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Foliar zinc uptake processes and critical factors influencing foliar Zn efficacy

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ABSTRACT

Zinc (Zn) deficiency in soils and plants is a global micronutrient deficiency problem in many cropping regions. Both soil and foliar application of Zn fertilizers have been commonly used to correct Zn deficiency and/or enhance Zn nutrition in crops. However, increasing foliar application of Zn fertilizers has been used to precisely deliver Zn when peak Zn demand could not meet by root Zn uptake at the late vegetative and early reproductive stages, where soil and climatic conditions inhibit adequate Zn uptake through roots. The present review has discussed key processes of foliar penetration of nutrient solutes and critical factors affecting the penetration rate of nutrient ions through the cuticular surfaces, including environmental factors, physiological status of leaves and plants, and physical and chemical properties of fertilizer chemicals used. In particular, the effects of leaf surface characteristics and chemical forms of foliar fertilizers on foliar nutrient uptake have been discussed in detail, with the aim to justify the potential of the newly developed nanocrystals of Zn compounds. Properties of different foliar Zn fertilizers have been compared and major shortcomings with traditional Zn foliar fertilizers have been identified, in relation to the justification to develop a new generation of foliar Zn technology.

Keywords:*zinc nutrition, foliar nutrients uptake, Zn foliar fertilizer.*

1. INTRODUCTION

Zinc deficiency in crops is a widespread problem around the world due to the wide distribution of low Zn in soils [1, 2]. Globally, almost half of the farmland soils cultivated for cereal crops are low in available Zn [3]. The low solubility of Zn in the soil solution, rather than low total Zn levels, is the main cause of the widespread occurrence of Zn deficiency in crop plants [4]. Zinc deficiency in plants causes stunted growth, shortened internodes and petioles, and small malformed leaves, which results in 'rosette' symptoms in young growth of dicotyledons and 'fan shaped' stem in monocotyledons [5, 6], leading to crop yield reduction and poor nutritional quality in fruits and grains.

Both soil and foliar application of Zn fertilizers have been used in field agronomic management of Zn nutrition in crops and fruit trees to increase yield and Zn density in seeds, grains and fruits [3, 7, 8]. In many parts of the world, including Australia and the USA, Zn deficient soils have been treated with routine Zn fertilizers, which may supply adequate Zn in soil for several years after application [1]. However, under some soil conditions, soil Zn fertilisation is not as effective as expected due to edaphic factors which decrease Zn solubility in soil solution, including high pH values, high carbonate contents and low organic matter contents [1]. Under these circumstances, foliar Zn application in crops and trees in these soils is an effective and efficient alternative to soil Zn fertilisation for the correction and prevention of Zn deficiency and yield loss. In intensive cropping systems, soil Zn application may not be adequate to meet the peak demand of Zn in plants during the peak of vegetative growth and extended floral and fruit developmental stages of many crop and fruit tree species. Therefore, foliar Zn application has been widely practiced by fruit growers particularly at the rapid reproductive growth stage, when hidden nutrient deficiencies may be present (such as flowering and young fruit development period) [9]. For example, grapefruit yield in plants with 60% of foliage affected by Zn chlorosis doubled when treated with foliar Zn one or two months before anthesis [10].Many studies have investigated the mechanisms of cuticular penetration of nutrients ions, foliar nutrient uptake, and nutrient translocation in the plants. This review focuses on the summary of the current knowledge of the key roles of Zn in plants, the existing foliar uptake mechanisms of nutrients, advantages of foliar Zn fertilisation, and main problems of current Zn foliar fertilizers, with the aim to develop a new generation of foliar Zn fertilizer technology. A recent example of Zn hydroxide nitrate nanocrystals has been used to illustrate the concept of slow-releasing and long-lasting foliar Zn fertilizers.

2. ZINC FORMS AND FUNCTIONS IN PLANTS

Zinc is the only metal represented in all six enzyme classes (oxidoreductases, transferases, hydrolases, lysases, isomerases, ligases) [5, 11]. In bio-membranes Zn concentration is higher than other micronutrients, due to the presence of many Zn-binding sites within the bio-membranes [12]. Some amino acids, such as Histidine (His), Glutamate (Glu), Aspartate (Asp) and Cysteine (Cys), provide ligands at these adsorption sites [12]. Zinc binding sites also occur in a wide range of other proteins, including the largest Zn binding protein (the Zn finger domain), membrane lipids and DNA/RNA molecules [5, 13]. In plant cells, Zn does not undergo valency changes and the majority of Zn in leaf cells is present in the forms of Zn^{2+} associated with low molecular weight complexes, storage metalloproteins, free ions, and insoluble forms associated with cell walls [14]. Approximately 58% to 91% of total cellular Zn may be soluble, but Zn may become physiologically unavailable within cells either by ligand chelation and/or by complexation with phosphate. Water-soluble Zn in plant cells is considered as the physiological active form, which is a better indicator of Zn status than total Zn content in plants [15]. In general, Zn in plant cells serves as structural, catalytic and cocatalytic roles in physiological and biochemical processes [11, 16].

In leaves Zn is mainly localised in epidermal cell wall, and in the intercellular spaces between epidermal and mesophyll cell walls in the upper and lower epidermis [17, 18]. Vacuoles in plant cells serve as a major site of Zn sequestration and detoxification [19, 20], in which Zn is not simply accumulated as free Zn^{2+} but bound to organic anions. For example, X-ray absorption spectroscopy analysis of vacuolar Zn in the Zn hyperaccumulator Arabidopsis halleri revealed that Zn was complexed primarily with phosphate and/or organic acids such as malate and citrate [21]. Zinc sequestered within the vacuole, in contrast, serves as a storage pool of Zn that can be mobilised into growing cells of shoot and root tips under Zn deficient conditions [22].

The concentration of Zn varied largely among different plant cells. An example from the species of ThlaspiCaerulescens is given in Table 1 below [17]. The highest Zn was up to 1136 mmol kg^{-1} dry weight in the vacuoles of epidermal cells while only around 10 mmol kg^{-1} dry weight in root cell walls. The highest concentration of Zn was found in the nodes of subclover stems and rice [23]. However, it is not clear what proportions of the cytoplasmic Zn are present as free Zn^{2+}, Zn bound to protein, amino acid, nucleotide and lipid ligands in organelles, respectively. These characteristics of Zn sequestration may enhance the steepness of the Zn concentration gradient across the cuticle against soluble Zn concentration at leaf surfaces.

Table 1. Zn concentrations (mmol kg-1 DW) in different leaf and root cells of *ThlaspiCaerulescens*

ThlaspiCaerulescens	Cells	Organelle	Cell wall	Vacuole	Chloroplast	Cytoplast
Leaf	Epidermal cells	49±11	177±36	1136±130		
	Guard cells	9±4	129±31	22±13		
	Subsidiary cells	37±10	147±55	46±19		
	Mesophyll cells		143±32	5±2	n.d.	
Root	Cortical cells		9±4	n.d.		n.d.

