

ENHANCING DECIDUOUS FRUIT AND TREE QUALITY THROUGH THE USE OF VARIOUS FOLIAR APPLICATIONS

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously, in its entirety or in part, been submitted at any university for a degree.

Signature

Date

Dedicated to my mother, Sylvia and my late father, Esslin

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SUMMARY

Fruit trees are exposed to various factors that can adversely affect the production of quality fruit. These factors can directly affect the fruit and the health of the tree and can be classified according to their biotic or abiotic nature, such as pathogenic attacks and drought stress respectively. One of the cultural practices used commercially to address these stresses is the application of fungicides and bactericides. The fruit production industry is under severe pressure from consumers, retailers and environmentalists, locally and internationally, to reduce chemical applications to fruit and fruit trees. The use of natural plant defence elicitor compounds and nutrients offer a potential alternative to fungicide and bactericide sprays and may also increase fruit quality and size as result of a reduction of plant stress. Trials were conducted to evaluate the efficiency of natural plant defence elicitors i.e. salicylic acid (SA) and flavonoids, in addition to mineral nutrients and bactericide/fungicides, on peach (incidence of *Xanthomonas* infection), plum (induced drought stress and Mg/Mn deficiencies) and apple (Mg/Mn deficiencies) fruit and trees against specified biotic or abiotic stress factors.

Trial on *Prunus persica* cv. 'Sandvliet' were conducted over two seasons (2008/2009 and 2011/2012) on a commercial site, Protea Farm, in the Worcester area in the Western Cape Province. During the 2008/2009 season the SA (AlexinTM, AlexiboostTM) containing treatments were applied first at 75% petal drop at concentrations of 125 and 250 ml. 100 L⁻¹. The copper (StCu, Cu)-containing treatment was applied at 50% petal drop, while dichlorophen (XanbacTM) treatments were applied at fruit set at concentrations of 150, 300 and 200 ml. 100 L⁻¹. The

flavonoid (Crophlife™) treatment was applied at the start of petal drop at a concentration of 150 ml.100 L⁻¹. During the 2011/2012 season, a new flavonoid (Cropbiolife™) treatment, as well as potassium (K-Max™) treatment, were incorporated into the trial and applied at concentrations of 150 and 500 ml. 100 L⁻¹ respectively. Additionally a SA (Alexin™) and dichlorophen (Xanbac™) treatments that performed well during the first season, were incorporated into the second season with application times and rates similar to the first season's protocol. In addition to fruit size and quality measurements, the percentage *Xanthomonas* infection was determined on the leaves and fruit of the experimental trees. The SA (Alexin™) containing treatment significantly reduced the incidence of *Xanthomonas* infection on leaves and fruit compared to the control in the first season. However, results varied between the two seasons, as no significant difference from the control could be obtained in the following season. The Alexin™ treatments also significantly increased the fruit size and quality. The flavonoid (Cropbiolife™) and K (K-Max™) containing treatments similarly reduced the *Xanthomonas* infection on leaves and fruit, as well as increasing the fruit size and quality in the second season. The dichlorophen (Xanbac™) containing treatment recorded varying results as it significantly reduced the *Xanthomonas* infection on the fruit only in the second season.

The plum trials were conducted over the 2011/2012 season on 'Laetitia' and 'Songold' plum trees, Welgevallen Experimental Farm, Stellenbosch University. Three SA (Alexin™, AlexSal and Rezist™) containing foliar treatments were applied on the 'Laetitia' trees. Only two SA (Alexin™, AlexSal) containing foliar treatments were applied on the 'Songold' trees. Additionally, a foliar treatment containing only K, Ca, Mg and B, was applied in both the 'Laetitia' and 'Songold' trials. All the treatments were first applied at 75% petal drop, at the same concentration of 250 ml.

100 L⁻¹. Additionally to fruit size and quality, the mineral nutrient content of the leaves and fruit was determined. The ascorbic acid and glutathione content was determined in fruit at harvest and again after storage. None of the treatments had a positive effect on the parameters measured, except the SA (AlexinTM) containing treatments which increased the titratable acidity (TA) in both at harvest and after storage. The treatments also did not alleviate the induced stress compared to the control.

The apple and plum tree trials were conducted over the 2011/2012 season in a semi-closed greenhouse, at the Welgevallen Experimental Farm, Stellenbosch. Magnesium (Mg) and Manganese (Mn) deficiencies were induced in one-year-old 'Royal Beaut' apple and 'Laetitia' plum trees planted in 10 L nursery bags, by omitting these nutrients from a standard Long Ashton soil application. Foliar treatments of Mg (MagMaxTM) and Mn (ManMaxTM) containing sprays were subsequently applied at concentrations of 250 and 75 ml. 100 L⁻¹ respectively, after deficiency symptoms for these nutrients were visually observed. Mineral nutrient analysis of the leaves were analysed on the 13th of February for the plums and 30th of March 2012, for the apples. The Mn (ManMaxTM) containing treatment successfully overcame the Mn induced deficiency. The Mg (MagMaxTM) containing treatment did not overcome the induced Mg deficiency and was probably due to the deficient nitrogen levels in the plants, caused by an error in the initial Long Ashton nutrient solution formulation.

In conclusion AlexinTM, K-MaxTM and CropbiolifeTM have shown their ability to decrease *Xanthomonas* infection in peaches. Additionally to their positive effect on fruit size and quality on the peaches. SA was not able to overcome the induced stress on plums, but had a positive effect on the fruit quality and size. The ManMaxTM been

proven to overcome the induced Mn deficiency, while MagMaxTM was unsuccessful to overcome the Mg deficiency.

OPSOMMING

Vrugtebome word blootgestel aan verskeie faktore wat die produksie van kwaliteit vrugte nadelig kan beïnvloed. Hierdie faktore kan 'n direkte invloed hê op die vrugte en op die gesondheid van die boom en kan geklassifiseer word op grond van hulle biotiese of abiotiese natuur, soos patogeen infeksie en droogte stres onderskeidelik. Van die produksie praktyke wat gebruik word sluit in die toepassing van verskillende swamdoders en bakterisiede. Die vrugtebedryf is onder geweldige druk van verbruikers, die kleinhandel en omgewingsbewustes om die toediening van chemikalieë aan vrugte en vrugtebome te verminder. Die gebruik van natuurlike plant verdediging stimulerende verbindings en nutriënte, bied 'n moontlike alternatief tot die spuit van swamdoders en bakterisiede, en kan ook moontlik 'n bydrae maak tot verbeterde vrugkwaliteit en -grootte. Proewe is uitgevoer om die effektiwiteit van die natuurlike plant verdediging stimulant, salisielsuur (SA) en flavonoïede, addisioneel tot verskillende voedingstowwe en bakterieële / swamdoders op perske, pruim en appels teen *Xanthomonas* infeksie, droogte stres en Mg / Mn tekorte as biotiese en abiotiese stres faktore onderskeidelik te evalueer.

