SOME FACTORS AFFECTING MAGNESIUM UPTAKE BY CITRUS LEAVES

A thesis presented in partial fulfilment of
the requirements for the Degree of
Master of Horticultural Science
at Massey University
Palmerston North
New Zealand

by

Shaligram Kumar Thapa

1971

A B S T R A C T

Pineapple sweet orange seedlings and rooted leaf bud cuttings of Meyer lemons were used to investigate the effects of some of the factors affecting magnesium uptake by leaves. Magnesium was determined by thiazole yellow method of Drosdoff and Nearpass (1948) and uptake was usually measured 24 hours after spray treatment.

It was shown that the addition of wetting agents to magnesium nitrate sprays significantly increased the uptake of magnesium by leaves. The nonionic wetter (Terric GN9) at the very low (0.01% a.i.) and high (0.08 - 0.1% a.i.) concentrations did not affect magnesium uptake, whereas at intermediate concentrations, magnesium uptake was increased.

Use of the humectant glycerine at 1 or 2 percent significantly increased the uptake of magnesium by leaves, compared with sprays to which no glycerine was added, but had no beneficial effect over sprays which contained a nonionic wetter (Terric GN9).

Magnesium uptake by leaves grown in 100% relative humidity for two weeks was greater than the uptake by leaves grown in average relative humidity of 71%

Both morning and the evening sprays resulted in greater uptake of magnesium by leaves, compared with afternoon sprays.

A significant increase in leaf magnesium concentration occurred after 2 hours of a magnesium nitrate spray application. Leaf magnesium concentration rose steeply for 24 hours after spraying, thereafter remaining constant. (Because it was not possible to measure the degree of magnesium transport out of the leaf, it is not clear whether magnesium uptake, in fact, stopped after 24 hours).

Of the three magnesium salts used, magnesium nitrate and magnesium chloride sprays resulted in greater magnesium uptake by leaves, compared with magnesium sulphate sprays.

Uptake varied with the concentration of magnesium in the leaves. The lower the concentration of magnesium in the leaves, the less the uptake of magnesium by leaves, and the higher the concentration of magnesium, the higher the uptake of magnesium.

Leaf nitrogen also affected uptake of magnesium by leaves. High leaf nitrogen (2.92% of dry weight) resulted in greater uptake of magnesium than the low leaf nitrogen (2.08% of dry weight). The average increase in the concentration of magnesium in the leaves of low nitrogen plants was 0.09% of dry weight, while in leaves of high nitrogen plants the increase was 0.19%.

Thus the increase in the % leaf concentration of magnesium in the high nitrogen plants was double that of the low nitrogen plants. This may be a direct effect of the low leaf nitrogen or an indirect one due to the induced low leaf magnesium in those plants.

———————*———————

A C K N O W L E D G E M E N T S

I gratefully acknowledge the continuous assistance and guidance of Mr D.A. Slade of the Department of Horticulture, Massey University in completing this project. My sincere thanks also go to Professor J.A. Veale of the Department of Horticulture for his useful suggestions and advice.

This study was made possible by financial assistance from the New Zealand Department of External Affairs under the Colombo Plan. I am very grateful having been given this opportunity.

TABLE OF CONTENTS

Page

Page

CHAPTER 4

CHAPTER 5 Page

LIST OF TABLES

LIST OF FIGURES AND PLATES

LIST OF APPENDICES

APPENDIX PAGE

CHAPTER 1

1. INTRODUCTION

Nutrient sprays, these days, are becoming increasingly important to supplement the mineral requirements of the crops to increase crop production.

Nutrient sprays may be important in two directions.

(i) Where soil application of fertilizers is not responsive or very slow.

(ii) To prevent the development of a deficiency symptom very soon before the trouble is expected or immediately it has appeared.

But the responses of nutrient sprays are influenced by environmental factors (both physical and chemical) and plant factors. Magnesium absorption is not an exception to these factors. Leaves of some plant species do not show responses to magnesium salt sprays, while others do. Soil application of magnesium salts on the other hand, has been slow in action or has not been effective or partially effective. Foliage application of magnesium salts appears to be superior to soil application in increasing the concentration of magnesium in the leaves and in reducing deficiency syptoms. But the responses are not consistent.

The present study, therefore, was undertaken to determine the degree to which a number of likely factors might

affect the magnesium absorption by citrus leaves. The literature review, description of the methods and the discussion of the results have been presented with the aim of providing as much background information as possible in order to facilitate further detailed studies of magnesium absorption. For this reason, the literature review has been made more extensive than otherwise would have been required. The discussion of the results includes some hypotheses and speculations which lack evidence to support them, but they may be of value for future work.

CHAPTER 2

2. FOLIAR APPLICATION OF NUTRIENTS

Among some of the early work on the use of mineral nutrients to plants as foliage application is that of Johnson (1924), on pineapple plants in Hawaii, where pineapple plants were grown on soil rich in manganese. Within three to six months after planting, plants developed a serious injury known as pineapple-yellows or "manganese yellows". Johnson was able to control that chlorosis by simply applying sprays of ferrous sulphate ($FeSO_4 7H_2O$) to the leaves. The response was quick greening of the sprayed leaves, which indicated that at least some of the iron had been absorbed by the leaves.

Much of the work on iron sprays has been done with a view to supplying iron to plants suffering from what is called lime induced chlorosis. But due to immobilization of iron once it has penetrated the leaf cells, none of this work has given satisfactory results. For example, Guest and Chapman (1949) tried more than thirty iron compounds in dipping tests, using orange, grapefruit and lemon leafy shoots, which had been affected by lime induced chlorosis. None of them caused complete recovery. Hilgeman (1969) also had disappointing results with ferrous sulphate and two iron chelate compounds, when these were sprayed on chlorotic leaves of citrus in the field.

Zinc sprays have given satisfactory responses to control zinc deficiency in a wide range of fruit crops. For example, the studies of Parker (1937) on mottle leaf of grapefruit, of Reed and Parker (1936) on mottle leaf of orange, of Dhingra and Others (1967) on citrus chlorosis, of Labanauskas and Puffer (1964) in correcting manganese and zinc deficiency in Valencia ornage, of Paulechova - Kralikova (1966) on little leaf of apples and of Hoffmann and Samish (1966) on control of zinc deficiency in apples, are all examples of investigations with responsive species.

Copper sprays have been satisfactory in correcting copper deficiency in many plants. The studies of Dunne (1938) on Wither tip or summer dieback of apple trees, of Badamin (1966), Tarasov and Kovalenko (1967) on the control of shoot dessication of apples, of Lee (1964) on copper deficiency in Venturia Country citrus and of Kiely (1966) on exanthema of citrus, have all shown that copper sprays have been satisfactory in correcting deficiency symptoms.

Manganese sulphate sprays have controlled manganes deficiency of many plants. Camp and Peach (1938) in less than 30 days, obtained completely green leaves when manganese deficient cirtus leaves were dipped in manganese sulphate solution. Parker and Southwick (1941), by the use of various manganese compounds as spray, corrected manganese deficiency symptoms in citrus. Labanauskas (1962) corrected manganese

deficiency of grapefruit by manganese sulphate sprays. Working with Newtown apple, Uriu and Koch (1964) corrected the symptoms for the entire year by zinc plus manganese sprays applied at least twice in early spring.

Boron deficiency of several fruit and vegetable crops has been controlled by the foliar sprays of borax or boric acid. Askew and Chittenden (1936), with a single spray of 1 per cent hydrated borax, obtained four to five fold increase in boron level in the fruit and prevented the internal cork symptom of born deficiency in apple. Early season sprays of boron on apple have resulted in substantial increase of boron in fruits and considerable amounts in leaves (Bramlage and Thomson, 1962).

Urea sprays have been studied extensively to furnish a considerable part of nitrogen needs of several crop plants. The response to urea sprays is variable. Urea sprays to apple trees have increased leaf chlorophyll and leaf total nitrogen as compared with unsprayed trees (Hamilton et al., 1943; Ludders and Bunemann, 1967), improved vegetative growth (Hilkenbaumer and Hohmann, 1964; Fisher et al., 1948; Fisher and Cook, 1950; and Fisher, 1952). However, urea sprays to grapes proved disappointing (Weinberger et al., 1949).

Burrell and Boynton (1943) doubled the potassium content of the apple leaf with six sprays of 1% potassium

Sulphate. Ganje et al., (1966) controlled soil induced potassium deficiency of citrus in green house, with potassium nitrate sprays.

Magnesium has been supplied both by soil and foliage application. Findings indicate that foliage application of magnesium is better than soil application in controlling magnesium deficiency in plants.

The specific examples cited above, provide some evidence that some type of leaves can, to some extent, absorb nutrients from sprays. If nutirent requirements can be satisfied and applied economically by some other means then there is no real need of supplying them as sprays. Since only low concentration of salts can usually be applied to the leaves without burning them, the most important use of such sprays can be made for the correction of minor element deficiencies.

CHAPTER 3

3. LITERATURE REVIEW

3.1. Pathways of penetration of nutrients, herbicides and other substances into the leaf

Both surfaces of the leaf are penetrable, but all areas of the leaf are not equally permeable. Preferential areas of foliar absorption named in the literature for various substances (herbicides, other pesticides, nutrients, and fluorochromes) are:

(a) directly over the veins,

(b) over anticlinal epidermal walls,

(c) glandular and non-glandular trichromes,

(d) open stomata,

(e) hydathodes, lenticels, and natural fissures,

(f) insect punctures and other imperfections in the cuticle.

Penetration may be classified as stomatal or cuticular, then cellular. Opinion is divided as to whether stomatal or cuticular entry is more important as a generalization. Both are known to occur under appropriate circumstances.

3.1.1. Entry through stomata

Stomata have been claimed to be primary sites of entry for Co^{60} (Gustafson, 1956; Gustafson and Schlessinger, 1956),

NAA (1-naphthaleneacetic acid) (Harley et al., 1957). This path was not indicated for P^{32}, K^{42}, Rb^{86} (Teubner et al., 1957) or urea, Rodney (1952), Volk and McAuliffe (1954).

Many workers claim that stomatal penetration does not occur unless the surface tension of the solution is reduced considerably by the addition of surfactants (Weaver and De-Rose, 1946; Turrell, 1947; Norman et al., 1950; van Overbeek and Blondeau, 1954). Only open stomata have been reported to be penetrable by oils (Rohrbaugh, (1934) Minshall and Helson, (1949); not, or only slightly by water and to varying degrees by aqueous solutions containing surfactants. Completely closed stomata can exclude all fluids (van Overbeek,1956). Sargent and Blackman (1962), on the other hand, presented a new theory as regards to penetration of 2, 4-D. According to them, 2,4-D does not need to have a passage between two guard cells of stomata, but can penetrate through guard and accessory cells.

3.1.2. Structure of the cuticle

3.1.2.1. Physical nature of the plant cuticle

According to Schieferstein and Loomis (1959), Brongniart (1934) has described the cuticle as the outer cellulose-free covering of leaves, distinct from the cuticularized layers that might be formed beneath it. In lower forms such as the mold Phycomyces, the cuticle is a

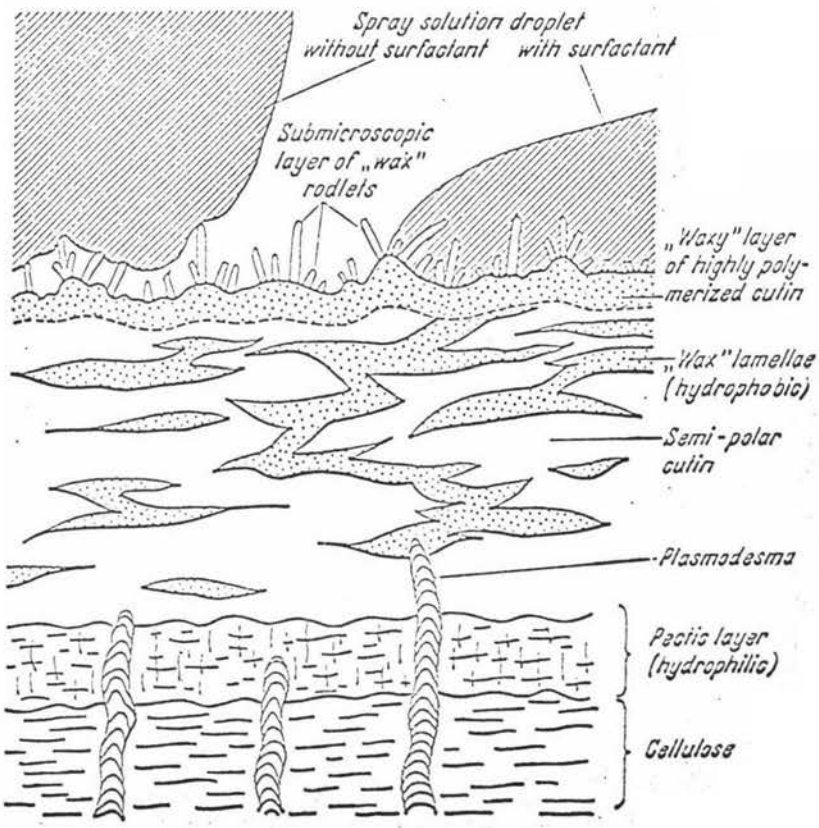
simple thin membrane of low elasticity and of poor adherence to the cell wall (Roelofsen 1950), according to van Overbeek (1956), and in higher plants the cuticle is usually complex as sketched in Fig. 1

The true cuticle, according to Lee and Priestley (1924), is formed by the oxidation of oily materials which generally permeate the walls of living cells. The oils are assumed to be products of cell metabolism, and to have diffused through the cellulose pectin structure of the cell wall. Lee and Priestley (1924) discussed the cuticle formation and concluded that various lipoidal substances, formed or mobilized in the epidermis, migrate to the surface of the plant. There they tend to oxidize, saturating the double bonds by polymarization or oxidation, and thereby cause a "varnish like" covering to form.

In addition to the cuticle, leaves may show marked accumulations of readily soluble surface waxes. The way in which wax is deposited on the surface of the leaf is not clear. In many species characteristically shaped projections of wax arise from the surface, but the specific sites from which these projections are extruded have not been found by Shieferstein and Loomis (1959). Their observations suggest that wax is extruded at random through the thin areas of the cuticle. But Hall and Donaldson (1962) claimed that epidermal cells of Brassica oleracea and Trifolium repens reveal pores from which

Fig. 1. Hypothetical structure of the functional aspects of the plant cuticle. The waxy rodlets of leaves having "bloom" may prevent contact of a spray droplet with the leaf surface

(Foy et al., 1967)



wax is extruded. They suggested that wax from a number of pores, may form a single wax particle. The configuration of these particles appears to be responsible for water repelling properties of a heavily bloomed surface. Cutin and waxy material have also been found on the free surfaces of the leaf mesophyll cells and on the inner walls of the epidermis where these are exposed to internal air spaces. The internal cuticle is continuous with the external cuticle through the stomatal apertures, whose bounding cells, the guard cells, are covered with a cuticle on their free surface.

Scott et al. (1948) found the outer epidermal walls of leaves of citrus sinensis Osbeck to be cutinized and coated with wax, which seemed to be excreted through minute canals similar to those in the fruit rind. By treating sections of leaves with IKI-H_2SO_4, they showed the canals to be lined with protoplasmic threads; and protoplasmic activities within these canals were attributed to the excretion and the maintenance of the waxy surface.

Environmental factors can influence the formation of cuticle. Stevens (1932) measured the cuticle thickness of several varieties of cranberry for three consecutive seasons and found up to 20% variation with the season, similar in all varieties. Lee and Priestley (1924) showed experimentally that exposure to certain atmospheric conditions is a primary requisite in the changing of fat and oil layers to the varnish-

like condition characteristic of a mature cuticle. Further, light and humidity were shown to affect the thickness and consistency of the cuticle by their influence upon the oxidation and condensation of fatty acids, a process involved in cuticle formation. Working with Nicotiana glauca and Hedera helix, Skoss (1955) observed that the deposition of cuticle was continuous until the leaf reached morphological maturity; beyond maturity no further deposition occurred. Leaves grown in the sun produced heavier cuticles of greater wax content than leaves grown in the shade. The temperature conditions under which plants were grown were shown to influence the deposition of cuticle and wax. The most cuticle was produced at a median temperature, and the greatest percentage of wax at a high temperature. Plants undergoing water stress produced cuticles containing a greater proportion of waxes than plants with more favourable moisture conditions.

3.1.2.2. Chemical nature of the plant cuticle

According to Foy et al. (1967), Frey-Wyssling (1948) has summarised the chemical nature of cutinized plant cell walls, which are composed of four distinct substances, all of which may vary in distribution within the wall. These substances are: (a) cutin, (b) cutin waxes, (c) pectin and (d) cellulose. All constituents contribute their own physio-chemical properties to the surface layer of the plant.

(a) Cutin:- Cutin has been described as the polymerisation and condensation of C_{18} hydroxy fatty acids which are synthesized in the protoplasm and penetrate the wall as procutin. Extruding on the surface, these precurssors are oxidized and polymerised, forming a sponge-like frame of submicroscopic dimensions. Cutins contain reactive end groups which enable them to form esters and ethers. Also, cutin may contain an appreciable amount of dicarboxylic acids and hydroxy carboxylic acids. Having many such polar groups such cutin may absorb water and swell and this increases permeability (van Overbeek, 1956).

Lee (1925) made a chemical study of cutin and showed it to be a complex mixture of fatty substances, consisting primarily of free fatty acids, fatty acids combined with monohydric alcohols, and soaps. Legg and Wheeler (1925) isolated two acids as major components of cutin and suggested the names "cutic acid" ($C_{26}H_{50}O_6$) and cutinic acid ($C_{13}H_{22}O_3$). Other acids were isolated in much smaller quantities.

(b) Cutinwaxes:- Crafts and Foy (1962) state that cutin waxes are short chain esters and alcohols of relatively low molecular weight, lacking reactive end groups and unable to polymerise. Cutinwaxes are optically negative, stainable in lipid dyes, melt above 220°C, and do not absorb ultra violet light.

Foy et al. (1967) state that waxes are hydrophobic in nature and hence resistant to wetting with pure water. The waxy rodlets of the leaves form a "bloom" which may prevent contact of a spray droplet with the leaf surface (Fig.1), but the addition of a suitable surfactant to the oil or aqueous sprays may facilitate the wetting of waxy leaf surfaces. Further enhanced wetting does not mean enhanced penetration, because surface wax deposits may interfere with wetting but little with penetration, provided good surface contact is ensured.

(c) Pectins:- Crafts and Foy (1962) state that pectins consist of long chain polygalacturonic acid molecules having side carboxylgroups which can form salts and impart to pectins base exchange properties. Polygalacturonic acid and its methylated derivative are soluble in water, but its calcium salt is insoluble. Pectin substances have little tendency to crystallize; they occur in an amorphous state in plant cell walls, and they are responsible for the strong water holding properties of the walls.

(d) Cellulose:- Foy et al. (1967) describe the cellulose as composed of long chain molecules which are relatively stable. These molecules are organised into micelles. The micelles are associated into microfibrils, and because of its microfibrillar organisation, cellulose imparts tensile strength and elasticity. Crafts and Foy (1962) indicate that

it is this property of cell walls that resists expansion and results in turgor. Turgor, in turn, enables the plants to grow erect against the force of gravity, to extend roots into the soil, to absorb water and nutrients, and to maintain its foliar organs in positions favourable for maximum absorption of carbondioxide and light.

3.1.2.3. The role of the cuticle

Epidermal cells of leaves and stems, unlike roots, are more or less covered with thick cuticle, which may be superimposed by wax excrusions. The lipid character of these waxes and cutin layer can produce an obstacle for the penetration of hydrolphilic substances. It is believed that the obstacle created by the cuticle is so great that the penetration of the hydrophilic substance occurs only through stomata. However, the stomatal passage allows solutions only to enter in stomatal chambers and intercellular spaces, but not in the cells, because the outer walls of the cells lining these cavities are also covered by an internal cuticle (Scott, 1950). Also, the hydrophobic nature of the cuticle lining the stomatal pores normally does not permit the passage of the aqueous solutions. Use of detergents may induce this passage (Dybing and Currier, 1961). Thus, under natural conditions, absorption of solutes must take its regular course by the penetration of the cuticle.

The question at once arises whether there is any pore in the cuticle, providing pathways for penetration. Electron-microscopy studies reveal that there are no pores in the cuticle other than local thin spots, punctures, breaks, and fissures made by insects. However, Hall and Donaldson (1962) have found true perforations in the epidermal cells of Trifolium repens and Brassica oleracea. Wax is said to be extruded through these pores.

Orgell (1955) has described cuticle as being imbricate arrangements of lipoid platelates cemented together by hydrophilic pectaneous substances. Thus, an intercuticular penetration should be possible for a polar solution. But there is no direct proof of such a pathway. Therefore, as a rule, in foliar absorption, substances to be absorbed have, in contrast to the situation in roots, to penetrate a lipid like layers. This means that an "intracuticular penetration" without pathways through distinct pores has to be performed before solutions can enter the cellulose walls (Wittwer and Teubner, 1959).

3.1.3. Structure and nature of the cell wall

According to Jensen (1967), cell walls vary greatly in composition and morphology. However, the cell wall consists of three layers, middle lamella, primary wall and secondary wall. The primary wall is frequently thin (1 to 3 /u thick)

and elastic. It increases in area as the cell grows. A secondary wall is formed between the cytoplasm and the primary wall, when the growth of the cell ceases. The secondary wall is often thick (5-10 /u) and rigid, providing great tensile strength to the cell. The primary walls of two cells are joined by a common layer called the middle lamella.

The cell wall when first formed is very thin, but increases in thickness through the deposition by the protoplasm of new particles upon those already present. Thickness of the cell wall sometimes becomes so great, that it almost fills the cell cavity (Anderson, 1927).

The chemical nature of the cell wall is less understood and thought to be a complex one. Miller (1938) provides a diagram which shows the substances that may compose the cell wall (Fig. 1a).

Fig. 1a. A diagram showing the substances that may compose the cell wall (From Miller, 1938).

Material that may enter into the composition of the cell wall	Cellulose	Normal, typical or true celluloses			α, β, γ types
		Hydrocelluloses			
		Oxycelluloses			
		Compound celluloses - Lignocelluloses			
		Hemicelluloses or Pseudocelluloses	Skeletal hemi celluloses	Pentosans xylan Araban Galactosans	In the cell walls of wood and seed coats.
			Reserve hemi celluloses	Pentosans xylan Araban Mannosans Galactosans	In the cell walls of endosperms & young fibres of wood & bark
	Suberin and Cutin				
	Pectic Substances	Pectic acids			
		Pectates			
		Pectose			
		Pectin			
	Other constituents	Resins, gums, tannis, minerals, Colouring matters, proteins, fats, phospholipides, ethereal oils and callose.			

Cellulose, composed of thousands of repeating glucose units, is the major structural element of the cell wall, especially in the case of primary wall (Jensen, 1967).

Cellulose has been reported to be loosely embedded in the martix in the form of microfibrils (Frey-Wyssling and Muhlethaler; 1965) The microfibrils, in the secondary wall which contains 60 to 94 per cent cellulose and only a little martix, are closely packed and interwoven into a net work. There exists interspaces between the microfibrils and between the elementary fibrils (Frey-Wyssling and Muhlethaler, 1965). The size of the intermicellar spaces between the elementary fibrils is about 10 A° and therefore they should be penetrable by small molecules such as water and halogen ions. The size of the interfibrillar spaces between the microfibrils however, has been reached up to 100 A°. Hence, larger molecules should pass freely if the spaces are not filled with non-cellulose material (Frey-Wyssling and Muhlethaler, 1965). Other components of the primary wall and the middle lamella are the pectic substances. These are large molecules made up of repeating units called hexuronic acids, which are derivatives of hexose sugars (Jensen, 1967). Still other compounds are found in the walls of many cells. Chief among them are the waxes, which constitute the surface layers of the plant.

Ectodesmata:- The discovery of extodesmata in the outer wall of the epidermal cells has provided a new outlook for foliage penetration. Franke (1961) has studied the occurrence and distribution of ectodesmata in the epidermis of Plantagomajor and Helxine soleirolii. Leaf structures, such as guard cells, conical hairs, anticlinal walls and the epidermal cells adjacent to the leaf veins have been observed to contain large numbers of ectodesmata. Since ectodesmata extend from the cuticle, which they do not perforate, through the wall to the lumina of epidermal cells, they seem to provide an almost direct connection of the protoplasts with the surrounding outside medium. Franke (1961) noticed that the solutions which formed visible crystals and precipitated in the outer wall, entered the epidermal cell wall in localized pathways and the localization of these bodies coincided with that of ectodesmata. Hence, he concluded that ectodesmata provide the principal pathways for transport of substances from outside to the interior of tissues and vice-versa. Nutrients applied to the leaf surfaces are assumed to follow the same pathways i.e. ectodesmata.

3.1.4. Structure of the plasma-membrane

The cytoplasm is surrounded by a layer called plasma-membrane or plasmalemma which is thin, flexible and not directly visible to the light microscope. The plasmamembrane, according to (Jensen, 1967) is composed of protein and lipid. Their molecules are arranged in the membrane in such a way that the protein molecules are aligned on the exterior of the membrane and are bound to the lipid molecules which occupy the centre.

Frey-Wyssling and Muhlethaler (1965) have mentioned a variety of functions which are performed by the plasmamembrane. It controls semipermeability, resorption, excretion and secretion, leading to the formation of slime and a whole series of cell-wall substances, and it is also capable of breaking down substances enzymically.

Jensen (1967) has mentioned the advantages of the semipermeability of the membrane in that it prevents the organic materials of the cell such as sugars and soluble proteins from leaking out of the cell while allowing water and salts to enter, and this maintains the integrity of the cell in relation to the surrounding environment.

The plasmamembrane provides no passage as such for the substances coming from outside and they are at first only

adsorbed in the plasmamembrane and then taken into the cytoplasm by a process requiring metabolic energy (Jensen, 1967).

3.1.5. Mechanisms of foliar penetration

3.1.5.1. Mechanisms of penetration in the cuticle

Many workers, in order to study the penetrability and mechanism of cuticular penetration, have examined the isolated cuticles (Skoss, 1955; Schieferstein and Loomis, 1959; Goodman and Addy, 1962; Darlington and Cirulis, 1963; Yamada, Wittwer and Bukovac, 1964, and Silva Fernandes, 1965). These studies have confirmed the penetrability of the cuticular membranes.