Data was summarized from Frey [17]. n.d.: not detected.

3. NUTRIENT PENETRATION MECHANISMS AT LEAF SURFACES AND FOLIAR ZN UPTAKE

The uptake of inorganic nutrients into leaves is a complex process, mainly consisting of foliar adsorption, cuticular penetration, diffusion in apoplastic and symplastic spaces, phloem loading into vascular veins and translocation out of the sprayed leaf tissues into other actively growing parts of the plants [24]. Nutrient ions at foliar surfaces must firstly penetrate across the cuticle barrier, then move along the apoplastic pathway of the cell walls of the epidermal and mesophyll cells, or be actively absorbed through the plasma membrane of the leaf cells and transported along the symplastic pathway within the cells [25, 26]. Subsequently, the translocation of foliar absorbed nutrients is dependent on their proportional loading into vascular veins in the leave and the phloem of main veins for translocation. Many biotic and abiotic factors can affect each step in the process of foliar nutrient penetration and translocation.

3.1. Cuticular Properties of Plants

The major barrier to penetration of foliar-applied substance (organic and inorganic) is represented by the cuticle layer. The penetration of substance through the cuticle is a diffusive process driven by the concentration gradient between solute concentration at leaf surface and soluble concentration in the apoplast space [26, 27]. Plant cuticles have separate diffusion paths for penetration of lipophilic non-electrolytes and hydrated ionic compounds. As the cuticles represent the main barrier to nutrient penetration at the leaf surface (Figure 1), the mechanisms involved in cuticle penetration of polar and nonpolar substances have been intensively investigated. To illustrate the separate pathways for organic and inorganic solutes, it is necessary to understand structure and biochemical basis of the cuticular leaf surfaces.

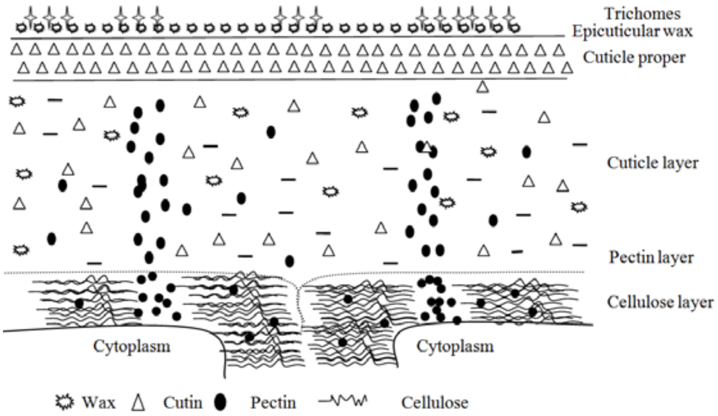

Figure 1. Schematic representation of the general structure of the plant cuticle (Adapted from Tagliavini, [27])

The cuticle is the outmost structure of the surface of aerial parts which helps to maintain and regulate water status for survival and reproduction even under adverse environmental conditions [28]. The cuticle covers all above-ground part, including flowers, stems, leaves, fruits and seeds of all higher (e.g. flowering plants) and lower plants (e.g. ferns) [28]. In specific environments, the cuticle serves as a multifunctional layer representing one of the largest interfaces between the atmosphere and biosphere [29].

The cuticle structure plays an important role in the penetration of foliar nutrients, so it is necessary to understand its fundamental structure and composition. Different plant species have different structural and biochemical characteristics of leaf surfaces, such as wax, which determines the retention and wettability of foliar applied droplets [30]. The average thickness of the leaf cuticle is about 2-4 µm and it consists of three layers, including the epicuticular wax layer, the cuticle proper and the cuticular layer [31, 32]. Epicuticular wax is the outmost layer of the cuticle and also the most hydrophobic component of the cuticle. This generates a certain degree of hydrophobicity at the outer surface of the leaves. The average mass of the wax layer is about 100 µg cm^{-2} [33]. In most species, waxes are composed of two major classes of substance, namely linear long-chain aliphatic compounds and cyclic terpenoids [31]. The cuticle proper lies under the epicuticular waxes and is made up almost entirely of cutin and/or cutan [24]. Cutin is insoluble in all solvents and free of polysaccharides [31]. This layer often has a thickness of about 50-150 nm [34]. The cuticle layer (Figure 1) is located under the cuticle proper and consists of cutin/cutan, pectin and hemicelluloses that increase the polarity of the layer due to the presence of hydroxyl and carboxylic functional groups [24]. Variable amounts of polysaccharide fibrils and pectin lamellae may extend from the cell wall, binding the cuticle to the underlying tissue such as epidermal cells. A gradual increase in negative charge from the epicuticular wax to the pectin layer creates an electrochemical gradient that may not only increase the movement of cations and water molecules from the leaf surface to the pectin layer, but also create a platform to prolong the remain of the positively charged suspension-based nutrient particles at leaf surface [35].

The natural lipophilic and hydrophobic properties of cuticle structural components make them an effective barrier against the diffusion of foliar-applied nutrients. However, the chemical properties of the foliar-applied nutrients can influence the penetration across the cuticle. The following sections will briefly discuss the current state of knowledge on the penetration mechanisms of polar solutes through the cuticle, in comparison with apolar lipophilic substances.

3.1.1. Cuticle penetration pathway I: polar pore pathway for ionic salts

Inorganic salts and other hydrophilic substances are highly soluble in water and practically insoluble in lipid phases such as cutin and cuticular wax. As a result, hydrated ions require an aqueous pathway (hydrophilic) across cuticles [36]. Many foliar-applied plant hormones, growth regulators, pesticides and nutrients are hydrophilic or ionic (e.g. Zn^{2+}), which require a hydrophilic pathway for their penetration through the leaf cuticles into the cells [37]. Foliar-applied nutrients are mostly found in the form of inorganic salts such as urea, synthetic chelates of metal micronutrients and weak acids such as boric acid [26]. In previous studies with different methods such as berberinesulphate, precipitation of $AgNO_3$ and $HgCl_2$, polar pores preferentially distribute around the sites of cuticular ledges of guard cells, cuticles over accessory cells, the base of trichomes and cuticles over anticlinal walls, suggesting that the absorption of polar substance could positively correlate with the density of stomata or trichomes on the leaf surface (e.g. abaxialvsadaxial) [36, 37].