Die *Prunus persica* 'Sandvliet' proewe is oor twee seisoene (2008/2009 en 2011/2012) op 'n kommersiële perseel, Protea Farm, in die Worcester-area in die Wes-Kaap Provinsie, uitgevoer. Gedurende die 2008/2009 seisoen is die SA (AlexinTM, AlexiboostTM) bevattende behandelings eers toegedien by 75% blomblaarval teen konsentrasies 125 en 250 ml. 100 L⁻¹. Die koper (StCu, Cu) bevattende behandeling is toegedien by 50% blomblaarval, terwyl die dichlorofen (XanbacTM) bevattende behandelings toegedien is by vrugset, teen konsentrasies van

150, 300 en 200 ml. 100 L⁻¹. Die flavonoïde (Croplife™) behandeling is toegedien by die begin van blomblaarval teen 'n konsentrasie van 150 ml. 100 L⁻¹. Gedurende die 2011/2012 seisoen was 'n nuwe flavonoïd (Cropbiolife™) en 'n kalium (K-Max™) behandeling toegevoeg tot die eksperiment, met 'n toediening teen konsentrasies van onderskeidelik 150 en 500 ml. 100 L⁻¹. Daarbenewens is die SA (Alexin™) en dichlorofen (Xanbac™) behandeling van die 2008/2009 seisoen herhaal teen dieselfde konsentrasies en toedieningstye soos in die protokol van die eerste seisoen. Behalwe vir die bepaling van vruggrootte en -kwaliteit, is die persentasie *Xanthomonas* infeksie op blare en vrugte ook bepaal. Die SA (Alexin™) bevattende behandeling het die voorkoms van *Xanthomonas* infeksie op die blare en vrugte betekenisvol verminder in vergelyking met die kontrole. Resultate het egter gewissel in die daaropvolgende seisoen en geen beduidende verskille tussen die behandelings is waargeneem nie. Hierdie SA-bevattende behandelings het ook tot 'n toename in vruggrootte en -kwaliteit gelei. Die flavonoïde bevattende behandelings, (Cropbiolife™) en K (K-Max™), het soortgelyke afnames in *Xanthomonas* infeksie op die blare en vrugte in die tweede seisoen getoon, sowel as 'n toename in vruggrootte en -kwaliteit. Die dichlorofen (Xanbac™) bevattende behandeling het variërende resultate getoon aangesien dit slegs tot 'n beduidende afname in *Xanthomonas* infeksie op die blare en vrugte in die tweede seisoen kon lei.

Pruim proewe is uitgevoer in die 2011/2012 seisoen op 'Laetitia' en 'Songold' pruimbome te Welgevallen Proefplaas, Universiteit van Stellenbosch. Drie SA (Alexin™, AlexSal en Rezist™) bevattende blaar behandelings is toegedien op die 'Laetitia' bome. Slegs twee SA (Alexin™, AlexSal) blaar behandelings is toegedien op die 'Songold' bome. 'n Verdere K, Ca, Mg en B blaar behandeling is ook toegedien in beide die 'Laetitia' en 'Songold' proewe. Al die behandelings se eerste toediening

het saamgeval met 75% blomblaarval, teen dieselfde konsentrasie van 250 ml. 100 L⁻¹. Addisioneel tot vruggrootte en –kwaliteit, is die mineraal element inhoud van die blare en vrugte bepaal. Die askorbiensuur en glutatioon inhoud is bepaal in die vrugte met oes asook na opberging. Geen behandeling het 'n positiewe uitwerking op die parameters wat gemeet is getoon nie, behalwe een van die SA (AlexinTM) bevattende behandelings wat die titreerbare sure (TS) verhoog het in beide kultivars. Die behandelings kon ook nie die geïnduseerde stres verlig in vergelyking met die kontrole nie.

Die appel- en pruim proewe is uitgevoer gedurende die 2011/2012 seisoen in 'n semi-geslote glashuis te Welgevallen Proefplaas, Universiteit van Stellenbosch. Magnesium (Mg) en Mangaan (Mn) tekorte is geïnduseer in een-jaar-oue 'Royal Beaut' appel en 'Laetitia' pruim bome, aangeplant in 10L kwekerysakke, deur dié elemente uit 'n toediening van standaard Long-Ashton voedingsoplossing aan die grond weg te laat. Mg (MagMaxTM) en Mn (ManMaxTM) bevattende blaarspuitte is daarna toegepas teen onderskeidelik konsentrasies van 250 en 75 ml. 100 L⁻¹. 'n Minerale analise van die blare is uitgevoer op 13 Februarie, op die pruime en 30 Maart 2012, op die appels. Die Mn (ManMaxTM) bevattend behandeling het die Mn-geïnduseerde tekort verlig. Die Mg (MagMaxTM) bevattende behandeling het nie die geïnduseerde Mg-tekort verlig nie. Dit is moontlik toe te skryf aan die stikstof tekort in die plante wat te wyte was aan 'n foutiewe Long Ashton voedingsoplossing formulاسie wat aanvanklik toegedien is.

Ten slotte het AlexinTM, K-MaxTM en CropbiolifeTM getoon dat hul die vermoë het om *Xanthomonas* infeksie te verminder, asook om vruggrootte en kwaliteit in perskes te verbeter. SA was nie in staat om die geïnduseerde stres op pruime te oorkom nie, maar het 'n positiewe uitwerking op die vruggrootte en kwaliteit gehad. ManMaxTM

het getoon dat dit 'n geïnduseerde, visuele Mn tekort kan oorkom, terwyl MagMaxTM onsuksesvol was om die Mg-tekort te oorkom.

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GENERAL INTRODUCTION

The management of tree health and the production of quality fruit require the management of various factors. These include the adequate management of the trees nutritional status, disease, pests and water status. This is achieved through the application of fungicides, bactericides, foliar feeds, organic substances and fertilizers to the tree and soil (Datnoff *et al.*, 2007; Gupta, 2011). However, in spite of these efforts to ensure better tree health and fruit quality the fruit industry is under severe pressure to reduce the amount of chemicals applied to both the tree and the soil and ultimately the fruit (Urquhart, 1999).

Foliar applications have been used in agriculture for many years, and it is a very useful tool to apply nutrients and chemicals directly to the leaves and fruit (Swietlik & Faust, 1984). A balanced nutrient status has also been shown to increase plant resistance to pests and diseases (Datnoff *et al.*, 2007). Salicylic acid (SA) has shown an elicitor of systemic acquired resistance, a resistance that may be helpful both for disease and stress resistance (Walter *et al.*, 2007; Durner *et al.*, 2007). Flavonoids a phenolic compound, can produce a barrier for pathogen infection through lignification of the cell wall; additionally they also function as antioxidants which scavenge reactive oxygen species (ROS) (Agati *et al.*, 2012). Reactive oxygen species are usually produced when the plant is under stress or pathogen attack. Additionally nutrients such as magnesium (Mg), calcium (Ca), potassium (K), boron (B) and manganese (Mn) play important roles in plants, and may help with resistance against diseases and abiotic stresses (Marschner, 1995; Datnoff *et al.*, 2007).

