-glandular trichomes decreased drastically with leaf expansion although their numbers per leaf remained constant or decreased

as they were shed (Schönherr and Schreiber, 2004). These results suggest that the final number of trichomes in a mature leaf is established early in development. Therefore the functional role of trichomes is likely to be most important at the early stages of leaf development when density is highest. However, since changes in trichome number occur simultaneous with changes in cuticular waxes, it is difficult to directly quantify the contribution of trichome density to foliar absorption as the leaf ages.

At the whole plant level, differences among species in patterns of leaf development, canopy expansion and bearing habit also affect the homogeneity of the leaf canopy by altering the leaf population at a given age at any point in time. For example, in peach (*Prunus persica* L. Batch) the canopy is borne mainly on long shoots with shoot growth and leaf expansion continuing throughout the growing season (Gordon and Dejong, 2007). On the other hand, the canopy of apple (*Malus domestica* L.) and almond (*Prunus amygdalus* L.) trees consists mainly of short shoots and development of the leaf canopy is essentially complete within a month (Lakso, 1980). There is also a distinct difference between evergreen and deciduous trees, with evergreen trees (eg. Citrus sp. *Olea europea*) maintaining their leaves for more than a year with growth occurring in flushes during discrete periods. In many annuals, including the major cereal crops, plant growth and leaf expansion continues from first leaf expansion through flowering until seed set, at which time leaf senescence commences in the oldest leaves and progresses to the youngest leaves.

Young trees growing under high levels of fertility and water availability show a longer period of shoot extension and persistence of the leaf canopy (Ramos *et al.*, 1984) while conversely total canopy area and size of leaves is adversely affected by nutrient deficiencies or water deficit (Chabot and Hicks, 1982). Variability in light intensity and spectral distribution within the canopy may also result in non-uniformity of the foliage within the canopy. The distribution of light within the canopy may be influenced by various cultural practices such as tree spacing, rootstocks and pruning (Jackson and Palmer, 1980). Also, leaf senescence is delayed in exposed compared to shaded parts of the canopy.

The uptake and redistribution of foliage-applied nutrients varies with the heterogeneity of leaves (Weinbaum, 1988). Experiments on foliar applications of urea in both almond (Weinbaum, 1988) and olive (Barranco *et al.*, 2010) made in June/July, or later, had a greater response than April applications. Fisher (1952) and Barranco *et al.* (2010) interpreted this effect as a result of a greater leaf area available for urea absorption. The export of N from foliage-applied urea to immature leaves is reduced when applied early in the season because of a greater incorporation of N into leaf protein in developing foliage. In contrast, N applied in the late season does not stimulate protein formation and shows increased mobility to other plant parts as leaf senescence occurs (Klein and Weinbaum, 1984). The effect of leaf age on the absorption and transport of foliar-applied nutrients can be directly attributed to the stage of leaf transition from being a sink for photosynthates produced in mature tissues to then becoming a source of photosynthates for newly developing sinks.

"The transition from sink to source status is one of the key events in leaf development. When a leaf is about half grown it stops importing phloem-mobile nutrients from the rest of the plant and begins to export its own products of photosynthesis. This shift in transport direction, which is largely irreversible, involves major changes in the way metabolites are transported to and from the leaf mesophyll through plasmodesmata and via transporters. The import of nutrients ceases when plasmodesmata in large veins are lost or narrowed preventing phloem-unloading. Export begins when the minor veins mature and begin to unload sugars and other compounds into the phloem. The uni-directional nature of loading is a consequence of sucrose transporter orientation in the plasma membrane of phloem cells, or of the trapping of raffinose family sugars in those species that load through plasmodesmata." (Turgeon, 2006)

In view of leaf ontogeny, leaves are not physically or physiologically capable of exporting nutrients until after they have matured and likewise old leaves are incapable of importing nutrients following maturation. This view is consistent with older literature in which radioactive ^{32}P was applied as a foliar treatment to bean leaves of sequentially younger age and the transport of the applied labelled P monitored 48 hours later by placing plants on X-ray films (Figure 4.1). The application of ^{32}P to mature leaves (A and B) resulted in rapid transport of the labelled P containing products to young developing leaves and roots. With application to successively younger leaflets (C), transport out of the treated leaf is reduced and restricted to the nearest sink tissue (apical shoot meristems) with no ^{32}P transported to root; while application to immature leaves (D) resulted in 100% retention of the labelled P in the treated leaf. While the timing with which leaves transition from sink to source varies between species and environments, the effect of this transition on the ability of leaves to export foliar-applied nutrients is a general principle that should be considered when designing and interpreting foliar applications.

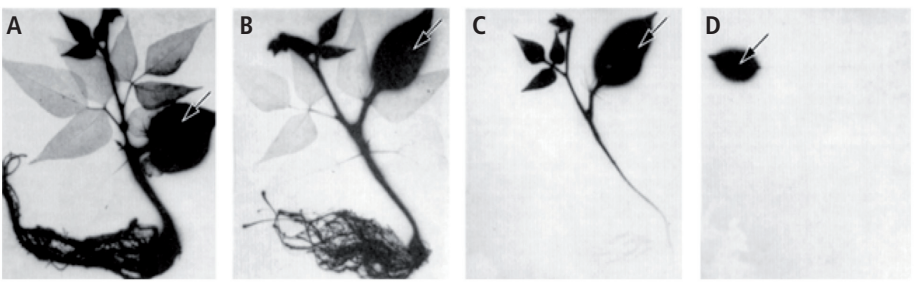

Figure 4.1. ^{32}P was applied to the indicated leaf (arrow) by immersion. 24 hours after exposure, plants were placed on X-ray film and the distribution of the labelled P was illustrated.

Collectively these results illustrate the difficulty in interpreting studies of leaf age, leaf cuticular structure and plant phenology on foliar absorption. In the absence of biotic and abiotic factors, it is apparent that (at the single leaf scale) the proportion of cuticular waxes on a leaf area basis decreases with time as leaves expand more quickly than new cuticular matter is synthesized. In field conditions, this pattern may be reversed as light, temperature, humidity, mechanical stresses and other abiotic and biotic factors stimulate cuticular synthesis while restricting leaf expansion. The complexity of the interactions between leaf age and foliar absorption are further complicated by simultaneous changes in leaf metabolism and nutrient export that occur during development, as well as patterns of crop phenology which determine leaf age distribution, canopy architecture and relative competition between organs. It is also very difficult to compare results from diverse experiments since true physiological leaf age is highly dependent on growth conditions and simultaneous changes in deposition of cuticular materials, leaf expansion and the accumulation of mechanical and biotic stresses (pathogens and herbivory) which all act to alter foliar absorption capacity. When rapid leaf export of a phloem mobile nutrient is occurring care should be taken to ensure quantification of the rate of absorption by measuring both nutrient recovery in the treated area as well as in sink organs.

- The influence of leaf age and the environment on the efficacy of foliar applications is complex and currently no universal principles can be discerned.
- Leaves of diverse species exhibit gross differences in cuticle structure, wax percentages and composition but these differences are poorly predictive of foliar absorption capacity.
- The effect of leaf age on the absorption and transport of foliar-applied nutrients can be directly attributed to leaf transition from a sink for photosynthates to a source of photosynthates for newly developing sinks.
- At the whole plant level differences in patterns of leaf development, canopy expansion and bearing habit affect the homogeneity of the leaf canopy and hence alter the population of leaves of a given age at any point in time.
- The rates of uptake of applied chemicals by leaves declines with leaf age from initiation to full expansion and may increase again during leaf senescence.

4.3. Plant species and variety

While there have been a large number of reports that demonstrate differences in foliar absorption between species, very few of these studies have identified the mechanism underlying them. Klein and Weinbaum (1985) examined urea absorption by the leaves of olive (*Olea europea* L.) and almond (*Prunus dulcis* Mill. D.A. Webb) and found that olive absorbed 15 times more urea than almond per unit leaf area. Amongst fruit trees, examples of varietal differences in response to foliar N application have been seen for

peach, plum, apple and citrus. Van Goor (1973) showed significant differences in Ca^{2+} absorption by apples of different varieties, with 'Cox's Orange Pippin' absorbing five times more Ca^{2+} than 'James Grieve'. Wojcik *et al.* (2004) indicated that an increase in apple fruit Ca^{2+} concentration as a result of foliar Ca^{2+} application depended on variety. For example, 'Idared' apples took up less Ca^{2+} than 'Jonagold' and 'Gloster'.

