

**Evaluation of the aerial application of micronutrients and adjuvants to  
citrus orchards**

**by**

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## DECLARATION 1: PLAGIARISM

I, Johannes Petrus Strydom, declare this as my own work, except where secondary material is used, and it has however been duly acknowledged. I also certify that no plagiarism was done in compiling this thesis.

A handwritten signature in black ink, appearing to read 'J.P. Strydom', is written over a horizontal line.

Signed: Johannes Petrus Strydom

Date: 14 December 2023

## ABSTRACT

Large farming operations make use of conventional tractor sprayers that spray high volumes of water. This is done to increase coverage and uptake of the active ingredient that is sprayed. However, time is of the essence to make applications at critical phenological growth stages of the citrus trees in order to make the micronutrient application as effective as possible. Due to the need for timeous applications, large farms make use of aerial applications that are more time efficient with lower volumes of water used. Thus, a trial was initiated in collaboration with Villa Crop Protection and the Citrus Research International (CRI) to evaluate the aerial application of micronutrients, and to test the value of an added adjuvant (Masterlock®) to improve application efficacy and nutrient uptake.

An experimental citrus orchard of McClean Valencia's was selected at Letaba Estates in the Limpopo province, and foliar Zinc (MAX-IN™ ZINC) and Boron (MAX-IN™ BORON) products were applied, as well as a patented product Masterlock®. The MAX-IN™ range consists of a surfactant and humectant to increase leaf uptake of the micronutrient, Masterlock® enhances droplet distribution throughout the canopy, as well as increasing the uptake of applied compounds through the leaf surface. The trial was carried out over two years, with micronutrient applications conducted in September of 2019 and 2020. Leaf analyses to quantify total B and Zn concentrations were conducted on leaf samples before application and two weeks after application. Droplet deposition was quantified by using two methods: 1. Water sensitive paper and 2. Dropsight. Deposition measured throughout the canopy to ascertain the efficacy of Masterlock® to improve distribution and wetting levels of the spray mixture. Fruit set was determined for both seasons as well as fruit size and brix for the 2019 season.

The aerial application of micronutrients significantly increased the total foliar nutrient concentration in the leaf. Micronutrients can be used to maintain the micronutrient nutrition status of trees throughout the growing season if multiple aerial or conventional applications are made. Compared to the producer's prescribed programme, where products were applied at a much lower concentration, the MAX-IN™ products, applied at a higher concentration were not more effective at increasing the foliar total B and Zn concentration when applied either aurally or conventionally. Both the MAX-IN™ product range and the farm practice were able to

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increase total Zn and B concentrations inside the leaf within the prescribed norms. Masterlock® significantly increased the droplet deposition and total wetted surface area throughout the tree canopy for aerial applications compared to the treatment where no Masterlock® was included. The better distribution throughout the canopy can be explained by an increase in deposition onto the leaf surface.

This study conclusively established that aerial application of foliar nutrients such as B and Zn are effective in citrus and can be used successfully. Masterlock® an adjuvant that consists of drift control characteristics and a surfactant will significantly improve the deposition of the spray mixture of aerially applied nutrient mixtures.

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## CHAPTER 1: INTRODUCTION

### **1.1 Rationale for the research of aerial applications of micronutrients in citrus orchards**

Large farming operations make use of conventional tractor sprays when applying micronutrients, this is because of the high volumes of water that can be sprayed onto citrus trees to ensure good coverage and uptake of the active ingredient. However, there are various constraints to this, i.e., there is a lot of traffic in the orchards, it is labour intensive, and applications at the critical phenological growth stages are often missed because this method of application is not time efficient. Consequently, some large farming operations have resorted to aerial applications to ensure that they are able to apply micronutrients to their trees in a timeous manner. However, the efficacy of these applications is questioned especially considering an adjuvant is seldom used and the water volume used is relatively low. It therefore needs to be determined if the current farm practice is effective and if the use of an adjuvant, which improves droplet adhesion, droplet size, reduces bounce off and increases droplet spreading will increase efficacy of aerial applications of micronutrients. These aerial applications of micronutrients consist of low water volumes that are sprayed per hectare, and since these micronutrients must be taken up by the leaf, good coverage and deposition is of critical importance for the efficacy of these applications.

### **1.2 Justification**

If aerial applications prove to be effective, this will enable large farming operations to reduce tractor traffic in orchards to avoid mechanical damage to fruit and to enable them to make applications at critical phenological stages of the citrus trees. An application at the ideal phenological growth stage will increase fruit set, quality, and yield. Boron (B) and zinc (Zn) ideally needs to be sprayed at pre-bloom, full bloom and fruit set. Not only can vast areas also be sprayed in a very short period, but aerial applications will also be less costly and reduce pressure on the labour force. This project aimed to establish whether aerial applications of foliar micronutrients are viable and effective. It also aimed to determine the effectiveness of various commercial foliar micronutrient products and adjuvants to increase efficacy of micronutrient uptake. There is limited data available regarding aerial applications of micronutrients, there is data present in tea, maize and wheat but is not compared to conventional application methods. These crop canopies are also a lot smaller than citrus trees, and should not be compared.

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### **1.3 Hypotheses**

1. Aerial application of micronutrients is equally or more effective than standard conventional tractor foliar nutrient applications, provided an adjuvant is added that increases canopy penetration by increasing droplet size and wetting of leaf surfaces.
2. Uptake of foliar applied micronutrients will increase as the area of leaf wetted by the solution increases.
3. Efficacy of nutrient uptake will be improved when a high-quality micronutrient formulation is applied, which consist of a surfactant as well as a humectant for both aerial and conventional tractor application methods.

### **1.4 Aims**

1. To establish to what extent canopy penetration, leaf surface coverage and foliar nutrient uptake of spray mixture is improved by Masterlock® for an aerial application of foliar micronutrients.
2. To determine if uptake is improved by utilizing a surfactant and humectant in the foliar micronutrient products in both conventional tractor and aerial applications.
3. Establish to what extent high concentration foliar micronutrient formulations increase concentration inside the leaf compared to low concentration formulations.
4. Establish if aerial applications are feasible.

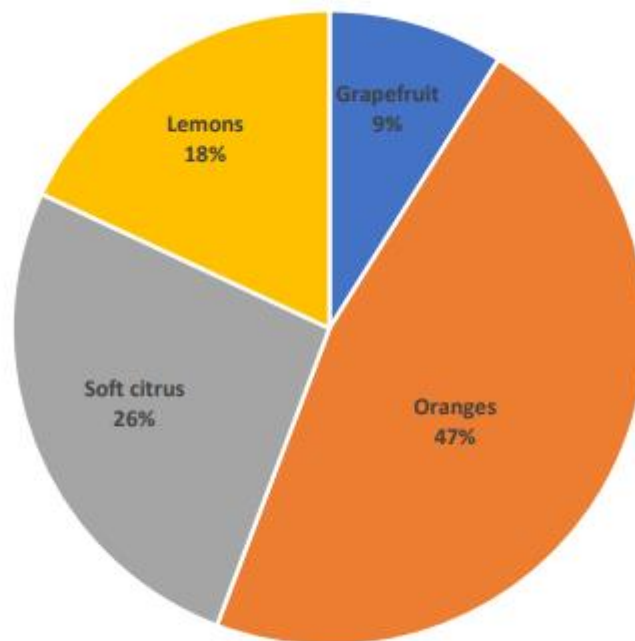
### **1.5 Objectives**

1. To spray Masterlock® in different tank mixtures aerially and conventionally by tractor sprayer and assess nutrient uptake through leaf analyses.
2. To assess the canopy penetration and deposition of Masterlock® sprays at different heights within citrus canopies.
3. To assess leaf surface area coverage as a result of Masterlock® sprays through the use of fluorescent dye for the aerial application method.
4. To assess zinc and boron nutrient uptake of different foliar micronutrient products with humectants and surfactants, as well as without the additives.

## CHAPTER 2: LITERATURE REVIEW

### 2.1 Introduction

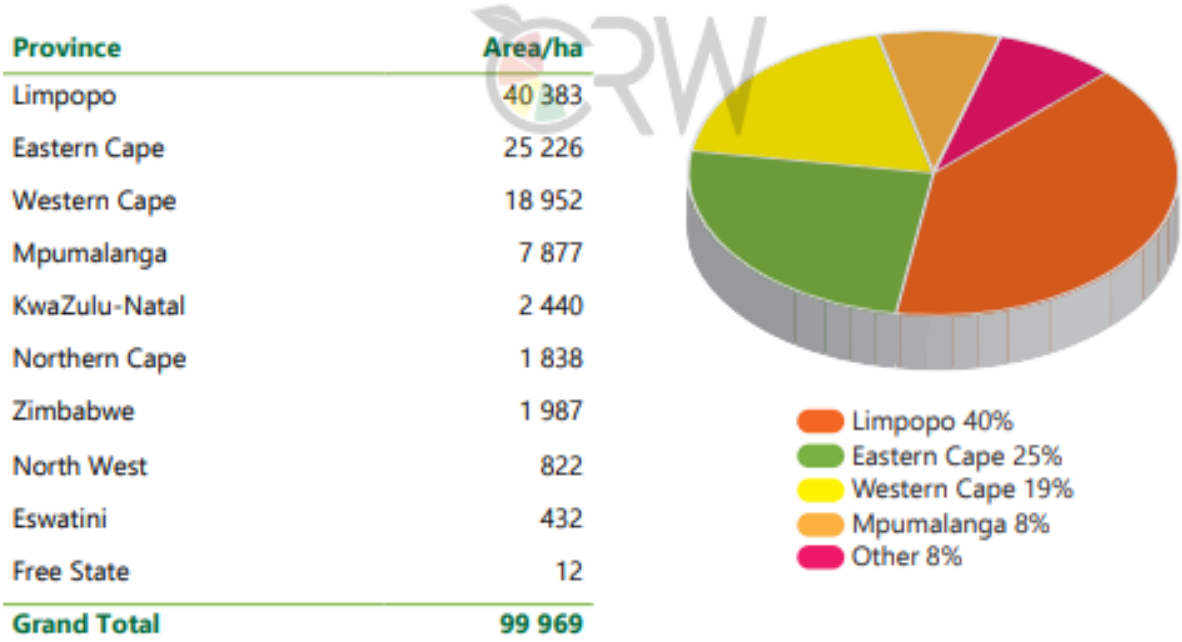
Citrus can be regarded as one of the most popular fruit crops in the world, and it is widely grown across multiple countries. Citrus fruit consist of photochemical and bioactive compounds such as carotenoids, flavonoids, minerals and vitamins. These phytonutrients act as antioxidants and stimulates the immune system as well induce protective enzymes in organs such as the liver (Okwu, 2008). These elements are essential for overall nutritional well-being for humans. Oranges are regarded as the most cultivated citrus fruit worldwide accounting for 50% of the world citrus production followed by tangerines, lemons, and grapefruit. As can be seen in Figure 1 oranges accounts for 47% of the total citrus that is produced in South Africa (Citrus Growers Association 2022).



**Figure 1. South Africa total citrus distribution percentages based on area planted (Citrus Growers Association 2022)**

Globally the citrus market has grown steadily in terms of exports, as compared to other fruits like mangoes or avocados. South Africa had the highest increase of tons exported (2092-2599 tons), Turkey was in second place (1602-1958 tons) while Spain (3935-3712 tons) and Egypt (2002-1928 tons) had a decreased in tons exported (Citrus Growers Association 2022). Citrus is widely grown in South Africa, with production occurring mainly in Limpopo, the Eastern Cape, Western Cape and Mpumalanga as can be seen in Fig 2. Nationally there has been a 6% increase in hectares from 2018 (81 603 ha), and 11% from 2019 (86 808 ha) to 2020 (96 230 ha). Between 2020 and 2022 (at 99969 ha) the growth levelled off, being only an 3.74% increase (Citrus Growers Association 2022). This is just below the global trend of 4.4%. The citrus industry is continuously growing because of the demand for exports and as a result large citrus farming operations continue to expand and plant more orchards as can be seen in Fig 3.

Globally, South Africa was ranked second in the 2022 season in terms of the value of citrus

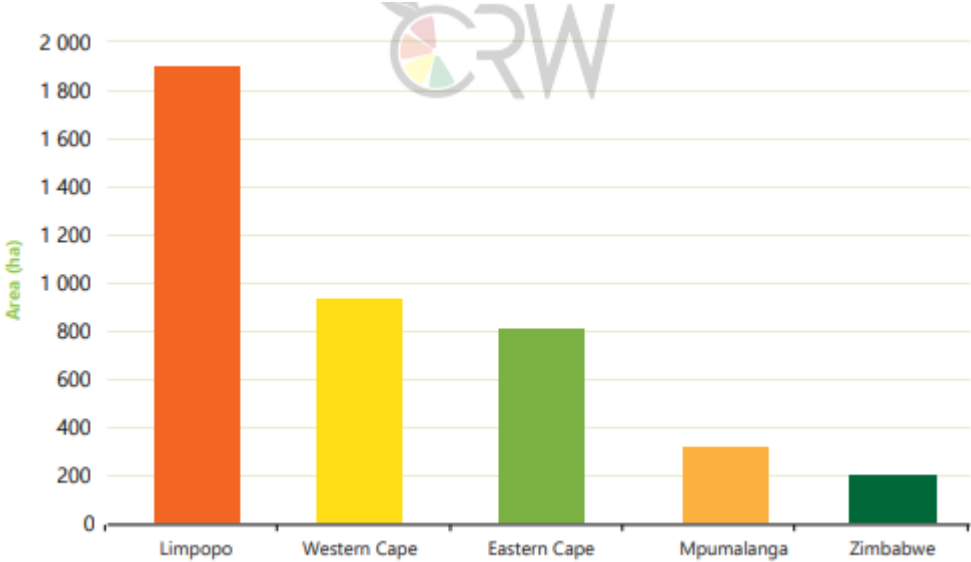


**Figure 2. Area planted to citrus in the different citrus producing regions in Southern Africa (Citrus Growers Association, 2022)**

exports. Historically South Africa exports excellent quality citrus to primarily Europe, the Middle East, the United Kingdom, and Russia (Citrus Growers Association, 2022). In the 2021/22 season South Africa set records for citrus exports by exporting 2.7 million tons (Citrus Growers Association, 2022). This was a result of new production areas as well as favourable



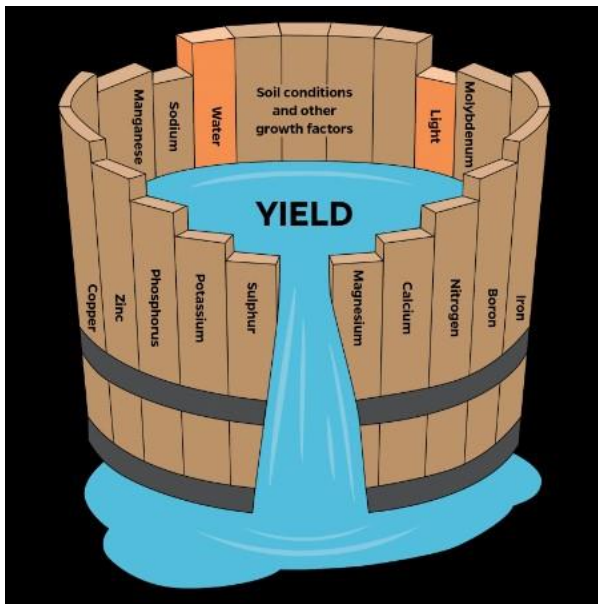
weather conditions and higher demand in some premium markets such as the United States. The citrus industry plays a critical role in the South African economy and in 2018 it contributed approximately R13.2 billion to the total fruit export value of R22 billion. (Dlikilili, and van Rooyen, 2018). It also employs 125 000 workers and contributes 27% to the total agricultural export value (Dlikilili, and van Rooyen, 2018). It is thus of utmost importance to sustain the citrus industry in order to strengthen the South African economy.



**Figure 3. New hectares of citrus planted per province in South Africa (Citrus Growers Association, 2022)**

As a result of the increase in new hectares as well as export demand, the citrus industry is always looking to increase in yield and fruit quality. One of the critical factors to help increase citrus fruit production and quality is to make use of nutritional products and applying certain elements at critical phenological growth stages of citrus trees.

The Law of minimum indicates that if there is a limiting factor or limiting nutrient present, that the plant or tree will not be able to obtain its potential yield (Fig 4) (De Baar, 1994). This limiting factor can be because of environmental conditions, soil or, water, amongst others which restricts uptake through the roots. If the limiting factor is a micronutrient deficiency a foliar applied nutrients application provides an opportunity to correct the limiting factor quickly to obtain potential yields.



**Figure 4. Von Liebig law illustrating that yield will be influenced by a limiting factor (Photo: Agri4Africa)**

The significance of nutrients applied on foliage relative to the nutrient demand of the fruit trees is influenced by the following six factors (Weinbaum, 1988):

- Timing of applications for specific growth stages and level of nutrient demand
- The absorption potential determined by the leaf surface and canopy size
- The concentration of nutrient applied that can lead to phytotoxicity
- The degree to which the surface area is wettened
- The phloem mobility of nutrients
- The restriction or inability of the tree roots to absorb sufficient quantities of nutrients from the soil to attain potential yield.

Macro elements such as nitrogen, potassium and phosphorous, are present in leaves at levels between 0.1 to 10% of leaf dry mass, whilst the micronutrients such as boron and zinc are required at concentration of 0.0001 to 0.01% leaf dry mass which is much lower than the macro elements (Weinbaum, 1988). The efficiency of foliar applied nutrients has been determined and the following needs to be considered:

- The availability of the nutrient after application, for example through pores or stomata and whether it will translocate to the phloem.
- The absorption percentage of the applied nutrient
- The actual amounts of nutrients absorbed
- Percentage recovery following the foliar applied nutrients, for instance salt stress caused by the application.

