THE IMPLEMENTATION AND VALIDATION OF REDUCED VOLUME AGROCHEMICAL APPLICATIONS IN THE SOUTH AFRICAN CITRUS INDUSTRY USING NOVEL TECHNOLOGY

by

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DECLARATION

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SUMMARY

The South Africa citrus industry is the third largest exporter in the world and is considered one of the most important horticultural crops due to high economic export value. However, citrus trees are susceptible to a wide range of insect pests and fungal diseases. This places pressure on producers to deliver high quality fruit that adhere to strict export requirements. The largest and most important export market of citrus is the European Union (EU), which have a zero-tolerance approach towards Citrus black spot [*Phyllostica citricarpa* (van der Aa)] and false coddling moth [*Thaumatotibia leucotreta* (Meyrick)]. This leads to high spray volume applications that are seen as insufficient and not sustainable. The high input costs relating to water, labour and equipment as well as the environmental impact is a result of these high demands for 100% clean fruit. Furthermore, these high volumes are determined without taking canopy density into account, which contributed to high volumes being lost to run-off.

The potential of reduced spray volumes has been investigated, however limited trials have been done on the feasibility, implementation and biological efficacy of these different spray volumes in a seasonal commercial spray program. Therefore, the aim of this study was firstly to evaluate the possible reduction of spray volumes in the South African citrus industry without compromising on the need to get 100% control of important pests and diseases. Secondly, to investigate the use of the novel technology (LiDAR) to characterize citrus tree canopy density.

For the first objective spray trials were conducted in the Limpopo, Western and Eastern Cape provinces on commercial citrus producing farms. Reduced volumes (750 to 3000 L/ha) were compared with the farm's standard spraying volume (4000 to 9000 L/ha) evaluating spray deposition parameters such as deposition quantity (FPC%), uniformity (CV%) and quality (ICD%). Furthermore, the pest and disease efficacy were also evaluated in terms of clean fruit.

For the second objective trials were conducted on three commercial farms in the Western Cape to determine the effect of three different pruning categories on FPC%, CV% and ICD% in combination with two different spray application volumes (1500 L/ha as the reduced volume and 3000 L/ha as the standard volume). In an attempt to develop a non-destructive technique to measure canopy

density use of LiDAR technology was investigated and compared with manual canopy measurements.

From this study it was concluded that higher spray volumes result in better control of pests and diseases due to better deposition uniformity values. Furthermore, the importance of the penetration of spray mixtures into the canopy to achieve adequate control of pests and diseases is also essential. The manual manipulation of canopy density by pruning proved to be beneficial for spray deposition in creating more ‘spray-friendly’ canopies. The potential of LiDAR to be used as a calibration tool, was seen in this study, detecting differences in canopy densities. However, the LiDAR parameters were poorly correlated with manual measurements. It is suggested that the application be simplified in future studies for better correlation.

OPSOMMING

Die Suid-Afrikaanse sitrusbedryf is die derde grootste uitvoerder ter wêreld en word beskou as ‘n belangrike gewas as gevolg van sitrus se hoë ekonomiese uitvoerwaarde. Sitrusbome is egter vatbaar vir 'n wye verskeidenheid van insekplae en swamsiektes. Dit plaas druk op produsente om vrugte van hoë gehalte te lewer wat aan streng uitvoervereistes voldoen. Die grootste en belangrikste uitvoermark van sitrus is die Europese Unie (EU), wat 'n zero toleransie benadering tot Sitrus Swart Vlek (*Phyllostica citricarpa* (van der Aa)) en vals kodlingmot [*Thaumatotibia leucotreta* (Meyrick)] het. Dit lei tot hoë chemiese spuitvolumes wat as onvoldoende en nie volhoubaar beskou word. Die hoë insetkoste met betrekking tot water, arbeid en toerusting, sowel as die omgewingsimpak, is die gevolg van die vereiste om 100% siekte- en plaagvrye vrugte te produseer. Verder word hierdie hoë volumes bepaal sonder om die boomdigtheid in ag te neem, wat daartoe bydra dat hoë volumes as afloop verlore gaan.

Die potensiaal van verminderde spuitvolumes is al voorheen ondersoek, maar beperkte proewe is gedoen oor die haalbaarheid, implementering en biologiese effektiwiteit van hierdie verskillende spuitvolumes in 'n seisoenale kommersiële spuitprogram. Die doel van hierdie studie was dus om eerstens die moontlike vermindering van spuitvolumes in die Suid-Afrikaanse sitrusbedryf te evalueer om steeds 100% beheer oor belangrike plae en siektes te kry. Tweedens, om die gebruik van die nuwe tegnologie (LiDAR) te ondersoek om sitrusboomdigtheid te karakteriseer.

Vir die eerste doelwit is spuitproewe in die Limpopo-, Wes- en Oos-Kaap provinsies op kommersiële sitrusplase uitgevoer. Verlaagde volumes (750 tot 3000 L/ha) is in vergelyking met die plaas se standaard spuitvolume (4000 tot 9000 L/ha) geëvalueer. Spuitafdeposisieparameters soos deposisie kwantiteit (FPC%), uniformiteit (CV%) en kwaliteit (ICD%) is ondersoek. Die plaag- en siekte-effektiwiteit is ook geëvalueer in terme van die persentasie skoon vrugte.

Vir die tweede doelwit is drie proewe op kommersiële plase in die Wes-Kaap uitgevoer om die effek van drie verskillende snoei kategorieë op FPC%, CV% en ICD% in kombinasie met twee verskillende toedieningsvolumes (1500 L/ha as die verminderde volume) en 3000 L/ha as die standaard volume) te bepaal. In 'n poging om 'n nie-destruktiewe tegniek te ontwikkel om die boomdigtheid te meet, is die gebruik van LiDAR-tegnologie ondersoek en vergelyk met fisiese metingsmetodes.

Uit hierdie studie is bevind dat hoër spuitvolumes beter beheer van plae en siektes tot gevolg het, as gevolg van beter deposisie unifomiteitswaardes. Verder is die belangrikheid van die penetrasie van spuitmengsels in die boom uitgelig om voldoende beheer oor plae en siektes te verkry. Die fisiese manipulasie van die boomdigtheid deur snoei het bewys dat dit voordelig is vir spuitdeposisie deur die skep van meer 'spuitvriendelike' bome. Die potensiaal van LiDAR om as 'n kalibreringsinstrument gebruik te word, is in hierdie studie beklemtoon, waarby verskille in boomdigtheid waargeneem is. Die LiDAR parameters was egter swak gekorreleer met fisiese metings. Daar word voorgestel dat in toekomstige studies ‘n eenvoudiger benadering gevolg word vir beter korrelasie.

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CONTENTS

CHAPTER 1

A review of the important economical pests and diseases of citrus in South Africa, agrochemical spray applications and the novel technology LiDAR

INTRODUCTION

The South African citrus industry is a fast growing, dynamic and competitive industry both in local and international markets. Citrus orchards cover 77 708 ha across the major production areas that include the Limpopo, Eastern Cape, Western Cape and Mpumalanga provinces. South Africa is the third biggest exporter of citrus in the world with 1.09 million tonnes exported in 2017 (CGA Annual report 2018). The industry employs 125 000 people, which comprises 14% of the agricultural job market in South Africa (CGA Annual report 2018).

Citrus trees are susceptible to a wide range of insect pests and fungal diseases. Due to the economic importance of fresh citrus export, producers rely heavily on agrochemical sprays to adhere to export requirements, which are in some cases a zero-tolerance towards certain pests and diseases. The result of these high demands are that spray volumes range from 9000 – 16 000 L/ha (Van Zyl *et al*., 2013). However, these high-volume spray applications are contributing to environmental pollution and high input costs relating to water, labour and equipment. A canopy-based tree-row-volume (TRV) needs to be developed for a more sustainable approach towards the use of agrochemicals in the citrus industry. This could possibly be achieved through the use of novel technology such as LiDAR (Light detection and ranging) to characterize citrus tree canopy density and topography. This will result in more accurate sprayer calibration and application of agrochemicals, suited to trees with different densities.

Previous studies done have indicated that reduced spray volumes have the potential to decrease input costs (van Zyl *et al.*, 2013). However, these studies also show that reduced volumes lead to poorer penetration as well as spray uniformity in comparison to high spray volumes. Furthermore, few trials have been done on the feasibility, implementation and biological efficacy of these different spray volumes when used in seasonal spray programmes.

Therefore, the aim of this study was firstly to evaluate the possible reduction of spray volumes in the South African citrus industry without compromising on the need to get 100% control of important pests and diseases. Secondly, to develop a TRV calibration system using the novel technology (LiDAR) to characterize citrus tree canopy density.

This chapter is aimed at providing an overview of certain important economically important pests and diseases, the current approach to the calibration of citrus agrochemical spray

applications in South Africa and the basic principles of LiDAR and the application thereof in the agricultural industry. It also includes the possible advantages and a conclusion regarding the need to develop a TRV calibration system with the use of LiDAR to improve orchard characterisation and therewith sprayer calibration.

IMPORTANT PESTS AND DISEASES IN THE SOUTH AFRICAN CITRUS INDUSTRY

RED SCALE

Aonidiella aurantii (Maskell), also known as the California red scale, is one of the most widespread citrus pests present in all citrus producing countries except Japan and Colombia (Grout and Moore, 2015). It has a wide range of host plants, which include different fruit trees, cultivated shrubs, indigenous plants, roses and several deciduous and subtropical fruits (Bedford, 1998; Grout and Moore, 2015). The common name, red scale, comes from the circular reddish-brown scale that is attached to the surface of the plant tissue which the insect uses as protection (Donovan, 2014). All citrus cultivars can become heavily infested, in descending order of susceptibility: lemons, grapefruit, navels, Valencia's and soft citrus (Bedford, 1998). Red scale is one of the most disruptive pests in the citrus industry and already in 1895 posed a threat to the South African industry (Bedford, 1998).

Damage

All parts of the citrus tree are susceptible to red scale infestation including fruit, branches, twigs and leaves (Forster *et al*., 1995). A speckled appearance can occur on fruit from severe infestations, which can make the fruit unmarketable to export countries that treat red scale as a quarantine pest (Donovan, 2014; Grout and Moore, 2015). If early infestation occurs before the fruit has reached full size, permanent pockmarking can be another reason for downgrading of the fruit (Grout and Moore, 2015). Serious infestation of the citrus tree leads to defoliation, discolouration, wilted leaves, deformed shoots and twig dieback, which can ultimately lead to the death of the tree itself (Donovan, 2014; Grout and Moore, 2015). Tree vigour and yield can be reduced in the long term due to mature fruit drop (Grout and Moore, 2015; Garcerá *et al*., 2011; Alfaro *et al*., 2003; Vanaclocha *et al*., 2009; Walker *et al*., 1991). The damage on young citrus trees can be severe when the entire trunk becomes heavily infested, with longitudinal cracking appearing on the bark (Bedford, 1998; Grout and Moore, 2015).

Life cycle

Both male and female red scale are described as small pale, yellow crawlers, 0.8 to 1.2 mm and 2.1 mm in diameter respectively (Kerns *et al*., n.d.; Grout and Moore, 2015). Without magnification, these crawlers are very difficult to see in the orchard (Grout, 2012).

The life-cycle of the red scale is divided into different life stages as described below:

First instar

This stage is known as the crawler stage (Bedford, 1998). The female gives birth to between 100 and 150 live, active crawlers (Kerns *et al.*, n.d.; Donovan, 2014). These crawlers will mostly move towards the light, settling on upper leaf surfaces and in the depressions of the oil glands of young fruit, within the first 6 hours after emerging (Bedford, 1998). When these crawlers settle they lose their mobility and a soft, white scale-like cover forms over the body (Grout and Moore, 2015). Within two days of the crawler settling, the first scale formation known as the nipple stage, occurs with a raised secretion of the whitecap in the centre (Bedford, 1998; Grout and Moore, 2015). Hard, red to orange-coloured covers will be the result of healthy and fully developed nymphal scales (Grout and Moore, 2015).

Second instar

Females have a second instar stage where the waxy covering will enlarge and it is referred to as the grey adult stage (Bedford, 1998). The male on the other hand has immobile pre-pupal and pupal stages which develop into winged male adults. At this stage the legs of the insect are no longer present (Bedford, 1998). After the first moult the male becomes elongated and has a reddish-brown colour, which results in a fragile two-winged insect (Bedford, 1998). Winged males are not usually observed in the orchards, but are found when they are trapped in pheromone traps (Grout, 2012).

Management

Monitoring

Red scales are always present in the orchards, with simultaneously overlapping cohorts (Grout, 2012). Monitoring of red scale between winter and early summer will provide the most accurate and valuable results, with the recommendation of one trap per hectare (Grout, 2012). Pheromone traps with synthetic red scale pheromone (Grout and Moore, 2015) are used to detect the presence of male red scale insects (Kerns *et al.*, n.d.). This detection will be an indication for the producer of the intensity of the infestation and if it will be necessary to apply chemical control agents. Microscopic examination and regular scouting and inspection of the fruit is vital to determine the level of parasitism and to decide on a corrective treatment or not (Grout and Moore, 2015). However, in South Africa pheromone traps have not proven to be reliable and is not recommended to be seen as the deciding factor to treat the orchard or not (Grout, 2012).

Biological control

Natural enemies of red scale include several species of parasitoid wasps and predators such as ladybirds (Grout and Moore, 2015). Other natural enemies include the aphelinids (*Aphytis coheni, A. melinus, A. africanus, A. chrysomphali and Encarsia lounsburyi*), the encrytids (*Habrolepis rouxi, Comperiella bifasciata*), predators (*Chilocorus* spp. *Rhyzobius lophanthae*), lacewing species (*Geocorus* spp.), and a predatory mite (*Cheletogenes ornatus*) (Grout and Moore, 2015).

Biological control must be treated with a multifaceted approach, by considering various consequences such as the effect of natural enemies on red scale (Grout, 2012). The use of natural enemies can be beneficial in controlling other pests and methods have to be harmonised for optimal results (Grout, 2012).

Chemical control

The use of pesticides to control red scale has changed over the years due to resistance to various products. Pesticides to which red scale are resistant include chlorpyrifos and methidathion which contain organophosphates (OPs) (Donovan, 2014). Since the first occurrence of OP resistance in the 1970's (Grout, 2012), the approach towards chemical control has had to change, not only for red scale but for other pests as well. OP resistance is assumed due to very few populations of red scale that are still susceptible, due to the constant spraying of OPs for other pests such as citrus thrips and mealybugs (Grout, 2012).

Other limitations of chemical control agents are that they have poor contact efficacy or residue restrictions and are also lethal to natural enemies such as the parasitoids, which can lead to secondary pest infestations (Donovan, 2014; Grout and Moore, 2015). Other options had to be considered, and this included the recent use of mineral oils and insect growth regulators to assist with the control of red scale. Two examples of products currently used are Buprofezin, which disrupts the molting process through chitin synthesis inhibition and pyriproxyfen, which sterilizes the adults and can cause nymphal mortality. (Kerns *et al*., n.d.; Grout, 2015).

Pesticides for red scale are applied as full cover sprays, but due to phytotoxic concerns, certain treatments are restricted to specific times of the season. These treatments are divided into two groups, namely preventative or corrective application. Preventative application can be applied at 80 – 100 % petal fall or 10 – 14 weeks after petal fall whereas with corrective application fruit should be inspected before treatment is done. If corrective sprays are necessary, it should be applied before 50% of the fruit are infested with one or more live nymphal or adult red scale. Inspection should start 8 - 9 weeks after petal fall and follow up

inspections should be done every 14 days (Grout, 2012). Where red scale infestations are high, corrective control is used, with methomyl the preferable option (Grout and Moore, 2015).

FALSE CODLING MOTH

False codling moth (FCM) was first described as a citrus pest in 1901 by Fuller as the "Natal codling Moth" and later by Howard in 1909 as the "orange codling moth" (Newton, 1998). FCM, *Thaumatotibia leucotreta* (Meyrick) (syn. *Cryptophlebia leucotreta*) (Stibick, 2010) is present in all of the major citrus producing areas in South Africa, although in various literature it is stated that FCM was not present before 1974 in the Western Cape (Citrusdal – Clanwilliam district) (Schwartz, 1981; Newton, 1998). Numerous factors contribute to the effect of FCM pressure present in orchards, which include management strategies, environmental conditions, climate of different production regions as well as the host type. This is why the occurrence and levels of FCM differ from season to season and should be managed accordingly (Moore and Hattingh, 2017). FCM has an extensive list of host plants which includes deciduous, subtropical and tropical fruits, acorns, olives, walnuts and macadamias amongst others (Newton, 1998).

FCM is an economically important pest due to its endemism to sub-Saharan Africa (Schwartz, 1981; Newton, 1998; Stibick, 2010), therefore certain export countries such as USA, South-Korea, China and the European Union consider FCM as a quarantine pest (Moore *et al*., 2015). FCM is an internal pest and may possibly be exported without detection and thus poses a threat to maintaining export markets (Grout and Moore, 2015). The susceptibility levels within different citrus types stated by Moore and Hattingh (2017), ranges from non-host status (lemons), through low susceptibility (Valencia cultivars and white grapefruit), moderate susceptibility (Midseason cultivars, Star Ruby grapefruit and Turkey Valencia's and certain mandarin types such as Satsumas), to more susceptible navel oranges (Grout and Moore, 2015).

Life cycle

A complete life cycle can range between 30 and 174 days depending on the environmental conditions (Stibick, 2010). FCM can remain active throughout the whole year if sufficient host plants are present (Stibick, 2010). Within a year there can be six overlapping generations with no winter diapause at any time (Grout and Moore, 2015). Individual life phases of FCM include egg, larvae, pupae and adult phases.

Egg

Eggs are laid singly on the surface of the fruit usually cryptically in depressions of the rind (Newton, 1998; Grout and Moore, 2015). Egg development can take between 2 and 22 days depending on the temperature. Temperatures below 0°C, over a 2-3 day period, can kill the eggs (Venette *et al*., 2003). It can be identified as a translucent white flat and oval shaped 1 mm egg (Stibick, 2010). Before hatching the eggs are translucent at first, but darken internally through a red stage to black stage (Moore, 2012).

Larvae

The larvae has a body colour of diffuse pink overall tending to orange yellow on the sides, top and legs; with a length of 12 - 20 mm (Stibick, 2010). If favourable conditions are present the eggs hatch and the neonate larva finds a suitable area on the fruit for penetration (Grout and Moore, 2015). The majority of the larval stage is spent inside the fruit (Venette *et al*., 2003), which includes the five instars of larval development (Grout and Moore, 2015). As soon as the last instar is ready to pupate, the larva exits the fruit via the frass-filled hole and drops to the ground or emerges after the fruit has fallen (Newton, 1998).

Pupa

On the ground the larva spins a silken cocoon, covered with trash particles and establishes in the top layer of the soil (Newton, 1998; Grout and Moore, 2015). The cocoon is now in the prepupal stage moulting into a pupa (de Jager, 2013). The pupae have a dark brown colour and are 10 mm in length (Moore, 2012). This pupal stage lasts anything between 2 – 33 days depending on the temperature (Venette *et al*., 2003). The moth will then emerge from the soil to continue its life cycle (Grout and Moore, 2015).

Adult

The adult moth is rarely seen in orchards (Grout and Moore, 2015) because they fly during the night (de Jager, 2013). The moth is dark-brown to variable mottled grey in colour with visible spiral grey scales on the dorsal side of the body (Newton, 1998; Grout and Moore, 2015). Adult moths have an average body length of 6 – 9 mm with a body width of 2.5 mm (Stibick, 2010). This adult stage can last between 14 and 70 days according to de Jager (2013) and in this period the female release pheromones to attract males for reproduction (de Jager, 2013).

Damage

Citrus fruit are susceptible to FCM infestation (Stibick, 2010) from pea size until harvest (Moore, 2012). The damage caused by FCM include fruit fall, tissue decay and post-harvest decay (Grout and Moore, 2015). During December to April high levels of fruit drop occurs

(Moore, 2012). As the larva penetrates into the fruit surface of the citrus, the area around the penetration hole differs from colour depending on the growth stage of the fruit (green fruit assumes a yellow colour and ripe orange fruit eventually becomes brown and sunken) (Grout and Moore, 2015). These fruits showing signs of infestation and damages lead to the rejection of export consignments (Moore and Kirkman, 2008).

As the larva matures, it gradually enlarges the original penetration hole to leave the fruit in search of a pupation site (Newton, 1998; Grout and Moore, 2015). Because of this, frass may remain on the fruit surface giving an indication of the presence of FCM (Hofmeyr, 1998). This leads to fruit decay, premature ripening and abscission (Newton, 1998). The damage caused by the larvae all contribute to fruit fall, which occurs three to five weeks after penetration by a larva (Moore, 2012) and thus a decrease in yield. Post-harvest decay can appear due to fruit infested shortly before harvest being difficult to identify in the packhouse (Grout and Moore, 2015). The infected fruit is also susceptible to fungal pathogens and thus secondary infection (Stibick, 2010).

Management

Due to the phytosanitary status of FCM for most export markets, a zero-tolerance of FCM presence should be the goal (Grout and Moore, 2015). Infestation can quickly intensify due to the fact that, during the fruiting period of citrus, FCM eggs are laid continually and, on hatching, the larvae bore into the fruit within a few hours, classifying FCM as an extremely difficult pest to control (Newton, 1998). A combination of management strategies has been implemented to control FCM in orchards, which include biological, cultural and chemical control methods and has been studied and documented, to be selected to align with the pest pressure in each production situation (Moore and Hattingh, 2017). The aim for control should be to suppress FCM from early in the season to avoid build–up of high population numbers (Moore, 2012). This approach will support other control methods that are applied later in the season such as chemical control (Moore, 2012).

Monitoring

Pheromone traps were used in the past to monitor population levels and to determine whether it was necessary to apply chemical sprays. This approach has, however, been modified due to the phytosanitary status of the pest and zero tolerance levels are now the target (Grout and Moore, 2015). Pheromone traps are now used for optimal timing of sprays and to compare activity levels between seasons, and between orchards to aid prioritisation of treatment application (Moore, 2012; Grout, 2015; Grout and Moore, 2015). Trap counting should be done weekly on the same day for optimum results and to try to determine a relationship between

trap catches and fruit infestation (Moore, 2012). It is important not to place traps within 200 m of each other, due to trap interaction that can lead to unreliable results (Moore, 2012).

Although pheromone traps can be seen as an effective long-term monitoring system for FCM (de Jager, 2013), fruit drop surveys are now considered to be the most important way of monitoring FCM (Moore, 2012). Such fruit surveys should be undertaken from January to harvest, and all dropped fruit should be collected and cut open to determine the cause of fruit drop (Moore, 2012). This is done as there are many possible causes of fruit drop in citrus orchards and it cannot be assumed that FCM is the only cause (Moore, 2012).

Sanitation

It has been confirmed that fruit infestation could be reduced by an average of 75% with weekly orchard sanitation (Moore and Kirkman, 2008). Sanitation, which is strongly improved by biological control, should be regarded as the basis of FCM control (Grout and Moore, 2015; Moore and Hattingh, 2017). Recommended orchard sanitation guidelines include the following: starting from November until fruit are harvested, all dropped fruit and hanging fruit that appear damaged, infested or decaying should be removed and destroyed. This should be done at least once a week and during the hotter months (January to March), orchard sanitation should take place at least twice a week (Moore and Kirkman, 2008). Appropriate orchard sanitation will eliminate the probability of FCM completing its life cycle over the winter period (Moore, 2012).

Biological control

The biological control agent proven to be the most effective is an egg parasitiod, *Trichogrammatoidea cryptophlebiae, which* is a natural enemy of FCM. (Newton, 1998; Grout and Moore, 2015; Moore and Hattingh, 2017). More than 80% of FCM can be parasitized if undisrupted (Moore, 2012). However, augmentative releases of *T. cryptophlebiae* cannot, by themselves, achieve the level of control needed in citrus and pesticide applications continue to be used (Newton, 1988). It is important that the parasitoids eggs should be released several times, especially when the fruit is susceptible to infestation (de Jager, 2013). Other natural enemies of FCM include natural occurring species such as the wasp, *Agathis bishopi* (Moore, 2012).

Chemical control

With a variety of chemical control agents registered for the control of FCM, the application of these insecticides should be used sensibly to avoid the possibility of resistance development (Grout and Moore, 2015). Keeping sustainability and the threat of pesticide application to biological control in mind, the use of pesticides should be minimized as much as possible. The

many life stages of FCM together with the persistent pest pressure that occurs throughout the fruiting season, contributes to the difficulty of chemical control of FCM (Newton, 1998). However, the recently registered chemicals such as methoxyfenozide and spinetoram, which should both be sprayed as a two-spray programme, 8 and then 4 weeks before harvest (Moore, 2012), are effective in controlling *T. leucotreta* infestation (Moore and Hattingh, 2017).

Mating disruption (MD) and "attract and kill"

A biorational approach of controlling FCM include mating disruption where a pheromone is used to attract and kill the males, resulting in reduced mating and therefore reduced FCM populations (de Jager, 2013). This method includes applying a synthetic female sex pheromone that either causes confusion, repel or habituate. This leads to the males not finding the females for mating (Moore, 2012). In studies done by Hofmeyer (2002) and Moore and Hattingh (2012), the effectiveness of MD has been proven, with reductions of 86% and 95%, respectively. The best results was obtained when MD was applied early in the season when FCM levels were still low (Moore, 2012).

Sterile insect technique (SIT)

SIT is an integrated pest management (IPM) approach towards FCM control that is non-toxic to the environment and can easily be integrated into area-wide programmes according to de Jager (2013). This method involves the release of sterile moths at the ratio of 10 sterile to 1 wild moth (Moore, 2012). This control method have proven to be effective in reducing moth catches (99%), fruit infestation (96%) and export rejections (89%) since the inception of an integrated programme in the Western Cape (Moore and Hattingh, 2017). The SIT method is seen as the most effective area-wide suppression technique available and can successfully suppress FCM populations (Moore, 2012).

MEALYBUG

There are several mealybug species attacking citrus in South Africa. For the purpose of this review the focus will be on the citrus mealybug, *Planococcus citri* (Risso). *P. citri* is an economically important pest throughout the world (Hattingh *et al*., 1998; Grout and Moore, 2015). It occurs in all citrus producing areas in South Africa and has increased in most regions since the 1990's (Moore and Hattingh, 2012). This pest has the potential to develop high population levels and cause severe damage (Hattingh *et al.*, 1998). Citrus mealybug has a wide range of host plants such as subtropical crops, deciduous fruits and various shrubs (Grout and Moore, 2015).