The mechanism by which species differ in response to foliar sprays was investigated by Picchioni *et al.* (1995) who showed that the rate of B absorption by apple leaves was two to three times higher than that of pear, plum and sweet cherry. Genotypic differences in shoot leaf surface characteristics, among the species tested, was found to influence greatly the amount of solution retained per unit leaf area. Leaf retention varied between species, with apple retaining significantly more B on a leaf area basis than pear, plum and sweet cherry (Figure 4.2). On average, apple shoot leaves retained, absorbed and exported at least twice as much labeled B per unit leaf area as prune and pear shoot leaves; and three to four times as much as sweet cherry shoot leaves. These differences in the quantity of B absorbed by the leaves and the amount of B exported from the leaves (Figure 4.3) may be attributed to the occurrence on the apple leaves of abundant epidermal hairs that help retain the applied solution (Picchioni and Weinbaum, 1995). These observations are in agreement with others (Brewer *et al.*, 1991; Fernandez *et al.*, 2011; Hesse and Griggs, 1950) who found a significant influence of trichomes upon the degree of surface wetting of the different plant surfaces.

The rate at which a nutrient is removed from the leaf in the phloem will also influence tissue nutrient levels observed hours or even days following foliar application. Boron application in apple and almond, for which there is a substantial remobilization of applied B out of the leaves and movement into fruiting tissues (Picchioni and Weinbaum, 1995), generally results in a lower long-term leaf B concentration than the application of an equivalent treatment to pistachio or walnut in which B is immobile. This difference in relative mobility of B is the result of the formation of specific B-sorbitol compounds in apple and almond but not in walnut or pistachio (Brown and Shelp, 1997). Therefore under these circumstances leaf tissue analysis for B content performed hours or even days after foliar treatment will result in the false conclusion that walnut and pistachio absorbed more B than apple or almond.

Differences in rate of re-mobilization of absorbed nutrients may also explain differential response of species to Fe-chelates. Schlegel *et al.* (2006) reported that penetration of Fe-IDHA into stomatous leaf surfaces differed among plant species. The decrease in slopes with time was most conspicuous with apple and grapevine leaves. Penetration plots were linear with broad bean and Madagascar jasmine but not with the other species tested. The previous authors suggested that if Fe-chelates accumulate under the cuticle and are not translocated rapidly then rate constants would decrease with time.

Species difference in utilization of foliar nutrients is undoubtedly related to the physical and chemical composition of the leaf surface. For example, the recovery and absorption of applied Zn by walnut was less than pistachio (Zhang and Brown, 1999b). This low effectiveness is associated with a highly hydrophobic cuticular wax layer in

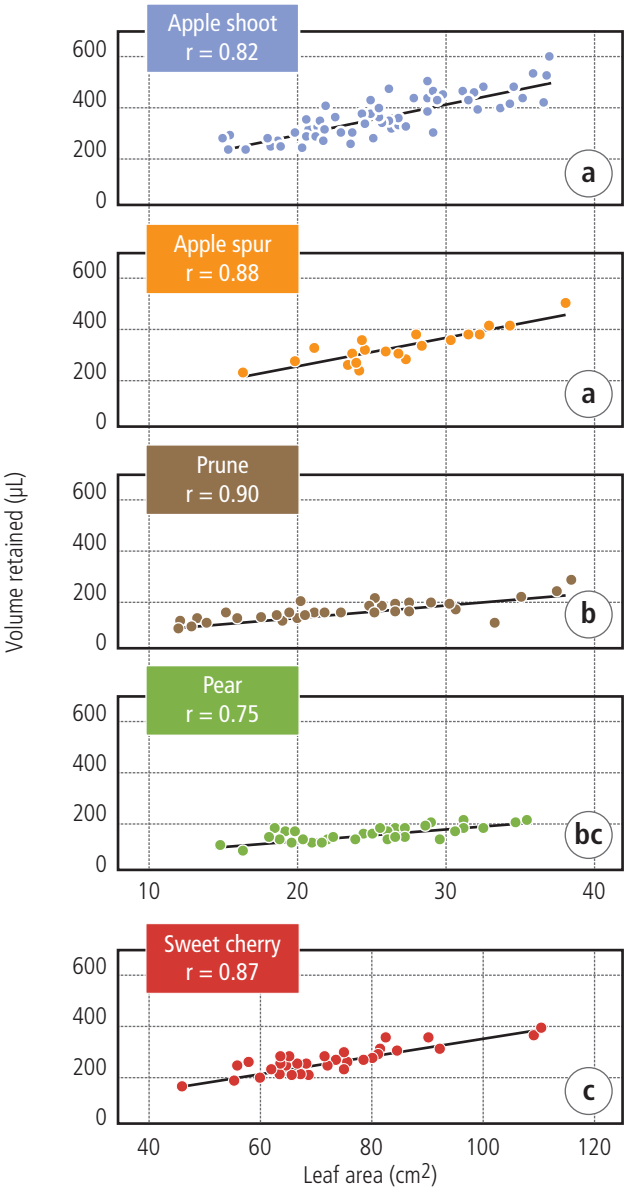

Figure 4.2. Relationship between leaf area per single leaf and the total volume of B treatment solution (1000 mg B/liter + 0.05% Triton X-100) retained per leaf. Cultivars from top: 'Red Delicious' Apple, 'French' prune, 'Bartlett' pear and 'Bing' sweet cherry. All data correspond to shoot leaves unless specified. Regression lines with the same letter have slopes that are not significantly different at P=0.05. Each correlation coefficient is significant at P=0.01(Adapted from Picchioni and Weinbaum, 1995).

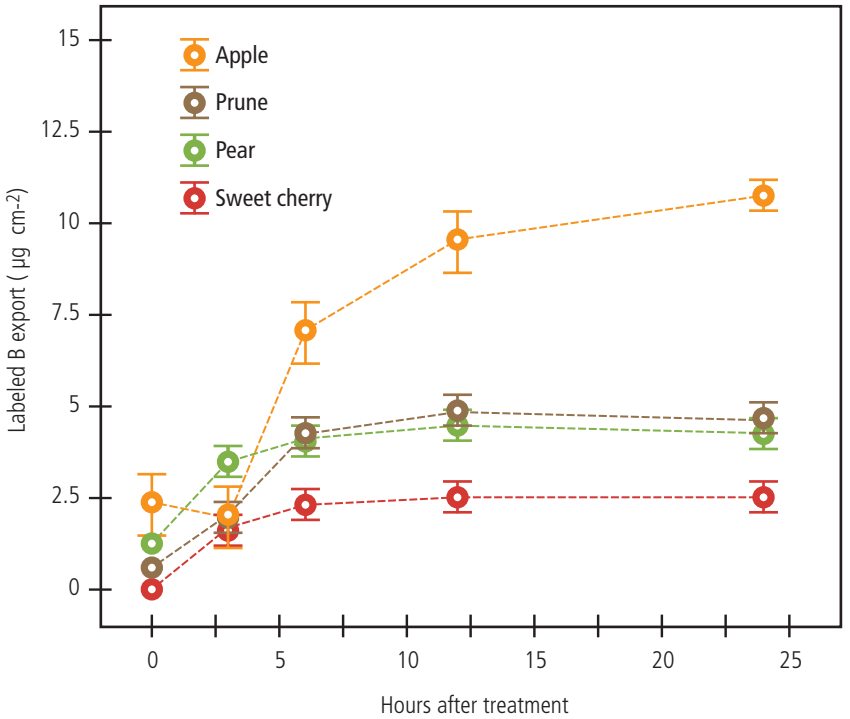

Figure 4.3. Export of foliar-applied, labelled B by shoot leaves expressed as absolute quantity. Time 0 h refers to 15-20 min following application (when the leaf had visibly dried). Export was calculated as the difference between the quantity of labeled B uptake and the quantity of labeled B measured in the leaf tissue extract at each time period (µg cm^{-2}). Cultivars are shown in Figure 4.2. Each value is the mean +/- standard error of five tree replicates of two leaves on a single shoot (Adapted from Picchioni and Weinbaum, 1995).

walnut, which limits Zn penetration, and a high capacity for fixation of the cationic Zn to exchange sites in the cell wall and cuticle (Zhang, 1999).