It was determined that foliar applications of the macro element nitrogen, can only increase the nitrogen concentration by 3% in fruit trees (Weinbaum, 1988). In contrast foliar applied B resulted in a 300% increase in the concentration of B in the leaf (Weinbaum, 1988). Thus, this indicates that a macro element is needed in higher quantities than a microelement. The quantities of nutrients that can be sprayed onto the leaf are very low, otherwise phytotoxicity can occur, leading to damaged leaves and reduced photosynthesis. These limitations indicate that soil applications of macro elements at higher quantities will be most cost effective, whilst foliar applications at lower quantities are cost-effective. However, several spray applications of micronutrients may be required throughout the year to ensure adequate concentration, which impacts cost.

In the different citrus production areas, there are large farming operations, where large blocks of similar varieties are produced in different regions. These large farming operations are under pressure to apply foliar nutrients and plant growth regulators (PGR's) in a short timeframe because these varieties have the same management practice that needs to be performed over large areas and completing this in time is an issue. The parallel need to simultaneously apply pest and disease control products, is a practical challenge (personal communication: large farming operations and chemical retailers). This is a logistical problem that farming practices face daily.

Aerial application of foliar nutrients is a novel approach to reduce tractor traffic in orchards and to ensure that applications are made at critical phenological stages. This will reduce compaction of the soil as well as fruit mechanical damage which can influence exports due to marks on the fruit. Not only can vast areas be sprayed in a very short period, but it is also regarded as less costly and reduces pressure on the labour force. Due to the small drop size generated during

aerial applications, the effective penetration of the micronutrients into the canopy, wetting of leaf surfaces and uptake of nutrients, is unclear. As a result, these large farming operations are currently unsure about the effectiveness of aerial application of foliar nutrients. If the droplets do not reach the target area and the optimal leaf surface coverage is not obtained, it is thought that there will not be sufficient uptake of the micronutrients to correct possible deficiencies, rendering the aerial spraying of micronutrients ineffective.

## **2.2 The role of zinc and boron in citrus**

Citrus is very dependent on nutrients for growth and production of good quality fruits, as is true for all crops. When a deficiency arises, it can result in serious nutritional disorders (Catara, 1987). The importance of Zn and B to ensure; yield and quality of citrus fruit has been evaluated in a number of studies (Dawood et al., 2001).

Boron plays an important role in pollen tube elongation, pollen grain germination and fruit set, and therefore is an early determinant of yield in citrus (Khan, et al. 2012). Boron deficiencies, which is more extensive than other micronutrients, often have an impact on yield and fruit quality (Zekri and Obreza 2003). Boron influences the external and internal cork formation in fruit and deficiencies will lead to small deformed cracked fruit (Shorrocks and Nicholson, 1979). Symptoms of B deficiencies can appear both in the reproductive as well as vegetative parts. Typical symptoms include defoliation of citrus trees (Fig 5), die off of twigs, gum formation in the bark, fruit abortion, and poor fruit set (Khan, et al. 2012).



**Figure 5. Early signs of boron deficiency in citrus. Twigs started to die off and some fruit was aborted with poor fruit set (Photo J.P. Strydom)**

Boron is widely distributed in the hydrosphere and lithosphere (Brdar-Jokanović, 2020). The most common sources of naturally occurring soil boron are borosilicate mineral tourmaline, volatile volcano emanations, geothermal streams, groundwater, and seawater (Brdar-Jokanović, 2020). The availability of B is dependent on the type of soil present in an orchard. Alkaline and calcareous soils typically have limited B availability. In addition, B can easily leach as water percolates through a soil profile (Khan, et al. 2012). This indicates that, depending on the soil type, B can be deficient in certain areas within an orchard depending on the type of soil present and water percolation through the profile. Due to the limited mobility inside the plant, B applications during the season are important to maintain nutritional status and ensure deficiencies do not develop. The roots promote loading and uptake of B into the xylem channel. However, B is immobile in the phloem for most plants, but certain B-formulations do move in the phloem (Richard, 2017). Growing tissues will rely on B supplied via the xylem and if present in the phloem, B will be complexed with compounds such as sugar alcohols, which will allow free mobility (Richard, 2017). Boron that was applied via foliar methods form a B-sucrose complex in the citrus plants can be transported in the phloem from the leaves to the roots (Du et al.2020).

There are various sources of B that can successfully be used including boric acid, water soluble sodium borate, borax and calcium borate. The foliar application of boric acid was proven most effective, while soil applied borax the most effective in citrus (Srivastava, et al; 2005).

Zinc is also an important micronutrient that is involved in the synthesis of tryptophan one of the essential amino acids in protein metabolism (Brown et al., 1993). Zinc is responsible for activities of enzymes, such as DNA and RNA polymerase and, may also have an important role in protein biosynthesis (Khan et al., 2012).

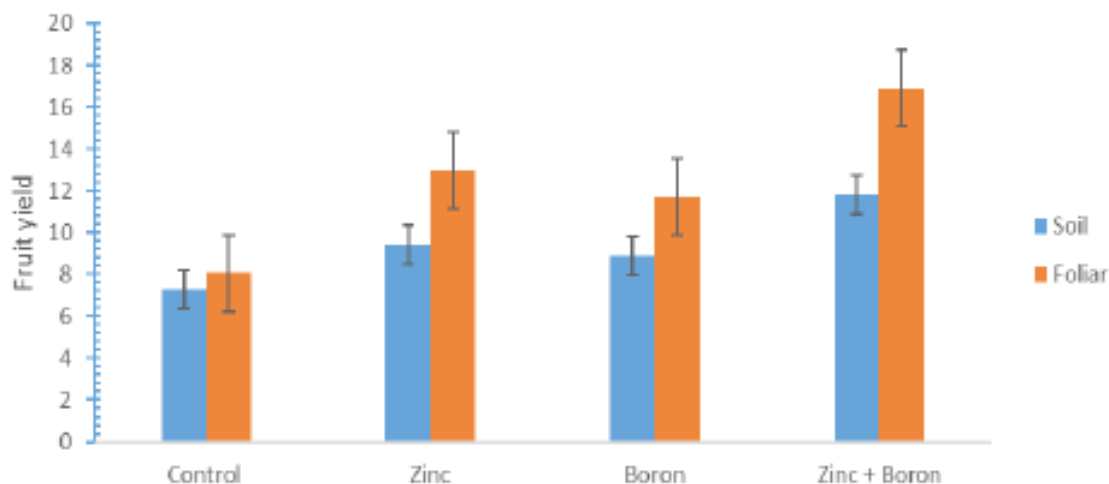
The photosynthetic rate ( $P_n$ ) and also chlorophyll content decreases as the Zn content reduces (Fei et al., 2016). Studies also confirmed that peroxidase (POD) and catalase (CAT) activity significantly increases when Zn content is not limiting in leaves. This suggests that if there is a Zn deficiency, the POD and CAT processes will be affected by it (Fei et al., 2016).

Zinc deficiencies in citrus include interveinal chlorosis (Fig 6), russetting as well as abnormal vegetative growth. Soils that have Zn deficiencies are usually at a pH of 6 and higher, and more often on sandy soils (Thorne, 1957). The most used sources for Zn are usually zinc nitrate, zinc oxide or zinc sulphate (Khan et al., 2012). A foliar application of zinc sulphate is the most effective way of keeping the nutritional status high within the citrus trees (Khan et al., 2012).



**Figure 6. Deficiency symptoms of zinc (Photo J.P. Strydom)**

Due to both Zn and B being phloem immobile in the plant (although Zn reported to move from in the phloem to the roots) (Haslett et al., 2001) and that they only needed in small quantities, the timing of application needs to be done at the correct phenological growth stage of the citrus tree for the best result. Zinc and B applications before flower initiation, fruit set and when the fruit size is at 10-20 mm stage were found to be important phenological growth stages to increase nutrient levels to prevent deficiencies (Sajid et al., 2010). In a previous study by Ruchal 2020 it was noted that a Zn and B foliar application on citrus at these growth stages significantly increased flowering, fruit set, as well as reducing flower and fruit drop. Depending on the time of application, foliar applied Zn and B was found to improve fruit set by as much as 42% (Ruchal, 2020). This can directly impact yield as can be seen in Fig 7 (Noor et al., 2019).



**Figure 7. Interactive effect of soil or foliar applications of B and Zn on fruit yield of citrus (Noor et al., 2019)**

According to previous studies the method of application is important (Noor et al., 2019). As can be seen from Fig 7, the foliar applications of Zn and B were more effective at increasing yield than soil applications. Translocation and absorption of micronutrient via the roots is slow because of poor mobility and availability of the elements due to the fact that these elements are dependent on upwards movement with the transpiration stream (Khan et al., 2012).

Foliar applied Zn and B also has a significant role in fruit set as can be seen from Table 1 (Ruchal, 2020). From the data it can be seen that at 66 days after application, the mean value of fruit set was 65.96% per branch. Foliar application of 0.04% B resulted in 82.09% fruit set and 0.1% Zn + 0.04% B in 78.19% fruit set, which was the highest fruit set per branch. The lowest fruit set per branch of 42.17% was found in the control with no Zn or B applications (Ruchal, 2020).

**Table 1. Effect of Zn and B on fruit set per branch on citrus orchards (Ruchal, 2020)**

Treatment	Percentage of fruit set at:				
	66 DAS	76 DAS	86 DAS	96 DAS	106 DAS
control	42,17 <sup>c</sup>	35,52 <sup>c</sup>	32,8 <sup>c</sup>	23,55 <sup>c</sup>	11,28 <sup>b</sup>
0,15% Zn	50,77 <sup>bc</sup>	67,28 <sup>ab</sup>	52,55 <sup>b</sup>	48,44 <sup>b</sup>	42,58 <sup>a</sup>
0,04% B	82,09 <sup>a</sup>	74,66 <sup>a</sup>	71,32 <sup>a</sup>	67,52 <sup>a</sup>	51,27 <sup>a</sup>
0,05% Zn + 0,02% B	76,55 <sup>ab</sup>	73,9 <sup>a</sup>	64,06 <sup>ab</sup>	55,82 <sup>ab</sup>	47,32 <sup>a</sup>
0,1% Zn + 0,04% B	78,19 <sup>a</sup>	49,1 <sup>bc</sup>	63,24 <sup>ab</sup>	55,58 <sup>ab</sup>	47,61 <sup>a</sup>
F-test	*	*	**	**	*
CV%	26,4	26,6	21	21,3	38,3
LSD	26,8	24,6	18,4	16,4	23,6
SEM	4,96	4,75	3,8	4,14	4,39
Grand mean	65,96	60,09	56,8	50,19	40,01

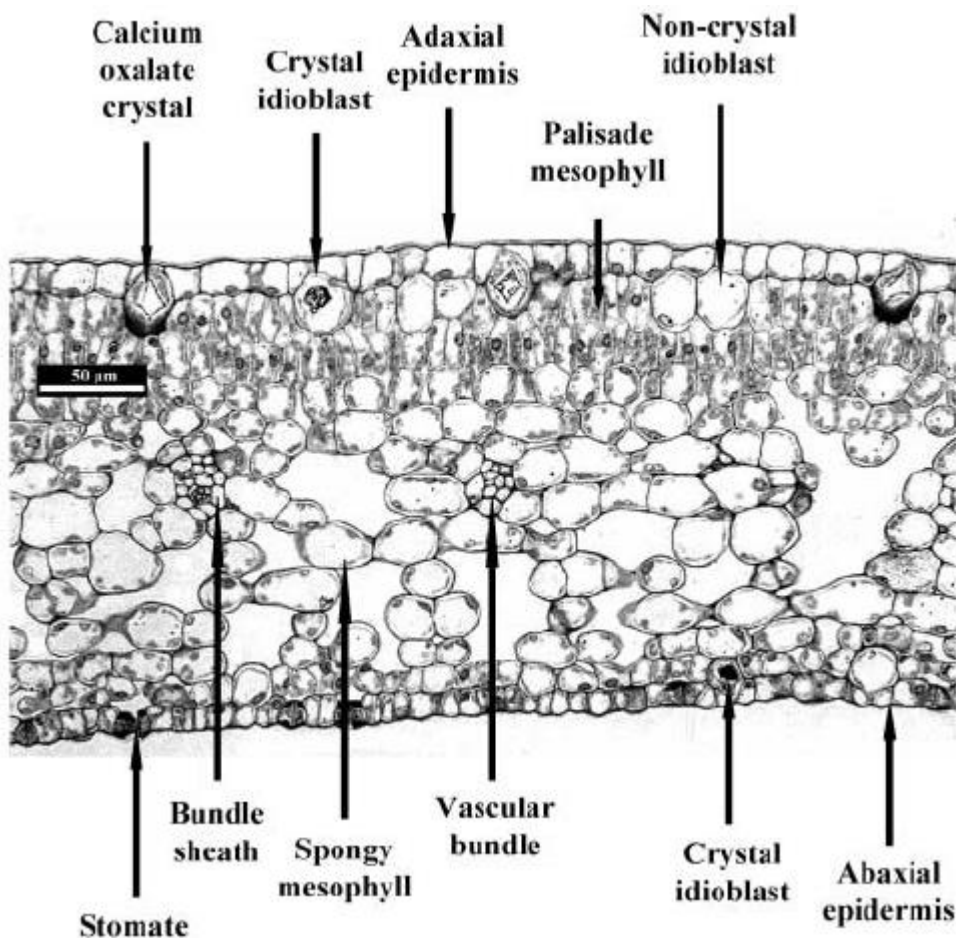
### 2.3 Uptake of micronutrients through the leaf surface

Despite the progress in foliar nutrition technology, many challenges are still encountered in the development of agricultural recommendations for this practice (Smolen, 2012). This is ascribed to the fact that the effectiveness of foliar nutrition is largely affected by the structural and chemical features of the leaf surface and the surrounding environment (Fernandez et al., 2013). Chemical features include nitrogen content, organic acids, non-structural carbohydrates and mineral substances, which acts as chelators to ensure increase in uptake. For successful uptake, the nutrient solution must first penetrate the leaf surface and cuticle before entering the cytoplasm of the cell within the leaf to fulfil its function in plant growth (Fernández & Eichert, 2009). Penetration of foliar nutrients can occur through several pathways, namely: the cuticle, the stomata, leaf hairs and other specialized epidermal cells which is all structural factors (Singh et al. 2013). The leaf surface characteristics as well as the presence of epicuticular waxes play an important role. Due to the presence of cuticular waxes, the inclusion of an adjuvant helps increase retention and absorption of the micronutrient, as well as wettability of the droplet (Khan et al., 2012).

Citrus leaves consist of an upper and lower epidermis. The upper epidermis is covered with a cuticle layer (consisting of wax, cutin and cellulose) (Khan et al., 2012). Large palisade cells



extend into the upper epidermis and are composed of two guard cells as well as a pore (Fig 8). Ridges of cuticle overhang the stomata opening which prevents solutes and water from penetrating through the stomatal pore (Khan et al., 2012). The cuticle covering the upper and lower epidermis makes the leaf waterproof and acts as the first layer of entry. Uptake of micronutrients occurs firstly through passive uptake through the leaf cuticle which is followed by active uptake through the leaf cells. Thus, uptake occurs through the cell wall before entering the epidermal cells. This is regulated by photosynthesis and respiration. Uptake can also occur through the stomata of the leaf (Khan et al., 2012).



**Figure 8. Anatomical structure of lemon leaf (Storey and Leigh, 2004)**

The uptake of Zn through the leaf surface was examined from fresh hydrated leaves of citrus (Du et al., 2015) and found to first enter into the leaf via passive diffusion through the surface of the leaf, i.e., the cuticle and cell wall of the epidermis. Secondly, once the Zn moves through

the leaf surface there are three processes that are followed for movement throughout the leaf: firstly, within the interveinal tissue there is redistribution of Zn, secondly there is movement from the interveinal tissue to the lower order veins, lastly there is Zn movement to the higher order veins where it will be redistributed through the leaf (Du et al., 2015). Overall, the Zn movement in the leaf was found to be restricted. As a result, there should be continuous supply when needed by the plant of Zn during the growing season (Coetzee, 2007).

Boron uptake through the leaf surface is also restricted and is influenced by the nutritional status in the plant (Boaretto et al., 2008). Boron mobility is highly dependent on the formation of polyol-B-polyol complexes that are translocated via the phloem. This occurs mainly in species that produces significant amounts of dulcitol, mannitol and sorbitol like citrus (Boaretto et al., 2005). These sugar alcohols products act as a product of photosynthesis and exists in the liquid form. The mineral nutrients that form complexes with sugar alcohols, their migration and mobility in the alkaline environment has been evaluated and found to be more mobile than nutrients that does not form a complex with sugar alcohols (Brown and Hu, 1996). Boron enters through a parallel pathway involving membrane channels that facilitates movement in plants and this is specific to phloem (Reid, 2014).

Importantly, the overall efficiency of the uptake process is determined by the degree to which the leaves are wet by the spray mixture. This relates to the physical and chemical properties of the cuticle, the nature of the applied solute and the use of adjuvants (Skoss, 1955; Fernández and Eichert, 2009). Adjuvants such as surfactants influence translocation and uptake by solubilizing or altering cuticle permeability or affecting the active or passive uptake (Skoss, 1955; Fernández and Eichert, 2009). In active uptake the molecules that are present moves against the concentration gradient, whereas in passive uptake the molecules move along with a concentration gradient.

#### **2.4 The role of adjuvants in spray mixture**

An adjuvant can be described as a material that is added to a tank mixture to modify or aid the physical characteristics of the mixture or action of an agrichemical product. Adjuvants can therefore be regarded as a key contributor that improves efficacy of a spray mixture (Winfield Solutions, LLC, 2014). There are various types of spray adjuvants that can be employed in

foliar fertilisation; to ensure the adhesion of aqueous sprays to leaf surfaces (e.g., wetters), to improve coverage (e.g., surfactants), to minimise weathering of deposits (e.g., stickers) and to increase foliar uptake (e.g., humectants, pH modifiers, and penetrants) (Winfield Solutions, LLC, 2014).