Polar pores or pathways in the cuticles may be dynamically formed by hydration of polar functional groups (COOH-, OH- and ester group) in the cuticle layer [38-41], which are charged and size selective [42]. For example, each Ca^{2+} must be accompanied with two of Cl^{-} ions and rate constants of penetration decreased with increasing molecular weight of Ca salts [37, 39]. This suggests that Zn^{2+} and other negative ions must penetrate in equal valence numbers to maintain electro-neutrality to cross the cuticle membrane, such as nitrate and sulphate anions in foliar fertilizer solutions. The rates of ion diffusion depend on the swelling of cuticles (or polar pores), which is closely regulated by ambient humidity around the leaf surfaces, being more open for penetration of water and solutes when higher humidity causes cuticle swelling [27]. However, no conclusive experimental evidence has been reported to support the presence of the so called ‘polar pores’ in cuticle as they are too small to be identifiable with current microscope technology [26].

Using isolated astomatous cuticles, the average pore radii were found to be 0.45-1.18 nm [36, 43], which was largely dependent on the species and external conditions [44]. A pore radius greater than 20 nm was found on the stomatous leaf surface of *Viciafaba* L., *Coffea Arabica* L. and *PrunusCerasus* L., which were probably located in the cuticle above the stomata [44]. The average pore radius of 4-5 nm was found on the astomatous leaf surfaces of *Coffea Arabica* L. and *Populus*x *Canadensis*Moench [44, 45]. The density of polar pores was reported around 1010 pores cm^{-2} with the frequency of aqueous pores in the cuticle decreasing during leaf expansion [37]. These differences undoubtedly cause the different penetration rates of foliar nutrient ions at foliar surfaces of different plant species.

3.1.2 Cuticle penetration pathway II: lipophilic pathway for non-ionic substances (apolar lipophilic substances)

The penetration mechanism of non-ionic compounds is different from that of ionic substances. Penetration of non-ionic substances through cuticles occurs via physical diffusion, in which apolar substances (neutral not-charged molecules) dissolve and diffuse in the lipophilic domains composed of cutin and amorphous waxes [26, 46]. This process consists of three steps: sorption into the cuticular lipids, diffusion across the cuticular membrane and finally desorption into the apoplast of the epidermal cells [47, 48]. Experimental evidence has shown that apolar solute mobility in the cuticle varies markedly between species and its diffusion rate decreases with increasing molecule size [49, 50]. In addition, the diffusion process for apolar substances within plant cuticles is closely and positively related to ambient temperature, because rising temperature enhances the fluidity of amorphous cuticular waxes [50]. For instance, the difference in solute mobility in ivy cuticles between IAA and tebuconazole amounts to factors of 24.5, 10.5, and 2.2 at 25ºC, 35ºC and 55ºC, respectively [49].

As a result, organic solvents may be used to aid or facilitate the penetration of inorganic solutes into leaves, when formulating foliar fertilizers in agricultural practice, such as adding adjuvants in inorganic solutes to increase foliage coverage area or surface contact ratio [51]. Adjuvants are required to accelerate and promote foliar penetration via enhancing leaf wetting, retention, and penetration. Sometimes, humectants are required to lower the point of deliquescence (POD) to prolong spray drying in foliar formulations and increase the frequency of dissolution process in water films around the fertilizer particles [52-54]. Optimised formulation is critical to the efficacy of foliar fertilizers in field application.

3.2. Locality of Solute Penetration at Foliar Surfaces: Stomata, Trichomes and Other Plant Surface Structures

At the organ level, leaf surface characteristics significantly influence the retention and penetration of nutrient ions from foliar fertilizers at leaf surfaces, which are visually represented by the presence and density of stomata and trichomes, apart from the waxiness property. The intrinsic functions of stomata are to regulate gas exchange and transpiration rate (water loss) in terrestrial plants [55]. Many studies have showed that a high density of stomata (such as in the case of abaxial surface) significantly increase the rate of foliar uptake, mainly under the conditions of stomatal opening during the light phase [56-60]. It has also been argued that cuticles extend through the stomatal aperture and into the sub-stomatal cavity [61], and thus remain the main barrier to the penetration of substances entering the stomatal aperture. However, the cuticular surfaces around the ledges of stomata are believed to contain higher density of hydrophilic pathways than the astomatous cuticles. Šantrůček presented evidence that the cuticle of the stomatous leaf surface of *Hedera helix* was 11 times as permeable as that of the astomatous surface for water penetration [62], because the surface area of cuticular penetration sites was greatly increased by the presence of stomata and trichomes, compared to that without any stomata and/or trichomes (e.g. the adaxial surfaces of citrus and eucalyptus leaves).

The cuticular penetration efficiency would also be greatly enhanced by the presence of abundant density of polar pores on guard and accessory cells, and the favourable humid conditions around the ledges [27]. The humidity within the stomata and sub-stomatal cavities is high enough to condense or maintain condensation for the supplied chemical [63]. The thickness of the water film connected through the stomata is approximately 100 nm or less [64]. Therefore, once the connection is established, the stomata will provide a continuous pathway for the flow of soluble solutes or ion along the diffusive gradient of concentrations, as long as the stomata are not completely closed. Thus water or water-based substances will be transported more rapidly and efficiently via hydraulic connection through the stomata than across the large cuticular resistance. Many studies have shown a significant rate increase of foliar uptake by the presence of stomata when open in contrast to uptake through the rest of the cuticle [56-58].

Nevertheless, the contribution of stomata to the uptake of foliar-supplied substances remains controversial. The argument against the direct role of stomata in cuticle penetration is that the infiltration of liquids into stomata in intact plant organs is unlikely due to the high surface tension of water or aqueous solutions, internalized gaseous pressure in the sub-cavity of stomata and the disability of stomata path after closure [26]. Although a stoma having an aperture below 0.5 µm is usually regarded as closed, Eicher’s group found even a smaller aperture (below 0.5 µm) could still enable the penetration of externally applied solutions through the pores [44]. Further studies using the leaves of *Coffeaarabica, Prunuscerasus*and*Viciafaba* show that the average radii of pores inside the stomatal cavity were greater than 20 nm; Confocal Laser Scanning Microscopy (CLSM) revealed the presence of nano-scale particles (43 nm) in the stomatal aperture, which reached the mesophyll cells, but particles over 1.1 µm did not [44, 45]. This evidence supports the role of stomatal surfaces in the direct uptake of nano-sized and foliar-applied solutes because diffusion in this pathway is faster and less size-limited than in the astomatous cuticle. However, the evidence by Eichert and Goldbach was produced from in vitro tissues rather than the intact organs using in vivo method [44]. The internal pressurisation may be substantially weakened or diminished as soon as the tissues were separated from the organ, leading to the direct penetration of nanoparticles into the stomatal apertures, which may not be possible when the internal pressures was established. However, an isolated cuticle membrane cannot be used to study the effect of guard cells and the glandular trichomes because it is impossible to have the integrity of cuticles covering guard cells or trichomes when isolating them. Their roles in the pathway of ionic solutes can only be evaluated on leaf discs through relative comparison of stomatous penetration during the diurnal phases [56].