Leaf longevity can also account for species difference in response to foliar fertilizers with evergreen trees such as citrus, banana, coffee and cacao plants being more efficient in their utilization of soil-derived and foliar-applied leaf nutrients because of their longer leaf life; and the cycles of leaf wetting and drying enhances the recycling of nutrients through the autumn fall and decomposition process (Aerts and Chapin, 2000).

Species differ remarkably in their response to foliar fertilizers as a consequence of differences in:

- Canopy architecture and distribution of leaves of different age.
- Leaf retention and absorption of foliar sprays.
- The rate of remobilization of applied nutrients.

4.4. Effect of the environment on efficacy of foliar-applied nutrients

Light, humidity and temperature can each affect foliar absorption in several ways: 1) through direct effects on the spray solution prior to leaf absorption (Chapter 3.2.); 2) through effects on the leaf developmental processes discussed above (Chapter 4.2); and 3) by altering photosynthesis, stomatal opening, respiration, leaf expansion and sink activity and consequently changing energy and metabolite availability for the uptake, assimilation and subsequent transport of foliar nutrients.

4.4.1. Light

Chemical ion uptake by leaves can be directly influenced by light as a result of physical and chemical changes in the cuticle and also from the direct involvement of light on the energy and metabolite availability on the uptake and assimilation of foliar-applied nutrients (Abadia, 1992; Alvarez-Fernandez *et al.*, 2004; Hundt and Podlesak, 1990; Jacoby, 1975; Muhling and Lauchli, 2000; Nobel, 1969; Nobel, 1970; Rains, 1968; Raven, 1971; Swader *et al.*, 1975).

The amount and composition of synthesized waxes and their arrangement on the surface is directly influenced by light including photosynthetically active radiation (Cape and Percy, 1993; Takeoka *et al.*, 1983), as well as by UV-B radiation (Barnes *et al.*, 1996; Bringe *et al.*, 2006). The thickness of the cuticle and the amount of cuticular waxes in various plant species was found to be higher on those grown under high rather than low light intensities (Macey, 1970; Reed and Tukey, 1982) and the development of secondary wax structures is increased by higher light intensities (Hull *et al.*, 1975). The influence of light is cumulative with exposure and Leece (1978) demonstrated that the seasonal build-up and development of secondary wax structures on the abaxial surface of plum leaves (*Prunus domestica* L.) positively corresponded with increasing light intensity. Leaves of apple trees grown outdoors can synthesize up to three times more cuticular wax per surface area as compared to the same species grown in the greenhouse (Hunsche *et al.*, 2004) and as much as 30 times greater than those grown under low light intensity and high humidity plant culture conditions (Bringe *et al.*, 2006).

Numerous researchers have also shown stimulatory effects of light on short-term absorption of nutrients (Bowen, 1969; Christensen, 1980; Nobel, 1969; Rains, 1968) while others have demonstrated that light does not affect absorption (Rathore *et al.*, 1970; Zhang and Brown, 1999b). Jyung *et al.* (1965) and Shim *et al.* (1972) showed positive relationships between light intensity and ability of apple and bean (*Phaseolus vulgaris* L.) leaves to take up urea, Rb and PO_4. Rains (1968) demonstrated increased uptake of K^+ by corn/maize (*Zea mays* L.) leaf slices in light; an effect that was seen under even low-light conditions and could be reversed with the addition of metabolic inhibitors. In corn/maize the light level required to maximize K absorption was significantly lower than that required for photosynthesis (Rains, 1968); while in tomato much higher light levels were required to maximize K absorption which was rapidly decreased in the dark and by inhibitors of photosynthesis and metabolic uncouplers as shown in Figure 4.4

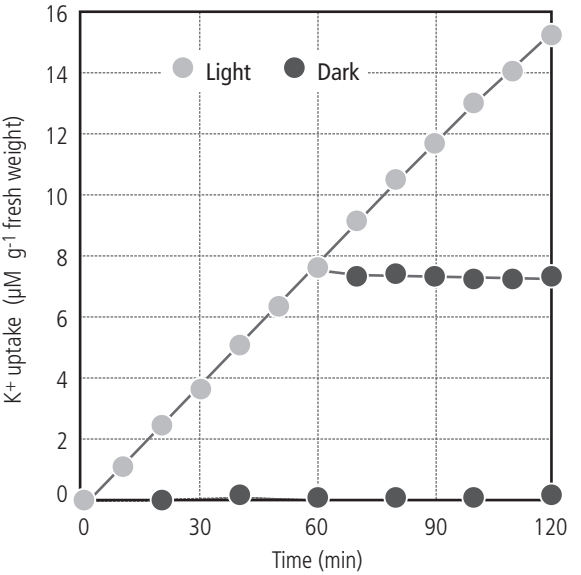

Figure 4.4. Effect of light and dark periods on uptake of K into leaf slices of tomato (Adapted from Nobel, 1969).

(Nobel, 1969). Schlegel and Schönherr (2002) reported that rates of $CaCl_2$ penetration from apple and pear leaf surfaces in the light were higher than in the dark.

While a positive effect of light on uptake of foliar applied elements has been frequently reported, many examples exist where light, metabolic inhibitors or reduced temperatures had no effect on nutrient absorption or transport (Rathore *et al.*, 1970; Zhang and Brown, 1999b). In pistachio and walnut Zn absorption at high concentrations (7.5 to 15 mM) was not affected by light or metabolic inhibitors (Zhang and Brown, 1999b) which supports earlier reports on Zn absorption in bean (*Phaseolus vulgaris*) (Rathore *et al.*, 1970). Zhang and Brown (199b) interpreted this result as evidence that Zn absorption was mostly determined by an ion exchange and/or diffusion process rather than a metabolically active one.

A direct effect of light on foliar absorption may also occur if the spray compound is unstable in the light. Several authors have demonstrated that Fe^{3+} chelates are UV-labile (Albano and Miller, 2001a; Albano and Miller, 2001b; Albano and Miller, 2001c) and as a consequence Schönherr *et al.* (2005) concluded that foliar penetration of chelated Fe preferentially occurs during the night and therefore foliar applications during the late afternoon are recommended. The potential for photochemical or temperature dependent degradation of foliar sprays exists for many commercial products and should be verified before widespread adoption. However, application of sprays under dark conditions may in turn limit uptake rates since the stomatal pathway may not be involved in the absorbtion process due to stomatal closure during the night.

The reported differences on the effect of light in short-term uptake of foliar- applied nutrients are likely to be the result of several interacting factors. At the cellular level the transmembrane transport, regulation and assimilation of most nutrients is directly or indirectly influenced by the metabolic status of the cell so light deprivation would be expected to reduce uptake. The only exception among the essential plant elements may be B, which is uncharged at normal pH, and sufficiently permeable through both leaf cuticle and cellular membranes to be absorbed and assimilated spontaneously into molecules of biological and metabolic importance. The finding that Zn absorption does not respond to the presence or absence of light may simply reflect a lack of sensitivity of the methodology employed or could be a consequence of the relatively small metabolic demand that (passive) Zn uptake would have on whole cell energetics which likely reflects the significant binding of Zn to cell wall materials by non-metabolic diffusion and exchange processes. The contrasting results obtained with the macronutrients, N, P and K, likely reflect the proportionally lower cell wall binding and the predominance of active uptake and transport processes for these more mobile elements. It is also probable that applications of relatively low concentrations of micronutrients do not represent a substantial metabolic cost while supplying higher concentrations of the macronutrients presents a significant metabolic cost for uptake and assimilation especially in experimentation using excised leaf slices.

