Foliar Iron Fertilization – A Critical Review

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ABSTRACT

Application of foliar iron sprays is a common means of correcting Fe deficiency of agricultural crops. However, variable plant responses to iron sprays ranging from defoliation to no effect have been often described in the iron fertilization literature. There is still limited knowledge concerning the mechanisms of penetration of a leaf applied Fe-containing solution and the role of Fe in the leaf. The complex and multi-disciplinary character of the factors determining the effect of iron sprays hinder the development of suitable foliar fertilization strategies, applicable under variable local conditions and for different plant types. The review is directed towards describing some key factors involved on the process of penetration of a leaf applied, iron containing solution as a prior step to briefly analyze the available foliar iron fertilization literature. Iron chemistry, leaf penetration and plant nutrition principles will be merged with the aim of clarifying the constraints, opportunities and future perspectives of foliar iron sprays to cure plant Fe deficiency.

Key words: Iron sprays, iron deficiency; foliar application; iron chelates; iron salts.

THEORETICAL BACK-GROUND

Iron in Plants

Iron (Fe) deficiency is a common disorder affecting plants in many areas of the world, which is chiefly associated with high pH, calcareous soils. Plant Fe deficiency has economic significance since crop quality and yields can be severely compromised and the use of expensive corrective methods is often required (Álvarez-Fernández et al., 2004). Despite the ubiquitous presence of iron in the earth's crust, the low solubility of Fe compounds in many soils prevents plant iron uptake and induces the development of Fe deficiency symptoms (Lindsay, 1984). Like other living organisms, plants developed root strategies to cope with Fe insolubility based on acidification, excretion of reductants or chelators and having an increased root reductase activity (Rogers and Guerinot, 2002). Plants exhibiting these mechanisms have been classified as Strategy I (dicotyledoneous and non-graminaceous monocotyledoneous species) and plants producing phytosiderophores are categorized as Strategy II (graminaceous species) (Marscher and Römheld, 1994).

Iron was shown to be transported from the root to aerial plant organs in the xylem as a ferric citrate complex (Tiffin, 1966). The physiological function of the non-proteinogenic amino acid nicotianamine is not fully understood (Reichman and Parker, 2002). The characteristics of the leaf cell, plasma membrane-bound ferric chelate reductase have been recently described (Brüggemann et al., 1993; Larbi et al., 2001). Fe(III) reduction in sugar beet leaf disks was shown to be pH dependent and markedly stimulated by light (Larbi et al., 2001). The role of the leaf apoplast in relation to symplasmic Fe uptake remains unclear. Fe deposition

as insoluble compounds in Fe deficient leaves, known as the “chlorosis paradox” (Römheld, 2000) has been suggested to occur and the significance of apoplastic pH changes in Fe-deficient plants is not fully understood (Kosegarten et al., 2001; Nikolic and Römheld, 2003).

Leaf Penetration Principles

The process of foliar penetration of a leaf applied solution is quite complex and depends upon an array of environmental and plant factors (Weinbaum and Neumann, 1977). Plant leaves are known to be organs specialized in organic compound production via photosynthesis and related mechanisms. As a result of several investigations, it was recognized that foliar-applied compound penetration would occur via the cuticle through cuticular cracks and imperfections, through stomata, leaf hairs and other specialized epidermal cells (Tukey et al., 1961). The importance of stomatal versus cuticular leaf absorption, particularly with regard to aqueous solutions is subject of much debate (Currier and Dybing, 1961; Eichert et al., 2002). Schönherr and Bukovac (1972) showed that liquids having surface tensions approaching that of pure water, would fail to spontaneously penetrate stomata unless some external force is applied. The capacity of certain wetting agents to lower the surface tension of sprays below 30 mN m^{-1} has been investigated in several instances, but problems of phytotoxicity were often described (Weinbaum and Neumann, 1977). More recently, Eichert et al. (1998) and Eichert and Burkhardt (2001) provided evidence of stomatal infiltration of 1 mM uranine (Na-fluorescein) solutions without the application of surfactants or pressure.

Most of the foliar uptake studies carried out in the last 30 years investigated the mechanisms of cuticular penetration. In contrast to roots, the outer walls of epidermal cells of all aerial plant organs are covered by a hydrophobic cuticle, which is the limiting barrier for the two-way transport of water and solutes. Leaf water repellence has been shown to be chiefly related to the epicuticular wax crystalloids which cover cuticular surfaces in a micro-relief (Barthlott and Neinhuis, 1997). Cuticular waxes embedded in the cutin/cutan matrix were found to be responsible for the barrier properties and diffusion of non-electrolytes. Neutral, non-charged molecules penetrate the cuticle by diffusion and dissolution in cuticular waxes (Schönherr, 2000). In contrast, the mechanism of cuticular penetration of water and ions is not fully understood but may occur due to the existence of aqueous pores (Schönherr and Schreiber, 2004).