Life cycle

The complete life cycle of the mealybug is dependent upon climatic conditions and can range between 4 weeks to several months (Hattingh *et al.*, 1998; Grout and Moore, 2015).

Egg

An adult female can lay between 300 and 600 eggs over a period of 6 – 14 days (Grout and Moore, 2015) in clumps of 5 - 20 eggs inside white egg sacs called ovisacs (Asiedu *et al*., 2014; Gill *et al*., 2016).

Larva

Environmental conditions will determine the hatching of the eggs, but it can last anything from 6 days to several weeks (Gill *et al.*, 2016). The first instar, called crawlers, are highly mobile (Hattingh *et al.*, 1998) and will settle on the undersides of leaves, young twigs or between touching fruit (Gill *et al.*, 2016). The instar phases and morphology of the male and female differ greatly. Male mealybugs have three instars and a pre-pupal stage, whereas the females have four instar stages and resemble the adult mealybug (Gill *et al.*, 2016).

Adult female

These are wingless and, depending on the host, live up to approximately 29 days (Gill *et al.*, 2016). A mealy wax secretion covers the body (Hattingh *et al.*, 1998). Adult females are 3 to 4 mm in length (Moore and Hattingh, 2012).

Adult male

The male can easily be distinguished from the female, being yellowish-brown and have hyaline wings (Hattingh *et al.*, 1998), making it possible for them to disperse to other trees for mating purposes (Gill *et al.*, 2016).

Damage

Damage to fruit is common when mealybugs feed under the calyx. (Grout and Moore, 2015). As mealybug feeds on the fruit it injects a toxic saliva while extracting plant sap (Kerns *et al*., n.d.). This leads to severe damage such as fruit discolouration, fruit splitting, fruit drop and the formation of dents and lumpy shoulders (Kerns *et al.*, n.d.; Grout and Moore, 2015). Indirect damage includes the build-up of sooty mould as a result of honeydew excreted by the mealybugs as they feed (Grout and Moore, 2015). Citrus mealybug also feeds on the roots, bark and leaves (Hattingh *et al.*, 1998). Mealybugs feed on the main vein of young leaves, resulting in a characteristic deformation (Moore and Hattingh, 2012).

Management

Monitoring

The use of pheromone traps can be used to determine mealybug activity, by only attracting male mealybugs and are an indication of early infection (Kerns *et al.*, n.d.). Tree trunks should be examined regularly for the presence of mealybugs as well as honeydew. Sooty mould and ants can be an indication of mealybug infestation (Kerns *et al.*, n.d.; Hattingh *et al.*, 1998).

Biological control

One of the most effective means of control is the use of natural enemies, when not disrupted by other chemical applications (Kerns *et al.*, n.d.). Natural enemies of the citrus mealybug include a complex of insects namely hymenopteran parasitoids, predatory fly larvae, lacewings, predaceous mites, syrphid flies and ladybird beetles (Kerns *et al.*, n.d.; Grout and Moore, 2015). If not disturbed by detrimental sprays, these natural enemies can control mealybugs to an large extent especially during the mid- to late season (Moore and Hattingh, 2012). Examples of commercially available insects for biological control is the Encyrtid wasp, *Anagyrus* spp. (Kerns *et al.*, n.d.; Grout and Moore, 2015).

Chemical control

Chemical sprays to control mealybug populations must be used with caution. The use of excess or incorrect chemicals can be deadly to the natural enemies as mentioned previously. This will result in a higher infestation of mealybugs and the unnecessary use of chemicals can also result in environmental damage. The use of chemical applications is furthermore difficult and can be insufficient because of the mealybugs hiding in crevices between fruit and leaves (Gill *et al.*, 2016). Therefore it is important to note that the highest level of coverage is required for successfully controlling mealybug and for this a wetting agent is advised (Moore and Hattingh, 2012).

Products registered for chemical control of mealybugs include organophosphates (e.g. chlorpyrifos, methidathion and malathion), carbamate, neonicotinoid and insect growth regulators [ex. buprofezin (Kerns *et al.*, n.d.; Grout and Moore, 2015)]. Timing of chemical application is important and it is recommended that it should be before the calyx closes and early in spring when mealybug populations are still low and shortly after beginning of the first crawler movement (Hattingh *et al.*, 1998; Moore and Hattingh, 2012). The debris and sooty mould from mealybug occurrence can act as a seal against spray applications if not done in time. It is important to note that mealybugs cannot be controlled by chemical control alone (Moore and Hattingh, 2012) and an integrated approach with biological control options should be taken.

CITRUS BLACK SPOT

Citrus black spot (CBS) is caused by *Phyllosticta citricarpa* and is one of the most economically important citrus diseases in South Africa. CBS originated in South-East Asia, and was first reported and described in 1895 by Benson in Australia (Smith *et al*., 1997; Truter, 2010) Although the disease is widespread in several countries, it does not occur in Europe, Central America and the Caribbean region (Archipelago, 2003; Carstens *et al*., 2012). The European Union represents 40% of the South-African export market (Citrus Growers' Association of Southern Africa, 2016) and the zero-tolerance for *P. citricarpa* on exported fruit requires South African producers to achieve complete control. In a recent study, *P. citricarpa* was found in Europe, but was not associated with disease symptoms, which showed that the pathogen can persist over time (Guarnaccia *et al*., 2017). The disease mainly occurs in summer rainfall areas with humid, warm and wet climates. In South Africa, citrus producing provinces with these favourable conditions where CBS is known to be present include KwaZulu-Natal, Mpumalanga, Limpopo, North West and the Eastern Cape (Carstens *et al.*, 2012).

Etiology

The casual organism of CBS is *Phyllosticta citricarpa* (van der Aa), which is an ascomycete fungus. This disease affects all citrus species excluding sour oranges (*C. aurantium*) and it's hybrids and Tahiti limes (Kotze, 1981; Archipelago, 2003; Carstens *et al.*, 2012). When *P. citricarpa* is found in a new area, it is usually first observed on lemons, which is considered most susceptible (Kiely, 1948; Kotzé, 1981). *P. citricarpa* is host specific and only occurs where susceptible citrus cultivars are grown and where the environmental conditions are suitable (Kotze, 1981; Carstens *et al.*, 2012; Yonow *et al*., 2013).

Epidemiology

The epidemiology of *P. citricarpa* is influenced by various factors, including environmental conditions, developmental stage of the fruit and the availability of inoculum sources (Kotzé 1981; Whiteside *et al*., 1993).

Ascospores and pycnidiospores are the sources of infection for *P. citricarpa* (Kotze, 1981). The pycnidiospores are mainly responsible for *P. citricarpa* dispersal within a tree, especially in high rainfall tropical conditions (Spósito *et al*., 2008). Ascospores which are borne in pseudothecia are considered the main source of inoculum for fruit infections in the field (Kotzé, 2000). These pseudothecia only occur on leaf litter on the orchard floor and they develop within 40 – 180 days after leaf drop depending on the frequency of wetting (rain or irrigation) and drying (Kiely, 1948; McOnie, 1964; Kotze, 1981; Fourie *et al*., 2013). If leaf litter is constantly wet or dry, pseudotheica formation and maturation is hindered. Because citrus

leaves drops all year round, mature ascocarps can occur anytime during the year (Kotze, 1981; Kotzé, 2000), but optimal conditions are required for ascospore discharge.

The period for fruit infection begins at fruit set and lasts for 4-5 months after fruit set (Kotzé, 2000). After being discharged, ascospores land on susceptible tissue where they adhere and germinate. A wetness period of 15 hours and optimal 27°C is needed for the ascospores to germinate and infect (Magarey *et al*., 2015). After germination, the ascospores produce a germ tube and appressorium which produces a thin infection peg that penetrates the cuticle(Kotzé, 1963; McOnie, 1965; Fourie, 2015) . A latent infection is then established as a small mass of mycelia developing between the epidermis wall and the cuticle (Kotzé, 2000). Symptom expression will occur once the fruit are fully matured (Kotze, 1981). As the fungus grows further into the rind tissue typical black spot symptoms will appear (Kotzé, 2000).

Pycnidiospores are formed in pycnidia that occur on the fruit lesions (and on dead twigs and leaves (Kotze, 1981; Fourie, 2015). This source of inoculum is considered secondary. The pycnidiospores are water-borne and thus can only contribute to infection when coming into contact with susceptible fruit, leaves or twigs following downward water-dispersal from lesions with pycnidia (Kotze, 1981). A wetness period of 12 hours along with optimal temperature of 25°C is needed for the pycnidiospores to germinate (Fourie, 2015).

Symptoms

Symptom development is enhanced by certain conditions to which the citrus tree is exposed. These include high temperatures, poor soil conditions, insufficient irrigation, poor tree vigour, high light intensity, nematodes and other diseases that may be present as well as the age of the tree (Kotzé, 2000; Schutte, 2009; Truter, 2010). Fruit, leaves and twigs of citrus trees can be infected by *P. citricarpa* and are briefly described below.

Infected leaves rarely show symptoms of disease (Schutte, 2009) and a number of lesions per leaf may range from a few to several spots (Wager, 1952). The younger lesions are reddish brown with lighter colour centres surrounded by a diffuse yellow halo, where the older lesions are sunken with a grey centre and dark-brown margin (Truter, 2010). Leaf symptoms are more common on lemons than any other cultivar (Schutte, 2009).

Fruit symptoms are more common and distinctive than leaf symptoms (Carstens *et al*., 2012). Identification of different symptoms can be very difficult due to these being very variable (Kotzé, 2000). Due to the latent nature of the infection, fruit symptoms only appear six months or more after fruit set, when fruit is mature (Schutte, 2009), however symptoms can also appear on immature fruit, specifically lemons (Wager, 1952). Fruit symptoms are divided into three main categories: Hard spot, freckle spot and virulent/spreading spot (Kiely 1948; Kotze, 1981). Hard spot is the most typical *P. citricarpa* symptom. Hard spot lesions can be identified

as small, round, sunken light-brown lesions that appear on maturing fruit, first on the sunny side of the fruit. Freckle spot or early virulent spot will appear on mature fruit and are small reddish, irregular shaped lesions that can develop further into either virulent spot or hard spot. Later in the season when fruit are fully developed and temperatures rise, virulent spot can develop as sunken necrotic lesions that are brown to brick red in colour (Kiely 1948; Kotzé, 1981, 2000; Burrow, 2014).

Even though symptoms on twigs have not been formally described, recent research shows that twig symptoms are commonly found on lemons in South Africa and can be a source of inoculum. Lesions found on actively growing twigs are round and slightly sunken, have a brown to black margin and a grey to light brown centre (Truter, 2010).

Management

Cultural practices

A number of cultural practices are used in the control or management of *P. citricarpa*. Orchard sanitation, which include the removal of remaining mature infected fruit after harvest, before the new crop develops, helps to reduce the source of inoculum, namely pycnidia that may be washed down onto young fruit (Kotze, 1981; Archipelago, 2003). Another method for inoculum control is to accelerate the decomposition of fallen leaves on the orchard floor or simply the removal thereof (Truter, 2010). If leaves are not removed from the orchard floor, mulching can also be a control option. Mulching, which should take place after leaf drop (August to September), will accelerate the decomposition of the leaves bearing the ascocarps, causing a decrease in inoculum (Schutte, 2009). From year one, the tree is susceptible and this increases with age as well as with declining health (Schutte, 2009). It is therefore important to maintain tree health, aeration and well managed canopies.

Chemical control

Commercially, there are a range of fungicides available for *P. citricarpa* control. It is very important to time the spray applications within the critical infection period and a knowledge thereof is important (Kotzé, 2000). In South Africa, fungicide protection is needed for 3-4 months after fruit set, between November and February, when fruit is most susceptible to infection (Kotze, 1981). Spray programmes constitute the use of both contact (copper and mancozeb) and systemic fungicides (benzimidazole or strobilurines).

Systemic fungicides have both protective and curative action and are capable of penetrating the epidermis and killing the mycelium present (Schutte, 2009) and thus can be used for corrective control of citrus black spot. The consistent use of fungicides can lead to resistance development, especially with systemic agents such as the benzimidazoles. For instance, in the case of benomyl, after a brief period of extensive use in the industry in the

1980's (Herbert and Grech, 1985). To avoid the risk of fungicide resistance developing a mixture of contact and systemic fungicides should be used (Schutte, 2009).

ALTERNARIA BROWN SPOT

Alternaria brown spot (ABS) is one of the more serious diseases of many soft citrus cultivars (Timmer *et al.*, 2006) and occurs worldwide, causing serious economic losses on susceptible cultivars (Mahmoudi, 2010). This disease was first described on Emperor mandarin in Australia in 1903 (Cobb, 1903; Pegg, 1966). In southern Africa the disease occurs in all citrus producing areas, but is more predominant in areas that have humid climatic conditions, which is followed by high rainfall (Schutte, 2003). The blemishes on the rind of the fruit can decrease the marketability of the fruit (Timmer, *et al*., 2000) and result in significant income loss due to a lower value product (Mahmoudi, 2010).

Etiology

The casual organism of Alternaria brown spot is *Alternaria alternata* Fr. (Keissl) (Timmer *et al.*, 2000). There are two different pathotypes for *A. alternata,* known as the "the rough lemon pathotype" and the "tangerine pathotype", which have been described based on host specificity and toxin production (Peever *et al*., 2002). The tangerine pathotype mainly affects tangerines and their hybrids and produces the ACT-toxin, which is responsible for the symptoms associated with Alternaria brown spot (Timmer *et al.*, 2003; 2006).

Epidemiology

The ABS pathogen infects fruit, twigs as well as young leaves. Infection is most importantly associated with environmental conditions and the age of the tissue where infection occurs, *i.e.* young leaves, shoots and fruits are susceptible. The pathogen reproduces by means of conidia that are produced on the surface of lesions on wilted twigs and on mature leaves (Timmer *et al.*, 2000; 2003). These conidia are disseminated by wind and water splash and infect susceptible tissues under optimal environmental conditions (Schultz *et al*., 2013). The optimal conditions for infections are between 20-27°C with a leaf wetness period of 10-12 hours (Timmer *et al.*, 2000) on susceptible host tissue. Rainfall and a sudden change in relative humidity results in the release of conidia (Timmer *et al.*, 2006). The first symptoms can appear with a short incubation period of 24 to 48 hours (Timmer *et al.*, 2006).

Symptoms

Symptoms can be seen on young leaves, twigs and fruit (Schutte, 2003; Fourie *et al*., 2009) and can appear within 24 hours of infection taking place (Ohtani *et al.*, 2009). Extensive leaf and fruit drop as well as twig dieback can occur following severe infections (Reis *et al*., 2006).

Initial leaf lesions occur on the tissue of young leaves as brown to black spots (Timmer *et al.*, 2000), which can range from small circular leaf spots to large necrotic blighted lesions

(Schutte, 2003). The ACT-toxin is responsible for the necrosis and expansion of lesions on the leaves (Ohtani *et al.*, 2009), including the spread of necrosis along the veins. Leaves are susceptible to infection from the time of formation until they are fully expanded and hardened off (Timmer *et al.*, 2000).

Symptoms on fruit include brown to black lesions and can vary in size. Normally the young infected fruit drop off, but if infected fruit remain on the tree, corky eruptions are formed, which can easily be dislodged to leave pockmarks on the surface (Timmer *et al.*, 2000; 2006). These symptoms are undesirable and therefore reduces the value of the fruit for the fresh fruit market (Reis *et al.*, 2006)

Symptoms on young shoots include lesions that are 1-10 mm in diameter (Timmer *et al.*, 2000) and affected shoots often dieback after infected leaves have dropped (Timmer *et al.*, 2006).

Management

Cultural

Cultural practices can support ABS control. Planting sites should be selected, taking into consideration that good air circulation and wind movement will decrease the incidence and severity of disease development (Timmer *et al.*, 2000).

Chemical

ABS is mainly controlled by fungicides, but this is expensive due to the many applications that have to be made throughout the season (Reis *et al*., 2007). Chemical management with plant protection products must coincide with possible infection periods in favourable environmental conditions (Schutte, 2003). Fungicides that are commonly used include iprodione, maneb, mancozeb, chlorothalonil, metiram, strobilurins and copper (Timmer *et al.*, 2000). To ensure that all fruit and leaf surfaces are wetted properly, a medium cover spray is recommended (Schutte, 2003). A disease prediction named the Alter-Rater model is being used in the USA for the timing of fungicide applications (Timmer *et al.*, 2003).

SPRAY APPLICATION IN CITRUS ORCHARDS

Since the 1940's the spray mechanisation has slowly developed, however due to the cost of labour, equipment and orchard's sizes expanding, the pace for spray technology development has accelerated (Jones *et al*., 2016). Being the third largest exporter of citrus fruit in the world, South Africa has access to more markets than any other citrus exporting country. The demand for high quality fruit, free from diseases and pests, places pressure on producers, resulting in high volume spray applications (Fourie *et al.*, 2013). These high volume applications are usually used by growers as a safety buffer and results in good disease and pest control despite the costs and risks involved (van Zyl *et al*., 2015). The excessive costs, which include high

water usage, labour and equipment, as well as the contribution to environmental pollution is a matter of concern to the citrus industry. Growers should aim towards more efficient spray applications in order to reduce non-target environmental contamination. The efficiency thereof will be dependent upon several factors such as cultivar differences, environmental conditions, canopy geometry and density, different spraying machines, spray technique, fungicide or pesticide used, influence of different adjuvants and lastly the complex interaction between all these contributing factors (Van Zyl *et al.*, 2013). Research carried out in an effort to reduce high-volume spray applications both in South Africa and internationally will be discussed below.

Spray deposition assessment

When evaluating the efficacy of spray application a quantitative method of deposition assessment is needed (Salyani and Whitney, 1988). Previous methods used to determine spray coverage did not give a good indication of the spray deposition especially on critical parts of the plant. The need for adequate and useful spray assessment techniques to determine spray application equipment efficiency was highlighted by Brink (2005) and stimulated the development of the spray assessment protocol also used in this study. Various methods have been developed and tested to evaluate spray deposition and the effectiveness thereof. The need to measure spray deposition in terms of quality and quantity was developed by van Zyl *et al.* (2013). This followed on previous work done by Brink *et al*. (2004, 2006), Fourie *et al*., (2009), Salyani and Hoffmann (1996) and van Zyl *et al*. (2010a; 2010b). A deposition assessment protocol was developed for the assessment of spray deposition to be used in future research especially in citrus spray application. This was initially developed to study efficient spray coverage for more effective disease management. This methodology was further improved by Fourie *et al.* (2009), Brink (2012) and van Zyl *et al.* (2010a, 2010b) and is briefly explained below.

With spray application assessment two main factors are considered to determine the efficacy of the spray application of a fungicide or pesticide namely deposition quantity and deposition quality (Brink, 2005, 2012; Fourie *et al*, 2009; van Zyl, *et al*., 2010). Yellow fluorescent pigment is used to trace the spray deposition, which was found to be equivalent in particle size to certain copper hydroxide formulations (Van Zyl *et al*., 2013). Therefore, spray deposition quantity and quality measurements should both be included in a spray assessment protocol (Brink *et al*., 2016).

Deposition quantity

This is measured as percentage fluorescent particle coverage (FPC %) of the target surface. This value gives an indication of the area covered by pigment particles per leaf or fruit surface.

A higher FPC% indicates more pigment that landed on the surface. A 3-dimensional natural target site does not consists of the same orientation and surface properties as artificial targets, such as water-sensitive papers, which is commonly used for spray assessment (Holownicki *et al*., 2002). The most common methods for measuring spray droplet and spray deposition is water sensitive paper (WSP) (Kesterson *et al*., 2015). A study done by Salyani and Whitney (1988) evaluated different deposition assessment methods such as the visual judgement of deposition parameters, string employed as a spray collector, fluorometry and colorimetry. This study highlights the efficacy of these different parameters in terms of reliability, sensitivity, time efficacy costs and versatility. A downside of WSP is that the paper can only be used when the application volume is low enough that overlapping stains do not saturate the entire paper, whereas fluorometry and colorimetry is seen as more accurate (Kesterson *et al.*, 2015). However, these methods do not always give a good indication of spray deposition quality, therefore a deposition quality parameter was developed.

Deposition quality and uniformity

This parameter is essential to evaluate with deposition quantity, indicating how effective and well distributed spray deposition is, which is vital to validate deposition quantity as explained by Frick (1970). Frick indicated that the measurement of deposition quality is an important aspect as "a high-level deposit badly distributed is less efficient than a low-level deposit well distributed". This value represents the quality of chemical distribution over the leaf or fruit surface. For this measurement, the area is divided into equally-sized squares of 150 x 150 pixels. Depending on the surface area, this amounted to a number of individual squares per leaf. Ranging from 10 to more than 110 squares per leaf, of which the percentage area covered by fluorescent pigment particle is determined for each square. The coefficient of variation of the mean value of all the blocks analysed per leaf (CV% = Standard Deviation x 100/mean) is used as a measure of deposition quality per leaf, *i.e.* uniformity of deposition on the leaf surface. Low CV% values are indicative of better deposition quality (Van Zyl *et al.*, 2013), due to less variation in deposition between squares.

It is important that deposition quantity and quality is measured and that both is used to determine the efficacy of the agricultural chemicals applied (van Zyl *et al.*, 2010). For instance, if the deposition quantity is adequate, but the quality of the deposited dosage is poor, this can lead to poor efficacy thereof (Brink *et al.*, 2016).

The effectiveness of reduced spray volumes for pest control

The study by Cunningham and Harden (1998) indicated that citrus growers in Australia are applying spray applications mostly at medium volumes (2000 – 7000 L/ha) but that high spray volumes (>7000 L/ha) are not unusual. To determine if lower volume pesticide application can

replace conventional high-volume pesticide application, two different spray machines, an air-assisted low profile sprayer and an air-assisted sprayer fitted with tower air conveyors were used. Spray volumes between 500 and 10 000 L/ha were used: 10 000 L/ha applied with the oscillating boom sprayer and 500, 1000, 1500, 3000 and 6000 L/ha were applied using the air-assisted sprayer. Biological evaluation was done for two pests namely California red scale (*Aonidiella aurantii* Maskell) and citrus mealybug (*Planoccoccus citri* Risso) to determine the biological efficacy of the different spray volumes. It was found that with the 6000 L/ha treatment a greater reduction in fruit infected with mealybug compared to the 10 000 L/ha treatment and all other sprayer treatments. This result can be due to the high spray deposit observed with the 6000 L/ha spray application and the lower percentage (14%) of run-off comparing to the 10 000 L/ha application with 28 % run-off losses. The general trend was decreasing canopy run-off with decreasing application volumes. For the red scale evaluation, the application of 6000 L/ha showed significantly higher percentage mortality than any other spray volume. In general, effective pest control is most likely due to high deposits of chemical and uniformity of spray deposition. It was concluded that low volume sprays for large canopy trees like citrus is possible (Cunningham and Harden, 1998). However, to achieve sufficient pest control, using correct spray equipment is important along with adjustment of application rates per volume of the spray mixture.

For better interpretation by the industry and the producers, a deposition benchmark of biological effective deposition was developed by van Zyl *et al.* (2013). They concluded that a linear relationship exists between fungicide concentration, leaf area covered with fluorescent pigment particles (FPC%) and copper fungicide residue on the leaves. They subsequently modelled ABS control on mandarin leaves against FPC% values. From these models, benchmarks were calculated for 50% and 75% disease control, *viz.* 2.07 FPC% and 4.14 FPC%, respectively. Even though this was developed for the control of (ABS), it serves as a basis for the interpretation of the deposition results obtained during spray application trials.

Sprayer calibration methods

When volumes are determined by only taking into consideration the treated area, and not the physical properties of the orchard, it can result in inappropriate application volumes resulting in either over-application which can lead to increased spray drift and run-off, or too low applications, which results in poor coverage and control (Furness *et al*., 1998). This method also does not take canopy volume and density into account, which is in itself dependent upon tree age, cultivar, rootstock, planting density and location, and is thus not a suitable approach. High spray volume applications which include pesticides and fungicides can range between 6000 to 16 000 L/ha independent of canopy density *(*van Zyl *et al*., 2014). Therefore, a more

accurate approach would be to use a calibration system based on canopy volume or the tree-row-volume (TRV).

The TRV method uses the surface target area to determine the amount of pesticide or fungicide needed for adequate coverage instead of the ground area (Manktelow and Praat, 1997). A study done by Silva Junior *et al*. (2016) shows that TRV-based sprays obtained efficient CBS control and contributed towards a more sustainable citrus spraying approach. Trials were carried out in Brazil, where average spray volumes range from 800 - 3000 L/ha (Silva Junior *et al.*, 2016). Even though these volumes are two to three times less than in South Africa, this study is an excellent example of the of the possible gain there is in reduced spray volumes. However, it must be kept in mind that Brazilian citrus is mostly grown for juice production, and compared with fresh fruit production, lower levels of disease and pest control is acceptable.

Spray volume on perennial tree crops (apple, stone fruit and citrus) are commonly based on canopy volume or TRV, and a more accurate and adequate calculation of spray volume can be determined. To calculate TRV, the average tree height (m) is multiplied by the average tree depth (m) and row length (m) per hectare. In citrus (Silva Junior *et al.*, 2016), it was determined that, irrespective of fungicide rate correction, a 40% reduction of CBS spray costs and water usage was achieved, while the financial return increased by up to 35%.

The use of canopy density as a factor of a calibration system

Due to the fact that canopy sizes and planting distances differ extensively in fruit orchards, the density and foliage area per hectare will also differ greatly. Growth characteristics of different citrus cultivars will also influence these parameters. It is therefore important that chemical sprays are applied accurately in dose/cm^2 of the targeted foliage and must be adapted for different canopy densities (Furness *et al.*, 1998).

In the study by van Zyl *et al.* (2014), three different spray volume applications were applied to navel oranges: low (3700 – 4800 L/ha), medium (6500 – 8500 L/ha) and high (9600 – 12 100 L/ha). In addition to different spray volumes, trials were conducted in two orchards with different canopy densities categorized using a 5-point scale. With the help of fluorometry, digital photomacrography and image analyses the quantity and quality of fungicide deposition could be determined. It was observed in both trials that deposition quantity increased as the spray volume increased, but better spray efficiency was achieved at lower volumes. Less dense and pruned canopies, together with low volume spray applications and the effect of adjuvants were noticed in terms of deposition quantity, efficiency and uniformity. This study highlights the importance of canopy management especially when spraying at reduced spray volumes.