The increase in the demand for quality fruit and the reduction in chemicals applied to fruit have led to the need for alternatives. The purpose of this study was to evaluate

alternatives for increasing fruit size, quality and tree health on peaches, plums and apples.

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ACHIEVING SYSTEMIC ACQUIRED RESISTANCE THROUGH FOLIAR APPLICATION OF SALICYLIC ACID

INTRODUCTION

Fruit production is becoming more and more challenging as pathogen resistance to chemicals increase (Russel, 1995). Benzimidazoles, dicarboximides and phenylamides are just a few agrochemical classes that are at risk of pathogens developing resistance. The continued pressure from South Africa's main fruit export markets to reduce the amount of chemical applications on fruit is further compounding this problem (Urquhart, 1999).

Salicylic acid (SA) has been found to be an elicitor for the development of the plants own inherited resistance mechanisms (Walters *et al.*, 2007). Systemic acquired resistance (SAR) is a plant response to current and future infection by a pathogen and the stress caused by the infection. Salicylic acid can be applied exogenously to crops in a natural compound making it a sustainable option in fruit production, opposed to the use of chemical alternatives, to increase natural plant resistance and decrease chemical control of diseases. Applying SA as a foliar application has been used successfully to induce systemic acquired resistance in specific cases. Mc Conchie *et al.* (2007) found that SA reduced the occurrence of *Fusarium* rot on rockmelon. Furthermore SA have also been found to increase fruit quality and yield, as Karlidag *et al.* (2009) recorded an increase in yield, total soluble solids (TSS) and fruit colour in strawberries.

Foliar applications have been well established in agriculture as a useful and effective tool for the application of nutrients and chemicals directly to the leaves and fruit (Swietlik & Faust, 1984). A balanced nutrient status has also been shown to increase plant resistance to pests and diseases (Datnoff *et al.*, 2007) and may be an alternative approach to increase SAR in fruit production systems.

This review aims to focus on the mode of action of foliar applied nutrients, the factors affecting the efficacy of foliar applications and to highlight possible links to why balanced nutrition may increase plant resistance naturally. Furthermore, induced resistance, especially relating to SAR, as well as the biosynthesis, signalling and mode of action of SA that induces resistance in plants, will be discussed.

FOLIAR NUTRITION

1.1 Uptake of foliar applied nutrients

The process of mineral nutrient uptake by plant leaves is firstly via penetration of the cuticle and epidermal walls through diffusion. Penetration of the cuticle is hampered by the structure of the cuticle. The cuticle is made up of two layers. The outer layer consists of cutin with epicuticular waxes, while the inner layer is made up of cellulose and pectic substances, encrusted with cutin. The epicuticular wax is hydrophobic, while the cutin is made up of hydrophilic polyesterified hydroxyl fatty acids (Swietlik & Faust, 1984).

Diffusion of the substances through the cuticle is affected by temperature and the concentration gradient across the cuticle. Diffusion of organic compounds is higher than that of inorganic compounds. Kannan & Wittwer (1965) reported that urea

diffused at a rate of 10 to 20 times higher than inorganic compounds. When the nutrients penetrate the cuticle, movement occurs via apoplastic or symplastic transport towards the vascular tissue. The nutrients are then loaded into the phloem, a process which is energy dependent, and subsequently transported out of the leaf or fruit as sources. Alternatively, the nutrients may also be actively transported across the plasmalemma to the leaf cells where incorporation into organic compounds may occur which would allow for easy transportation throughout the plant (Haynes & Goh, 1977).

Nutrients or foliar sprayed chemicals may also enter the leaves via the stomatal pores (Norris & Bukovac, 1968). The stomatal pores are in fact cuticular invaginations and come into direct contact with foliar applications (Norris & Bukovac, 1968). Trichomes are another path by which foliar applied nutrients may enter the leaves; their effect on absorption is a function of the leaf age and plant species (Hull *et al.*, 1975). In addition to these structure that grant access to foliar applied minerals, the presence of pores and canals in the cuticle of the apple fruit that may facilitate uptake of foliar applied nutrients was demonstrated in a study by Miller (1982).

1.2 Factors that affect the uptake of foliar applied nutrients

1.2.1 Environment

The environment can have different effects on the absorption of foliar applied nutrients because it impacts on the development of the cuticle and directly affects physiological processes in the plant (Flore & Bukovac, 1982). Leece (1978) found that as the season progressed, the secondary wax structure increased on the abaxial side of plum leaves. This was ascribed to the associated increasing light intensity that

prevailed towards the end of the growing season. Results from this study thus suggest that the absorption of foliar applications early in the season may be more effective, where leaves are formed under environmental conditions where the thickening of the cuticle may not yet have occurred. In addition, higher temperatures increased both the formation of waxes and leaf expansion in this study on plums, which in turn lead to less wax per unit surface as leaf expansion is faster than the formation of waxes (Leece, 1978). Although such a cuticular structure would allow for better absorption, the stomata are usually closed under higher temperatures, which in turn will lead to a reduction in stomatal absorption. Foliar applications during high temperatures and light intensity conditions may also damage the leaves (Swietlik & Faust, 1984) by raising the concentration of the foliar chemicals on the leaves, which in turn cause physical damage (Swietlik & Faust, 1984).

1.2.2 Leaf anatomy

Fisher & Walker (1955) showed that phosphate (P) absorption was better in younger than in older apple leaves. This may be due to a less developed cuticle structure in the younger leaves. However, Leece (1978) showed that wax structure development in plum leaves was not determined by the physiological age of the leaves, but rather by the phenological stage of the leaf within the growing season. It was determined that leaves of the same physiological age, where some developed earlier in the season, had no abaxial waxes opposed to those that developed later in the season. The lower surface of a leaf has been observed to absorb greater quantities of product than the upper surface of a leaf, mainly due to a thicker cuticle associated with the adaxial side of the leaf (Cook & Boynton, 1952).

1.2.3 Fruit Crop

The degree of absorption of foliar-applied nutrients differs between different fruit species. Plum leaves are less effective at absorbing foliar applied nutrients than apples and citrus leaves (Swietlik & Faust, 1984). This phenomenon can be attributed to the difference in leaf structure, as plum leaves have a wax layer on both sides of the leaf (Leece, 1978). Additionally the guard cell are also covered with wax, and as mentioned in section 1.1 these natural openings play an important role in the absorption of foliar applied nutrients (Leece, 1978).