Darlington and Cirulis (1963) regarded, leaf cuticular penetration, as a diffusion process influenced by temperature, concentration and relative solubility in organic solvents. Penetration increased with the increased temperature and with the higher lipophilic character of the compounds, and was directly proportional to the concentration. The different compounds applied penetrated the isolated apricot cuticle in the range of 1.5 to 3.3% during 48 hours at 25°C. Yamada, Wittwer and Bukovac (1964) on the other hand, observed that only 0.2 to 2% of the applied cations and anions penetrated the isolated tomato fruit and onion leaf cuticles, but 80% passed through the dialysing membrane after 40 hours. Hence, their results suggest that this may not be simple diffusion. The lower penetration might have resulted from the lower permea-

bility of the isolated cuticles, brought about by the changes in physical and chemical characteristics of isolated cuticles, during the process of their isolation by chemical means.

Some permeability, surface binding, and ion exchange characteristics have been reported for enzymically isolated cuticalar membranes (Yamada, Wittwer and Bukovac, 1964; Yamada, Bukovac and Wittwer, 1964). They include the stomatous green onion leaf cuticle and the astomatous tomato fruit cuticle. Cations and anions were bound to the inner surfaces of cuticles to much greater extent than on the outer surfaces. With dialyzing membranes, no such differences between the two surfaces occurred. Approximately, three times more cations were bound on the inner surface (7.89 m/u mole per cm^2) of the tomato cuticle than the outer surface (2.73 m/u mole/cm^2). The possible explanation of such differential ion binding by surfaces of isolated culicular membranes has been given as the morphological differences in the cuticular surfaces. The outer surfaces were smooth, while the inner surfaces were irregular, having protrusions and cellular fragments. A further possible explanation for the differential ion binding by the two surfaces as given by Yamada, Bukovac and Wittwer (1964), has been related to the physio-chemical properties of the two surfaces. The outer cuticular surface is believed to be composed of layers of highly polymerized and oxidized fatty acids. While the inner surface represents a more heterogeneous chemical composition.

It has been reported that cuticular penetration from outside to inside is greater than from inside to outside. This type of behaviour was first observed in the stomata free cuticles of Hedera helix leaves in which more water penetrated inward, i.e. towards the protoplast than outward (Schieferstein and Loomis, 1959).

A similar type of result was obtained for the penetration of radioactive cations (Ca^{45} Rb^{86}) and anions $S^{35}O_4$, Cl^{36}) and also for undissociated organic compounds through enzymically isolated cuticles of astomatous tomato fruit and stomatous green onion leaves (Yamada, Wittwer and Bukovac, 1964; 1965). Again the penetration of cations from outside to inside was more pronounced than the anions and the rate of penetration through different cuticular surfaces was directly related to the extent of ion binding on the surface which was opposite the site of the initial entry (Yamada, Wittwer and Bukovac, 1964).

Crafts and Foy (1962) have shown the evidence of a gradient from low polarity on the exterior to a relatively high polarity in the layers bordering the epidermal cell-wall. In any case the negative charges characteristics of cuticular membranes offer reasonable explanations for permeability differences between cations and anions as well as the greater binding of cations on the inner surfaces as compared with anions.

However, Goodman and Addy (1962) obtained an opposite result for the penetration of a number of organic compounds through apple cuticles i.e. the penetration of organic compounds from inside to outside was significantly greater than from outside to inside. In this case the cuticles were isolated with ammonium oxalate and oxalic acid. Such chemically separated cuticular membranes might be different from those isolated enzymically and their structure might have been altered, thus causing the reversed behaviour. Further, the permeability to organic compounds might be different from that of inorganic ions.

Urea, as reported by Yamada, Wittwer and Bukovac (1965) penetrates the cuticular membrane, with a higher velocity than could be expected from the simple diffusion. The penetration of urea, thus, through the isolated tomato fruit cuticle was 10-20 times greater than the penetration of inorganic ions (Rb^{+}, Ca^{++}, Cl^{-}, SO_4^{--}) They concluded that urea might have penetrated through the facilated diffusion. There is supporting evidence for facilated diffusion in that urea promotes the uptake of other nutrients similtaneously applied (Wittwer and Teubner, 1959; Labanauskas and Puffer, 1964). The effect of urea on the permeability as reported by Yamada, Wittwer and Bukovac (1965), is based on the loosening of the membrane structure by changing ester, ether, and diether bonds between the macromolecules of cutin.

Thus, finally, it seems that both inorganic, organic ions

and undissociated molecules can penetrate the isolated cuticular membranes by means of simple diffusion, while urea seems to penetrate by a process of facilated diffusion. In intact tissues similar physical processes might be involved during penetration.

3.1.5.2. Mechanisms of penetration in the cell wall

As soon as the substances penetrate the cuticle and cuticular layers, they reach the cellulose layers of the wall. The interfibrillar spaces between the cellulose fibrils are thought to be large enough for the penetration by diffusion. Still it is believed that wall penetration takes place through separated pathways. These pathways are ectodesmata. Franke (1961) strongly believes that nutrients follow these pathways.

However, Sutcliffe (1962) states that the contents of the cell wall such as cellulose, hemicellulose, polyuronides and phospholipids are capable of binding salts in the forms which are readily exchangeable, and hence the wall acts as a Donan system containing an appreciable concentration of negative charges. The movement of ions takes place from the external medium, by diffusion and exchange, to the surface of the cytoplasm.

3.1.5.3. Mechanisms of penetration in the Plasma-Membrane

Substabces coming from outside have to overcome a third

barrier, the plasmalemma on reaching the protoplasts. According to Jensen (1967) the plasmamembrane provides passage for physical penetration of water molecules only and a large number of substances are incorporated by a process requiring metabolic energy. This process may involve a carrier system which involves the picking up, at the external surface of the plasmamembrane, of a substance to be transported by a carrier into the internal surface of the membrane.

Jensen (1967) provides the evidence of another mechanism, which may be responsible for the active uptake of the substances. This is called Pinocytosis, which involves the invaginations of cell membrane and formation of vesicles within the cytoplasm of the cell. The vesicle may break down and the material contained in it is finally utilised by the cell.

The evidence of active absorption of rubidium and phosphate by the primary leaves of the bean plant has been demonstrated by Jyung and Wittwer (1964) and for tobacco leaves by Jyung et al., (1965).

The energy required for active absorption may be obtained from respiration and photosynthesis. If the energy is provided by respiration, lack of oxygen should reduce the uptake, and if the energy is provided by photosynthesis the presence of CO_2 and light should accelerate the uptake. The fact that the light improves the uptake of several substances has been demonstrated by several workers (Sargent and Blackman, 1962,

1965; Jyung et al., 1965; Middleton and Sanderson, 1965).

Energy is thought to be mostly provided in the form of ATP. Hence, the addition of this compound must increase the uptake. This has been demonstrated for the uptake of Rb^+ by the cells enzymically isolated from tobacco leaves, where addition of ATP, increased Rb^+ uptake by 13% (Jyung et al., 1965). Also, application of high energy products of photosynthesis along with the substances to be absorbed, has increased the uptake of the latter. For example, feeding with sucrose enhanced the uptake of leucine by apple leaves in the dark (Kamimura and Goodman, 1964). Similarly, instead of oxygen, the addition of intermediates of the respiratory metabolism, enhances the uptake of the substance to be absorbed. Thus, feeding with succinate increased the Rb^+ uptake by cells of enzymically isolated from tobacco leaves in the dark and light (Jyung et al., 1965).

From the details described above, it appears that the following stages are involved in the overall process of foliar uptake.

I. In the first stage, substances applied to the surface of the leaves penetrate the cuticle, and the cell wall through a physical process of diffusion.

II. In the second stage, substances are adsorbed in the surface of the plasmamembrane.

III. In the third stage, substances are taken into the cytoplasm, by the processes requiring metabolic energy.

During stage I and II, substances only fill up the free space and merely adsorb. They are not taken up into the cytoplasm. This has been shown from the fact that during these stages, substances are again washed out (Vickery and Mercer, 1964).

The fact that the overall foliage penetration involves the above three stages can be seen by illustrating the work of Vickery and Mercer (1964) and Allen (1964). Vickery and Mercer (1964) recogise three stages of uptake of sucrose by bean leaf. (i) an initial rapid uptake of sucrose. (ii) a linear phase (iii) a phase in which uptake decreases. Phase (i) and (ii) may correspond to the stage I and II of the cell wall penetration and adsorption, and the third phase may correspond to the active uptake, which requires metabolic energy.

Allen (1964) has provided much information on these various phases of uptake of metallic ions by apple leaves. He recognises four phases of uptake of copper by apple leaves. His results confirm that in phase (i) and (ii), the process involved is only adsorption. In phase (iii) there is decrease in uptake, which corresponds to active uptake requiring metabolic energy. In phase (iv), there is again increase in the uptake and

material from within the leaf is simultaneously released into the solution due to the breakage of some barrier to diffusion.

Finally, after the incorporation of the substances into the protoplasts of the epidermal cells, a translocation of the absorbed materials to the other areas of the leaves or to other plant parts takes place regularly.

3.2. Factors affecting penetration and movement

3.2.1. Plant factors

3.2.1.1. Physio-chemical nature of the plant cuticle

Plant species differ in the physio-chemical nature of the cuticle. Environmental factors play an important role in the structure and composition of the cuticle. For example, the cuticle, is generally thicker on leaves which have developed in bright sunlight than which developed in shade, and the wax deposition in the former has been found to be greater than in the latter (Skoss, 1955). The point is that in the case of sun grown plants, there is high rate of transpiration and hence plants go through a period of water stress, which causes heavier cutinization of leaves.

Crafts and Foy (1962) state that developmentally the cuticle advances from a thin hydrophobic covering of young stems and leaves to a considerably thicker layer at leaf maturity. With age the cuticle oxidizes and becomes less permeable. But weathering, cracking, insect punctures and

abrasion may deteriorate the cuticle and make it imperfect as a layer.

Silva Fernandes (1965) observed that the extent of cuticle thickness and wax deposition has an important effect in the penetration. No penetration of copper solutions from copper acetate or sulphate or mercury from solution of phenylmercuric acetate occurred through astomatous membranes containing 0.1 mg/cm^2 or more of cutin. Wax offered an important barrier for the penetration of mercury through the membrane, but the effect of the wax depended on its composition. Penetration of mercury increased by an increase in the percentage of esters in the wax.

Crafts and Foy (1962) have mentioned that both inward and outward movement of aqueous solutions is restricted by the heavy and more or less continuous deposits of cuticular wax. The irregular surface wax deposits influence the wetting and contact angle between droplet of solution and leaf surface.

The contact angle between water and the waxy component will be higher than between water and the cutin component i.e. water wets cutin better than wax. This is only because of binding capacity of polar groups for water through hydrogen bonds, while hydrocarbon chains only attract water through the much weaker van der Waal's forces. (van Overbeek, 1956). Since plant species differ in the amount and pattern of depo-

sition of cutin components, they also differ in wettability and this may affect penetration.

3.2.1.2. Age of the leaf

There is an almost universal agreement that the younger leaves show greater absorption than the older ones. The nutrients include urea, Cain (1956); phosphorus, Fisher and Walker (1955); Koontz and Biddulph (1957, and Thorne (1958); Magnesium, Oland and Opland (1956) and Zinc, Wallihan and Heymann-Herschberg (1956). The reason for the greater absorption by the younger leaves may be thinner cuticles and less wax deposition. In advancing age leaf cuticles become thicker, and thereby may inhibit penetration. But the deposition of cuticle as reported by Skoss (1955) takes place only to a certain stage of morphological maturity, beyond that no further deposition occurs.

3.2.1.3. Leaf surfaces and morphology

Horizontal, hairy, pubescent leaves retain more spray than the vertical and smooth leaves (Ennis et al., 1952); and this results in greater absorption in the former type of leaves than in the latter. Upper and lower surfaces of leaves also exhibit different rate of absorption. Usually the lower surfaces are more penetrable than the upper surfaces to a large number of substances including herbicides as well as nutrients (Went and Carter, 1948; Fogg, 1948; Guest and

Chapman, 1949; Cook and Boynton, 1952; Volk and McAuliffe, 1954; Bennett and Thomas, 1954; Gustafson, 1956; Cain, 1956; Gustafson and Schlessinger, 1956).

This is perhaps due to the presence of thinner cuticle, numerous stomata, prominent veins and roughness of the lower surface of the leaves. However, species differ as to which surface functions most in absorption (Gustafson, 1957).

3.2.1.4. Mineral status of the leaf

Apple leaves having higher status of nitrogen have shown greater absorption of urea, Cook and Boynton (1952), magnesium, Forshey (1959; 1963) than the leaves of lower status of nitrogen. This increase in absorption has been partly attributed to the difference in growth of the plants because in above studies, plants grown in high nitrogen conditions produced rapid and quicker growth of the plants and leaves were comparatively larger and greener than the low nitrogen plants. Under these conditions the cuticle might be expected to be thinner than on the slower growing smaller leaves of low nitrogen trees and thus, more easily penetrable.

On the other hand, Wittwer and Teubner (1959) state that plants deficient in phosphorus absorb foliar applied P^{32} more rapidly than those grown in phosphorus rich media. However, the results of Koontz and Biddulph (1957) indicate that plants grown in rich phosphorus media translocate more phos-

phorus than those grown in phosphorus deficient media. However, the results of Koontz and Biddulph (1957) indicate that, plants grown in rich phosphorus media translocate more phosphorus than those grown in phosphorus deficient media.

3.2.2. External factors

3.2.2.1. Light

Light may affect the absorption both directly or indirectly. It may favour the absorption directly by stimulating the opening of stomata or indirectly by supplying energy through photosynthesis. Light may also affect the structure of leaves and the condition of stomata and thereby may affect absorption. The fact that light intensity and quality improve the rate of absorption of various substances has been demonstrated by several workers. (Sargent and Blackman, 1962; 1965, Kamimura and Goodman, 1964; Middleton and Sanderson, 1965).

Middleton and Sanderson (1965) state that the effects of light on absorption may not simply be related to stomatal opening or the transport of the carbohydrate. They may be caused in part by effects on the formation of carbohydrate. Also, light may affect both permeability of the cytoplasm and active transport processes.

3.2.2.2. Temperature

Currier and Dybing (1959) state that warm temperatures, if not excessive enhance penetration by affecting physio-chemical processes (increased rate of diffusion, lowered viscosity etc.), physiological factors (acceleration of photosynthesis, phloem translocation, accumulation processes, protoplasmic streaming and growth).

Teubner et al. (1957) found increased mineral absorption and transport by bean and tomato leaves when the temperature was increased from 50°F to 70°F. With increasing temperature the penetration of 2, 4-D (Barrier and Loomis, 1957; Rice, 1948) and NAA (Harley et al., 1957) also increased.

It has been pointed out by van Overbeck (1956) that the penetration through the layers of oriented fatty molecules is highly temperature dependent. If fatty molecules oriented in layers as micelles are in the solid state they have a lower permeability. In the liquid state they have a higher permeability; when totally disorganised, as at lethal temperatures, such layers can no longer serve as permeation barriers. Since this temperature effect is on the barrier itself, the principles stated by van Overbeek (1956) must hold for any material, regulator or otherwise, penetrating through the plasmamembrane or any other structure composed of fatty molecules in micellar orientation.

3.2.2.3. Humidity

A relatively high humidity has increased the penetration of a number of foliage applied substances. Currier and Dybing (1959) have stated the effects of relatively high humidity on penetration. It delays drying of the spray deposits, prevents water stress in the plant and may increase cuticular permeability.

Increased hydration of cutin martix, according to van Overbeck (1956), may spread the wax components further apart; because the wax components are not elastic as is the spongy cutin framework. This, in turn, will increase the permeability of the cuticle. High turgidity of epidermal cells and other underlying tissues would have a similar result. Conversely, lower water content of cutin and underlying tissue will cause shrinkage, and the wax units will be pulled close together and this will decrease the cuticle permeability. This proposed "valve system" has great significance for the water balance of the leaf. When the cuticle is dry, the valve effect restricts water loss; when the cuticle is wet, the valve system allows water uptake.

Similarly, various substances are known to penetrate the cuticle most efficiently in saturated atmosphere. For example, the penetration of maleic hydrazide has been increased in high humidity (Zukel et al., 1956) and the penetration of phenoxy-acetic acid has stopped on crystallization in a spray droplet

(Rice, 1948; Holly, 1956).

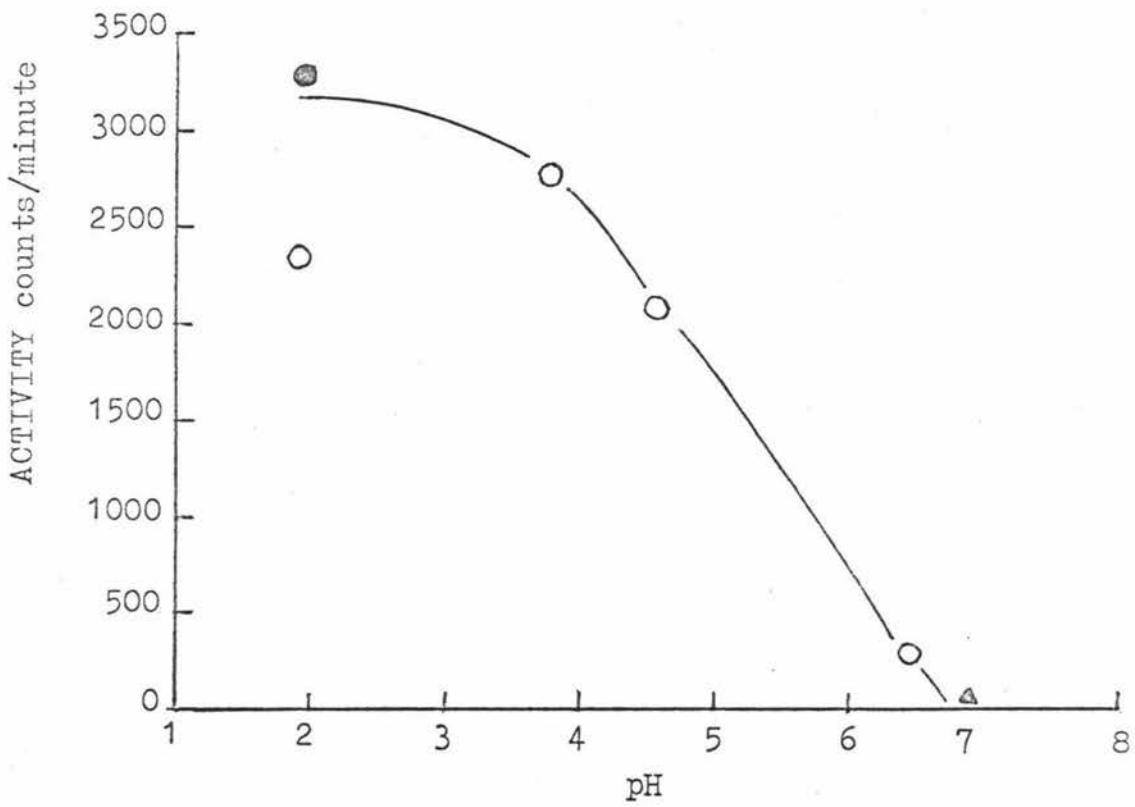
High humidity also has significantly increased the uptake of strontium - 89 and caesium 137 than low humidity (Middleton and Sanderson, 1965). However, no effect of humidity has been found on the uptake of sugar by tomato leaves (Went and Carter, 1948).

3.2.2.4. pH of the spray solutions

The effects of solution pH on permeability and penetration are complicated by the facts that whether the results are caused by effects on degree of dissociation of solutes, or whether the changes in permeability are brought about by the membrane itself. In cases, where the latter influence may be more prominent, there is still the question of whether the effect is due to hydration of the plasma-membrane or to a hydrolysis effects on membrane components.

The results of Cook and Boynton (1952) suggest that the per cent urea absorbed by apple leaf at pH 5.6 is greater than at pH 8. Similarly a pH of 2 to 3 as compared to higher pH of applied phosphate solution facilitated more rapid uptake by leaves (Silberstein and Wittwer, 1951; Swanson and Whitney, 1953; Fisher and Walker, 1955). As shown in Fig. 2 the amount of P^{32} translocated from the blade of a bean plant is a function of the pH of the applied drop and the amount absorbed and translocated is greater at low pH.

Fig.2. The level of P^{32} activity found in the petiole following a 4-hr. period of translocation from the blade as a function of pH of the applied solution. Each point an average of at least 3 plants. Data represented by the symbols O, ●, and ▲ taken from separate experiments (Swanson and Whitney, 1953).



The role of pH in promoting absorption has been attributed to two factors, the effect of high acidity in suppressing the dissociation of phosphoric acid and a possible direct effect of pH on the permeability of epidermal and subjacent tissues (Swanson and Whitney, 1953).

In case of divalent ions (Ca^{++}, Mg^{++}) the effects of pH on relative absorption does not appear to have been elaborately studied. More investigations in this line are needed.

3.2.2.5. Surfactants

Surfactants are used to increase the effectiveness of spray solutions applied to the foliage. According to Currier and Dybing (1959), the response to surfactants may be due to one or more of the following:

(a) improving coverage;
(b) removing air films between spray and leaf surface;
(c) reducing interfacial tension between relatively polar and apolar submicroscopic regions of the cuticle;
(d) enducing stomatal entry;
(e) increasing the permeability of the plasma-membrane through stimulation or incipient toxicity;
(f) facilitating cell-wall movement in the region of the wall cytoplasm interface;
(g) acting as a cosolvent;
(h) interacting directly with chemical some manner;
(i) acting as humectants,

The response of surfactants, however, vary depending on the nature of solute to be absorbed and plant species. Triton x-100 for example, depressed magnesium absorption and improved phosphorus absorption in apple (Fisher and Walker, 1955). Tween 20 was ineffective in phosphorus absorption in bean (Teubernal et al., 1957), but increased urea absorption in apple (Cook and Boynton, 1952).

On the other hand, concentration of surfactant to be used has influenced the penetration of solutes applied to the foliage. In herbicide studies higher concentration of surfactants has increased the herbicidal activities (Freed and Montgomery, 1958; McWhorter, 1963; Foy and Smith, 1965). This has been attributed to the increased penetration of herbicides due to higher concentration of surfactants. But the concentration of surfactant increasing to a certain stage only has shown progressively increasing penetration; beyond this there may not be an additional effect. One reason may be micelle formation by the surfactant molecules when the surfactant concentration exceeds a critical level in an aqueous system. This critical micelle concentration (cmc) is associated with abrupt changes in many characteristic properties of the surfactant (Furmidge, 1959; and Jansen et al., 1961). These changes in properties may affect such phenomena as penetration, translocation and biological activites of the growth regulators.

Also, it has been recognised that toxic substances whether additives or herbicides, if used in higher concentration, can

block phloem translocation (Leonard and Crafts, 1956; and Leonard, 1958).

Lowering of surface tension has resulted in greater absorption (Cook and Boynton, 1952; Dorschner and Buchholtz, 1956; Freed and Montgomery, 1958; Hughes and Freed, 1961). This may be due to increased wettability of the leaves. However, the results of Freed and Montgomery (1958), Hughes and Freed (1961), Foy and Smith (1965) indicate that the lowering of surface tension has not always enhanced the penetration, rather interaction of surfactant with the species of molecule to be absorbed is more important. Wetting seems to be important for penetration, but on some leaves (B. vulgaris and Bean) spray load can be reduced by the use of wetting agents (Blackman, 1952; Koonz and Biddulph, 1957). The thinner adherent films also dry more quickly (Koontz and Biddulph, 1957).

3.2.2.6. Humectants

Humectants increase penetration by preventing the drying of spray deposits and also by increasing cuticular permeability (Holly, 1956; Sivadjian, 1956; and Gray, 1956). Use of glycerine has increased the absorption of phosphorus by apple leaf; (Fisher and Walker, 1955) bean leaf, (Koonz and Biddulph, 1957); and has increased the absorption of chlorophenoxyacetic acids by leaves of sun flower and oats (Holly, 1956). The use of glycerine also has increased the herbicidal activities of

sodium arsenate, ammonium sulfamate and sodium trichloro phenoxyacetate. The glycerine used at the concentration of 2.5% gave much kill as 10%, while 1% glycerine gave somewhat poor result, but was better than without glycerine.

Use of carbowax has increased the penetration of 2, 4-D (Rice, 1948; Ennis and Boyd, 1946), and molasses of DNOC (Fogg, 1948).

Gray (1956) found glycerine an excellent agent in increasing the penetration of streptomycin in the leaves of a number of crop plants (bean, tomato, pepper and tobacco). Sorbitol, diethylene glycol, and other polyhydroxy alcohols were also effective in increasing the concentration of streptomycin in bean leaves, but they were not as effective as glycerine. Glycerine also increased the effectiveness of streptomycin in controlling common blight disease of beans in the greenhouse. Absorption increased with time from sprays containing glycerine, while there was little increase in absorption with time from sprays containing no glycerine. This indicated that glycerine kept streptomycin in solution for a longer period of time and hence resulted in greater absorption, while streptomycin solution without glycerin dried quickly and resulted in less absorption.

Gray (1956) has explained the possible mechanism by which glycerine has increased the absorption of streptomycin. According to him, the increased absorption of streptomycin

is due to the capability of glycerine in keeping streptomycin in the solution and bringing in close contact with leaf surface for a longer period of time and also by increasing the cuticular permeability.

About the concentration of glycerine, use of 1% glycerine resulted a 10 fold increase in absorption of streptotrycin in a 24 hour absorption period, the use of 0.5% glycerine caused a 6.1 fold increase and 0.25% glycerine caused a 4 fold increase.

3.2.2.7. Solute characteristics

Generally, plants membranes are more permeable to non-polar compounds than to the polar compounds. Inorganic compounds, acids and bases and salts are characteristically polar. Among organic compounds, those having "polar groups" are polar in behaviour. These polar groups include - OH, - COOH, - NH_2, - CHO, - CN, - $CONH_2$ - SH, and NCS and also any group that contains double or triple bonds. The alkyl groups - CH_3, - C_2H_5, etc. are typically non-polar compounds and of course the hydrocarbons are among the most non-polar compounds. Membrane permeability is also closely related to polarity that the substitution of a non-polar group for a polar group within the molecule may greatly increase the rate of penetration, although the molecular size may be then increased (Curtis and Clark, 1950).