In contrast, the functions of trichomes are much less investigated in the uptake of foliar applied solutes than stomata. The presence and density of trichomes on leaves and other aerial organs of plants depend on the plant species and shoot organs where the trichome develops. In most cases, trichomes are not connected to the vascular system of the plant, but are rather appendages extending from the epidermis and covered by the cuticle layer [65]. However, studies with tobacco plants found that root Zn supplement significantly increased the density of the trichomes on leaf surfaces and high Zn accumulation in the base of trichomes detected by Confocal Laser Scanning Microscopy, suggesting the role of trichomes in Zn detoxification [66]. This indirectly implies that there may be higher density of polar pathways at the base of trichomes for cationic Zn exchange at foliar surfaces, in comparison to those without trichomes. In many plant species, trichomes are also presented in some areas where the entry of nutrients is facilitated due to their low cutinisation [27]. Some species of *Tillandsia* have a highly specialised water uptake system at leaf surfaces that is based on elaborate trichomes which can absorb water from the moist air [67, 68].

Intact fruit, fruit skin discs or isolated fruit cuticle membranes have been used for estimating direct penetration (without the influences of stomata and trichomes) of nutrient solutes through the cuticle, such as Ca solutions [58, 69-71].

Although foliar-applied Ca is positively related to the early developmental stages of fruits, the permeability of fruit surfaces was significantly reduced without the presence of stomata and trichomes [58]. The evaluation of stomatal contribution in foliar nutrient penetration may be carried out by controlling the stomatal opening in diurnal phases. The direct role of trichomes in nutrient penetration at leaf surface may be verified by using trichome-less mutants and their wild types in foliar nutrient uptake tests [68].

4. PHLOEM LOADING OF FOLIAR-ABSORBED NUTRIENTS IN LEAVES

The physiological efficacy of foliar-absorbed nutrients to achieve their functions in treated plants eventually depends on whether they can be transported from the fed leaves into other growing parts, such as fruits, grains and new growth points [72]. After the penetration of foliar nutrients across the cuticle layer, the nutrient ions diffuse into the phloem of minor veins through the apoplastic and/or symplastic pathway [25, 26]. Foliar uptake rate differs among different forms of Zn applied at the leaf surface, but it is not clear whether these differences are directly translated into different rates of phloem loading and re-translocation from treated leaves to untreated growing parts [73]. Despite early research on the botanical characteristics of the leaf vascular structure [74], there has been very limited research investigating whether foliar-absorbed nutrients are transported differently from root-absorbed nutrients before loading into phloem. Before we can discuss about mechanisms involved in vascular loading and phloem transport of foliar absorbed nutrients, it is necessary to review the structure of the veins, which are considered to be critical to the initial diffusions and phloem loading of the foliar absorbed nutrients [74].

4.1. Leaf Veins and Transfer Cells

Leaf vascular bundles (or leaf veins) are key structural components of vascular system in plants, which are responsible for transportation of water, nutrients and photosynthate [74, 75]. Leaf veins are arranged in a branching pattern, with successively smaller veins branching from somewhat larger ones. In general, the veins are divided into different orders: the middle vein (or midrib) is the first order (1º) and all veins associated with the midrib are the 2nd order vein or major veins (2º). The small veins or minor veins are further branched from the 2nd order vein and embedded in the mesophyll tissue, which are not associated with midrib and are collectively referred as 3º, 4º, 5º etc. [76, 77].

The primary functions of minor veins are to retrieve (or gather) and transport photo-assimilates successively into larger veins and eventually to the midrib [77]. Since minor veins are structurally developed with phloem cells, they play an important role in the initial phloem loading for photosynthate translocation from mesophyll cells into sieve elements. The minor veins are structurally evolved with special transfer cells, which are specialized cells with wall ingrowth in minor veins and are known to function in short-distances to transport solutes at the beginning or the end of the transport pathway in plants [78]. Transfer cells of minor veins are abundant in dicotyledons and especially well developed to handle solute transport and re-translocation in leaf tissues [78]. The presence of minor vein transfer cells in leaves may facilitate the retrieval of foliar absorbed nutrients (in organic or chelate-complex forms) from apoplastic and symplastic spaces due to the larger surface area of the wall ingrowth of the cells and further transport into the sieve elements for phloem transport towards growing points. As a result, functional loading of foliar absorbed nutrients into transfer cells in minor veins would be critical to their translocation efficiency out of the fed leaves, but direct evidence on this hypothesis is not available and requiring further studies.

4.2. Movement of Foliar-Absorbed Nutrients in Apoplast or Symplast

The transformation and diffusion of foliar-absorbed nutrients in and through the apoplastic space has yet been examined [79]. The apoplast is generally defined as all the compartments beyond the plasmalemma, including the interfibrillar and intermicellar space of the cell wall, xylem, the gas- and water-filled intercellular space [80]. Therefore, the total volume of leaf apoplast may be broadly defined as the space between the cuticle layer and the cell plasmalemma continuum. There has been very limited understanding of the fate of foliar-absorbed nutrients in the apoplastic space in leaves. Foliar absorbed cations, such as Zn^{2+}, Cu^{2+}, Fe^{3+} and Ca^{2+}, are expected to be firstly adsorbed by negative charges in the apoplastic space (such as cell walls), which may limit their diffusion into the veins and translocation into other plant organs [79]. It is reasonable to speculate that factors regulating the synthesis of chelating compounds and functional groups in cell walls may influence the adsorption of cationic nutrient ions such as Zn^{2+}. Zinc deficiency may cause the accumulation of polyphenol compounds in cell walls, which can bind the newly absorbed soluble Zn in cell walls [12]. Another example is that foliar-absorbed, non-charged Fe-chelates may be transported in the apoplast more readily than ionic Fe substances [57]. However, the results showed very limited mobility of all supplied Fe chemicals, even within the treated leaves, suggesting different metal ions may be adsorbed by different factors or complexing groups in cell walls [57, 81].

In general, phloem mobility of essential nutrients has been classified as highly mobile (N, P, K, Mg, S, Cl, Ni), intermediately or conditionally mobile (Fe, Zn, Cu, B, Mn) and immobile (Ca, Mn) [6]. Foliar-absorbed elements with higher mobility are theoretically more likely to induce systemic responses in plants than the immobile nutrients [82]. In many species, most of the foliar-absorbed Zn, Cu, Mn and Ca are retained in the treated leaves with limited proportions of them exported out of the sprayed leaves [24]. However, foliar spray of these elements may still have significant physiological benefit to the treated leaves and other plant parts in the short-term, despite the limited transport out of the treated leaves, which may be influenced by their concentrations in leaf tissues at the beginning. It is likely that the proportion of translocation of foliar absorbed nutrients increases with background nutrient status (i.e. their endogenous concentrations in the leaves before foliar application of the nutrients concerned).