To determine structural and geometrical parameters such as vegetative volumes, manual measurements are usually used, however these methods are time consuming, costly and destructive. LiDAR can be used as an possible alternative (Polo *et al*., 2009).

LIGHT DETECTION AND RANGING (LIDAR)

Light detection and ranging (LiDAR) is novel technology that is being used in various fields of study, but is usually used for high-accuracy mapping and digital terrain models (DTMs) (Mohd *et al*., 2011). It is a cost-effective procedure considering the amount of data generated (http://lidar-uk.com/index.php). It was developed over 40 years ago and originally used for mapping particles in the atmosphere (Carter *et al*., 2012). LiDAR surveying was made possible in the 1980's when GPS (Global Positioning Systems) could be used for precise positioning of airborne LiDAR and the development for aircraft and satellite uses were therefore possible (http://lidar-uk.com/index.php). This technology is popular in forestry and is used to retrieve forest structural parameters and is an effective tool for forest inventories (van Leeuwen and Nieuwenhuis, 2010). This technology has advanced rapidly over the years. It has numerous advantages such as being highly accurate, providing high point density measurements, has the capability to cover large areas and the ability of users to resample areas quickly and efficiently (Carter *et al*., 2012). It has also been shown that LiDAR can precisely estimate tree LAI (leaf area index) (Lefsky *et al*., 2002).

LiDAR fundamentals

Based on the same principles as radar scanning, LiDAR also exploits electromagnetism for the detection of spatial objects, using optics for the refraction of these electromagnetic waves (van Leeuwen and Nieuwenhuis, 2010). However, unlike radars, LiDAR scanners are unable to penetrate clouds, rain or dense weather (mist) and ideal weather conditions for scanning would be a clear, sunny day, especially when airborne LiDAR is being used (Carter *et al.*, 2012). A basic measurement is made by the LiDAR device and is explained in laminas terms by Lefsky *et al.*, (2002) as the distance between the sensor and a target surface, obtained by determining the elapsed time between the emission of a short duration laser pulse and the arrival of the reflection of that pulse (the return signal) at the sensor's receiver. Multiplying this time interval by the speed of light results in a measurement of the round-trip distance travelled. Dividing that figure by two yield the distance between the sensor and the target. These characteristics classifies LiDAR as an active system, because of the system generating and directing energy towards a target and subsequently detecting the radiation that is reflected back (Carter *et al.*, 2012).

For the purposes of this study, a tree canopy would be measured to determine tree density and, according to Lefsky *et al*., (2002), "the laser pulse returned after intercepting a morphologically complex surface, such as a vegetation canopy, will be a complex combination of energy returned from surface at numerous distances."

Types of LiDAR

There are three different platform types that laser scanning operate from namely ground-based, airborne and space borne LiDAR (van Leeuwen and Nieuwenhuis, 2010). For purposes of this review, ground-based LiDAR will be discussed along with airborne LiDAR, which is mainly used in forestry mapping.

Airborne LiDAR

Airborne laser scanning systems also referred to as ALS are the most popular type of LiDAR sensors (Wulder *et al*., 2012). ALS is mostly used for large area retrieval of forest structural parameters (van Leeuwen and Nieuwenhuis, 2010). ALS generally consist of the LiDAR sensor, a GPS receiver, an inertial measurement unit (IMU), an on-board computer and a data storage device (http://lidar-uk.com/index.php). Airborne LiDAR is popular for scanning large areas of forest structural parameters, and also due to its cost-efficiency (van Leeuwen and Nieuwenhuis, 2010). Airborne LiDAR improved immensely during the early 1990's as the IMU was developed to have the ability to begin achieving decimetre accuracies (Carter *et al.*, 2012).

Ground based LiDAR

Ground based LiDAR is similar to airborne LiDAR, but an IMU is not required. The LiDAR sensor is mounted on a tripod, a vehicle or mobile robot on which the LiDAR sensor rotates 360 degrees while also recording the return pulses and the distance between the sensor and the object (in this case the citrus tree canopy) is calculated (http://lidar-uk.com/index.php). For smaller areas and where higher densities of objects are required, ground based LiDAR is ideal (Carter *et al.*, 2012).

LiDAR applications

This technology is applied for various uses such as flood and pollution modelling, mapping and cartography, urban planning, coastline management, archaeology, oil and gas exploration, navigation, architecture, geology and meteorology to name a few (http://lidar-uk.com/index.php). It is widely used in the agricultural industry especially in the field of forestry as a tool for the 3D measurement of plant shapes and canopy structures (Omasa *et al*., 2007) and to accurately measure both the vertical and horizontal vegetation structure in detail

(Wulder *et al*., 2012). Other measurements include canopy height, plant growth and shape changes, physiological responses and substances in leaves (Omasa *et al*., 2007). Various LiDAR applications and studies have been done indicating the potential of this technology in the agricultural industry. For example, Ehlert *et al*., (2008, 2010) and Saeys *et al*. (2009) used a laser system for measuring crop biomass and crop density in cereals. Gebbers *et al*. (2011) also used laser sensors to map LAI in broad acre crops. Measurement of wood volume by means of a LiDAR sensor has been proposed by Keightley and Bawden (2010) for grapevine biomass analysis (Arnó *et al*., 2013).

In recent studies LiDAR has also been used to determine the LAI of vegetative structures. LAI (leaf area index) is generally used in agriculture to determine the total area of a leaf surface, commonly applied in viticulture and in forest-monitoring applications (van Leeuwen and Nieuwenhuis, 2010; Carter *et al*., 2012). LiDAR technology can be used to determine the LAI in a non-destructive method and will give an indication of the canopy density measurement and this will be used to assist in this study.

CONCLUSION

High spray volumes have become the norm in the South African citrus industry. These volumes have several negative impacts on the environment and the high costs involved are undesirable and possibly unnecessary. In reducing these spray volumes lies various potential advantages, not only for local producers but also for the South African citrus industry in general.

Results from spray application research in South Africa has shown that the current spray methods are not the most effective approach, even though with existing spray machines, reduced volumes can lead to adequate coverage. There is therefore a need for research to verify that reduced volume sprays can be applied in South African citrus orchards and still provide acceptable coverage for protection against pests and diseases. The outcomes of this study will be used to determine if reduced volume spray applications for certain pests and diseases such as citrus black spot, Alternaria brown spot, red scale, mealybug and false codling moth will result in acceptable levels of control.

Due to high volumes being based on leaf wall area (LWA) calibration method, which does not take canopy density into account, a need for a non-destructive calibration method is required. This will be researched with the help of novel technology namely LiDAR. A new tree-row-volume (TRV) calibration system would be developed and refined for use in citrus production in South Africa. The LiDAR system would be developed to characterize citrus tree canopy density and topography that can be used in the industry by producers themselves.

Not only do TRV-adjusted spray volumes have the potential to reduce costs, but it will also have a positive impact from an environmental point of view. If the same approach to take canopy volume into consideration was used in South Africa, the financial as well as environmental implications would be vast. This will assist to develop a calibration system specifically for citrus trees that are based on TRV and canopy density.

Spray application research under South African conditions for citrus specifically is still a topic with several potential possibilities. The investigation towards novel technology such as LiDAR and its non-destructive methods will form the backbone of this study. The focus will be on reducing spray volumes as much as possible but still maintaining adequate coverage for certain pests and diseases especially for the requirements set out for the exporting market.

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CHAPTER 2

Evaluation of reduced volume fungicide and pesticide sprays for control of important pests and diseases in the South African citrus industry

ABSTRACT

Citrus trees are susceptible to a wide range of insect pests and fungal diseases and therefore spray volumes of pesticides and insecticides are commonly pre-determined despite the orchard's characteristics. Chemicals and water are therefore often wasted when too high volumes are applied. These high volumes are the result of strict regulations by economical important countries such as the European Union (EU) and their phytosanitary restrictions towards diseases such as Citrus black spot (CBS) and False coddling moth (FCM). High spray volumes have become an industry norm in South Africa, ranging from 6000 – 16 000 L/ha; much higher compared with other citrus producing countries. Previous spray deposition research has demonstrated the potential of reduced spray volumes but highlighted the importance of canopy management to improve penetration. The biological efficacy of reduced volume sprays was also not previously evaluated. In order to evaluate these factors, various spray trials were conducted in the Limpopo, Western and Eastern Cape provinces on commercial citrus producing farms. Spray deposition parameters and pest and disease control, following spray programmes applied at reduced volumes (750 to 3000 L/ha) were compared with the farm's standard spraying volume (4000 to 9000 L/ha). The deposition quantity (FPC%) results obtained in this study, showed that the reduced volume applications generally achieved higher deposition quantity values on fruit and leaves in comparison with the high volume applications. However, the higher spray volumes achieved better deposition uniformity results (CV%) and had higher biological control in terms of clean fruit. Therefore, when comparing the biological efficacy and the spray deposition results, it was seen that deposition uniformity values are directly proportional to the biological efficacy. Furthermore, the effect of canopy density and spray penetration was clear and had a direct effect on deposition parameters. Spray deposition parameters on the inner canopy positions were poorer than the outer canopy positions, which also correlated with biological efficacy. This study supports the importance of penetration of spray volumes into the tree canopy as well as the potential of reduced spray volumes.

INTRODUCTION

Agriculture is the largest single user of water in South Africa, using approximately 60% of the total withdrawals (Donnenfeld *et al*., 2018). With the current water crisis in South Africa, increasing water scarcity and rainfall variability is a reality, placing producers in the agriculture sector under immense pressure. This will require agriculture to adapt their practices to use less water in the production process. In the citrus industry, spray volumes of pesticides are commonly pre-determined despite the orchard characteristics, resulting in the wastage of chemicals and water (Silva Junior *et al.*, 2016).

High spray volumes have become an industry norm in the South African citrus industry, ranging from 6000 – 16 000 L/ha (van Zyl *et al.*, 2014). Worldwide South Africa is ranked as one of the countries with the highest spray volumes, compared to other citrus producers such as Spain,Florida and Brazil (Vincent *et al*., 2009; Dewdney *et al*., 2015; Silva Junior *et al.*, 2016).

High spray volumes are the result of strict export requirements especially for the European Union (EU) market, such as the zero-tolerance approach towards Citrus black spot [caused by *Phyllostica citricarpa* (van der Aa)] and false coddling moth [*Thaumatotibia leucotreta* (Meyrick)] (CGA, 2017; Guarnaccia *et al*., 2017). The EU is the largest and most important export market of citrus for SA, making up to 44% of the total exported fruit in the 2016/2017 growing season (CGA, 2017). Therefore, quality disease free fruit is needed to satisfy this economical important international market.

Despite the costs and risks involved, growers also use high volume applications as a safety buffer for good disease and pest control (van Zyl *et al.*, 2015). However, high spray volumes are costly and large proportions are lost due to drift or run-off. High volume applications are also time consuming and costly in terms of labour, water, chemicals and equipment (Furness *et al*., 1998).

The spray machine characteristics, tree size, plant protection product (PPP), nature of the spray target, spraying time, as well as the weather conditions during spraying all contribute to the success of the application (Salyani and McCoy, 1989). It is furthermore always important to keep in mind that a fungicide is only as effective as its application (Claassen, 2015). Therefore, a proper combination of the application factors can result in substantial savings in material, labour, fuel and machinery costs while providing optimal pest control with minimal environmental pollution (Salyani and McCoy, 1989).

Seen in other studies, mature citrus trees retain a maximum amount of 2300 L/ha before the point of runoff (Cunningham and Harden, 1998, 1999), proving that spraying past the point of runoff leads to inefficient application and high volumes of waste. Silva *et al.* (2016) studied

spray volume and fungicide rates for the control of citrus black spot, and found no significant differences between different volumes, with the lower volumes performing just as effectively as the higher volumes, but at higher efficiency rates. In another study by Cunningham and Harden (1999), where different sprayers were evaluated to reduce spray volumes in mature citrus trees in conjunction with biological efficacy of the California red scale (*Aonidiella aurantii* Maskell) as well as Citrus mealybug *(Planococcis citri* Risso), it was found that the high volume treatments provided effective pest control, which was attributed to the evenness of spray distribution within the canopy. Garcerá *et al*. (2011) indicated that it is necessary to know how far spray volumes can be reduced without affecting the efficacy of applications. They investigated the effect of spray volume on coverage and on mortality of California red scale and found that higher volumes did not result in better efficacy, concluding that reduced spray volumes can be evaluated under field conditions.

Numerous studies of deposition obtained by different spray volumes have been done in the South African citrus industry, which indicated the potential of lower volume applications (Van Zyl *et al*., 2013). However, few trials have been done on the feasibility, implementation and biological efficacy of these different spray volumes when used in seasonal spray programmes. Reduced spray volumes have the potential of significantly decreasing the cost; however, these applications require more attention to sprayer calibration, technique and maintenance (van Zyl *et al.*, 2013).

Deposition assessment of spray coverage on leaf and fruit surfaces are commonly used to evaluate spray application, however, the evaluation of biological efficacy will give a more critical and applied outcome (Jones *et al*., 2000). Therefore, a need arose to evaluate reduced spray volume application in terms of disease and pest control in the South African citrus industry under commercial conditions, especially in light of the need to obtain 100% control of important phytosanitary pests and diseases. The aim of this research chapter was to investigate reduced spray volumes in the South African citrus industry in terms of deposition parameters and biological efficacy.

MATERIALS AND METHODS

Trial sites and layout

Trial sites included four commercial farms in South Africa, each located in different citrus growing regions, *i.e.* Groblersdal and Marble Hall (Limpopo), Citrusdal (Western Cape) and Patensie (Eastern Cape).

Groblersdal

A uniform 8.5 ha Midnight Valencia (*Citrus sinensis*) on Swingle citrumelo rootstock orchard was selected for the first trial. The orchard had a tree spacing of 6 × 3 m with an average tree

height of 3.5 m. The average tree density was determined visually following similar guidelines as van Zyl *et al.* (2014) on a 5-point scale (1 - very sparse leaf concentration, heavily aerated; 2 - sparse leaf volume, well aerated; 3 - good balance between leaf volume and canopy aeration; 4 - dense canopy, sparsely aerated; 5 - very dense leaf concentration, poorly aerated with no pruned canopy windows). The tree density for this trial site was estimated as a 4 on the 5-point scale. For this trial two different spray machines were used, namely an Ultima (2000 L tank) sprayer (Johnson Advanced Machinery; www.citro.co.za) for high volume application and the Martignani KWH Turbo 2 electrostatic (1500 L tank) sprayer [S. Agata sul Santerno (RA), Italy] for low volume spray application. Applications were applied for the period from October 2016 to January 2017 following the farm's standard spray programme for pest [red scale (*Aonidiella aurantii* (Maskell)), mealybug (*Planococcus citri* (Risso)] and false codling moth [*Thaumatotibia leucotreta* (Meyrick)] and disease [Citrus black spot (*Phyllosticta citricarpa*)] control (Table 1).

The three spray volume treatments, tractor speed and spray pressure for this trial was as follows: 750 L/ha with the Martignani sprayer; 3.10 km/h; 15 bar (Treatment 1), and 4900 L/ha; 2.74 km/h; 40 bar (Treatment 2) and 8900 L/ha; 3.36 km/h; 20 bar (Treatment 3) applied with the Ultima sprayer. Power take-off (PTO) speed of the tractor was kept constant at 540 rpm. This trial was repeated in the 2017/2018 season (Table 2). At the last seasonal spray, spray deposition was determined for each treatment using the methods described by van Zyl *et al.* (2013, 2014). Each of the spray treatments contained yellow fluorescent pigment [South Australian Research and Development Institute (SARDI) Yellow Fluorescent Pigment, 40% EC (SARDI, Loxton, South Australia); at 1 ml/L; Ultima 1 (1×), Ultima 2 (2×) and Martignani (4×)]. This made it possible to visualize and measure the deposition on the leaf and fruit surfaces. In this trial no trees were left untreated, due to the phytosanitary pest and disease status of false codling moth and citrus black spot. For each spray treatment, a minimum of five rows were sprayed from both sides, thereafter a single row-section of 10 trees was marked, at least 3 rows away from the next treatment to avoid the influence of spray drift. This marked section was used to select data trees from which we collected the leaf and fruit samples for spray deposition analysis as well as the biological efficacy data.

Marble Hall

A 5.7 ha Delta Valencia (*Citrus sinensis*) on Swingle citrumelo rootstock orchard was selected with a tree spacing of 7 × 3.5 m, average height of 3.4 m and a tree density of 4 determined according to the 5-point scale as describe above. For this trial, the Jacto Arbus [Tualatin, Oregon, USA (2000 L tank)] spray machine for high volume application, and the Cima Blitz 55 [Rovic & Leers, South Africa; (2000 L tank)] spray machine for low volume application was used. Applications were applied for the period from October 2016 to January 2017 following

the farm's standard spray programme for pest (red scale, mealybug and false codling moth) and disease (Citrus black spot) control (Table 3). The two spray volume treatments, tractor speed and spray pressure for this trial was 6000 L/ha; 2.03 km/h; 20 bar with the Arbus sprayer and 2000 L/ha; 2.55 km/h; 1.5 bar with the Cima sprayer. Power take-off (PTO) speed of the tractor was kept constant at 540 rpm. At the last seasonal spray, pigment was added for deposition analysis as described above [at 1 ml/L; Arbus (1×), Cima (3×)]. No trees were left untreated, due to the phytosanitary pest and disease status of false codling moth and citrus black spot. A similar plot layout and selection of data trees were planned as described above in the Groblersdal trial.

Citrusdal

A 3.4 ha Fairchild tangerine (*Citrus reticulata* Blanco) orchard, with a tree spacing of 6 x 4 m was selected. Tree density was estimated at 3 as determined according to the 5-point scale (van Zyl *et al.*, 2014).Three different spray volume treatments were applied in this trial using a Nieuwoudt oscillating tower sprayer [Nieuwoudt Sprays, Citrusdal, South Africa; (2000 L tank)] for a high volume (8800 L/ha) and medium volume (4000 L/ha) treatment. Tractor speed and spray pressure for the high volume application was 3.0 km/h; 30 bar and 6.0 km/h for the medium volume application respectively. For the lower volume application, a Cima Blitz 55 [Rovic & Leers, South Africa; (2000 L tank)] spraying machine was used at 3000 L/ha with a tractor speed of 2.70 km/h and spray pressure of 2 bar. Power take-off (PTO) speed of the tractor was kept constant at 540 rpm. Following the farm's standard spray programme for Alternaria brown spot (*Alternaria alternata)* control, the applications were applied from September 2017 to March 2018 (Table 4). At the last seasonal spray, pigment was added for deposition analysis as described above at [1 ml/L; Nieuwoudt at 8800 L/ha (1_x), Nieuwoudt at 4000 L/ha (2_x) and Cima (3_x)]. Plot layout and selection of data trees were similar as in the case of Groblersdal trial site as described above. In this trial a total of five trees were left untreated as a control treatment to determine disease pressure in the orchard. These trees were located in the first row of the beginning of the orchard, to minimize the influence of spray drift from other treatments.

Patensie

A Nadorcott mandarin (*Citrus reticulata*) orchard with a tree spacing of 5 x 2 m was selected. Tree density was estimated at 4 according to the 5-point scale (van Zyl *et al.*, 2014). Two spray machines were used, namely the Ultima Narrow (2000 L tank) for a 6700 L/ha application at a tractor speed of 1.80 km/h; 30 bar spray pressure and a Martignani KWH Turbo 2 electrostatic sprayer [S. Agata sul Santerno (RA), Italy; (1500 L tank)] for 1000 L/ha applied at two different tractor driving speeds (1.4 km/h and 3.4 km/h) and spray pressure of

15 bar. Power take-off (PTO) speed of the tractor was kept constant at 540 rpm. Following the farm's standard spraying programme for pest (red scale, mealybug and false codling moth) and disease (Citrus black spot and Alternaria brown spot) control. The applications were applied from October 2017 to March 2018 (Table 5). At the last seasonal spray, pigment was added for deposition analysis as described above [at 1 ml/L; Ultima (1×), Martignani at 1.4 km/h and 3.4 km/h (4×)]. In this trial no trees were left untreated, due to the phytosanitary pest and disease status of false codling moth and citrus black spot. A similar plot layout and selection of data trees were planned as described above in the Groblersdal trial.

Sampling of field trials

Leaves

For each treatment, 3 uniform trees, as treatment replicate trees, were randomly selected within the orchard section that received a specific spray volume treatment. Within each replicate tree, 6 positions were selected in the canopy: three vertical positions (top, middle and bottom part of each tree canopy) and two horizontal positions namely inner (30 to 50 cm into the tree canopy) and outer canopy (leaves and fruit on the outside of the tree canopy) positions. From each position separately, 12 undamaged leaves (72 leaves per replicate tree) were collected in plastic bags, labelled and transported back to the laboratory for spray deposition analyses. The leaves were stored at cool (4°C) and dry conditions until deposition analysis was done.

Fruit

From the same replicate trees used to sample leaves, fruit was sampled at the same six positions as mentioned previously. Five fruit were sampled from each position (30 fruit per replicate tree). Each fruit's side that faced the spray machine was marked with a black dot using a permanent marker pen. The fruit were collected in carton containers, labelled and transported back to the laboratory for spray deposition analyses. The fruit were stored at cool (4°C) and dry conditions until deposition analysis was done.

Deposition analysis

Similar to the methodology used by van Zyl *et al*. (2013), leaves and fruit were subjected to deposition analysis to determine the deposition quantity and quality of the chemicals applied at different spray volumes. Images of leaves and fruit were taken in a dark room with an illuminated ultra-violet light source (UV-A, ≈365 nm, Labino Mid Light; www.labino.com) for the fluorescent pigment to be clear on images.

First, petioles were removed from leaves at the base of the leaf blade using a pair of scissors. A single leaf was positioned in the middle of a back-illuminated red Perspex box to

reduce any shadowing and to enhance edges of leaves. A glass pane (200 × 200 × 2 mm) was used to cover and flatten the leaf. A Canon EOS 40D camera equipped with a 60 mm macro lens was mounted on a tripod in a fixed position directly above the Perspex box and was used to take digital photos in RAW file format (.CR2 ≈ 10 MB) of the upper and lower leaf surfaces of each leaf. Similar methods were applied for the digital analysis of the fruit, except that the fruit was placed on a small plastic stand in the middle of the Perspex box. The digital photos were taken from the fruit surface that faced the spray machine (marked with a black marker when samples were collected in the field) and away from the spray machine (the opposite side of marked side). For leaves an aperture setting of F10 and an ISO setting of 100 was used, while for fruit an aperture setting of F14 and ISO setting of 160 was applied. RAW images were converted to 8-bit Exif-TIFF (.TIF ≈ 30 MB) with Digital Photo Professional version 3.1.0.0 (CANON INC.; www.canon.com) files for digital analysis to determine the deposition parameters (Van Zyl *et al*., 2013).

To assess the deposition quantity and quality per leaf or fruit, similar methodology used by (van Zyl *et al.*, 2013) was again used in this study. Spray deposition assessment involved digital image analysis with Image Pro Plus software version 7.0 (Media Cybernetics, www. mediacy.com) to determine the deposition quantity and quality per leaf. Deposition quantity was measured as percentage of the leaf or fruit area covered by pigment particles (percentage fluorescent particle coverage, FPC%). For the deposition quality assessment, the leaf or fruit area was divided into equally-sized squares [100 x 100 pixels (10 000 pixels)]. Depending on the leaf or fruit size, this amounted to as few as 20 to more than 250 individual squares per leaf, of which the percentage of area covered by fluorescent pigment particles was determined for each square. The Interquartile Coefficient of Dispersion (ICD %) per leaf or fruit was used as a measure of deposition quality per leaf or fruit, *i.e.* uniformity of deposition on the leaf or fruit surface. Low ICD values were indicative of better deposition quality. Deposition uniformity between leaves or fruit was calculated as the uniformity in pigment deposition in a 12 leaf or 5 fruit batch (standard deviation/mean × 100). Deposition efficiency was expressed as deposition quantity normalised to FPC % per 1000 L/ha.

Biological evaluation

False codling moth, mealybug, red scale

For the pest evaluation, 16 data trees were selected on a zig zag basis down the centre of two rows of each orchard section that received a specific spray volume treatment. From each tree, 6 fruit from each of abovementioned six positions were selected. Each of the fruit were rated according to the infestation level (1: infested and 0: not infested) (Grout, T., Personal communication, 2017). The presence of each pest was evaluated accordingly. For mealybug

the presence of sooty mould, honey dew or debris under the calyx or in the stylar opening would indicate infestation even from earlier stages. To determine if false coddling moth was present, fruit showing surface damage were cut at the site of damage to inspect the internal damage. For red scale evaluation, a head-loupe or Optivisor was used to do red scale counts.

Citrus black spot

To evaluate the occurrence of citrus black spot at the Groblersdal, Marble Hall and Patensie trial sites, 16 data trees were selected on a zig zag basis down the centre of two rows of each orchard section that received a specific spray volume treatment. From each tree, 6 fruit were again evaluated at abovementioned six positions in the tree canopy. Each fruit was evaluated for the presence of CBS lesions and rated according to the following 3-point index that was used by Schutte *et al*., (2003) and which was described by Kellerman and Kotzé (1977): no lesions (0), 1-3 lesions (1) and 4+ lesions (2).

Alternaria brown spot

Alternaria brown spot evaluation was done in the Western and Eastern Cape trials, based on the disease incidence and severity by counting the number of infected fruit. From each treatment three uniform data trees were selected as replicates down a row that was sprayed from both sides with the specific volume. From each tree, 6 fruit from the outside of the canopy (fruit from the outside of the tree), were selected from each of the following three positions: top, middle and bottom. The same three positions were used to rate 6 fruit each from the inner canopy (30 to 50 cm into the tree). Disease incidence was rated using a scale of 0 (no symptoms present) and 1 (symptoms present). These ratings were expressed as percentage of the clean fruit. Ratings were done on a monthly basis (from September 2017 to June 2018), prior to each Alternaria spray until harvest.