1.2.4 Interactions between nutrients

The nutritional status of plants may also play a key role in the uptake of foliar application as many nutrients may have a synergistic effect on one another's absorption (Fisher & Walker, 1955). One example being MgSO_4 sprays, found to alleviate magnesium (Mg) deficiencies in apples with an adequate nitrogen (N) status. The chemical formulation of mineral nutrients within the foliar application is also an important factor that affects nutrient absorption. Fisher & Walker (1955) found that after 24-hours, apple leaves absorbed up to 71% of applied Mg from $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 66% from $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 32% from $(\text{CH}_3\text{COO})_2\text{Mg} \cdot \text{H}_2\text{O}$, 8% from $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 4% from $\text{Mg}(\text{H}_2\text{PO}_4)_2$ respectively. Chelated forms of mineral nutrients have also been showed to increase absorption, and has been attributed to the increased mobility of these nutrients in this formulation (Kannan & Wittwer, 1965).

1.2.5 Solution pH

The absorption of some nutrients by leaves is pH dependent. The optimum absorption of Ca^{2+} for example, applied as a CaCl_2 solution, occurred at a pH 7 in sweet cherry fruit (Lidster *et al.*, 1979). The optimum absorption of urea by apple leaves have been reported between pH 5.4 and 6.6 (Cook & Boynton, 1952).

1.2.6 Surfactants

Surfactants have been developed to increase absorption of foliar-applied nutrients by lowering the surface tension of the applied solution and by reducing the contact angle between the leaf surface and the liquid (Leece, 1976). A contact angle of zero allows for complete wetting and is known as the critical surface tension (Schönherr & Bukovac 1972). Such a critical surface tension of a plum leaf is $22\text{-}24 \text{ mN}\cdot\text{m}^{-1}$, and will facilitate stomatal infiltration due to the low tension (Schönherr & Bukovac, 1972).

1.3 Translocation of foliar absorbed nutrients

According to Bukovac & Wittwer (1957), foliar applied nutrients can be classified into three categories: mobile nutrients, partially mobile nutrients and immobile nutrients. As such potassium (K), sodium (Na), phosphor (P), chlorine (Cl) and sulfur (S) was classified as mobile nutrients; magnesium (Mg), zinc (Zn), copper (Cu), manganese (Mn), iron (Fe) and molybdenum (Mo) as partially mobile nutrients; and calcium (Ca) as an immobile nutrient (Bukovac & Wittwer, 1957). The following examples illustrate the differences in translocation of some of these elements.

Mg is partially mobile and is transported from older leaves to younger leaves and fruit. Studies conducted on 'McIntosh' apples showed that 37 % of the absorbed Mg was found in permanent structures of the tree such as the roots and woody tissues. This indicated that the plants utilize these reserves to promote new growth.

B is considered as partially immobile, seeing that most of the B absorbed by leaves remain in the treated leaves (Chamel *et al.*, 1981). However, in fruit that produces sorbitol such as apple trees, B is mobile.

Low Ca levels in fruits are frequently related to the Ca immobility in the phloem, which challenges the transport of sufficient Ca concentrations to the developing fruit during critical stages (Swietlik & Faust, 1984). This results in many physiological disorders, e.g. bitter pit in apples (Swietlik & Faust, 1984). To reduce the incidence of such physiological disorders, Ca has to be applied directly to the fruit, and not only to the leaves.

Fe is classified as partially mobile, but in sorghum as much as 60 % of leaf absorbed Fe has been recorded to be translocated out of the treated leaf within 50 hours of treatment (Eddings & Brown, 1967). However this high level of translocation has not been found in other species and the norm has been determined to be 25 % (Eddings & Brown, 1967).

1.4 Effect of nutrient foliar applications on physiological processes

Photosynthesis (Pn) is usually expected to increase after foliar application of nutrients. This has been found to be the case when N was applied to apple and peach trees (Swietlik & Faust, 1984). In a study by Swietlik & Faust (1984), the Pn, as well

as stomatal and mesophyll conductance, decreased after foliar application of nutrients on apple seedlings. In this study CaCl_2 had the most negative effect on Pn and stomatal conductance in particular compared to other formulations and physiological processes. Foliar applications of KCl lead to increased stomatal conductance which in turn led to increased transpiration.

Nitrogen fertilization of the rhizosphere has been used for many years to increase vegetative growth (Marchner, 1995). This can be further increased by supplementing soil N application with foliar applications (Fisher *et al.*, 1948). Swietlik & Faust (1984) reported that urea foliar application on nursery plums, apples and pear trees led to increased budding and prolonged the activity of the cambium. Foliar urea sprays has also been found to increase trunk circumference in sour cherry trees however, similar sprays had little effect on the vegetative growth of peaches (Weinberger *et al.*, 1949; Swietlik & Faust, 1982).

Mg foliar sprays have been found to decrease excessive vegetative growth however, Mg levels in trees has to be kept at optimal levels in relation to N to achieve this decrease in vegetative growth (Greenham & White, 1959), to achieve the optimal balance between reproductive and vegetative growth

Boron plays an important role in pollen germination and pollen tube growth (Batjer & Thompson, 1949). Sufficient B in fruit trees leads to the sufficient germination and subsequently higher yields.

INDUCED RESISTANCE

2.1 What is induced resistance?

Plants are known to be resistant to most diseases in nature, a condition referred to as, non-host resistance (Walters *et al.*, 2007). Plants have also developed mechanisms to defend themselves against pathogens, a state known as induced resistance (Walters *et al.*, 2007). This identification, attack and defence against pathogens, as well as herbivorous insects can be passive and/or active. Passive resistance is a defence mechanism that is located within the plant, such as enforced cell walls. Active resistance rather refers to defence mechanisms that develop after the pathogen attack or an infection occurred.

Active resistance can be either pathogen race-specific or have a broad pathogen spectrum resistance. Race-specific resistance occurs when a plant possesses the resistant gene (R-gene), which codes for a response, that recognizes the matching avirulence (Avr) gene in the pathogen (Walters & Heil, 2007). Race-specific resistance is activated rapidly, which leads to a faster defence response. In the absence of the Avr gene, broad spectrum resistance takes over, also known as polygenic or basal resistance (Walters & Heil, 2007).

Induced resistance can occur systemically or locally. Systemic induced resistance develops away from the point of infection, where local induced resistance develops at the same point as the infection. Three plant hormones have been identified for their central role in induced resistance namely salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). Induced resistance can be divided further into induced systemic resistance (ISR) and systemic acquired resistance (SAR). JA and ET are important signalling compounds for the development of ISR (Fig. 1).

2.1.1 Systemic acquired resistance (SAR)

SAR is effective against a broad range of virulent pathogens which includes fungi, bacteria and viruses (Walters & Heil, 2007). SAR is characterised by the accumulation of SA, which prevents the spread of disease, by causing the formation of necrotic lesions of the infected tissue known as a hypersensitive response.