Both the upper and the lower leaf surface are involved in the process of penetration of an applied solution. However, several studies reported an increased penetrability of the lower epidermis versus the upper, due to stomatal and cuticular variations between both leaf sides. Structure and composition of the cuticle as well as the morphology, distribution and size of the stomata differ among plant species and will play a role regarding the penetration of foliar sprays. Factors related to the physiological state of the plant such as root temperature, root osmotic potential, age of leaf or the current nutrient status modulate the effectiveness of foliar fertilization (Weinbaum, 1996). Environmental factors such as relative humidity, light and temperature play a role concerning the penetration of a leaf applied solution. Relative humidity and leaf water status are key factors governing foliar uptake. At a high relative humidity drying of the salt deposit is

delayed and cuticular permeability may increase through hydration (Currier and Dybing, 1959; Schönherr, 2001).

The prevailing environmental conditions also affect the physical-chemical properties of the applied solution. According to Schönherr (2001), the choice of an adequate electrolyte carrier compound is the only strategy to favor the penetration of ions through plant leaves. Penetration occurs when the applied salt solution is dissolved and liquid phase between the leaf surface and the solid salt residue exists. Suitable element carriers for foliar fertilization should preferentially have a high solubility and a low molecular weight (Schönherr, 2001).

Plant cuticles are poly-electrolytes and were shown to have isoelectric points around 3 (Schönherr and Bukovac, 1972; Schönherr and Hüber, 1977). As a consequence, the ion exchange capacity of the cuticle can be expected to be altered by pH fluctuations (Chamel, 1996). Thereby, leaf applied solution pH values higher than 3, will render the cuticle negatively charged (Schönherr and Hüber, 1977).

The leaf surface micro-relief formed by epicuticular wax crystalloids largely influences spray retention and leaf wettability. Subsequently, drops of a pure aqueous solution on non-wettable leaf surface can only be expected to make contact with the tips of wax crystalloids. Intimate contact between an aqueous solution and the epicuticular waxes of the leaf surface can be achieved by the addition of suitable surfactants in the spray formulation (Schönherr, 2000). Surfactant composition and concentration are key factors ruling leaf penetration of agrochemicals (Stock and Holloway, 1993).

Phytotoxicity and alteration of the epicuticular wax fine structure associated with surfactant solution applications has been often described (Tamura et al., 2001). On the other hand, Uhlig and Wissemeier (2000) observed a reduction in phytotoxicity of non-ionic surfactant (Triton X-100 and Genapol) by the presence of divalent cations.

Early Fe spray investigations included Vatsol OT (Na-dioctyl-ester of Na-sulfosuccinic acid, an anionic sulfosuccinate ester) and Triton X-100 (octylphenoxy- polyethoxy ethanol; a non-ionic) surface active agents in formulations (Guest and Chapman, 1949; Wallihan et al., 1964). A 0.1% Vatsol OT concentration was found to reduce the surface tension of aqueous solutions to 28.5 mN m^{-1} . However, the anionic surfactant molecules can be expected to interact with the electrolyzed cations of the Fe-salt solution, forming large molecules which may block cuticular pores and interfere with the process of leaf Fe penetration. Several foliar Fe application studies included the organosilicone surfactant Silwet L-77 (tri-siloxane poly-ethoxylate), which can reduce the equilibrium surface tension of the solution to approximately 20 mN m^{-1} , and has been often related to promotion of stomatal infiltration.^[22] However, Knoche et al. (1991) observed that Silwet L-77 and Silwet L-7602 degraded in acid (pH 2 to 5) and alkaline (pH 9 to 10) spray solutions, resulting in a loss of surface tension over time. Schönherr (2001) recorded an increased cuticular penetration of solutions containing alkyl polyglucoside surfactants (Glucopon 215 CSUP, Agrimul PG 2069 and Plantacare 1200 UP). In contrast to ethoxylated alcohols, alkyl polyglucosides are not phytotoxic (Schönherr, 2001).

Key iron chemistry facts

After aluminium, Fe is the second most abundant metal in the earth's crust (Lee, 1991). The only relevant oxidation states in the ordinary aqueous and related chemistry of Fe are II and III (Cotton et al., 1999).