Statistical analysis

The data from each trial were analysed separately. Deposition quantity (FPC%), quality (ICD %), uniformity (CV%) and biological evaluation data were subjected to appropriate analysis of variance (ANOVA). The skewing effect of outliers was negated by using median FPC% values of the 12 leaves or 5 fruit for deposition analysis. Data from upper and lower leaf surfaces was analysed separately but were combined when describing the results. The Student's t-test for least significant difference (P = 0.05) was used to compare means. SAS (Statistical Analysis System) version 8.2 statistical software (SAS institute Inc., 1999) was used for analysis.

Benchmark modelling

Alternaria brown spot disease control levels were compared with those predicted using the Alternaria Brown Spot benchmark model developed by van Zyl *et al.* (2013). This model uses

deposition quantity values to predict expected control of Alternaria brown spot on mandarin leaves. Two benchmarks, FPC_{50} (2.07 FPC%) and FPC_{75} (4.14 FPC%), were highlighted to give an indication of deposition quantity levels on leaves that would results in 50% and 75% disease control, respectively.

RESULTS

Groblersdal

2016/2017 season

Biological efficacy

Analysis of variance (ANOVA) of the biological evaluation data showed a significant treatment × horizontal canopy position × vertical canopy position interaction for mean percentage fruit clean from red scale (RS) (P = 0.0395) (Table 6). This interaction was attributed to the Martignani treatment giving significantly better RS control on the inner canopy than the outer canopy in the tops of trees (results not shown), while the percentage clean fruit for RS was generally higher on outer canopy fruit (89.72%) than inner canopy fruit (86.41%) (P = 0.0606), and lower in tops and middles of trees (85.46 and 87.59%, respectively) than in bottoms of trees (91.13%) (P = 0.0934). The Ultima 2 treatment performed significantly (P < 0.0001) better (99.48%) than the Martignani treatment (75.19%), with the Ultima 1 treatment achieving intermediate results (88.72%) (Table 7).

For mealy bug (MB), a significant horizontal × vertical canopy position interaction was observed in the ANOVA (P = 0.0056) (Table 6). The percentage clean fruit was generally higher and at similar levels at outer canopy positions (78.72 to 83.33%), but significantly lower at inner canopy positions in the tops and bottoms (75.89 and 74.11 %, respectively) and particularly in the middle of trees (55.67%). A significant treatment effect was seen for red scale (RS) (P < 0.0001) (Table 6) indicating that the Ultima 2 treatment yielded significantly more fruit clean from MB (88.89%) than the Ultima 1 (68.58%) and Martignani (65.93%) treatments (Table 7).

For FCM and CBS no infestation or lesions were observed during trial evaluation in the 2016/2017 season.

Deposition quantity – leaves

Analysis of variance (ANOVA) of FPC% (deposition quantity) data on leaves from this trial showed a significant treatment × horizontal canopy position interaction (P < 0.0001) as well as vertical canopy position effect (P < 0.0001) (Table 8). The significant vertical canopy position effect indicated that regardless of treatment a significantly higher mean FPC% value was recorded in the bottom of trees (7.19 FPC%) followed by the middle (6.94 FPC%) and the top of trees (4.90 FPC%). The treatment × horizontal canopy position interaction indicated the

Martignani spray deposited significantly higher quantities of pigment (12.84 FPC%) on outer canopies, and the Ultima 1 spray significantly lower quantities on inner canopies (3.58 FPC%) than the other treatment × position combinations (4.99 to 6.05 FPC%), which did not differ significantly from each other. In general, the Martignani (8.81 FPC%) deposited higher quantities than the Ultima 1 (4.29 FPC%) and Ultima 2 (5.94 FPC%) treatments (Table 9). All the FPC% means of the treatments were higher than the FPC_{75} benchmark of 4.14 established by van Zyl *et al*. (2013) for the control of Alternaria Brown Spot.

Deposition uniformity – leaves

The ANOVA of deposition uniformity (CV%) data showed a significant effect for horizontal canopy position ($P = 0.0184$) as well as a significant treatment effect ($P < 0.0001$; Table 8). All the treatments performed poorer in terms of deposition uniformity at the inner canopy positions (55.03 CV%) compared with outer canopy positions (47.22 CV%). The Martignani treatment had the highest CV% (poorest uniformity) of 62.66 CV%, which was significantly poorer than the Ultima 1 (48.33 CV%) and Ultima 2 (42.39 CV%) treatments (Table 9).

Deposition quality – leaves

The ANOVA of the deposition quality (ICD%) results on leaves showed a significant treatment effect ($P = 0.0003$) (Table 8). The results showed similar mean deposition quality (ICD%) values for the Martignani and Ultima 1 treatments (43.55 ICD% and 43.45 ICD%, respectively), while the Ultima 2 treatment had a significantly poorer deposition quality (50.98 ICD%) (Table 9).

Deposition quantity – fruit

In the ANOVA of deposition quantity data from fruit, a significant effect was observed for treatment ($P = 0.0222$) (Table 8). The highest mean deposition quantity on fruit (FPC%) was achieved by the Martignani treatment (13.15 FPC%), similar to the Ultima 2 treatment (9.15 FPC%), and significantly better than the Ultima 1 treatment (5.95 FPC%) (Table 9).

Deposition uniformity – fruit

The ANOVA of CV% data on fruit indicated a significant treatment effect ($P = 0.0484$) (Table 8). Furthermore, a significant vertical canopy position effect ($P = 0.0258$) and horizontal canopy position effect ($P = 0.0417$) were observed. The best deposition uniformity was generally achieved at the bottom canopy position (48.78 CV%) followed by the middle canopy position (53.04 CV%) and the top canopy position (62.50 CV%), while deposition on the inner canopy positions was generally poorer (59.04 CV%) in comparison with the outer canopy position (50.57 CV%). The best uniformity results were achieved by the Ultima 2 treatment, but with a mean CV% similar to the Martignani treatment (47.55 and 54.22 CV%, respectively)

(Table 9). The poorest (highest) CV% was achieved by the Ultima 1 treatment (62.47 CV%), not differing statistically from the Martignani, but significantly poorer than the Ultima 2 treatment.

Deposition quality – fruit

For the ICD% (deposition quality) data on fruit, a significant interaction for treatment × vertical canopy position (P = 0.0003) was seen in the ANOVA (Table 8). From this interaction it was observed that the Martignani treatment at the bottom and middle canopy positions performed the best with the lowest ICD% for fruit (54.84 ICD% and 55.25 ICD%, respectively). However, the top canopy position of the Martignani along with the top, middle and bottom positions of both the Ultima treatments performed similarly with mean deposition quality values ranging from 68.98 – 75.73 ICD% (results not shown). In general, the Martignani treatment had significantly better deposition quality (60.77 ICD%) than the Ultima 1 and -2 treatments (70.83 and 74.04 ICD%) (Table 9).

2017/2018 season

Biological efficacy

The ANOVA of the biological efficacy results for percentage fruit clean from red scale (RS) showed a significant treatment × vertical canopy interaction (P = 0.0019) (Table 10). The treatments generally performed the best at the bottom canopy position (91.15 to 96.67% clean fruit), followed by the middle (87.50 to 93.23%) and top (58.33 to 82.29%) canopy positions (Table 11). At these positions, the treatments did not differ significantly, except at the top canopy position where the Ultima 2 (82.29%) and Martignani (72.22%) resulted in significantly better control than the Ultima 1 treatment (58.33% clean fruit) (Table 11).

For MB control a significant treatment × horizontal × vertical canopy position interaction was observed (P = 0.0098; Table 10). This 3-factor interaction was largely attributed to variable levels of MB control in tops of trees (results not shown), and the significant treatment × vertical canopy position interaction (P = 0.0002) is presented (Table 11). The Martignani treatment resulted in significantly more clean fruit (81.67%) in the tops of trees than the two Ultima treatments (51.56% and 47.92%, respectively). Likewise, the Martignani treatment resulted in significantly more clean fruit (67.22%) in the middle of trees than the Ultima 2 treatment (50.52%), which performed significantly better than the Ultima 1 treatment (38.02% clean fruit). At the bottom tree positions, the Martignani (62.78%) and Ultima 2 (61.46%) treatments performed significantly better than the Ultima 1 treatment (31.77%; Table 11). For the horizontal canopy effect (P = 0.0031) the outer canopies were generally better protected than inner canopies, with 66.67% *vs.* 42.20% clean fruit.

Deposition quantity – leaves

Analysis of variance of deposition quantity (FPC%) on the leaves showed a significant vertical canopy position effect (P = 0.0032) as well as a significant horizontal canopy position effect (P = 0.0003) (Table 12). Deposition quantity was generally higher in bottoms of trees (3.16 FPC%), compared with the middle (2.87 FPC%) and tops of trees (1.78 FPC%), while higher deposition quantities (3.26 FPC%) were measured on outer canopies, than inner canopies (1.94 FPC%). No significant treatment effect was observed (P = 0.2650), but the highest deposition was achieved by the Ultima 1 treatment (3.19 FPC%), compared with the Martignani (2.07 FPC%) and Ultima 2 treatment (2.55 FPC%) (Table 13). Deposition quantities were intermediate between the FPC_{75} and FPC_{50} benchmarks (van Zyl *et al*., 2013).

Deposition uniformity – leaves

The deposition uniformity (CV%) showed a significant treatment × horizontal canopy position interaction (P = 0.0101) (Table 12). Both the Ultima treatments achieved significantly better deposition uniformity on the outer canopy (63.85 and 67.91 CV%, respectively) than the Martignani (117.72 CV%), while similar uniformity levels were recorded on the inner canopies (91.04 to 110.09 CV%). In general, the Ultima 1 and Ultima 2 treatments gave better deposition uniformity (Table 13).

Deposition quality – leaves

The ANOVA of the deposition quality (ICD%) showed only a meaningful treatment effect (P = 0.0595; Table 12). The Martignani performed significantly better (48.77 ICD%) than the Ultima treatments (*c.* 58 ICD%) (Table 13).

Deposition quantity – fruit

Analysis of variance (ANOVA) for the deposition quantity indicated a significant horizontal canopy effect (P = 0.0002) and a non-significant treatment effect (P = 0.6586) (Table 12). The horizontal canopy position effect indicated that the best deposition was achieved in the tops of trees (3.28 FPC%) followed by the bottom (2.90 FPC%) and middle of trees (2.50 FPC%). No statistical differences were observed between the treatments in terms of deposition quantity, although the Ultima 1 treatment performed the best (3.11 FPC%) followed by the Ultima 2 treatment (2.90 FPC%) and Martignani treatment (2.75 FPC%) (Table 13).

Deposition uniformity – fruit

For CV% data, the ANOVA showed a significant vertical canopy position effect (P = 0.0450), a meaningful horizontal canopy position effect (P = 0.0511), and a non-significant treatment effect (P = 0.1620; Table 12). Uniformity was generally better on outer than inner canopy fruit (66.82 *vs.* 79.17 CV%), and better in bottoms (62.94 CV%) than middle and tops of trees

(82.44 and 73.60 CV%, respectively). The Ultima 2 treatment performed the best (with the lowest CV%; 67.21 CV%) but almost similar to the Ultima 1 treatment (67.21 CV%) and the poorest was the Martignani treatment (82.40 CV%) (Table 13).

Deposition quality – fruit

The ANOVA of the deposition quality showed no significant interactions or main effects (Table 12). All treatments had mean deposition quality ratings of *c.* 86 ICD% (Table 13).

Marble Hall

Biological efficacy

In the ANOVA of percentage clean fruit data for RS control a significant vertical canopy position effect was observed ($P = 0.0236$) as well as a significant treatment effect ($P < 0.0001$) (Table 14). The highest percentage clean fruit was recorded in bottoms of trees (96.88%), with the middle canopy position (91.41%) intermediate levels and the top canopy position the lowest RS control levels (90.89% clean fruit). The Arbus treatment had significantly better control (97.22%) than the Cima treatment (88.89% clean fruit) (Table 15).

Analysis of variance (ANOVA) of the percentage clean fruit data of different treatments for mealybug (MB) control showed a significant treatment × vertical canopy position interaction ($P = 0.0146$), as well as a significant horizontal canopy position effect ($P < 0.0001$) (Table 14). MB control was generally better on outer canopy fruit than inner canopy fruit (84.7 *vs.* 67.0% clean fruit). The reduced volume Cima treatment resulted in similar levels of MB control across vertical canopy positions (*c.* 69.6% clean fruit), similar to that of the higher volume Arbus treatment in the middle of the tree (69.8%), but significantly lower than the Arbus in tops (85.4%) and bottoms (91.1%) of trees. The Arbus treatment generally performed the best with a mean percentage clean fruit of 82.12%, differing significantly from the Cima treatment with only 69.62% control of MB (Table 15).

Deposition quantity – leaves

Analysis of variance (ANOVA) of the deposition quantity data from this trial for the leaves indicated that there was a significant treatment × horizontal canopy position interaction ($P = 0.0063$). The Cima treatment had the best deposition quantity at the outer canopy position (4.04 FPC%) and the inner canopy position of the Arbus treatment had the poorest (0.97 FPC%). The Cima treatment at the inner canopy position and the Arbus on the outer canopy positions showed intermediate results (3.03 and 2.16 FPC% respectively). The Cima treatment (3.53 FPC%) generally achieved better results than the Arbus treatment (1.57 FPC% (Table 17), with deposition quantities above the FPC_{50} benchmark of 2.07 FPC% (van Zyl *et al.*, 2013).

Deposition uniformity – leaves

The ANOVA of the deposition uniformity (CV%) data on leaves indicated a significant horizontal canopy position effect ($P < 0.0001$) as well as a significant treatment effect ($P = 0.0097$) (Table 16). Deposition uniformity was generally better on outer canopies than inner canopy positions (52.21 *vs.* 86.75 CV%). The Arbus achieved better uniformity (62.21 CV%) than the Cima treatment (76.76 CV%) (Table 17).

Deposition quality – leaves

For the deposition quality results, the ANOVA indicated a significant treatment × vertical canopy position interaction *(*$P = 0.0207$) (Table 16). Deposition quality was generally poor at the top, middle and bottom canopy positions of the Arbus treatment (73.22, 71.61 and 71.28 ICD%, respectively) and the top canopy position of the Cima (67.82 ICD%). The bottom and middle canopy positions of the Cima treatment had the better deposition quality values, having similar values (55.59 and 55.18 ICD% respectively). Overall, the Cima treatment performed the best in terms of deposition quality (59.53 ICD%), which was significantly better than the Arbus treatment (72.04 ICD%) (Table 17).

Deposition quantity – fruit

Analysis of variance (ANOVA) of the deposition quantity data from this trial for the fruit indicated that there was a significant horizontal × vertical canopy position interaction ($P = 0.0296$) as well as a significant treatment effect ($P = 0.0006$) (Table 16). The inner middle canopy position had the poorest mean deposition quantity (3.20 FPC%), while the outer bottom position had the highest deposition quantity (10.74 FPC%). Furthermore, on inner and outer canopy positions in tops of trees similar mean FPC% (10.84 FPC% and 10.69 FPC, respectively) were recorded. The Cima treatment performed significantly better (11.11 FPC%) than the Arbus treatment (6.54 FPC%) (Table 17).

Deposition uniformity – fruit

For the deposition uniformity results the ANOVA indicated a horizontal canopy position effect ($P = 0.0086$) (Table 16) and a non-significant treatment effect ($P = 0.2929$). Outer canopy positions had better deposition uniformity (43.91 CV%) than inner canopy positions (64.48 CV%). The Cima treatment (50.90 CV%) performed similar to the Arbus treatment (57.49 CV%) (Table 17).

Deposition quality – fruit

The ANOVA indicated no significant interactions or effects but a meaningful treatment effect ($P = 0.0624$). The Cima and Arbus treatments performed similarly (59.84 and 64.79 ICD%, respectively) (Table 17).

Citrusdal

Biological efficacy

The ANOVA of percentage fruit free from Alternaria Brown Spot (ABS) indicated a significant horizontal canopy position effect (P = 0.0062) as well as a significant treatment effect (P = 0.0019) (Table 18). Significantly more clean fruit were recorded on inner (90.74%) than outer canopies (86.42%). The best control was achieved by the Nieuwoudt 2 treatment (89.82% clean fruit), significantly better than the Cima (75.93%) and Nieuwoudt 1 (74.07%) treatments. All spray treatments led to significantly more fruit free from ABS compared to the unsprayed control (Table 19).

Deposition quantity – leaves

The ANOVA indicated a significant horizontal canopy position effect (P = 0.0001) as well as non-significant, but meaningful, effects for vertical canopy position (P = 0.0652) and treatment (P = 0.0963) (Table 20). The outer canopy position generally had higher deposition quantity values (6.98 FPC%) compared with the inner canopy position (4.43 FPC%). For the vertical canopy effect, bottom and middle positions had similar deposition quantities (6.20 and 6.15 FPC%, respectively) with lower deposition in the top canopy positions (5.66 FPC%). The Cima treatment deposited significantly more pigment (7.57 FPC%) than the Nieuwoudt 1 treatment (2.88 FPC%), but similar to the Nieuwoudt 2 treatment (6.53 FPC%) (Table 21). In comparison with the deposition benchmark of van Zyl *et al*. (2013), the Cima and Nieuwoudt 2 treatments achieved mean FPC% values higher than the FPC_{75} benchmark of 4.14 FPC%. The Nieuwoudt 1 treatment achieved an FPC% value higher than the FPC_{50} benchmark of 2.07 FPC%.

Deposition uniformity – leaves

Analysis of variance (ANOVA) of the deposition uniformity data indicated a significant effect for vertical canopy position (P = 0.0010), horizontal canopy position (P < 0.0001) and treatment (P = 0.0056) (Table 20). Mean CV% was the poorest in tops of trees (82.57 CV%) followed by the bottom canopy position (63.75 CV%) while the best mean CV% was observed at the middle canopy position (61.54 CV%). The deposition uniformity was better on the outer canopy position (55.02 CV%) than the inner canopy position (83.55 CV%). The Nieuwoudt 2 treatment had the best deposition uniformity (58.30 CV%), significantly better than the Cima (70.57 CV%) and Nieuwoudt 1 (78.99 CV%) treatments (Table 21).

Deposition quality – leaves

The ANOVA indicated a significant horizontal canopy position effect (P <0.0001) as well as a significant treatment effect (P = 0.0035) (Table 20). Deposition quality was better on outer

canopies (69.91 ICD%) than inner canopies (79.46 ICD%). The Cima achieved significantly better deposition quality (57.61 ICD%) than both the Nieuwoudt treatments (87.23 and 79.20 ICD%, respectively) (Table 21).

Deposition quantity – fruit

A significant treatment effect (*P* = 0.0406) was seen in the ANOVA (Table 20). The Nieuwoudt 2 treatment performed the best with the highest deposition quantity of 9.55 FPC%, significantly higher than the Cima (4.96 FPC%) and Nieuwoudt 1 treatments (6.02 FPC%) (Table 21). In terms of the deposition benchmark of van Zyl *et al*. (2013), all the treatments achieved mean FPC% values higher than the FPC_{75} benchmark for ABS control of 4.14 FPC%.

Deposition uniformity – fruit

The ANOVA for the deposition uniformity (CV%) data showed a significant horizontal canopy position effect (*P* = 0.0185) as well as a significant treatment effect (*P* = 0.0302) (Table 20). Deposition uniformity was better on the outer canopy position (56.39 CV%) than the inner canopy position (70.50 CV%). The Nieuwoudt 2 treatment achieved the lowest mean deposition uniformity value (44.44 CV%), similar to the Nieuwoudt 1 treatment (65.75 CV%), but significantly better than the Cima treatment (80.15 CV%) (Table 21).

Deposition quality – fruit

The ANOVA indicated a significant horizontal canopy position effect of *P* = 0.0098, as well as a meaningful treatment effect (*P* = 0.0501) (Table 20). Deposition quality were better on the fruit in outer canopies (75.41 ICD%) than inner canopies (79.95 ICD%). The Nieuwoudt 2 treatment performed the best in terms of deposition quality (71.53 ICD%), similar to the Nieuwoudt 1 treatment (77.27 ICD%), but significantly better than the Cima treatment (87.24 ICD%) (Table 21).

Patensie

Biological efficacy

The biological efficacy data showed very low disease and pest pressure in the area, with only a few incidences of mealybug (MB) and Alternaria brown spot (ABS) observed on fruit, while no CBS or FCM or RS infected or infested fruit were observed. The ANOVA of the biological efficacy with regards to mealybug (MB) control showed a significant horizontal canopy position effect (*P* = 0.0318), as well as a significant treatment effect (*P* = 0.0010) (Table 22).

The MB control with the three different treatments was similar in terms of percentage clean fruit, although the Martignani 1 treatment (100.0%) and the Ultima treatment (99.13 % clean fruit) performed significantly better than the Martignani 2 treatment (97.4%) (Table 23).

For the Alternaria brown spot (ABS) control, the ANOVA indicated a horizontal canopy position effect (P = 0.0286) and a meaningful (P = 0.0702) treatment effect (Table 22). Poorer control was achieved on the inner canopy position (98.96% clean fruit) than on the outer canopy position (99.77% clean fruit). All treatments performed similarly with 98.78 to 99.83% clean fruit (Table 22).

Deposition quantity – leaves

Analysis of variance (ANOVA) of the deposition quantity data indicated a significant horizontal canopy position effect (P = 0.0002) and a significant treatment effect (P = 0.0426) (Table 24). The mean FPC% was highest at the outer canopy position (1.66 FPC%) than at the inner canopy position (0.95 FPC%). The Martignani 2 treatment achieved the highest deposition quantity on the leaves (1.87 FPC%), significantly better than the Ultima treatment (1.07 FPC%) and Martignani 1 (0.90 FPC%) treatments (Table 25). Deposition quantities of all treatments were lower than the FPC_{50} benchmark for ABS control (van Zyl *et al*., 2013).

Deposition uniformity – leaves

For deposition uniformity data the ANOVA indicated no significant interactions or effects (Table 24). The treatments performed statistically the same, although the Ultima had the lowest mean CV% value (83.90 CV%) compared with the Martignani 2 treatment with a mean of 91.54 CV% and the Martignani 1 treatment (101.64 CV%) (Table 25).

Deposition quality – leaves

The ANOVA of the deposition quality data indicated a significant treatment effect (P = 0.0012) (Table 24). The Martignani 2 treatment (77.11 ICD%) and Martignani 1 treatment (90.35 ICD%) performed significantly better than the Ultima treatment (92.78 ICD%) (Table 25).

Deposition quantity – fruit

The ANOVA for deposition quantity data indicated a significant horizontal × vertical canopy position interaction (P = 0.0059) and a non-significant treatment effect (P = 0.3426) (Table 24). The outer canopy positions generally had better mean deposition quantity values (0.57 – 1.25 FPC%) than the inner canopy positions (0.16 – 0.56 %), with the top and bottom positions achieving similar deposition quantity values. Treatments did not perform significantly different from each other in terms of mean FPC% and ranged from 0.55 to 0.67 FPC% (Table 25).

Deposition uniformity – fruit

The ANOVA of the deposition uniformity (CV%) on the fruit data indicated a significant horizontal canopy position × treatment interaction (P = 0.0350), which was ascribed to the Martignani 2 treatment achieving better uniformity on inner than outer canopy leaves, while

the other treatments generally had poorer uniformity on inner canopy leaves. The Martignani 2 (68.97 CV%) and Ultima treatment (72.95 CV%) performed significantly (P = 0.0323) better than the Martignani 1 treatment (87.12 CV%) (Table 25).

Deposition quality – fruit

The ANOVA of the deposition quality results on fruit showed a significant horizontal × vertical canopy interaction (P = 0.0083) (Table 24), but deposition quality was generally similar (97.53 to 100.0 ICD%) (results not shown). No significant differences were seen between treatments (P = 0.3099) (Table 25).

DISCUSSION

In this study, reduced spray volumes showed promising results in terms of spray deposition parameters. However, high spray volumes achieved better control of pests and diseases. Various other studies have investigated the potential of low volume spray applications, the environmental impact thereof as well as the efficacy of spray applications for the control of pests and diseases (Cunningham and Harden, 1998, 1999; Furness *et al.*, 1998; Fourie *et al*., 2013; van Zyl *et al.*, 2014; van Zyl *et al*., 2015; Silva *et al*., 2016). The potential of reduced volume applications for citrus was highlighted in these studies, however the validation thereof by means of biological efficacy evaluation was lacking. This specific study contributes to the further evaluation of reduced spray volumes in the South African citrus industry, and specifically also investigated the biological efficacy of these reduced volume sprays in commercial orchards.

The deposition quantity (FPC%) results obtained in this study showed that the reduced volume spray applications achieved higher deposition quantity values on fruit and leaves in comparison with the high volume applications. This could be attributed to the maximum spray retention ability of fruit and leaves being exceeded at high spray volumes, leading to spray mixture being lost to run-off (Fourie *et al.*, 2009). This agrees with the results of studies done by Cunningham and Harden (1998, 1999) that found spraying mature citrus trees with application volumes above 2000 L/ha was ineffective as the result of spray volume being lost to run-off, as well as exo- and endo-drift. High spray volumes were also regarded as excessive due to waste (due to run-off or drift) of water and chemicals, which also supports the use of lower volume applications (Furness *et al*., 1998).

In trials where the same spray machines were used at different spray volumes, significant differences were seen between the treatments. At the Groblersdal trial site, an Ultima spray machine was used to apply two different volumes (4900 and 8900 L/ha) for two consecutive seasons. The higher volume application achieved higher deposition quantity values than the low volume application in the 2016/2017 season; however, for the 2017/2018 season the

opposite result was observed. The Ultima spray machine produces a high air volume and the relatively poor performance at low volume might be attributed to sprayer setup not being ideally calibrated for low volume application, specifically pressure and air volume, which might have resulted in small droplets being blown off the targets. Similar results were seen at the Citrusdal trial site where a Nieuwoudt spray machine was used to apply two different volumes (4000 and 8800 L/ha), and the higher volume application achieved better deposition quantity results. The Nieuwoudt spray machine does not have air-assistance and uses hydraulic pressure through venturi nozzles on oscillating towers only. These spray machines require high hydraulic pressure to penetrate the tree canopy and is most effective at high volumes. At lower spray volumes the hydraulic pressure is reduced, which might explain poorer deposition. This limitation is evident in all the deposition parameters where the 4000 L/ha treatment had poorer deposition quantity, uniformity and quality on the leaves and fruit than the higher volume treatment with the same machine.