The accumulation of SA leads to the systemic expression of pathogenesis-related (PR) protein genes (Fig. 1). The mechanism by which these PR genes lead to the expression of resistance is unknown. However, for the expression of the PR genes, the functioning of the NPR1 regulatory protein is required (Walters & Heil, 2007).

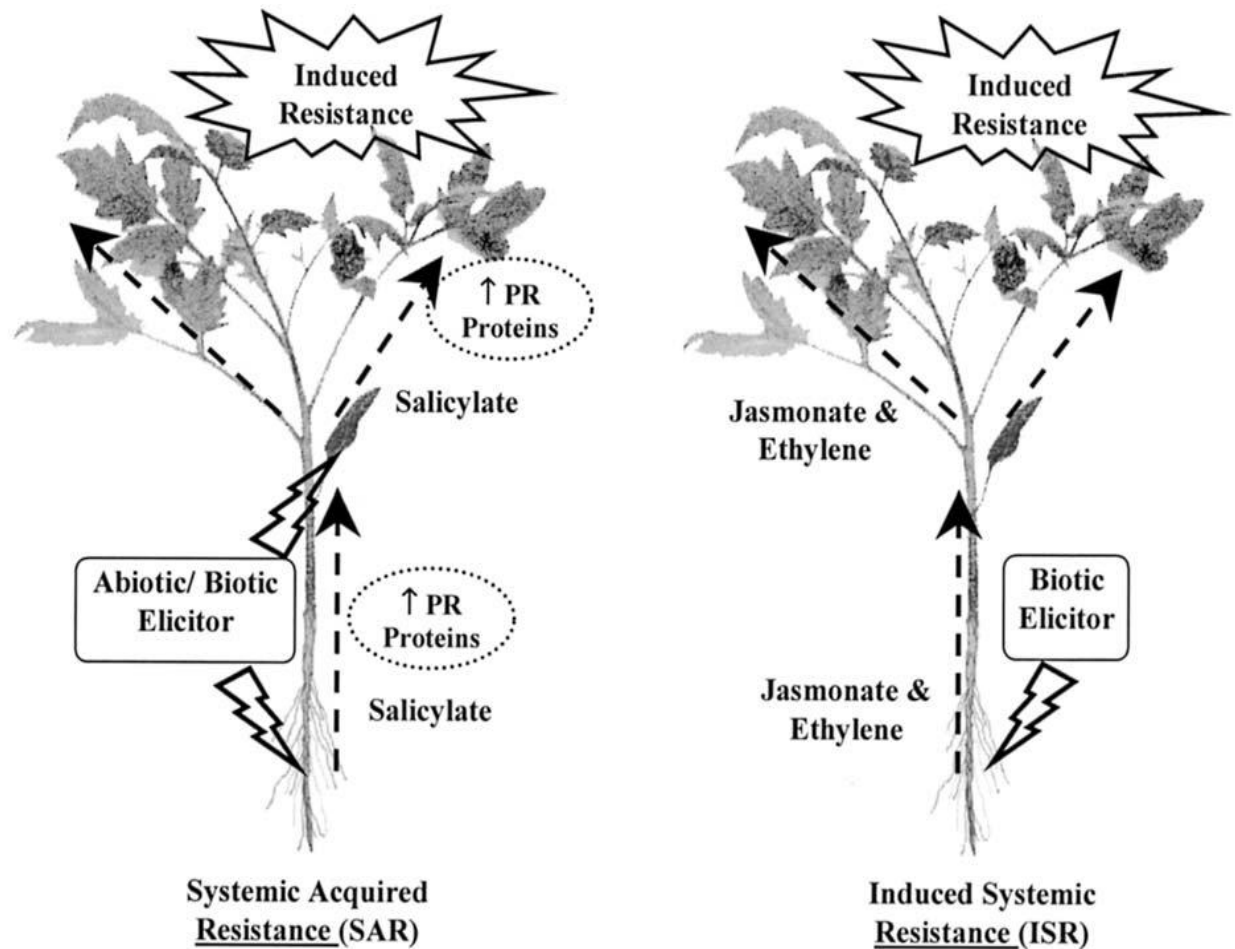


Fig. 1. A comparison between two forms of induced resistance Systemic acquired resistance (SAR) induced by both abiotic or biotic elicitors, resulting in the accumulation of salicylate such as salicylic acid leading to the expression of pathogen related (PR) genes. Induced systemic resistance (ISR) induced by a biotic elicitor, which is a specific strain of plant growth-promoting rhizobacteria, requires the accumulation of jasmonate and ethylene, and is independent of salicylate and the expression of PR genes (Vallad & Goodman, 2004).

2.1.2 Cost of Systemic Acquired Resistance (SAR)

SAR can result in various expenses to the plant and its surroundings such as allocation costs, ecological costs and genetic costs (Walters & Heil, 2007). Plants have limited

resources that have to be divided between growth, reproduction and defence. SAR may result in less allocation of resources for reproduction or growth, e.g. every nitrogen atom that is used to synthesize a PR protein, is one lost for reproduction and growth. This may be justified when the plant is under pathogenic attack, but is unlikely to be the case under normal conditions. In studies carried out by Smedegaard-Petersen & Tolstrup (1985) barley yield decreased 7 % when the crop was exposed to fungi, due to increased respiration to achieve resistance. Studies by Heil *et al.* (2000), where artificial SAR was achieved without a virulent pathogen after applications of ASM (a SAR elicitor agent), showed weaker growth and lower yields was reported in wheat (*Triticum aestivum*, cv. 'Hanno'). Further observations and studies indicated that the intensity of resistance is subject to the availability of resources (Walters *et al.*, 2007).

In addition to the efficacy of SAR against a broad-spectrum of pathogens, plants also rely on mutualistic interactions with micro-organisms to survive (Walters *et al.*, 2007). An example of plants interacting with micro-organisms is the legume family and the nitrogen-fixing *Rhizobia* bacteria, which improves the fertility of soil, and is used for agriculture (Walters *et al.*, 2007). Furthermore Mycorrhizal associations have become very important in modern day agriculture, where these interactions are actively promoted (Walters *et al.*, 2007). Plant-growth promoting rhizobacteria (PGPR) is both beneficial for plant growth and plant defence (Walters *et al.*, 2007). Artificially inducing SAR may have a negative effect on these beneficial interactions as rhizobacteria has to overcome the plant's inherent resistance to establish functioning nodes (Walters *et al.*, 2007). Laboratory studies, which have been widely used to research SAR, have mostly overlooked these mutual interactions (Walter &

Heil, 2007; Walters *et al.*, 2007) however, in the field; these interactions may be of critical importance.

The plant has many resistance mechanisms, many of which are yet to be discovered or deciphered (Walters *et al.*, 2007). The presently known mechanisms are highly interconnected and may have various effects on each other (Walters *et al.*, 2007). SAR is one of these mechanisms in a plant, in response to pathogen attacks. In reaction to this defence mechanism, pathogens also have the ability to respond to the defence adaptations of the plants via evolution and counter-adaptations (Gould, 1991). Agriculture has experienced these counter-adaptations when resistance to many of the regular pesticides, fungicides and bactericides have been introduced by pest and pathogen adaptations which rendered them resistant to existing chemistry.