To understand the initial and short-distance diffusion behaviour of foliar absorbed nutrients such as Zn, a non-destructive mapping method with high spatial resolution and highly sensitive detection is required. The method of radioactive isotopic labelling has been widely used to trace the movement of foliar-absorbed nutrients (e.g. N, P, Zn, etc.) in plants [83-85]. This technique provides a quick visual analysis of the general direction of transport out of the sprayed leaves and the distribution of the absorbed nutrients in a whole plant basis, but not micro-distance diffusion. In addition, this technique is not capable of semi-quantitatively and spatially resolving the short-distance diffusion of the absorbed nutrients in the treated leaves at the micrometre scale. Other tissue analysis methods require oven-drying the leaf tissues, which alters the original state of nutrient distribution in fresh and hydrated tissues, let alone the high spatial resolution required over a short-distance (miro/milli-meter scales). Until recently, an advanced technique- the synchrotron-based X-ray fluorescence microscope (μ-XRF) - has become available to study the uptake and distribution of some trace elements (such as Zn) in hydrated plant tissues, permitting in situ examination of the distribution gradient of foliar-absorbed nutrients in fresh, non-dehydrated leaf samples to understand the diffusion characteristics [86-88]. As a result, the μ-XRF technique can be used to spatially map the distribution of Zn in the sprayed leaves of varying species, age and Zn nutritional status, and their response to the application of Zn^{2+} in both soluble and suspension forms. This non-destructive mapping permits semi-quantitative characterisation of the initial diffusion of foliar absorbed Zn towards leaf veins. The examination of transfer cell roles in this diffusion and phloem loading requires further innovative methodology and foliar model systems.

5. KEY FACTORS AFFECTING FOLIAR NUTRIENT PENETRATION: CHEMICAL PROPERTIES OF SPRAY SOLUTION, PHYSIOLOGICAL AND ENVIRONMENTAL FACTORS

The response of plants to foliar application of nutrients is broadly determined by three major aspects: physicochemical properties of nutrient chemicals (and formulation agents), plant physiological status (e.g. nutrient adequacy vs deficiency), and ambient environmental conditions (e.g. relative humidity and temperature). Understanding these effects is critical to ensure optimised efficacy of foliar fertilizers in field applications. The key properties of foliar fertilizer solutions may include the solubility (or concentration) of target nutrients in saturated aqueous phase, and point of deliquescence (POD) of the nutrient chemical used, in addition to other factors in the overall formulation of foliar fertilizers, such as pH buffer, surfactant, adjuvants and humectant. In the meantime, plant physiological factors would also influence the efficacy of foliar-applied nutrients in both foliar uptake and translocation, such as leaf surface characteristics and leaf nutrient status (e.g. abundance vs. deficiency) [79]. Environmental factors, such as relative humidity in the ambient air around leaves, light intensity and ambient temperature, may affect foliar nutrient uptake directly or indirectly, such as through influencing the solubility of fertilizer chemical and nutrient ion concentration gradient at leaf surfaces, and cuticle properties and hydrophilic pathway resistance [31, 37]. These effects and interactions have been discussed below, by particularly using Zn fertilizers or compounds as examples.

5.1. Chemical Properties of Foliar-Applied Nutrient Solutions

5.1.1. Total and soluble nutrient concentrations of foliar fertilizer in solution

Foliar nutrient solutions are generally aqueous solutions, which not only contain the chemical compounds of specific nutrients concerned but also other ingredients used in its formulation (such as adjuvant, surfactants and/or humectants). The concentration gradient of soluble nutrient ions across the cuticular surface is the driving force for ion diffusion across the cuticles [26]. A positive relationship between the cation concentration and the penetration rate has been demonstrated with Ca in isolated cuticles [89] and Zn in intact leaves [90]. However, a negative correlation has been observed between the Fe concentration and the penetration rate (expressed as a percentage of the amount of applied) through isolated cuticles and intact leaves [56, 91]. Similarly, negative results were also observed with potassium (K) [92], 137Cs [59] and 2, 4-D [93]. Therefore, the relationship between concentration of the foliar-applied solution and the penetration rates may not be straightforward, but rather depend on other abiotic and biotic factors.

5.1.2. Solubility

In general, foliar-applied fertilizers are dissolved or suspended in water, based on their formulation styles. The chemical compounds containing nutrients may be inorganic salts (e.g. Zn nitrate), chelates or complexes (e.g. ZnEDTA) and sparingly soluble minerals (e.g. Zn oxide) [94]. The solubility of these salts, chelates or minerals in water at a specific temperature range is a physical property defined by their chemical composition. The available nutrient concentration in the aqueous phase would reach a maximum when the chemicals are saturated in the limited volume of water at the foliar surfaces. Aqueous solubility of a foliar nutrient compound is an important factor determining foliar penetration of the nutrient concerned, as the absorption of nutrient ions only occurs when the nutrient chemical is dissolved in the aqueous phase on a leaf surface [26]. The solubility in the aqueous phase is closely related to the intrinsic property of nutrient chemicals, i.e. POD above which the fertilizer chemicals start to dissolve in the aqueous phase.

5.1.3. Point of deliquescence (POD)

Point of deliquescence (POD) is a physical property of a chemical compound which determines the processes of hydration and dissolution at a given temperature [89]. POD refers to the threshold humidity at which a solid salt slowly dissolves as the ambient relative humidity rises above the POD, or gradually crystallizes from the saturated solution when the ambient relative humidity declines below the POD [89]. Deliquescent salts are hygroscopic substances which are capable of trapping water from the surrounding atmosphere and dissolving once a critical threshold of relative humidity has been attained [26]. The POD does not vary much with temperature, thus the lower the POD the lower the threshold relative humidity required to induce the dissolution of a nutrient fertilizer chemical, which could be used in dryer climate conditions and vice versa [89]. When relative humidity is above the POD, the applied fertilizer chemical dissolves to release nutrient ions for diffusion and penetration on the leaf surface with the maximum ion concentration at this point. While much work on foliar nutrient uptake processes has been based on the responses to soluble fertilizer chemicals [79], the concentration of dissolved nutrient ions from suspension-based mineral particles in the aqueous phase at foliar surfaces has been less investigated and characterised. Suspension-based foliar fertilizers contain sparingly soluble minerals (such as ZnO, MnO, etc), irrespective of its POD, which no doubt give rise to much lower concentration of nutrient ions at foliar surfaces than those of soluble chemicals. However, at lower concentration of nutrient ions in the saturated aqueous phase at leaf surfaces, the suspension-based fertilizer may generate a prolonged supply of the nutrient concerned over a much longer period of growth stages, than that of highly soluble fertilizer chemicals. In addition, suspension-based chemical particles deposited on the leaf surface would create an intact interface similar to the saturation state [95, 96].