In this study spray deposition analyses were done on leaves and fruit, while previous studies using this deposition assessment protocol focussed on leaves only (van Zyl *et al.*, 2012). When comparing spray deposition on leaves and fruit, a good correlation can be seen in most trials. This was seen for most trials except for the Citrusdal trial, where the treatment that performed the best in terms of leaf deposition results, had poorer deposition results on fruit. However, the fruit deposition results correlated well with the percentage fruit clean of ABS. This agrees with the study done by Schutte *et al*. (2012) showing that fruit is more reliable for fluorometry analyses with a better correlation with copper residues retained. Whilst deposition assessment on leaves has been demonstrated to be very convenient and effective (Fourie *et al.*, 2013; van Zyl *et al.*, 2014), it appears that deposition assessment results on fruit will be more applicable in the context of fruit diseases or pests. This supports the statement by Holownicki *et al*., (2002) that deposition research should be done on biological targets in their natural environment, as was done in this study. In this study and due to fruit showing symptoms of ABS or CBS along with signs of infestation by insects such as mealybug, red scale or FCM.

In terms of deposition uniformity (CV%) the higher spray volume applications generally performed better than the lower spray volumes. Deposition uniformity results on leaves and fruit indicated varying results between trials, with no clear correlation between the two. The horizontal canopy effect showed similar results in all trials. It showed that poorer results were generally achieved at the inner canopy positions than at the outer canopy positions for all treatments. Here the effect of canopy density and spray penetration was clear and had a direct effect on deposition uniformity, similar to results seen by van Zyl *et al.* (2014). From this the importance of canopy management is highlighted. Following the density estimation

determined according to the 5-point scale (van Zyl *et al.*, 2014), in the Citrusdal trial site the density was rated as “3”, which indicates a good balance between leaf volume and canopy aeration, while the other trial sites had a rating of “4” which is described as a dense canopy that is sparsely aerated. This could also be a possible reason that deposition quantity levels were higher at the Citrusdal trial site on the inner canopy position in comparison with the other trial sites with denser and less “spray friendly” canopies.

It is expected that proper canopy management through pruning of spray windows should improve spray deposition by increasing the amount of spray mixture penetrating as well as improving air-movement within the canopy. The effect of pruning will be investigated in Chapter 3. Deposition uniformity was generally poorest at the top canopy positions, and better in the bottom and middle canopy positions. This correlated with the deposition quantity observations.

For deposition quality (ICD%) it was observed that the low volume applications had better ICD% values, which can be attributed to less run-off of spray volume. Similar results were seen between the leaf and fruit data for the Groblersdal 2017 and Marble Hall trial sites; however, the same trend was not observed in the other trial sites. The horizontal canopy effect generally showed that at the inner canopy position, poorer deposition quality was achieved compared to the outer canopy positions, with the only exception being the Groblersdal 2016/2017 trials. The phenomenon of the variation of spray deposition quality can again be attributed to the difference in air flow through the canopy, with the use of different spray machines. As previously discussed, the high turbulence caused by an Ultima spraying machine and the need for hydraulic pressure needed for the Nieuwoudt spraying machine can influence deposition parameters.

In terms of biological efficacy, the same results were seen across all the trials, with the higher spray volumes achieving better results in terms of the percentage clean fruit. Better control was always achieved at the outer canopy, always outperforming the inner canopy positions. For the vertical canopy positions the bottom positions had the best control in all the trials based on percentage clean fruit in terms of red scale and mealybug. These results support the findings by Cunningham and Harden (1998), where biological efficacy was higher with high volume treatments due to higher deposits of spray volume and evenness of spray distribution. Therefore, when comparing the biological efficacy and the spray deposition results, it was seen that deposition uniformity values are directly proportional to the biological efficacy control of red scale and mealybug. This indicates that better control was achieved at better uniformity values, and not better deposition quantity as expected. These results are similar to the findings obtained by Garcerá *et al.* (2011), which showed that greater coverage does not necessarily result in better biological efficacy, as expected. However, it could possibly

be contributed to deposition uniformity and the evenness of spray deposition within a canopy. These findings indicated that deposition uniformity is a very important parameter of spray deposition assessment, specifically in the context of disease and pest control. As stated by Furness *et al.* (1998), the need for spray machines with good coverage and uniformity is essential when applying reduced spray volumes.

At the Citrusdal trial site, ABS symptoms were seen on fruit from the trees with different spray treatments as well as on the unsprayed control trees. These results give valuable insights into the effects of spray volume on ABS control. The results indicated that the higher spray volume treatment performed the best in terms of percentage clean fruit and therefore better control of ABS. Differences between inner and outer canopy positions were observed, where better control was achieved at the outer canopy positions, irrespective of the application volume. This could possibly be the result of weak spray penetration or that the young flush, which are more susceptible to ABS infection, are usually located on the outer part of the tree canopy (Schutte, 2003). Naturally, fewer ABS symptoms will therefore be seen at inner canopy positions and the better spray deposition quantity and uniformity on the outer canopy position will have a greater effect on ABS control.

The spray deposition benchmarks developed by van Zyl *et al.* (2013) was compared with deposition quantity and ABS control achieved in our study. At the Citrusdal trial site, all the spray volume treatments that had a mean FPC% above 4.14%, achieved 75% or more control of ABS, except for the Nieuwoudt 4000 L/ha treatment that achieved a mean percentage clean fruit of 74.07% with a mean deposition quantity of 2.88 FPC%. These biological efficacy results confirm the findings by van Zyl *et al.* (2013) and highlights the usefulness of such deposition parameter benchmarks for other diseases and pests. The higher the deposition quantity achieved on the leaves and fruit, the better the control of disease can be expected. At the Patensie trial site deposition quantity values on leaves and fruit were all below the ABS benchmark, but little to no ABS symptoms were observed. It is therefore uncertain whether lower levels of ABS control would have been the result if the disease pressure was higher.

This study highlights the importance of the evaluation of reduced spray deposition parameters in combination with control of pests and disease in a commercial orchard. Spray deposition analyses of only the fruit would be sufficient for future research, as it is more time efficient and an accurate representation of the spray deposition parameters. Furthermore, this study supports the importance of penetration of spray mixtures into the canopy to achieve adequate control of pests and diseases. Results from the various trials also indicated that higher spray volumes result in better control of pests and diseases due to better deposition uniformity values. Reduced spray volumes achieved poorer levels of control in spite of better deposition quantity values, but with poorer uniformity values. Reduced spray volumes remain

a beneficial option to eliminate high volumes of water and chemicals lost due to run-off, but canopy management is paramount to ensure adequate spray penetration and deposition uniformity.

Spray deposition parameters with high and reduced volume applications in citrus orchards can be improved by utilizing different techniques, such as the use of adjuvants that reduce the surface tension and improve wetting, spreading and dispersing of the spray mixture (van Zyl *et al.*, 2014). Even though high spray volumes achieved better control, the use of reduced spray volumes can be as effective if the correct adjustments to the equipment used for the specific application are made to improve spray uniformity. Spray machine calibration factors such as the alteration of hydraulic pressure by using different nozzles and the adjustment of the tractor driving speed is also critical for adequate penetration and distribution when applying reduced spray volumes on dense citrus canopies. Nozzles sizes can also affect the droplet size and therefore influence the deposition. Canopy management such as pruning is important to help to reduce the effect of canopy density, by creating spray windows in the tree canopy that allow for effective spray penetration, therefore improving spray deposition parameters. The need to investigate the effectiveness and validation of reduced spray volumes on canopies that are more ‘spray-friendly’, will be addressed in the next chapter.

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TABLES AND FIGURES

Table 1. Spray programme for Groblersdal for the 2016/2017 season indicating the active ingredient of the product sprayed, the target organism or function of the product, the dilute concentration (g or ml/100L), 2×, 4× (g or ml/100L) as well as the spray dates for volumes sprayed at 750 L/ha (Martignani), 4900 L/ha and 8900 L/ha (Ultima).

Spray	Spray date	Active ingredient	g/L	Target organism/function	1× (g or ml/100L) Ultima @8900 L/ha	2× (g or ml/100L) Ultima @4900 L/ha	4× (g or ml/100L) Martignani @ 750 L/ha
1	Middle June	E8 Dodecen	73	False codling moth	27	54	108
2	Begin July	*Cryptophlebia leucotreta* granulovirus (CrleGV)	330	False codling moth	250	500	1000
3	Begin October	Mancozeb	1000	Citrus black spot	750	1500	3000
		Polyether-polymethylsiloxane-copolymer	4	Wetting agent	30	60	120
4	Middle October	Mancozeb	1000	Citrus black spot	750	1500	3000
		Polyether-polymethylsiloxane-copolymer	4	Wetting agent	30	60	120
5	End October	Buprofezin	200	Mealybugs	1500	3000	6000
		Paraffinic complex	1333	Wetting agent	10	20	40
		Cryptophlebia leucotreta granulovirus (CrleGV)	330	False codling moth	250	500	1000
		Mancozeb	1000	Citrus Black spot	750	1500	3000
		Trifloxystrobin	930	Citrus Black spot	70	140	280
		Spirotetramat	131	Red scale	1000	2000	4000
6	End November	Sulfoxaflor	960	Red scale	72	144	288

Spray	Spray date	Active ingredient	g/L	Target organism/function	1× (g or ml/100L) Ultima @8900 L/ha	2× (g or ml/100L) Ultima @4900 L/ha	4× (g or ml/100L) Martignani @ 750 L/ha
		Cryptophlebia leucotreta granulovirus (CrleGV)	330	False codling moth	250	500	1000
		Mancozeb	1000	Citrus Black spot	750	1500	3000
		Trifloxystrobin	930	Citrus Black spot	70	140	280
7		Spirotetramat	131	Red scale	1000	2000	4000
8	Begin December	E8 Dodecen	73	False codling moth	27	54	108
		Ammonium sulphate	1000	Wetting agent	1.5	3.0	4.5
9	Middle December	E8 Dodecen	73	False codling moth	27	54	108
10	End December	E8 Dodecen	73	False codling moth	27	54	108
11	Middle January	E8 Dodecen	73	False codling moth	27	54	108
12	End January	E8 Dodecen	73	False codling moth	27	54	108
13	Begin February	E8 Dodecen	73	False codling moth	27	54	108

Table 2. Spray programme for Groblersdal for the 2017/2018 season indicating the active ingredient of the product sprayed, the target organism or function of the product, the dilute concentration (g or ml/100L), 2×, 4× (g or ml/100L) as well as the spray dates for volumes sprayed at 750 L/ha (Martignani), 4900 L/ha and 8900 L/ha (Ultima).

Spray	Spray date	Active ingredient	g/L	Target organism/function	1× (g or ml/100L) Ultima @8900 L/ha	2× (g or ml/100L) Ultima @4900 L/ha	4× (g or ml/100L) Martignani @ 750 L/ha
1	Middle June	E8 Dodecen	73	False codling moth	27	54	108
2	Begin July	*Cryptophlebia leucotreta* granulovirus (CrleGV)	330	False codling moth	250	500	1000
3	Begin October	Mancozeb	1000	Citrus black spot	750	1500	3000
		Polyether-polymethylsiloxane-copolymer	4	Wetting agent	30	60	120
4	Middle October	Mancozeb	1000	Citrus black spot	750	1500	3000
		Polyether-polymethylsiloxane-copolymer	4	Wetting agent	30	60	120
5	End October	Buprofezin	200	Mealybugs	1500	3000	6000
		Paraffinic complex	1333	Wetting agent	10	20	40
		Cryptophlebia leucotreta granulovirus (CrleGV)	330	False codling moth	250	500	1000

Spray	Spray date	Active ingredient	g/L	Target organism/function	1× (g or ml/100L) Ultima @8900 L/ha	2× (g or ml/100L) Ultima @4900 L/ha	4× (g or ml/100L) Martignani @ 750 L/ha
		Mancozeb	1000	Citrus Black spot	750	1500	3000
		Trifloxystrobin	930	Citrus Black spot	70	140	280
		Spirotetramat	131	Red scale	1000	2000	4000
6	End November	Sulfoxaflor	960	Red scale	72	144	288
		Cryptophlebia leucotreta granulovirus (CrleGV)	330	False codling moth	250	500	1000
		Mancozeb	1000	Citrus Black spot	750	1500	3000
		Trifloxystrobin	930	Citrus Black spot	70	140	280
7		Spirotetramat	131	Red scale	1000	2000	4000
8	Begin December	E8 Dodecen	73	False codling moth	27	54	108
		Ammonium sulphate	1000	Wetting agent	1.5	3.0	4.5
9	Middle December	E8 Dodecen	73	False codling moth	27	54	108
10	End December	E8 Dodecen	73	False codling moth	27	54	108
11	Middle January	E8 Dodecen	73	False codling moth	27	54	108
12	End January	E8 Dodecen	73	False codling moth	27	54	108
13	Begin February	E8 Dodecen	73	False codling moth	27	54	108

Table 3. Spray programme for Marble Hall for the 2016/2017 season indicating the active ingredient of the product sprayed, the target organism or function of the product, the dilute concentration (g or ml/100L), 3× (g or ml/100L) as well as the spray dates for volumes sprayed at 2000 L/ha (Cima) and 6000 L/ha (Arbus).

Spray	Spray date	Active ingredient	g/L	Target organism/function	1× (g or ml/100L) Arbus @6000 L/ha	3× (g or ml/100L) Cima @2000 L/ha
1	Mid Oct 2016	Mancozeb	435	Citrus black spot	200	600
		Zinc oxide	4.7			
		Borax	10	Improved deposition	50	50
		Orange oil	50			
		Buffer	390	pH correction	100	100
		Carbendazim		Citrus black spot	55	165
		Chlorfenapyr	240	Thrips	36	108
		Buprofezin	500	Mealybug	30	90
2	Mid Nov 2016	Mancozeb	750	Citrus black spot	200	600
		Borax	10	Improved deposition	50	50
		Orange oil	50			
		Acetamiprid		Thrips/Mealybug	150	450
		Buffer	390	pH correction	50	150
3	Begin Dec 2016	Mancozeb	750	Citrus black spot	150	450
		Borax	10	Improved deposition	50	50
		Orange oil	50			
		Buprofezin	500	Mealybug	30	105

Spray	Spray date	Active ingredient	g/L	Target organism/function	1× (g or ml/100L) Arbus @6000 L/ha	3× (g or ml/100L) Cima @2000 L/ha
		Buffer	390	pH correction	50	50
		Pyroclostrobin	250	Citrus black spot	10	30
		Abamectin	84	Thrips	6	18
		Pyraclostrobin	100	Citrus red scale	30	90
4	Mid Jan 2017	Mancozeb	150	Citrus black spot	150	450
		Borax	50	Improved deposition	50	50
		Orange oil				
		Pyraclostrobin	10	Citrus black spot	10	30
		Abamectin	6	Thrips	6	18
		Buffer	50	pH correction	50	50

Table 4. Spray programme for Citrusdal for the 2017/2018 season indicating the active ingredient of the product sprayed, the target organism or function of the product, the dilute concentration (g or ml/100L), 2×, 3× (g or ml/100L) as well as the spray dates for volumes sprayed at 8800 L/ha and 4000 L/ha (Nieuwoudt) and 3000 L/ha (Cima).

Spray	Spray date	Active ingredient	g/L	Target organism/function	1× (g or ml/100L) Nieuwoudt @ 8800 L/ha	2× (g or ml/100L) Nieuwoudt @ 4000 L/ha	3× (g or ml/100L) Cima @ 3000 L/ha
1	Sept	Copper Hydroxide	180	Alternaria spot brown	18	36	54
2	Oct	Copper Hydroxide	180	Alternaria spot brown	18	36	54
3	Nov	Copper Hydroxide	180	Alternaria spot brown	18	36	54
		Mineral oil	840	Adjuvent	125	250	375
		Azoxystrobin	250	Alternaria spot brown	250	500	750
4	Des	Copper Hydroxide	180	Alternaria spot brown	18	36	54
5	Jan	Copper Hydroxide	180	Alternaria spot brown			
		Azoxystrobin	250	Alternaria spot brown	250	500	750
		Mineral oil	840	Adjuvent			
6	Feb	Copper Hydroxide	180	Alternaria spot brown	18	36	54
7	Mar	Copper Hydroxide	180	Alternaria spot brown	18	36	54

Table 5. Spray programme for Patensie for the 2017/2018 season indicating the active ingredient of the product sprayed, the target organism or function of the product, the dilute concentration (g or ml/100L), 4× (g or ml/100L) as well as the spray dates sprayed at 1000 L/ha (Martignani) and 6700 L/ha (Ultima).

Spray	Spray date	Active ingredient	g/L	Target organism/function	1× (g or ml/100L) Ultima @6700 L/ha	4× (g or ml/100L) Martignani @1000 L/ha (1.4 km/h)	4× (g or ml/100L) Martignani @1000 L/ha (3.4 km/h)
1	Mid Oct 2017	Mancozeb	800	Citrus black spot	200	800	800
		Abamectin	18	Thrips	20	80	80
		Mineral oil	840	Citrus black spot	100	400	400
2	Begin Nov 2017	Mancozeb	800	Citrus black spot	150	600	600
		Buprofezin	500	Mealybug	30	120	120
		Pyraclostrobin	250	Citrus black spot	10	40	40
		Spirotetramat	240	Red scale	10	40	40
		Abamectin	18	Thrips	20	80	80
		Mineral oil	840	Citrus black spot	250	1000	1000
3	Mid Dec 2017	Mancozeb	800	Citrus black spot	150	600	600
		Buprofezin	500	Mealybug	30	120	120
		Trifloxystrobin	500	Citrus black spot	10	40	40
		T.leucotreta granulovirus	2 x 1010 occlusion bodies/mL	False codling moth	3.3	13.2	13.2
		Spirotetramat	240	Red scale	10	40	40
		Abamectin	18	Thrips	20	80	80
		Mineral oil	840	Citrus black spot	250	1000	1000

Spray	Spray date	Active ingredient	g/L	Target organism/function	1× (g or ml/100L) Ultima @6700 L/ha	4× (g or ml/100L) Martignani @1000 L/ha (1.4 km/h)	4× (g or ml/100L) Martignani @1000 L/ha (3.4 km/h)
4	End Jan 2018	Mancozeb	800	Citrus black spot	200	800	800
		T. leucotreta granulovirus	2 x 1010 occlusion bodies/mL	False codling moth	3.3	13.2	13.2
		Abamectin	18	Thrips	20	80	80
		Mineral oil	840	Citrus black spot	250	1000	1000
5	Mid Feb 2018	Mancozeb	800	Citrus black spot	200	800	800
		Polyether-polymethylsiloxane-copolymer	1000	Wetter	3	12	12

Table 6. Analyses of variance (ANOVA) of mean percentage clean fruit in terms of red scale and mealybug ratings at different vertical and horizontal canopy positions on trees sprayed at 750 L/ha using a Martignani spay machine and 4900 and 8900 L/ha using an Ultima spray machine at the Groblersdal trial for the 2016/2017 season.

Source	% Clean fruit (Red scale)			% Clean fruit (Mealybug)		
	DF*	SS**	SL***	DF	SS	SL
Treatment	2	27477.85	<0.0001	2	29854.78	<0.0001
Treatment (Rep)	44	17236.50	0.0470	44	37146.80	0.0006
Horizontal canopy position	2	1544.52	0.0606	2	7842.79	<0.0001
Treatment × horizontal canopy position	4	582.02	0.7101	4	2718.94	0.1710
Vertical canopy position	1	772.26	0.0934	1	10450.16	<0.0001
Treatment × vertical canopy position	2	208.83	0.6816	2	1752.58	0.1268
Horizontal × vertical canopy position	2	1048.07	0.1480	2	4458.23	0.0056
Treatment × horizontal × vertical canopy position	4	2784.49	0.0395	4	3402.10	0.0922
Error	220	58819.06		280	92477.04	
Corrected total	281	111473.60		281	190103.43	

*Degrees of Freedom, **Sum of squares, ***Significance level

Table 7. Mean percentage fruit clean from mealybug (MB) and red scale (RS) on leaves and fruit on trees at the Groblersdal site for 2016/2017 season that were sprayed at 750 L/ha using a Martignani spray machine and 4900 and 8900 L/ha using an Ultima spray machine.

Treatment	% clean fruit (Red scale)	% clean fruit (Mealybug)
Martignani @750 L/ha	75.19 c[1]	65.93 b
Ultima 1 @4900 L/ha	88.72 b	68.58 b
Ultima 2 @8900 L/ha	99.48 a	88.89 a
LSD	5.821	8.546

[1] Means for each pest followed by the same letter are not significantly different at $P = 0.05$

Table 8. Analyses of variance (ANOVA) of deposition quantity (FPC%), uniformity (CV%) and quality (ICD%) data measured on leaves and fruit from trees at the Groblersdal trial in the 2016/2017 season sprayed with 750 L/ha using Martignani spraying machine and 4900 and 8900 L/ha using an Ultima spraying machine.

		Deposition quantity (FPC%)			Deposition uniformity (CV%)			Deposition quality (ICD%)		
	Source	**DF***	**SS****	**SL*****	**DF**	**SS**	**SL**	**DF**	**SS**	**SL**
Leaves	**Treatment**	2	376.91	0.0183	2	7 814.53	<0.0001	2	1342.90	0.0003
	Treatment (Rep)	6	134.73	0.0299	6	2 216.26	0.1638	6	492.44	0.2876
	Horizontal canopy position	1	256.89	<0.0001	1	1 646.17	0.0184	1	5.84	0.7580
	Treatment × horizontal canopy position	2	347.57	<0.0001	2	797.16	0.2382	2	251.32	0.1424
	Vertical canopy position	2	114.04	0.0001	2	1 113.83	0.1397	2	304.26	0.0973
	Treatment × vertical canopy position	4	41.35	0.0867	4	1 287.32	0.3249	4	547.44	0.0853
	Horizontal × vertical canopy position	2	5.14	0.5767	2	516.31	0.3888	2	440.82	0.0381
	Treatment × horizontal × vertical canopy position	4	3.51	0.9412	4	1 483.96	0.2574	4	201.06	0.5100
	Error	36	302.21		36	8093.39		36	2228.04	
	Corrected total	107	2140.69		107	4218.57		107	12225.10	
Fruit	**Treatment**	2	934.98	0.0222	2	3918.87	0.0484	2.00	3391.35	0.0002
	Treatment (Rep)	6	365.45	0.0146	6	2245.85	0.6397	6.00	219.42	0.4514
	Horizontal canopy position	1	70.82	0.0949	1	2028.72	0.0417	1.00	71.04	0.3068
	Treatment × horizontal canopy position	2	61.59	0.2889	2	987.95	0.3446	2.00	226.33	0.1961
	Vertical canopy position	2	11.05	0.7939	2	3719.84	0.0258	2.00	573.89	0.0219
	Treatment × vertical canopy position	4	62.66	0.6251	4	418.69	0.9168	4.00	1951.62	0.0003
	Horizontal × vertical canopy position	2	16.69	0.7068	2	1027.56	0.3307	2.00	66.26	0.6089
	Treatment × horizontal × vertical canopy position	4	182.98	0.1329	4	796.43	0.7747	4.00	247.34	0.4538
	Error	35	681.49		35	18314.05		35	1301.98	
	Corrected total	105	3730.66		105	69069.98		105	18172.73	

*Degrees of Freedom, **Sum of squares, ***Significance level

Table 9. Mean deposition quantity (FPC%), uniformity (CV%) and quality (ICD%) realised at the Groblersdal trial for the 2016/2017 season on leaves for different treatments at the horizontal canopy position (inner and outer canopy) and sprayed at 750 L/ha using a Martignani spray machine and 4900 and 8900 L/ha using an Ultima spraying machine.

		FPC% (Deposition quantity)	CV% (Deposition uniformity)	ICD% (Deposition quality)
Leaves	**Martignani @750 L/ha**	8.81 a[1]	62.66 a	43.55 b
	Ultima 1 @4900 L/ha	4.29 b	48.33 b	43.45 b
	Ultima 2 @8900 L/ha	5.94 b	42.39 b	50.98 a
	LSD	2.733	1.338	5.225
Fruit	**Martignani @750 L/ha**	13.15 a	54.22 ab	60.77 b
	Ultima 1 @4900 L/ha	5.95 b	62.47 a	70.83 a
	Ultima 2 @8900 L/ha	9.15 a	47.55 b	74.04 a
	LSD	4.545	11.267	3.522

[1] Means in each column followed by the same letter are not significantly different at $P = 0.05$

Table 10. Analyses of variance (ANOVA) of mean percentage clean fruit in terms of red scale and mealybug ratings at different vertical and horizontal canopy positions on trees sprayed at 750 L/ha using a Martignani spay machine and 4900 and 8900 L/ha using an Ultima spray machine at the Groblersdal trial for the 2017/2018 season.

Source	% Clean fruit (Red scale)			% Clean fruit (Mealybug)		
	DF*	SS**	SL***	DF	SS	SL
Treatment	2	7034.56	0.0017	2	45565.70	<0.0001
Treatment (Rep)	44	20892.94	0.0009	44	85143.52	<0.0001
Horizontal canopy position	1	2175.93	0.0031	1	42207.45	<0.0001
Treatment × horizontal canopy position	2	779.13	0.2030	2	621.26	0.5120
Vertical canopy position	2	25711.19	<0.0001	2	1513.00	0.1973
Treatment × vertical canopy position	4	4274.92	0.0019	4	10912.92	0.0002
Horizontal × vertical canopy position	2	782.11	0.2018	2	1229.31	0.2670
Treatment × horizontal × vertical canopy position	4	828.23	0.4927	4	6321.61	0.0098
Error	220	53365.16		220	101777.78	
Corrected total	281	115844.17		281	295292.55	

*Degrees of Freedom, **Sum of squares, ***Significance level

Table 11. Mean percentage fruit clean from mealybug (MB) and red scale (RS) on leaves and fruit at three vertical canopy positions (Top, middle and bottom) as well as two horizontal canopy positions (Inner and outer canopy) on trees at the Groblersdal site for 2017/2018 season that were sprayed at 750 L/ha using a Martignani spray machine and 4900 and 8900 L/ha using an Ultima spray machine.