Hydrated Fe(II) salts are pale green and most of them darken in the presence of air due to oxidation (Nicholls, 1973; Silver, 1993). In the absence of complexing agents, solutions of Fe(II) contain the pale green hexaquo ion, $[\text{Fe}(\text{H}_2\text{O})_6]^{2+}$, which is easily converted to Fe(III) by molecular oxygen (Silver, 1993). Oxidation is more favorable in basic solution and neutral and acid solutions of Fe(II) oxidize less rapidly with increasing acidity (Nicholls, 1973). Ferrous Fe forms stable complexes with certain N-hetero-cyclic derivatives such as 1,10-phenanthroline or 2,2'-di-pyridyl, which are used as redox indicators. However, the most important Fe(II) ligands are porphyrins, which were found to occur in many enzyme systems (Cotton et al., 1999). Complexes originating from ethylene-diamine, tartrate, citrate, oxalate and in general, poly-hydroxylated organic compounds are not stable in aqueous solution (Burriel et al., 1992).

The mixture of Fe^{2+} and H_2O_2 or $\text{S}_2\text{O}_8^{2-}$ is called Fenton's reagent. Ferrous Fe oxidizes due to the presence of $\text{OH}\cdot$ and $\text{SO}_4\cdot$ radicals (Cotton et al., 1999). High levels of free Fe^{2+} are responsible for the generation of oxygen radicals from O_2 reduction. The hydroxyl radicals are extremely harmful for almost any biological molecule (Laulhére et al., 1995).

Ferric Fe is the most common oxidation state of Fe since it forms strong complexes with a wide array of oxygen ligands of major biological significance (Silver, 1993). One of the most characteristic features of Fe(III) in aqueous

solution is its tendency to hydrolyze and/or to form complexes (Cotton et al., 1999). The Fe(III) ion is colorless but most ferric solutions are frequently yellow-brown due to the presence of colloidal Fe oxide and basic species (Cotton et al., 1999).

Ferric Fe salt solutions often contain the $[\text{Fe}(\text{H}_2\text{O})_6]^{3+}$ aqua ion, which is pale purple in color (Lee, 1991). The hydrolysis of $[\text{Fe}(\text{H}_2\text{O})_6]^{3+}$ in non-complexing media is complicated and condition dependent. At $\text{pH} < 1$, the sole species is the aqua ion, but above $\text{pH} 1$ hydrolysis gradually occurs. In the range of $\text{pH} 1$ to 2 still other types of oxo-species may result. At $\text{pH} > 2$ more condensed species and colloidal gels are formed, leading to precipitation of the red brown gelatinous hydrous oxide (Cotton et al., 1999). At $\text{pH} 4$ to 5 the hydroxo species polymerizes to a dimer which forms a brownish solid. At even higher pH values, a reddish brown precipitate of the hydrous oxide is formed (Lee, 1991).

Ferric Fe shows a preference for forming complexes with ligands which coordinate through O as opposed to N (Lee, 1991). Chelating N ligands such as dipyridyl or 1,10-phenanthroline are formed, but are less stable than their Fe(II) counterparts (Lee, 1991). Oxygen ligands have a high affinity for Fe(III) and complexes are formed by phosphate, organic phosphates and oxalate, chelating amines (e.g. EDTA) glycerol, sugars, diketones and salicylic acid derivatives (Burriel et al., 1992; Cotton et al., 1999). Iron chelation by siderophores, defined as low molecular weight organic chelators with a very high and specific affinity for Fe(III), is the most common mechanism of microbial Fe acquisition (Pierre et al., 2002).

LEAF IRON APPLICATION

An analysis of the available literature reveals that most of the studies to date investigated the effect of FeSO_4 foliar treatment and its rate of cuticular and leaf penetration as described by Abadía et al. (2002) and Fernández (2004). The number of publications concerning leaf Fe penetration is limited, but some conclusions can be drawn:

There is evidence that the phenomenon of plant Fe-chlorosis and its correction via leaf applications was a subject of interest as early as the first half of 19th Century (Gris, 1843). Some authors were clear about the advantages of using Fe-chelates versus inorganic Fe compounds (Basiouny and Biggs, 1971; Leonard, 1967). However, more recent reports observed similar effects after spraying chlorotic plants with FeSO_4 and Fe-chelates (Álvarez-Fernández et al. 2004; Rombolà et al., 2000; Tagliavini et al., 2000).