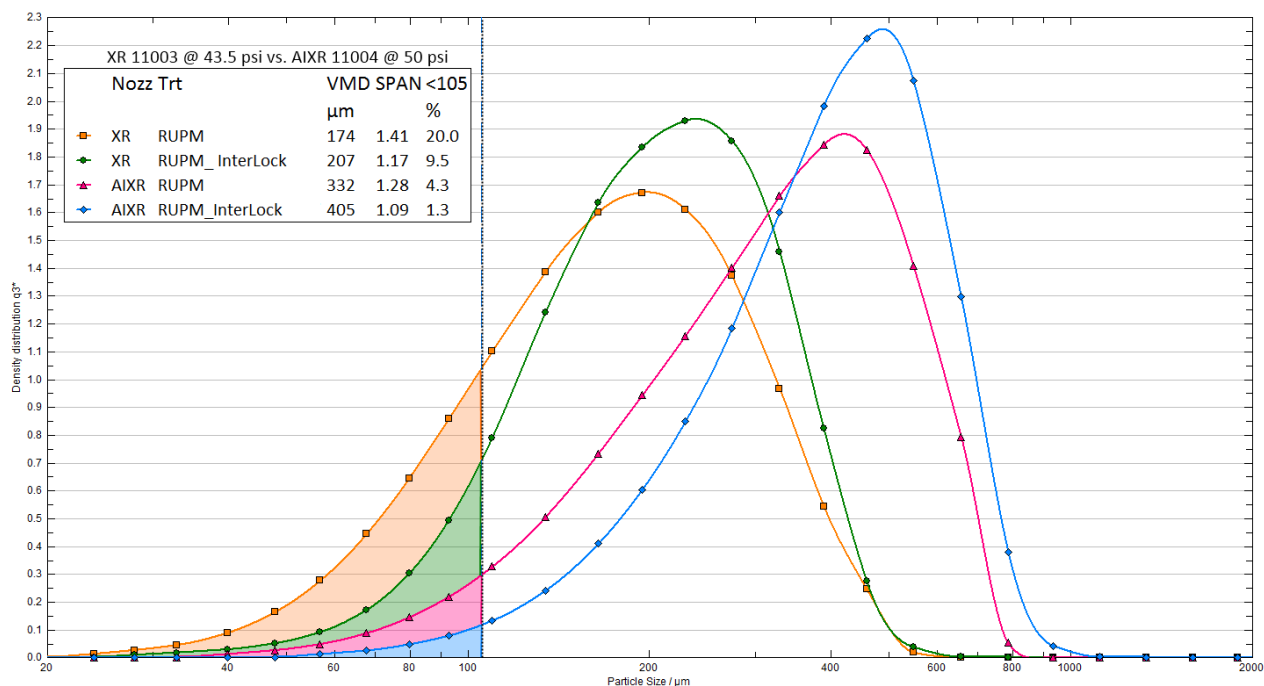
Surfactants decrease droplet bounce on impact with leaves or fruit, while increasing spreading for optimal coverage. Surfactants can also be described as a surface acting agent that breaks the surface tension of the leaf for optimal coverage and uptake (Spanoghe et al., 2007). The absorption process of systemic products is then enhanced by the hydration of the waxy layers on the leaf surface. The surfactant can also assist certain contact products by temporarily containing them in a more favourable environment for optimal efficacy, this includes high humidity and optimum temperatures. There are different types of adjuvants that include (Spanoghe et al., 2007):

1. Oils
  - a. Mineral oils
  - b. Vegetable Oils
  - c. Oil derivatives
2. Surfactants
  - a. Anionic surfactants
  - b. Cationic surfactants
  - c. Non-Ionic surfactants
  - d. Organosilicon surfactants
3. Waxes
4. Polymers
5. Solvents
6. Terpenes
7. Alcohols
8. Phospholipids
9. Inorganic salts
10. Inorganic fillers
11. Humectants

It is important to determine which adjuvant is best suited to foliar applications of micro nutrients or chemicals that are used, so that a synergism is achieved. Some adjuvants also have a significant other benefit, such as reducing drift by manipulating the droplet size and deposition (Spanoghe et al., 2007). Oil emulsion adjuvants, for example, have these characteristics which result in increased in droplet size and deposition.

There is a relationship between density distribution (Y-axis) and the particle size (X-Axis) of the droplet (Fig 9). Droplets smaller than 105  $\mu\text{m}$  have the ability to be mobile in air and can result in drift depending on relative humidity, temperature and wind speed (Winfield Solution, LLC, 2013). Drift is the movement of a droplet away from the target area because of wind. A spray analysis system was used to measure what happens to droplets exiting the nozzle using a laser-based model. As can see from Figure 9 the volume medium diameter increased from 174  $\mu\text{m}$  to 207  $\mu\text{m}$  resulting in an increase in droplet size and VMD. This is because of the addition of the adjuvant Interlock. The volume medium diameter (VMD) refers to the midpoint of droplet size, where half of the volume is larger than the mean and the other half of the volume is smaller than the mean.

The oil emulsion adjuvant (Interlock) reduced drift from 20% to 9.5% while using an XR (Extended range) nozzle. The volume medium diameter of the AIXR (Air induction extended range) nozzle also increased from 332  $\mu\text{m}$  to 405  $\mu\text{m}$ . The oil emulsion adjuvant (Interlock) decreased drift from 4.3% to 1.3%. The use of a drift control adjuvant like Interlock can therefore improve deposition and uptake of foliar applied micronutrients because of the improved deposition (Winfield Solution, LLC, 2013). In this instance the data shows the importance of an oil emulsion adjuvant to increase droplet size in order to increase deposition onto the leaf, it also shows the importance of nozzle selection that will also increase droplet size and deposition.



**Figure 9. Modification of droplet size using an adjuvant measured using the Winfield United spray analysis system to determine the impact of droplet size on density distribution (Winfield Solution, LLC, 2013)**

Another important adjuvant is a humectant. Humectants, such as alkyl polyglycolides, increase the drying time of the droplets which allows the active ingredient to be available in solution form for a longer period of time on the leaf surface (Polli et al., 2022). This results in increased uptake of the active ingredient that is sprayed. When temperatures are high with low humidity, this adjuvant has a significant effect on efficacy of uptake (Polli et al., 2022). A humectant will retain moisture from the air in order for the droplet to stay wet for longer periods of time (Ramsey et al., 2005).

Since a wide range of adjuvants are available for foliar nutrition, selection of a suitable adjuvant for regular application is complex, even more so if the droplet size of the spray solution is particularly small and applied from a distance, as per aerial sprays. Furthermore, mature citrus trees can present formidable application challenges due to their tall, broad and dense canopies. In addition, aerial applications do not create the turbulence required to penetrate the canopy of the trees, so it is unclear whether wetting of the full leaf surface can be achieved via aerial applications (Salyani and Cromwell., 1992). Although it was found that, when used at the prescribed concentration,  $\text{SO}_4^{2-}$ -formulations of foliar products (“straights”) are more effective

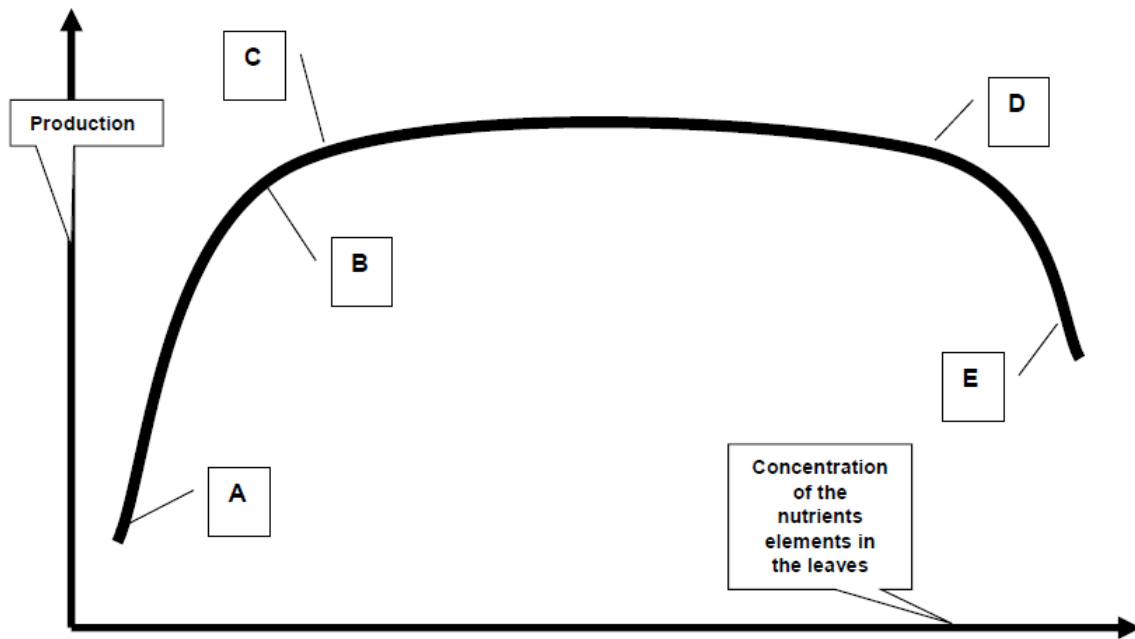
than chelated products (Van Der Merwe, 2015), the efficacy of these applications is proposed to be lower compared to foliar spray treatments that are co-formulated with adjuvants (Fernandez et al., 2013).

Although many adjuvants are commercially available for use in combination with agrochemicals, their compatibility and effectiveness with foliar applied nutrients is often unknown (Rodriguez-Lucena et al., 2010). This causes uncertainty amongst producers regarding the efficacy of the products they use. Furthermore, despite possible improvements in penetration obtained by various adjuvants, some adjuvants used in commercial products can cause injury to leaves and fruits, which can cause phytotoxicity (Horesh and Levy, 1981). Various studies found that the magnitude of the on-canopy deposition and spray losses are mainly a result of the equipment design, operational parameters, weather conditions and the tank mix (Hoffman and Salyani, 1996). This highlights the need to make a distinction between beneficial, or effective, foliar micro-nutrient products and adjuvants that can be used to effectively spray different products onto the leaves.

## **2.5 Citrus leaf analyses and norms**

Nutritional status of citrus trees can be determined through leaf analyses that allows the determination of the concentrations of essential minerals inside leaf tissue in relation to the behaviour of the plant (Menino, 2012). Leaf analyses is a way of establishing what the nutrient requirements are for a specific crop, and it can be assumed that there is a positive relation between leaf nutrient content, yield and quality and nutrient supplied. Studies suggest that leaf analyses are the best method of identifying the need for a nutrient application in perennial crops because actual nutrient levels are determined (Menino, 2012). Through leaf analysis it is possible to determine if the nutrient requirements of the citrus trees are more than what the soil can provide.

The following correlations was illustrated by Coetzee, 2007 between the concentration of the nutrient elements inside the citrus leaf and production as can be seen in Fig 10.



**Figure 10. Generalised production curve as affected by the concentration of the nutrient elements in the leaves (Coetzee, 2007).**

The concentration status of a nutrient in a leaf is correlated to production, i.e., when it is very (A) low a large response in production is obtained as the concentration increases. If higher nutrient levels are maintained (B, C), the best results in terms of nutritional status and production are observed. When an even higher nutrient concentration in the leaves occurs, very little effect on yield and quality is obtained (D), being referred to as the region of luxury consumption. There will be a decrease in the nutritional and production status of the tree if the maximum concentration is increased further, resulting at times in toxicity symptoms (E) (Coetzee, 2007).

The optimal range for B in citrus leaves is between 75-200 ppm (Table 2) This indicates that a higher response is expected at <75 ppm and lower response after application at >200 ppm. Higher response refers to better uptake and translocation of the element because of the deficiency present. The optimal range for Zn is between 25-100 ppm, i.e., a higher response after application can be expected at <25 ppm and a lower response >100 ppm. It is important

to stay within the optimal ranges, as previous studies suggest that concentrations of nutrients above or below these values will affect the productivity and growth of the plant (Menino, 2012).

**Table 2. Norms used for boron and zinc in South Africa for citrus trees (Coetzee, 2007)**

Growth Stage	Nutrient Type Id	Nutrient Classification Type Id	Nutrient (ppm)
April - March	B	Deficient	<40
April - March	B	Optimal	75-200
April - March	B	Excessive	>300
April - March	Zn	Deficient	<15
April - March	Zn	Optimal	25-100
April - March	Zn	Excessive	>200

Efficacy of aerial applications of micronutrients with regards to uptake and deposition has not yet been studied. An aerial application of micronutrients will enable growers to do a micronutrient application at the correct phenological growth stage of citrus trees and be less time consuming compared to conventional tractor methods. This study aimed to determine if the aerial application of micronutrients is viable and effective, by making use of adjuvants to increase deposition and coverage in combination with Zn and B products.

A review of literature has shown that this is yet to be determined or studied and is thus a shortcoming in this field of agriculture.



## CHAPTER 3: Materials and methods

### 3.1 Experimental Site

The trials were conducted during 2019 and 2020 on an eight-year-old 'Mclean' Valencia with a Swingle Citrumelo (SC) rootstock at Letaba Estates, Letsitele (-23.848728 30.299157) (Figs 11 and 12). The row spacing was 6.0 m x 3.0 m with total orchard size of 12 ha. The trees had a height of 4.2 m and a diameter of 3.6 m. The summer (September-April) average maximum temperature was 30°C, with an average minimum temperature of 20°C, while the winter (May-August) maximum average temperature was 26°C and average minimum temperature of 12°C. A METOS®SA weather station with instruments from Pessl, was also present on the farm to supply valuable information regarding environmental conditions, i.e., temperature, humidity, windspeed, rainfall, as well as leaf wetness, during the times of application.



**Figure 11. Google earth image of the experimental orchard H75 at Letaba Estates**



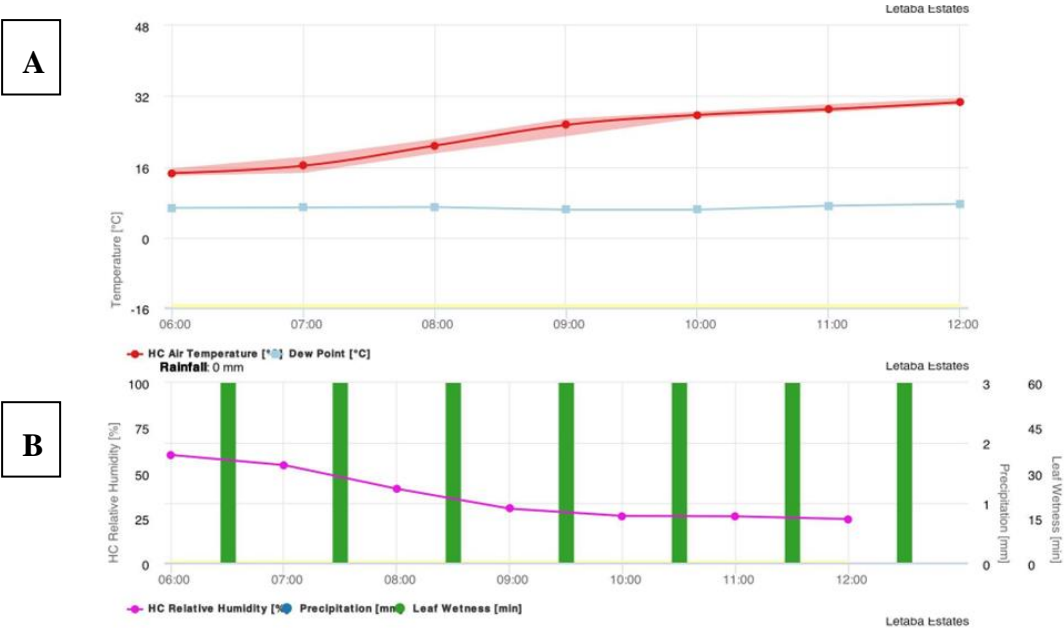
**Figure 12. Image of the Mclean Valencia trees**

### **3.2 Experimental conditions**

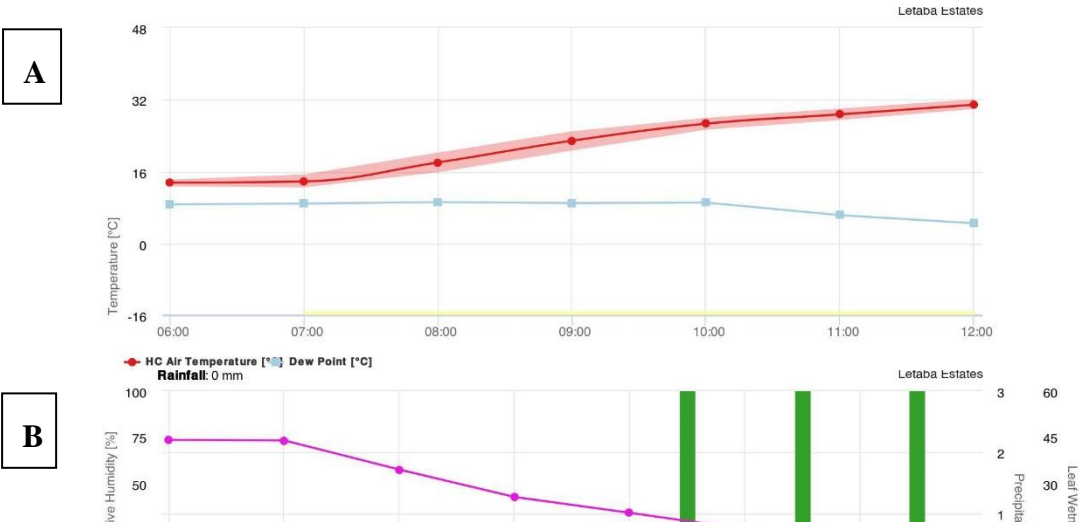
A weather station was used to determine critical environmental conditions for spraying foliar micronutrients, which were relative humidity and temperatures. The weather station gave the opportunity to identify ideal conditions for doing an application, which included relative humidity above 50% and temperatures below 30°C. Since rainfall will induce runoff and result in a lower application concentration for both B and Zn, application times were selected without any rain. Higher humidity is also favourable, since the period that the leaf remains wet is directly correlated with total time available for uptake, i.e., if the droplet remains for a longer period of time on the leaf surface, the micronutrient has a longer period to be absorbed by the leaf surface.