SALICYLIC ACID AND SYSTEMIC ACQUIRED RESISTANCE

3.1 The role of salicylic acid in Systemic Acquired Resistance (SAR).

Salicylic acid (SA) is a SAR elicitor and essential to achieve local and systemic acquired resistance in plants (Durner *et al.*, 1997). Evidence supporting this hypothesis has shown that the level of SA in both tobacco and cucumber increased several hundred-fold after infection by a pathogen (Malamy *et al.*, 1990).

The analyses of transgenic plants which express the *nahG* gene showed no accumulation of free SA (Gaffney *et al.*, 1993). The *nahG* encodes salicylate hydroxylase, the enzyme that is responsible for catalysis of the conversion of SA to catechol (Gaffney *et al.*, 1993). This conversion causes reduced levels of free SA in the plants, and thus prevents the expression of SAR. This indicated that SA is required for the induction of SAR.

3.2 Biosynthesis of salicylic acid.

Two distinct and compartmentalized pathways have been suggested for the biosynthesis of SA, namely the phenylpropanoid route that initiates from phenylalanine in the cytoplasm and the isochorismate pathway in the chloroplast (Fig. 2). Earlier studies indicated that SA is synthesized from phenylalanine (León *et al.*, 1993). Phenylalanine is firstly converted to Trans-cinnamic acid (t-CA) and this step is catalysed by phenylalanine ammonia-lyase (PAL). Trans-cinnamic acid is then converted to benzoic acid (BA) via chain shortening, followed by hydroxylation in the C-2 position to produce SA (Yalpani *et al.*, 1993). The conversion of BA to SA is catalysed by a cytochrome P450 mono-oxygenase, namely benzoic acid 2-hydroxylase (BA2H) (León *et al.*, 1993). Many possible rate-limiting steps may exist, but the conversion of t-CA to BA seems to be the most likely rate-limiting step (León *et al.*, 1993) (Fig. 2).

The alternative route, SA is synthesized from chorismate which is a product of the shikimic acid pathway (Shah, 2003). Chorismate is converted to Isochorismate via SID2-encoded isochorismate, which is then converted to SA via isochorismate pyruvate lyase (IPL) (Fig. 2).

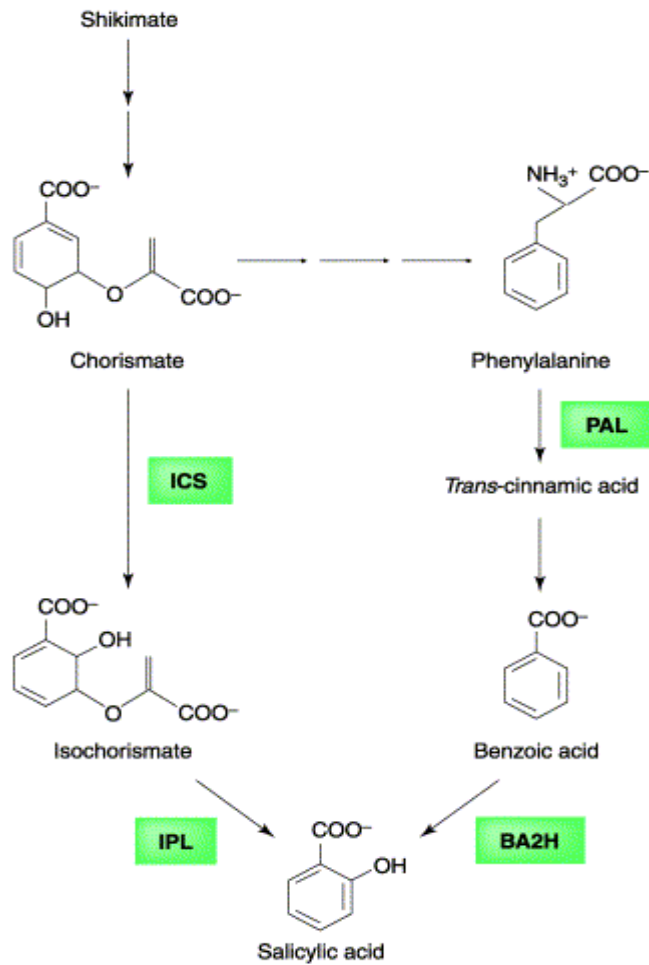


Fig. 2. Proposed pathways for the biosynthesis of SA in plants. In the shikimate pathway: chorismate is converted to isochorismate catalysed by isochorismate synthase (ICS) after which isochorismate pyruvate lyase (IPL) catalyzes the conversion of isochorismate to salicylic acid (SA). Alternatively phenylalanine is converted to *trans*-cinnamic acid catalysed by phenylalanine ammonia lyase (PAL). Secondly *trans*-cinnamic under goes chain shortening to form benzoic acid, after which benzoic acid is converted to SA catalysed by benzoic-acid-2-hydroxylase (BA2H) (Shah, 2003).

3.3 Salicylic acid signalling

In *Arabidopsis thaliana* a signal is produced to initiate the production of SA when a pathogen infection occurs (Shah, 2003). Alternatively, SA production can also be stimulated by the R-gene. Both signals can lead to the development of lesions that are associated with the hypersensitive response. The Enhanced Disease Susceptibility 1 (EDS1) gene is required for both the development of the HR and for the activation of the defence signalling mediated by the toll-interleukin-2 receptor-nucleotide-binding site-leucine-rich repeat (TIR-NBS-LRR) type R-genes. Encoding of the EDS5 and salicylic-acid-induction deficient2 (SID2) genes lead to the biosynthesis of SA. EDS1 and phytoalexin deficient4 (PAD4) are required for basal resistance and for increased SA accumulation in response to increased pathogens attack. SA leads to the activation of pathogenesis-related (PR) gene expression and resistance via two mechanisms. Firstly via the NPR1 required pathway with its associated TGA-element binding protein 2 activates the expression of the PR-1 gene. The other mechanism is where SA with ET, JA and a SFD1 derived lipid leads to the expression of PR-1 genes. These PR genes ultimately lead to the development of resistance against either a pathogen or a stress (Shah, 2003).

CONCLUSION

Foliar application is an effective method to increase both the nutrient content and improving tree health of fruit crops (Swietlik & Faust, 1984). An increasing number of studies have used the foliar application of SA to successfully induce SAR (Karlidag *et al.*, 2009). Understanding the factors that affect the foliar applications has

facilitated with the development of adjuvants, adequate timing of sprays and increased the effectiveness of foliar applications (Swietlik & Faust, 1984).