5.1.4. Other chemical properties of fertilizer solutions

In addition to nutrient ion concentrations, chemical form and other chemical formulation agents, pH of the fertilizer solution can also affect the efficacy of nutrient penetration through leaf surfaces, as they can significantly influence the solubility of nutrient ions in water [26]. For example, the Ca penetration rate through the cuticle membrane of pear leaves is always higher in the form of Ca chloride and Ca nitrate than the forms of Ca propionate, Ca lactate and Ca acetate, within the same humidity range of 70% to 100% (Schönherr, 2001). As cuticles have isoelectric points around pH=3, the cuticle exhibits negative charges when solution pH is > 3, and the carboxylate groups in the cuticles readily bind positively charged cations (e.g. K^+, Ca^{2+}, Zn^{2+}) [40, 97]. In most cases, the pH of foliar fertilizer solutions tends to be near neutral. Therefore, it is reasonable to expect that positively charged chemical molecules or particles would adhere better onto leaf surfaces. In addition, the dissolution of foliar nutrient chemicals in water may alter the pH in the aqueous phase (or water film) at the leaf surface, which may in turn regulate the solubility of chemical compounds applied. For example, low pH values are considered optimal for metal ions (Zn), 3.3-5.2 for Ca [98], 5.4 - 6.6 for urea [99, 100] and 7-10 for K-Phosphate [101]. As a result, solution pH conditions should be standardised when comparatively evaluating the efficacy of foliar applied fertilizer compounds.

5.2. Biotic Factors of Leaves Affecting Foliar Uptake

5.2.1 Leaf surface characteristics

Leaf surface characteristics vary among different species and are also different on the adaxial and abaxial surface of the same leaf [61]. The most distinctive feature between adaxial and abaxial leaf surfaces is the presence and/or the relative density of stomata, which is higher on the abaxial than the adaxial surface [61]. The density of stomata at the leaf surface also varies dramatically with the leaf age [61]. It has been well documented that the abaxial surface takes up mineral nutrients more rapidly than the adaxial leaf surface, which has been attributed to the higher stomatal density at the abaxial leaf surface. The greater nutrient absorption on the abaxial leaf surface may be caused by the presence of a thinner cuticular membrane and a larger number of stomata [102]. The abaxial leaf surface would provide a larger contact area and higher density of hydrophilic pathways for the penetration of nutrient ions released from fertilizer chemicals, particularly during the day when stomata are open [24]. As a result, total nutrient uptake from both sides of leaf surfaces should be considered when evaluating the efficacy of a foliar fertilizer. In the field, aerosol droplets are sprayed onto the whole canopy, particularly cover the abaxial surface to achieve improved nutrient uptake.

5.2.2 Effects of leaf age on foliar nutrient uptake

Leaf age or developmental stage is one of the most important biological factors which may affect foliar uptake of nutrients as leaves of different age have a different biochemical composition of cuticular waxes and leaf surface characteristics (e.g. density of stomata and trichomes), leading to different densities and properties of hydrophilic pathways for ionic nutrients. The lifespan of leaves varies from days to weeks depending on plant species concerned [31]. The wax density significantly increases with the degree of leaf expansion, but the difference in wax abundance or form between adaxial and abaxial surface has yet to be investigated [103]. The thickness of the wax film also varies significantly with leaf age. Wax thickness of young *Prunus. laurocerasus*adaxial leaf surfaces was 30-40 nm, while 130-160 nm in a fully developed (more than one year) wax film was found in the same species [104, 105]. In peach plants, the composition of wax changes as leaves expanded. Triterpenoid acids are major components (84-95%) of waxes in the youngest leaves, but the proportions of these constituents decrease as a leaf expands or ages [72]. The abaxial surface waxes of the oldest leaves contain the highest proportions of hydrocarbons, whilst the wax from the adaxial leaf surface of the corresponding leaves contain the largest amount of ester [106]. The hydrophilic pathways in the cuticle are formed by polar groups, but the composition and proportion of polar groups of the wax on the leaf surface changes with leaf age. These changes in biochemical composition of waxes may be related to the development of hydrophilic pathways in the cuticles and thus foliar nutrient penetration rate.

In general, the penetration rate of nutrient ions in young or partially expanded leaves is higher than that of fully expanded leaves [56, 60, 107]. For example, Fe penetration rates were the highest in young leaves of grapevine and peach (adaxial) at the unfurling stage, but the rates in peach leaves declined to 40% and in grapevine leaves to 5% as leaves expanded, compared to the initial rates [56]. The rate of penetration into stomatousabaxial leaf surfaces is also higher in young and unfolding leaves than that of older leaves. For example, the maximum of 15N absorption reached 80% of the total amount applied in 20-day-old leaves, compared with 27 to 38% for 60-day-old leaves [108]. As young leaves expanded, 15N absorption decreased and the total wax concentration increased [108]. However, *Prunuslaurocerasus* leaves had a higher permeability of both isolated cuticle and leaf disk from old leaves than that in young leaves [109]. In studies with isolated cuticles from Marsh grapefruit (*Citrus paradise*Macfad), trans-cuticular movement of urea declined as leaf age increased from week 3 to 7 since its emergence, while the permeability increased again when the leaves became older than 9 weeks [110]. On the basis of the above evidence, the relationship between foliar nutrient uptake and leaf age seems to follow a parabola curvature, starting with a high rate at immature stage, declining with the rate of leaf expansion until the fully expanded stage and increasing again when the leaves commencing senescence. However, the total absorption in the leaves would be much higher at the fully expanded stage albeit the rate of absorption declines. In addition, the immature leaves or young flower/buds may be prone to the toxicity of soluble ions when the foliar nutrients are applied at high concentrations. Further research is required to assess the physiological responses of young and old leaves to a range of soluble nutrient concentrations. The potential toxicity of nutrient ions in young and immature leaves may be alleviated through suspension-based fertilizer chemicals of low solubility, such as the Zn hydroxide nitrate [$Zn_5(OH)_8(NO_3)_2$•$2H_2O$] (ZnHN) nanocrystals [111].

5.2.3. Effects of plant nutrient status on foliar nutrient uptake

A range of structural and metabolic changes may be expected in leaves in response to nutrient deficiencies, depending on the severity of the deficiency and nutrients concerned. For examples, boron and zinc deficiencies can cause malformation of leaf surface structure (e.g. stomata size) and the accumulation of secondary metabolites (e.g. phenolics and polyphenols) [6]. As a result, foliar nutritional status may affect foliar nutrient uptake through: (1) structural and functional changes of leaf surfaces induced by nutrient deficiency, which may influence the penetration process, (2) the accumulation of metabolites that may chelate nutrient ions (such as trace metals, e.g. Fe, Zn, Cu, etc), and (3) the internal concentration of soluble nutrient ions and the gradients relative to the external concentration at leaf surfaces.