The penetration of inorganic ions varies with different tissues and depends on the particular salts used. In general, however, monovalent cation and anions such as K^+, Na^+, Br^-, and Cl^- penetrate faster than divalent ones such as Sr^{++}, Ca^{++}, and SO_4^{--}, and the latter more rapidly than trivalent ones. With acid and bases, the strongly dissociated HCl and KOH penetrate slowly, while weak ones such as H_2CO_3 and NH_4OH move into the cell rather rapidly.

3.3. The citrus leaf

An explanation of the morphological and anatomical considerations of citrus leaves is necessary to understand the penetration of spray material into them. Webber and Batchelor (1946) have described the orange and lemon leaf as a compound, unifoliate type, oval to oblong in shape, and remaining on the tree for two seasons or more, while the size of the orange leaf at maturity may be 3 to 5 cm in breadth and 7 to 12 cm in lenght.

Scott et al. (1948) have given a detail account of the development of the Valencia orange leaf. Growth in blade length and width has been found to start during the spring flush growth and continued up to 130 days. During this time, two distinct phases of increased growth have been observed. The first one after 13 days, then there is a short period of decrease in rate of leaf expansion, which is of 6 days. Then a second period of rapid leaf expansion starts and continues up

to 128 days. These two phses of rapid expansion coincide with the spring and fall growth flushes. It has been observed that the rate of increase in blade lenght is greater than the rate of increase in blade width. The rate of leaf thickness during the first 65 days is relatively more rapid than the rate of increase in either blade length or width. Full thickness is attained in 80 days.

Anatomically, the general structure of the lemon and orange leaf, according to Webber and Batchelor (1946), is similar to that of dicotyledonous plants. The outer walls of the epidermal cells are cutinized and coated with wax (Turrell, 1947), which is secreted through a minute cannals similar to those found in fruit rind. Often several layers of mesophyll cells are noticed. Palisade cells generally develop large oil glands and calcium oxlate crystals. Halma (1929) observed that the ratio of the thickness of palisade tissue to the thickness of the leaf is rather constant for any variety and species of citrus. However, the ratio differs from species to species. For example, leaves of lemon groups have 20% higher value than that of orange leaves.

Stomata have been found mostly on the lower surfaces of the leaf and occasionally on the upper surfaces, especially over the major veins. Stomata develop in the very early stage of leaf growth and the production of stomata stops when the young leaves attain $\frac{1}{4}$th of their final size (Reed and Hirano, 1931).

The density of stomata has been found to be dependent on climatic conditions. Citrus species or varieties grown in hot and arid regions have greater number of stomata in the leaves than those grown in cool regions (Bahgat, 1923), according to Webber and Batchelor (1946).

Hirano (1931) has made an extensive study of the density of stomata in the leaves of various species of citrus and related genera. His observations indicate that the citrus species growing in the tropics except grapefruit and lemon have more than 500 stomata/sq.mm, while those outside the tropics have lower density of stomata/sq.mm., except c. aurantofolia and c. cambrovioxora. Reed and Hirano (1931), on the other hand, examined the distribution of stomata on the leaf surfaces of lemons and oranges and found the greatest density in the middle of the blade and the least at the base. Also, the density of stomata was greatest on the leaves near the apex and the least on the leaves situated 1/3rd of the distance from the base to the apex.

Turrell (1947) has studied the detailed structure of citrus stomata, their composition and pore size in relation to the penetration of liquids. His drawing indicate that the resinous stomatal plug nearly fills the cutinized stomatal chamber and the space remaining between the plug and the chamber wall is about 0.5 µ. At the bottom of the chamber, a stomatal tube connects it to the substomatal chamber. The measurement of the

pore size (Length and breadth) indicate that the citrus species differ in pore size (Table I). Also individual leaves, depending on their size, differ in pore size. For example, a middle size Eureka lemon has a greater pore area than that of a small size Eureka lemon (Table I)

Turrell (1947) has pointed out that from the considerations of contact angles and capillarity, there is little or no penetration of rain water through the stomata of citrus leaf. However, oil sprays, and the sprays of inorganic salts containing suitable wetting agents may penetrate readily through stomata.

Table I. Stomatal pore sizes of citrus leaves (Turrell, 1947).

Kind of leaf	Number of pores measured	Leaf Size	Pore length (/u)			Pore width (/u)			Average pore perimeter* (/u)	Average pore area* (/u^2)
			Average	Maximum	Minimum	Average	Maximum	Minimum		
Eureka lemon	50	Medium	7.04±1.73+	12.4	3.5	3.08±.97+	5.9	1.2	17.1	17.0
Eureka lemon	50	Small	7.13	12.4	2.4	2.07	5.9	1.2	16.5	11.6
Marsh grape fruit	50	Medium	8.06±1.59	12.0	4.7	2.03±.50	3.5	1.2	18.5	13.2
Valencia orange	70	Medium	8.91±3.10	14.2	5.3	3.80±.98	6.5	1.8	21.5	26.6
Washington Navel orange	50	Medium	4.78±0.86	8.3	3.5	2.32±.82	4.7	1.2	11.8	8.7

* Considering the stomatal pore as an ellipse, + standard error

3.4. Uptake of magnesium by leaves of citrus and other crops

3.4.1. Glass house and Laboratory studies

3.4.1.1. The effect of different magnesium salts on magnesium absorption

Fisher and Walker (1955) grew one year-budded McIntosh apple plant in a glasshouse. The plants were grown in pots in a sand culture. Only one bud was grown to obtain fairly large size leaves. A single spray of a known amount of magnesium compounds on the lower surfaces of the leaves was done. After a given interval of time, the leaves were removed from the tree, washed and the wash water was analysed. The amount not received was considered to be absorbed by the leaf. The data was presented as apparent percentage absorption, which designated the percentage of applied material not recovered by the wash solution and assumed to have been absorbed by the leaf.

Among five magnesium compounds tested, $MgCl_2.6H_2O$, $(CH_3COO)_2.MgxH_2O$ or $Mg(NO_3)_2.6H_2O$ showed high rate of absorption than $MgSO_4.7H_2O$ and $Mg\ H_4(PO_4)_2$ (Table 2). Magnesium absorption from $Mg\ H_4(PO)_2$ tended to be slower than $MgSO_4.7H_2O$.

Table 2. The effect of different magnesium salts on magnesium absorption. All sprays have the magnesium equivalent of a 5% $MgSO_4.7H_2O$ solution (apparent % magnesium absorption 24 hours after application). Fisher and Walker (1955).

	Replications		Average
	I	II	
$MgSO_4.7H_2O$	9.1	7.7	8.4
$MgCl_2.6H_2O$	63.9	69.3	66.6
$(CH_3COO)_2.MgxH_2O$	29.8	34.2	32.0
$Mg(NO_3)_2.6H_2O$	62.3	79.7	71.0[a]
$Mg\ H_4\ (PO_4)_2$	0.0	7.0	3.5
[a] leaf injury			

Hagler (1957), in order to test the uptake of different magnesium compounds, used Mucadine grapes, which were grown in a glasshouse. Plants were sprayed thoroughly to cover both the sides of the leaf. Samples were taken 20 hours after spraying and again one month later. Sampled leaves were washed in a one per cent hydrochloric acid solution, rinsed with distilled water and analysed for magnesium by the Titan Yellow method of Peach and English (1944). His results indicate that magnesium uptake from $Mg(NO_3)_2.6H_2O$ and $MgCl_2.6H_2O$ were higher than $MgSO_4.7H_2O$ and other sources of magnesium. After 20 hours of spraying the concentration of magnesium in the leaves of the

plants sprayed with $Mg(NO_3)_2.6H_2O$ and $MgCl_2.6H_2O$ was 0.51 and 0.54% (dry wt. basis) respectively and that of $MgSO_4.7H_2O$ was only 0.10.

Thus, the results of Fisher and Walker (1955), and Hagler (1957) indicate that the rate of uptake of magnesium from $Mg(NO_3)_2.6H_2O$ and $MgCl_2.6H_2O$ was higher than that of $MgSO_4.7H_2O$.

3.4.1.2. The effect of different spreaders and hygroscopic agents on magnesium absorption.

Fisher and Walker (1955) used apple plants grown in a manner described in the section 3.4.1.1. Spraying, sampling and analysis of magnesium techniques were the same as mentioned in that section. Plants were sprayed with a 5% $MgSO_4.7H_2O$ solution in which Tween 85, Triton X100 and Tergitol were included. The results given in Table 3 indicate that the Triton X100 almost completely prevented magnesium absorption, while the Tween 85 and Tergitol both resulted in about 25 per cent apparent absorption in a 24 hour period.

Table 3. The effect of three different spreaders on magnesium absorption from a 5 per cent $MgSO_4.7H_2O$ spray application (apparent percentage of magnesium absorption). Fisher and Walker (1955).

Treatment	Replications						Average
	1	2	3	4	5	6	
Tween 85	34.5	33.4	28.4	25.8	14.2	14.1	25.1
TritonX100	1.0	7.4	3.0	1.1	0.0	0.0	2.1
Tergitol	29.5	34.4	19.9	25.5	19.7	19.3	24.7

On the other hand, the inclusion of hygroscopic agents (glycerine, carbowax, or methyl cellosolve to a mixture containing Tween 85 and $MgSO_4.7H_2O$ did not increase the magnesium absorption (Table 4).

Thus, it seems that the hygroscopic agents had no effect on absorption if a spray contained Tween 85. But there is quite a possibility that these hygroscopic agents might have had effect in absorption if Tween 85 could not have been included in the spray mixture.

Table 4. The effect of glycerine, carbowax, and methyl cellosolve on magnesium absorption from a 5 per cent $MgSO_4.7H_2O$ spray application (apparent percentage of magnesium absorption). Fisher and Walker (1955).

Treatment	Average
Tween 85, 1 drop/50ml (check)	20.4 [a]
Carbowax 1%	
Methyl cellosolve 1%	16.6
Glycerine 2%	21.1

[a] Differences between treatments not significant.

3.4.1.3. The rate of magnesium absorption.

In this study too, apple plants grown in a manner described in the section 3.4.1.1. were used by Fisher and Walker (1955). Spraying, sampling and magnesium analysis techniques were the same as mentioned in that section. Within an hour after application, 20 per cent of magnesium from $MgSO_4.7H_2O$, was absorbed by leaves (Table 5). Absorption was very slow after the first hour and there was no apparent increase in absorption up to the six day period (Fig. 3). Inclusion of urea in a 5 per cent $MgSO_4.7H_2O$ spray had no effect on magnesium absorption (Table 5).

Fig. 3. The rate of absorption of nitrogen, phosporus, and magnesium from sprays applied to the lower surface of McIntosh apple leaves(Fisher and Walker,1955).

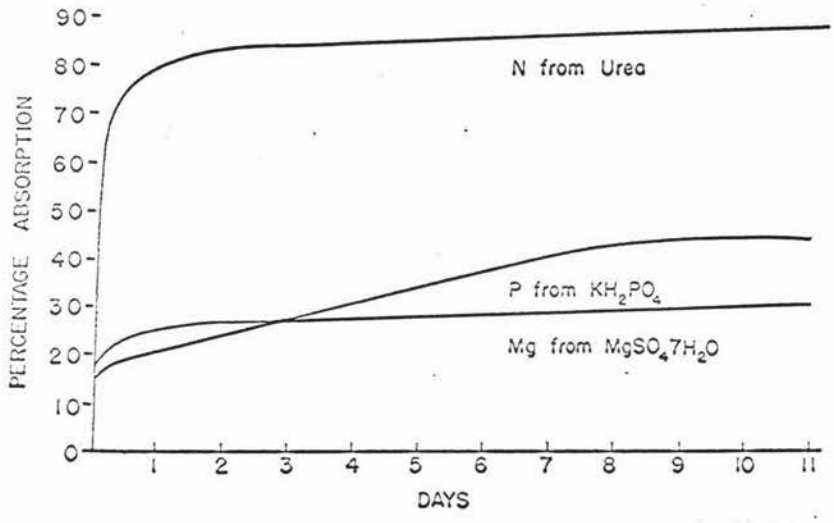


Table 5. The rate of magnesium absorption over a 12 day period (apparent percentage of magnesium absorption). Fisher and Walker (1955).

Absorption time	5% $MgSO_4.7H_2O$				5% $MgSO_4.7H_2O$ + 66% urea			
	I	II	III	Ave.	I	II	III	Ave.
1 hour	21.0	19.6	19.0	19.2	17.5	17.8	16.5	17.3
5 hours	19.0	19.6	25.5	21.4	20.5	20.5	24.5	21.8
8 hours	23.6	22.5	27.5	24.5	20.5	17.0	18.3	18.6
28 hours	19.4	17.8	21.5	19.6	19.4	20.5	22.9	20.9
6 days	14.7	6.6	11.1	10.8	18.3	11.7	-[a]	-
12 days	22.7	30.0	44.4	32.6	33.6	43.4	-	-

[a]One of the 5 leaves was broken off before the desired time interval.

3.4.1.4. The effect of spraying at different hours of the day

Oland and Opland (1956), in order to investigate the effect of spraying at different hours of the day, used the Norwegian apple variety Torstein. Plants were grown partly in the nursery and partly in greenhouse, when it was necessary to avoid rain on the plants. Each single leaf used in the experiments was sprayed separately with a hand atomiser. Both upper and lower surfaces of the leaves were sprayed with a 5 per cent $MgSO_4.7H_2O$ solution. Before analysis, the leaves were wiped with a dot of cotton wool repeatedly wetted, and rinsed in distilled water. Then leaves were dried at 75°C to a constant wt.

and analysed for magnesium by titration, used by Cheng and Bray (1951).

Spraying had been done either in the early afternoon or in the evening. No significant difference in absorption of magnesium by the leaves was noticed when $MgSO_4.7H_2O$ was sprayed during the day, but the evening spraying showed a large and highly significant uptake (Table 6).

The large uptake of magnesium by apple leaves from evening sprays led Oland and Opland (1956 to conclude that ion exchange as a mechanism is involved in the absorption; therefore the diurnal shift in acid production might influence magnesium uptake. Internal release of hydrogen ions for exchange would be most likely to occur after the probable shift in the production of organic acids during the evening.

Besides the hydrogen ion exchange hypothesis of Oland and Opland (1956) for the large uptake of magnesium from evening sprays, the high humidity during the hours that followed may also be responsible for large uptake of magnesium.

Table 6. The effect of $MgSO_4.7H_2O$ solution applied at different hours during the day (Oland and Opland, 1956).

Time of spraying	Control	2.30 P.M.	9.00 P.M.	L.S.D.
Absorption time hours	0	23	23	
Mg content, m.e/100g dry matter	11.1	13.2	24.3	4.38

3.4.1.5. The effect of nitrogen level

Plants adequately supplied with nitrogen absorb more magnesium both from soil and foliage application than those supplied with less nitrogen. The work of Forshey (1963) would illustrate this point. A sand culture experiment with 2N and 3 Mg treatments in factorial combination was conducted. High and low nitrogen (1N and $\frac{1}{8}$ N respectively) and Mg as none, foliage, and soil treatments (OMg, FMg, and SMg, respectively) were applied as follows.

Symbol	Nitrogen	Magnesium
1/8 N - OMg	2.5 me/liter	None
1/8 - F Mg	2.5 me/ "	5 Epsom salts sprays
1/8N - SMg	2.5 me/ "	Same amount in the culture solution as applied to the foliage of the FMg trees.
1 N - 0 Mg	20 me/liter	None
1 N - FMg	20 me/ "	5 Epsom salt sprays
1 N - SMg	20 me/ "	Same amount in the culture solution as applied to the foliage of the FMg trees.

One year-old McIntosh apple trees on EM XIII root stocks were planted in 4 - gallon plastic pails in silica sand. All shoots were removed after planting and the trunks were cut to uniform height. Each treatment consisted of six replications and the trees were arranged in randomized block design on the benches of an unheated glasshouse.

Trees were fed twice daily with culture solution containing all essential elements except magnesium. Spraying of magnesium sulphate was not done till sufficient leaf area had developed to make spraying practical. Trees were sprayed with a 1.5% $MgSO_4.7H_2O$ solution once a week and altogether 5 sprays were done. After each application the average volume of the $MgSO_4.7H_2O$ applied per tree was calculated and corrected for run-off and the same volume of $MgSO_4.7H_2O$ solution was added to the culture solution of each of the SMg trees. Sampled leaves were washed with distilled water containing mild detergent and analysed for N, Mg, K, P and Ca.

Results indicate that leaves of high nitrogen trees (1 N trees), on an average, contained 2.14% of N (dry wt. basis) while low nitrogen trees ($\frac{1}{8}$ M trees) only 1.36% of N (dry wt. basis). In total, high nitrogen trees contained more than three times as much as the low nitrogen trees and this

difference in nitrogen content resulted in difference in dry weight of 60.8%. Leaves and shoots were found to be differed greatly in growth and the weight of both in high nitrogen treatment was three times that of those in low nitrogen treatment.

This difference in nitrogen status had effect on magnesium absorption. Low nitrogen trees absorbed very little magnesium from the culture solution and did not absorb a measureable amount from the Epsom salt sprays. While high nitrogen trees absorbed considerable magnesium from both soil and foliage applications.

The increase in magnesium absorption by high nitrogen trees has been attributed to the increased growth of high nitrogen trees. But Forshey (1963) indicates that, it does not give full answer to the difference because the dry weight of the high nitrogen trees was 67.1% greater than that of the $\frac{1}{8}$N trees, but the increase in magnesium content was 111%. However, Forshey (1963) concluded that the greater response of magnesium sprays by high nitrogen trees may be due to a combination of three factors.

First and foremost, the high nitrogen trees absorbed more magnesium from sprays than low nitrogen. Second, the low nitrogen trees were incapable of absorbing considerable amount of magnesium from sprays, and thirdly, the greater absorption of magnesium by high nitrogen plants might be attributed to the

increased concentration of magnesium in the leaves.

3.4.1.6. The effect of magnesium level

In general, low supply of magnesium in culture solution results in low concentration of magnesium in the foliage (Ford, 1966; 1968 and Smith et al., 1954). But increasing concentration of magnesium in culture solution increases magnesium uptake to a certain stage only; after that the curve obtained is less steep. This can be seen by citing Ford's work (1966). Ford (1966) grew one year-rooted shoots of apple M.VII root stocks for a single season by supplying 4 levels of magnesium (<3 ppm, 10 ppm, 45ppm and 135 ppm). These were designated as a severe deficiency level ($Mg_{(0)}$) a borderline deficiency level ($Mg_{(1)}$) and adequate level ($Mg_{(2)}$) and a high level ($Mg_{(3)}$) respectively. The treatments affected growth form and also mineral composition of the trees. $Mg_{(0)}$ plants developed severe magnesium deficiency and the growth was depressed. $Mg_{(1)}$ plants also showed depression in growth, but the magnesium deficiency was milder. $Mg_{(2)}$ plants had normal growth and foliage. $Mg_{(3)}$ plants had darker and greener foliage than $Mg_{(2)}$ plants. No symptoms of toxicity developed in $Mg_{(3)}$ plants, but the mean dry matter accumulated by these plants was less than $Mg_{(2)}$ plants. The results indicate that magnesium uptake increased from $Mg_{(0)}$ to $Mg_{(2)}$ but above this level the gradient of magnesium uptake/in relation to supply was less steep and this led to the conclusion that its supply was no longer a limiting factor and an excess

uptake caused the reduction in growth at this high level of magnesium.

Ford (1967) studied the effect of different levels of soil magnesium on uptake of magnesium from foliage application. He grew one year-rooted shoots of M.VII apple root stock for a single season by spraying their roots continuously with nutrient solutions containing either < 3 ppm Mg ($Mg_{(0)}$) or 45 ppm ($Mg_{(2)}$) to give respectively very deficient or healthy plants. The new shoots of half the plants in each of these treatments were dipped periodically in a 2% solution of $MgSO_4.7H_2O$ plus non-ionic wetter. The $Mg_{(2)}$ plants absorbed more magnesium than ($Mg_{(0)}$ plants i.e. the magnesium in the leaves of $Mg_{(2)}$ plants was increased by approximately 0.25% of dry weight, while the concentration of magnesium in the leaves of $Mg_{(0)}$ plants was increased by 0.16% (Table 7).

This led to the conclusion that the $Mg_{(0)}$ plants were either less capable of absorbing magnesium than those whose roots were well supplied with magnesium or they translocated more of the absorbed magnesium.

Table 7. Mean concentration of elements (% dry wt.) in leaves (Ford, 1967).

Element		$Mg_{(0)}$		$Mg_{(2)}$		Sig. diff.
		Undipped	Dipped	Undipped	Dipped	
Per cent	Mg	0.09	0.25	0.27	0.52	0.06
"	N	3.36	3.89	3.68	3.81	NS
"	K	1.90	1.57	1.64	1.38	NS
"	Ca	0.67	0.71	1.06	0.85	NS
ppm	Fe	186	129	120.0	98	68
"	Mn	47.9	58.0	48.1	42.7	NS
"	Cu	6.90	54.45	4.50	4.50	1.65

3.4.2. Plant responses to field conditions

3.4.2.1. Responses to citrus

In citrus, magnesium has been applied both by soil and foliage applications and responses are variable.

In acid soils of Florida, soil applications of dolomite and magnesium supohate were effective in correcting magnesium deficiency of citrus (Camp, 1947; Reitz et al., 1954). But in California, the use of dolomite in stony sandy loam soils was not effective and did not increase the magnesium in leaves of Valencia orange, while magnesium sulphate was effective and increased the concentration of magnesium in the leaves (Embleton et al., 1956). McColloch et al., (1957) was able

to increase magnesium in the leaves by soil application of magnesium sulphate to the light textured soils in California. However, in heavier textured soil (higher exchange capacity), even massive application of Epsom salts (195 lbs per tree) was not effective in increasing magnesium in the leaves adequately. Haas (1948) obtained only temporary or no effect by the application of $MgSO_4.7H_2O$ to the light textured soil in California.

In South Africa, soil application of dolomite and magnesium sulphate produced very little response (de Villiers, 1942).

Coming down to the foliage application of magnesium salts in Citrus, Epsom salt spray has only little effect in correcting magnesium deficiency in citrus (Haas, 1948). According to Embleton and Jones (1959), the results obtained by Heymann-Herschberg in Palestine and Trait in Florida were also the same as Haas (1948). However, in South Africa, a 2 per cent suspension of finely ground burnt dolomite (calcium magnesium carbonate) was effective as a spray (de Villiers, 1942). In South Africa, zinc and magnesium deficiency often occur in the same orchard. In such cases, zinc and magnesium are applied in the same spray (5 to 10 lbs of $ZnSO_4$ and 20 lbs of dolomite/ 100 gals of water).

On the other hand, magnesium nitrate sprays have been quite effective in increasing magnesium concentration in the

leaves and reducing visual symptoms of deficiency. Two sprays of magnesium sulphate at 10 lbs plus calcium carbonate 10 lbs/100 gals, increased leaf magnesium in mature Valencia orange leaves from 0.14 to 0.19 per cent of dry weight (Strauss, 1963), and when applied annually for 2 to 3 years at 600-1000 gals/acre when the spring growth was 2/3rds fully expanded, corrected magnesium deficiency (Beutal, 1964).

Embleton and Jones (1959) were able to correct magnesium deficiency of Valencia orange trees by $Mg(NO_3)_2.6H_2O$ spray applied at the rate of 10 lbs/100 gals of water, when the spring flush of growth had about 2/3rds expanded. They found substantial increase of magnesium in the leaves from such a spray (Table 8). Magnesium deficiency symptoms in leaves were practically removed in six months and leaves became a deeper green in colour. Magnesium chelate at the rate of 4 to 5 lbs/100 gals was not as effective as magnesium nitrate at 10 lbs/100 gals in increasing magnesium concentration in the leaves or in eliminating visual symptoms of magnesium deficiency.

Table 8. Effects of magnesium treatments on the concentration of magnesium in the leaves of Valencia orange (Embleton and Jones, 1959).

Treatment No.	Treatment	Mg as % dry wt. of leaves [a]		
		7/2/59	10/25/59	5/14/58
1	Check	0.18	0.20a[c]	0.16[a c]
2	$MgSO_4.7H_2O$ on soil	0.19	0.23b	0.16[a]
3	$Mg(NO_3)_2.6H_2O$ spray	0.20	0.33d	0.22c
4	Mg-chelate - $Mg(NO_3)_2.6H_2O$ spray	0.18	0.24bc	0.18[b]
5	Micronutrient - Mg chelate - $Mg(NO_3)_2.6H_2O$ spray	0.17	0.25c	0.20bc
6	Micronutrient spray	0.17	0.21a	0.15a
	Significance[d]	NS	**	**

a Each value is the mean from six four-tree plots

c Subscript letters a, b, c and d after mean values - indicate populations at the 1% level of probability. Mean values are statistically different if they do not have a common script letter after the values.

** Indicates statistical significance at the 1% level or higher.

3.4.2.2. Miscellaneous responses

Magnesium sprays have been applied to a number of fruit trees as well as vegetable crops in order to supply their magnesium requirements, and to reduce or prevent the magnesium deficiency. Experience with apple trees indicates that the Epsom salt ($MgSO_4.7H_2O$) sprays have been quite promising in increasing magnesium in the leaves and in reducing visual symptoms of deficiency (Boynton et al., 1943; Southwick and Shaw, 1944; Boynton, 1945; Chuka et al., 1945; Southwick and Smith, 1945; Woodbridge, 1955; Ford, 1964; Ford et al., 1965; and Nagai et al., 1966)

Weber (1966) working with a number of fruit and vegetable crops, was able to correct potassium induced magnesium deficiency in the fields by both foliage and soil application of magnesium sulphate. Apples, cherries, tomatoes, cucumbers, cauliflowers and broccolli had all been responsive to Epsom salt sprays.

Nichols (1948) prevented magnesium deficiency in tomatoes by five applications of two per cent Epsom salt sprays.

Scott and Scott (1951) were able to prevent magnesium deficiency in several Eastern Bunch grape varieties by 4% $MgSO_4.7H_2O$ sprays; while soil application of 2 lbs/tree of $MgSO_4.7H_2O$ was not effective. The concentration of magnesium in all the sprayed vines was higher and the soil application

had no effect on the concentration of magnesium in the leaves (Table 9).