4.4.2. Temperature

Temperature can influence foliar absorption through its effect on the rate of drying of the spray application; the nutrient solution physico-chemistry; as well as its impact on leaf cuticles; and on plant metabolism, ion uptake and assimilation. The most immediate effect of high temperature is increasing the drying rate of the spray droplets which will directly reduces absorption. However, increased absorption by leaves over prolonged high temperatures has been recorded in several species (Cook and Boynton, 1952). This may be the result of the high temperature during leaf development altering the amount and composition of synthesized waxes and their arrangement on the surface (Baker, 1974; Reed and Tukey, 1982) which then influences absorption (Norris, 1974). Reed and Tukey (1982) claimed that under conditions of persistent high air temperature surface wax components adopt a vertical configuration and hence their leaf surface coverage decreases which consequently allows increased nutrient absorption. Lurie *et al.* (1996) reported that even slight alterations in the molecular configuration of surface waxes can significantly affect nutrient absorption rate. Temperature will also have a direct effect on the rate of leaf development and hence influence foliar absorption through effects on leaf phenology and sink:source relations (Chapter 4.1.).

Over a short period, the prevailing temperature during and immediately following foliar application has varied effects depending on species and mineral element applied. In pistachio, Zn absorption after application ranged from 9 to 14% as the temperature increased from 8 to 31°C over a 24 hour period. Within the same temperature range Zn absorption in walnut only increased from 4 to 6% (Zhang and Brown, 1999b). The temperature coefficient (Q10) is perhaps the most classical of all indices for separating active and passive uptake processes by plant tissues (Zhang and Brown, 1999b).

According to Wittwer and Teubner (1959) the Q10 for active nutrient uptake processes in plants is usually above 2. The average Q10 of between 1.2 to 1.4 for Zn absorption observed by Zhang and Brown (1999b) is consistent with a Q10 of 1.2 as reported by Rathore *et al.* (1970). This lack of a strong temperature dependence suggests that foliar Zn absorption was largely non-metabolic dominated by ion exchange and/or diffusion processes. Furthermore lower Zn uptake by leaves under lower temperatures has also been attributed to an increased viscosity of the aqueous ambient solution which probably results in a decreased rate of diffusion of Zn ions (Rathore *et al.*, 1970). The slight temperature dependence observed in these experiments is in contrast to the strong temperature dependence reported by Bowen (1969) who found a Q10 of >2.5 using sugarcane leaf slices. In Marsh grapefruit (*Citrus paradisi* Macfad.) the permeability of isolated leaf cuticles to urea within the first 4 to 6 hours after application increased as temperature was raised from 19 to 28°C but there was no further increase at 38°C (Orbovic *et al.*, 2001).

Further evidence that the effects of temperature on cuticular penetration are not due to changes in metabolism has been presented (Schönherr, 2001; Schönherr *et al.*, 2005; Schönherr and Luber, 2001). When temperature increased from 15 to 30°C rates of penetration of Ca and K did not increase (Schönherr, 2001); and in the range of 15 to 35°C the rate constants of penetration of chelated Fe^{3+} did not depend on temperature (Schönherr *et al.*, 2005). In field situations temperature will interact with humidity to affect the physico-chemical characteristics and solubility of deposited materials.

4.4.3. Humidity

As with light and temperature, humidity can affect multiple processes that ultimately influence the rate of foliar absorption of aerial applied fertilizers. The key processes affected by humidity are 1) the reaction of the foliar application during aerial transport and once deposited on the plant surface 2) the effect of humidity of leaf cuticle structure and stomatal function and 3) the effect of humidity on leaf metabolism and transport processes. In the short term the effect of humidity on nutrient absorption by leaves is primarily related to the rate of drying of droplets during aerial transit to the plant surface and their persistence once deposited on the plant surface (Gooding and Davies, 1992). High relative humidity favors uptake as it delays rapid solution drying which can lead to crystallization on the leaf surfaces (Gooding and Davies, 1992). Additionally, high air humidity causes the swelling of the cuticular membrane which favors the absorption of hydrophilic compounds (Schönherr and Schreiber, 2004) (Chapter 3). Therefore humidity at the time of foliar application affects the velocity of penetration by two independent mechanisms: a) swelling of the cuticle; and b) dissolution of salt as related to the point of deliquescence (POD) which is defined as the relative humidity value at which the salt becomes a solute (Chapter 3.1.4.). Over longer time frames (days to weeks) the amount and composition of synthesized waxes and their arrangement on the surface is influenced by relative humidity which consequently may alter the rate at which foliar applications can penetrate the plant surface (Baker, 1974). Post absorption humidity can have general effects on plant responses to foliar nutrients by affecting xylem and phloem transport processes. Eichert and Goldbach (2010) reported that,

at high relative air humidity, B applied to the cotyledons of *Ricinus communis* L. was transported to hypocotyls and roots; whereas at low relative humidity no translocation of B was detectable. It is concluded that ambient air humidity influences phloem mobility of B *via* its effect on the xylem flow rate; if the xylem flow rate is low or interrupted then foliar-applied B becomes modestly mobile.

Various studies have demonstrated the effect of humidity on physico-chemical properties of different spray solutions and their interactions with plant surfaces (Schönherr and Schreiber, 2004) (Chapter 3). In their classical earlier work, Wittwer and Bukovac (1959) showed that the uptake of P by bean leaves was doubled when the treated surface was kept moist compared with similar treatments in which leaf surfaces were allowed to dry. Salts with points of deliquescence (POD's) above the prevailing relative humidity, when applied in the leaves, theoretically remain as solutes and leaf penetration is prolonged. This principle has been clearly demonstrated in isolated cuticular membrane studies of Ca, K and Fe compounds under varied humidity regimes

Table 4.2. Point of deliquescence (POD) of various salts (Schönherr, 2002).

Compound	POD (%)
$CaCl_2 \cdot 6H_2O$	33
$Ca(NO_3)_2 \cdot 4H_2O$	56
$MgCl_2 \cdot 6H_2O$	33
$Mg(NO_3)_2 \cdot 6H_2O$	56
$MgSO_4$	90
$Zn(NO_3)_2 \cdot 6H_2O$	42
$ZnSO_4$	90
KCl	86
KNO_3	95
K_2SO_4	98
$K_2CO_3 \cdot 2H_2O$	44
K_2HPO_4	92
KH_2PO_4	95
NH_4NO_3	63
Ca-propionate $\cdot H_2O$	95
Ca-lactate $\cdot 5H_2O$	97
Ca-acetate	100
$FeCl_3 \cdot 6H_2O$	44
$Fe(NO_3)_3 \cdot 9H_2O$	54
$Mn(NO_3)_2 \cdot 4H_2O$	42
$MnCl_2 \cdot 4H_2O$	60

membrane penetration followed first order kinetics. When humidity is above the POD the salt residue on the cuticle dissolves while penetration ceases when humidity falls below the POD. Above the POD there is often a linear increase in penetration as humidity increases, though the exact nature of this relationship is mineral salt and species specific (Schönherr, 2001; Schönherr and Luber, 2001).

Anomalies in the relationship between humidity, POD and penetration can be the result of undisturbed layers at the leaf surface which would increase effective humidity on the leaf surface. High humidity may also alter stomatal opening and have non-linear effects on aqueous pore formation (Schlegel *et al.*, 2005; Schlegel *et al.*, 2006; Schönherr, 2006). Van Goor (1973) demonstrated that an increased penetration of Ca^{2+} through the cuticular membrane of apple fruit correlated with decreasing air humidity in the period of time just after application. This phenomenon is explained by an increase in droplet Ca^{2+} concentration resulting from their drying and the consequent increase in the concentration gradient for diffusion. However, despite initial enhanced absorption dynamics at low air humidity the final uptake rates of nutrients from salts of low hygroscopicity are decreased because of rapid salt crystallization once humidity drops below the POD (Wojcik, 2004).