Favourable conditions for the application of Zn and B occurred during both the 2019 and 2020 seasons, with temperatures between 17°C -and 20°C at time of application in 2019 and between 18°C -and 22°C in 2020. Relative humidity was 60% and 50% during applications in the 2019 and 2020 seasons (Figs 13 and 14). During both years, wind speed was between 4 and 8 km h<sup>-1</sup> at the time of application (ideal wind speed when spraying is below 15 km h<sup>-1</sup>). The applications were conducted on 3 September 2019 and 15 September 2020. In 2019 the approximate rainfall during the growing seasons was 560 mm, while it was much more in 2020, i.e., 720 mm. No rain was recorded on the day of application, and neither one day after

application for 2019. For the 2020 season, there was also no rain recorded on the day of application, but there was 1 mm of rain the day after application.



**Figure 13. The Metos®SA weather station application, FieldClimate was used to capture the data on Letaba estates on 3 September 2019. A) Minimum and maximum temperatures at time of application. B) Relative humidity and, precipitation**



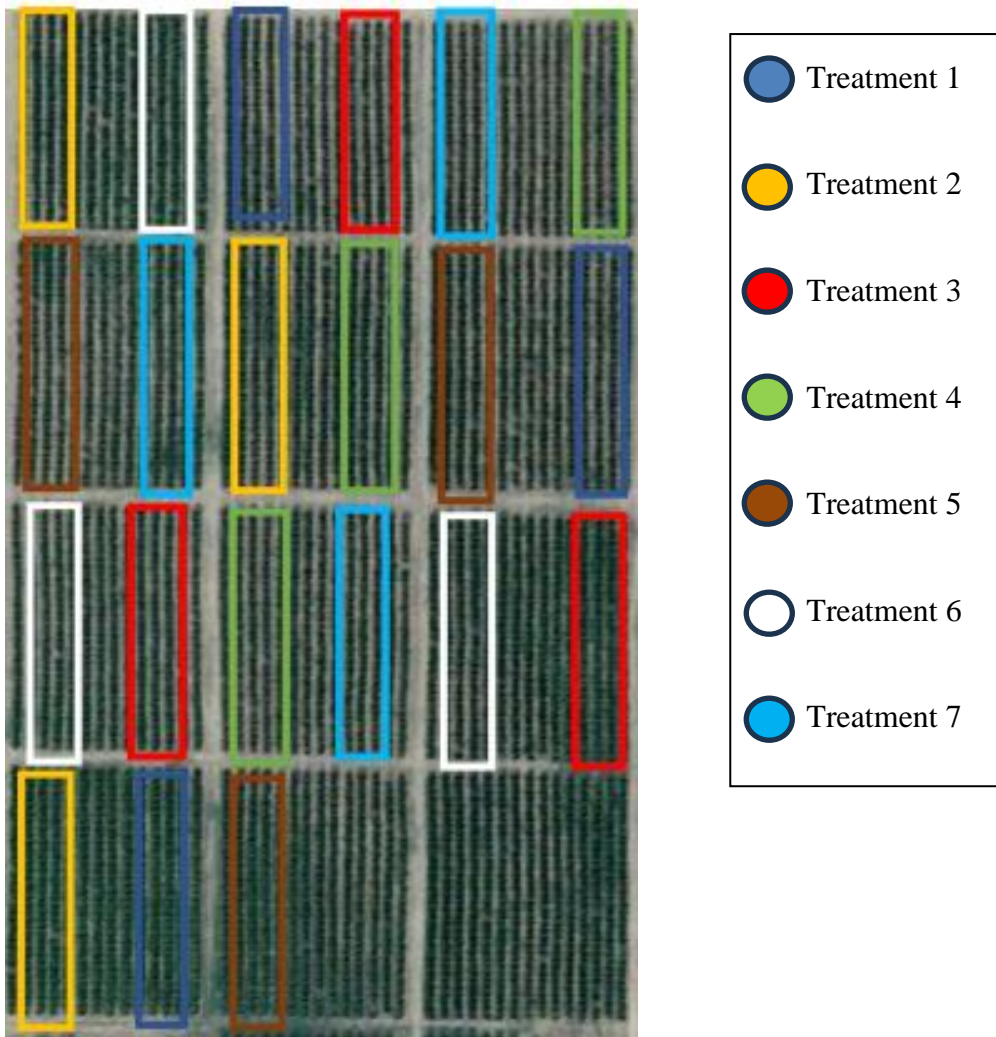
**Figure 14. The Metos®SA weather station application, FieldClimate was used to capture the data on Letaba estates on 15 September 2020. A) Minimum and maximum temperatures at time of application. B) Relative humidity and, precipitation**

### 3.3 Experimental design

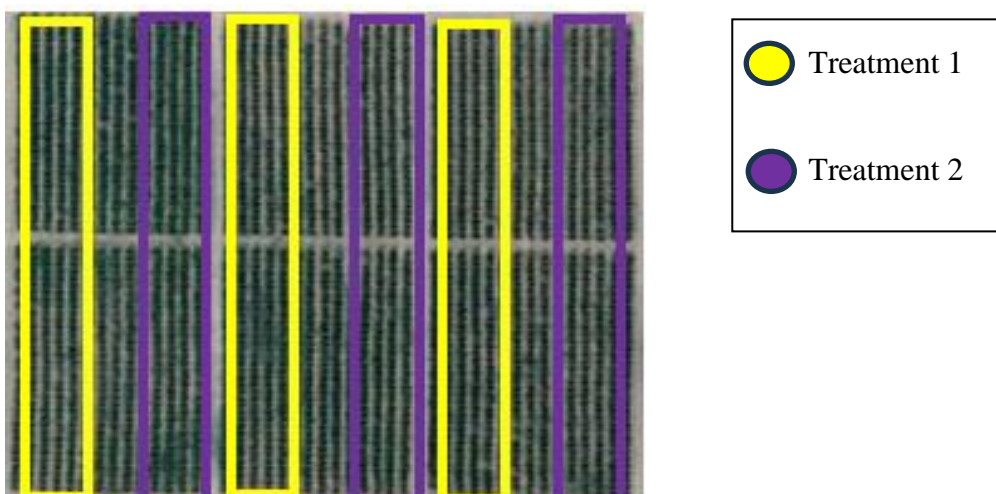
The experimental design was a completely randomized block design consisting of nine treatments, replicated three times (Figs 16 and 17). The 27 experimental plots, each 0.22 ha in size, consisted of four rows, 6 m apart and 100 m in length and in a North-South direction. All the trees were similar in vigour and canopy structure. Between each experimental plot, four buffer rows were left untreated to avoid drift or contamination between the plots. Treatments one to seven were applied during full bloom (Fig 15), while treatments eight and nine took place a month later in both seasons with different methods (water sensitive paper and Dropsight) to assess the effect of Masterlock® on leaf droplet coverage and canopy penetration. The wind speed at the time of application of treatment 8 and 9 was between 6-9 km h<sup>-1</sup>.



**Figure 15. Full bloom stage at which the micronutrient applications took place**



**Figure 16. Completely randomized trial layout for treatment 1-7 on orchard H75**



**Figure 17. Trial layout for Treatment 8 and 9 on orchard H75**

### 3.4 Treatments

In Table 4 details regarding the treatments is provided. The aerial applications were done with a light aeroplane (Table 3) and a water volume of 100 L ha<sup>-1</sup>. For the conventional tractor applications, a water volume of 2000 L ha<sup>-1</sup> was used, which is considered to be a low-medium water volume.

Both the light aeroplane and conventional tractor sprayer were calibrated before the application started to ensure accurate application rates. The calibration variables were pressure, speed of the tractor/aeroplane, boom length, number of nozzles, type of nozzles. The products were thoroughly mixed in a pre-mixing tank before being pumped into the aeroplane or the tractor sprayer (Fig 18).

**Table 3. Specifications of the aeroplane used to spray the aerial applications**

Engine Type:	P&W PT6A-65AG
Hopper Capacity:	3.028 L
Fuel Capacity:	961 L
Wingspan:	18,04 m
Wing Area:	37,29 m <sup>2</sup>



**Figure 18. Pre-mixing tank**

**Table 4. Treatments 1-7 that were applied at full bloom on the ‘Mclean’ Valencia citrus trees at Letaba Estates in September 2019 and September 2020 and Treatments 8 and 9 that were applied a month later.**

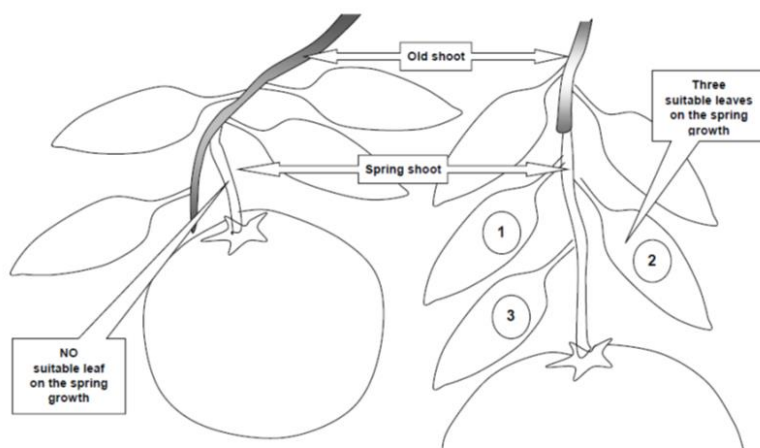
TREATMENT		Description of treatments					
		Products and volumes used	Volume water used (L ha <sup>-1</sup> )	Total applied (g/ha)		Concentration in spray mixture (mg/L)	
				Zn	B	Zn	B
<b>1</b>	Aerial	MAX-IN™ BORON 3.40L/ha + MAX-IN™ ZINC 4.5L/ha	100	329.4	326.4	3294	3264
<b>2</b>	Conventional	MAX-IN™ BORON 3.40L/ha + MAX-IN™ ZINC 4.5L/ha	2 000	329.4	326.4	164.7	163.2
<b>3</b>	Aerial	Masterlock® Rate 1% + MAX-IN™ BORON 3.40L/ha + MAX-IN™ ZINC 4.5L/ha	100	329.4	326.4	3294	3264
<b>4</b>	Conventional	Masterlock® Rate 0.1% + MAX-IN™ BORON 3.40L/ha + MAX-IN™ ZINC 4.5L/ha	2 000	329.4	326.4	164.7	163.2
<b>5</b>	UTC	Untreated Control	2 000	0	0	0	0
<b>6</b>	Aerial Farm	Aerial Farm Practice	100	5.2	6.8	52	68
<b>7</b>	Conventional	Conventional Farm Practice	2 000	5.2	6.8	2.6	3.4
<b>8</b>	Aerial	Masterlock Rate 1%	100	N/A			
<b>9</b>	Aerial	Without Masterlock 1%	100	N/A			

### 3.5 Product formulations

The MAX-IN™ product formulation is boric acid for MAX-IN™ Boron and zinc oxide for MAX-IN™ Zinc. It was supplied by Villa Crop Protection. These products contain a sugar alcohol base or fructose which acts as complexing agent. The current farm practice were boric acid and zinc sulphate, with an organic amino acid complex which was supplied by an anonymous supplier. Masterlock® is modified vegetable oil which is supplied by Villa Crop Protection.

### 3.6 Sampling procedure

One day before treatment applications, and 10 to 14 days after treatment applications, six trees, situated in the middle of the experimental plots, to eliminate drift or contamination from other treatments, were randomly selected per replication for sampling. All measurement trees were clearly marked for repeated sampling. From these trees, combined samples were obtained from six positions, i.e., from the inside and outside of the canopy at three different heights, namely top, middle and bottom of the tree. These samples were all from fruit bearing terminals (Figure 19). Fifty to 70 leaves, picked from fruit bearing terminals (i.e., five to nine months old) were collected per replication. The samples were placed in paper bags and inside cooler boxes before they were couriered to the laboratory for analysis one day after collection.



**Figure 19. Illustration of the leaves from fruit bearing terminal, suitable for sampling for leaf analyses (Coetzee, 2007)**



### **3.7 Leaf analysis**

The leaf analyses were done at a commercial agricultural laboratory (Intertek Agricultural Laboratories situated in Bapsfontein, Gauteng). After sampling, the leaf blades were washed with a Teepol solution, rinsed with de-ionised water and dried over night at 70 °C in an oven. The dried leaves were then milled and ashed at 480 °C, shaken up in a 50:50 HCl (32%) solution for extraction through filter paper (Campbell & Plank, 1998 and Miller, 1998). The cation and micro-nutrient (B, Fe, Zn, Cu, Mn) content of the extract was measured with a Varian ICP-OES optical emission spectrometer. Total N content of the ground leaves was determined through total combustion in a Leco N-analyser.

### **3.8 Fruit set**

Fruit set was determined by the number of flowers per tree estimated by counting the number of flowers within the limits of a 0.5 × 0.5 × 0.5 m frame during full bloom in September, as well as a fruit count in December for both season. These were the same data trees as used for the leaf sampling. The tree canopy was divided into an Eastern and Western sector and an upper and lower half. A flower count was performed in each of these four respective quadrants per tree. The total number of flowers was estimated by extrapolating the mean number of flowers per frame to the total tree volume. The canopy volume [V (m<sup>3</sup>)] was calculated according to the following formula (Burger et al., 1970):

$$V = r^2(\pi h - 1.046r)$$

r = canopy radius;

h = height of the fruit bearing canopy.

### **3.9 Deposition and surface area coverage of the droplets onto the leaf surface**

Two methods were used to evaluate deposition of the spray mixture. The first was water sensitive paper (Syngenta) to evaluate deposition and leaf surface coverage of the droplets sprayed by the light aeroplane and the conventional tractor method. This was done to establish the wetting pattern of the spray solution. The water sensitive paper is specially coated with a yellow surface which stained dark blue when any droplet lands on the paper. There were two

treatments (Treatment 8 and 9) each with three replications, and six trees were evaluated per replication. Treatment 8 was an aerial application with the inclusion of Masterlock® and Treatment 9 was an aerial application without Masterlock®. The treatments were sprayed a month later completely separate from treatments 1-7.

The water sensitive paper was placed at three different heights in the tree that included the top, middle and bottom, and were also placed on the inside and outside of the tree canopy at the same locations where the leaf samples were taken, giving a total of six samples per tree. After the aerial and tractor applications the water sensitive paper was left to dry and then the droplets were analysed using an automatic image analyser application, SnapCard®. This analysis was only done in the 2019 season. SnapCard was developed by The University of Western Australia and the Department of Agriculture and Food, Western Australia. The application is available on play store.

The second method, used in the 2020 season, was to use a fluorescent dye (Uview™) to evaluate surface coverage and droplet size using a fluorescent light (Figure 20). Fifteen leaves were sampled per treatment inside and outside of the canopy at three different heights namely: top, middle and bottom of the tree canopy. The percentage coverage was, in this case, determined using the Dropsight application (<https://dropsight.ag/>).

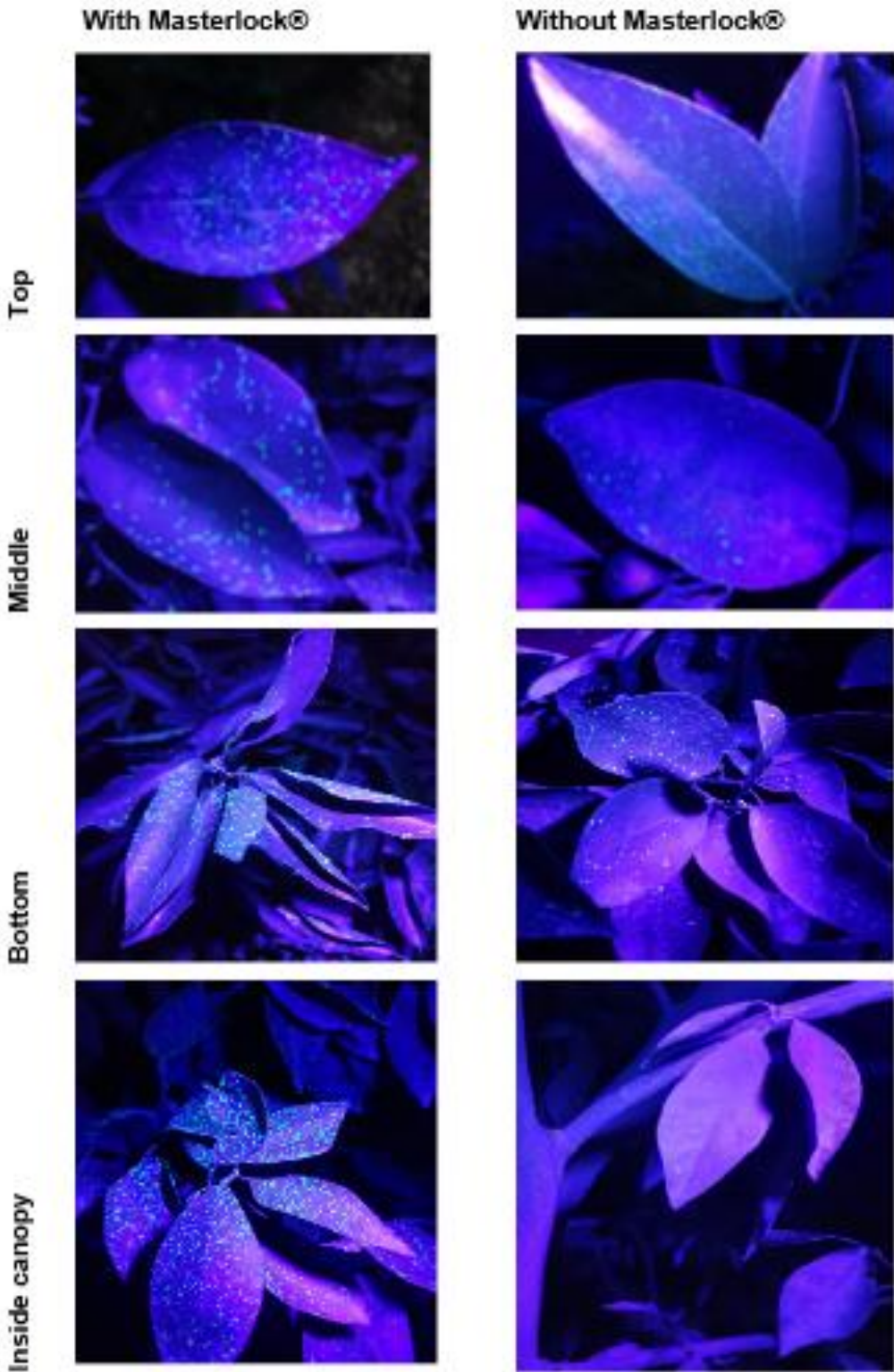


Figure 20. Distribution and size of droplets as a result of aerial applications with and without Masterlock® at different positions in the tree canopy as shown with fluorescent dye.