However, the detailed mechanisms involved in the interaction of nutrient status with foliar uptake have not been reported in the literature. In pear and peach plants, iron deficiency decreased stomatal aperture and stomatal conductance, cuticle weight per unit surface in Fe-chloric pear leaves and contents of soluble cuticular lipids in Fe-deficient peach leaves [112]. In nitrogen (N)-deficient olive plants, leaves absorbed more foliar applied N than those with adequate N status [113]. Similarly, in citrus plants, foliar N uptake decreased with the increasing total shoot N concentration [114]. Plants response to foliar boron (B) supply was found to be affected by B status: i.e. B-deficient leaves had significantly lower B absorption rates than B-sufficient leaves, whichprobably due to B-deficiency induced reduction in the density of stomata and polar pathways [115]. Plants without root B supply exhibited 30% of foliar B absorption, compared to plants with root B supply, which could be attributed to the limitation of foliar B uptake likely caused by the reduced leaf surface permeability in B-deficient leaves [115]. From the above evidence, alteration of leaf surface characteristics caused by nutrient deficiency is one of the critical factors affecting foliar nutrient penetration and uptake, such as cuticular structure and biochemical composition, the density of stomata and trichomes, and specific leaf surface area.

Surprisingly, very little has been reported about Zn deficiency induced changes in structure of leaf surface structure in literature, apart from the limited leaf expansion, reduced stomata density and stomata aperture. In a study with peanut plants, Zn treatments resulted in an increase in the thickness of lamina, upper epidermis and palisade tissues [116]. The stomata density on both sides of the leaf decreased under Zn deficiency conditions [116], which could result in decreased nutrient uptake through leaf surfaces. Although there is still insufficient understanding of the effects of nutrients deficiency at leaf structure level, the Fe and Zn deficiency-induced changes on leaf surface suggests that they may induce effects at the epidermal level with potential implications for penetration of foliar applied nutrients. Further investigations should evaluate the effects of Zn deficiency on foliar Zn uptake.

5.3. Environmental Factors Affecting Foliar Uptake on Leaf Surface

5.3.1. Relative humidity in ambient atmosphere

The relative humidity in the ambient atmosphere is probably one of the most important environmental factors, which affects: (1) the deposition and retention of foliar applied nutrient chemicals at the leaf surface, (2) the dissolution and crystallisationof the nutrient chemicals at leaf surface, and (3) leaf cuticle structure and stomatal function. Once the nutrient solution is applied onto leaf surfaces, the effect of high relative humidity on foliar nutrient absorption is primary because the sprayed droplets can maintain longer periods of hydrated form for absorption [117]. The dissolution and/or crystallisation of deposited fertilizers at leaf surfaces are related to the POD of fertilizer chemicals and other agents used to make up the foliar fertilizer formulation [79]. Chemicals with low PODs are easily redissolved because the frequency of ambient humidity above the POD is higher and the amount of nutrient ions dissolved from foliar fertilizer chemicals is larger. Although the initial enhancement of absorption at low ambient humidity may happen due to the drying of droplets which causes consequent increase in the concentration gradient for diffusion at the leaf surface [118], the continual uptake rate of nutrients from chemicals with low hygroscopicities decline rapidly as the chemicals quickly undergo crystallisation when the relative humidity fall below the POD [119]. Therefore, the addition of humectants in foliar fertilizer formulation may be necessary for the deployment of high POD fertilizer chemicals in relatively dry climatic conditions.

In addition, the amount of wax per leaf area and wax crystal morphology changed under the high relative humidity, which may subsequently affect nutrient ion penetration into the cuticles [120]. High relative humidity increases ion penetration rates because it decreases the hydrophobic properties of the cuticle surface and increases cuticle swelling. For example, increasing relative humidity from 50% to 90% doubled the rate constants of Ca (in chloride solution) penetration [39]. Similarly, relative humidity effects on ion penetration were consistent, regardless of chemical forms of Ca, such as $Ca(NO_3)_2$ and organic Ca salts [89]. High humidity may enhance the transport capacity of hydrophilic pathways (or polar pores) by increasing the size or number of polar pores in the cuticle [89]. High humidity may also alter stomatal opening and have non-linear effects on the formation of hydrophilic pathways across the cuticles [36, 56]. As a result, it is important to reveal the relationship between ambient relative humidity and uptake rate of nutrient ions when estimating the uptake efficacy of foliar fertilizer chemicals, to provide the basis for further formulation of the fertilizer solutions to match typical climatic conditions in target regions.

5.3.2. Light-induced changes in leaf surface characteristics and foliar uptake of nutrients

Exposure to light may influence the uptake of nutrient ions at leaf surfaces through: (1) altering physical and chemical characteristics of waxes on leaf surface and (2) influencing the stability of applied chemical compounds [79]. The amount and composition of synthesised wax and its arrangement on the leaf surface are directly influenced by the exposure to light, including photo-synthetically active radiation and UV-B radiation [121, 122]. Long-term exposure to high light intensity increases the thickness of the wax layer and the amount of wax on leaf surface in plants, compared to those exposed to low light intensity [123, 124]. It has been reported that outdoor-grown apple leaves contain more than three times of wax per surface area than those grown under glasshouse conditions [125], and thirty times more than those grown under high humidity and low light intensity conditions [122]. Therefore, leaves exposed to high intensity of light for long periods may result in high penetration resistance to foliar nutrient uptake because of the increased cuticle thickness or the amount of wax developed [126]. These changes in leaf surface characteristics caused by exposure to high solar radiation suggest that foliar nutrient efficacy may vary with climatic conditions even for the same crop or horticultural species.

Regarding short-term uptake, many researchers have shown the positive effects of light on nutrient absorption through leaf surfaces [127-129]. Exposure to light increases permeability of cuticular membranes over the guard cells of the stomata, the opening of which is stimulated by light. Guard cells directly respond to light, which facilitates the foliar penetration process by increasing stomata opening [130]. Studies on the foliar uptake of naphthaleneacetamide acid (NAA) in pear leaves, Zn in citrus leaves and Fe (III) in broad bean leaves, provided evidence for the major role of light in promoting foliar penetration by inducing stomatal opening on abaxial leaf surfaces [56, 59, 131].

It has been found that a direct effect of light on foliar absorption might occur when the applied nutrient compounds are sensitive to light, such as Fe-chelates [132, 133]. In studies on foliar Fe uptake from Fe-chelates, exposure to light conditions induced significant photo-degradation of Fe-EDTA to a yellow-tan precipitate [132, 133]. Schönherr et al. concluded that Fe penetration on leaf surfaces preferentially occurred at night and foliar application of Fe-chelates should therefore be carried out in the late afternoon, which is to minimise or avoid photo-oxidation in leaves [91]. However, a disadvantage is that, low light or darkness induces stomatal closure and decreases the stomatal pathway for nutrient absorption. Therefore, foliar fertilizers with high sensitivity of photo-degradation should be avoided by spiking with other stabilising agents when creating foliar fertilizer formulations for field application.