Loft (1948; 1952), prevented late summer chlorosis of James and Scuppernong grape variety, by injecting $MgSO_4.7H_2O$ solution into the vines.

Bingham (1963) was not able to observe responsive effects on avocado trees, by the use of 1.2% magnesium nitrate sprays application; however, higher concentration i.e. 3.6 to 4.8% of $MgSO_4.H_2O$ sprays was effective.

Table 9. Magnesium concentration in grape leaves as affected by spray and soil application of $MgSO_4 \cdot 7H_2O$ (Scott and Scott, 1951).

Seletion No.	Treatment	Per cent magnesium in leaves (dry wt. basis)		
		July 29	Sept. 6	Oct. 4
US. 519 - 6	Check	0.09	0.19	0.07
	Mg spray	0.13	0.23	0.12
	Mg Soil	0.07	0.09	0.05
US. 519 - 10	Check	0.10	0.10	0.04
	Mg spray	0.05	0.17	0.10
	Mg soil	0.11	0.10	0.06
US. 4017-22	Check	0.24	0.30	0.23
	Mg spray	0.29	0.50	0.40
	Mg soil	0.23	0.26	0.26
US. 520 - 2	Check	0.13	0.10	0.07
	Mg spray	0.22	0.35	0.16
	Mg soil	0.17	0.16	0.09
US. 4032-16	Check	0.16	0.14	0.08
	Mg spray	0.15	0.28	0.30
	Mg soil	0.16	0.18	0.18
Mean 5 selec-	Check	0.14	0.17	0.10
tions	Mg spray	0.17	0.29	0.22
	Mg soil	0.15	0.6	0.13

L.S.D. 5% level between individual reading 0.13 between treatment means 0.06

3.4.2.3. The effect of nitrogen level.

Plants have been reported to absorb more magnesium both from soil and foliage application of magnesium salts if they are adequately supplied with nitrogen. Increasing the soil nitrogen level, even without addition of magnesium in any form, has resulted in increase of magnesium in the leaves of apple (Boynton and Compton, 1944; Cain and Boynton, 1948; Weeks et al., 1952, 1958 and Cain, 1953), of peach (Ritter, 1956) and of strawberry (Victor et al., 1967). While differential supply of nitrogen fertilizers in the soil along with magnesium salt resulted in greater uptake of magnesium by leaves of citrus plants, which were supplied with high nitrogen, and the growth of plants slightly increased from those supplied with low nitrogen (Reuther and Smith, 1950; 1952), Forshey (1959) observed that level of nitrogen in soil had no effect on the concentration of magnesium in the foliage of McIntosh apple trees that received no magnesium salt spray. However, in the treatments that received even one spray of Epsom salt, increasing nitrogen supply in the soil increased the concentration of magnesium in the foliage (Table 10).

3.4.2.4. The effect of magnesium level

Differential supply of magnesium fertilizer in soil has affected the uptake of magnesium in citrus. Plants highly supplied with magnesium fertilizers have also shown high concentration of magnesium in leaves (Reuther and Smith,

1950; 1952). Differential supply of dolomite, however, did not increase magnesium concentration of Valencia orange leaves, but Epsom salt did increase (Embleton et al., 1956).

Table 10. Nitrogen and mineral content of McIntosh apple leaves from trees receiving three level of nitrogen fertilization and three Epsom salts spray treatments in factorial combination (Forshey, 1959).

Treatments	% dry wt. basis				
	N	Mg	K	Ca	P
N_1Mg_1	1.76	0.12	1.41	0.70	0.21
N_2Mg_1	1.97	0.13	1.25	0.82	0.19
N_3Mg_1	2.30	0.13	1.22	0.71	0.18
N_1Mg_2	1.86	0.13	1.44	0.75	0.19
N_2Mg_2	2.12	0.17	1.30	0.78	0.19
N_3Mg_2	2.44	0.18	1.15	0.71	0.19
N_1Mg_3	1.90	0.15	1.34	0.72	0.20
N_2Mg_3	1.99	0.19	1.33	0.72	0.20
N_3Mg_3	2.33	0.22	1.26	0.72	0.20
L.S.D. 5%	0.18	0.02	NS	NS	NS
1%	0.27	0.03			

Symbols:-	N in lbs.	Epsom salt sprays
N_1Mg_1	0	0
N_2Mg_1	½	0
N_3Mg_1	1½	0
N_1Mg_2	0	1
N_2Mg_2	½	1
N_3Mg_2	1½	1
N_1Mg_3	0	3
N_2Mg_3	½	3
N_3Mg_3	1½	3

3.5. Magnesium mobility studies

Many workers support or claim magnesium mobility in plants, while others do not. Findings are contradictory. Phillis and Mason (1942) and Ford (1968) claim that frequent magnesium deficiency in loder leaves is due to redistribution of magnesium from older leaves to younger ones. Ford (1966) on the other hand, stated that external supply of magnesium affected redistribution of magnesium in apple plants. Magnesium moved from, or accumulated in, the lower leaves of the shoots according to the level of magnesium supplied to the plants. In deficient plants magnesium moved from lower leaves, while a high supply of magnesium led to an accumulation of magnesium especially by the lower or middle leaves. Increased external supply of magnesium caused pronounced effect on magnesium concentration in the leaves, stems and roots (Table 11)

The concentration of magnesium in the leaves was increased five fold, in the stems two fold and in the roots almost four fold. Forshey (1963) observed that nitrogen status of leaf not only affected the magnesium absorption, but also affected its distribution.

However, Ruck and Gregory (1955) reported that magnesium was not readily transported from the leaflets of rooted potato leaves to the tissues of a shoot arising from the bud subtended by each leaf. Neales (1958) could not find significant translocation of magnesium from the older leaflets of clover to the younger leaflets formed on stolons after the transfer to the magnesium deficient medium. Thus, Neales (1958) concluded that in the leaf growing under magnesium deficient conditions, there is little or no addition to the active component of leaf magnesium by translocation, chlorophyll degradation proceeds, and the chlorosis typical of magnesium deficiency appears. In order to prevent chlorophyll breakdown, Garner et al. (1930) concluded that total magnesium content of the tobacco leaf must be several times greater than the quantity present in the chlorophyll. Batzer et al. (1952) concluded that magnesium was not transported from apple leaves, the concentration of magnesium in these leaves at leaf fall was 0.38% dry weight of leaf which is considered to be high level. Buckovac et al. (1960) could not find translocation of foliar applied Mg^{28} out of treated primary leaves of seedling or fruiting bean plants within 24 hours. Buckovac et al. (1960) concluded that magnesium deficiency may develop in

Table 11. Mean concentration of elements (% or ppm dry wt.) in leaves, stems and roots (Ford, 1966).

Element	Leaves					Stems					Roots				
	$Mg_{(0)}$	$Mg_{(1)}$	$Mg_{(2)}$	$Mg_{(3)}$	Sig diff p = 0.05	$Mg_{(0)}$	$Mg_{(1)}$	$Mg_{(2)}$	$Mg_{(3)}$	Sig diff p = 0.05	$Mg_{(0)}$	$Mg_{(1)}$	$Mg_{(2)}$	$Mg_{(3)}$	Sig diff p = 0.05
per cent Mg	0.10	0.16	0.26	0.52	0.08	0.05	0.06	0.09	0.11	0.02	0.06	0.12	0.16	0.22	0.03
" N						0.76	0.31	0.96	0.69	0.19	3.00	3.39	3.33	3.41	
" P	0.37	0.26	1.27	0.25	0.09	0.16	0.11	0.13	0.11		0.31	0.37	0.33	0.32	0.04
" K	1.92	1.57	1.37	1.03	0.30	<024	<0.24	<0.24	<0.24		1.83	1.91	1.56	1.51	
Ca	0.76	1.30	1.00	0.90	0.24	0.50	0.47	0.40	0.32	0.10	0.37	0.50	0.42	0.35	0.15
(ppm) Fe	62.3	70.0	70.0	58.8		18.8	22.8	50.8	29.8	20.2	284.0	215.5	284.3	368.5	124.8
" Mn	66.5	69.8	72.0	53.8	14.5	13.3	14.8	16.3	11.0	6.6	158.0	68.8	75.3	72.0	59.8
" Cu	2.5	2.0	2.3	2.3		4.0	3.0	3.5	3.5		7.3	5.8	7.0	8.5	1.8

older bean leaves without any appreciable loss of magnesium from these organs through redistricution to young leaves or to fruits. The following reason has been offered for the development of magnesium deficiency. Under the situation where magnesium is deficient, the plants magnesium pool available for the maintenance of optimum chlorophyll synthesis becomes limited as the leaf matures. Reserves may be further depressed by the incorporation of leaf magnesium into the cell wall constituents (e.g. magnesium pectate) or into other unavailable forms; this results in degradation of chlorophyll and magnesium deficiency symptoms appear.

3.6. Literature review summary

The following points can be derived from the discussion and conclusions which have been covered in the literature review.

1. Nutrients, herbicides and other substances can enter the leaf when applied to its suface as sprays.
2. Nutrient sprays are effective in some species in certain conditions, while with others, few or no responses have been achieved.
3. Likewise, magnesium sprays have been responsive to some species, while with others little or no responses have been noted.
4. Foliage application of magnesium salts appear to be superior to soil application in increasing the concentration of magnesium in the leaves and in reducing the

visual symptoms of deficiency.

5. In citrus, Epsom salt ($MgSO_4.7H_2O$) sprays have only little effect in correcting deficiency symptoms. Sprays with magnesium nitrate ($Mg(NO_3)_2.6H_2O$), on the other hand, have been satisfactory in increasing the concentration of magnesium in the leaves and in reducing visual symptoms of deficiency.
6. Variations in environments, physiological and morpholgical states of sprayed leaves affect the absorption.
7. Conflicting views exist about the mobility of magnesium in plants. Some support the mobility of magnesium, while others do not.

CHAPTER 4

4. MATERIALS AND METHODS

4.1. The experiments

Nine experiments were done with a view to investigating the effects of some of the factors on magnesium uptake by citrus leaves. The factors studied were the following:

(i) different wetting agents

(ii) different concentrations of a nonionic wetting agent (Terric GN9)

(iii) different concentrations of glycerine

(iv) humidity

(v) spraying at different times of the day

(vi) rate of uptake of magnesium

(vii) different magnesium salts

(viii) leaf magnesium level

(ix) leaf nitrogen level

The effects of these factors on magnesium uptake was measured as differences in the concentration of magnesium in the leaves after treatments.

Plants used were of two types:

(i) Pineapple sweet orange seedlings and

(ii) rooted leaf bud cuttings of Meyer lemons.

The nutrient solution used in all experiments was that developed by Hewitt (1966) at Long Aston Research Station for the nutrient culture of fruit trees. This has been successfully used for growing a large number of crops. The composition of this nutrient solution is given in Appendix I, but was, however, changed for experiment IX in which a high and low level of nitrogen were to be supplied to the plants. This was achieved by replacing calcium nitrate with ammonium nitrate and calcium chloride (Appendix 2). A low level of nitrogen was achieved by omitting ammonium nitrate from the feed solution.

4.2. Composition and preparation of concentrated nutrient stock solution for experiments.

The concentrated nutrient stock solution for experiments I, II, III, V, VI, VII and VIII was prepared from the salts given in Appendix I, while the salts given in Appendix 2 were used for experiment IX. Each major nutrient was dissolved in a separate two-litre jar. All minor nutrients, on the other hand, except ferric citrate, were dissolved in one jar of two litres' capacity. The ferric citrate was stocked in a separate two-litre jar.

4.3. Techniques used in growing pineapple sweet orange seedlings.

4.3.1. Sowing of seeds

Seeds of pineapple sweet orange were imported from California in the last week of June, 1969. Seeds were sown on three dates, the first sowing was done in July, 1969, the second in August, 1969, and the third in September, 1969. Each time enough seeds were sown to have sufficient plants for experiments and for the selection of uniform plants. 2' x 1' plastic trays were used for this purpose; sand and peat (1:1) was used for germinating seeds, which were germinated in closed propagating chambers, the temperature of which ranged from 70 to 75°F. Seeds started germinating two weeks after sowing.

4.3.2. Planting out

Forty days later, when seedlings had attained, on an average, two and a half to three inches in height, the uniform and healthy looking seedlings were selected and transplanted in 4" plastic pots using *sterilized sand and peat (1:1). Only nucellus seedlings were used; embryonic seedlings which were weaker and smaller were culled out.

* Sterilized by heating electrically to 185°F for 15 to 20 minutes.

4.3.3. Feeding the plants

For experiments I, II, III, V, VI, VII and VIII, the feed solution was prepared from the concentrated nutrient stock solutions of Appendix 1 and for experiment IX, the concentrated nutrient stock solutions of Appendix 2 were used.

In general, plants were fed thrice weekly with the feed solution, sometimes freshly prepared and sometimes prepared beforehand and stored in a metal drum painted with bitumen paints. A 100 ml of feed solution was applied and found to be just enough to allow a small loss due to drainage. It was considered that this leaching would prevent the accumulation of salt residues and would also provide adequate moisture for plant growth. Heavy leachings were done occasionally to ensure adequate removal of any salt residues.

4.4. Growing of Meyer lemon leaf bud cuttings

Four to six months old, green, uniform and healthy looking leaves of Meyer lemons were planted in sand in the plastic trays (2' x 1' size); after treatment with Seradix 3*. These leaves were kept under closed propagating chambers for rooting. After two weeks rooting started and extensive roots were formed within four weeks. The length of the roots varied from $1\frac{1}{2}$ to $2\frac{1}{2}$ inches.

* Seradix 3 contains 0.8% indobutyric acid, formulated as a wettable powder.

Each rooted leaf was transplanted in a $2\frac{1}{2}$" plastic pot. Sand was used as the medium. Potted leaves were kept on the benches of the glasshouse. Watering was done every day.

4.5. Experimental methods

4.5.1. Spraying techniques

Ten days before the spray treatment, feeding with magnesium solution was stopped to make sure that no magnesium would be taken by the plants through the roots. Two days before spraying, the leaves of each plant were wiped carefully with cotton wool soaked in water to remove all the dirt and foreign materials adhering to the leaf surfaces. Each pot was covered with waterproof plastic paper and the trunk was wrapped at the base with cotton wool to prevent the possibility of contaminating the surface soil of the pots by spray droplets.

Unless otherwise mentioned, spraying to the run off was done in the morning with a 2.5% $Mg(NO_3)_2.6H_2O$ solution with the inclusion of a nonionic wetting agent (Terric GN9) at the concentration of 0.03% of active ingredient. A small hand sprayer was used for spraying the plants. The concentration of $Mg(NO_3)_2.6H_2O$ used was 2.5% W/V, because above this concentration foliage injury occurred. Also, $Mg(NO_3)_2.6H_2O$ used at the concentration of 2.5%, resulted in a more highly significant increase in the uptake of magnesium as measured by its concentration in the leaves, compared with lower concentration (preliminary experiment).

4.5.2. Experimental design

Plants of experiments I, II, III and V to VIII were arranged in randomized complete block design on the benches of the glasshouse, while those of experiments IV, VIII and IX were arranged in split plot design. Each treatment in all experiments had four replications with a single plant per plot.

Experiment I

The effect of different wetting agents on the uptake of magnesium by leaves.

Plants used in this study were of two types:

(i) Pineapple sweet orange seedlings

(ii) rooted leaf bud cuttings of Meyer lemons.

This experiment consisted of two parts. In the first part, pineapple sweet orange seedlings were used to investigate the effects of three wetting agents on magnesium uptake by leaves. These three wetting agents were:

(i) didecylmethylbromide (Deciquam 222, cationic)

(ii) sodium dodecylbenzene sulphonate (Gardilene 30, anionic)

(iii) nonyl phenol ethylene oxide condensate (Terric GN9, nonionic)

As these three wetting agents were new products of unknown effects on uptake, each was tried at one selected concentration. As the results showed a positive effect of each on magnesium uptake the effects of these three wettings

as well as a forth one, phthalic glycerol alkyl resin (Triton B-1956, nonionic) were further investigated in the second part.

Here a standard curve of wettability* of citrus leaves for each material (Fig. 5) was prepared with the help of a standard wetting chart made for citrus leaves (Fig. 4) by using the techniques adopted by Conibiar and Furmidge (1965) for the Laurel leaves. Each material was then used at the concentration to give the same degree of wettability (80%). This enabled one to compare the effect of these wetting agents on magnesium uptake. To investigate this, rooted leaf bud cuttings of Meyer lemons were used. Leaves were dipped in the treatment solution and sampled after 24 hours. Before the leaves were dipped, the pot of each individual leaf was covered with a piece of waterproof plastic paper to prevent the contamination of treatment solution with the surface soil of the pot.

* Wettability refers to the proportion of a leaf surface that remains covered with liquid film after the excess of the liquid has drained off. It involves the combination of wetting and spreading of liquids on the leaf surface. The same material often possesses both wetting and spreading properties but some material are better wetting agents than they are spreaders, while others are better spreaders than wetting agents. It should be remembered that spreading pertains to the extension of a liquid over a surface and wetting pertains to the retention of the liquid by the surface. A good spreader is not necessarily a good wetting agent and vica versa.

Fig.4. STANDARD WETTING CHART FOR CITRUS LEAVES

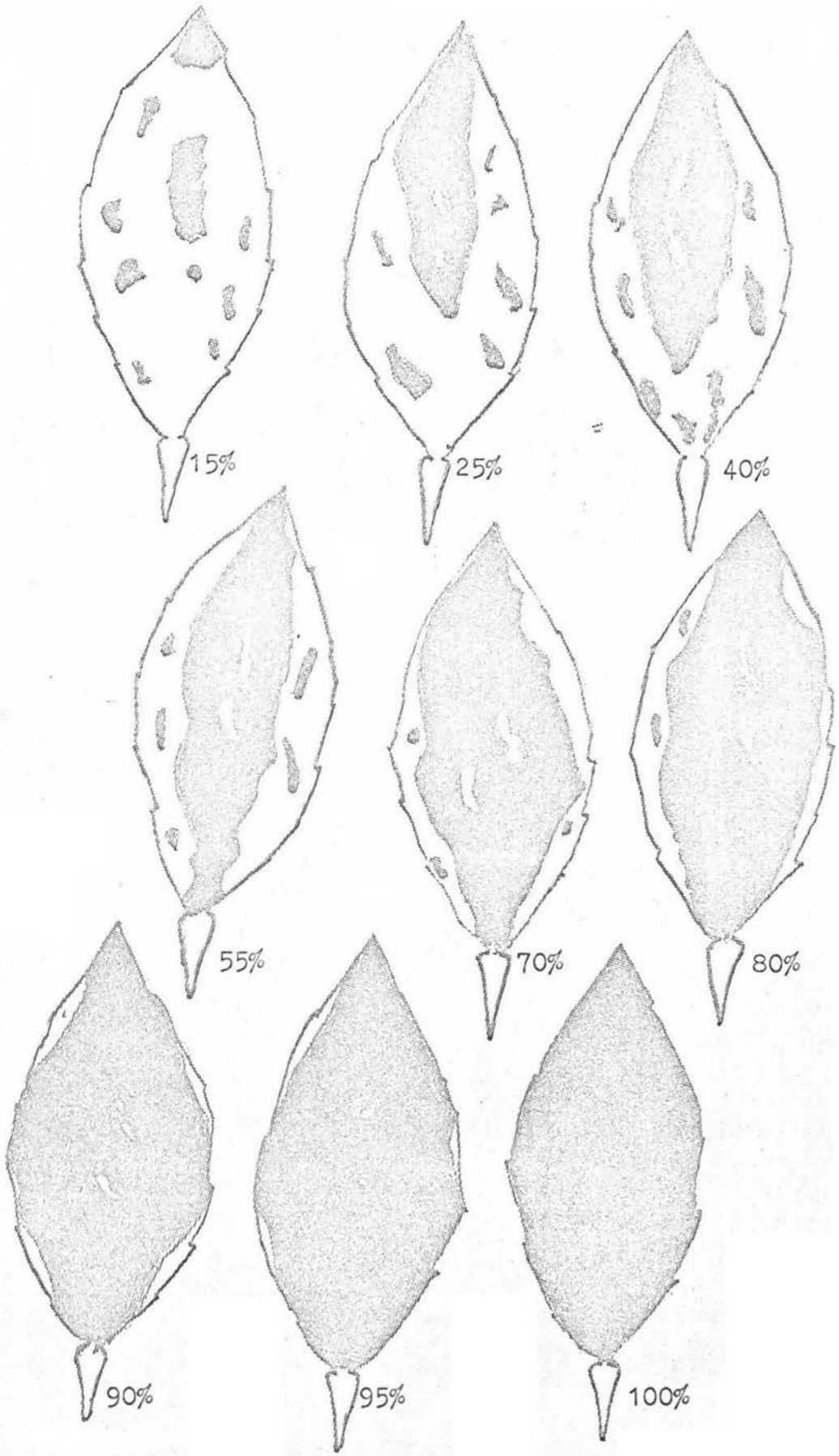
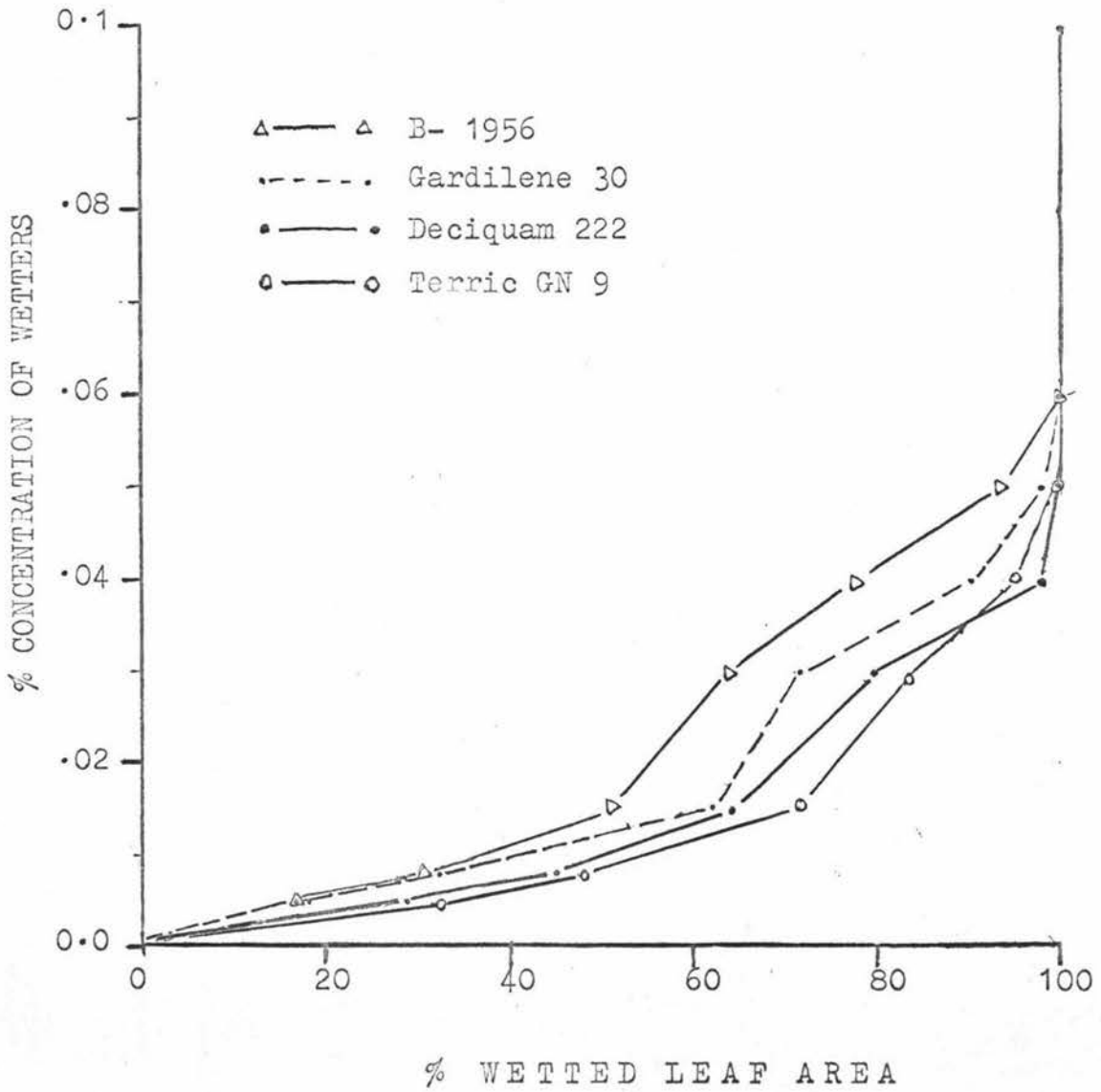


Fig.5. The standard curve of wettability of the upper surface of the citrus leaves for different wetting agents.



Experiment II

The effect of different concentrations of a nonionic wetter (Terric GN9) on the uptake of magnesium by leaves.

Pineapple sweet orange seedlings were used to investigate the effect of the different concentrations of Terric GN9 on magnesium uptake. The Terric GN9 was used at the concentrations of 0.01, 0.03, 0.05, 0.08 and 0.1 per cent of the active ingredient. The wettability of the wetter at the various concentrations used was determined by the same procedure as used in the experiment I.

Experiment III

The effect of different concentrations of glycerine on the uptake of magnesium by leaves.

Experiment III was conducted to see the effects of different concentrations of glycerine on the uptake of magnesium by leaves and compare it with that of Terric GN9. Pineapple sweet orange seedlings were used for this purpose. The glycerine was used at the concentrations of 1 and 2 per cent and the Terric GN9 at the concentration of 0.03% of active ingredient.

Experiment IV

The effect of humidity on the uptake of magnesium by leaves.

In this study, rooted leaf bud cuttings of Meyer lemons were used. Just after potting leaves were kept in two humidity conditions. One set of leaves was allowed to remain on the benches of the glasshouse, the relative humidity of which averaged 71%. This figure was obtained by averaging day to day humidity, measured three times a day (9.00 a.m., 2.00 p.m. and 5.00 p.m.)

Other sets of leaves were subjected to 100% relative humidity, which was obtained by enclosing the potted leaves with a sheet of plastic paper and putting a tray of water inside.

After two weeks leaves were dipped in a 2.5 per cent $Mg(NO_3)_2.6H_2O$ solution and sampled after 24 hours.

Experiment V

The effect of spraying at different times of the day on the uptake of magnesium by leaves.