	RS (Red scale)			MB (Mealybug)		
	Top	**Middle**	**Bottom**	**Top**	**Middle**	**Bottom**
Martignani @750 L/ha	72.22 d[1]	89.44 abc	96.67 a	81.67 a	67.22 b	62.78 b
Ultima 1 @4900 L/ha	58.33 e	87.50 bc	91.15 ab	47.92 de	38.02 ef	31.77 f
Ultima 2 @8900 L/ha	82.29 cd	93.23 ab	96.35 a	51.56 cd	50.52 d	61.46 bc
LSD		7.758			10.714	

[1] Means for each pest followed by the same letter are not significantly different at $P = 0.05$

Table 12. Analyses of variance (ANOVA) of deposition quantity (FPC%), uniformity (CV%) and quality (ICD%) data measured on leaves and fruit from the Groblersdal trial for the 2017/2018 season sprayed at 750 L/ha using Martignani spray machine and 4900 and 8900 L/ha using an Ultima spray machines.

		Deposition quantity (FPC%)			Deposition uniformity (CV%)			Deposition quality (ICD%)		
	Source	**DF***	**SS****	**SL*****	**DF**	**SS**	**SL**	**DF**	**SS**	**SL**
Leaves	**Treatment**	2	11.52	0.2650	2	10716.36	0.2041	2	1134.30	0.0595
	Treatment (Rep)	6	20.68	0.0432	6	15344.60	0.0062	6	726.09	0.2458
	Horizontal canopy position	1	23.43	0.0003	1	4821.18	0.0119	1	0.13	0.9695
	Treatment × horizontal canopy position	2	0.15	0.9471	2	7234.25	0.0101	2	22.21	0.8797
	Vertical canopy position	2	19.18	0.0032	2	3281.89	0.1043	2	430.85	0.0992
	Treatment × vertical canopy position	4	6.63	0.3273	4	2064.20	0.5550	4	109.64	0.8638
	Horizontal × vertical canopy position	2	0.64	0.7943	2	625.76	0.6325	2	179.58	0.3654
	Treatment × horizontal × vertical canopy position	4	2.47	0.7710	4	1320.25	0.7425	4	146.35	0.7898
	Error	30	41.13		30	20117.21		30	2586.94	
	Corrected total	53	125.83		53	65585.65		53	5336.08	
Fruit	**Treatment**	2	1.35	0.6586	2	2431.60	0.162	2	102.11	0.3833
	Treatment (Rep)	6	9.03	0.2697	6	2914.04	0.4593	6	271.11	0.2690
	Horizontal canopy position	1	20.74	0.0002	1	2058.45	0.0511	1	83.55	0.1254

	Deposition quantity (FPC%)			Deposition uniformity (CV%)			Deposition quality (ICD%)		
Source	**DF***	**SS****	**SL*****	**DF**	**SS**	**SL**	**DF**	**SS**	**SL**
Treatment × horizontal canopy position	2	2.16	0.3926	2	57.39	0.9442	2	62.56	0.4055
Vertical canopy position	2	5.68	0.0964	2	3432.34	0.0450	2	152.98	0.1202
Treatment × vertical canopy position	4	8.46	0.1387	4	3219.87	0.1962	4	107.69	0.5343
Horizontal × vertical canopy position	2	0.06	0.9715	2	475.87	0.6250	2	23.97	0.7030
Treatment × horizontal × vertical canopy position	4	2.87	0.6381	4	769.39	0.8169	4	99.41	0.5727
Error	30	33.65		30	14951.01		30	1008.63	
Corrected total	53	84.02		53	30309.96		53	1912.00	

*Degrees of Freedom, **Sum of squares, ***Significance level

Table 13. Mean deposition quantity (FPC%), uniformity (CV%) and quality (ICD%) realised at the Groblersdal trial for the 2017/2018 season on leaves and fruit for different treatments and sprayed at 750 L/ha using Martignani spray machine and 4900 and 8900 L/ha using an Ultima spraying machine.

		FPC% (Deposition quantity)	CV% (Deposition uniformity)	ICD% (Deposition quality)
Leaves	**Martignani @750 L/ha**	2.07 a[1]	111.38 a	48.77 b
	Ultima 1 @4900 L/ha	3.19 a	77.45 a	58.79 a
	Ultima 2 @8900 L/ha	2.55 a	89.00 a	58.16 a
	LSD	1.514	41.248	8.973
Fruit	**Martignani @750 L/ha**	2.75 a	82.40 a	86.78 a
	Ultima 1 @4900 L/ha	3.11 a	69.38 a	84.05 a
	Ultima 2 @8900 L/ha	2.90 a	67.21 a	87.12 a
	LSD	1.001	17.975	5.480

[1] Means in each column followed by the same letter are not significantly different at $P = 0.05$

Table 14. Analyses of variance (ANOVA) of mean percentage clean fruit in terms of red scale and mealybug ratings at different vertical and horizontal canopy positions on trees sprayed at 2000 L/ha using a CIMA spay machine and 6000 L/ha using an Arbus spray machine at the Marble Hall trial for the 2016/2017 season.

	% Clean fruit (Red scale)			% Clean fruit (Mealybug)		
Source	**DF***	**SS****	**SL*****	**DF**	**SS**	**SL**
Treatment	1	3333.33	<0.0001	1	7500.00	0.0040
Treatment (Rep)	30	2962.96	0.9756	30	23188.66	0.0036
Horizontal canopy position	1	144.68	0.3760	1	15052.08	<0.0001
Treatment × horizontal canopy position	1	5.79	0.8593	1	92.59	0.6255
Vertical canopy position	2	1409.14	0.0236	2	4490.74	0.0037
Treatment × vertical canopy position	2	269.10	0.4820	2	3368.06	0.0146
Horizontal × vertical canopy position	2	610.53	0.1929	2	14618.06	<0.0001
Treatment × horizontal × vertical canopy position	2	37.62	0.9026	2	1817.13	0.0991
Error	150	27523.15		150	58061.34	
Corrected total	191	36296.30		191	128188.66	

*Degrees of Freedom, **Sum of squares, ***Significance level

Table 15. Mean percentage fruit clean from mealybug (MB) and red scale (RS) on leaves and fruit at three vertical canopy positions (Top, middle and bottom) as well as two horizontal canopy positions (Inner and outer canopy) on trees at the Marble hall site for 2016/2017 season that were sprayed at 2000 L/ha using a Cima and 6000 L/ha using an Arbus spraying machine.

	% clean fruit (Red scale)	% clean fruit (Mealybug)
Cima @ 2000 L/ha	88.89 b[1]	69.62 b
Arbus @ 6000 L/ha	97.22 a	82.12 a
LSD	2.93	8.195

[1] Means in each column followed by the same letter are not significantly different at $P = 0.05$

Table 16. Analyses of variance (ANOVA) of deposition quantity (FPC%), uniformity (CV%) and quality (ICD%) data measured on leaves and fruit from trees at the Marble hall trial in the 2016/2017 season sprayed at 2000 L/ha using a Cima and 6000 L/ha using an Arbus spraying machine.

		Deposition quantity (FPC%)			Deposition uniformity (CV%)			Deposition quality (ICD%)		
	Source	**DF***	**SS****	**SL*****	**DF**	**SS**	**SL**	**DF**	**SS**	**SL**
Leaves	**Treatment**	1	21.68	0.0107	1	3810.18	0.0097	1	2815.68	0.0040
	Treatment (Rep)	1	0.14	0.8262	4	705.21	0.8786	4	320.12	0.2166
	Horizontal canopy position	2	38.70	0.0048	1	21463.70	<0.0001	1	72.19	0.3483
	Treatment × horizontal canopy position	2	36.05	0.0063	1	308.45	0.3110	1	34.79	0.5126
	Vertical canopy position	2	11.69	0.1443	2	530.79	0.4111	2	522.81	0.0560
	Treatment × vertical canopy position	2	1.33	0.7866	2	1150.17	0.1596	2	741.55	0.0207
	Horizontal × vertical canopy position	2	11.69	0.0238	2	160.14	0.7584	2	180.78	0.3352
	Treatment × horizontal × vertical canopy position	2	1.33	0.6134	2	121.25	0.8105	2	72.81	0.6347
	Error	24	31.99		24	14364.27		24	1229.85	
	Corrected total	71	413.01		71	56002.67		71	8334.81	
Fruit	**Treatment**	1	374.61	0.0006	1	780.71	0.2929	1	442.02	0.1287
	Treatment (Rep)	4	14.88	0.7833	4	2133.17	0.0496	4	484.34	0.0554
	Horizontal canopy position	1	186.90	0.0031	1	7613.31	0.0086	1	280.25	0.1865
	Treatment × horizontal canopy position	1	43.65	0.1192	1	115.57	0.7235	1	38.71	0.6167
	Vertical canopy position	2	217.99	0.0062	2	406.82	0.7993	2	957.74	0.0624
	Treatment × vertical canopy position	2	16.08	0.6209	2	834.01	0.6351	2	149.15	0.6151

Horizontal × vertical canopy position	2	139.02	0.0296	2	1771.62	0.3903	2	136.69	0.6400
Treatment × horizontal × vertical canopy position	2	15.20	0.6369	2	960.36	0.5939	2	67.58	0.8000
Error	24	206.10		24	4600.14		24	1080.77	
Corrected total	71	3153.24		71	40975.42		71	17465.98	

*Degrees of Freedom, **Sum of squares, ***Significance level

Table 17. Mean deposition quantity (FPC%), uniformity (CV%) and quality (ICD%) realised at the Marble hall trial for the 2016/2017 season on leaves and fruit for different treatments and sprayed at 2000 L/ha using a Cima and 6000 L/ha using an Arbus spraying machine.

		FPC% (Deposition quantity)	CV% (Deposition uniformity)	ICD% (Deposition quality)
Leaves	**Cima @ 2000 L/ha**	3.53 a[1]	76.76 a	59.53 b
	Arbus @ 6000 L/ha	1.57 b	62.21 b	72.04 a
	LSD	2.776	3.689	5.854
Fruit	**Cima @ 2000 L/ha**	11.11 a	50.90 a	59.84 a
	Arbus @ 6000 L/ha	6.54 b	57.49 a	64.79 a
	LSD	1.262	15.112	7.201

[1] Means in each column followed by the same letter are not significantly different at $P = 0.05$

Table 18. Analyses of variance (ANOVA) of mean percentage clean fruit in terms of Alternaria Brown spot ratings at different vertical and horizontal canopy positions on trees sprayed 3000, 4000 and 8800 L/ha using Martignani and Ultima spray machines as well as a control treatment with nothing sprayed.

	% Clean fruit (Alternaria brown spot)		
Source	**DF***	**SS****	**SL*****
Treatment	3	6990.74	0.0019
Treatment (Rep)	8	1419.75	0.6118
Horizontal canopy position	1	1867.28	0.0062
Treatment × horizontal canopy position	3	632.72	0.4292
Vertical canopy position	2	609.57	0.2678
Treatment × vertical canopy position	6	1550.93	0.3493
Horizontal × vertical canopy position	2	378.09	0.4372
Treatment × horizontal × vertical canopy position	6	918.21	0.6636
Error	40	8950.62	
Corrected total	71	23317.90	

*Degrees of Freedom, **Sum of squares, ***Significance level

Table 19. Mean percentage fruit clean from Alternaria brown spot (ABS) on leaves and fruit at the Citrusdal trial for the 2017/2018 season sprayed at 3000 L/ha using a Cima spraying machine and 4000 and 8000 L/ha using a Nieuwoudt 1 spraying machine.

	% clean fruit (Alternaria brown spot)
Cima @ 3000 L/ha	75.93 b[1]
Nieuwoudt 1 @ 4000 L/ha	74.07 b
Nieuwoudt 2 @ 8000 L/ha	89.82 a
Control	62.04 c
LSD	10.240

[1] Means in each column followed by the same letter are not significantly different at $P = 0.05$

Table 720. Analyses of variance (ANOVA) of deposition quantity (FPC%), uniformity (CV%) and quality (ICD%) data measured on leaves and fruit from trees at the Citrusdal trial in the 2017/2018 season sprayed at 3000 L/ha with a Cima spraying machine and 4000 L/ha and 8000 L/ha sprayed with a Nieuwoudt spraying machine.

		Deposition quantity (FPC%)			Deposition uniformity (CV%)			Deposition quality (ICD%)		
	Source	**DF***	**SS****	**SL*****	**DF**	**SS**	**SL**	**DF**	**SS**	**SL**
Leaves	**Treatment**	2	218.48	0.0963	2	3897.01	0.0056	2	8447.77	0.0035
	Treatment (Rep)	6	184.88	0.0002	6	841.25	0.7937	6	1510.21	0.0010
	Horizontal canopy position	1	93.86	0.0001	1	10992.24	<0.0001	1	1230.62	<0.0001
	Treatment × horizontal canopy position	2	3.75	0.6761	2	53.37	0.9073	2	50.13	0.6067
	Vertical canopy position	2	28.31	0.0652	2	4807.72	0.0010	2	36.87	0.6912
	Treatment × vertical canopy position	4	20.83	0.3738	4	1240.10	0.3593	4	142.43	0.5837
	Horizontal × vertical canopy position	2	5.64	0.5568	2	492.64	0.4168	2	9.26	0.9106
	Treatment × horizontal × vertical canopy position	4	36.49	0.1311	4	744.25	0.6108	4	272.88	0.2633
	Error	30	141.77		30	8200.23			30	49.32
	Corrected total	53	734.02		53	31268.82			53	
Fruit	**Treatment**	2	208.36	0.0406	2	11616.94	0.0302	2	1458.18	0.0501
	Treatment (Rep)	6	109.13	0.0818	6	5253.06	0.0939	6	851.87	0.0055
	Horizontal canopy position	1	18.63	0.1521	1	2688.99	0.0185	1	278.27	0.0098
	Treatment × horizontal canopy position	2	3.69	0.8086	2	65.69	0.9272	2	27.83	0.6869
	Vertical canopy position	2	28.11	0.2131	2	183.00	0.8109	2	37.08	0.6076
	Treatment × vertical canopy position	4	17.52	0.7305	4	2398.15	0.2633	4	187.71	0.2988
	Horizontal × vertical canopy position	2	2.54	0.8638	2	931.13	0.3544	2	54.22	0.4852
	Treatment × horizontal × vertical canopy position	4	23.64	0.6079	4	53.86	0.9980	4	48.66	0.8539
	Error	30	258.88		30	13002.70		30	1097.64	
	Corrected total	53	670.51		53	36193.51		53	4041.45	

*Degrees of Freedom, **Sum of squares, ***Significance level

Table 21. Mean deposition quantity (FPC%), uniformity (CV%) and quality (ICD%) realised at the Citrusdal trial for the 2017/2018 season on leaves and fruit sprayed at 3000 L/ha with a Cima spraying machine and 4000 L/ha and 8000 L/ha sprayed with a Nieuwoudt spraying machine.

		FPC% (Deposition quantity)	CV% (Deposition uniformity)	ICD% (Deposition quality)
Leaves	**Cima @ 3000 L/ha**	7.57 a[1]	70.57 a	57.61 b
	Nieuwoudt @ 4000 L/ha	2.88 b	78.99 a	87.23 a
	Nieuwoudt @ 8000 L/ha	6.53 ab	58.30 b	79.20 a
	LSD	4.528	9.660	12.940
Fruit	**Cima @ 3000 L/ha**	4.96 b	80.15 a	87.24 a
	Nieuwoudt @ 4000 L/ha	6.02 b	65.75 ab	77.27 ab
	Nieuwoudt @ 8000 L/ha	9.55 a	44.44 b	71.53 b
	LSD	3.479	24.134	9.719

[1] Means in each column followed by the same letter are not significantly different at $P = 0.05$

Table 22. Analyses of variance (ANOVA) of mean percentage clean fruit in terms of red scale and mealybug ratings at different vertical and horizontal canopy positions on trees sprayed at 1000 (at two different tractor speeds: 1.4 km/h and 3.4 km/h) and 6700 L/ha using Martignani and Ultima spray machines at the Patensie trial for the 2017/2018 season.

Source	% Clean fruit (Alternaria brown spot)			% Clean fruit (Mealybug)		
	DF*	SS**	SL***	DF	SS	SL
Treatment	2	54.01	0.0702	2	337.58	0.0010
Treatment (Rep)	45	431.13	0.5070	45	943.29	0.4540
Horizontal canopy position	1	47.26	0.0286	1	96.45	0.0318
Treatment × horizontal canopy position	2	30.86	0.2072	2	71.37	0.1800
Vertical canopy position	2	13.50	0.5009	2	25.08	0.5458
Treatment × vertical canopy position	4	84.88	0.0722	4	50.15	0.6579
Horizontal × vertical canopy position	2	13.50	0.5009	2	59.80	0.2373
Treatment × horizontal × vertical canopy position	4	73.30	0.1144	4	50.15	0.6579
Error	225	2190.39		225	4646.99	
Corrected total	287	2938.85		287	6280.86	

*Degrees of Freedom, **Sum of squares, ***Significance level

Table 23. Mean percentage fruit clean from Alternaria brown spot (ABS) and mealybug (MB) on leaves and fruit at three vertical canopy positions (Top, middle and bottom) as well as two horizontal canopy positions (Inner and outer canopy) on trees at the Patensie site for 2017/2018 season that were sprayed at 1000 (at two different tractor speeds: 1.4 km/h and 3.4 km/h) and 6700 L/ha using Martignani and Ultima spray machines.

	% clean fruit (Alternaria brown spot)	**% clean fruit (Mealybug)**
Martignani @1000 L/ha (1.4 km/h)	98.78 b[1]	100.00 a
Martignani @1000 L//ha (3.4 km/h)	99.83 a	97.40 b
Ultima @ 6700 L/ha	99.48 ab	99.13 a
LSD	0.100	1.331

[1] Means in each column followed by the same letter are not significantly different at $P = 0.05$

Table 24. Analyses of variance (ANOVA) of deposition quantity (FPC%), uniformity (CV%) and quality (ICD%) data measured on leaves and fruit from trees at the Patensie trial in the 2017/2018 season sprayed at 1000 (at two different tractor speeds: 1.4 km/h and 3.4 km/h) and 6700 L/ha using Martignani and Ultima spray machines.

	Source	Deposition quantity (FPC%)			Deposition uniformity (CV%)			Deposition quality (ICD%)		
		DF*	SS**	SL***	DF	SS	SL	DF	SS	SL
Leaves	Treatment	2	9.75	0.0426	2	2849.11	0.2033	2	2559.44	0.0012
	Treatment (Rep)	6	5.23	0.0310	6	4065.50	0.4445	6	306.89	0.5160
	Horizontal canopy position	1	5.88	0.0002	1	445.60	0.4241	1	182.29	0.0854
	Treatment × horizontal canopy position	2	0.65	0.3739	2	299.11	0.8035	2	158.53	0.2681
	Vertical canopy position	2	0.54	0.4399	2	168.01	0.8840	2	18.45	0.8528
	Treatment × vertical canopy position	4	2.04	0.2005	4	860.72	0.8643	4	112.17	0.7453
	Horizontal × vertical canopy position	2	1.80	0.0754	2	682.29	0.6099	2	291.08	0.0968
	Treatment × horizontal × vertical canopy position	4	1.52	0.3368	4	1093.67	0.8050	4	221.16	0.4438
	Error	30	9.59		30	20358.61		30	1728.22	
	Corrected total	53	37.02		53	30822.63		53	5578.23	
Fruit	Treatment	2	0.13	0.3426	2	3276.36	0.0323	1	3.01	0.3099
	Treatment (Rep)	6	0.30	0.7413	6	1529.94	0.7506	4	8.93	0.4307
	Horizontal canopy position	1	4.05	<0.0001	1	669.02	0.2308	1	13.73	0.0222
	Treatment × horizontal canopy position	2	0.01	0.9175	2	3360.76	0.0350	1	3.01	0.2590
	Vertical canopy position	2	0.26	0.2338	2	124.84	0.8703	2	27.45	0.0083

Source	Deposition quantity (FPC%)			Deposition uniformity (CV%)			Deposition quality (ICD%)		
	DF*	SS**	SL***	DF	SS	SL	DF	SS	SL
Treatment × vertical canopy position	4	0.22	0.6372	4	618.44	0.8448	2	6.02	0.2819
Horizontal × vertical canopy position	2	1.03	0.0059	2	1488.84	0.2062	2	27.45	0.0083
Treatment × horizontal × vertical canopy position	4	0.20	0.6673	4	791.92	0.7767	2	6.02	0.2819
Error	30	2.53		30	13413.78		30	44.63	
Corrected total	53	8.73		53	252573.89		53	140.27	

*Degrees of Freedom, **Sum of squares, ***Significance level

Table 25. Mean deposition quantity (FPC%), uniformity (CV%) and quality (ICD%) at the Patensie trial for the 2017/2018 season, sprayed at 1000 L/ha (at two different tractor speeds: 1.4 km/h and 3.4 km/h) and 6700 L/ha using Martignani and Ultima spray machines.

		FPC% (Deposition quantity)	CV% (Deposition uniformity)	ICD% (Deposition quality)
Leaves	**Martignani @1000 L/ha (1.4 km/h)**	0.90 b[1]	101.64 a	90.35 b
	Martignani @1000 L//ha (3.4 km/h)	1.87 a	91.54 a	77.11 b
	Ultima @ 6700 L/ha	1.07 b	83.90 a	92.78 a
	LSD	0.762	21.231	5.833
Fruit	**Martignani @1000 L/ha (1.4 km/h)**	0.55 a	87.12 a	99.09 a
	Martignani @1000 L//ha (3.4 km/h)	0.67 a	68.97 b	99.67 a
	Ultima @ 6700 L/ha	0.57 a	72.95 b	99.26 a
	LSD	0.181	13.024	1.273

[1] Means in each row followed by the same letter are not significantly different at $P = 0.05$

CHAPTER 3

Effect of pruning on spray deposition and evaluation of a light detection and ranging (LiDAR) system to characterize citrus tree canopy density

ABSTRACT

Inadequate disease and pest control in citrus orchards are often attributed to poor spray application of plant protection products, largely due to the complex shape and high density of a citrus tree. Seasonal pruning of citrus trees has various advantages such as canopy management for improved spray penetration. The characterization of tree canopy density is multifaceted and can be a laborious task. Remote sensing offers a non-destructive, time efficient method to characterise tree canopies. Spray trials were conducted on three commercial farms in the Western Cape to determine the effect of different degrees of pruning on spray deposition quantity, uniformity and quality following sprays with a Cima (Blitz 55 low volume) spraying machine at two different spray application volumes (1500 L/ha as the reduced volume and 3000 L/ha as the standard volume). In an attempt to develop a non-destructive technique to measure canopy volume and density use of LiDAR (Light Detection and Ranging) technology was investigated. Canopy density was measured by manual measurements, individually measuring trees and comparing this to 3D images obtained from scanning trees with the LiDAR. Results indicated that LiDAR successfully observed the changes in tree canopy density after pruning, but the LiDAR parameter developed correlated poorly with manual measurements. At higher spray volumes, pruning had no to little effect on spray deposition parameters. However, when applying lower spray volumes, it was shown that light pruning had a marked effect on spray deposition, improving it markedly in comparison to the results seen for unpruned or heavily pruned trees. It was shown that if lower spray volumes are employed, tree canopy manipulation through pruning must be done to get adequate spray deposition.

INTRODUCTION

The development of measures to minimize the impact of pesticides on the environment and to reduce the risks associated with their application is a very important issue (Garcer *et al*., 2017). It is primarily important that the correct amount of plant protection product (PPP) must be applied on foliar and fruit targets for the control of pests and diseases (Hall, 1991). However, due to increased concerns about environmental pollution, resistance development, and production costs, it is important that recent advances in low volume spray application technology should be practically implemented. In South Africa, citrus trees are sprayed at high volumes (8000 – 16 000 L/ha) to achieve high quality export fruit that are free from important phytosanitary pests and diseases. However, this leads to high input costs in terms of water, plant protection products, labour, time and equipment (Fourie *et al*., 2013). These applications of agrochemicals generally do not match the individual profile of the tree or orchards and would result in over- or even under-application (Zamahn and Salyani, 2004).

Spray volumes are commonly calculated irrespectively of orchard characteristics, such as canopy size, density and height (Silva Junior *et al*., 2016). Furthermore, the target organism or plant part in an orchard is complex and may be an insect, pathogen, mite, the leaves, the fruit etc., and may differ in their position within the tree canopy (Hall, 1991). Citrus trees have large canopies with variable density, and uniform spray coverage is therefore particularly difficult (Stover *et al*., 2002). Important factors contributing to variable spray deposition are canopy shape as well as canopy density and volume (Stover *et al*., 2002). The canopy volume is considered as the entire canopy of a tree from the base of the crown to the highest point and from the centre of the crown out to the furthest tips (Verna *et al*., 2016).

It is not suitable to apply the same dosage of PPP in orchards with different canopies characteristics. Matching chemical rates and sprayer outputs to three-dimensional canopies have always been complex (Furness *et al*., 1998). Non-uniform density throughout canopies are common, citrus being an example of a crop with a dense outer shell of foliage while being almost barren in the centre (Furness *et al*., 1998). In a dense crop such as citrus, the density of the surface area in the periphery can be ten times higher than in the centre of the canopy, suggesting that adjustments are needed in terms of application technique (Hall, 1991). However, the canopy needs to be characterised to allow for these adjustments.

Previously there were no recommendations available on how to adjust spray volumes to account for parameters such as canopy size and leaf area density of the canopy (Byers *et al*., 1989; Hall, 1991). However, recent studies in Brazil (Scapin *et al*., 2015; Silva Junior *et al*., 2016) have investigated the potential of spray volumes specifically calibrated to take the tree characteristics such as tree volume into consideration.

For sprayer calibration and spray volume calculations in practice, there are several methods to use. These are based either on two (leaf wall area) or three (tree row volume) dimensional factors

that are related to canopy structure (Walklate *et al*., 2011). The Leaf Wall Area (LWA) is measured in square meter (m^2) treated area/ha and considers the vegetation as a vertical wall facing the spray machine (Garcer *et al.*, 2017). The Tree Row Volume (TRV) system determines application volumes based on the convention that each row of trees is a rectangular box whose volume could be used to calculate the volume of space occupied by foliage per unit of ground surface (m^3 of foliage per hectare). Optimum application volumes are considered as the spray volume to reach the run-off point of the vegetation during application (Garcer *et al.*, 2017).

The potential of TRV-adjusted spray volumes to reduce the input costs has been verified in previous studies (Cunningham & Harden, 1998, 1999; Silva Junior *et al.*, 2016), which concluded that it can contribute to sustainable citrus production by reducing environmental impact, while achieving effective disease control. However, neither the TRV or LWA methods considers canopy density. Van Zyl *et al.* (2014) demonstrated the important effects of tree canopy density on spray deposition and recommended that pruning and canopy management is important to allow the effective implementation of reduced spray volume application.