### **3.10 Satellite imagery**

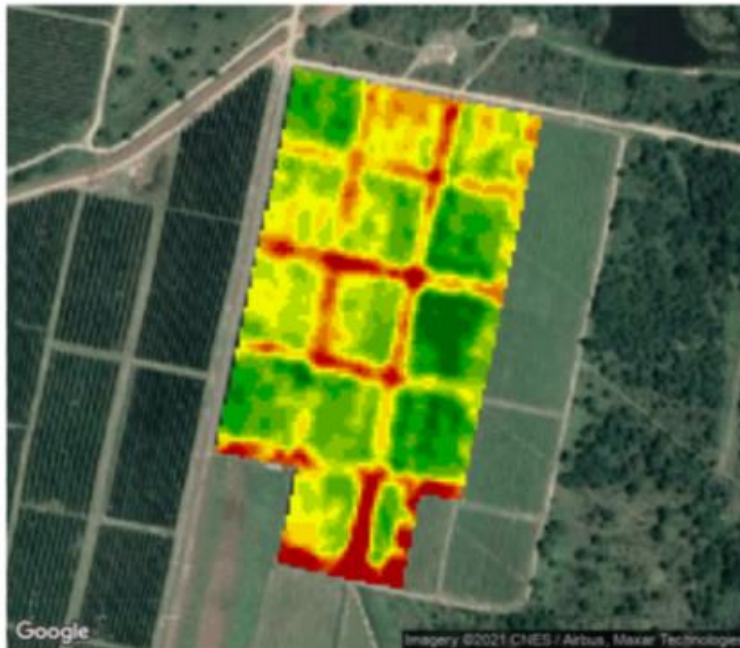
Two vegetation indices, i.e., normalised difference vegetation index (NDVI) and enhanced vegetation index (EVI), were used to assess tree vigour post-application of micronutrients. Geosys, (a company that supplies real time weather and satellite technology) supplied the NDVI and EVI imagery, which was then accessed through the CROPTICAL application (which provided the Geosys satellite data in workable form). Geosys has satellites that take images on a daily basis, this builds layers on top of each other to form these EVI and NDVI images over time (<http://www.urthecast.com/geosys>). CROPTICAL application is the route to accessing the images at any given time from the past.

The plant vigour status minimum increased from 0.2 before application (Fig 21) to 0.25 after application (Fig 22) for the treatments combined. The plant vigour status maximum increased from 0.4 before application to 0.46 after application. The average vigour status also increased from 0.3 before application to 0.39 after application. The EVI images were also used to determine the layout of the trial because of the difference in vigour throughout the orchard as well as the slope present that can lead to leaching of water and nutrients with a maximum elevation of 586 m and a minimum of 566 m (Fig 23). The red areas between the treatments are roads with low vigour as can be seen on Fig 21 and Fig 22. The 3D elevation was determined also via Geosys and CROPTICAL as explained for the NDVI and EVI images.

REPORT  
EVI

SEASON 2021  
GROWER LETABA  
FARM D6662  
FIELD NAME H75  
AREA 13 HA

CROP SOFT CITRUS  
VARIETY  
SOWING DATE 05/03/2021  
IRRIGATED  
USAGE GRAIN



CREATION DATE  
▶ 01/06/2021

IMAGE DATE  
▶ 13/09/2020

MIN VALUE  
▶ 0.2

AVG VALUE  
▶ 0.35

MAX VALUE  
▶ 0.41

INDEX	[Color Scale: Red to Green]													
EVI	0.2	0.29	0.3	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.4	0.41
AREA (HA)	0.47	0.26	0.38	0.59	0.95	1.30	1.82	1.91	1.56	1.67	1.26	0.54	0.34	
AREA (%)	3.6%	2%	2.9%	4.5%	7.2%	10%	13.9%	14.6%	12%	12.8%	9.7%	4.2%	2.6%	

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Figure 21. Enhanced Vegetative Index image of H75 orchard pre application

# REPORT EVI

**croptical**<sup>®</sup>  
farm monitoring tool

SEASON **2021**

CROP **SOFT CITRUS**

GROWER **LETABA**

VARIETY

FARM **D6662**

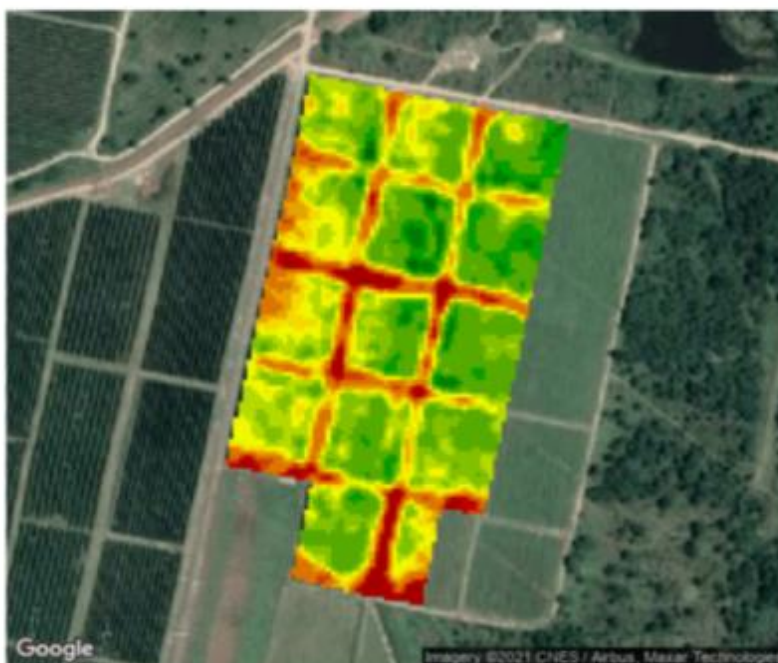
SOWING DATE **05/03/2021**

FIELD NAME **H75**

IRRIGATED

AREA **13 HA**

USAGE **GRAIN**



CREATION DATE

▶ **03/06/2021**

IMAGE DATE

▶ **21/05/2021**

MIN VALUE

▶ **0.25**

AVG VALUE

▶ **0.39**

MAX VALUE

▶ **0.46**

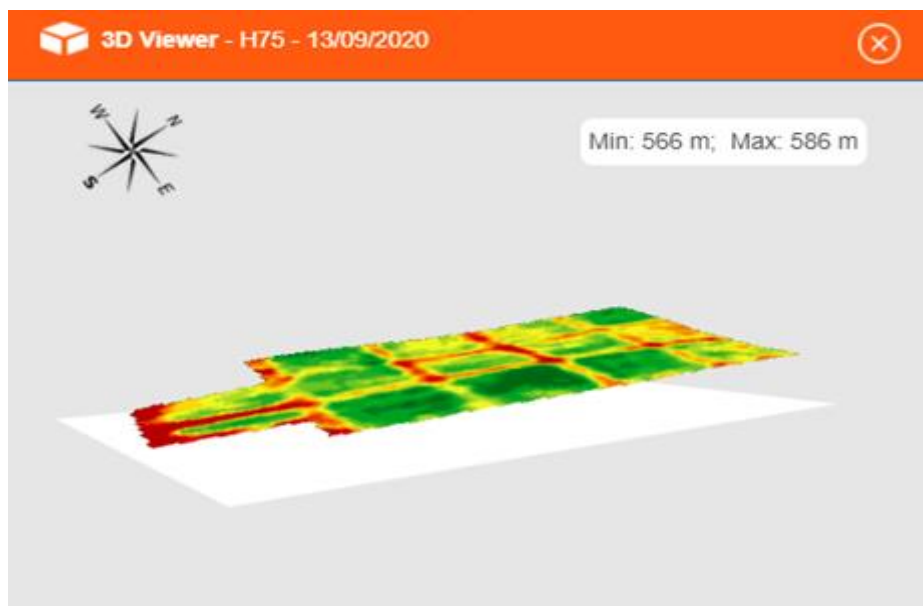
INDEX	[Color Scale: Red to Green]													
EVI	0.25	0.32	0.33	0.34	0.36	0.37	0.38	0.39	0.4	0.41	0.43	0.44	0.45	0.46
AREA (HA)	0.41	0.23	0.35	1.20	0.74	0.96	1.49	1.84	1.86	2.96	0.73	0.22	0.03	
AREA (%)	3.1%	1.8%	2.7%	9.2%	5.7%	7.4%	11.4%	14.1%	14.3%	22.7%	5.8%	1.7%	0.2%	

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**Figure 22. Enhanced Vegetative Index image of H75 orchard post application**



**Figure 23. 3D Elevation of the study orchard (H75) that illustrate the slope that is present**

### **3.11 Total soluble solids (Brix) and Fruit size**

In the 2019 season the total soluble solids (TSS, %Brix) was determined by sampling 9 fruit per treatment at three different heights from the outside of the tree, which included top, middle and bottom of the three. After picking the fruit, they were placed in insulated bags and couriered overnight to the CRI. %Brix value indicates the total sugar content of the fruit, thus the higher the Brix value the sweeter the fruit. These samples were taken a month after the treatment applications. For the purpose of the trial, it was included to determine if there were any differences between the treatments. Juice from all fruit per treatment were pooled and one measurement was made per treatment. The same 9 fruit per treatment was measured to determine average size per treatment. Each market requires different fruit sizes thus making it economically important to evaluate.

### **3.12 Scanning Electron Microscope (SEM) analysis**

Leaf samples were taken from inside and outside of the tree canopy at three different heights (top, middle and bottom) 7 days after application. The leaves that were sampled were placed

inside small glass containers. The glass containers were then couriered in a polystyrene cooler box. Analysis was accomplished using a Zeiss EVO® MA15 Scanning Electron Microscope at Stellenbosch University. Prior to analysis the samples were coated with a thin layer of gold in order to establish conductivity.

The analyses of cell layers of the leaf in cross sections (adaxial epidermis, mesophyll, abaxial epidermis) were taken in area mode and quantified by energy dispersive spectrometry and wavelength dispersive spectrometry (Zn 60 sec Peak/Background and B 60 sec Peak/Background) using an Oxford Instruments® X-Max 20 mm detector and Oxford INCA software.

### **3.13 Statistical analysis**

The leaf analyses, deposition and fruit set data were subjected to averages per treatment as well as standard deviation. The averages were subjected to Analysis of Variance technique (ANOVA) to observe the differences between the treatment as well as their interactions (Noor, et al., 2019). In cases where the differences were significant ( $P < 0.05$ ), the means were further assessed by using the Tukey's HSD post hoc test. For all the statistical analysis Microsoft Excel was used.

### **3.14 Research Methodology**

- Ethical aspects

This study consisted of a few ethical aspects. External people were involved in doing the application for both aerial application method and conventional tractor method. There are two companies involved regarding funding and data capturing: Villa Crop Protection as well as the CRI. There is also another company's product used for the farm practice treatment that will not be announced in this writing.



## CHAPTER 4: Results and Discussion

### 4.1 Results

#### 4.1.1 Weather data

The weather results of the 2019 season indicate precipitation of 0.2 mm two weeks prior to application and 3.8 mm two weeks after application. The average maximum temperature for this period was 29.6°C. For 14 out of the 30 days the maximum temperature was above 30°C. The average minimum temperatures for this period were 13.3°C. The average relative humidity for this duration was maximum 78.3% and minimum 23.5%. The day of application the maximum temperature was 28.0°C and the minimum temperature was 12.14°C with a maximum relative humidity of 75.3% and minimum of 31.3%. There was no precipitation on the day of application (Table 5), but a small amount of rainfall (0.2 mm) was recorded the next day.

**Table 5. 2019 Weather data (two weeks prior and two weeks after application)**

Date/Time	Air temperature [°C]			Relative humidity [%]			Daily Precipitation [mm]
	avg	max	min	avg	max	min	
2019/08/20	18,2	20,8	15,2	64,2	80,0	41,6	0,0
2019/08/21	17,5	24,7	11,9	58,9	87,0	29,4	0,0
2019/08/22	16,8	25,0	10,4	60,7	83,3	31,7	0,0
2019/08/23	18,3	28,5	10,5	50,2	81,8	18,3	0,0
2019/08/24	18,1	28,3	9,0	48,4	76,2	19,9	0,0
2019/08/25	20,5	30,5	12,6	45,9	73,1	18,4	0,0
2019/08/26	23,2	34,0	14,5	33,0	59,3	9,8	0,0
2019/08/27	23,7	34,2	14,5	28,6	51,2	10,0	0,0
2019/08/28	21,6	30,3	13,3	44,9	65,9	26,1	0,0
2019/08/29	20,5	28,0	14,9	59,5	84,9	34,4	0,0
2019/08/30	22,5	34,1	13,8	41,9	73,1	11,0	0,0
2019/08/31	23,6	33,2	15,2	37,7	69,4	6,1	0,0
2019/09/01	19,6	25,4	14,6	53,4	75,1	9,7	0,0
2019/09/02	19,9	30,2	10,3	48,2	90,5	13,9	0,2
<b>2019/09/03</b>	<b>19,2</b>	<b>28,0</b>	<b>12,1</b>	<b>53,4</b>	<b>75,3</b>	<b>31,3</b>	<b>0,0</b>

2019/09/04	22,5	34,2	12,5	40,2	79,6	8,0	0,0
2019/09/05	21,7	29,8	12,9	40,5	70,2	22,7	0,0
2019/09/06	16,7	20,0	14,1	75,6	88,3	62,3	3,0
2019/09/07	14,8	20,4	12,5	75,3	95,6	43,8	0,8
2019/09/08	15,3	22,8	9,6	56,7	86,3	28,4	0,0
2019/09/09	17,9	29,2	7,7	51,4	85,9	20,1	0,0
2019/09/10	19,2	29,9	10,0	50,5	84,4	22,6	0,0
2019/09/11	21,7	33,5	11,7	43,4	72,5	15,1	0,0
2019/09/12	24,7	36,6	14,9	30,3	56,9	7,3	0,0
2019/09/13	26,2	38,6	16,0	25,2	70,0	5,5	0,0
2019/09/14	20,6	27,2	16,9	67,6	85,6	42,0	0,0
2019/09/15	20,9	28,8	17,2	74,1	96,4	43,4	0,0
2019/09/16	22,5	31,0	15,8	66,3	90,3	37,3	0,0
2019/09/17	24,0	33,4	16,1	56,4	85,8	26,0	0,0
2019/09/18	25,7	36,7	17,5	43,0	76,5	9,0	0,0

In 2020 there was 38.8 mm of precipitation two weeks prior to application and 11.6 mm two weeks after application. The average maximum temperature for this period was 27.47°C. For 9 out of the 30 days the maximum temperature was above 30°C. The average minimum temperatures for this period were 14.52°C. The average relative humidity for this duration was maximum 85.76% and minimum 38.85%. The day of application the maximum temperatures was 32.33°C and minimum 13.98°C, with a maximum relative humidity of 73.58% and minimum 41.36%. There was no precipitation on the day of application, but 1.2 mm fell in the two days after application (Table 6).