This experiment was conducted to investigate the effect of spraying at different times of the day on magnesium uptake. Pineapple sweet orange seedlings were used for this purpose. Spraying with $Mg(NO_3)_2.6H_2O$ was done at 9.00 a.m., 12.30 p.m., 2.30 p.m. and 7.30 p.m. The relative humidity and temperature at each spraying time was recorded. The relative humidity at 9.00 a.m., 12.30 p.m., 2.30 p.m. and 7.30 p.m. was 81, 71, 73 and 83 per cent

respectively, the temperature 69°F, 72°F, 73°F and 68°F respectively.

Experiment VI

The rate of magnesium uptake by leaves.

Experiment VI was conducted to study the rate of uptake of magnesium by leaves from a 2.5% $Mg(NO_3)_2.6H_2O$ spray application. Pineapple sweet orange seedlings were used for this purpose. After spraying, samples were collected at 0 hour, 2 hours, 8 hours, 24 hours, 96 hours and 168 hours.

Experiment VII

The effect of different magnesium salts on the uptake of magnesium by leaves.

In this study, pineapple sweet orage seedlings were used. Three magnesium salts, namely $MgSO_4.7H_2O$, $MgCl_2.6H_2O$ and $Mg(NO_3)_2.6H_2O$ were tried. All sprays had magnesium equivalent of a 2.5 per cent $Mg(NO_3)_2.6H_2O$ solution. The relative humidity immediately before spray was 80% after 5 hours i.e. at 2p.m. 75%, after 10 hours i.e. 7p.m. 81% and after 22 hours i.e. the next morning 86%.

Experiment VIII

The effect of leaf magnesium level on the uptake of magnesium by leaves.

In this study, pineapple sweet orange seedlings which were fed with four levels of magnesium (4ppm, 12ppm, 36ppm and 72ppm), were used. The purpose of this feeding was to

Plate 1. Pineapple sweet orange seedlings supplied with high and low level of nitrogen. Low nitrogen (57ppm) plant on the left is showing nitrogen deficiency; while high nitrogen (250ppm) plant on the right is taller, with larger and greener leaves.

provide plants with different leaf magnesium levels and to see if these influenced the uptake of magnesium from sprays. Magnesium deficiency developed in 4ppm plants, and growth was depressed; the 12ppm plants also showed magnesium deficiency. The 36ppm plants were healthier and leaves were greener in colour, while the leaves of the 72ppm plants were a deeper green in colour, and there was no sign of magnesium toxicity.

Experiment IX

The effect of leaf nitrogen level on the uptake of magnesium by leaves.

In this study, pineapple sweet orange seedlings were supplied with two levels of nitrogen (57ppm and 250ppm). These were called low level and high level nitrogen respectively. The 57ppm plants developed nitrogen deficiency and the growth was stunted. Leaves were yellowish in colour and smaller than the 250ppm plants which were taller, with larger and deeper green leaves (plate 1).

4.6. Leaf samples and analysis

4.6.1. Sampling techniques

Each sample consisted of twelve leaves from each individual plant. This size of the sample was found to be adequate for analysing magnesium by the method used by Drossdoff and Nearpass (1948). The sampled leaves were collected in separate plastic bags, taken to the laboratory and immediately washed and dried. The sampled leaves were 4 to 6 months old. Though previously, leaves of various ages had been tried (Cameron

et al., 1952, and Wallace et al., 1952). In the present study the leaf age was selected as 4 to 6 months, because Smith (1966) indicated that at this age magnesium and other minerals are stable. Leaves younger than this are less stable in their mineral content.

4.6.2. Cleaning

The common methods employed to remove surface contaminants on leaves include washing the leaves in water containing detergent or hydrochloric acid, or to wipe them with cotton wool or a cloth. But leaching of nutrients might occur, if the leaves are dipped in water. Mann and Wallace (1952) have reported leaching losses of potassium as high as 99% if immersion was continued long enough. Richards (1932) stated that even rain could cause loss of potassium through leaching. Nicholas (1948) did not wash tomato leaves sprayed with magnesium sulphate before analysis. Boynton et al. (1943) working with apple leaves, depended on the washing effect of heavy rain.

Many workers, however, found that the washing of leaves prior to analysis is necessary to remove surface contaminants. Jacobson (1945) found washing in 3N HCl followed by thorough rinsing with distilled water to be effective in the removal of iron contaminations arising from dust and spray residues. Titus and Boynton (1953) adopted a similar procedure. Smith et al. (1950), in a study of various cleaning treatments for

the preparation of orange leaves for analysis, reported no loss of any element, when the leaves were subjected to any of several cleaning treatments, including immersion in a detergent solution and scrubbing for as long as six minutes.

Working with apple leaves, Taylor (1956) tried five cleaning procedures. Wiping the individual leaf with a damp cheese cloth was superior to other methods (Detergent + EDTA, Detergent distilled water, and not cleaned), for the removal of iron contamination not arising from spray material. While cleaned or uncleaned samples showed no significant difference in the concentration of N, P, K, Ca, Mg, Mn, Cu and Boron. Bhan et al. (1959) found no loss of K, Mg, Ca or P from leaching, when samples of mature orange leaves were shaken or immersed in distilled water. Labanauskas (1966) found no significant leaching of N, P, K, Ca, Cl, Zn, Mn, B, Fe and Mg from orange leaves due to the various washing techniques used. Dipping of detergent washed leaves in acidulated hydrochloric solution was not necessary if the leaves were properly washed in the detergent solution.

The above examples support the views of Tukey et al. (1958), that considerable variability exists among plant species with regard to their susceptibility to leaching losses of minerals. It can, however, be concluded that at least citrus leaves, prior to analysis, can safely be washed in water containing detergent, without the loss of minerals, and there is no need for dipping of detergent-washed leaves in acidulated hydro-

chloric acid solution.

Thus, in the present studies, the following procedures were used for cleaning the leaves. Leaves were agitated in 0.1% Teepol solution for approximately 10 secs, then wiped individually on both sides with cotton wool soaked in Teepol. They were then quickly swilled in three lots of distilled water and dried between sheets of blotting paper. Dry leaves were placed in a 150 ml beaker and dried for 40 to 48 hours at 70° to 75°C. Dried leaves were ground in a Wiley Mill and kept at 65°C for 30 hours.

4.6.3. Ashing

Approximately 2 grams of dried leaves were weighed and ashed in a crucible at 450°C. The ashing was completed within 10 to 12 hours at the above temperature.

4.6.4. Techniques of leaf magnesium analysis

Kidson (1946) has described the quantitative procedure for determining small amounts of magnesium in plant material by using titan yellow. Gillam (1941) described a titan yellow method for determining magnesium in fertilizers and soil extracts. In both methods, iron, aluminium, phosphorus and calcium have to be removed and ammonium salts destroyed by time consuming processes. Peech and English (1944) by using titan yellow, developed a direct rapid semiquantitative method

for the determination of magnesium in soil extracts in the presence of calcium, iron, aluminium and manganese. By using their procedure as a basis, and thiazole yellow as recommended by Mikkelsen and Toth (1947), instead of titan yellow, Drosdoff and Nearpass (1948) have developed a rapid quantitative colorimetric method for the determination of magnesium in tung leaves, and this has been recommended for other plant tissues. Fisher and Walker (1955) used the same method as adopted by Drosdoff and Nearpass (1948) for determining magnesium in apple leaves.

In the present studies, therefore, the method adopted by Drosdoff and Nearpass (1948) was used for determining magnesium in citrus leaves. Standard curves were run for each solution prepared. Nitrogen was determined by the Kjeldhal method (White et al., 1948). The details of procedures of magnesium determination and nitrogen determination have been given in Appendix 3 and 4 respectively.

4.7. Statistics

Analysis of variance of the data have been presented in a summarised form in Appendices 5 to 14.

CHAPTER 5

5. RESULTS

5.1. Experiment I

The effect of different wetting agents on the uptake of magnesium by leaves.

The data presented in Table 12 indicate that all three wetting agents, when included in sprays, increased the uptake of magnesium by leaves, compared with the control in which no wetting agent had been added. However, the cationic wetter (Deciquam 222) and anionic wetter (Gardilene 30) were less effective in increasing the uptake of magnesium than the nonionic wetter (Terric GN9). The average increase in the concentration of magnesium in the leaves by Terric GN9 was 0.10% (dry wt. basis), while by Deciquam 222, and Gardilene 30 was 0.05 and 0.06% (dry wt. basis) respectively. These increases in the concentration of magnesium in the leaves were significant at the 5 per cent level.

The further investigations on the effect of magnesium uptake by these three wetting agents as well as Triton B - 1956, indicated that these wetting agents differed in their effects on magnesium uptake, though they had been used at the concentrations which resulted the same wettability (80%). The anionic (Gardilene 30) was superior to cationic (Deciquam 222) and nonionic (Triton B - 1956) in increasing the uptake of magnesium, but not to the nonionic (Terric GN9). See Table 12a. Thus, this result indicated that wettability can

not be the sole criterion for the increased penetration of magnesium in the leaves but other factors such as the effects of wetting agents on cuticular permeability or interaction with the species of molecule to be absorbed, may be more important.

Table 12. The effect of different wetting agents on the uptake of magnesium by leaves from a 2.5 per cent $Mg(NO_3)_2.6H_2O$ spray application (absorption period 24 hours).

Treatments	Mg as % dry weight of leaves				Mean[x]
	I	II	III	IV	
Control $Mg(NO_3)_2.6H_2O$ spray only	0.38	0.34	0.36	0.37	0.36a
$Mg(NO_3)_2.6H_2O$ + Cationic (Deciquam 222) 0.015%	0.43	0.40	.39	0.42	0.41b
$Mg(NO_3)_2.6H_2O$ + Anionic (Gardi-lene 30) 0.015%	0.42	0.43	0.40	0.49	0.42b
$Mg(NO_3)_2.6H_2O$ + Nonionic (Terric GN9) 0.03%	0.46	0.48	0.45	0.47	0.46c
C.V. = 3.17%					

[x] Means with the same letter are not significantly different at the 5 per cent level as measured by Duncan's multiple range test (Federer, 1955).

Table 12a. The effect of different wetting agents used on the uptake of magnesium by leaves from a 2.5 per cent $Mg(NO_3)_2.6H_2O$ spray application, at 80% wettability of the leaf area (absorption period 24 hours).

Treatments	% leaf area wetted	Mg as % dry weight of leaves				Mean[x]
		I	II	III	IV	
Control $Mg(NO_3)_2.6H_2O$ Spray only	80	0.19	0.16	0.17	0.20	0.18a
$Mg(NO_3)_2.6H_2O$ + Deciquam 222(Cationic) 0.03%	80	0.21	0.23	0.20	0.24	0.22b
$Mg(NO_3)_2.6H_2O$ + Terric GN9 (nonionic) 0.026%	80	0.24	0.26	0.22	0.25	0.24bc
$Mg(NO_3)_2.6H_2O$ + Triton B - 1956 (nonionic) 0.042%	80	0.25	0.21	0.24	0.23	0.23b
$Mg(NO_3)_2.6H_2O$ + Gardilene 30 (anionic) 0.035%	80	0.26	0.24	0.28	0.27	0.26c
C.V. = 7.62%						

[x]Means with the same letter are not significantly different at the 5 per cent level as measured by Duncan's multiple range test (Federer, 1955).

5.2. Experiment II

The effect of different concentrations of a non-ionic wetter (Terric GN9) on the uptake of magnesium leaves.

The data presented in Table 13 and Fig. 6 indicate that the Terric GN9 at concentrations of 0.03 and 0.05% of active ingredient, having 82.50 and 100% wettability respectively, had shown significantly greater increase in the uptake of magnesium by leaves than the other concentrations used. There was no significant difference in the uptake of magnesium among 0.01, 0.08 and 0.1% of Terric GN9 and the control in which no wetter had been added.

The wettability results given in Table 13a and Fig. 6a show that as the concentration of the Terric GN9 had been increased, the percentage wettability of the leaves had also been increased, and 100% wettability was obtained as soon as the concentration of the wetter exceeded 0.04% of its active ingredient.

Table 13. The effect of different concentrations of a non-ionic wetter (Terric GN9) on the uptake of magnesium by leaves from a 2.5 per cent $Mg(NO_3)_2.6H_2O$ spray application (absorption period 24 hours).

Treatments	Mg as % of dry weight of leaves				Mean[x]
	I	II	III	IV	
Control $Mg(NO_3)_2.6H_2O$ spray only	0.35	0.36	0.37	0.41	0.38a
$MgNO_3)_2.6H_2O$ + 0.1% Terric GN9	0.34	0.38	0.40	0.36	0.37a
$Mg(NO_3)_2.6H_2O$ + 0.08% Terric GN9	0.35	0.36	0.41	0.39	0.37a
$Mg(NO_3)_2.6H_2O$ + 0.05% Terric GN9	0.45	0.43	0.46	0.47	0.45 b
$Mg(NO_3)_2.6H_2O$ + 0.03% Terric GN9	0.46	0.45	0.43	0.42	0.44 b
$Mg(NO_3)_2.6H_2O$ + 0.01% Terric GN9	0.42	0.39	0.37	0.42	0.40a
C.V. = 6.07%					

[x] Means with the same letter are not significantly different at the 5 per cent level as measured by Duncan's multiple range test (Federer, 1955).

Fig.6. The effect of different concentrations of a nonionic wetter (Terric GN 9) on the uptake of magnesium by leaves.

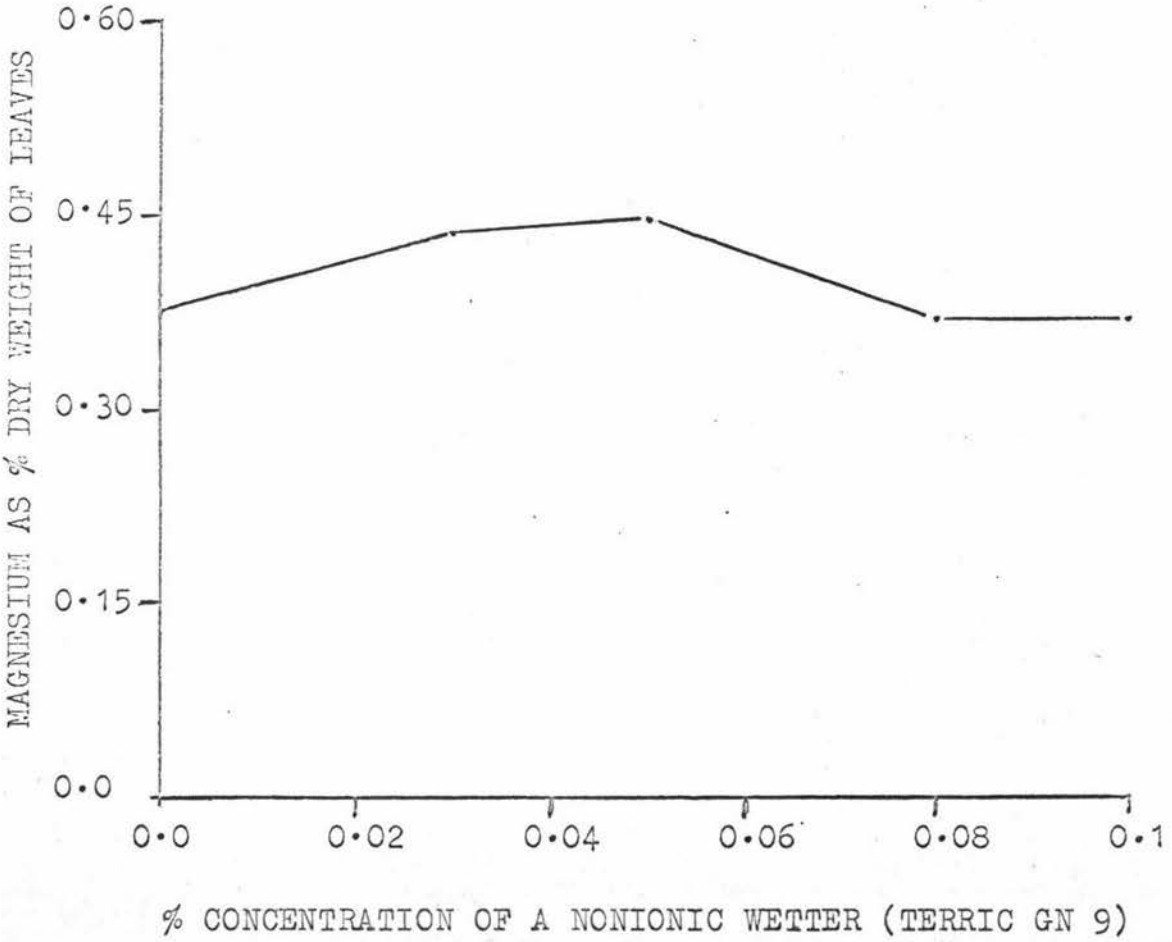
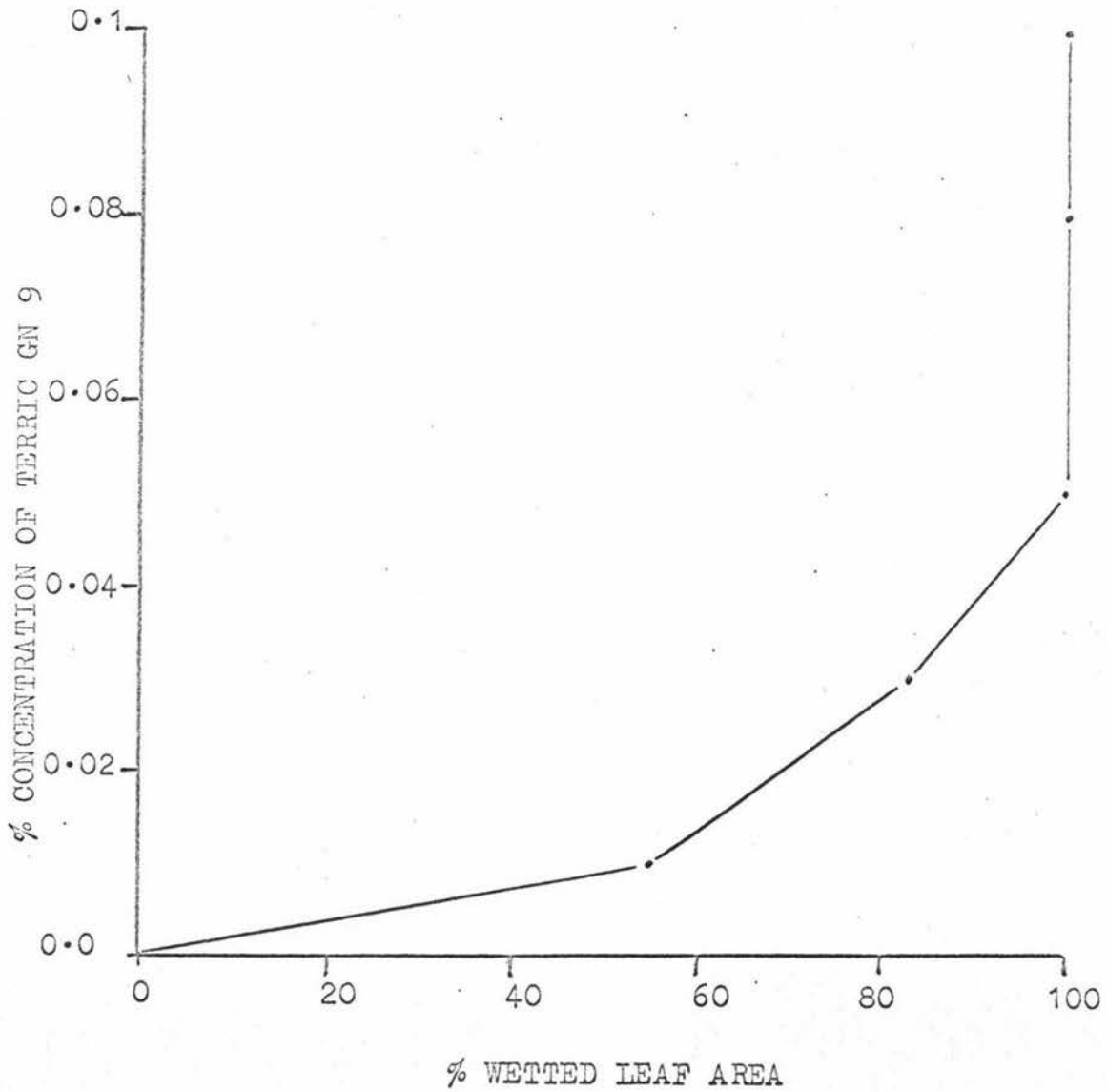


Table 13a. The visual assessment of the wetting of the upper surface of the citrus leaves by Terric GN9.

Wetter	Concn. %	% Wetting								Mean[x]
		I	II	III	IV	V	VI	VII	VIII	
Terric GN9 (non-ionic)	0.01	55	40	70	55	70	40	50	55	55a
"	0.03	80	90	80	80	80	90	80	80	82.5b
"	0.05	100	100	100	100	100	100	100	100	100 c
"	0.08	"	"	"	"	"	"	"	"	100 c
"	0.1	"	"	"	"	"	"	"	"	100 c
C.V. = 6.69%										

[x] Means with the same letter are not significantly different at the 5 per cent level as measured by Duncan's multiple range test (Federer, 1955).

Fig.6a. The effect of different concentrations of a nonionic wetter (Terric GN 9) on the wetting of the upper surface of the citrus leaves.



5.3. Experiment III

The effect of different concentrations of glycerine on the uptake of magnesium by leaves.

The result presented in Table 14 indicates that the inclusion of the humectant glycerine in the spray solution had significantly increased the uptake of magnesium by leaves than the control in which neither glycerine nor a wetter had been added. However, the inclusion of 1 or 2% glycerine in the spray solution was not better than the Terric GN9 used at the concentration of 0.03% of the active ingredient. Also, 2% glycerine was not more effective than the 1% glycerine. The average increase in the concentration of magnesium in the leaves by 0.03% Terric GN9, 1 and 2% glycerine was 0.06, 0.08 and 0.07% (dry weight basis) respectively. These increases in the concentration of magnesium in the leaves were significant at the 5 per cent level.

Table 14. The effect of different concentrations of glycerine on the uptake of magnesium by leaves from a 2.5% $Mg(NO_3)_2.6H_2O$ spray application (absorption period 24 hours).

Treatments	Mg as % dry weight of leaves				Mean[x]
	I	II	III	IV	
Control $Mg(NO_3)_2.6H_2O$ spray only	0.35	0.37	0.33	0.36	0.35 a
$Mg(NO_3)_2.6H_2O$ + Terric GN9 0.03%	0.41	0.45	0.39	0.42	0.41 b
$Mg(NO_3)_2.6H_2O$ + Glycerine 1%	0.42	0.40	0.44	0.46	0.43 b
$Mg(NO_3)_2.6H_2O$ + Glycerine 2%	0.39	0.43	0.42	0.44	0.42 b
C.V. 5.27%					

[x] Means with the same letter are not significantly different at the 5 per cent level as measured by Duncan's multiple range test (Federer, 1955)

5.4. Experiment IV

The effect of humidity of the uptake of magnesium by leaves.

Regardless of the humidity, the spray significantly increased the uptake of magnesium by leaves (Table 15). The 100% relative humidity had increased the magnesium concentrtion by 0.11% (dry weight basis), while 71% relative humidity had increased only by 0.07%. These increases were significant at the 5 per cent level. The greater increase in the concentration of magnesium in the leaves of the 100% relative humidity can be attributed to the increased permeability of the cuticle due to its hydration.

Table 15. The effect of humidity on the uptake of magnesium by leaves from a 2.5 per cent $Mg(NO_3)_2.6H_2O$ spray application (absorption period 24 hours).

Treatments / Reps.	No spray		Mean of sub total	Spray with 2.5% $Mg(NO_3)_2.6H_2O$		Mean of sub total
	100% relative humidity	71% relative humidity		100% relative humidity	71% relative humidity	
	Mg as % dry wt. of leaves	Mg as % dry wt. of leaves		Mg as % dry wt. of leaves	Mg as % dry wt. of leaves	
I	0.18	0.14		0.28	0.23	
II	0.17	0.15		0.29	0.19	
III	0.14	0.17		0.25	0.24	
IV	0.15	0.13		0.26	0.22	
Mean**	0.16	0.15	0.15	0.27	0.22	0.24*

* L.S.D. between means of overall spray and no spray at the 5 per cent level is 0.013

** L.S.D. between similar humidity conditions comparing unsprayed blocks at the 5 per cent level is 0.031

5.5. Experiment V

The effect of spraying at different times of the day on the uptake of magnesium by leaves.

The data in Table 16 indicate that regardless of the spraying time, all treatments had significantly increased the uptake of magnesium by leaves, compared with the control. Spraying in the morning (9.00 a.m.) and in the evening (7.30 p.m.) had significantly increased the uptake of magnesium by leaves, compared with the spraying at 12.30 p.m. and 2.30 p.m. No significant differences in the uptake of magnesium by leaves were found between the 9.00 a.m. and 7.30 p.m. or between the 12.30 p.m. and 2.30 p.m. spray applications.

Table 16. The effect of spraying at different times of the day on the uptake of magnesium by leaves from a 2.5 per cent $Mg(NO_3)_2.6H_2O$ spray application (absorption period 24 hours).

Time of spraying	Absorption time hrs.	Mg as % dry wt. of leaves				Mean[x]
		I	II	III	IV	
Control	0	0.28	0.26	0.27	0.27	0.27a
9.00 a.m.	24	0.41	0.44	0.40	0.43	0.42c
12.30 p.m.	24	0.37	0.39	0.38	0.35	0.37b
2.30 p.m.	24	0.38	0.40	0.36	0.39	0.38b
7.30 p.m.	24	0.42	0.45	0.44	0.46	0.44c
C.V. = 4.03%						

[x] Means with the same letters are not significantly different at the 5 per cent level as measured by Duncan's multiple range test (Federer, 1955)

5.6. Experiment VI

The rate of uptake of magnesium by leaves.