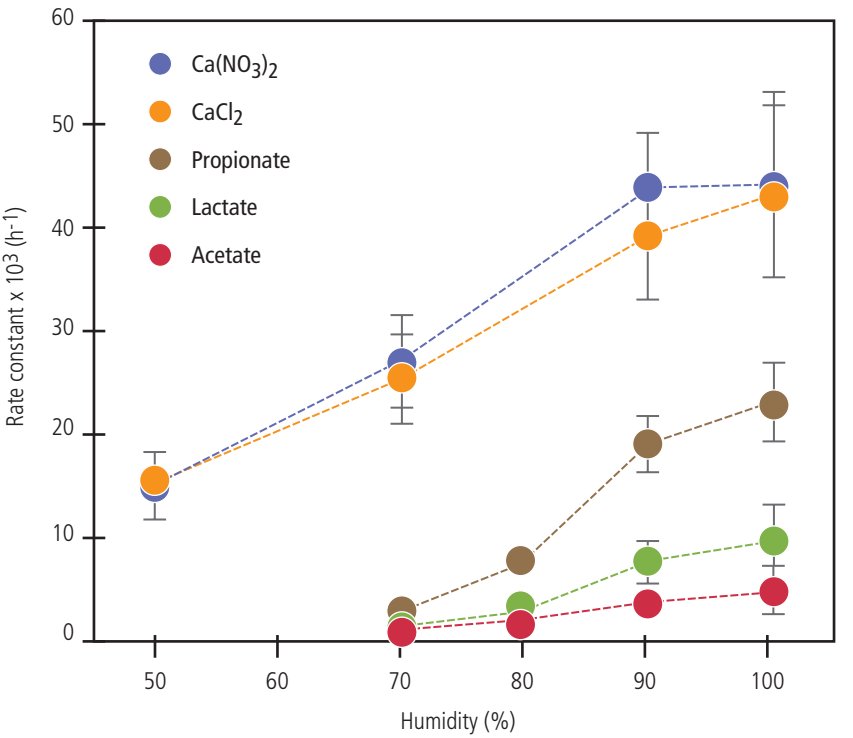

Figure 4.5. Influence of humidity and anions on penetration of Ca salts through isolated cuticles of apple fruit (Adapted from Schönherr, 2001).

While it is clear that the POD of the foliar applied chemical and the humidity during the application period have an important effect on penetration, knowledge of the POD alone is often insufficient to predict the efficacy of a mineral salt as a foliar fertilizer. For example, the apparent ease of penetration of $Ca(NO_3)_2$ or $CaCl_2$ (Figure 4.5) demonstrated by Schönherr (2001) and Schönherr and Luber (2001) does not explain the great difficulty many growers have encountered in correcting field Ca deficiencies; and there are many reports of formulations with purported efficacy that an analysis of POD alone would not support. While a POD less than ambient humidity is required for uptake to occur, it is not always a guarantee since many other factors (e.g. associated with plant physiology or the prevailing environmental conditions) may hinder the absorption process under field conditions. Efficacy of salts with a high POD can be enhanced through the use of adjuvants with humectant properties (Chapter 3). In addition, and as suggested by Burkhardt (2010) for hygroscopic aerosols deposited onto plant surfaces, salts with low POD's may either act as desiccants or simply increase nutrient uptake rates. Consequently, salts with low PODs may be more effective but may be more likely to cause phytotoxicity.

In summary, humidity influences foliar absorption primarily through its effect on droplet size and persistence on the leaf surface in the liquid state. Humidity also alters leaf cuticular composition, its physical and chemical characteristics and has direct effects on leaf physiology and transport processes.

Light, humidity and temperature can affect foliar absorption: 1) through direct effects on the spray solution prior to leaf absorption; 2) through effects on the leaf development processes; and 3) by altering photosynthesis, stomatal opening, respiration, leaf expansion and sink activity which consequently changes energy and metabolite availability involved in the uptake, assimilation and subsequent transport of foliar applied nutrients.

- Light and temperature influence foliar absorption primarily through their effects on the physical and chemical characterisitics of the foliar solution as well as the development of the cuticle.
- Direct effects of light or temperature on leaf metabolism influencing foliar fertilizer efficacy are not highly significant.
- Humidity alters both leaf structure and the rate at which foliar fertilizer solutions dry on leaf surfaces.

4.5. Summary of the effects of the environment on plant response to foliar fertilization

Over longer periods (weeks) the environment in which a plant develops can alter the cuticle and other physical characteristics of leaves as well as crop phenology and metabolism. Environmental stress has been observed to either enhance foliar uptake,

through the disruption of leaf cuticular integrity, or reduce uptake and utilization, by impairing leaf expansion, sink activity and metabolism. In the shorter term (hours or days) optimal environmental conditions maximize photosynthetic activity, stomatal opening and metabolic performance of crops and therefore enhance the potential for uptake, translocation and the plant response to foliar-applied nutrients. This effect is greatest for nutrients that are readily permeable; are applied in metabolically significant quantities; and are rapidly assimilated by the plant. For such nutrients unfavourable environmental conditions (especially low light or sub-optimal temperatures) may limit the availability of adequate energy and metabolic substrates to drive the uptake, transport and assimilation processes. Examples of fertilizer nutrient sources that may be impacted by sub-optimal environmental conditions are urea and other soluble and permeable macronutrient formulations of N, P, K, S and Mg. For elements that interact strongly with the cuticle and cell wall components and are applied at concentrations that would not be expected to represent a substantial energetic or metabolic cost (predominantly the micronutrients) for their absorption, a direct effect of temperature on metabolic processes (and hence absorption) is unlikely. In this case any effect of the environment is likely to be a result of physical and not biological influences.

In addition to its direct effects on metabolic absorption and transport processes, temperature determines the pattern of droplet drying and leaf surface distribution, which also has a direct effect on the efficacy of foliar applications (Chapters 2 and 3). Ultimately, it is the combination of effects of environment on the plant prior to foliar application and on the biology of the plant during and post-absorption that determine its impact on the efficacy of foliar fertilization.

4.6. Nutrient mobility and transport

The efficacy of foliar nutrient sprays depend not only on the absorption of the nutrients but also on the transport of these nutrients to other plant parts like fruit, grains, young leaves, etc. (Bukovac and Wittwer, 1957). Knowledge of the ability of an element to be transported from the site of application can provide insight into the longevity and potential nutritional impact of foliar application on non-sprayed tissues. Non-sprayed tissues include roots, new growth that develops after spray application, and tissues that were not directly in contact with the spray solution. This includes internal tissue of fruit, dormant buds, enclosed reproductive tissues (such as wheat ears within the leaf sheath) as well as vascular and storage tissues.

While the ability of an element to be transported from the site of application to other plant parts (roots, storage tissues, reproductive organs) increases the potential for the whole plant to benefit, it must be emphasized that transport from the site of application is not essential for foliar efficacy. Indeed, it is likely that most Zn, Mn, Ca and Fe sprays are local in their effect with only very limited transport out of the sprayed tissues. Nevertheless, these sprays may still have a significant local benefit and even a relatively small transport out of treated leaves and tissues may have a short-term, critical benefit to the plant. Development of foliar fertilizers and application techniques

that optimize transport of nutrients from site of application remain one of the most important challenges to the industry. Currently, there is only limited information to suggest that foliar-applied nutrients are transported differently or have a different physiological impact than soil-derived nutrients. Similarly, while it has been shown that the chemical form in which a nutrient is applied can influence its rate of absorption, it has not been confirmed if the nutrient provided can then influence the transport of the absorbed nutrient from the site of application. These are questions of great importance to the science and practical field application of foliar fertilizers.

Marschner (1995) classifies nutrients into three groups with regard to their phloem mobility: highly mobile (N, P, K, Mg, S, Cl, Ni); intermediate or conditionally mobile (Fe, Zn, Cu, B, Mo); and low mobility (Ca, Mn). Furthermore, Epstein and Bloom (2005) also classify nutrients with regard to their phloem mobility (Table 4.3). The former authors classify B as having high or low mobility depending on species as described by (Brown and Hu, 1998).

The plant species and phenological stage have critical effects on the mobility of all elements but these are particularly important for the intermediate or conditionally mobile ones.

In particular the mobility of micronutrients within plants is an important characteristic that determines plant growth and survival under conditions of limited nutrient availability. Three factors combine to determine the overall phloem mobility of a nutrient: a) the ability of the nutrient to enter the phloem; b) the ability of the nutrient to move within the phloem; and c) the ability of the nutrient to move out of the phloem into sink tissues.