Measuring canopy density is complex. Garcer *et al.* (2017) proposed the calculation of the total leaf surface area per tree (m^2 leaf/tree). This was done by multiplying the volume of the tree expressed as m^3/tree (as the apparent volume of trees considering that the canopy of the citrus has an ellipsoidal shape) with the leaf area density (LAD) measured in m^2 leaf/m^3 canopy. Measurement of LAD is, however, very time-consuming and often destructive. Sensors and modelling such as Light Detection and Ranging (LiDAR) offer the potential to measure tree volume and density in a non-destructive manner (Bjugstad, 2014).

LiDAR is a remote sensing technique based on measuring the time a laser pulse takes between the sensor and the target, which in the case of a vegetative structure such as a citrus tree canopy, includes the leaves and branches (Polo *et al*., 2009). It is widely used in the agricultural industry, especially in the field of forestry, as a tool for the 3-dimensional measurement of plant shapes and canopy structures (Omasa *et al*., 2007). Various LiDAR applications and studies have been done indicating the potential of this technology in the agricultural industry. Polo *et al.* (2009) demonstrated a good correlation between manual and sensor-based measurements of the vegetative volume of tree-row plantations, indicating that this is potentially an effective, non-destructive and accurate tool for measuring tree volume.

Therefore, there is a need to investigate the potential of calibration or measurement systems that takes canopy density into consideration, and ideally these systems should be non-destructive, practical, accurate and time efficient. The aim of this research chapter is firstly to determine the effect of pruning on spray deposition in the citrus tree canopy and secondly to evaluate the ability of LiDAR to accurately determine canopy density for the potential incorporation in a TRV calibration system.

MATERIALS AND METHODS

1. Pruning treatments, spray application and deposition assessment

1.1. Trial layout

Trial sites (orchards) were identified on three different farms in South Africa, located in the Western Cape. The first farm is in Agter-Paarl (a 8-year-old Bears lime orchard on Rough Lemon rootstock tree with a spacing of 5 × 3 m) and the other two farms in Citrusdal, named Citrusdal 1 (a 30-year-old own rooted Midnight Valencia orchard with a spacing of 7 × 3 m) and Citrusdal 2 (a 63-year-old Washington Navel on Rough Lemon rootstock orchard with a spacing of 6 × 3 m).

1.2. Pruning treatments

Trees in the trial orchards were pruned using the following categories, no-pruning, light-pruning and heavy-pruning. Light-pruning entailed the removal of one secondary branch (a side branch secondary to the parent/primary branch) on each side of the canopy, and for heavy-pruning two secondary branches from each side of the tree canopy were removed. Each pruning category was repeated on six trees that were randomly selected from 18 trees in two rows within each trial orchard. These pruning treatments were done with the aim to create spray windows in the canopy as will be done with normal seasonal pruning. The average tree density for each trial was determined visually following similar guidelines as van Zyl *et al.* (2014) on a 5-point scale (1 - very sparse leaf concentration, heavily aerated; 2 - sparse leaf volume, well aerated; 3 - good balance between leaf volume and canopy aeration; 4 - dense canopy, sparsely aerated; 5 - very dense leaf concentration, poorly aerated with no pruned canopy windows.

Prior to pruning, the average leaf area (LA; cm^2), tree canopy volume (m^3) and leaf area/wet leaf mass (cm^2/kg) ratio were determined for each orchard on six separate trees. The average leaf area in a trial orchard was determined as follows: a steel frame with a volume of 0.375 m^3 was inserted into the canopy of a selected tree at eye level. Within this frame, all leaves were picked and placed into plastic bags. The total LA from the picked leaves were measured with a leaf area index sensor (WD3 WinDIAS Leaf Image Analysis System, Delta T Devices Ltd, Cambridge, UK). The wet weight (kg) of the picked leaves was also determined. This allowed for the calculation of average LA and leaf area density (LAD) in m^2/m^3 of the tree, as well as the LA/wet mass ration in m^2/kg.

The canopy volume for each tree was also calculated by measuring the height (H) and width (W) of the tree with a measuring stick and using the following equation: $\left(\frac{H}{2} x \frac{W}{2}\right) x \left((H - 0.5)\ x\ 3.14\right) - 1.046\ x\ (\frac{W}{2})$ (K. Breytenbagh, personal communication, 2017). These measurements were done for 6 uniform trees in the orchard and the average from the 6 trees was consequently determined. This value was used in later calculations following the pruning of the trees.

After pruning, leaves were removed from the pruned branches and collected in plastic bags. The wet mass of the leaves from pruned branched was determined for each tree. From these weights the total leaf area removed from a tree could be calculated.

1.3. Spray applications

The two rows subjected to the different pruning treatments were sprayed with either 3000 L/ha or 1500 L/ha using a CIMA [(Blitz 55 low volume) Rovic & Leers, South Africa] spray machine. The trees were sprayed from both sides with a mixture of water and fluorescent pigment [South Australian Research and Development Institute (SARDI) Yellow Fluorescent Pigment, 40% EC (SARDI, Loxton, South Australia) at a concentration of 1 ml per litre. However, at the lower spray volume dosage per hectare was kept the same as for the 3000 L/ha application, and 2× the pigment concentration was applied at 1500 L/ha. The pigment made it possible to visualize and measure the deposition on the leaf and fruit surfaces. The same spraying machine was used in all three trials.

1.4. Sampling of leaves

After the spray mixture had dried on the trees, leaves were collected from each of the six trees subjected to the different pruning treatments, and from 6 canopy positions: three vertical canopy positions (top, middle and bottom part of each tree canopy) and two horizontal canopy positions [inner (>30 to 50 cm into the tree canopy) and outer canopy (leaves from the outside of the tree)]. From each position, 12 undamaged leaves were collected separately in plastic bags (in total, 72 leaves per replicate tree), labelled and transported to the laboratory where the leaves were stored at cool (4°C) and dry conditions until deposition analysis was done.

Deposition analysis

Similar to the methodology used by van Zyl *et al*. (2013), leaves were subjected to deposition analysis to determine the deposition quantity and quality of the chemicals applied at different spray volumes. Images of leaves were taken in a dark room with an illuminated ultra-violet light source (UV-A, ≈365 nm, Labino Mid Light; www.labino.com) for the fluorescent pigment to be clear on images.

First, petioles were removed from leaves at the base of the leaf blade using a pair of scissors. A single leaf was positioned in the middle of a back-illuminated red Perspex box to reduce any shadowing and to enhance edges of leaves. A glass pane (200 × 200 × 2 mm) was used to cover and flatten the leaf. A Canon EOS 40D camera equipped with a 60 mm macro lens was mounted on a tripod in a fixed position directly above the Perspex box and was used to take digital photos in RAW file format (.CR2 ≈ 10 MB) of the upper and lower leaf surfaces of each leaf. For leaves an aperture setting of F10 and an ISO setting of 100 was used. RAW images were converted to 8-bit Exif-TIFF (.TIF ≈ 30 MB) with Digital Photo Professional version 3.1.0.0 (CANON INC.; www.canon.com) files for digital analysis to determine the deposition parameters (Van Zyl *et al*., 2013).

To assess the deposition quantity and quality per leaf, similar methodology used by (van Zyl *et al*., 2013) was again used in this study. Spray deposition assessment involved digital image analysis with Image Pro Plus software version 7.0 (Media Cybernetics, www. mediacy.com) to determine the

deposition quantity and quality per leaf. Deposition quantity was measured as percentage of the leaf area covered by pigment particles (percentage fluorescent particle coverage, FPC%). For the deposition quality assessment, the leaf area was divided into equally-sized squares [100 x 100 pixels (10000 pixels)]. Depending on the leaf size, this amounted to as few as 20 to more than 250 individual squares per leaf, of which the percentage of the area covered by fluorescent pigment particles was determined for each square. The Interquartile Coefficient of Dispersion (ICD %) per leaf was used as a measure of deposition quality per leaf, *i.e.* uniformity of deposition on the leaf surface. Low ICD values were indicative of better deposition quality. Deposition uniformity between leaves was calculated as the uniformity in pigment deposition in a 12-leaf sample per canopy position (standard deviation/mean × 100). Deposition efficiency was expressed as deposition quantity normalised to FPC % per 1000 L/ha.

2. LiDAR evaluation

2.1. LiDAR scanning

In order to determine canopy density of the citrus trees subjected to abovementioned pruning treatments, a four-wheeled robot (called “Dassie”) mounted with a LiDAR and camera system was used.

To detect the movement of the LiDAR in the environment, visual odometry was used. A ZED Stereo camera with an Nvidia Jetson TX1 (NVIDIA Corporation, 2788 San Tomas Expressway, Santa Clara, CA 95051, USA) mounted with the LiDAR on the robot, was used to calculate any movement the robot made. This was then translated into LiDAR movements within the robot operating system (ROS) where data capture and visualisation were done.

The Dassie Robot uses a LMS LiDAR [SICK Automation Southern Africa (Pty) Ltd., Lanseria Corporate Estate, 1748 Lanseria Republic of South Africa] to generate point cloud data from the surrounding environment. The LiDAR is mounted in a vertical position as it has a 2D sweeping beam and can therefore be used in a push-broom configuration. As described above, Dassie has the ability to measure its own movement within an environment and with that, generate a 3D point cloud as it moves past an object. The Dassie Robot maintains a constant speed during the scanning process.

In this case, the point cloud generated, consisted of scanned canopies of trees. Each point represents a leave or branch that was “hit” by die LiDAR. By scanning the tree canopies, the aim was to determine a possible correlation between tree canopy density as measured by the LiDAR and manual tree canopy density measurements. The point clouds scanned by the Dassie were imported to Cloud Compare software (v2.7.0; www.scribd.com). The cloud compare software was used to manually select individual trees and exporting the data to VoxR software (Lecigne *et al*., 2015). However, these point clouds generated are ‘unstructured data that must be reconstructed by using dedicated software programs to provide information’ (Dassot *et al*., 2011). The “VoxR” software package was used to quantify the canopy structure from the LiDAR data using the “surface” function. The “surface” function was used to first generate voxels around the existing points.

A voxel is defined as a 3D cube with a central point as seen in Figure 1. The voxelization algorithms used by VoxR are usually based on an iterative process that aims to classify points in a three-dimensional regular grid of voxels (Figure 1) (Fernández-Sarría *et al*., 2013). The voxelization algorithm returns the x, y and z coordinates of each voxel's centre point and the number of points present within the voxel of the point cloud. These voxel cube sizes (resolution) can be defined to manipulate the regular cube grid size of voxels and only filled voxels are considered by the algorithm. This is done to simplify the point cloud geometry and the subsequent analysis. The location where the tree is denser will correspond to the location were the most points in a specific voxel will be found (Lecigne *et al*., 2018).

This is also used to determine the surface ratio (SR), which is the analysis of the distribution of the ratio between the number of points and the number of voxels and helps to normalise the deviations in density caused by measurement irregularities. This is done by projecting the new voxel grid towards the ground plane using the "level" function. All voxels that overlap or are stacked on top of each other's points are summated and stored to generate density classes. Density is seen as the number of points within each voxel in the projection. The "surface function" then runs through each level of the voxel grid as seen in Figure 1 and generates a value signifying the structure of the outside of the canopy, comparing each voxel's density level with the next. A higher surface ratio value would indicate that the position where the voxel was situated contained a grated number of points of the point clouds, therefore indicating a higher canopy density.

The flow of data can be presented as:

2.2. Preliminary LiDAR scans

Preliminary LiDAR scans with Dassie were done in a Nules Clementine orchard outside of Stellenbosch. In this orchard, large canopy volume and density variation, due to soilborne disease, was present. In the scans, seven groups of trees that contained trees with canopy densities ranging from dense to very sparse, were scanned once from both sides, therefore one pass per tree side was made. The data from these scans were used to group trees into density groups based on LiDAR scans to determine if the LiDAR scans could distinguish between trees with visual differences in canopy density. These preliminary scans led to the following conclusions:

- The LiDAR platform was mounted 1.5 m above the ground and was subject to interference from overhanging branches leading to it not being able to reach the top of the trees in some cases.
- The laser beams do not reach beyond leaves, which limits the ability to accurately predict the density in some cases.
- Only one side of the tree could be scanned at a time with odometry from the robotic platform, which was not perfect in an environment where the LiDAR was not scanning the entire tree. All of this resulted in point clouds that could not properly be combined. The point clouds of two halves of one scanned tree is therefore seen as one repetition.

2.3. LiDAR scanning of pruned canopies at the Citrusdal 2 trial site.

Prior to pruning and subsequent to the pruning treatments (as described above), the trees were scanned with the LiDAR to collect pre- and post-pruning LiDAR data. Using the pre-pruning average LA measurement and LA/wet mass ratio described above, the pre-pruning and post-pruning leaf area density (LAD) of each of the six selected trees of each of the three pruning categories could be determined. These measurements and LiDAR scans were repeated for each spray volume treatment (36 trees in total). The pre-prune and post-prune LAD values were correlated with the surface ratio (SR) values of the same trees obtained following the pre-prune and post-prune LiDAR scans. This was done to determine if there was a meaningful relationship between the SR values and the manually determined LAD values of the trees. Additionally, the statistical relationship between SR and deposition parameters was also investigated.

2.4. Statistical analysis

The data from each trial were analysed separately. Deposition quantity (FPC%), quality (ICD %) and uniformity (CV%) data were subjected to appropriate analysis of variance (ANOVA). The skewing effect of outliers was negated by using median FPC% values of the 12 leaves for deposition analysis. Data from upper and lower leaf surfaces were analysed separately but were combined when describing the results. The Student's t-test for least significant difference (P = 0.05) was used to compare means. Pearson's correlation analyses (Snedecor and Cochran, 1967) were conducted to determine the correlation between the pre- and post-prune data for SR, deposition parameters (FPC%, CV% and ICD%) and LAD. Scatter-plots were also drawn to visually assess any non-linear relationship between these variables. The analyses were done using XLSTAT 2015 (https://www.xlstat.com/en/; Addinsoft, 28 West 27th Street, Suite 503 New York, NY 10001) and SAS version 8.2 statistical software (SAS institute Inc., 1999).

RESULTS

Pruning treatments, spray application and deposition assessment

Agter-Paarl

Tree density in this orchard was manually assessed before pruning using the 5-point scale as a category 2 - sparse leaf volume, well aerated.

Deposition quantity

Analysis of variance (ANOVA) of FPC% (deposition quantity) data on leaves indicated a spray volume × horizontal canopy position × vertical canopy position interaction ($P = 0.0349$) as well as a spray volume × pruning treatment × vertical canopy position interaction ($P < 0.0001$) (Table 1). The highest deposition quantities were achieved by the 1500 L/ha volume application at the middle and bottom canopy positions where no-pruning treatment was applied (0.39 and 0.42 FPC%, respectively), similar to the middle canopy position of the heavy-pruning treatment (0.34 FPC%) (Table 2). The 1500 L/ha spray deposited lower quantities on middle and bottom canopy positions for the other pruning treatments (0.19 to 0.26 FPC%), and the lowest quantities in the tops of trees (0.05 to 0.14 FPC%). Relatively poor deposition quantity was generally seen following the 3000 L/ha spray application (0.05 to 0.13 FPC%). However, deposition quantities for the 3000 L/ha were fairly uniformly distributed throughout the canopy, whilst significantly lower quantities were deposited following the 1500 L/ha spray in the outer (0.15 FPC%) and inner (0.02 FPC%) positions in the tops of trees, than the middle and bottom positions (0.25 to 0.41 FPC%) (results not shown).

Deposition uniformity

Analysis of variance (ANOVA) of CV% (deposition uniformity) data on leaves indicated a significant spray volume × pruning treatment × vertical canopy position interaction ($P < 0.0001$; Table 1). For this deposition parameter very high mean CV% values (poor uniformity) were observed for all spray volumes, pruning treatments and vertical canopy positions, which mostly did not differ significantly (103.32 to 132.21 CV%), except for the deposition uniformity values in tops of trees at the lower spray volume, which were significantly poorer (175.39 to 222.76 CV%) (Table 2).

Similar ($P = 0.2268$) deposition uniformity results were generally observed on outer (129.66 CV%) and inner canopy positions (136.78 CV%).

Deposition quality

For the deposition quality results, the ANOVA indicated a significant horizontal × vertical canopy position interaction ($P = 0.0048$) (Table 1). Deposition quality at the top inner canopy positions was the poorest (100.00 ICD%), with the best deposition quality realised at the outer middle canopy position (96.06 ICD%). A significant treatment effect was observed ($P < 0.0001$) (Table 1), and the no-pruning treatment resulted in the best deposition quality (96.41 ICD%) followed by the heavy-pruning treatment (98.02 ICD) and the light-pruning treatment (99.64 ICD%). However, compared

with previous research (Chapter 2), these differences were not practically meaningful (results not shown).

Citrusdal 1

Tree density in this orchard was manually assessed before pruning using the 5-point scale as category 3 - good balance between leaf volume and canopy aeration.

Deposition quantity

Analysis of variance (ANOVA) of FPC% data on leaves showed a significant pruning treatment × horizontal × vertical canopy interaction (P = 0.0326; Table 3). This interaction was due to higher deposition values achieved on the outer canopy positions of all the pruning treatments than on the inner canopy position (8.22 and 4.82 FPC%, respectively). However, the bottom canopy positions on the outer canopy position of the all the pruning treatments had higher deposition quantity values than the middle and top positions, 13.90 FPC% for no-pruning, 16.09 FPC% for light-pruning and 16.89 FPC% for heavy pruning. A meaningful pruning treatment × horizontal canopy position (P = 0.1178) and significant spray volume × horizontal canopy position (P < 0.0001) interaction were also observed (Tables 4 and 5, respectively). The lowest deposition quantity was seen on the inner canopy positions of the no-pruning treatment (4.74 FPC%), significantly lower than on light- and heavy-pruning treatments (8.21 and 6.35 FPC%, respectively). The highest deposition quantities were seen on the outer canopy positions, with higher quantities on the light-pruning and heavy-pruning treatments (11.72 and 11.13 FPC%), than on the no-pruning treatment (10.03 FPC%) (Table 4). Mean deposition quantity on the outer canopy position following the 1500 L/ha application (14.07 FPC%) was significantly better than at the inner canopy deposition of the same volume application (5.65 FPC%), with deposition quantity following the 3000 L/ha volume application achieving intermediate results (7.22 to 7.85 FPC%), not differing significantly from each other (Table 5).

Deposition uniformity

The ANOVA indicated a significant pruning treatment × horizontal canopy position interaction (P = 0.0347; Table 3) and spray volume × horizontal canopy position interaction (P < 0.0001), while vertical canopy position did not have a significant effect (P = 0.5377) (Table 3). In outer canopies, uniformity values were better in the light-pruning and heavy-pruning treatments (44.91 and 45.45 CV%, respectively) than the no-pruning treatment (54.16 CV%), which did not differ from inner canopy uniformity in the heavy- and light-pruning treatments (60.48 and 57.35 CV%, respectively) (Table 4). Uniformity of inner canopies of the no-pruning treatment was the poorest (78.65 CV%). Deposition uniformity was significantly better on the outer canopies of both the 3000 L/ha and 1500 L/ha volume application (47.87 and 48.47 CV%, respectively) than on inner canopies of the high (56.83 CV%) and lower volume sprays (74.14 CV%) (Table 5).

Deposition quality

Analysis of variance (ANOVA) of ICD% data showed the following (Table 3): a significant vertical canopy position effect ($P < 0.0001$) and a significant spray volume × pruning treatment × horizontal canopy position interaction ($P = 0.0062$). This interaction was ascribed to better deposition quality for all pruning treatments on the inner canopies for reduced sprayed volumes (35.06 for no-pruning, 27.16 for light-pruning, 29.36 for heavy-pruning ICD% respectively), while deposition quality was generally poorer at high volume sprays and more so on outer canopies and in unpruned trees (60.38 ICD%). For the vertical canopy position effect, the bottom canopy position had the best deposition quality (35.58 ICD%), followed by the middle canopy position (37.23 ICD%) and the top canopy position having the poorest quality (41.91 ICD%) of all the positions.

Citrusdal 2

Tree density in this orchard was manually assessed before pruning using the 5-point scale as category 4 - dense canopy, sparsely aerated.

Deposition quantity

A significant spray volume × pruning treatment × horizontal canopy interaction was also observed following the ANOVA ($P = 0.0076$; Table 6). For both spray volumes, it was seen that the FPC% values were generally significantly higher on the outside of the tree canopy versus the inside of the tree canopy (Table 7). In the case of the 3000 L/ha sprays, the mean FPC% values were similar on the inside of the tree canopy for all the pruning treatments (0.91 to 1.13 FPC%), as well as for the outer canopy (2.65 FPC% to 2.85 FPC%). At 1500 L/ha, deposition quantities were higher in inner canopies of pruned trees (1.54 to 1.71 FPC%) compared with unpruned trees (1.01 FPC%), while deposition quantity were similar on outer canopies of unpruned and lightly pruned trees (4.17 and 4.29 FPC%, respectively) and significantly lower on heavily pruned trees (2.83 FPC%) (Table 7). Analysis of variance also showed a significant vertical canopy effect ($P < 0.0001$) (Table 5). The highest deposition quantity was achieved at the middle canopy position (2.75 FPC%) followed by the top canopy position (2.16 FPC%) and the bottom canopy position having the lowest deposition quantity (1.81 FPC%).

Deposition uniformity

A meaningful spray volume × pruning treatment × horizontal canopy interaction was also observed following the ANOVA ($P = 0.0586$) (Table 5). Deposition uniformity was better on the outer canopies (68.39 to 77.76 CV%) compared to inner canopies (91.37 – 116.82 CV%). At 1500 L/ha, inner canopies of lightly pruned trees (91.37 CV%) had significantly more uniform deposition than unpruned trees (110.29 CV%), with heavily pruned trees with intermediate uniformity (98.21 CV%) (Table 7). Conversely, at 3000 L/ha, deposition on inner canopies of lightly pruned trees (116.82 CV%) and heavily pruned trees (108.19 CV%) was significantly less uniform than unpruned trees (97.89 CV%). The ANOVA also indicated a significant vertical position effect ($P <0.0001$; Table 5):

the middle canopy position generally had the best deposition uniformity (74.58 CV%) followed by the top canopy position (86.60 CV%) and the bottom canopy position (101.13 CV%).

Deposition quality

For the deposition quality results, the ANOVA indicated no significant interactions, but the following significant effects: a vertical canopy position effect ($P < 0.0001$), a horizontal canopy position effect ($P < 0.0001$) as well as a spray volume effect ($P = 0.0121$; Table 5). The inner canopy position (77.83 ICD%) had the poorest ICD% in comparison with the outer canopy position (70.71 ICD%), while the middle canopy position had the best ICD% with the lowest deposition quality value (70.49 ICD%), followed by the bottom canopy position (74.15 ICD%) and the top canopy position (78.43 ICD%). For the volume effect, it was seen that the 3000 L/ha application (77.20 ICD%) had the poorest deposition quality in comparison with the 1500 L/ha (71.58 ICD%).

3. LiDAR evaluation

LiDAR preliminary scans

From the scan data generated during preliminary scans of trees that contained trees with canopy densities ranging from dense to very sparse, “filled volume” values and images were derived from the number of points remaining after cutting the point cloud of a scanned tree in half. Therefore, the more porous a tree looks in the image, the more laser beams travels through the canopy, *i.e.* not hitting any object. A higher SR value as indicated in Figure 2 indicates a less porous, or denser tree canopy; values ranged from 11 942 – 21 386. From these scans it was therefore clear that the LiDAR could also detect visual canopy density differences between different tree canopies.

LiDAR scanning of pruned canopies

Results from the Pearson’s correlation analysis indicated a positive and strong correlation between pruning class and LAD removed ($r = 0.823$). A negative and poor correlation between pruning class and LAD post pruning was seen ($r = - 0.161$) as well as a negative poor correlation between pruning class and SR post pruning ($r = - 0.152$) (Table 8). The correlation between the LAD pre-prune and LAD post prune shows an $r = 1.000$, indicating that values of these two parameters were very similar to each other.

SR post-prune values and LAD post-prune values correlated poorly ($r = 0.084$; $P = 0.625$). LAD post-prune values were positively, but poorly correlated with mean FPC% values ($r = 0.243$; $P = 0.152$).

For deposition parameters a negative, but strong correlation was seen between deposition quantity (FPC%) and deposition uniformity (CV%) ($r = -0.734$) and deposition quality ($r = -0.725$) (Table 8).

DISCUSSION

In this study a robot-mounted LiDAR system was evaluated to determine if it can be used to specifically determine citrus tree canopy density. If successful, this could replace laborious manual canopy density measurements. Having a quick and easy method of density determination in an orchard would allow for the inclusion of canopy density as a factor in citrus spray calibration. This research contributes to the ongoing advances in using remote sensing for tree density measurement as a fast and inexpensive tool. Furthermore, the effect of manual manipulation of the tree canopy density through pruning was evaluated to determine its effect on spray deposition quantity, uniformity and quality. This was done to investigate the effects of tree canopy density of spray deposition and also the possibility to use low volume spray applications to reduce environmental pollution and production costs without compromising on the level of control of pests and diseases. The ultimate aim was therefore to show that with proper pruning practices, the use of low volume spray application is a viable alternative to South Africa's culture of high-volume spray application.

The effect of pruning on canopy density and spray penetration was evident in this study, with deposition results generally indicating that better penetration of the canopy was achieved with the creation of spraying windows and decreasing the canopy density on the outer side of the tree. This result is attributed to the positive effect of pruning on the canopy geometry, which allows for more even and better spray penetration (Hall, 1991).