In terms of research on SAR and SA, most of the investigations were performed in laboratories, disregarding the possible effects of the environment and plant variation under field conditions (Walters *et al.*, 2007). This may result in contradictory results for field trials. The long-term effects of artificially induced SAR via SA may have negative effects on the mutual interactions the plant has with symbionts such as mycorrhizae. This loss of beneficial interactions may lead to a decrease in yield and vegetative growth.

Though several biochemical and molecular studies on the mechanism of SA and its efficacy report on annual crops such as tobacco (Yalpani *et al.*, 1993) and *Arabidopsis*, research on perennial plants is lacking and needs to be addressed. In depth studies into both the signalling and biosynthesis of SA in higher plants may open the door for further use of SA in fruit tree production.

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IMPROVING FRUIT QUALITY AND TREE HEALTH OF *PRUNUS PERSICA* CV. 'SANDVLIET' THROUGH FOLIAR APPLICATIONS

INTRODUCTION

Modern agriculture is a business with the main goal to make a profit. The desire for higher yields is always strongly linked with the demand for better fruit quality to ensure greater financial returns. To obtain export grade fruit have to comply with specifications of size, colour, appearance and taste. To achieve this goal, producers apply fungicides, bactericides, foliar feeds, organic substances and fertilizers to the tree and soil (Datnoff *et al.*, 2007; Gupta, 2011). However, in spite of these efforts to produce high quality fruit for the consumer, the fruit industry is continuously under severe pressure to reduce the amount of chemicals applied to both the tree, soil and ultimately the fruit (Urquhart, 1999). One possible solution to meet this challenge would be a foliar application for the combined purpose of crop protection and enhanced quality.

Flavonoids and salicylic acid have been shown to be important compounds in plant resistance (McConchie *et al.*, 2007; Agati *et al.*, 2012), but the effect of these compounds on fruit size and quality is not very well documented.

Salicylic acid (SA) is an elicitor of systemic acquired resistance (SAR) (Malamy *et al.*, 1990). SA causes an expression of pathogen-related (PR) genes. Products of these genes support the plant in the development of resistance by the production of antimicrobial enzymes and secondary metabolites, also including the cell wall polymers, lignin and suberin as well as phenylpropanoids and phytoalexins (Durner

et al., 1997). Tareen *et al.* (2012) showed that SA as a postharvest application can contribute in the reduction of fruit weight loss, softening and pH. Additionally they found an increase in antioxidant enzymes such as superoxide dismutase, catalase and peroxidase activity. SA has also led to increase in total soluble solids (TSS), titratable acidity (TA), fruit colour and yield on strawberries (Karlidag *et al.*, 2009). Flavonoids produce a physical barrier for pathogen infection through lignification of the cell wall; additionally they also function as antioxidants which scavenge reactive oxygen species (ROS) (Agati *et al.*, 2012). Reactive oxygen species are produced when the plant is under stress or pathogen attack.

Elements included in foliar spray formulations mostly contain Calcium (Ca), Magnesium (Mg), Boron (B) and Potassium (K). These elements have been well documented to play important roles in the metabolic and physiological functions of plants, including in mechanisms of plant defence (Datnoff *et al.*, 2007). The positive effect of K on fruit size in many fruit species has been well documented. In addition potassium has also been reported to facilitate higher disease resistance as Matthee & Daines (1969) found that K led to the reduction in *Xanthomonas pruni* in peaches. Potassium is vital for root growth, water uptake, builds cellulose and regulates up to 60 different enzymes in plants (Datnoff *et al.*, 2007). Ca is well known to play an important role in cell wall development (Taiz & Zeiger, 2010). Research further indicated that Ca is an important element in managing pathogen infections, as Ca have been known to cause a reduction in many important crop diseases (Datnoff *et al.*, 2007). Research by Biggs *et al.* (1994) showed that Ca resulted in a reduction of *Leucostoma personii* disease severity on peach twigs. Magnesium is an essential element in plants as it is structurally important to the chlorophyll molecule and plays a vital role in photosynthesis, and thus plant health and the ability to produce quality

fruit. On the other hand Fulmer (1918) as cited by Datnoff *et al.* (2007) found that Mg is also important for microbial growth in soil, but although this is the case, Mg has correspondingly led to reduction in certain crop disease (Datnoff *et al.*, 2007). Boron is listed as an essential micro-element in fruit crops; yet its role is not fully understood. It is however now recognized that B plays an important role in primary cell wall activity where borate forms stable esters with cell wall saccharides such as mannose and its derivatives to maintain structural integrity of cell walls. Boron is also known to be involved in cell division and cell elongation as well as pollen tube growth in fruit (Marschner, 1995). Many research reviews showed that B reduced pathogenic infections, including that of *Xanthomonas* in cauliflower (Kumar & Sharma, 1997).

Xanthomonas is a bacterial disease that has grown in economic importance in the stone fruit industry in South Africa and the rest of the world (Labuschagné, 1994). The disease was first reported in South Africa in 1956 and 1958 in the Villiersdorp, Franschhoek, Ceres and Stellenbosch areas, from where it spread to all stone fruit producing areas (Du Plessis, 1988). The disease is a sporadic occurring disease in South African stone fruit producing areas and is prominent under wet, wind-driven rain conditions (Du Plessis, 1988). The disease is most severe and common in areas with sandy soils under humid and warm conditions (Battilani *et al.*, 1999). This disease causes small spots on fruit which further develops into sunken and dark brown, black lesions (Du Plessis, 1988). As fruit growth continues, these lesions ultimately results in cracks in the fruit. In addition, *Xanthomonas* causes lesions on the leaves, which eventually leads to chlorosis and defoliation. Not only is there a direct loss of fruit production due to cosmetic damage, but the defoliation early in the season leads to a reduction of fruit size and inevitably, fruit quality (Werner *et al.*,

1986). This disease causes summer canker, which forms the primary inoculum for the following season. Bacteria extruded from the surface of these cankers, are forced by splashing water which in turn spread bacteria into natural openings and wounds or scars (Du Plessis, 1988). The control of this disease is further complicated by the banning antibiotics in crop production and the phytotoxicity of many copper compound containing chemicals (Zaccardelli *et al.*, 1992).

The purpose of this study was to determine the efficacy of various commercial foliar formulations to improve fruit quality e.g. fruit mass, diameter, height, total soluble solids, titratable acidity and firmness to enhance tree health and reduce the severity of *Xanthomonas* infections of *Prunus persica* cv ‘Sandvliet’.

MATERIAL AND METHODS

Plant material

The trial were conducted over two seasons (2008/2009 and 2011/2012) on a commercial site, Protea Farm, in the Worcester area in the Western Cape Province, South Africa (33° 34’ S, 19° 17’ E; ca. 461 m a.s.l).