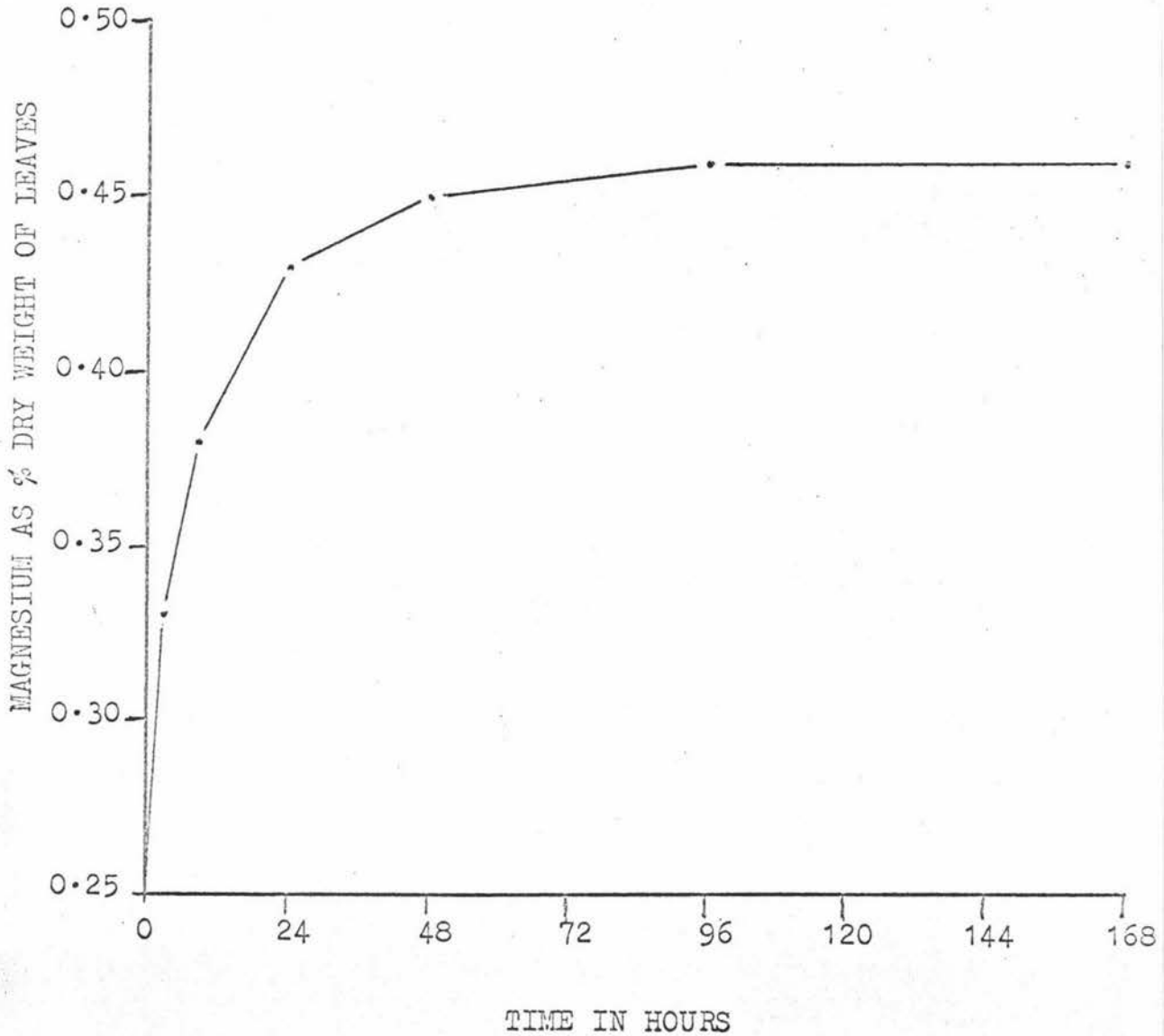
The data presented in Table 17 and Fig. 7 indicated that a significant uptake of magnesium occurred after two hours of magnesium nitrate spray application. The rate of magnesium uptake by leaves increased significantly up to 24 hours after spray application, thereafter the increase was nonsignificant. Since it was not possible to measure the degree of magnesium transport out of the leaf, it is not clear whether magnesium uptake, in fact, stopped after 24 hours of spray application.

Table 17. The rate of uptake of magnesium by leaves from a 2.5 per cent $Mg(NO_3)_2.6H_2O$ spray application.

Absorption time	Mg as % dry weight of leaves				Mean[x]
	I	II	III	IV	
0 hour	0.27	0.26	0.24	0.28	0.26a
2 hours	0.34	0.32	0.33	0.35	0.33b
8 hours	0.37	0.39	0.36	0.40	0.38c
24 hours	0.44	0.42	0.40	0.46	0.43d
48 hours	0.46	0.44	0.48	0.42	0.45d
96 hours	0.48	0.46	0.44	0.45	0.46d
168 hours	0.47	0.48	0.46	0.44	0.46d
C.V. = 4.96%					

[x] Means with the same letter are not significantly different at the 5 per cent level as measured by Duncan's multiple range test (Federer, 1955).

Fig. 7. The rate of uptake of magnesium by leaves.



5.7. Experiment VII

The effect of different magnesium salts on the uptake of magnesium by leaves.

Sprays of all the three magnesium salts significantly increased the uptake of magnesium by leaves than the control in which no sprays had been applied (Table 18). However, $Mg(NO_3)_2.6H_2O$ and $MgCl_2.6H_2O$ sprays had shown greater increase in the uptake of magnesium by leaves than the $MgSO_4.7H_2O$ sprays. The increase in the concentration of magnesium in the leaves by $Mg(NO_3)_2.6H_2O$ and $MgCl_2.6H_2O$ sprays was 0.17 and 0.16% (dry weight basis) respectively, while by $MgSO_4.7H_2O$ sprays the increase was only 0.06%. There was no significant difference in the concentration of magnesium between $Mg(NO_3)_2.6H_2O$ and $MgCl_2.6H_2O$ sprays.

Table 18. The effect of different magnesium salts on the uptake of magnesium by leaves. All sprays have the magnesium equivalent of a 2.5 per cent $Mg(NO_3)_2.6H_2O$ solution (absorption period 24 hours).

Treatments	Mg as % of dry weight of leaves				Mean[x]
	I	II	III	IV	
Control	0.25	0.26	0.27	0.26	0.26a
$MgSO_4.7H_2O$	0.34	0.31	0.32	0.33	0.32b
$MgCl_2.6H_2O$	0.42	0.39	0.43	0.44	0.42c
$Mg(NO_3.6H_2O$	0.42	0.43	0.46	0.41	0.43c
C.V. = 4.66%					

[x] Means with the same letter are not significantly different at the 5 per cent level as measured by Duncan's multiple range test (Federer, 1955).

5.8. Experiment VIII

The effect of leaf magnesium level on the uptake of magnesium by leaves.

Regardless of magnesium level in the leaves, sprays significantly increased the uptake of magnesium by leaves (Table 19). However, a higher level of leaf magnesium had shown a higher uptake of magnesium by leaves while a lower level of leaf magnesium showed a lower uptake of magnesium by leaves from a 2.5% $Mg(NO_3)_2.6H_2O$ spray. Also a high level of magnesium in the feed solution had increased magnesium concentration in the leaves comparatively more than the lower level of magnesium in the feed solution. However, magnesium concentration in the leaves increased from 4ppm to 36ppm, but above this level the gradient of increase in relation to supply was less steep (Fig. 8). Similar type of curve was obtained for the uptake of magnesium by leaves from sprays (Fig. 8).

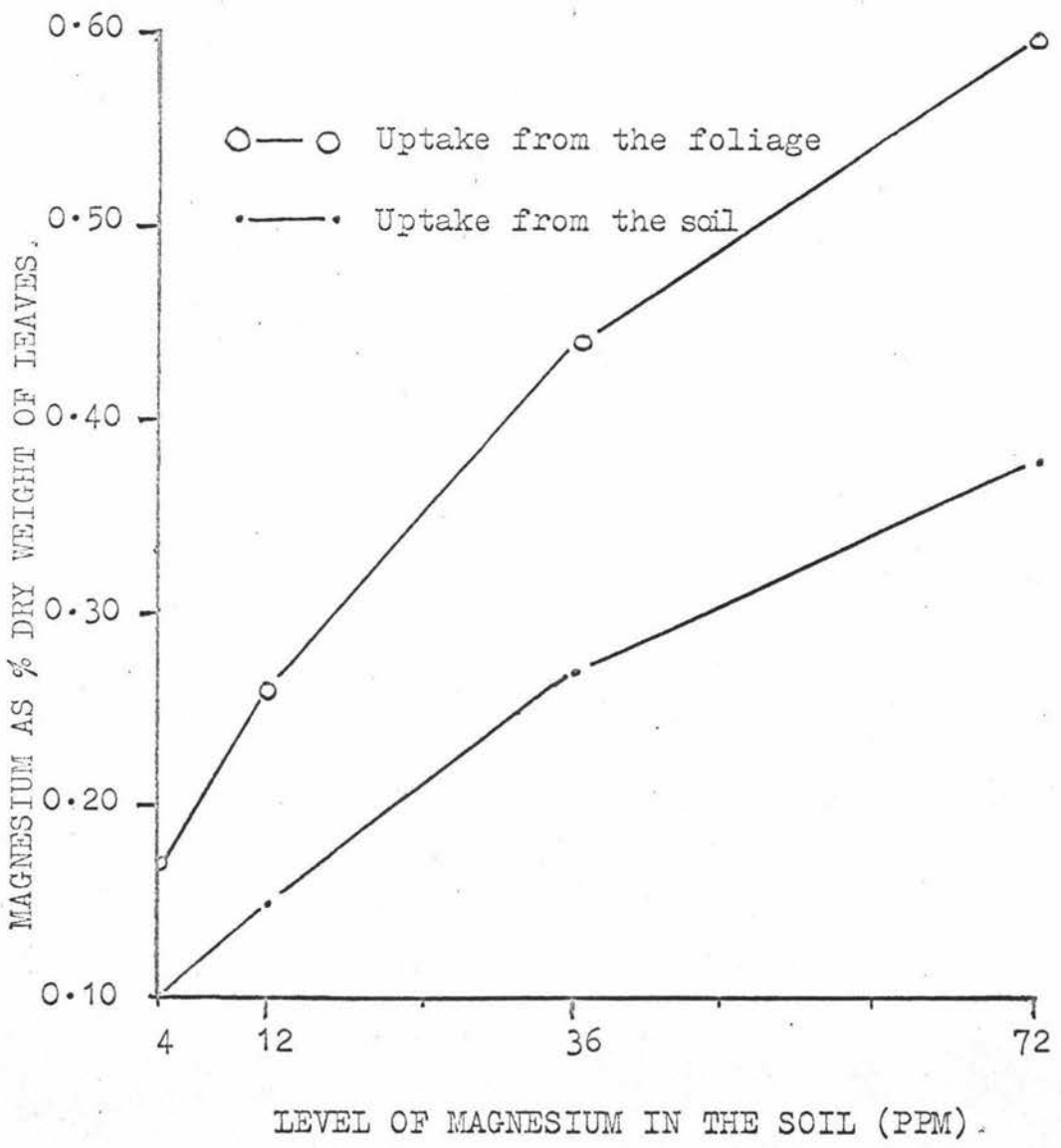
Table 19. The effect of leaf magnesium level on the uptake of magnesium by leaves from a 2.5 per cent $Mg(NO_3)_2.6H_2O$ spray application (absorption period 24 hours).

Treatments / Reps.	No spray				Mean of Sub Total	Spray with 2.5 per cent $Mg(NO_3)_2.6H_2O$				Mean of Sub Total
	4 ppm	12 ppm	36 ppm	72 ppm		4 ppm	12 ppm	36 ppm	72 ppm	
I	0.11	0.14	0.28	0.38		0.19	0.25	0.45	0.61	
II	0.12	0.17	0.27	0.37		0.17	0.29	0.46	0.60	
III	0.09	0.15	0.29	0.40		0.15	0.24	0.44	0.58	
IV	0.10	0.16	0.26	0.39		0.18	0.27	0.42	0.63	
Mean**	0.10	0.15	0.27	0.38		0.17	0.26	0.44	0.60	
					0.23					0.37*

* L.S.D. between means of overall spray and no spray at the 1 per cent level is 0.023

** Means of similar soil treatments comparying the unsprayed with sprayed blocks are not significantly different at the 1 per cent level as measured by Duncan's multiple range test, if they have the same letter (Federer, 1955)

Fig. 8. The effect of leaf magnesium level on the uptake of magnesium by leaves.



5.9 Experiment IX

The effect of leaf nitrogen level on the uptake of magnesium by leaves.

The data presented in Table 20 indicate that the nitrogen status of low nitrogen (57 ppm) plants was markedly lower than that of the high nitrogen (250 ppm) plants. The average leaf concentration of the former was 2.08% dry weight of the leaves as compared with 2.92% dry weight in the latter. This difference in nitrogen status resulted in the difference in magnesium uptake by leaves. The high nitrogen plants (250 ppm) had shown significantly greater increase in the uptake of magnesium by leaves than the low nitrogen (57 ppm) plants. The average increase in the concentration of magnesium in the leaves of high nitrogen plants was 0.19% (dry wt. basis), while the increase in the leaves of low nitrogen plants was only 0.09%. Thus, the increase in the concentration of magnesium in the leaves of high nitrogen plants was double that of low nitrogen plants.

Table 20. The effect of leaf nitrogen level on the uptake of magnesium by leaves from a 2.5 per cent $Mg(NO_3)_2.6H_2O$ spray application (absorption period 24 hours).

Treatments / Reps.	No spray		Mean of Sub Total	Spray with $Mg(NO_3)_2.6H_2O$				Mean of Sub Total
	High N (250ppm)	Low N (57ppm)		High N (250ppm)		Low N (57ppm)		
	Mg as % dry wt. of leaves	Mg as % dry wt. of leaves		N % dry wt of leaves	Mg as % of dry wt of leaves	Mg as % of dry wt of leaves	N % dry wt. of leaves	
I	0.29	0.22		2.80	0.45	0.35	2.10	
II	0.27	0.26		3.08	0.48	0.37	1.96	
III	0.30	0.21		2.94	0.51	0.40	2.24	
IV	0.32	0.24		2.87	0.49	0.33	2.03	
Mean**	0.29	0.23			0.48	0.36		
			0.26					0.42*

* L.S.D. between means of overall spray and no spray at the 1 per cent level is 0.088

** L.S.D. between similar soil treatments comparing unsprayed with sprayed blocks is 0.096

CHAPTER 6

6. DISCUSSION OF THE DATA

Leaf surfaces are often difficult to wet with pure aqueous sprays. The inclusion of a suitable surfactant improves wetting and spreading of liquids to leaf surfaces. Adequate wetting of leaf surfaces is essential to making spray applications effective. But enhanced wetting is not always associated with enhanced penetration (Foy et al.,1967). Some compounds may also, by acting as humectants, (Currier and Dybing, 1959) increase penetration, while others may facilitate the penetration by increasing cuticular permeability (Jansen et al., 1961). Accordingly experiments to measure these effects were designed.

The fact that the addition of the wetting agents in the spray solution increased the magnesium uptake agrees with the results reported by Guest and Chapman (1949), who obtained greater and more uniform response to iron sprays. It also agrees with Cook and Boynton (1952) for the urea absorption, Fisher and Walker (1955) for magnesium absorption by McIntosh apple leaves, and with results from a number of other workers who obtained higher and enhanced herbicidal activities when the wetting agents were included in the spray solutions. (Barrier and Loomis, 1957; Jansen et al., 1961 and McWhorter, 1963). However, the use of wetting agents was ineffective in

increasing the absorption of foliar applied phosphorous (Koontz and Biddulph, 1957; Barrier and Loomis, 1957). Thus, it appears that the wetting agents do increase the penetration of some substances, while not of others. What makes them behave so differently? According to Parr and Norman (1965), the work of Orgell (1957) indicates that the chemical changes that take place after the wetting agents are included in the spray solutions play an important role in the penetration. For example, Orgell (1957) found that both cationic and anionic surfactants hindered the absorption of acid dyes and 2, 4-D at low pH values, while non-ionic surfactants had little effect. On the other hand, some surfactants may have direct effects on the cuticular membranes. Hydration of the cuticle takes place with increased permeability and this favours the penetration (Jansen et al., 1961).

In the present study, the increased uptake of magnesium by leaves, when the wetting agents were included in the spray solution, can not be attributed to the adequate wettability only. Since the wetting agents, even when used at the concentrations resulting the same wettability (80%), still differed in their effects on magnesium uptake. Thus, this result confirmed the statements of Freed and Montgomery (1958) and Hughes and Freed (1961), that wettability alone may not be the sole critarion to explain the effect on penetration, but their interaction with the species of molecule to be absorbed may be more important.

However, increasing of the nonionic wetter(Terric GN9)to a certain stage only has shown increased penetration of magnesium in the leaves; beyond this there is a decrease in penetration (Fig. 6). One reason for this may be micelle formation by the surfactant molecules when the surfactant concentration exceeded a critical level in an aqueous system. This critical micelle level (cmc), according to Furmidge (1959) and Jansen et al. (1961), is associated with abrupt changes in many characteristic properties of the surfactant. These changes in properties may influence such phenomena as penetration and translocation. Also, toxic substances (additives or herbicides), if used at a higher concentration, can block phloem translocation (Leonard and Crafts, 1956; Leonard, 1958).

It has been pointed out by Blackman (1952), Koontz and Biddulph (1957) that the wetting of the leaf surface is essential, but it should be remembered that on some leaves spray load can be reduced by the use of wetting agents. The thinner adherent films also dry more quickly (Koontz and Biddulph, 1957).

The retention of the spray materials is important. Fogg (1947) names the contact angle as one of the major factors affecting the amount of solution retained on the leaf surface, because it determines the form of the droplets. If the contact angle is very high, as that of water on Triticum leaves (130°), the droplet is nearly sperical and is easily displaced. If rather low, as with water on Sinapsis leaves (72°), there

results a squat form drop with a large area of contact and with a low centre of gravity, making for stability so far as displacement is concerned; thus, the maximum quantity will be retained by the leaf. If the contact angle is very low so that drainage is no longer prevented by discontinuities in the water phase, there will be very little retention, even though the leaf surface is quite uniformly wetted. Thus, Fogg(1947) states that addition of the wetting agents may cause large changes in water retention due to variations in contact angles.

In the present study, it is quite possible that the higher concentration of the nonionic wetter (Terric GN9) might have resulted in low contact angle which is not effective in retaining the solution on the leaf surface (Fogg, 1947), thereby resulting in less penetration. Also excessive run off might have occurred, and caused less penetration.

When the effect of a nonionic wetter (Terric GN9) was compared with that of glycerine, it was found that the use of glycerine had the same effect as that of Terric GN9 on the uptake of magnesium by leaves (Table 14). The fact that the use of glycerine increased the uptake of magnesium, agrees with the results reported by Fisher and Walker (1955) for the absorption of phosphorus by apple leaves, Koontz and Biddulph (1957) by bean leaves, and Gray (1956) for the absorption of streptomycin by the leaves of a number of crop plants (bean, tomato, pepper and tobacco etc.). These results, however, contradict the findings of Fisher and Walker (1955) on absorption of

magnesium by apple leaves. But Fisher and Walker (1955) had included glycerine in the spray solution which already contained the spreader (Tween 85). Thus, it seems that glycerine had no additional effect over Tween 85, but it might have increased the magnesium absorption due to its humectant properties, if it could have been included in the spray solution alone.

The importance of glycerine in the application of chlorinated phenoxyacetic acids was demonstrated in experiments on sunflowers and oats. Penetration during the first hour or so was the same in the presence or absence of the glycerine. However, as soon as the drops without glycerol dried out, penetration ceased. In the presence of glycerine, the drops stayed moist and penetration continued for many hours (Holly, 1956).

While the intimacy of the contact of the chemical with the leaf surface is important in absorption, the state i.e. liquid or solid of the chemical may also influence the ease of penetration. Consideration of the physical processes involved shows that a chemical will be absorbed with relative ease from the liquid phase in contrast to the same chemical as a crystal on the surface of the leaf. Either humid conditions following application or the addition of hygroscopic agents may help to maintain a liquid state, thus facilitating the penetration (Hughes and Freed, 1961).

The mechanism by which the use of glycerine in the present study has increased the magnesium absorption can be attributed to its property of keeping magnesium salt in solution form and in close contact with leaf surface for a longer period of time. The low volatility of the glycerine permits it to remain on the leaves for several days (Gray, 1956).

If the function of glycerine is to keep spray droplets in moist condition for a longer period of time and to prevent them from drying quickly, then the conditions providing high humidity should have the same effect. The data in Table 15 indicate that the concentration of magnesium in the leaves of 100% relative humidity was increased by 0.11% of dry weight while in leaves of 71% relative humidity it was increased by 0.07% of dry weight. Thus, the 100% relative humidity had resulted in greater uptake of magnesium from the sprays than the 71% relative humidity. These results agree with Zukel et al. (1956) for the absorption of maleic hydrazide. They also agree with Rice (1948) and Holly (1956) for the absorption of phenoxyacetic acids. Penetration of phenoxyacetic acids has been found to cease on crystallization of phenoxyacetic acids in spray droplets. These results, however, disagree with Went and Carter (1948) who found the sucrose uptake by tomato plants was independent of humidity.

The increased penetration of chemicals due to high humidity in other studies, and of magnesium in the present study may be due to a variety of reasons. A relatively higher

humidity prevents water stress in the plant, delays drying of spray deposit, and increases cuticular permeability due to hydration of the cuticle. On the other hand, extended dry periods may increase cuticular thickness, which causes less permeability, resulting in less penetration.

Time of spraying has also shown significant differences in the uptake of magnesium by leaves. Spraying in the morning (9 a.m.) and the evening (7.30 p.m.) has resulted in the greater uptake of magnesium by leaves than spraying at 12.30 and 2.30 p.m. These results agree with Oland and Opland (1956) who obtained highly significant incease in the uptake of magnesium by apple leaves from $MgSO_4.7H_2O$ spray, when the spraying was done in the evening, than those done in the afternoon. The large uptake after spraying in the evening led them to conclude that ion exchange as a mechanism is involved in the absorption. Hence, the diurnal shift in acid production might influence magnesium uptake. Internal release of hydrogen ions for exchange would be most likely to occur after the probable shift in the production of organic acids during the evening. These results, however, contradict Teubner et al. (1957), who found higher uptake of P, K, and Rb from morning application than from the evening application, and they concluded that daylight might have favoured the stomatal openings and thereby increased the penetration.

In the present study the increased uptake of magnesium by leaves from the morning spray can be attributed to the

influence of light favouring the stomatal entry, or to high humidity (83%) at the time, and the high uptake of magnesium by leaves from the evening spray can be attributed to the hydrogen ion exchange hypothesis of Oland and Opland (1956) involving a diurnal fluctuation in the organic acid content, or to higher humidity (81%) at the time or during the hours that followed.

The rate of uptake of magnesium, presented in Fig. 7, indicates that the concentration of magnesium in the leaves has increased rapidly after two hours of spray application. Leaf magnesium concentration has risen steeply for 24 hours after spraying, thereafter remaining constant. These results agree with those reported by Fisher and Walker (1955) for the absorption of magnesium by apple leaves. They found that absorption was slow after the first hour of spray application of $MgSO_4.7H_2O$ and no apparent increase in magnesium absorption up to six days was noticed. Allen (1960), also found no significant increase in magnesium uptake from magnesium nitrate and chloride after two hours of application. The possible reason for slow and little increase in the concentration of magnesium in the leaves after two hours of application may be the drying of spray droplets. Another reason for the nonsignificant increase in the concentration of magnesium in the leaves with relation to time may be its mobility to other plant parts, if the claims on magnesium mobility made by Phillis and Mason(1942), and Ford (1966;1968) can be taken for granted.

The effect of different magnesium salts on the uptake of magnesium by leaves indicates that $Mg(NO_3)_2.6H_2O$ and $MgCl_2.6H_2O$ sprays have shown greater responses than $MgSO_4.7H_2O$. The data given in Table 18 indicate that $Mg(NO_3)_2.6H_2O$ and $MgCl_2.6H_2O$ sprays have increased the concentration of magnesium in the leaves by 0.11 and 0.10% (dry wt. basis) respectively over the $MgSO_4.7H_2O$ spray. These results agree with those reported by many workers. Fisher and Walker (1955) reported that after 24 hours 71% of the magnesium from $Mg(NO_3)_2.6H_2O$ sprays applied to the lower surface of apple leaves was apparently absorbed while with comparable sprays of $MgSO_4.7H_2O$ only 8.4% of the magnesium was absorbed. However, $Mg(NO_3)_2.6H_2O$ resulted in some foliage injury. Hagler (1957) showed that $Mg(NO_3)_2.6H_2O$ sprays on grapes were more effective in increasing the concentration of magnesium in the leaves and in reducing visual deficiency symptoms than the $MgSO_4.7H_2O$ sprays. Embleton and Jones (1959) obtained a substantial increase in concentration of magnesium in citrus leaves by the use of 1% $Mg(NO_3)_2.6H_2O$ spray and they eliminated the visual symptoms of deficiency. Ford et al. (1965) with five post blossom sprays of 0.83% $MgCl_2.6H_2O$ (containing only half the amount of magnesium) produced similar effects as obtained by 2% $MgSO_4.7H_2O$ sprays. Thus, the above findings and the present results indicate that $Mg(NO_3)_2.6H_2O$ and $MgCl_2.6H_2O$ sprays are superior to $MgSO_4.7H_2O$ sprays in increasing the concentration of magnesium in the

leaves and in reducing visual symptoms of deficiency. What makes these salts behave so differently, i.e. why is magnesium from $Mg(NO_3)_2.6H_2O$ and $MgCl_2.6H_2O$ taken up more rapidly than from $MgSO_4.7H_2O$?

It has been suggested that magnesium salts in the crystal form on the leaf surface are inaccessible to the plant. To be absorbed, they must be in liquid form on the leaf surface. Depending on the atmospheric conditions, magnesium salts differ in the length of the time during which they can remain in liquid form. $Mg(NO_3)_2.6H_2O$ and $MgCl_2.6H_2O$, as reported by Allen (1960), can remain in a liquid state over a wide range of atmospheric conditions, due to their deliquiscent nature, while $MgSO_4.7H_2O$ cannot. Perhaps this difference in the behaviour of the salts might have resulted in difference in magnesium absorption. Allen's work (1959) also indicates that the rate of magnesium uptake from $Mg(NO_3)_2.6H_2O$, $MgCl_2.6H_2O$ and $MgSO_4.7H_2O$ is the same, if the leaves are immersed in the solutions for a fixed period of time. But when leaves are momentarily dipped and allowed to dry up, the rate of magnesium uptake from these salts differs. This has been attributed to the physical nature of the deposits left behind them.