The degree of mobility of a particular element occurs to varying degrees throughout the plant's life and this may vary significantly between species. The developmental stages that affect micronutrient mobilization include seed germination, vegetative and reproductive growth, leaf senescence and the onset of new growth in perennial species. Nutrient mobilization during flower and seed formation as well as following seed germination are the most critical phases. Indeed mobilization of stored nutrients during seed germination, particularly in infertile and arid soils, is important for supplying

Table 4.3. Classification of nutrients with regard to their phloem mobility (Epstein and Bloom, 2005).

Mobile	Intermediate or conditional mobility	Low mobility
Potassium	Sodium	Calcium
Nitrogen	Iron	Silicon
Sulfur	Zinc	Manganese
Magnesium	Copper	Boron (species dependent)
Phosphorus	Molybdenum	
Boron (species dependent)		
Chlorine		

micronutrients to the young seedlings prior to their developing a sufficient root system to enable significant soil uptake. During leaf senescence, re-mobilization of nutrients from the leaves to the reproductive tissues represents an important source of nutrients for the fruits and seeds. Recent evidence suggests that the nutrient content of the seeds can be enhanced using appropriate well-timed foliar applications with subsequent benefits for human consumption (of 'treated' grain) and subsequent (following planting out) seed germination (Cakmak *et al.*, 2010; Dordas, 2006; Ozturk *et al.*, 2006).

In general, there is low potential for re-mobilization of foliar absorbed nutrients until the potential binding sites for that element within the leaf are saturated, thus nutrient deficiency can reduce nutrient mobility since there will be many unsaturated binding sites to be filled (saturated). Nutrient mobility may also be low until the structural integrity of the leaf begins to decline during senescence thereby releasing previously tightly bound nutrients. This effect is particularly prominent for nutrients that are found in permanent structures such as the cell wall which exhibit low turnover rates for elements including some micrantrients e.g. Zn, B and Cu. When grown in deficient or marginally adequate levels of nutrient supply, more than 90% of the micronutrients, Cu, Zn and B, are present within permanent structures particularly the cell wall (Brown and Bassil, 2011; Brown *et al.*, 2002; Zhang and Brown, 1999a). In species with limited B mobility (Brown and Hu, 1998) foliar applications of B are most effective at enhancing translocation when B is sufficient in the tissue at the time of application (Hanson, 1991; Leite *et al.*, 2007; Will *et al.*, 2011). A similar response was hypothesized for Zn (Erenoglu *et al.*, 2002; Zhang and Brown, 1999a) with optimum re-translocation of applied Zn to grain being observed when a combination of soil and foliar Zn was used. Furthermore during grain development in wheat Cu-sufficient flag leaves lost more than 70% of their Cu compared to only 20% in Cu-deficient plants (Hill *et al.*, 1979a; Hill *et al.*, 1979b). This relationship between nutrient status and re-mobilization does not occur with the more mobile elements N, P, K, S, Mg, B (in polyol producing species), Cl, and Ni since a small portion of the cellular content of these elements is associated with permanent structures and each of these elements is also phloem mobile. In general, deficiency of N, P, K, Mg and S enhances senescence and speeds nutrient re-mobilization.

The mobility of micronutrients has a significant effect on the occurrence, expression and correction of deficiencies. Phloem mobile elements can move from organs of relative abundance to growing tissues so that plants do not immediately exhibit nutrient deficiency or depressed plant growth when the demand for a particular nutrient is higher than its uptake rate. Understanding the precise mechanism of phloem mobility is important as it can provide a basis for the selection, or genetic engineering, of plants with enhanced phloem mobility. Improved tolerance to a short-term micronutrient deficiency has recently been demonstrated for B (Brown *et al.*, 1999). Knowledge of the chemical form in which nutrients are transported in the phloem is important when developing foliar fertilizer formulations that mimic the naturalal plant process. The development of polyol-based fertilizers for transport of B as well as those based on amino acids have been rationalized on this understanding though scientific proof of transport of the element in its esterified form is not yet available.

Any discussion of phloem transport must recognize that transport is strongly affected by plant genotype and various external and internal factors though a few broad

generalizations may be made. Nitrogen, P, K, Ni, Mg, S, Cl and B in polyol transporting species (Brown and Bassil, 2011) are thought to be phloem mobile in all species with transport rates determined by local leaf nutrient status and source:sink relationships. The elements Ca, B (in polyol non-transporting species) and Mn are immobile in the vast majority of plants except in a few species (Ca and Mn are mobile in lupin) and during senescence in some (Brown and Shelp, 1997; Graham *et al.*, 1988; Jeschke *et al.*, 1987). The intermediate or conditionally mobile elements (Zn, Fe, Cu and Mo) can be immobile or relatively mobile depending on phenology and supply which will be discussed later.

The typical ranges for components of xylem and phloem saps in higher plants are given in Table 4.4.

Because of the generally high mobility of N, P, K, Ni, Mg, S and Cl little discussion of species or phenology-specific re-mobilization will be presented here. Nitrogen, K, P,

Table 4.4. Comparison of concentrations of organic and inorganic solutes in the phloem (stem incision, pH 7.9-8.0) and xylem (tracheal, pH 5.6-5.9) exudates of *Nicotiana glauca* (Marschner, 2012).

	Phloem	Xylem	Ratio phloem/xylem
		(mg L^{-1})	
Dry matter	170-196	1.1-1.2	155-163
Sucrose	155-168	nd	
		(µg mL^{-1})	
Amino compounds	10,808	283	38.2
Nitrate	nd[1]	na[2]	
Ammonium	45.3	9.7	4.7
K	3,673.0	204.3	18.0
P	434.6	68.1	6.4
Cl	486.4	63.8	7.6
S	138.9	43.3	3.2
Ca	83.3	189.2	0.44
Mg	104.3	33.8	3.1
Na	116.3	46.2	2.5
Fe	9.4	0.60	15.7
Zn	15.9	1.47	10.8
Mn	0.87	0.23	3.8
Cu	1.20	0.11	10.9

From Hocking, 1980b.
[1]nd: not detectable
[2]na: not available

S, Mg, Ni, Cl and B (in polyol producing species) are highly mobile in the phloem with transport driven mostly by source:sink relationships and tissue senescence. Phloem mobile nutrients frequently follow a circuitous path through the leaves (Figure 4.6) and are preferentially mobilized from the leaves to the fruit *via* the phloem rather than proceeding directly to sink tissues and fruit in the transpiration stream (Jeschke and Hartung, 2000).

Phloem mobility can become particularly high during seed maturation in annual plants with the majority of nutrients being supplied by re-translocation from the leaves to the seeds (Neumann, 1982). The extent of re-mobilization is highly element specific (Table 4.5) with a large percentage of final seed nutrient content being derived from leaf re-mobilized nutrients and not from 'new' uptake (Marschner, 2012). In many species the N requirements of developing seeds exceed the supply capacity of the roots and the resulting N deficit triggers catabolism (breakdown of leaf proteins) and the transfer of the resulting N and other nutrients to the seed. Catabolism of leaf protein also has a positive effect on the availability of Cu and Zn for re-mobilization to reproductive tissues (Hill *et al.*, 1979b; Kutman *et al.*, 2011).

Factors that influence the relative re-mobilization of the poorly mobile phloem elements have the greatest relevance to studies on the efficacy of foliar applications.

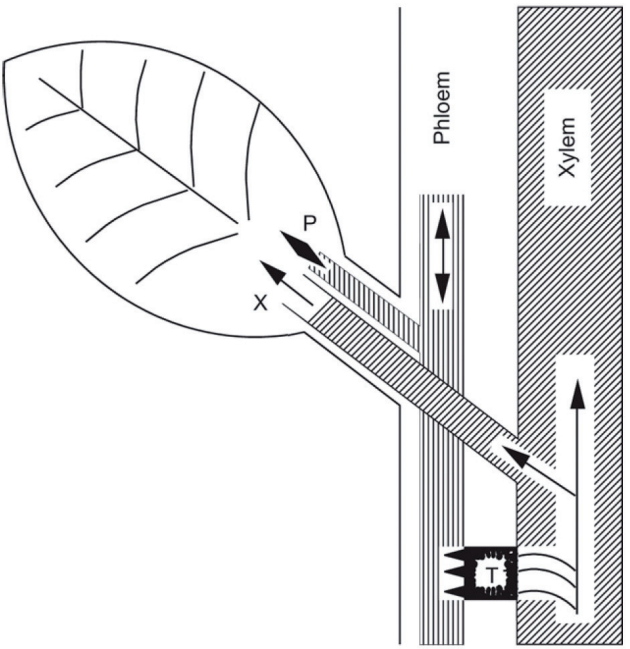

Figure 4.6. Schematic diagram of long distance transport in xylem (X) and phloem (P) in a stem with a connected leaf; and xylem-to-phloem transfer mediated by a transfer cell (T) (Marschner, 2012).