5.3.3. Temperature effects on leaf surface characteristics and nutrient uptake

Long-term exposure to altered ambient temperature can affect physical properties of leaf surface in plants at cellular, organ and even whole plant level [134]. This not doubt, will affect foliar uptake rates of nutrient ions. In a short-term, temperature may affect foliar absorption through speeding up the drying rate of an applied nutrient solution and physico-chemical property of the nutrient solution (e.g. viscosity and solubility), in addition to temperature-dependent metabolism of some nutrients in plants [26]. In the foliar Zn absorption studies, Zn uptake of pistachio (*Pistachio vera* L.) leaves increased from 9 to 14% when temperature increased from 8 to 31ºC, while only a 4 to 6% increase in uptake for walnut (*Juglansregia* L.) leaves within the same temperature range [135]. Other studies with bean (*Phaseolus vulgaris*) leaves pointed out that low Zn uptake at low temperature was attributed to an increased viscosity of the aqueous ambient solution, and therefore a decreased rate of Zn diffusion on the leaf surface [136]. Thus in a short period (immediately after spray), the effects of the prevailing temperature around the sprayed leaves on foliar nutrient uptake may be more likely caused by temperature-induced changes in the physico-chemical properties of fertilizer chemicals after being deposited at leaf surface. Warm conditions may stimulate foliar penetration of nutrients indirectly, by increasing the rate of physiological processes such as photosynthesis and nutrient translocation in plants [137].

Nevertheless, the direct effects of temperature on the cuticular penetration of nutrient ions may be minimal, since this process is passive diffusion rather than active transport process. Tests with Ca^{2+} (at 15 - 30ºC), xylose (at 15 - 35ºC) and K^+ (at 10 - 25ºC) did not find significant effects of temperature on their penetration rates through leaf surfaces [39, 43, 138]. Foliar Zn uptake is only slightly affected by ambient temperature, because it is dominated by ion-exchange and/or diffusion process, rather than active one [135]. As a result, the dissolution of suspension-based Zn chemicals (e.g. ZnO or ZnHN crystals) and the concentration of soluble Zn at leaf surface may be more likely influenced by the ambient temperature, rather than the Zn uptake process per se.

6. CHEMICAL FORMS OF FOLIAR ZN FERTILIZERS

Zinc foliar fertilizers may be generally grouped into two broad categories based on the solubility in water: soluble salts and chelates (such as Zn-EDTA, $ZnSO_4$ and $Zn(NO_3)_2$, $ZnCl_2$ etc.) and sparingly soluble Zn compounds or minerals (such as Zn phosphate, ZnO) [79]. Zinc sulphate ($ZnSO_4$) is the most widely used inorganic source of Zn for foliar application, due to its high solubility and low cost [1]. Synthetic Zn chelates (e.g. Zn-EDTA) are also known for being more effective in foliar applications, but they are considerably more expensive than inorganic compounds and relatively unstable during storage [1]. However, zinc deficiency in rice was corrected more efficiently by spraying Zn-EDTA than $ZnSO_4$ [139]. In citrus plants, ZnCl2 was found more efficient in casing plant responses in Zn status than ZnSO4, although ZnCl2 may cause phytotoxicity symptoms in the sprayed leaves [140]. Foliar application of highly soluble Zn chemicals at moderate-high concentrations may cause extensive damages (or phytotoxicity) in young leaves and floral organs, due to specific ion toxicity and osmotic stresses, which could cause yield losses if applied at the critical reproductive stage [141, 142]. This high risk of phytotoxicity may be alleviated by multiple applications of foliar Zn across sensitive plant growth stages, such as from early flowering to early fruiting/seeding stages. However, multiple applications require time and intensive labour and thus uneconomical. These thus require development of long-lasting Zn fertilizers to satisfy peak Zn demands during the extended period from late vegetative to early reproductive stages in many horticulture species.

The intention of Zn-mineral suspension (e.g. ZnO) is to generate long-lasting Zn supply with low risk of phytotoxicity, in which the mineral particles are finely ground and suspended in water, together with many other agents such as surfactant and adjuvant [90, 135]. However, Zn solubility of the suspended ZnO in either fine powder or granular form is very low (< 3 mg Zn L^{-1}) and the release rate of Zn^{2+} may be too low to meet Zn requirements in plants, particularly when plant demand for Zn is high in the sensitive growth stages [111]. To compensate this shortcoming, very high concentrations of ZnO are often required in foliar spray, in order to achieve some degree of Zn uptake in the leaves and plants. However, the residues of ZnO suspension at the leaf surface may be washed off by rainfall or heavy dew because of poor binding onto the leaf surface [143]. The efficacy of these sparingly soluble trace metal minerals (such as ZnO, CuO) is thus unpredictable at leaf surfaces. In addition, running off of excessive oxide minerals from leaf surfaces to soil may cause in situ accumulation of trace metals and ecotoxicity to soil microorganisms, compounding the low efficiency of trace metal minerals in crop production [144]. Moreover, the grinding of these oxides into more uniform micro-particles for the suspension preparation is an energy-intensive process.

Therefore, there is a significant need to continue the development of Zn materials to increase Zn mobility and enhance the longevity of foliar Zn application, particularly to meet the peak Zn demand during the extended periods of later vegetative growth to early reproductive development [79]. These limitations in existing fertilizers have triggered the research to synthesise a series of new Zn compounds with defined Zn solubility and prolonged Zn supply potential, without the involvement for expensive and sophisticated chemical engineering. For example, purposely synthesized zinc hydroxide nitrate (ZnHN) nanocrystals, which have a controlled solubility (30-50 mg Zn L^{-1}) and carry positive charges may be a potential foliar Zn fertilizer with less risk of phytotoxicity, even at very high concentrations of total Zn at leaf surfaces [111, 145].

Research findings so far have demonstrated that foliar application of the ZnHN suspension at total Zn concentrations up to 400 mg Zn L^{-1} can generate much higher Zn uptake than the ZnO and the foliar absorbed Zn can be translocated into other growing parts of the treated plants [146].

4. CONCLUSIONS

From the information reviewed above, it is important to consider various effects of biotic and abiotic factors on foliar penetration of nutrient ions concerned when evaluating the efficacy of a compound or chemical as a foliar fertilizer. The nutrient ion concentration gradient across the cuticle boundary of leaves is the driving force of passive diffusion of nutrient ions through the cuticles. The chemical forms and intrinsic physico-chemical properties of the chemical closely determine the solubility of nutrient ions and thus the concentration gradient across the cuticle boundary, which influences foliar uptake rate, . nutrient efficacy and longevity in plants. Among the physiological factors, plant nutrient status is particularly important when considering foliar nutrient efficacy as nutrient deficiency may drastically change leaf surface characteristics and vascular transport systems of interconnecting veins in leaves. These key aspects of interactions among nutrient chemicals, foliar surfaces and ambient climatic conditions must be systematically investigated when evaluating a candidate fertilizer compound as foliar fertilizer.

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