It would be thus expected that as long as the immersed leaves are wet or subject to conditions similar to those obtained when they were dipped, magnesium would be taken up equally readily from three salts. However, evaporation occurs and sometimes proceeds till the magnesium salts crystallize

out, at which point a difference would arise between the salts, which might account for the observed differences.

Allen (1960) reported that at 20°C the relative humidity of the air in equilibrium with saturated solutions of magnesium sulphate, acetate, nitrate and chloride is 82, 65, 55 and 33% respectively. This means that if the relative humidity of the air is greater than that of the air in equilibrium, with a saturated solution, the latter (or even the crystals from which it is derived) would take up water vapour from the air. If it is less, the solute is eventually crystallized out. Thus, at 20°C relative humidity of 32% would cause solutions of all four salts to crystallize out.

In the present study, therefore, a consideration of relative humidity mentioned in section 4.5.2. (experiment VII) indicates that $Mg(NO_3)_2.6H_2O$ and $MgCl_2.6H_2O$ might have remained in the solution on the leaf surface throughout that experiment. The $MgSO_4.7H_2O$ solution on the other hand, would have been dried out at once, and only brought into the solution overnight and the following morning when the humidity was high. Assuming therefore that the magnesium is only taken up by the leaf from solution, penetration from the $MgSO_4.7H_2O$, would in this instance have been halted during the daytime, and only resumed when the relative humidities were high in the night and the following morning.

The effect of leaf magnesium level on the uptake of magnesium from $Mg(NO_3)_2.6H_2O$ sprays, presented in Table 19, indicates that the concentration of magnesium in the leaves of 4 ppm, 12 ppm, 36 ppm and 72 ppm plants was significantly increased by 0.07, 0.11, 0.17 and 0.22% (dry wt. basis) respectively. Thus, increasing the magnesium level in the soil has also increased the uptake of magnesium from sprays. The fact that the leaves of 4 ppm and 12 ppm plants showed little uptake of magnesium from aprays may be due to a lesser capability of these leaves to absorb magnesium than the leaves of those plants whose roots were well supplied with magnesium, or it may be due to translocation of more of the absorbed magnesium.

These results agree with those reported by Ford (1967) who grew apple plants by feeding them with two levels of magnesium (45 ppm and <3 ppm). The leaves of 45 ppm plants when dipped in a 2% $MgSO_4.7H_2O$ solution showed a higher uptake of magnesium than the leaves of <3 ppm plants. However, the uptake of magnesium by leaves from sprays in present study increased from 4 ppm to 36 ppm, and above this level the gradient of magnesium uptake was less steep (Fig. 8). A similar type of curve was also obtained for the absorption of magnesium from the soil (Fig. 8).

Not only the leaf magnesium level, but also the leaf nitrogen level has shown marked difference in the uptake of magnesium from sprays. The difference in nitrogen status was

associated with the substantial difference in magnesium concentration in the leaves. Low nitrogen plants absorbed comparatively little magnesium both from culture solution and sprays. In contrast, the high nitrogen plants absorbed considerable magnesium from both soil and sprays. The average increase in the concentration of magnesium in low nitrogen plants was 0.09% of dry weight, while in high nitrogen plants, it was 0.19% of dry weight (Table 20). Thus, the per cent increase in the concentration of magnesium (dry weight basis) in the leaves of high nitrogen plants was double that of the low nitrogen plants. These results agree with those reported by Forshey (1959, 1963) for the absorption of magnesium by apple. Forshey (1963) found that high nitrogen apple plants absorbed considerable amounts of magnesium both from soil and foliage application, while low nitrogen plants absorbed an unmeasurable amount of magnesium from the foliage application and very little from the culture solution. Reuther and Smith (1950, 1952) obtained greater concentration of magnesium in the leaves of citrus plants highly supplied with nitrogen than in those supplied with low nitrogen. Increasing the nitrogen supply, even without addition of magnesium in any form, has resulted in a greater increase of magnesium in the leaves of apples (Boynton and Compton, 1944; Cain and Boynton, 1948; Weeks et al. 1952, 1958, and Cain, 1953), of peaches (Ritter 1956) and of strawberries (Victor et al., 1967).

The increase in magnesium concentration associated with high nitrogen treatment can be attributed to increased growth.

But Forshey (1963) argued that difference in growth cannot entirely explain the difference in magnesium absorption by high and low nitrogen plants, because the dry weight of high nitrogen plants was only 67% greater than that of low nitrogen plants, but on the other hand, increase in magnesium content was 111%.

Thus, increased growth of high nitrogen plants may not be the only cause for the greater uptake of magnesium from sprays, but some other factors might have been involved. High nitrogen trees might be more capable of absorbing magnesium than the low nitrogen plants. In the present study, it has been noticed that the supply of high nitrogen produced rapid and quicker growth of the plants, leaves were comparatively larger and greener than those of low nitrogen plants(Plate 1). Under these conditions the cuticle might be expected to be thinner than on the slower growing smaller leaves of low nitrogen plants, and thus more easily penetrable. On the other hand, with the larger cells there would be a fewer vertical epidermal walls/unit area and if they provide a pathway for the entrance of the chemical, as postulated by Wylie (1943) and Roberts et al. (1948), then theoretically the effect would be a decrease rather than an increase in absorption. Larger cells, however, would result in larger stomatal openings and thus permit easier and faster entry by this path.

CHAPTER 7

7. CONCLUSION

Magnesium is the key metallic substance in chlorophyll and therefore it is of prime importance in crop production. Its deficiency has been reported in citrus orchards in various parts of the world. Magnesium deficiency has affected not only the growth of citrus plants, but also fruit yield and quality. Thus, for successful citrus fruit production the requirements of magnesium cannot be overlooked.

The slow, slight, and sometimes minimal effect of soil application of magnesium salts leaves no other means quicker than foliage application for furnishing the magnesium requirements of citrus plants.

But the results obtained from foliage application of magnesium salts are not consistent and this raises a number of questions regarding factors affecting magnesium absorption.

The present study indicates that the use of wetting agents is effective in increasing the uptake of magnesium by leaves. But the responses are variable depending on the type of wetting agents used. The variable results obtained from the wetting agents even when used at concentrations resulting in the same wettability show that factors other than wettability are also involved. The interaction of wetters with the species

of molecule to be absorbed, sorption of the wetter to the cuticle changing its charge and polarity, solubilization of the penetrant and the reaction with the cuticle may also be more important and require further investigation.

The beneficial effect of glycerine on the uptake of magnesium by leaves indicates that the glycerine can be used as a spray supplement. Glycerine, even if used at lower concentrations than the concentrations (1 and 2%) used in the present study, may have the same effects. Therefore, further investigations are desirable regarding different concentrations of glycerine and their effects on the uptake of magnesium by leaves. Other hygroscopic agents (carbowax, sorbitol diethylene glycol, and other polyhydroxy alcohols) should be tried.

The conclusion that the morning and evening sprays were superior to mid-day and afternoon sprays, can not be made since in this investigation the temperature and humidity were not controlled. High relative humidity in the morning and evening might have resulted in increased penetration of magnesium. This has been demonstrated by subjecting leaves to high and low humidity conditions. Thus it may be that it is the relative humidity before and after the spray which is important, but not the time of the day. Because similar relationships occur in practice, this finding may be of practical significance but the exact reason remains obscure until it is examined further.

The beneficial effect of $Mg(NO_3)_2.6H_2O$ and $MgCl_2.6H_2O$ sprays over $MgSO_4.7H_2O$ in increasing the uptake of magnesium by leaves can possibly replace $MgSO_4.7H_2O$ in controlling magnesium deficiency of the citrus plants.

The slighter uptake of magnesium by leaves having initially low level of magnesium, whether due to their inability to absorb or to some other reason is not known. Ford's work (1968) indicates that the lack of magnesium in the initial stage of leaf development eventually develops necrosis, even though a high supply is restored to them while still green. A leaf therefore requires adequate magnesium during its initiation, especially during its expansion, and thereafter, a constant supply.

The increased uptake of magnesium by leaves of high nitrogen plants indicates that the use of nitrogen fertilizers on the plants prior to magnesium sprays can be of practical significance in reducing visual symptoms of magnesium deficiency. The data in Table 20 indicate that the leaves which had high nitrogen content (1.92% of dry weight of leaves), absorbed twice as much of magnesium (% dry wt. basis) as that of leaves having low nitrogen content (2.08%). But high level of nitrogen may have antagonistic effects with some nutrients (P and K).

A P P E N D I C E S

APPENDIX 1

Composition of concentrated nutrient stock solutions for experiments I, II, III, V, VI, VII, and VIII.

Salt	Gms/litre stock solution in jar	For feed soln /litre use	ppm
Major nutrients			
KNO_3	202	2 ccs	K 156, N 57
$Ca(NO_3)_2.4H_2O$	472	2 ccs	Ca 160, N 113
$MgSO_4.7H_2O$	185	2 ccs	Mg 36, S 48
$NaH_2PO_4.2H_2O$	104	2 ccs	Na 31, P 41
Minor nutrients			
* $MnSO_4.4H_2O$	2.23	1 ccs	Mn 0.55
* $CuSO_4.5H_2O$	0.25		Cu 0.064
* $ZnSO_4.7H_2O$	0.29		Zn 0.065
* $H_3 B O_3$	1.86		B 0.33
* $(NH_4)_6 Mo O_{24}.4H_2O$	0.088		Mo 0.048
Ferric Citrate ($Fe C_6H_5O_7.5H_2O$	2.99	10 ccs	Fe 5.6

* These salts were combined as one concentrated nutrient stock solution.

APPENDIX 2

Composition of Concentrated nutrient stock solution for experiment IX

Salt	gms/litre stock solution in jar	For feed soln /litre use	ppm
Major nutrients	202 gms	2 ccs	K 156, N 57
KNO_3	202 gms	2 ccs	K 156, N 57
$CaCl_2$	222 "	2 ccs	Ca160, cl 284
$NH_4\ NO_3$	276	2 ccs	N 193
$Na\ H_2PO_4.2H_2O$	104	2 ccs	Na 31, P 41
$MgSO_4.7H_2O$	185	2 ccs	Mg 36, S 48
Minor nutrients			
* $MnSO_4.4H_2O$	2.23	)	Mn 0.55
* $CuSO_4.5H_2O$	0.25	)	Cu 0.064
* $ZnSO_4.7H_2O$	0.29	)	Zn 0.065
* $H_3\ BO_3$	1.86	) 1 cc	B 0.33
* $(NH_4)_6Mo_7O_{24}.4H_2O$	0.088	)	Mo 0.048
Ferric citrate ($FeC_6H_5O_7.5H_2O$)	2.99	10 cc	Fe 5.6

* These salts were combined as one concentrated nutrient stock solution.

APPENDIX 3

Quantitative micro-determination of magnesium in plant tissue and soil extract.

A rapid colorimetric method.

Reagents

(1) Thiazole yellow 0.10%. Dissolve 0.10 gm of thiazole yellow in 100 ml of distilled water and store in a dark bottle. This reagent will keep at least 2 months under ordinary conditions.

(2) Hydroxylamine Hydrochloride, 5%. Dissolve 25 gms of hydroxylamine Hydrochloride in distilled water and dilute to 500 ml and store in a dark-coloured bottle.

(3) Sodium Hydroxide, 2-5N. Dissolve 100 gms of sodium hydroxide in distilled water and dilute to 1 litre.

(4) Starch Solution, 2%. To 2 gms of C.P. soluble starch add slowly, while stirring, the remainder of 100 ml of hot water. Filter it. This reagent should be prepared freshly as needed.

(5) Compensating Solution: Dissolve 3.7 gms of calcium chloride ($CaCl_2.2H_2O$), 0.74 gram of aluminium sulphate

$Al_2(SO_4)_3.18H_2O$, 0.36 gram of manganous chloride ($MnCl_2.4H_2O$), and 0.60 gram of sodium phosphate (Na_3PO_4) in about 500 ml of water containing 10 ml of concentrated hydrochloric acid. Dilute to 1 litre.

(6) Starch Compensating Reagent - Mix equal volumes of the starch solution and compensating solution, prepare daily as needed.

(7) Standard Magnesium Solution: (25 ppm of magnesium). Dissolve 250 mg of reagent garde magnesium metal in dilute hydrochloric acid solution (150 ml of distilled water plus 10 ml. of concentrated hydrochloric acid) and bring to volume in a 250 ml volumetric flask. Dilute this solution 1 to 40 for the working standard of 25 ppm of magnesium.

Procedure

Ash 2 grams of oven dried samples, dissolve the ash in dilute hydrochloric acid and make up to 100 ml by adding distilled water. A 1 ml aliquot of this solution is satisfactory to cover the range 0.10 to 0.70% of magnesium in the leaves on a dry basis. For smaller or larger concentrations than this, the aliquots should be adjusted accordingly. Transfer the aliquot to a 50 ml volumetric flask and add enough to bring the volume to about 25 ml.

Add 1 ml of the hydroxylamine hydrochloride solution and 5 ml. of starch compensating solution and mix the solution. Add exactly 1 ml of thiazole yellow solution and mix the solution, and then add 5 ml of the sodium hydroxide solution. Bring the flask to volume with distilled water; mix thoroughly the contents, and allow them to stand for 10 minutes before reading on a photo electric colorimeter using a green filter. The colour is stable for several hours. The concentration of magnesium is obtained from a standard curve as described below. An Hitachi 101 spectrophometer with the green filter 530 was used in this work. The colorimeter is set to read 100% transmittance with a blank solution that is run by the same procedure used with the samples and standards.

Standard Curves

When 0, 1, 2, 3, 4 and 5 ml. aliquots of the standard solution of 25 ppm of magnesium were analysed exactly as described above and the readings plotted on semilog graph paper a straight line relationship was obtained. A number of curves in advance, one or two on each sheet of graph paper were prepared in order to use the appropriate curve for each set of determinations.

APPENDIX 4

Nitrogen analysis

Digestion

Weigh accurately about 0.10 gms oven-dried ground sample and transfer to urea tube calibrated at 50 mls. Add approximately 1.4 gms of mixed catalyst and 5 mls of 98% W/W nitrogen-free sulphuric acid.

Place tubes in digestion block, and digest for 60-80 minutes, after samples have cleared. Cool, dilute to 50 mls with distilled water, mix thoroughly and leave to recool.

(Note: 2 reagent blanks, should also be digested).

Distillation:

Add 5 mls of 2% boric acid solution and 4 to 5 drops of mixed indicator to 100 ml receiver flask. Rinse a 5ml. pipette with the solution to be tested and then transfer 5 mls to the jacketted tube of the Markham apparatus. Rinse in with a little distilled water, add 10 mls of strong Na OH solution, rinse twice with distilled water and close top.

Raise the receiver flask until the condenser tube just dips below the surface of the boric acid solution and commence

steaming. Continue distillation for 2 minutes after colour has changed, and 10-15 mls distillate collected.

Lower receiver flask, wash down tube with a little distilled water (Two aliquots of each sample to be distilled).

Tritration

Tritrate against the standard acid to just clear grey, comparing each sample with refrence blank colour.

Reagents

1. Mixed Indicator

Dissolve separately 0.25 gms brom-cresol green in 250 mls of 95% alcohol, and 0.05 gms of methyl red in 50 mls of 95% alcohol (i.e. each solution is 0.1%). Mix 40 mls of brom-cresol-green with 8 mls of methyl red, in bottle with Capillary dropper.

2. 2% Boric acid

Dissolve 40 gms boric acid crystals in 2000 mls boiling distilled water. After cooling transfer to glass-stoppered bottle.

3. Strong NaOH

Dissolve 1000 gms NaOH pellets in 2,300 mls distilled water keeping stirring. Filter through glass wool into rubber stoppered bottle.

4. Catalyst

Mix 10 gms selenium with 500 gms R Potassium Sulphate, using mortar and pestle.

5. Standard acid

Take 8.5 mls conc. AR HCl and made to 100 c.c.
Take 10 cc of this and make to 1 litre (0.010N).

APPENDIX 5

Analysis of variance of the effect of different wetting agents on the uptake of magnesium by leaves.

Source	s.s.	d.f.	M.S.	F.	Result
Treatment	0.02135	3	0.00711	37.85	* *
Replication	0.00155	3	0.00051	2.74	N.S.
Error	0.00170	9	0.00018		
		15	0.02460		

APPENDIX 6

Analysis of variance of the effect different wetting agents used on the uptake of magnesium by leaves, at 80% wettability of the leaf area.

Source	s.s.	d.f.	M.S.	F.	Result
Treatment	0.01515	4	0.00378	12.58	*
Replication	0.00101	3	0.00033	1.12	N.S.
Error	0.00361	12	0.00030		
Total	0.01977	19			

APPENDIX 7

Analysis of variance of the effect of different concentrations of a nonionic wetter (Terric GN9) on the uptake of magnesium by leaves.

Source	s.s.	d.f.	M.S.	F.	Result
Treatment	0.02438	5	.00488	8.13	*
Replication	0.00096	3	.00032	0.53	N.S.
Error	0.00899	15	.00060		
Total	0.03433	23			

APPENDIX 7a

Analysis of variance of the visual assessment of the wetting of the upper surface of the citrus leaves by Terric GN9.

Source	s.s.	d.f.	M.S.	F.	Result
Treatment	12400.00	4	3100.00	90.41	**
Replication	90.00	7	12.86	0.38	N.S.
Error	960.00	28	34.29		
Total	13450.00	39			

APPENDIX 8

Analysis of variance of the effect of different concentrations of glycerine on the uptake of magnesium by leaves.

Source	s.s.	df.	M.S.	F.	Result
Treatment	0.01505	3	0.00501	11.29	*
Replication	0.00215	3	0.00050	1.12	N.S.
Error	0.00400	9	0.00044		
Total	0.02120	15			

APPENDIX 9

Analysis of variance of the effect of humidity on the uptake of magnesium by leaves.

Factor Main Plot	s.s.	df.	M.S.	F.	Result
Replication	0.000619	3	0.000206	2.82	N.S.
Spray and no spray	0.033307	1	0.033307	456.26	* *
Error (a)	0.000218	3	.000073		
Split plot					
Level of humidity	0.003907	1	0.003907	6.93	*
Spray and no spray x level of humidity	0.001405	1	0.001405	2.49	N.S.
Error (b)	0.003384	6	0.000564		
Total	0.042894	15			

APPENDIX 10

Analysis of variance of the effect of spraying at different times of the day.

Source	s.s.	df.	M.S.	F	Result
Treatment	0.07055	4	0.01763	75.37	* *
Replication	0.00101	3	0.00033	1.44	N.S.
Error	0.00281	12	0.00023		
Total	0.07437	19			

APPENDIX 11

Analysis of variance of the rate of uptake of magnesium by leaves.

Source	s.s.	df.	M.S.	F.	Result
Treatment	0.13628	6	0.02271	58.39	* *
Replications	0.00112	3	0.00037	0.96	N.S.
Error	0.00700	18	0.00038		
Total	0.14441	27			

APPENDIX 12

Analysis of variance of the effect of different magnesium salts on the uptake of magnesium by leaves.

Source	s.s.	df.	M.S.	F.	Result
Treatment	0.07887	3	0.02629	93.89	* *
Replication	0.00102	3	0.00034	1.21	N.S.
Error	0.00248	9	0.00028		
Total	0.08237	15			

APPENDIX 13

Analysis of variance of leaf magnesium level on the uptake of magnesium by leaves.

Factor Main plot	s.s.	df.	M.S.	F.	Result
Replication	0.00079	3	0.00025	0.65	N.S.
Spray and no spray	0.15821	1	0.15821	416.34	* *
Error (a)	0.00113	3	0.00038		
Split plot					
Level of magnesium	0.60644	3	0.20215	777.50	* *
Spray and no spray x level of magnesium	0.02693	3	0.00898	34.53	* *
Error (b)	0.00460	18	0.00026		
Total	0.79810	31			

APPENDIX 14

Analysis of variance of the effect of leaf nitrogen level on the uptake of magnesium by leaves.

Factor Main plot	s.s.	df.	M.S.	F.	Result
Replication	0.00157	3	0.00052	0.57	N.S.
Spray and no spray	0.10080	1	0.10080	100.76	* *
Error (a)	0.00272	3	0.00091		
Split Plot					
Level of nitrogen	0.03330	1	0.03330	65.29	* *
Spray and no spray x level of nitrogen	0.00332	1	0.00332	6.50	*
Error (b)	0.00303	6	0.00051		
Total	0.14474	15			

B I B L I O G R A P H Y

Allen, M. 1959. Role of the anion in magnesium uptake from foliar applications of its salts on apple. Nature, 184 : 995.

Allen, M. 1960. The uptake of metallic ions by leaves of apple trees. II. The influence of certain anions on uptake from magnesium salts. J. hort. Sci. 35 : 127-135.

Allen, M. 1964. Uptake of metallic ions by leaves of apple trees. III. The uptake of copper by leaves immersed in solutions of its salts. J. hort. Sci. 39 : 308-324.

Anderson, D.B. 1927. A microchemical study of the structure and development of flax fibres. Am. J. Bot. 14 : 187-212.

Askew, H.O., and E. Chittenden. 1936. The use of borax in the control of internal cork of apples. Part III. Effect of borax sprays on the boron status of fruit and incidence of internal cork in apples. J. Pomol. 14 : 242-245.

Badmin, P.A., 1966. The control of shoot dessication (Russian) Sadovodstvo, No. 3, p. 18 in Hort. Abstr. 36 : 6087

Barrier, G.E., and W.E. Loomis. 1957. Absorption and translocation of 2,4-dichlorophenoxyacetic acid and P^{32} by leaves. Pl. Physiol. 32 : 225-231.

Batzer, L.P., B.L. Rogers, and A.H. Thomson. 1952. Fertilizer applications as related to nitrogen, phosphorus, potassium,

calcium and magnesium utilization by apple trees. Proc. Am. Soc. hort. Sci. 60 : 1-6

Bennett, S.H., and W.D.E. Thomas. 1954. Absorption, translocation and breakdown of schradan applied to the leaves, using P^{32} lavelled material. II. Evaporation and absorption. Ann. appl. Biol. 41 : 484-500.

Beutal, J.A. 1964. Magnesium nitrate sprays proving effective. Int. Fert. Corresp., reprinted in Kenya Coffee, 29 : 71-73 in Hort. Abstr. 34 : 5558.

Bhan, K.C., A. Wallace, and O.R. Lunt. 1959. Some mineral losses from leaves by leaching. Proc. Am. Soc. hort. Sci. 73: 289-293

Bingham, F.T. 1963. Magnesium nutrition of avocado under sand culture conditions. Proc. Soil Sci. Soc. Am. 27: 58-61

Blackman, G.E. 1952. Studies in the principles of phytotoxicity. J. exp. Bot. 3: 1-27.

Boynton, D. 1945. Studies on control of magnesium deficiency in New York apple orchards. Proc. Am. Soc. hort. Sci. 46: 1-5.

Boynton, D., and O.C. Compton. 1944. The influence of differential fertilization with ammonium sulphate on the chemical composition of McIntosh apple leaves. Proc. Am. Soc. hort. Sci. 45: 9-17.

Boynton, D., J.C. Cain, and J. Van Geluwe. 1943. Incipient magnesium deficiency in some New York apple orchards. Proc. Am. Soc. hort. Sci. 42: 95-100.

Bramlage, W.J., and A.H. Thomson 1962. The effects of early season sprays of boron on fruit set, colour, finish, and storage life of apples. Proc. Am. Soc. hort. Sci. 80: 64-71.

Buckovac, M.J., F.G. Teubner, and S.H. Wittwer. 1960. Absorption and mobility of magnesium in the bean (P.Vulgaris. L.). Proc. Am. Soc. hort. Sci. 75: 429-434.

Burrell, A.B., and D. Boynton. 1943. Response of apple trees to potash in the Chaplain Valley. III. Proc. Am. Soc. hort. Sci. 42: 61-64.

Cain, J. 1956. Absorption and metabolism of the urea by leaves of coffee, cocao and banana. Proc. Am. Soc. hort. Sci. 67: 279-286.

Cain, J.C. 1953. The effect of nitrogen and potassium fertilizers on the performance and mineral composition of apple trees. Proc. Am. Soc. hort. Sci. 62: 53-66.

Cain, J.C., and D. Boynton. 1948. Some effect of season fruit crop and nitrogen fertilization on the mineral composition of McIntosh apple leaves. Proc. Am. Soc. hort. Sci. 51: 13-22.

Cameron, B.H., R.T. Mueller; A. Wallace, and E. Sartori. 1952. Influence of age of leaf, season of growth, and fruit production on the size and inorganic composition of Valencia orange leaves. Proc. Am. Soc. hort. Sci. 60: 42-50.

Camp, A.F. 1947. Magnesium deficiency in citrus. Soil Sci. 63: 43-51.

Camp, A.F., and M. Peach. 1938. Manganese deficiency in citrus in Florida. Proc. Am. Soc. hort. Sci. 36: 81-85.

Cheng, K.L., and R.H. Bray. 1951. Determination of calcium and magnesium in soil and plant material. Soil Sci. 72: 449-458.

Chuka, J.A., J.H. Waring, and O.L. Wyman. 1945. Magnesium deficiency in main apple orchards. Proc. Am. Soc. hort. Sci. 46: 13-14.

Conibear, D.I., and C.G.L. Furmidge. 1965. Physio-chemical studies on agricultural sprays. VII. The visual assessment of spray coverage. J. Sci. Fd Agric. 16: 144-149.

Cook, J.A., and D. Boynton. 1952. Some factors affecting the absorption of urea by McIntosh apple leaves. Proc. Am. Soc. hort. Sci. 59: 82-90.

Crafts, A.S., and C.L. Foy. 1962. The chemical and physical nature of plant surfaces in relation to the use of pesticides

and to their residues. pp. 112-139 in Residue reviews. Vol. I. Academic Press, Inc., New York.

Currier, H.B., and C.D. Dybing. 1959. Foliar penetration of herbicides. Review and present status. Weeds. 7: 195-213

Curtis, O.F., and D.G. Clark. 1950. An introduction to plant physiology. 1st ed., pp. 329 - 334 McGraw-Hill, New York.