Table 4.5. Re-mobilization of nutrients in a pea crop between flowering and ripening (Marschner, 2012).

	N	P	K	Mg	Ca
	Content in stems and leaves (kg ha^{-1})				
Harvest					
June 8 (flowering)	64	7	53	5	31
June 22	87	10	66	8	60
July 1	60	7	61	8	69
July 12 (ripening)	32	3	46	9	76
	Increase or decrease after June 22 (%)				
	-63	-73	-30	+10	+21
	In seeds (% of total shoot content)				
	76	82	29	26	4

Based on Garz, 1966.

Calcium is generally immobile in the phloem as a consequence of its low concentration in cytoplasm and phloem of between 0.1 to 10 µM (White and Broadley, 2003) as well as the negative impact of Ca on phloem sieve callose formation and plugging (Marschner, 2012). Phosphate is the major anion in the phloem sap and the translocation of cations such as Ca^{2+}, which form phosphates of low solubility, are limited in the sieve tubes by the solubility product of the phosphate salt. Many physiological disorders of fruits are associated with low Ca levels and because of its phloem immobility foliage-applied Ca is not re-distributed from sprayed leaves to the fruit (Swietlik and Faust, 1984). Because of the importance of Ca on the storage quality of fruits and vegetable, and as a consequence of the prevalence of Ca deficiency disorders, there has been considerable research effort on methods to enhance fruit Ca content (Blanco *et al.*, 2010; Koutinas *et al.*, 2010; Kraemer *et al.*, 2009b; Lotze *et al.*, 2008; Neilsen *et al.*, 2005a; Peryea *et al.*, 2007; Val and Fernandez, 2011). Evidence for the very low degree to which Ca is re-mobilized from sprayed leaves to fruits is provided in numerous publications and it is generally accepted that multiple sprays as well as the concurrent application of Ca to leaves, stems and fruit are required. Early season sprays appear to be more effective than late season sprays (Lotze *et al.*, 2008; Peryea *et al.*, 2007) probably as a consequence of different leaf characteristics, or benefits of direct spray application to young fruits, or because it provides the time available for multiple applications. Though evidence suggests that the choice of Ca formulation can affect the amount of Ca in the fruit it is not clear if this is a result of enhanced absorption or better transport (Lotze *et al.*, 2008; Rosen *et al.*, 2006).

Iron is generally considered to be an intermediately mobile nutrient in higher plants and can be re-translocated in small amounts from the old leaves to the younger ones (Abadia *et al.*, 2011; Fernandez *et al.*, 2006; Fernandez *et al.*, 2009). A typical concentration of Fe in the phloem is 9.4 μg ml^{-1} which is too low to supply plant demand (Hocking, 1980) and the pH of the phloem is about 7.8-8.0 which favours Fe^{3+} insolubility (Mass *et al.*, 1988). This suggests that mechanisms must exist to actively load the phloem and that Fe must be present in a complexed form (Palmer and Guerinot, 2009; Waters and Sankaran, 2011; White and Broadley, 2009). The degree of mobility of Fe clearly varies with species, stage of plant growth and Fe supply amongst other factors (Garnett and Graham, 2005; Shi *et al.*, 2011). Garnett and Graham (2005) observed very high levels of Fe and Cu, and moderate levels of Mn and Zn, re-mobilization during shoot senescence and grain filling in wheat. These results contrast with field studies in which very little Fe or Mn re-mobilization was observed (Hocking, 1994; Pearson and Rengel, 1994). Examples of limited mobility of foliar-applied Fe in both herbaceous plants and citrus shoots towards new, expanding leaves is a function of several factors, but most importantly the specific Fe source (Abadia, 1992; Abadia *et al.*, 2011; Fernandez *et al.*, 2009). In this regard, some reports have suggested a better plant translocation of Fe chelates versus inorganic salts (Basiouny and Biggs, 1976; Basiouny *et al.*, 1970; Fernandez and Ebert, 2005; Fernandez *et al.*, 2009).

Zinc is frequently more mobile than either Mn or Fe in most species (Nowack *et al.*, 2008; Zhang *et al.*, 2010) and Swietlik (2002). Longnecker and Robson (1993) suggested that Zn movement out of old leaves coincides with their senescence and the re-mobilization of Zn from the flag leaf to the grain in wheat has been confirmed (Herren and Feller, 1994). In subterranean clover (*Trifolium subterraneum*) total Zn in leaves and petioles decreased as the reproductive structures accumulated Zn (Riceman and Jones, 1958; Riceman and Jones, 1960). Application of ^{65}Zn labelled fertilizer to 'Pera' orange demonstrated that after 120 days 14% of the total absorbed Zn was translocated from the applied leaves to other plant parts (Sartori *et al.*, 2008). In other studies utilizing citrus species (Swietlik and Laduke, 1991) found limited or no evidence of Zn movement from sprayed leaves using ^{65}Zn isotope.

These results suggest that significant Zn re-mobilization can occur in some species during normal growth and in many species during senescence. Zinc concentrations in the phloem sap range from 3 to 170 μM (White and Broadley, 2009) and under conditions of normal supply only a small portion of Zn can be supplied *via* the phloem. While Zn re-mobilization in the phloem appears to be possible the quantity appears to be limited to about 5 to 20% across all experiments. The very limited re-translocation of Zn observed following foliar applications can be attributed to either the poor penetration or the high binding capacity of leaf tissues for Zn (Zhang and Brown, 1999a), but does not imply that phloem mobility was limited (Figure 4.7).

Manganese is generally considered to have low mobility in most species but can be influenced by the supply of Mn in the growth medium (Brown and Bassil, 2011). Early studies concluded that Mn has intermediate mobility with less mobility than P and

Figure 4.7. Zinc is highly immobile in pistachio trees. The right side of this tree received foliar fertilizer at the rate of 10 kg $ZnSO_4$ per 500 litres applied to drip in September (early post-harvest) with a hand gun sprayer each year for five years. The left half of the tree received no foliar applications. Repeated Zn applications effectively (but inefficiently) corrected deficiency on the sprayed right side. However, no significant benefit of the Zn applications was perceived on the un-sprayed left side (Courtesy Kiyoto Uriu).

more mobility than Ca. Experimental evidence by Romney and Toth (1954) showed that radioactive ^{54}Mn can be partially translocated from leaves when foliar applied. In addition Nable and Loneragan (1984) used ^{54}Mn and demonstrated that the isotope remained in the cotyledons and old leaves with little or no Mn exported from them and limited re-mobilization or re-translocation of Mn from mature leaves. $MnSO_4$ foliar sprays were applied by Swietlik and Laduke (1991) for four years in 'Valencia' orange (*Citrus sinensis* Osbeck) and 'Ruby Red' grapefruit (*Citrus paradisi* Macf.) resulting in a very small but measurable increase in Mn in new leaves of 2 to 5 ppm. Hocking *et al.* (1977) reported variation in Mn accumulation among various lupin species with the concentration in *Lupinus albus* L. being consistently higher than that of *Lupinus angustifolium* L. and the differences were also expressed in the relative extent to which the species mobilize Mn from pods to seeds. In wheat Mn contents increased throughout the entire leaf life and did not even decline during senescence (CF and Graham, 1995; Pearson and Rengel, 1994; Pearson *et al.*, 1995). Everett and Thran (1992) also reported that mobilization of Mn from leaves was limited as evidenced by the increase in amount of Mn in the needles of pinyon (*Pinus monophylla*) with time. However there is evidence to suggest that Mn is mobile in the phloem of *Ricinus communis* (Van Goor and Wiersma, 1976).