**Table 6. 2020 Weather data (two weeks prior and two weeks after application)**

Date/Time	HC Air temperature [°C]			HC Relative humidity [%]			Precipitation [mm]
	avg	max	min	avg	max	min	sum
2020/09/01	12,7	13,5	12,3	96,9	97,8	89,3	32,8
2020/09/02	14,0	15,6	12,5	94,6	98,2	84,9	6,0
2020/09/03	18,4	26,1	13,7	75,2	96,7	42,4	0,0
2020/09/04	18,2	24,8	13,4	70,6	94,6	45,0	0,0
2020/09/05	18,4	25,0	13,5	67,7	86,7	39,3	0,0
2020/09/06	16,2	23,7	11,9	70,8	91,4	37,5	0,0
2020/09/07	17,1	25,3	11,0	66,3	87,4	36,1	0,0
2020/09/08	18,4	26,2	12,5	64,5	88,3	39,0	0,0
2020/09/09	18,8	26,3	13,9	63,7	87,7	35,2	0,0
2020/09/10	18,7	27,4	12,0	62,0	87,4	31,2	0,0

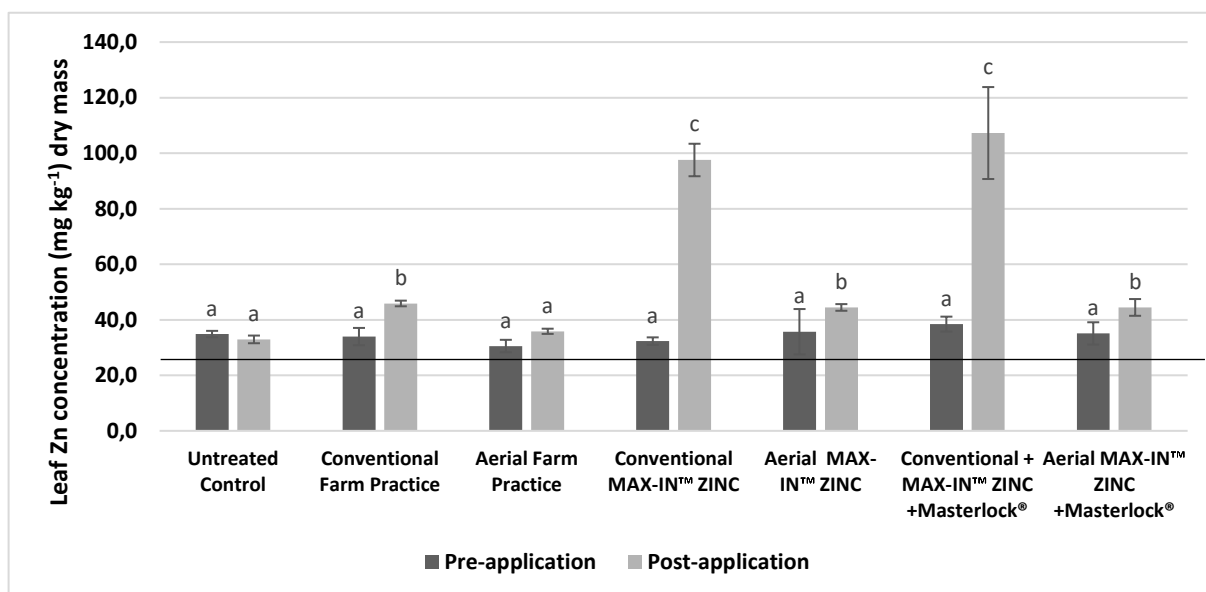
2020/09/11	19,2	27,0	11,8	57,5	87,9	29,3	0,0
2020/09/12	19,3	27,3	14,3	63,9	94,2	29,6	0,0
2020/09/13	20,8	29,7	12,5	55,2	85,7	23,2	0,0
2020/09/14	22,9	32,7	15,3	42,9	67,1	15,9	0,0
<b>2020/09/15</b>	<b>23,9</b>	<b>32,3</b>	<b>14,0</b>	<b>41,4</b>	<b>73,6</b>	<b>21,6</b>	<b>0,0</b>
2020/09/16	23,3	31,0	18,5	64,6	90,5	34,9	1,0
2020/09/17	21,3	26,8	16,2	69,0	89,5	52,8	0,2
2020/09/18	20,5	27,5	16,3	72,3	91,4	47,6	0,0
2020/09/19	25,2	36,2	16,1	51,8	87,0	21,1	0,0
2020/09/20	22,8	32,5	16,8	64,9	94,0	26,1	0,0
2020/09/21	23,5	34,8	16,4	48,2	72,3	12,7	0,0
2020/09/22	16,9	18,9	15,1	65,7	73,7	54,7	0,0
2020/09/23	19,1	27,1	14,8	59,8	77,0	35,4	0,0
2020/09/24	21,6	31,2	13,8	52,5	81,3	25,2	0,0
2020/09/25	24,5	36,1	14,5	44,4	78,1	16,2	0,0
2020/09/26	27,9	41,3	16,7	37,6	68,3	9,5	0,0
2020/09/27	20,0	26,4	16,9	62,4	75,1	42,4	0,0
2020/09/28	21,5	28,3	17,1	65,1	85,4	41,0	0,0
2020/09/29	20,2	24,2	17,2	72,7	87,2	58,5	0,0
2020/09/30	16,4	19,3	15,0	89,9	97,6	79,8	10,4

#### 4.1.2 Leaf analyses in the 2019 season

Pre-application zinc (Zn) (Fig 24) and boron (B) (Fig 25) leaf concentrations were within the accepted local norms for the 2019 season as proposed by Coetzee (2007). This is illustrated by the horizontal lines on the Fig 24 and 25. For the majority of the various treatments there was a significant increase in leaf Zn concentration post-application, although not all differences were significant.

Whilst the aerial application of the farm practice Zn product did not significantly increase leaf Zn and was not significantly different to the untreated control, the conventional farm practice was able to increase Zn leaf levels significantly (Figure 24). However, there was a significant increase in leaf Zn concentration post application when MAX-IN™ ZINC alone or with Masterlock® was applied aerially. The two conventional applications of the MAX-IN™ Zinc products with and without Masterlock® increased leaf Zn. However, once again there was no significant difference in Zn concentration between treatments with or without Masterlock®. The conventional post application with Masterlock® was higher aerial application, but no

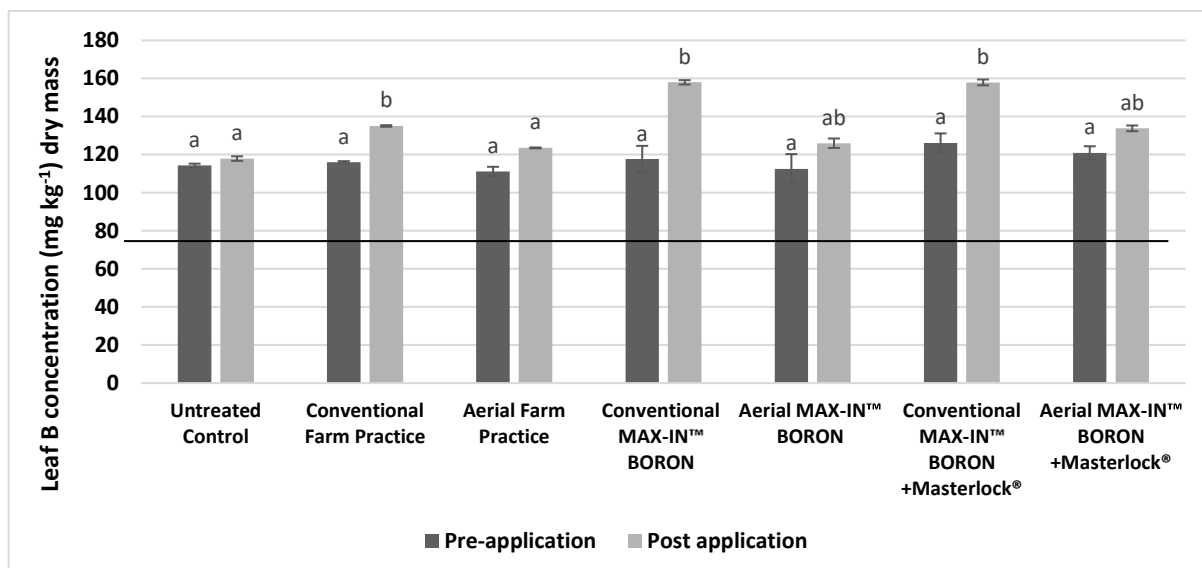
significant difference with or without Masterlock® between conventional and aerial was observed. Overall, the addition of Masterlock® compared to the other treatments either pre or post did not cause significant differences, but tended to be a bit higher with aerial applications.



**Figure 24. Comparison of pre-application and post-application Zn concentrations of Valencia leaves as affected by foliar Zn nutrition and adjuvant application applied in September 2019. The horizontal line indicates the minimum acceptable Zn leaf concentration. Statistical analysis was performed using one way ANOVA and Tukey's post hoc test. Bars with different letters are significant difference from each other with  $P < 0.05$ . There were also significant differences between treatments pre-and post-application with  $P < 0.0083$**

As observed for Zn, the pre-application and post application leaf concentration for B for the untreated control and the aerial farm practice in the 2019 season did not differ significantly (Figure 25). However, in contrast to Zn, there were also no significant difference between pre-and post-application B levels for aerial MAX-IN™ BORON with or without Masterlock®.

Significant increases in leaf B following applications were only found for the conventional applications in the 2019 season, which included the farm practice and MAX-IN™ BORON with or without Masterlock®. Importantly, these levels were significantly higher than the untreated control, but were not significantly higher than the aerially applied MAX-IN™ BORON with or without Masterlock®.



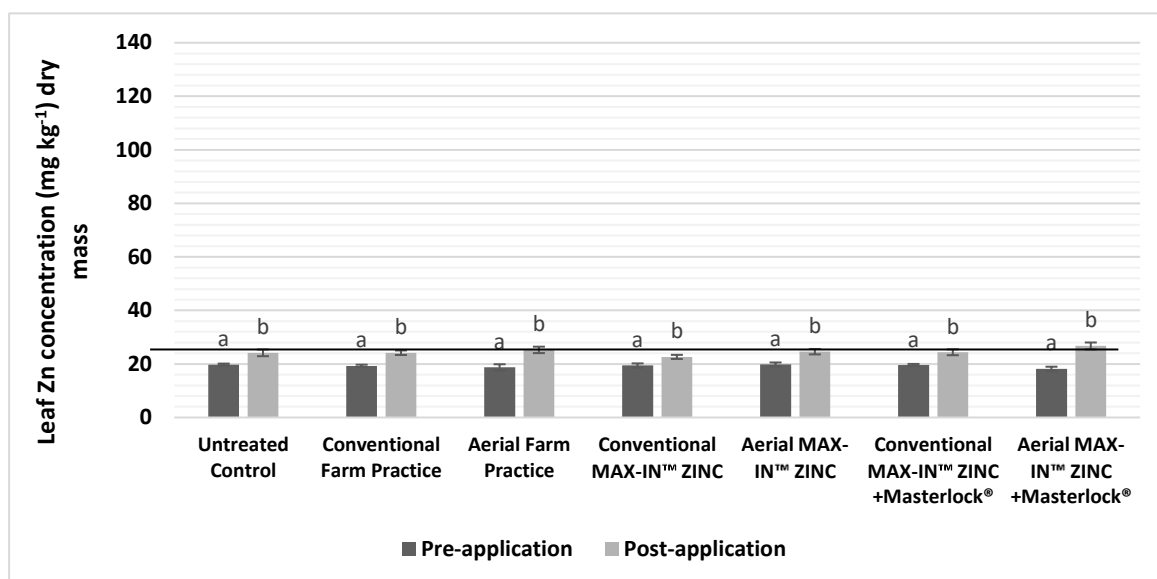
**Figure 25. Comparison of pre-application and post-application B concentrations of Valencia leaves as affected by foliar B nutrition and adjuvant application applied in September of 2019. The horizontal line indicates the minimum acceptable B leaf norm. Statistical analysis was performed using one way ANOVA and Tukey’s post hoc test. Bars with different letters are significant difference from each other with  $P < 0.05$ . There were also significant differences between treatments pre-and post-application with  $P < 0.0083$**

#### 4.1.3 Leaf analyses in the 2020 season

Pre-application boron (B) leaf concentrations were within the accepted local norms proposed by Coetzee (2007) for the 2020 season (Figure 27). However, the zinc (Zn) concentrations pre-application samples were below the norm (Figure 26).

The post-application Zn concentration was significantly higher than the pre-application leaf concentration for all the treatments, including the untreated control and as a result all treatments post application fell within the norm for Zn for citrus.

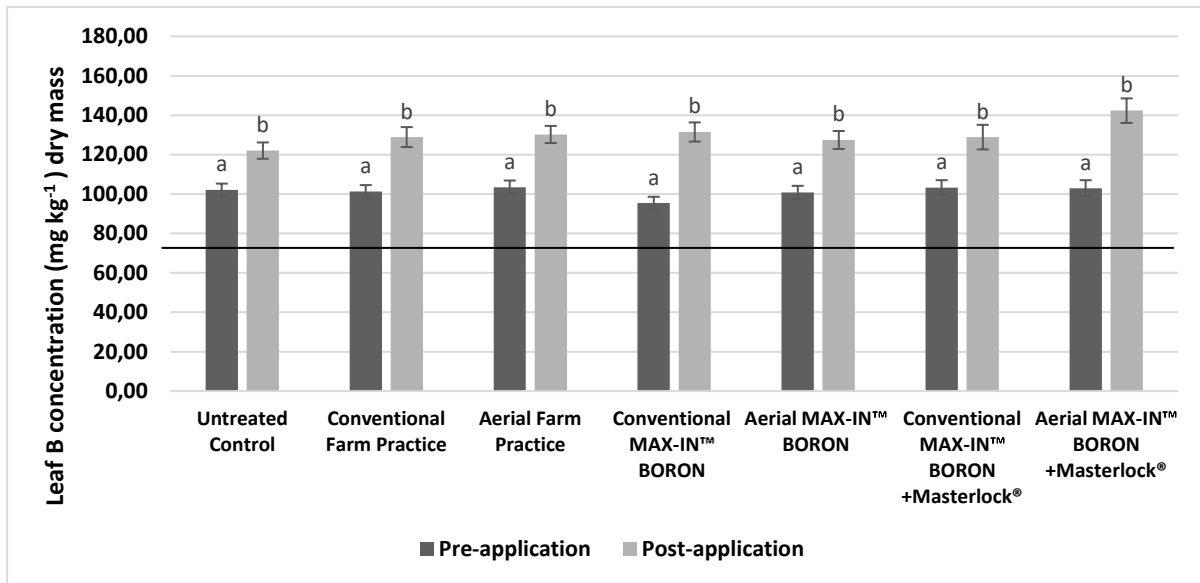
There were no significant differences between any of the treatments post application.



**Figure 26. Comparison of pre-application and post-application Zn concentrations of Valencia leaves as affected by foliar Zn nutrition and adjuvant application applied in September of 2020. The horizontal line indicates the minimum acceptable Zn leaf. Statistical analysis was performed using one way ANOVA and Tukey’s post hoc test. Bars with different letters are significant difference from each other with  $P < 0.05$ . There were also significant differences between treatments pre-and post-application with  $P < 0.0083$**

As observed for Zn, the post-application B leaf concentration of all treatments was significantly higher than the pre-application leaf concentration, including the untreated control (Figure 27).

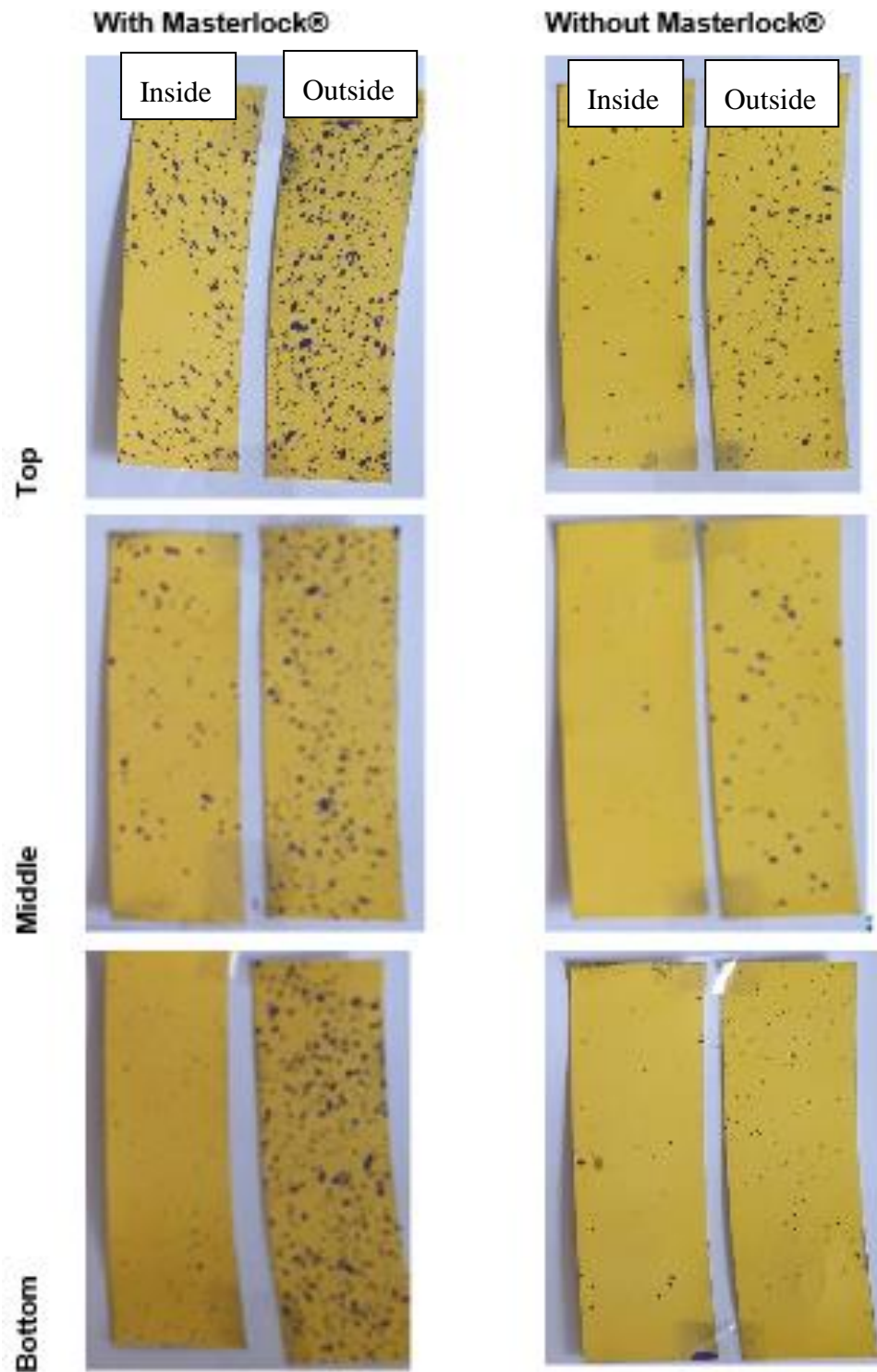
Also as observed for Zn, the aerial application treatment of MAX-IN™ BORON with the inclusion of Masterlock® also had the highest B leaf concentration although not significantly different to the other treatments. There were no significant differences between any of the treatments post application.