Evaluation of Fe salts as foliar sprays under different conditions and plant species has been the most common practice. In most cases, leaf Fe application was reported to have a re-greening effect associated with increased chlorophyll and Fe content. Addition of substances to the Fe formulations such as DMSO, urea or diluted methanol was found to cause variable effects in chlorotic plants (Reed, 1988; Nonomura et al, 1995). Similarly, several investigations observed variable physiological responses of Fe-deficient plants to diluted acids and chelators such as citric acid (Álvarez-Fernández et al., 2004; Tagliavini et al., 1995). Foliar applied Fe was shown to translocate both in herbaceous plants and citrus shoots towards new growing leaves as a function of several factors, chiefly the specific Fe source (Basiouny et al., 1970; Brown et al., 1965; Rediske and Biddulph,

1953). In this regard, some reports referred to a better plant translocation of Fe-chelates versus Fe-salts (Basiouny and Biggs, 1971; Hsu and Ashmead, 1984; Fernández et al., 2005). Variable Fe and surfactant concentrations have been supplied to plants, but often very high amounts of Fe were given which induced leaf burn and defoliation (Leonard, 1967). An optimal Fe concentration threshold for the supply of sufficient levels of Fe could not be elucidated from the literature. The range of applied Fe varies from 1 mM to 29 mM Fe (Leonard, 1967; Rombolá et al., 2000).

There is not much awareness about the importance of achieving an intimate contact between the applied solution and the leaf surface for increasing the chance of leaf penetration via surfactant application. On the other hand, degradation of the Silwet L-77 molecules at low solution values as shown by Knoche et al. (1991) poses an obstacle to interpret results concerning stomatal infiltration of leaf applied, Fe-containing solutions (Álvarez-Fernández et al., 2004; Levy and Horesh, 1984; Neumann and Prinz, 1974; Neumann and Prinz, 1975).

The significance of stomata and environmental factors such as light in the penetration of foliar sprays and the subsequent translocation of Fe in the plant remains unclear (Fernández et al., 2005; Fernández et al., 2003). The use of Fe(III)-salts in Fe penetration studies opens more questions about the mechanisms of Fe leaf absorption than it clarifies (Eddings and Brown, 1967; Kannan, 1969). Similarly, application of Fe(II)-sources renders a way of supplying Fe in an unprotected manner which will favor Fe oxidation, precipitation, oxidative damage and interaction with other spray components compromising the success of foliar sprays.

CONCLUSIONS AND FUTURE PERSPECTIVES

The process of penetration of a leaf applied Fe containing solution proves complicated due to the many factors involved. The relevance of leaf wetting and the physical chemical properties of the applied solution have already been stressed. A foliar applied solution has to penetrate the leaf, which is a prerequisite for leaf cell iron utilization. The properties of the applied solution (e.g. pH, surface tension or spreading ability) will largely influence the chances for leaf penetration. Similarly, the overall chemistry of Fe is a key factor to understand plant Fe physiology and to develop efficient techniques to cure plant Fe deficiency. The great pH and redox dependency of Fe poses many difficulties in terms of successful Fe carrier selection and Fe physiology (e.g. Fe immobilization).

Successful use of Fe(II) and Fe(III) salt solutions as foliar sprays appears unlikely in theoretical terms, since the reality of the field is far from laboratory conditions. Ferric Fe salts will yield insoluble hydrous oxide polymers and sprays should be optimally prepared and supplied at a very acid pH (e.g. < pH 2) which may induce leaf tissue damage. On the other hand, Fe(II) salts will rapidly oxidize upon exposure to ambient air, the reaction occurring more readily with increasing pH values. A further detrimental factor is the easiness of Fe ions to penetrate the leaf under optimal conditions, which may lead to Fe toxicity damage as shown by Fernández et al. (2005). Therefore, after spraying salt solutions results may range from no apparent effect to necrosis or defoliation according to an array of variable factors (e.g. local water pH or age of the salt solution). Foliar treatment with ionic Fe sources may also interfere with leaf penetration and movement in the apoplast,

since plant cuticles and cell walls are negatively charged as shown by Schönherr and Huber (1977) and Grignon and Sentenac (1991). Subsequently, application of non-charged or negatively-charged Fe-chelates for foliar sprays seems to be the most reasonable alternative as suggested by Fernández (2004) and Fernández et al., (2005). Further, the use of Fe chelates will minimize interactions with spray components and allows treatment at optimal pH values for penetration purposes (Fernández et al., 2004).

Consideration of the factors involved in leaf penetration following a holistic approach will help improving the efficiency of foliar Fe sprays (Figure 1). For instance, light is known to influence plant physiology, foliar penetration and the activity of the leaf plasma membrane Fe(III)-chelate reductase. Similarly, plant physiology aspects such as circadian rhythms may affect the penetration and translocation of leaf applied, Fe containing compounds (Fernández, 2004; Fernández et al., 2005).

In summary, more knowledge concerning the role of iron in plants, the influence of environmental factors, plant physiology and morphology, and a multi-disciplinary approach is required for the development of efficient spray formulations in the future.

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Figure 1. Summary of factors influencing the success of Fe sprays. Major interactions are represented with arrows.

Figure 1