Darlington, W.A., and N. Circulis. 1963. Permeability of apricot leaf cuticle. Pl.Physiol. 38: 462-467.

de Villiers, J.I. 1942. Trace element deficiencies in citrus. Results obtained in Eastern Transval. Fmg. S. Afr. 17: 337-340.

Dhingra, D.R., and Others. 1966. Effect of different concentrations of zinc sulphate sprays on the influence of citrus chlorosis. Punjab. hort. J. 6: 83-87 in Hort. Abstr. 37: 5693

Dorschner, K.P., and K.P. Buchholtz. 1956. Wetting ability of aqueous herbicidal sprays as a factor influencing stands of alfalfa seedlings. Agron. J. 48: 59-63.

Drosdoff, M., and D.C. Nearpass. 1948. Quantitative micro-determination of magnesium in plant tissues and soil extracts. A rapid colorimetric method. Analyt. Chem. 20: 673-674.

Dunne, T.C. 1938. Wither tip or summer dieback a copper deficiency disease of apple trees. J. Agric. West Aust. 15: 120-126.

Dybing, C.D., and A.B. Currier. 1961. Foliar penetration by chemicals. Pl. Physiol. 36: 169-174.

Embleton, T.W., and W.W. Jones. 1959. Correction of magnesium deficiency of orange trees in California. Proc. Am. Soc. hort. Sci. 74: 280-288.

Embleton, T.W., J.D. Kirkpatric, W.W. Jones, and C.B. Cree. 1956. Influence of application of dolomite, potash, and phosphate on yield and size of fruit and on composition of leaves of Valencia orange trees. Proc. Am. Soc. hort. Sci. 67: 183-190.

Ennis, JR., W.B., and F.T. Boyd. The response of kidney bean and soybean plants to aqueous spray application of 2,4 dichlorophenoxyacetic acid with and without carbowax. Bot. Gaz. 107: 552-559.

Ennis, JR., W.B., R.E. Williamson, and K.P. Dorschner. 1952. Studies on spray retention by leaves of different plants. Weeds 1: 274-286.

Federer, W.T. 1955. Experimental design, pp. 26-28. MacMillan, New York.

Fisher, E.G. 1952. The principles underlying foliage applications of urea for nitrogen fertilization of the McIntosh apple. Proc. Am. Soc. hort. Sci. 59: 91-98.

Fisher, E.G., D. Boynton, and K. Skodvin. 1948. Nitrogen fertilization of the McIntosh apple with leaf sprays of urea. Proc. Am. Soc. hort. Sci. 51: 23-32.

Fisher, E.G., and J.A. Cook. 1950. Nitrogen fertilization of the McIntosh apple with leaf sprays of urea. II. Proc. Am. Soc. hort. Sci. 55: 35-40.

Fisher, E.G., and D.R. Walker. 1955. The apparent absorption of phosphorus and magnesium from sprays applied to the lower surface of McIntosh apple leaves. Proc. Am. Soc. hort. Sci. 65: 17-24.

Fogg, G.E. 1947. Quantitive studies on the wetting of leaves by water. Proc. R. Soc. (London). Ser. B. 134: 503-522.

Fogg, G.E. 1948. The penetration of 3:5 dinitro-o-cre-sol into leaves. Ann. appl. Biol. 35: 315-330.

Ford, E.M. 1964. The control of magnesium deficiency in apple rootstock stool beds. J. hort. Sci. 39: 212-223.

Ford, E.M. 1966. Studies in the nutrition of apple root stocks. III. Effects of level of magnesium nutrition on growth form and mineral composition. Ann. Bot. N.S. 30: 639-655.

Ford, E.M. 1967. Studies in the nutrition of apple rootstocks. IV. Effect on growth and mineral composition of supplying magnesium through leaves. Ann. Bot. N.S. 31: 113-119.

Ford, E.M. 1968. The development of magnesium deficiency symptoms in relation to magnesium supply. Ann. Bot. N.S. 32: 45-56.

Ford, E.M., G.C. White, and M. Allen. 1965. The response of magnesium deficient Edward VII apple trees to variations in the timing and composition of foliar sprays. J. hort. Sci. 40: 351-360

Forshey, C.G. 1959. The effect of nitrogen status of magnesium deficient McIntosh apple trees on response to Epsom salts sprays. Proc. Am. Soc. hort. Sci. 73: 40-45.

Forshey, C.G. 1963. The effect of nitrogen status of McIntosh apple trees in sand culture on the absorption of magnesium from Epsom salts spray. Proc. Am. Soc. hort. Sci. 83: 21-31.

Foy, C.L., and L.W. Smith. 1965. Surface tension lowering, wettability of paraffin and corn leaf surfaces and herbicidal enhancement of Dalapon by seven surfactants. Weeds 13: 15-19.

Foy, C.L., W. Whitworth, T.J. Muzik, and H.B. Currier. 1967. The penetration, absorption, and translocation of herbicides, pp. 3-16. in Freed V.H., and R.O. Morris, ed., Environmental

and other factors in the response of plants to herbicides. Stn. tech. Bull. 100, Ore. agric. Exp. Stn.

Franke, W. 1961. Ectodesmata and foliar absorption. Am. J. Bot. 48: 683-691.

Freed, V.H., and M. Montgomery. 1958. The effect of surfactants on foliar absorption of 3-amino-1, 2, 4-triazole. Weeds 6: 386-389.

Frey-Wyssling, A., and K. Muhlethaler. 1965. Ultra structure plant cytology, pp. 138-157 Elsevier, Amsterdam.

Furmidge, C.G.L. 1959. Phsio-chemical studies on agricultural sprays. II. The phytotoxicity of surface active agents on leaves of apple and plum trees. J. Sci. Fd Agric. 10: 274-282.

Ganje, T.J., A.L. Page, and J.P. Martin. 1966. Foliar application of potassium nitrate. Calif. Citogr. 51: 493-496, 500-501.

Garner, W.W., J.E. McMurtrey, JR., J.D. Bowling, JR., and E.G. Moss. 1930. Magnesium and Calcium requirements of the tobacco crop. J. agric. Res. 40: 145-168.

Gillam, W.S. 1941. A photometric method for the determination of magnesium. Ind. Engng Chem. analyt. Edn. 13: 499-501.

Goodman, R.N., and S.K. Addy. 1962. Penetration of excised apple cuticle by radioactive organic and inorganic compounds. Phytopath. 52: 11.

Gray, R.A. 1956. Increasing the absorption of streptomycin by leaves, flowers with glycerol. Phytopath. 46: 105-111.

Guest, P.L., and H.D. Chapman. 1949. Investigations on the use of iron sprays dusts and soil applications to control iron chlorosis of citrus. Proc. Am. Soc. hort. Sci. 54: 11-21.

Gustafson, F.G. 1956. Absorption of Co^{60} by leaves of young plants and its translocation through the plant. Am. J. Bot. 43: 157-160.

Gustafson, F.G. 1957. Comparative absorption of cobalt^{60} by upper and lower epidermis of leaves. Pl. Physiol. 32: 141-142.

Gustafson, F.G., and M.J. Schlessinger, JR. 1956. Absorption of cobalt-60 by leaves of bean plants in the dark. Pl. Physiol. 31: 316-318.

Haas, A.R.C. 1948. Magnesium deficiency and its effects on citrus. Calif. Citogr. 33: 134, 146-148, 150.

Hagler, T.B. 1957. Effect of magnesium sprays on Mucadine grapes. Proc. Am. Soc. hort. Sci. 70: 178-182.

Hall, D.M., and L.A. Donaldson. 1962. Secretion from pores of surface wax on plant leaves. Nature, 194: 1196.

Halma, F.F. 1929. Quantitative difference in palisade tissue in citrus leaves. Bot. Gaz. 87: 319-324.

Hamilton, J.M., D.H. Palmiter and L.C. Anderson. 1943. Preliminary tests with uramon in foliage sprays as a means of regulating the nitrogen supply in apple trees. Proc. Am. Soc. hort. Sci. 42: 123-126.

Harley, C.P., H.H. Moon, and L.O. Regeimbal. 1957. Effect of the additive Tween 20 and relatively low temperatures on apple thinning by naphthaleneacetic acid sprays. Proc. Am. Soc. hort. 69: 21-27.

Hewitt, E.J. 1966. Sand and water culture methods used in the study of plant nutrition. pp. 431-432. East Malling. Tech. Commun. Commonw. Bur. Hort. Plantn Crops.

Hilgeman, R.H. 1969. Correction of iron chlorosis with iron chelates in Arizona. Calif. Citogr. 54: 406, 426-428.

Hilkenbaumer, F., and G. Hohmann. 1964. The effects of urea sprays on the performance of apple spindle bushes. ErwObstb., 6: 101-105 in Hort. Abstr. 35: 2771.

Hoffman, M., and R.M. Samish. 1966. The control of zinc

deficiency in apples. Israel J. agric. Res. 16: 105-114. in Hort. Abstr. 37: 2335.

Holly, K. 1956. Permeation of chlorinated phenoxyacetic acids into leaves. Ann. appl. Biol. 44: 195-199.

Hughes, R.E., and V.H. Freed. 1961. The role of surfactants in the foliar absorption of indole-3-acetic acid (IAA) Weeds 9: 54-59.

Jacobson, L. 1945. Iron in the leaves and chloroplasts of some plants in relation to their chlorophyll content. Pl. Physiol. 20: 233-245.

Jansen, L.L., W.A. Gentner, and W.C. Shaw. 1961. Effects of surfactants on the herbicidal activity of several herbicides in aqueous spray systems. Weeds 9: 381-405.

Jensen, W.A. 1967. The plant cell, pp. 9-24. MacMillan, London.

Johnson, M.O. 1924. The influence of manganese on growth of pineapples. Hawaii agric. Exp. Stn. Bull. 52, pp. 1-14.

Jyung, W.H., and S.H. Wittwer. 1964. Foliar absorption an active uptake. Am. J. Bot. 51: 437-444.

Jyung, W.H., S.H. Wittwer, and M.J. Bukovac. 1965. Ion uptake by cells enzymically isolated from green tobacco leaves. Pl. Physiol. 40: 410-414.

Kamimura, S., and R.N. Goodman. 1964. Evidence of an energy requirement for absorption of carbon^{14} labelled streptomycin and leucine by apple leaves. Phytopath. 54: 1467-1474.

Kidson, E.B. 1946. A method for determining small amounts of magnesium in plant materials by means of titan yellow. N.Z. Jl. Sci. Technol. (A) 27: 411-413.

Kieley, T. 1966. Exanthema of citrus. Agric. Gaz. N.S.W. 77: (5) 289-292.

Koontz, H., and O. Biddulph. 1957. Factors affecting absorption and translocation of foliar applied phosphorus. Pl. Physiol. 32: 463-470.

Labanauskas, C.K. 1962. Correction of manganese deficiency in grapefruit trees by foliar sprays in desert areas of Southern California. Proc. Am. Soc. hort. Sci. 80: 268-273.

Labanauskas, C.K. 1966. Effects of orange leaf washing techniques on removal of surface contaminants and nutrient losses. Proc. Am. Soc. hort. Sci. 89: 201-205.

Labanauskas, C.K., and R.E. Puffer. 1964. Effects of foliar applications of manganese and zinc and urea on Valencia orange yield and foliage composition. Proc. Am. Soc. hort. Sci. 84: 158-164.

Lee, B. 1925. The plant cuticle. II. A microchemical study. Ann. Bot. N.S. 39: 755-768.

Lee, B.W. 1964. Copper deficiency in Venturia citrus. Calif. Citogr. 49: 327-328

Lee, B., and J.H. Priestly. 1924. The plant cuticle. I. Its structure, distribution, and function. Ann. Bot. N.S. 38: 525-545.

Legg, V.H., and R.V. Wheeler, 1925. Plant cuticles. I. Modern plant cuticles. J. chem. Soc. 127: 1412-1421.

Leonard, D.A. 1958. Studies on the absorption and translocation of 2,4-D in bean plants. Hilgardia 28: 115-160.

Leonard, D.A., and A.S. Crafts. 1956. Translocation of herbicides. III. Uptake and distribution of radio-active 2,4-D by brush species. Hilgardia 26: 366-415.

Lott, W.L. 1948. Magnesium injection in Mucadine grape vines. Proc. Am. Soc. hort. Sci. 52: 283-287.

Lott, W.L. 1952. Magnesium deficiency in Mucadine grape vines. Proc. Am. Soc. hort. Sci. 60: 123-131.

Ludders, P., and G. Bunemann. 1967. The possibilities and limits for post harvest and winter spraying of pome fruit

trees with urea. ErwObstb., 9: 6-9 in Hort. Abstr. 37: 4377.

Mann, C.E., and T. Wallace. 1924. The effects of leaching with cold water on the foliage of the apple. J. Pomol. 4: 146-161.

McColloch, R.C., F.T. Bingham, and D.G. Aldrich. 1957. Relation of soil potassium and magnesium of citrus. Proc. Soil Sci. Soc. 21: 85-88.

McWhorter, C.G. 1963. Effects of surfactants on the herbicidal activity of foliar sprays of diuron. Weeds 11: 265-269.

Middleton, L., and J. Sanderson. 1965. The uptake of inorganic ions by plant leaves. J. exp. Bot. 16: 197-215.

Mikkelsen, D.S., and S.J. Toth. 1947. Thiazole yellow for determining the magnesium content of the soil extracts. J. Am. Soc. Agron. 39: 165-166.

Miller, E.C. 1938. Plant Physiology. 2nd ed., pp. 1 - 24. McGraw-Hill, New York.

Minshall, H., and V.A. Helson. 1949. The herbicidal action of oils. Proc. Am. Soc. hort. Sci. 53: 294-298.

Nagai, K. and others. 1966. Studies on magnesium deficiency of apple trees. I. Injection and foliar application of magnesium for the diagnosis and control of magnesium deficiency.

J. Jap. Soc. hort. Sci., 35: 207-217. in Hort. Abstr. 37: 6300.

Neales, T.F. 1958. The movement of leaf magnesium during the growth of white clover plants. Aust. J. biol. Sci. 11: 343-348.

Nicholas, D.J.D. 1948. Experiments on correcting magnesium deficiency in glasshouse tomatoes. J. Pomol. 24: 1-18.

Norman, A.G., C.E. Minarik, and R.L. Weintraub. 1950. Herbicides. A. Rev. Pl. Physiol. 1: 141-168.

Oland, K., and T.B. Opland. 1956. Uptake of magnesium by apple leaves. Physiologia Pl. 9: 401-411.

Orgell, W.H. 1955. The isolation of plant cuticle with enzymes. Pl. Physiol. 30: 78-80.

Parker, E.R. 1937. Effect of zinc application on the crop of grapefruit trees affected with mottle leaf. Hilgardia 11: 35-53.

Parker, E.R., and R.W. Southwick. 1941. Manganese deficiency in citrus. Proc. Am. Soc. hort. Sci. 39: 51-58.

Parr, J.F. and A.G. Norman. 1965. Considerations in the use of surfactants in plant systems: A review. Bot. Gaz. 126: 86-96.

Paulechova Kralikova, K. 1966. Contribution to the study of little leaf of apples. Biologia, Bratisl. 21: 356-364 in Hort. Abstr. 37: 349.

Peach, M., and L. English. 1944. Rapid microchemical soil tests. Soil Sci. 57: 167-195.

Phillis, E., and T.G. Mason. 1942. On diurnal variations in the mineral content of the leaf of the cotton plant. Ann. Bot. N.S. 6: 437-442.

Reed, H.S., and E. Hirano. 1931. The density of stomata in citrus leaves. J. agric. Res. 43: 209-222.

Reed, H.S., and E.R. Parker. 1936. Specific effects of zinc applications on leaves and twigs of orange trees affected with mottle leaf. Jour. agric. Res. 53: 395-398.

Reitz, H.J., C.D. Leonard, J.W. Sites, W.F. Spencer, I. Stewarts, and I.W. Wander. 1954. Recommended fertilizers and nutritional sprays for citrus. Fla agric. Exp. Stn. Bull. 536, pp.1-15.

Reuther, W., and P.F. Smith. 1950. A preliminary report on the relation of nitrogen, potassium, and magnesium fertilization to yield, leaf composition, and the incidence of zinc deficiency in oranges. Proc. Am. Soc. hort. Sci. 56: 27-33.

Reuther, W., and P.F. Smith. 1952. Relation of nitrogen, potassium, and magnesium fertilization to some fruit qualities of Valencia orange. Proc. Am. Soc. hort. Sci. 59: 1-12.

Rice, E.L. 1948. Absorption and translocation of ammonium 2,4 dichlorophenoxyacetate by bean plants. Bot. Gaz. 109: 301-314.

Richards, F.J. 1932. Physiological studies in plant nutrition. III. Further studies of the effect of potash deficiency on the rate of respiration in leaves of barley. Ann. Bot. N.S. 46: 367-388.

Ritter, C.M. 1956. Effects of varying rates of nitrogen fertilization on young Elberta trees. Proc. Am. Soc. hort. Sci. 68: 48-53.

Roberts, E.A., M.D. Southwick, and D.H. Palmiter. 1948. A microchemical examination of McIntosh apple leaves showing relation of cell-wall constituents to penetration of spray solutions. Pl. Physiol. 23: 557-559.

Rodney, D.R. 1952. Entrance of nitrogen compounds through the epidermis of apple leaves. Proc. Am. Soc. hort. Sci. 59: 99-102.

Rohrbaugh, P.W. 1934. Penetration and accumulation of petrolium spray oils in the leaves, twigs and fruit of citrus trees.

Pl. Physiol. 9: 699-730.

Ruck, V.C., and F.G. Gregory. 1955. Mobility of manganese, magnesium and potassium in leaf tissues. Nature, 175: 378.

Sargent, J.A., and G.E. Blackman. 1962. Studies on foliar penetration. I. Factors controlling the entry of 2,4-dichlorophenoxyacetic acid. J. exp. Bot. 13: 343-368.

Sargent, J.A., and G.E. Blackman. 1965. Studies on foliar penetration. II. The role of light in determining the penetration of 2,4-dichlorophenoxyacetic acids. J. exp. Bot. 16: 24-47.

Scott, F.M. 1950. Internal suberization of tissues. Bot. Gaz. 111: 378-394.

Scott, L.E., and D.H. Scott. 1951. Response of grapevines to soil and spray applications of magnesium sulphate. Proc. Am. Soc. hort. Sci. 57: 53-58.

Scott, F.M., M.R. Schroeder, and F.M. Turrell. 1948. Development, cell shape, suberization of internal surface and abscission in the leaf of the Valencia orange, Citrus sinensis. Bot. Gaz. 109: 381-410.

Shieferstein, R.H., and W.E. Loomis. 1959. Development of the cuticular layers in the angiosperm leaves. Am. J. Bot. 46: 625-635.

Silberstein, O., and S.H. Wittwer. 1951. Foliar application of phosphatic nutrients to vegetable crops. Proc. Am. Soc. hort. Sci. 58: 179-190.

Silva Fernandes, A.M.S. 1965. Studies on plant cuticle. IX. The permeability of isolated cuticular membranes. Ann. appl. Biol. 56: 305-313.

Sivadjian, J. 1956. Action of glycerol on the transpiration of leaves and on the cuticular permeability. Rend, Accd. Sci. 242: 2478-2479 in Chem. Abstr. 50: 14891.

Skoss, J.D. 1955. Structure and composition of plant cuticle in relation to environmental factors and permeability. Bot. Gaz. 117: 55-72.

Smith, P. 1966. Leaf analysis of citrus, pp. 208-288 in Childers, N.F., ed., Temperate to Tropical Fruit Nutrition. Rutgers - State University, New Brunswick, New Jersey.

Smith, P.F., W. Reuther, and A.W. Specht. 1950. Mineral composition of chlorotic orange leaves and some observations on the relation of sample preparation techniques to the interpretation of results. Pl. Physiol. 25: 496-505.

Smith, P.F., W. Reuther, A.W. Specht, and G. Harnciar. 1954. Effect of differential nitrogen, phosphorus, and magnesium supply to young Valencia orange trees in and culture on mineral

composition especially of leaves and roots. Pl. Physiol. 29: 349-355.

Southwick, L., and J.K. Shaw. 1944. Some results in correcting magnesium deficiency in apple orchards. Proc. Am. Soc. hort. Sci. 44: 8-14.

Southwick, L., and G.F. Smith. 1945. Further data on correcting magnesium deficiency in apple orchards. Proc. Am. Soc. hort. Sci. 46: 6-12.

Stevens, N.E. 1932. Thickness of cuticle in Cranberry fruits. Am. J. Bot. 19: 432-435.

Strauss, G.F. 1963. Magnesium nitrate sprays proving effective. Calif. Citogr. 48: 299.

Sutcliffe, J.F. 1962. Mineral salts absorption in plants. pp. 96-106. Oxford, Pergamon Press.

Swanson, G.A., and J.B. Whitney, JR. 1953. Studies on the translocation of foliar-applied P^{32} and other radioisotopes in bean plants. Am. J. Bot. 40: 816-823.

Tarasov, V.M., and V.F. Kovalenko. 1967. Dissication of apple shoots. Sadovodstvo, No 3, 18-20 in Hort. Abstr. 37: 6296.

Taylor, G.A. 1956. The effectiveness of five cleaning procedures in the preparation of apple leaf samples for analysis. Proc. Am. Soc. hort. Sci. 67: 5-9.

Teubner, F.G., S.H. Wittwer., W.G. Long, and H.B. Tukey. 1957. Some factors affecting absorption and transport of foliar applied nutrients as revealed by radio-active isotopes. Mich. agric. Exp. Stn. Q.B. 39: 398-415.

Thorne, G.N. 1958. Factors affecting uptake of radioactive phosphorus by leaves and its translocation to other parts of the plant. Ann. Bot. N.S. 22: 381-398.

Titus, J.C., and D. Boynton. 1953. The relationship between soil analysis and leaf analysis in eighty New York McIntosh apple orchards. Proc. Am. Soc. hort. Sci. 61: 6-26.

Tukey, JR., H.B., H.B. Tukey, and S.H. Wittwer. 1959. Loss of nutrients by foliar leaching as determined by radioisotopes. Proc. Am. Soc. hort. Sci. 71: 496-506.

Turrell, F.M. 1947. Citrus leaf stomata: Structure, composition, and pore size in relation to penetration of liquids. Bot. Gaz. 108: 476-487.

Uriu, K., and E.C. Koch. 1964. Response of yellow Newtown apple leaves to foliar application of manganese and zinc. Proc. Am. Soc. hort. Sci. 84: 25-31.

van Overbeek, J. 1956. Absorption and translocation of plant regulators. A. Rev. Pl. Physiol. 7: 355-369.

van Overbeek, J., and R. Blondeau. 1954. Mode of action of phytotoxic oils. Weeds 3: 55-65.

Vickery, R.S. and F.V. Mercer. 1964. The uptake of sucrose by bean leaf tissue. Aust. J. biol. Sci. 17: 338-347.

Victor Voth, K. Uriu., and R.S. Bringhurst. 1967. Effect of high nitrogen applications on yield, earliness, fruit quality, and leaf composition of California strawberries. Proc. Am. Soc. hort. Sci. 91: 249-256.

Volk, R., and C. McAuliffe. 1954. Factors affecting the foliar absorption of N^{15} labelled urea by tobacco. Proc. Soil Sci. Am. 18: 308-312.

Wallace. A., R.T. Mueller, and M.G. Squier. 1952. Variability in orange leaves of the same age and collected from a single tree. Proc. Am. Soc. hort. Sci. 60: 51-54.

Wallihan, E.F., and L. Haymann-Herschberg. 1956. Some factors affecting absorption and translocation of zinc in citrus plants. Pl. Physiol. 31: 294-299.

Weaver, R.J., and H.R. DeRose. 1946. Absorption and translocation of 2,4-dichlorophenoxyacetic acid. Bot. Gaz. 107: 509-521.

Weber, A. 1966. Magnesium deficiency. III. Experiments and investigations during 1958-1962. Tidsskr. Planteavl, 69: 494-510 in Hort. Abstr. 37: 37.

Webber, H.J., and L.D. Batchelor. 1946. The citrus industry. Vol. I, pp. 671-675. Univ. Calif. Press. Berkeley and Los Angeles.

Weeks, W.D., F.W. Southwick, Mack Drake, and J.E. Stockel. 1952. The effect of rates and sources of nitrogen, phosphorus and potassium on the mineral composition of McIntosh foliage and fruit colour. Proc. Am. Soc. hort. Sci. 60: 11-21.

Weeks, W.D., F.W. Southwick, Mack Drake, and J.E. Stockel. 1958. The effect of varying rate of nitrogen and potassium on the mineral composition of McIntosh foliage and fruit colour. Proc. Am. Soc. hort. Sci. 71: 11-19.

Weinberger, S.H., V.E. Prince, and L. Harris. 1949. Tests on foliar fertilization of peach trees with urea. Proc. Am. Soc. hort. Sci. 53: 26-28.

Went, F.W., and M. Carter. 1948. Growth response of tomato plants to applied sucrose. Am. J. Bot. 35: 95-106.

White, E.P., F.B. Thompson, and N. Brice. 1948. Application of the Dumas micro method to pasture nitrogen analysis. Analyst. 73: 146-148.

Wittwer, S.H., and F.G. Teubner. 1959. Foliar absorption of nutrients. A. Rev. Pl. Physiol. 10: 13-32.

Woodbridge, C.G. 1955. Magnesium deficiency in apple in British-Colombia. Can. J. agric. Sci. 35: 350-357.

Wylie, R.B. 1943. The role of the epidermis in foliar organizations and its relations to the minor variation. Am. J. Bot. 30: 273-280.

Yamada, Y., S.H. Wittwer, and M.J. Bukovac. 1964. Penetration of ions through isolated cuticles. Pl. Physiol. 39: 28-32.

Yamada, Y., M.J. Bukovac, and S.H. Wittwer. 1964. Ion binding by surfaces of isolated cuticular membranes. Pl. Physiol. 39: 978-982.

Yamada, Y., S.H. Wittwer, and M.J. Bukovac. 1965. The effects of urea on ion penetration through isolated cuticular membranes and ion uptake by leaf cells. Proc. Am. Sci. hort. Sci. 87: 429-432.

Zukel, J.W., A.E. Smith, G.M. Stone, and M.E. Davies. 1956. Effect of some factors on the rate of absorption of maleic hydrazide. Pl. Physiol. 31 : (Suppl.) xxi.