**Figure 27. Comparison of pre-application and post-application B concentrations of Valencia leaves as affected by foliar B nutrition and adjuvant application applied in September of 2020. The horizontal line indicates the minimum acceptable B leaf. Statistical analysis was performed using one way ANOVA and Tukey’s post hoc test. Bars with different letters are significant difference from each other with  $P < 0.05$ . There were also significant differences between treatments pre-and post-application with  $P < 0.0083$**

#### **4.1.4 Droplet deposition of aerially applied micronutrient products**

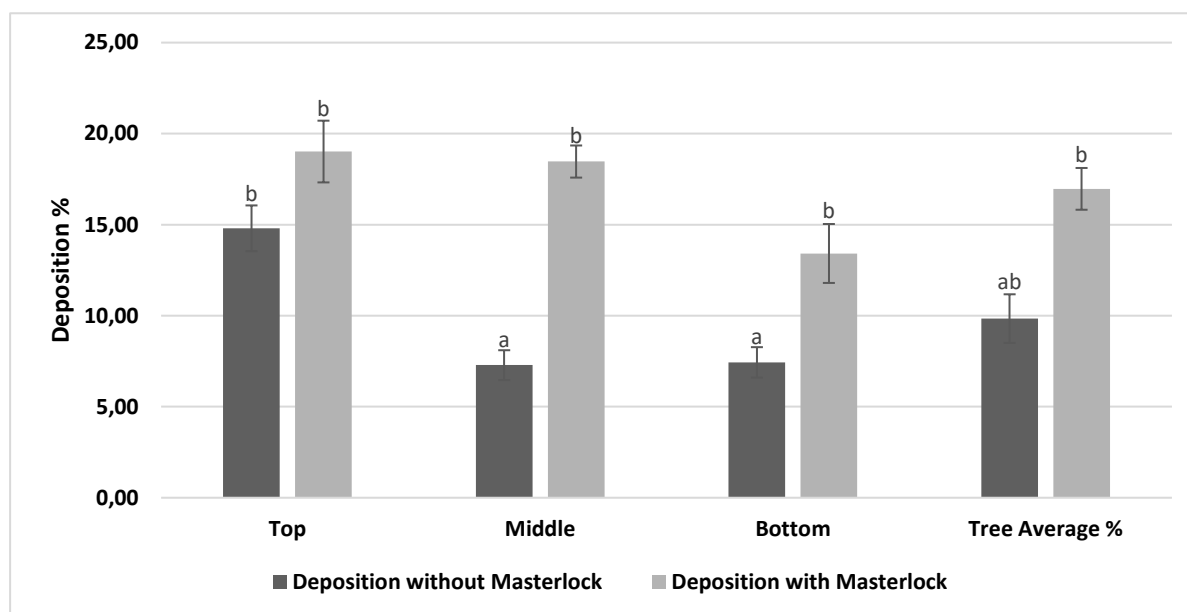
Droplet deposition was analysed with two different methods. The first method was using water sensitive paper to evaluate deposition. In Figure 28, the deposition is illustrated on the water sensitive paper that was used. This is a visual deposition representation with and without Masterlock® in different heights of the tree canopy. Masterlock® significantly increased deposition on total leaf surface at the middle and bottom parts of the tree canopy. More droplets reached the water sensitive paper with the inclusion of Masterlock®.



**Figure 28. Difference in distribution of droplets (wetting pattern) between treatments containing Masterlock® and without Masterlock®, at different positions in the tree canopy as shown with water sensitive paper**

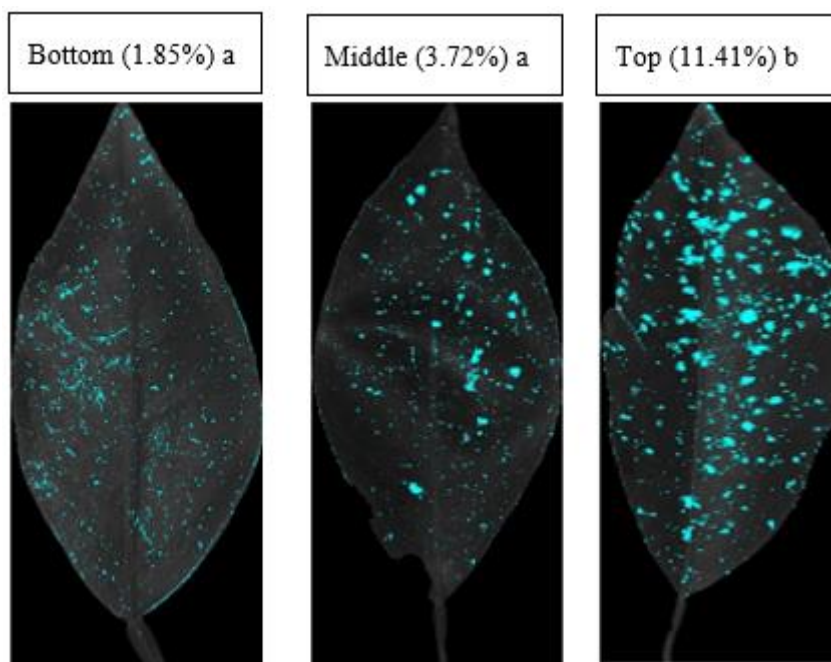


From the quantitative data of the water sensitive paper that was used for analyses, it can be seen that there was a significant difference between the treatments with and without Masterlock®, with deposition increasing significantly with the inclusion of Masterlock® at the bottom and middle parts of the tree canopy as well as the total average deposition % for the tree. Masterlock® had no significant difference in deposition at the top part of the tree canopy compared to the treatment without Masterlock® (Figure 29).

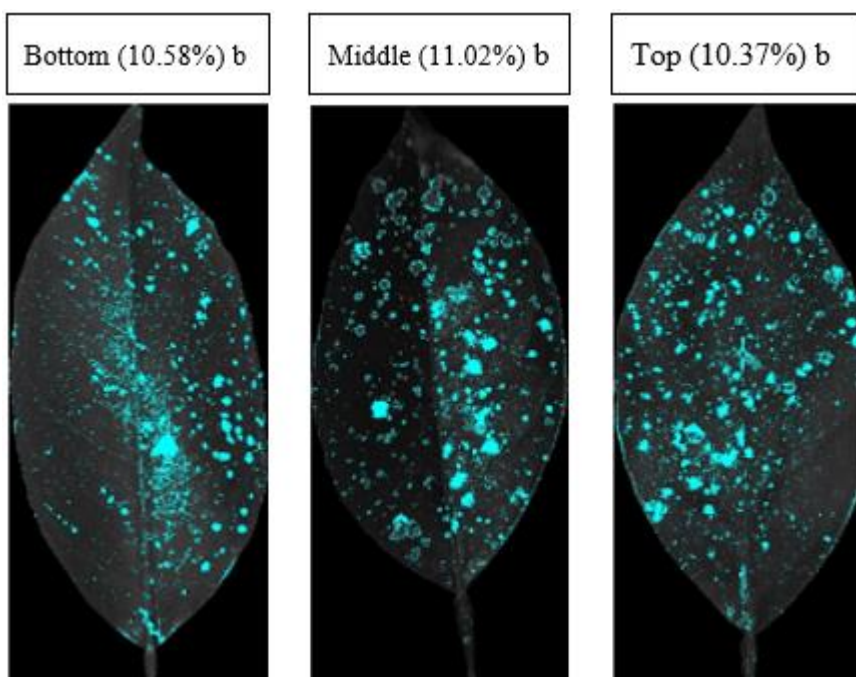


**Figure 29. Deposition of Valencia leaves with and without Masterlock®. The spray volume was applied to the orchard by a light aeroplane and analysed using water sensitive paper. Statistical analysis was performed using one way ANOVA and Tukey’s post hoc test. Bars with different letters are significant difference from each other  $P<0.05$ . There were also significant differences between treatments pre-and post-application with  $P<0.0167$**

The second method consisted of fluorescent dye and the Dropsight application that was used for analyses. As can see in Figure 30 (Without Masterlock®) and Figure 31 (With Masterlock®) there were clear visual differences in the deposition of the spray mixture when Masterlock® was included, supposed to when no Masterlock® was included. This was also depended on the level of the canopy from where the leaves were harvested. The treatment without Masterlock®, the top parts of the tree canopy had sufficient deposition, although the middle and bottom parts, the deposition decreased significantly. The treatment with Masterlock®, had sufficient Fluorescent Particle Coverage (FPC %) in the top, middle and bottom parts of the tree canopy.

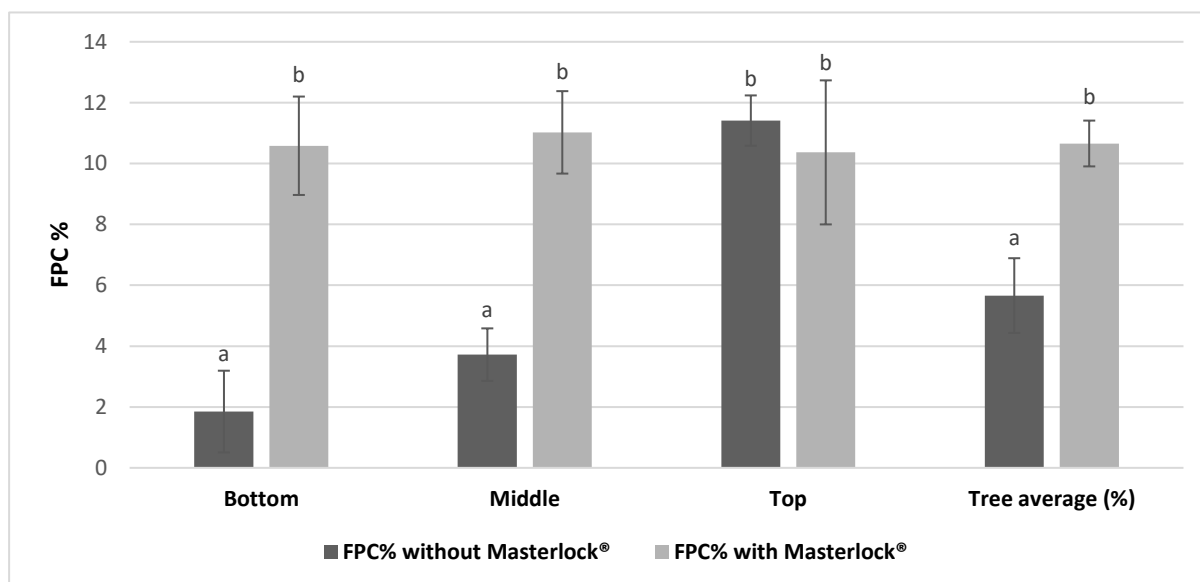


**Figure 30. Images of difference in distribution of droplets and their sizes between aerial applications without Masterlock®, at different positions in the tree canopy as shown with fluorescent dye and analyses with Dropsight**



**Figure 31. Images of distribution of droplets and their sizes between aerial applications with Masterlock®, at different positions in the tree canopy as shown with a fluorescent dye and analysis with Dropsight**

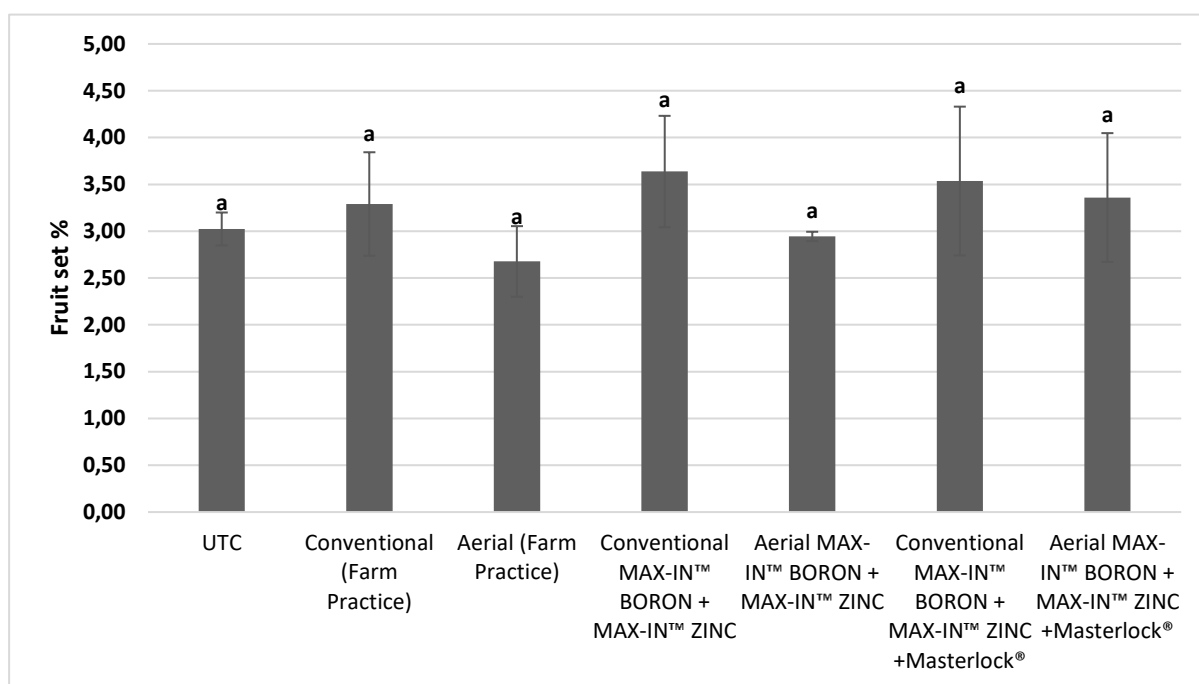
The qualitative results were quantified and the FPC % increased significantly with the inclusion of Masterlock®, at the bottom and middle parts of the tree canopy, as well as the total average FPC % for the tree (Figure 32). However, there were no significant difference in FPC% at the top part of the tree canopy for the Masterlock® treatments as compared to the treatment without Masterlock®.



**Figure 32. Fluorescent Particle Coverage (FPC)% of Valencia leaves with and without Masterlock®. The fluorescent dye was applied to the orchard by a light aeroplane and analysed using Dropsight. Statistical analysis was performed using one way ANOVA and Tukey’s post hoc test. Bars with different letters are significant difference from each other with  $P < 0.05$ . There were also significant differences between treatments pre-and post-application with  $P < 0.0025$ .**

#### 4.1.5 Fruit set

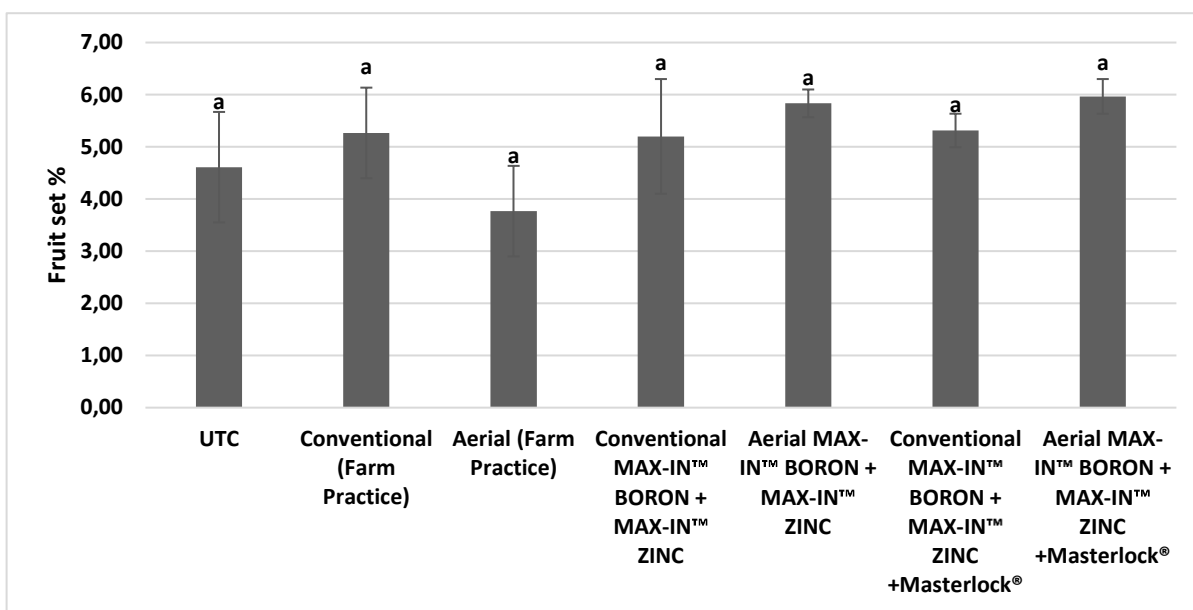
There were no significant differences in fruit set in the 2019 season for any of the treatments (Figure 33). The conventional application of MAX-IN™ ZINC and MAX-IN™ BORON with and without Masterlock® yielded the highest fruit set % although this was not significantly to any of the other treatments. Fruit set was lowest in the farm practice aerial application compared to the similar treatment using conventional farm practice, although not significantly.



**Figure 33. Fruit set percentage for the 2019 season. Statistical analysis using one way ANOVA and Tukey’s post hoc test indicated no significant difference between treatments with  $P>0.05$**

As observed in the 2019 season, there were also no significant differences in fruit set % between any of the treatments in the 2020 season (Figure 34). Fruit set % was higher for all the treatments in the 2020 season as compared to the 2019 season. Although not significant, fruit set was slightly higher when Masterlock® was included in the spray treatments for both the aerial and conventional application methods, although not significantly.

Fruit set in the aerial farm practice treatment was once again the lowest compared to the other treatments. In contrast to 2019, the aerial application treatments with MAX-IN ZINC™ and MAX-IN BORON™, with or without Masterlock® resulted in the highest fruit set although not significantly compared to the rest of the treatments.