Molybdenum exhibits high phloem mobility in soybean, rice and bean (Brown and Bassil, 2011; Kannan, 1986) but lower mobility in many other species (Masi and Boselli, 2011; Williams *et al.*, 2004). Bukovac and Wittwer (1957) reported that Mo has intermediate mobility and observed that in Mo-sufficient plants there was re-mobilization from leaves during flowering and pod filling; while in Mo-deficient plants content increased or remained constant in leaves which suggests that there was no or little re-mobilization (Jongruaysup *et al.*, 1994; Jongruaysup *et al.*, 1997).

Boron is a unique element in that phloem B mobility is strongly species- ependent (Brown and Bassil, 2011; Brown and Shelp, 1997). In most plant species, B is largely xylem transported and exhibits marked immobility once deposited in the leaf. It is suggested that this immobility is a consequence of either the incompatibility of B with the phloem or 'solute' trapping in which the high trans-membrane mobility of B favors the movement of any B in the phloem back to the less concentrated xylem stream (for discussion see Brown and Bassil, 2011; Brown *et al.*, 2002; Brown and Shelp, 1997). Limited phloem mobility may occur in certain species under conditions of low B supply (Huang *et al.*, 2008; Shelp *et al.*, 1996; Stangoulis *et al.*, 2001; Stangoulis *et al.*, 2010), perhaps suggesting that deficiency induced transporters are up-regulated.

In contrast to the majority of plants, re-mobilization of B from mature leaves can easily take place in species that primarily transport polyols (sugar alcohols) and foliar B applications have long been known to be an effective means of enhancing bud and flower concentrations resulting in increased fruit set and yield in *Malus*, *Prunus*, *Olea*, *Coffea* and *Pyrus* species. Using ^{10}B, Brown and Hu (1996) demonstrated that re-mobilization of B can occur when B forms esters with a sugar alcohols (e.g. sorbitol, mannitol ordulcitol) which are stable when sugar alcohol to B ratios exceed 100:1. Plants where B is mobile are far less susceptible to transient shortages of B since foliar-applied B can be re-mobilized and supply untreated tissues. This principle that phloem mobility impacts susceptibility to deficiency and enhances plant response to foliar fertilizers is equally relevant for all elements.

Phloem immobility increases susceptibility to short-term nutrient withdrawal but can be corrected with foliar fertilization. Wild type tobacco, in which B is immobile, and transgenic tobacco containing a gene that induces phloem B mobility, were grown in nutrient solutions containing adequate B for 38 days (Figure 4.8). B in the rooting medium was removed on day 39 and the oldest mature leaf of both cultivars was supplied with daily spray application of foliar B at 250 ppm. Within 24 hours of B removal from the rooting media wild type tobacco plants rapidly exhibit B deficiency symptoms including abortion of flowers, inhibition of shoot elongation and chlorosis (Figure 4.8a). Transgenic tobacco, in which B is mobile, did not exhibit B deficiency due to enhanced ability to translocate B from old to young tissue (Figure 4.8b) (Brown *et al.*, 1999a).

At normal pH values B is present as the uncharged low molecular weight borate molecule (H_3BO_3) with a relatively high cuticle and membrane permeability which is readily absorbed by leaves (Picchioni and Weinbaum, 1995). Therefore all species show a rapid uptake of B into the sprayed organ. However in species that transport polyols

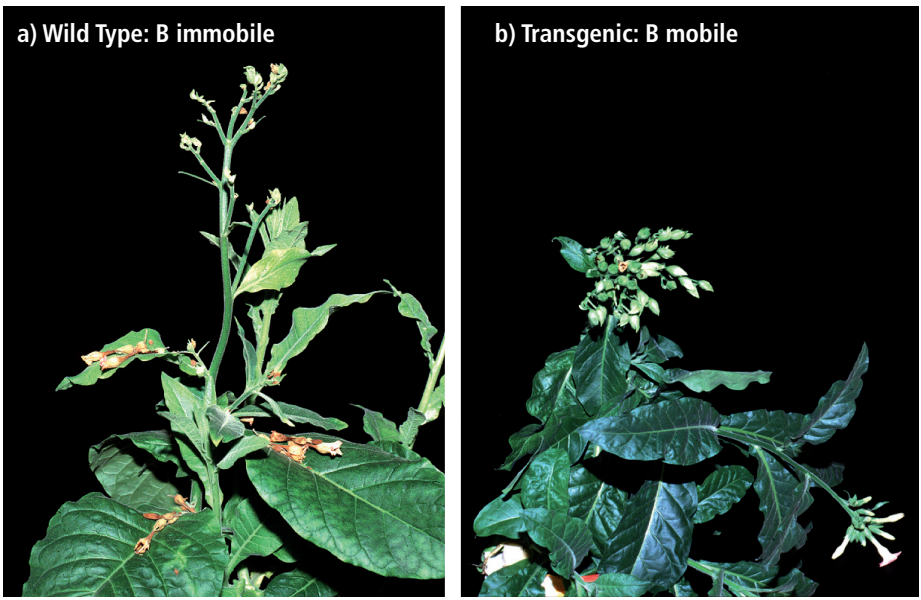

Figure 4.8. Boron is immobile in the natural 'Wild Type' cultivars of tobacco (a) and highly mobile in transgenic (b) cultivars into which the gene for sorbitol production has been inserted (Brown *et al.*, 1999). Plants were grown for 45 days with adequate boron then transferred to boron free media for 72 hours. After 72 hours, significant boron deficiency, including flower abscision was observed in wild-type but not in transgenic cultivars. The phloem mobility of boron in the transgenic species prevented the occurrence of deficiency by allowing for re-use of previously acquired B.

foliar applications of B are also rapidly transported out of the leaf in the phloem stream and move toward sink tissues including roots, young expanding leaves, reproductive organs and fruits, thus demonstrating the relevance of phloem mobility of crop response to foliar fertilization.

Phloem mobility has a profound effect on the ability of plants to absorb, translocate and benefit from foliar fertilizers and therefore it has an important role in determining their efficacy.

Phloem-immobile nutrients:

- Foliar application of phloem-immobile nutrients only benefit the tissues that directly receive the foliar spray.
- Measurement of nutrient status in sprayed leaves may be problematic due to the presence of residues from un-absorbed nutrients on the leaf surface.
- While development of foliar nutrient formulations with greater mobility is a worthwhile endeavor, a more immediate need is to maximize the efficacy of these materials on the sprayed tissues.

Phloem-mobile nutrients:

- Foliar application of mobile nutrients has the potential for systemic and long-term benefit.
- Limitations to the quantity of nutrient that can be applied and rapid dilution of the applied nutrients from mobilization within the plant reduce the potential benefit of foliar sprays of phloem-mobile nutrients.
- Therefore measuring the impact and benefit of phloem-mobile foliar nutrients is complicated by their mobility and dilution within the plant.

For both mobile and immobile nutrients the most relevant role of foliar sprays is to prevent immediate and transient deficiencies that cannot be addressed quickly by soil applications.

4.7. Conclusions

This chapter has highlighted the complex interactions between the environment, plant species, growth stage and conditions and timing of application on the efficacy of foliar fertilizers. While there are very few specific 'rules' that can be applied to every specific situation an understanding of the general principles that affect foliar fertilization will help make more informed decisions.

Certainties

- Species differ markedly in the characterisitics of their leaf surfaces and prediction of crop response to any formulation is currently impossible.
- The environment affects every aspect of foliar fertilization; from physical and chemical reactions of spray materials; to plant architecture; to leaf cuticular composition; and fate of the nutrients once they enter the plant.
- Plant phenology also has a large effect on leaf cuticular composition and therefore the efficacy of foliar fertilization.

- Phloem mobility has a profound effect on the manner in which foliar nutrients are utilized by treated plants.

Uncertainties

- Current knowledge of the factors that determine plant cuticular composition and response to foliar application is insufficient to predict, or manipulate, plant response to a foliar application.
- It is an unknown if foliar-applied nutrients, once they enter the cellular space, are more or less 'available' or mobile than soil acquired nutrients.

Opportunities

- Improved understanding of the principles that govern the movement of foliar-applied nutrients through the cuticle into the living leaf cellular spaces is essential to the development of improved foliar fertilizer formulations and practices.