In terms of deposition quantity (FPC%) and uniformity (CV%), diverse results were seen between the three trial sites. At the Citrusdal 1 trial site, the pruned treatments had better deposition quantity than the unpruned treatments, with light-pruning also demonstrating better canopy penetration than heavy-pruning with higher deposition quantity on inner canopy leaves of pruned trees. The latter difference might be anomalous, particularly as no difference was observed in deposition uniformity between the two pruning treatments at this trial site. At the Citrusdal 2 trial site, the pruning treatments promoted deposition quantity and uniformity at the lower spray volume only. At higher spray volumes, deposition quantity levels were similar in pruned and unpruned trees, but uniformity was poorer on inner canopy leaves of pruned trees. Poorer deposition on dense (unpruned) canopies can be attributed to the "leaf-wall" effect, which leads to run-off and loss of spray deposition, as also reported by van Zyl *et al.* (2014). The denser the exterior of the tree, the more spray solution will be blocked from reaching the inner canopy positions (Stover *et al.*, 2002). Previous research on citrus using these methods (van Zyl *et al.*, 2014) demonstrated better spray penetration of dense canopies, with concomitantly better deposition quantity and uniformity values, at higher spray volumes (6500 to 8500 L/ha). Results at the Citrusdal 2 trial site were therefore unexpected: poorer deposition uniformity was recorded at higher spray volume in inner canopies of pruned trees, but not in unpruned trees, whereas deposition uniformity was better in inner canopies of pruned trees at lower spray volumes. The reason for this observation in unclear. At the Agter Paarl trial site, very low deposition quantity values were observed, which was attributed to an incorrect dosage calculation

which lead to inadequate pigment application. Marginally better quantity and uniformity results were obtained on unpruned trees at the lower spray volume, whilst no significant differences were observed at the higher spray volume. The density estimation for this trial site was rated as “2”, which indicates that the tree canopy had a sparse leaf volume, but well aerated, and it is possible that the sprayer calibration (wind speed) was not optimal for this sparse canopy. Citrus trees are generally large and dense, and spray penetration into the canopy is expected to be difficult, with poorer deposition at the inner canopy positions. The effect of pruning and the benefits thereof were demonstrated to some extent in this study, but future work should also include other spray machines and should also investigate options for sprayer calibration relative to the tree canopy density as modified by the pruning treatments.

The preliminary LiDAR scans of the citrus tree canopies were able to differentiate between tree canopies with different canopy densities and this was expressed as different ”surface ratio” values, which increased with increasing canopy density, as was proposed by (Polo *et al.*, 2009). This parameter showed potential to be used as a LiDAR based indication of citrus tree canopy density. However, poor correlation between LiDAR and manual measurements were obtained, indicating that the 3D measurement of citrus canopies does not match manual measurements and pruning classes. It should also be noted that the conventional density parameter, LAD, also poorly correlated with the pruning classes. Pruning class correlated well with the removed-LAD, but poorly with post-pruning LAD, which might be attributed to the small proportion of canopy pruned relative to the tree size. Pruning treatments were not severe enough to make a significant difference in the tree LAD. It therefore appears that LAD on its own is also not a sufficiently sensitive parameter to determine tree canopy density for sprayer calibration.

Large variation was also observed between LiDAR scans with the SR values of some of the pre-pruned trees being lower than the post pruned trees. Between these scanning passes a variation in the SR values of the unpruned control trees could also be observed. These variations can be the result of abiotic factors, such as wind and temperature, which causes the canopies to change between scans. This study does not discredit LiDAR as a potentially valuable tool in measuring a citrus tree density, but rather highlights the complexity of this measuring system. In a study done by Gil *et al.* (2014) three different canopy parameters (crop height, crop width and leaf area index) of grapevines were manually measured, as well as by LiDAR. Good correlation between the two measurement techniques were observed and it was suggested that in future studies a similar protocol must be followed in order to simplify the measurements and to determine where variation occurs. However, this study was done on vines, which has a more simplified canopy structure in comparison with a citrus tree.

This study shows the inherent difficulties that accompanies the LiDAR technology, and some suggestions to improve future studies can be made. It could also serve as a good example of the pitfalls associated with being too ambitious in using advance technology for a novel use. The LiDAR

results obtained varied greatly. This was as a result of a multitude of factors including wind, impedance from over hanging branches, single scanning passes, not being able to include return passes, distance from canopy and the small number of trees scanned. The single return module was too sensitive to variations on the outside surface of the trees, which deems the LiDAR to be inaccurate for a single return application.

It is suggested that for future studies a multiple return LiDAR module be used. This module is used in the forestry industry and would increase the accuracy as well as give more insight into the inner canopy of the tree (Hsieh *et al*., 2014). More trees should be scanned in order to create a more accurate model to determine the density of each tree. This would decrease the variability as a result of the unique shape, size and volume of each tree. This variation could also be mitigated by doing multiple passes of the same row and scanning the same tree multiple times. Ideally, these repetitions could also be used to fill in details that was missed by the original scan or resolve variations between the scans. The number of scanning passes required before the change in SR value can also then be determined. However, the Cloud Compare software is not capable of merging the point clouds of different passes and an alternative method of combining the point clouds would therefore be needed. Alternatively, the mean or median SR value of each tree from the point clouds of the different passes could be used. These methods and suggestions should be investigated as means to mitigate the variability found in this study and to ensure good quality data to be used in the investigation of a possible correlation between the SR values and manual canopy density measurements.

For future investigations it would be suggested that small scale tests be conducted first. For example, doing a stationary test on a single dense tree in a controlled environment. Pruning a constant amount each time and taking multiple scans in between the pruning treatments to find a possible correlation between the LiDAR data and an actual measurable decrease in density. This could be used to create an initial model. To ensure repeatability, this model should be implemented on many trees in a moving test. However, this should be done with multiple passes as well as the return pass to gather as much information as possible. Accuracy could also be increased by having scanning passes at different heights from the ground to ensure no hidden detail is missing from the point cloud. Additionally, the LiDAR sensor should be raised to different heights, or to the mid-point of the tree, to negate the effects of overhanging branches.

The manual manipulation of canopy density by pruning was shown to be beneficial for spray deposition in creating more ‘spray-friendly’ canopies. The use of LiDAR as a valuable tool and the potential thereof for the measurement of citrus tree density is highlighted in this study. However, the accuracy in comparison with traditional manual methods of tree density determination is still yet to be realised. The use of this non-destructive tools for the future of spray volume calibration is significant for sustainable approach and should be investigated further.

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TABLES AND FIGURES

Table 1. Analyses of variance of mean deposition quantity (FPC%), uniformity (CV%) and quality (ICD%) values on leaves at different vertical and horizontal canopy positions in trees subjected to three different pruning treatments before being sprayed with fluorescent pigment at either 3000 L/ha or 1500 L/ha at the Agter-Paarl trial site.

Source	Deposition quantity (FPC%)			Deposition uniformity (CV%)			Deposition quality (ICD%)		
	DF*	SS**	SL***	DF	SS	SL	DF	SS	SL
Volume	1	0.87	<0.0001	1	53983.08	<0.0001	1	21.37	0.2395
Treatment	2	0.29	0.0403	2	6633.95	0.2298	2	375.22	0.0001
Volume × Treatment	2	0.13	0.2160	2	6474.82	0.2376	2	17.79	0.5557
Volume ×Treatment (Rep)	30	1.22	0.0012	30	64400.68	0.2805	30	445.25	0.3952
Horizontal canopy position	1	0.13	0.0104	1	2736.01	0.2268	1	2.05	0.7029
Volume × Horizontal canopy position	1	0.08	0.0445	1	50.79	0.8689	1	2.05	0.7030
Treat × Horizontal canopy position	2	0.05	0.2886	2	3355.52	0.4074	2	29.76	0.3485
Volume ×Treatment × Horizontal canopy position	2	0.02	0.5609	2	1416.12	0.6837	2	1.74	0.9399
Vertical canopy position	2	0.66	<0.0001	2	83684.81	<0.0001	2	316.79	<0.0001
Volume × treatment × vertical canopy position	2	0.51	<0.0001	2	57161.57	<0.0001	2	12.39	0.6437
Horizontal canopy position × vertical canopy position	2	0.10	0.0680	2	103.50	0.9725	2	155.15	0.0048
Volume × horizontal canopy position × vertical canopy position	2	0.13	0.0349	2	3.41	0.9991	2	1.32	0.9540
Treatment × horizontal canopy position × vertical canopy position	4	0.09	0.3168	4	5951.04	0.5263	4	74.23	0.2636
Volume × treatment × horizontal canopy position × vertical canopy position	4	0.12	0.1639	4	3859.89	0.7215	4	1.82	0.9980
Error	150	2.80		150	278580.98		150	2102.44	
Corrected total	215	7.33		215	578674.45		215	3752.13	

*Degrees of Freedom **Sum of squares ***Significance level

Table 2. Mean deposition quantity (FPC%) and uniformity (CV%) values realised by water and yellow fluorescent pigment sprays at 3000 L/ha or1500 L/ha at the top, middle and bottom position of tree canopies subjected to three different pruning treatments at the Agter-Paarl trial site.

		Cima @ 3000 l/ha			Cima @1500 l/ha		
	Pruning category	**Top**	**Middle**	**Bottom**	**Top**	**Middle**	**Bottom**
FPC%	**No-pruning**	0.12 def[1]	0.11 def	0.13 def	0.14 def	0.39 a	0.42 a
	Light-pruning	0.09 ef	0.09 ef	0.09 ef	0.05 f	0.26 bc	0.22 cd
	Heavy-pruning	0.05 f	0.13 def	0.11 def	0.08 ef	0.34 ab	0.19 cde
CV%	**No-pruning**	123.91 c	131.48 c	103.32 c	175.39 b	124.73 c	107.38 c
	Light-pruning	126.46 c	113.70 c	120.66 c	222.76 a	129.00 c	132.21 c
	Heavy-pruning	115.90 c	118.94 c	102.31 c	199.87 ab	121.48 c	128.42 c

[1] Means for FPC% and CV% followed by the same letter were not significantly different at $P = 0.05$ (LSD 0.110 and 34.763 respectively).

Table 3. Analyses of variance of mean deposition quantity (FPC%), uniformity (CV%) and quality (ICD%) values on leaves at different vertical and horizontal canopy positions in trees subjected to three different pruning treatments before being sprayed with fluorescent pigment at either 3000 L/ha or 1500 L/ha at the Citursdal 1 trial site.

Source	Deposition quantity (FPC%)			Deposition uniformity (CV%)			Deposition quality (ICD%)		
	DF*	SS**	SL***	DF	SS	SL	DF	SS	SL
Volume	1	7359.65	<0.0001	1	4319.48	0.0382	1	15802.39	<0.0001
Treatment	2	2164.74	0.0014	2	10017.94	0.0096	2	4399.30	0.0007
Volume ×Treatment	2	633.92	0.1078	2	2655.06	0.2517	2	1183.93	0.0972
Volume ×Treatment (Rep)	30	3960.24	0.0019	30	27559.77	<0.0001	30	7041.67	<0.0001
Horizontal canopy position	1	9945.83	<0.0001	1	16202.60	<0.0001	1	757.69	<0.0001
Volume × Horizontal canopy position	1	2629.69	<0.0001	1	3757.47	<0.0001	1	1168.80	<0.0001
Treat × Horizontal canopy position	2	272.92	0.1178	2	1448.53	0.0347	2	32.84	0.6942
Volume ×Treatment × Horizontal canopy position	2	14.45	0.8915	2	154.73	0.6934	2	472.81	0.0062
Vertical canopy position	2	493.02	0.0219	2	262.55	0.5377	2	1410.47	<0.0001
Volume × treatment × vertical canopy position	2	7.48	0.9423	2	66.37	0.8544	2	23.87	0.7668
Horizontal canopy position × vertical canopy position	4	220.32	0.4800	4	679.61	0.5230	4	119.48	0.6168
Volume × horizontal canopy position × vertical canopy position	4	198.36	0.5343	4	730.14	0.4858	4	64.74	0.8363
Treatment × horizontal canopy position × vertical canopy position	2	440.69	0.0326	2	373.31	0.4146	2	44.72	0.6086
Volume ×treatment × horizontal canopy position × vertical canopy position	2	221.81	0.1750	2	261.28	0.5394		57.24	0.7152
Error	150	9432.76		150	31610.06		150	6731.27	
Corrected total	215	39171.90		245	102594.25		215	39571.54	

*Degrees of Freedom **Sum of squares ***Significance level

Table 4. Mean deposition quantity (FPC%) and uniformity (CV%) values realised by yellow fluorescent pigment sprays at either 3000 L/ha or1500 L/ha at inner and outer position of tree canopies subjected to three different pruning treatments at the Citrusdal 1 trial site.

	Pruning treatment	Inner	Outer
FPC%[1]	**No-pruning**	4.74 e	10.03 b
	Light-pruning	8.21 c	11.72 a
	Heavy-pruning	6.35 d	11.13 ab
CV%[1]	**No-pruning**	78.65 a	54.16 b
	Light-pruning	57.35 b	44.91 c
	Heavy-pruning	60.48 b	45.45 c

[1] Means for FPC% and CV% followed by the same letter were not significantly different at $P = 0.05$ (LSD 3.693; 6.761 respectively)

Table 5. Mean deposition quantity (FPC%), uniformity (CV%) and quality (ICD%) values realised by water and yellow fluorescent pigment sprays at 3000 L/ha or1500 L/ha at inner and outer position of tree canopies subjected to three different pruning treatments at the Citrusdal 1 trial site.

		Cima @ 3000 L/ha		Cima @1500 L/ha	
	LSD	**Inner**	**Outer**	**Inner**	**Outer**
FPC%	**3.016**	7.22 b[1]	7.85 b	5.65 c	14.07 a
CV%	**5.520**	56.83 b	47.87 c	74.14 a	48.47 c
ICD%	**2.547**	40.82 b	49.22 a	28.36 c	27.46 c

[1] Means for FPC%, CV% and ICD% followed by the same letter were not significantly different at *P* = 0.05 (LSD 3.016; 5.520; 2.547 respectively).

Table 6. Analyses of variance of mean deposition quantity (FPC%), uniformity (CV%) and quality (ICD%) values on leaves at different vertical and horizontal canopy positions in trees subjected to three different pruning treatments before being sprayed with fluorescent pigment at either 3000 L/ha or 1500 L/ha at the Citrusdal 2 trial site.

Source	Deposition quantity (FPC%)			Deposition uniformity (CV%)			Deposition quality (ICD%)		
	DF*	SS**	SL***	DF	SS	SL	DF	SS	SL
Volume	1	52.50	0.0015	1	658.60	0.5865	1	5034.31	0.0121
Treatment	2	10.63	0.3028	2	219.78	0.9509	2	2781.48	0.1561
Volume ×Treatment	2	13.58	0.2208	2	6283.34	0.2524	2	1205.04	0.4343
Volume ×Treatment (Rep)	30	128.19	0.0185	30	65357.78	0.2100	29	20356.18	<0.0001
Horizontal canopy position	1	454.22	<0.0001	1	116703.70	<0.0001	1	7507.76	<0.0001
Volume × Horizontal canopy position	1	9.24	0.0418	1	2794.67	0.1531	1	238.10	0.2363
Treat × Horizontal canopy position	2	12.22	0.0648	2	453.82	0.8460	2	513.32	0.2213
Volume ×Treatment × Horizontal canopy position	2	22.11	0.0076	2	7839.27	0.0586	2	27.04	0.9229
Vertical canopy position	2	64.24	<0.0001	2	50140.02	<0.0001	2	7200.86	<0.0001
Volume × treatment × vertical canopy position	4	7.26	0.5094	4	8493.30	0.1862	4	342.36	0.7295
Horizontal canopy position × vertical canopy position	2	10.58	0.0930	2	2887.60	0.3473	2	426.28	0.2851
Volume × horizontal canopy position × vertical canopy position	2	5.83	0.2675	2	556.85	0.8145	2	281.13	0.4360
Treatment × horizontal canopy position × vertical canopy position	4	7.21	0.5127	4	2103.28	0.8170	4	616.00	0.4572
Volume × treatment × horizontal canopy position × vertical canopy position	4	5.00	0.6847	4	6843.75	0.2874	4	366.33	0.7036
Error	179	204.20		179	157181.21		163	12899.94	
Corrected total	430	1605.61		430	689714.24		400	90565.26	

*Degrees of Freedom **Sum of squares ***Significance level

Table 7. Mean deposition quantity (FPC%) and uniformity (CV%) values realised by yellow fluorescent pigment sprays at either 3000 L/ha or1500 L/ha at inner and outer position of tree canopies subjected to three different pruning treatments at the Citrusdal 2 trial site.

		Cima @ 3000 l/ha		Cima @1500 l/ha	
	Pruning treatment	**Inner**	**Outer**	**Inner**	**Outer**
FPC%	**No-pruning**	1.13 cd[1]	2.65 b	1.01 d	4.17 a
	Light-pruning	0.91 d	2.82 b	1.71 c	4.29 a
	Heavy-pruning	0.99 d	2.85 b	1.54 cd	2.83 b
CV%	**No-pruning**	97.89 bc	69.55 e	110.29 ab	68.39 e
	Light-pruning	116.82 a	70.67 e	91.37 cd	70.74 e
	Heavy-pruning	108.19 a	68.70 e	98.21 bc	77.76 de

[1] Means for FPC% and CV% followed by the same letter were not significantly different at $P = 0.05$ (LSD 0.690; 17.167 respectively)

Table 8. Correlation matrix with Pearson correlation coefficients (above the diagonal) and significance values (below the diagonal) between the pre- and post-pruned manual and (LAD) and LiDAR (SR) values as well as spray deposition parameters (FPC%, CV%, ICD%).

Variables	Pruning class	LAD pre-pruning (m2/m3)	Pruned LAD removed (m2/m3)	LAD Post-pruning (m2/m3)	SR pre-prune	SR post-prune	FPC%	CV%	ICD%
Pruning class	**1**	-0.140	0.823	-0.161	-0.291	-0.152	-0.075	-0.186	0.326
LAD[1] pre-pruning (m^2/m^3)	0.414	**1**	0.163	1.000	0.133	0.080	0.243	-0.039	-0.098
Pruned LAD removed (m^2/m^3)	0.823	0.342	**1**	0.139	-0.326	-0.149	0.034	-0.194	0.251
LAD Post-pruning (m^2/m^3)	-0.161	1.000	0.418	**1**	0.141	0.084	0.243	-0.034	-0.104
SR[2] pre-prune	-0.291	0.133	-0.326	0.411	**1**	0.083	0.018	0.054	-0.280
SR post-prune	-0.152	0.080	-0.149	0.084	0.629	**1**	0.179	0.028	-0.239
FPC%[3]	-0.075	0.243	0.034	0.243	0.018	0.297	**1**	-0.734	-0.725
CV%[4]	-0.186	-0.039	-0.194	-0.034	0.054	0.028	<0.0001	**1**	0.409
ICD%5	0.326	-0.098	0.251	-0.104	-0.280	-0.239	-0.725	0.013	**1**

Values in bold are different from 0 with a significance level alpha=0.05

1. LAD (Leaf area density) manually measured

2. SR (Surface ratio) measured by LiDAR

3. FPC% (Deposition quantity)

4. CV% (Deposition uniformity)

5. ICD% (Deposition quality)

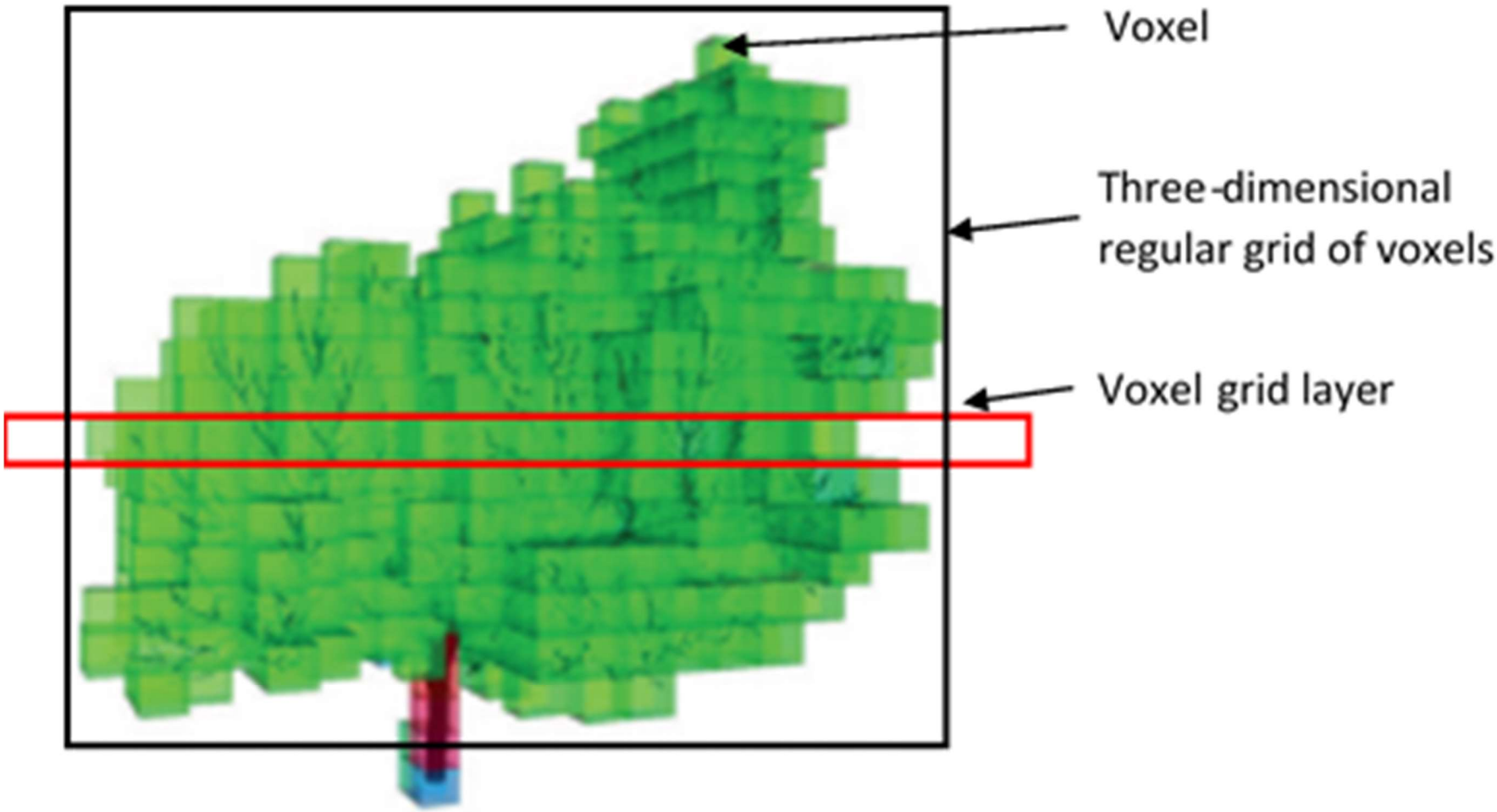

Figure 1. Example of voxelized point cloud of data generated by a tree scanned by LiDAR, demonstrating a single voxel, a three-dimensional regular grid of voxels and a voxel grid layer. (Lecigne, *et al*., 2017)

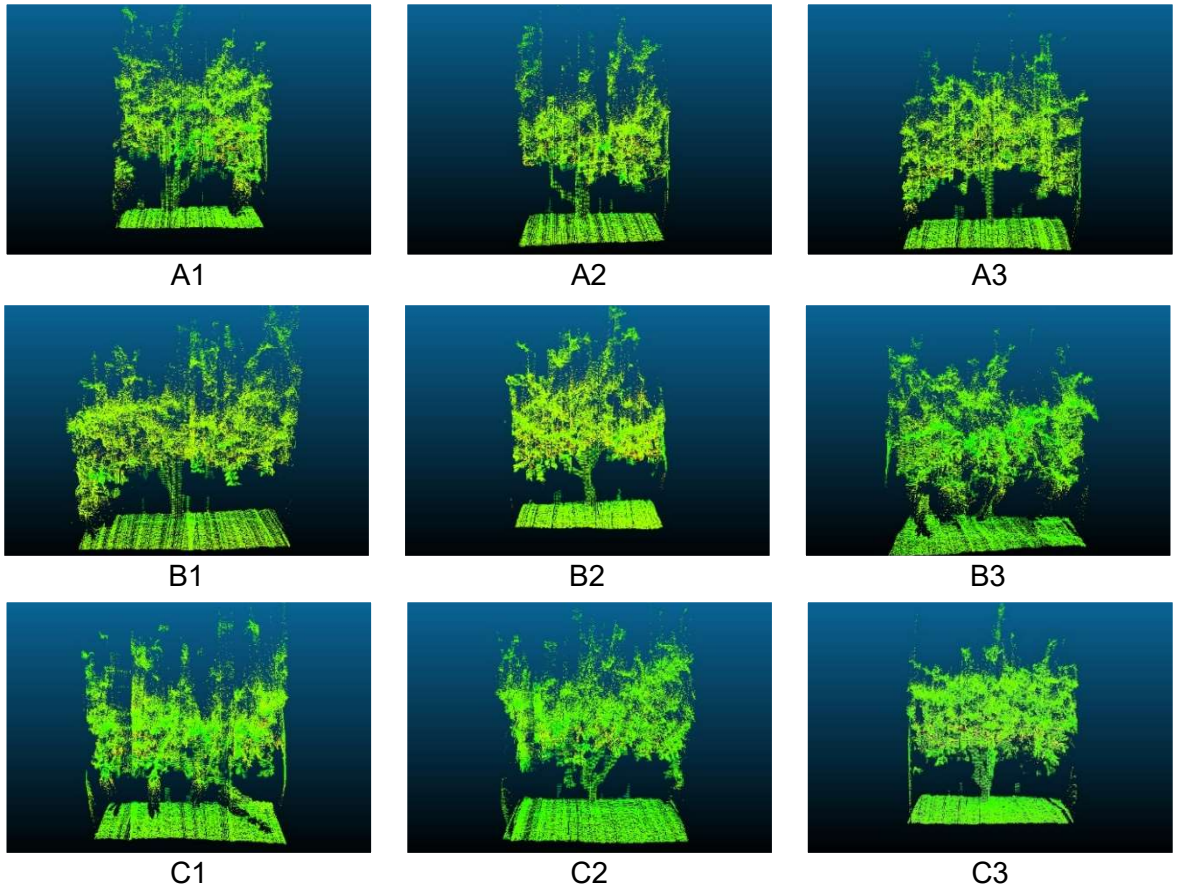

Figure 2. Nules Clementine trees scanned with the LiDAR. Each green point represents a hit and forms part of the point cloud used to determent the SR values. These SR values were compared to the conventional LAD values. SR readings were observed as follows: A1 = 11942, A2 = 13144, A3 = 13272, B1 = 14474, B2 = 16563, B3 = 16680, C1 = 17008, C2 = 21371 and C3 = 21386.