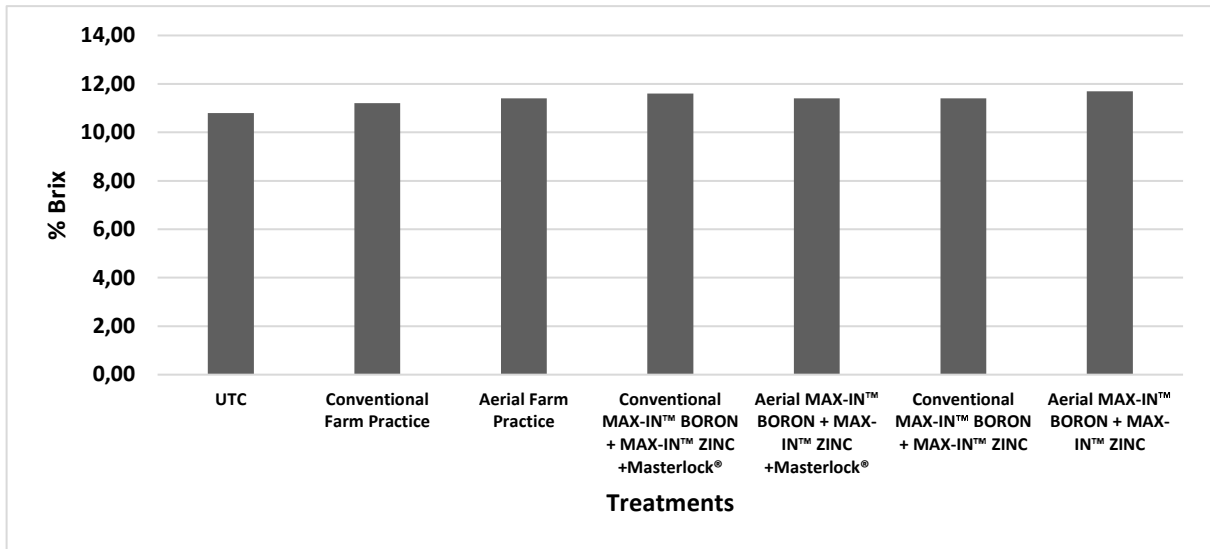


**Figure 34. Fruit set % for season 2020. Statistical analysis using a one-way ANOVA and Tukey’s post hoc test indicated no significant difference between treatments with  $P>0.05$**

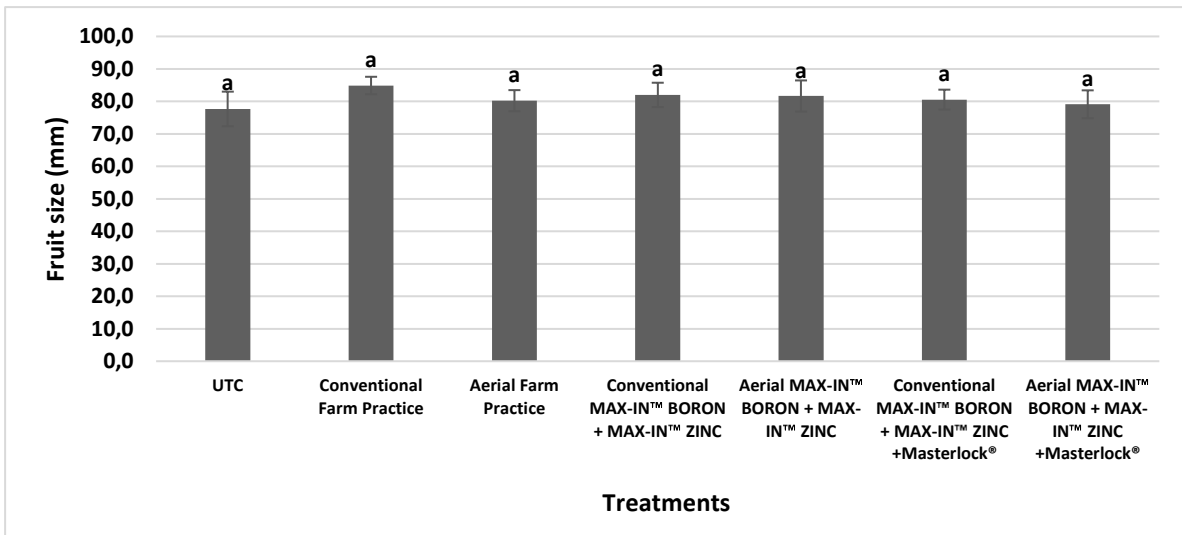
#### 4.1.6 Total soluble solids (%Brix) and Fruit Size

In the 2019 season the average %Brix was determined for a pooled sample from nine fruit per treatment and as a result no statistics could be performed (Figure 35). Fruit from the aerial application of MAX-IN™ products without Masterlock® had the highest Brix value of 11.7%, with fruit from the UTC having the lowest Brix value of 10.8%. All of the treatments increased the Brix value after application compared to the UTC, but this was unlikely to be statistically significant. The addition of Masterlock® only slightly increased the Brix value for the conventional treatments of the MAX-IN™ products. The export standard for Valencia’s citrus varieties is 8.5%, therefore all treatment was within export standard.

There were no significant differences in fruit size between the treatments (Figure 36).



**Figure 35. Total Brix value for all treatments for the 2019 season**



**Figure 36. Fruit size for all the treatments for the 2019 season. Statistical analysis using one way ANOVA and Tukey's post hoc test indicated no significant difference between treatments with  $P > 0.05$**

#### 4.1.7 Scanning Electron Microscope (SEM) analysis

No B was detected in the leaf tissues in the SEM analyses (ranging from 500 mg kg<sup>-1</sup> to 1000 mg kg<sup>-1</sup>, indicated as 0.5-1%), while Zn (ranging from 200 to 400 mg kg<sup>-1</sup>, indicated as 0.2% - 0.4%) was only detected in two of the 21 samples (replicates) (Table 7). Zinc was detected in two of the Aerial Farm Practice (T6) replications.

**Table 7. The determination of boron and zinc concentration in leaves from the various aerial and conventional applications using SEM and energy dispersive spectrometry and wavelength dispersive spectrometry**

	Weight% B	Weight% Zn	Weight% Total
Treatment			
Aerial MAX-IN™ BORON + MAX-IN™ ZINC	0.00	0.00	100.00
	0.00	0.00	100.00
	0.00	0.00	100.00
Conventional Farm Practice	0.00	0.00	100.00
	0.00	0.00	100.00
	0.00	0.00	100.00
UTC	0.00	0.00	100.00
	0.00	0.00	100.00
	0.00	0.00	100.00
Conventional MAX-IN™ BORON + MAX-IN™ ZINC	0.00	0.00	100.00
	0.00	0.00	100.00
	0.00	0.00	100.00
Aerial MAX-IN™ BORON + MAX-IN™ ZINC + Masterlock®	0.00	0.00	100.00
	0.00	0.00	100.00
	0.00	0.00	100.00
Aerial Farm Practice	0.00	<b>0.02</b>	100.00
	0.00	<b>0.04</b>	100.00
	0.00	0.00	100.00
Conventional MAX-IN™ BORON + MAX-IN™ ZINC + Masterlock®	0.00	0.00	100.00
	0.00	0.00	100.00
	0.00	0.00	100.00

## 4.2 Discussion

The weather conditions for both seasons differed to some extent and as a result of this, the leaf analyses as well as fruit set results varied. Previous studies suggest that the most effective temperatures for flower induction appear to be between 10 to 15°C (Albrigo, 2004). In the 2019 season the average temperatures were 2°C colder than the 2020 season. The lower temperature could have had an effect on the number of flowers, which directly correlates with fruit set.

Very high temperature during bloom, and for the following 2 months without good moisture could also initiate fruit drop (Albrigo, 2004). In the 2019 season no rainfall was present during full bloom and fruit set, in comparison in the 2020 season, there was rainfall present at full bloom, with extensive follow up rainfall at fruit set. Although no significant differences between treatments were observed regarding fruit set for both seasons, the environmental conditions could have had an impact on the increase fruit set percentage in the 2020 season.

The difference in rainfall mentioned for both seasons could also have had an effect on nutrient uptake. There was not enough data to statistically test whether weather conditions were different and how it impacted the results. In the 2019 season where rain was scarce during full bloom and fruit set, the citrus trees only received water via irrigation. Previous studies suggests that significant reductions in brix, citric acid, and suspended solids with increased frequency of irrigation, although in this study it was not recorded (Cruse et al., 1982). Rainfall increased surface area that is wettened, which increases total area available for nutrient interception and uptake by the roots. Zn and B availability is mostly confined to rhizosphere soil (Marschner, 1993). Changes in the rhizosphere are of importance for Zn uptake. The supply or application of ammonium nitrogen to the soil will acidify the soil and Zn will become more mobile and available for uptake (Zekri et al; 2014).

In the 2020 season the UTC values for both Zn and B increased significantly from pre-application to post-application, this could be due to rainfall at full bloom and fruit set. The 2020 season Zn and B values started at a lower baseline and this could also induce root uptake as described in the literature. Zinc and B uptake through the roots could have increased due to interception. This could also be because of pH that changed the rhizosphere and became more acidic. There was an ammonium nitrogen application made by the farm few days before treatment applications, which could have acidified the rhizosphere pH, which results in increase



availability of Zn (Marschner, 1993). Rain water pH was also recorded as slightly acidic (MLekouch et al., 2010). There was also a Solubor (20.5%) application to the soil, two months before treatment applications. The rainfall the day after application in the 2020 season could have initiated B uptake through the roots which could explain B leaf concentration that increased for the UTC.

The total leaf concentration for the 2019 season for both Zn and B were within the proposed norms by Coetzee, (2007) before application, and increased significantly after application. For the 2020 season, B leaf concentrations was also within the proposed norms before application, but the Zn leaf concentrations was below the norm. Coetzee, (2007) suggested that a higher response in production is expected (when nutrients are applied) if the concentration is below the norm, compared to if the concentration is above the norm. However, leaf analysis data from this study indicated that even if the concentration is above the norm, nutrient uptake can still occur, but a response to the higher level of nutrients is not often evident.

Zinc deficiencies are reported to result in the deformation of chloroplast structure, which includes loss of lamellae structure and the expansion of the matrix zone (Fei et al; 2016). Additionally, a significant decline in leaf chlorophyll content has been observed with a decline in leaf Zn content (Fei et al., 2016). Boron deficiencies, which are more extensive than other micronutrients, often have an impact on yield and fruit quality (Zekri and Obreza 2003). Boron influences the external and internal cork formation in fruit and deficiencies will lead to small deformed cracked fruit (Shorrocks and Nicholson, 1979). It was therefore important to keep the Zn and B concentration above the norm and to increase uptake. Zinc and B deficiencies were identified in the untreated control treatment two months after application in the 2019 and 2020 season, the other treatments did not present the visual deficiency symptoms (personal observation).

The aerial application of micronutrients was assessed and the leaf analyses data supports the hypothesis that aerial application were effective at increasing nutrient uptake of both Zn and B and increasing leaf concentrations. Only in the 2019 season was it observed that the aerial farm practice treatment could not significantly increase total Zn and B leaf concentration, as for all of the other treatments for both seasons the aerial applications significantly increased leaf Zn

and B concentration. The aerial application water volumes were at 100L ha<sup>-1</sup> which is low compared to the conventional tractor method of 2000L ha<sup>-1</sup>, however as the water volume decreases the concentration of the micronutrient in the spray volume increases. The higher the concentration of the solute that can be applied to a leaf surface, the longer the time it remains in an active state on leaf surface, and as a result, the greater the amount of penetration through the membrane (Oosterhuis, 2009). This is in accordance with Fick's law (Oosterhuis, 2009).

The conventional tractor method treatments significantly increased uptake in all the treatments for both seasons from pre-application to post-application. The significant increase could be because of the increase in coverage with the increase in water volume per ha<sup>-1</sup>, although the concentration in the spray mixture decreases. Previous studies already established that a conventional tractor sprayer Zn and B application at fruit set stage effectively improved the B and Zn level in the leaves, vegetative growth, productivity and fruit quality of Feutrell's Early mandarin (Khan et al., 2012).

The MAX-IN<sup>TM</sup> products consisted of a much higher concentration of Zn and B compared to the farm practice treatments. It was only observed in the 2019 season, that there were significant differences between the MAX-IN<sup>TM</sup> treatments and the farm practice treatments. The data suggested that although the farm practice products had a much lower concentration of Zn and B, compared to the MAX-IN<sup>TM</sup> treatments, that there was no significant difference between the treatments. As suggested by previous studies, Zn and B are only needed in small amounts (Zekri, 2003).

Zinc and B in the MAX-IN<sup>TM</sup> products form complexes with sugar alcohols, and the migration and mobility of these products in an alkaline environment have been found to be more mobile than nutrients that do not form a complex with sugar alcohols (Brown and Hu, 1996). As a result, uptake into the leaf and transport within the leaf would be higher with these complexed micronutrients. In comparison, the current farm practice products were boric acid and zinc sulphate, with an organic amino acid complex. These organic complexes were also studied and found to be an effective mechanism for micronutrient uptake (Kochian, 1991). The SEM data would have quantified the distribution in the leaf, this data would have allowed a better

understanding of distribution. This study concludes no significant difference between the sugar alcohol formulation complexes and the amino acid formulation complexes regards to uptake.

Due to the fact that much of the applied product can remain within the leaf's cuticle and is therefore not taken up to become metabolically active, as well as the fact that higher solute concentration can increase uptake of cations (Schreiber, 2005), analyses of cell layers of the leaves were done in cross sections (adaxial epidermis, mesophyll, abaxial epidermis) to quantify the B and Zn concentration within the leaf tissue. This was done by means of energy dispersive spectrometry and wavelength dispersive spectrometry using SEM (central analytical facility of Stellenbosch University (CAF)). The results were disappointing since it was only established after analysis that the detection limit of the method is  $100 \text{ mg kg}^{-1}$ , while total dry mass Zn and B concentrations typically is in the range of  $25\text{-}100 \text{ mg kg}^{-1}$  and  $75\text{-}200 \text{ mg kg}^{-1}$ , respectively. The variation in analysis results of the macro-nutrients, e.g., Na, K, Ca, Mg, is large and follows no meaningful pattern (data not presented). These analyses therefore could not determine the distribution of applied Zn and B in the leaves. However, it is of critical importance to quantify the Zn and B uptake through the leaf surface, this would indicate if there is any differences between different product formulations.

Previous studies established that surfactants decrease droplet bounce on impact with leaves or fruit, while increasing spreading for optimal coverage (Spanoghe et al; 2007). Masterlock® which consist of a non-ionic surfactant did not significantly increase the Zn and B leaf concentrations from any of the treatments for both seasons. The data from this study suggests that Masterlock® had a significant impact on increasing deposition and Fluorescent Particle Coverage percentage (FPC%) onto the leaf surface for the middle and bottom parts of the tree canopy. Masterlock® significantly increased the ability of the droplets to penetrate into the tree canopy and decrease drift. This could be because of the ability of Masterlock® to manipulate droplet size and to break the surface tension of the leaf surface (Spanoghe et al., 2007, Winfield Solution, LLC, 2013).

If the wind speed increased at time of application, it should be considered that Masterlock® could have had a more augmented effect on deposition and uptake (this was not tested). The wind speed during application for each season was within acceptable levels (between 4 and 8

km h<sup>-1</sup>) at time of application of treatments 1-7. The wind speed increased at time of application for treatment 8 and 9 (between 6-9 km h<sup>-1</sup>) where Masterlock® had a more significant effect on deposition.

Masterlock® in combination with Zn and B had no significant effect on fruit set in this study, but other studies found that depending on the time of application, foliar applied Zn and B was found to improve fruit set by as much as 42% (Ruchal, 2020). The timing of the foliar applied Zn and B, regards to fruit set is pre-bloom, full bloom and fruit set growth stages. Noor et al (2019) reported that a Zn application significantly enhances fruit set (%) especially in combination with B. The fruit set percentage was also significantly higher for all treatments in season 2020, this could be because of the increase in rainfall for that season as well as cooler temperatures pre-bloom. Previous studies suggests that fruit drop and fruit set is negatively influenced in an increase in temperatures and drought (Sato, 2015). However, for this study, there was no significant difference between treatments with regards to fruit set.

There were no significant differences in final fruit size between the treatments, All the treatments had an increase in fruit size compared to the UTC, although not significantly. Previous studies suggest that foliar increased concentrations of Zn were positively correlated with big and medium fruit production and negatively correlated with small fruit production (Rodríguez, 2005).

## **CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH**

### **5.1 Introduction**

This study concluded from the leaf analyses data, that both the aerial and conventional Zn and B applications significantly increased leaf concentrations and are effective and viable. Thus, this study supports the hypothesis that aerial application is feasible. The significance of the aerial application of micronutrients is that it enables large farming operations to reduce tractor traffic in orchards to avoid mechanical damage to fruit and to enable them to make applications at critical phenological growth stages of the citrus trees. Not only can vast areas also be sprayed in a very short period, but aerial applications will also reduce pressure on the labour force. This study also concluded these micronutrient applications can be used successfully even at conventionally prescribed rates. This is significant because only small amounts of Zn and B was needed to increase leaf concentrations, which will be less costly for the farmer. The fruit set (%) and fruit size (mm) data concluded no significant differences, probably due to the Zn and B concentration being above the norm, except for Zn in the 2020 season. The addition of Masterlock® to a spray mixture, significantly increased droplet deposition. This results in less drift and more of the spray mixture penetrating into the middle and bottom parts of the tree canopy. However, the significant increase in deposition did not correlate with an increase in uptake of Zn and B. It is, however, important to further investigate this correlation when wind speeds are above the acceptable norm at time of application. The use of SEM technology, as employed in this study period, was unfruitful. It is, however, also very important that the actual uptake of the micronutrients be quantified since this will indicate the efficacy of the MAX-IN™ technology compared to other products.

### **5.2 Future research**

Method development for the proper quantification of the actual uptake of foliar applied nutrients will increase the value of future research in this field. Evaluate if an aerial application of Zn and B will increase yield. Evaluate the correlation between B and Zn concentrations in

the soil and in the leaf pre-and post-application. Establish to what extent carbohydrates values are increased with Zn and B applications.

### **5.3 Final comments and summary conclusions**

Aerial application of foliar nutrients is effective and can be used successfully, even at the conventionally prescribed rates. Masterlock® will significantly improve the deposition of the spray mixture of aerially applied micronutrient mixtures, however Masterlock™ will not significantly increase uptake of the micronutrient.

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