‘Sandvliet’ peach trees planted in 1990, on a Kakamas rootstock with a tree spacing of 5.0 x 2.0 m, were selected for the experiment. The orchard was irrigated for 6 hours per week during the summer, with 26 liters per hour delivered per micro jet. Fertilizing and pest and disease control were applied according to commercial practice. Fruit was hand thinned at the end of September according to commercial standard at 100 fruit.100 mm⁻¹ stem diameter. The fruit was harvested on 1st of February 2009 during the 2008/2009 season (season 1), which was the commercial

harvest date. During the 2011/2012 season (season 2) the fruit was harvested on the 16th of January 2012 on the commercial harvest date.

Standard orchard management continued throughout the experimental period, but with the exception of those applications that could counter bacterial and fungal diseases or could influence fruit size such as K applications was omitted.

Experimental design and treatments

The experimental design was a randomized complete block design with twelve treatments and four replicates, where a single tree represented a block. Buffer trees were used within the row and between rows to reduce spray drift between treatments. During the first season twelve different treatments were applied at different intervals and concentrations as indicated in Table 1. All treatments were applied as foliar sprays using a Stihl mist blower (SR420, Germany) until the point of runoff in the mornings, following the manufacturer's recommendations for the ideal rate, interval and tree physiological stage. The products used for the treatments were supplied by UAP Crop Care (now Nulandis, Johannesburg, South Africa). Alexin-125-28 [product-rate (ml.100L⁻¹)-interval (days)], Alexin-250-28, Alexin-250-14, Alexin-250-infection period and Alexiboost-250-14 were first applied at 75% petal drop (19th of August 2008). Cu-300-14 + Alexin-250-14, StCu-150-14 and Cu-300-14 were first applied at 50% petal drop (12th of August 2008), while Xanbac-200-14 and Xanbac-200-14 + Alexin-250-14 was first applied at fruit set (2nd of September 2008). Croplife-150-14 was first applied at the start of petal drop on the 1st of August 2008. The untreated control received a foliar application of water corresponding to dates for Cu-300-14 + Alexin-250-14, StCu-150-14 and Cu-300-14 treatments. AlexinTM is an

organic foliar nutrient complex which contains salicylic acid, Ca, Mg, B and K. Croplife™ is an organic carbon complex which contains phenolic flavonoid compounds. Xanbac™ is a broad spectrum fungicide and bactericide with dichlorophen as active ingredient. ‘StCu’ and ‘Cu’ are both copper (Cu) containing foliar fungicide and bactericides. Alexiboost™ is combination of Alexin™ and Maxiboost™ (Nulandis, Johannesburg, South Africa) which, additional to Alexin™, also contains iron (Fe), zinc (Zn), Cu, molybdenum (Mo), sulphur (S) and the plant growth regulators, cytokinin and auxin.

During the second season, only two treatments from season 1 were re-applied (Alexin-250-14 and Xanbac-200-14) and compared to two new products: Cropbiolife™ and K-Max™ (Table 2). This trial comprised of five treatments and six replicates. The treatments were as previously applied until the point of run-off using a Stihl mist blower (SR420, Germany). All foliar treatments were applied in the early mornings between 8 am and 11 am. Buffer trees were used within the row and between rows to reduce spray drift between treatments. Cropbiolife™ was first applied at the start of petal drop (10th of August 2011), while Alexin™ was first applied at 75% petal drop (29th of August 2011). Xanbac™ and K-Max™ were applied at fruit set (6th of September 2011), and the control received water on dates that coincided with the Cropbiolife™ application times. Cropbiolife™ is an improved formulation of ‘Croplife’ used during season 1. K-Max™ is a liquid organic K-complex, while Alexin™ and Xanbac™ are the same products used in season 1.

Data collection and sampling

Season 1: Each experimental tree was inspected seven days before commercial harvest for the presence of *Xanthomonas* infection symptoms. Values from 0 to 10 were allocated per tree, 0 indicating a healthy tree with no leaf loss and 10 indicating no leaves on tree and transformed to % *Xanthomonas* infection on leaves. At harvest (1st February 2009), two branches (one on the sun side and the other on the shade side of the tree) was selected and 25 fruit per shoot evaluated visually for signs of *Xanthomonas* infection and transformed to % *Xanthomonas* infection on fruit. Also at harvest, a randomly selected sample of 15 fruit per tree was collected and transported to the Department of Horticultural Sciences at Stellenbosch University. The following fruit quality parameters were determined the following day: fruit mass, diameter, height, firmness, skin colour, as well as the total soluble solids (TSS) and titratable acid (TA). Maturity and quality indexing was done on 2nd of February 2009. The skin colour was determined visually using a grading system. Values of 1, 2 and 3 was assigned to fruit with 1, indicating a greenish background colour, 2 indicating a dim yellowish colour and 3, indicating a yellow ripe coloured fruit. The fruit's height were determined with a Mitutoyo Corporation caliper (CD-6, Japan), while the fruit's diameter (across the seam), mass and firmness (after a small piece skin was removed from opposite sides of the fruit) were determined with a GÜSS fruit texture analyzer (FTA-409, Switzerland). A collective sample of fruit sections per block was then juiced in an AEG electric juicer (DE-107, Germany) and the TSS determined with an Atago Paletta Refractometer (PR-32, Japan). TA of a subsample was determined with a Metrohm electronic titrator (719, Switzerland).

Season 2: Trees and fruit were inspected on the 16th of January 2012, four days before harvest, for the presence of the symptoms of *Xanthomonas* infection, similar to the

protocol during the first season. Additionally, on the 20th of January 2012 (commercial harvest date), 50 fruit per tree was randomly selected and assessed for *Xanthomomas* infection. Additionally in season two the shoot lengths and stem diameters were measured. Four randomly selected shoots per tree were measured to determine representative shoot length. Stem diameters were also determined half way between the orchard floor and first branches. The stem diameter was used to calculate the yield efficiency, by dividing it with the yield. The yield was determined during the harvest period by weighing all the fruit harvested per experimental plot to gain the average yield per treatment. At harvest, a randomly selected sample of 20 fruit per tree was collected and transported to the Department of Horticultural Sciences, Stellenbosch University. Ten fruit per tree was used for maturity indexing at harvest and a further ten fruit per tree stored for 28 days at -0.5°C. Fruit quality parameters were determined as previously mentioned for season 1 at harvest on 17th of January 2012 and after storage, on 15th of February 2012. The TA was not determined during season two. The shriveling was also determined after cold storage; the fruit either showed or did not show shriveling.

Statistical analysis

Data were analyzed using the General Linear Means Procedure (GLM) of the Statistical Analysis Systems (SAS) (SAS Institute Inc., Cary, NC 2004). Means were separated with the least significant difference (LSD) test at $p \leq 0.05$ or $p \leq 0.10$. Logit transformation was performed on all data expressed as percentages, prior to statistical analysis.

RESULTS

Xanthomonas infection of leaves

Season 1: Most of the foliar applications significantly reduced *Xanthomonas* infection on leaves and leaf loss compared to the control (Table 3). The Alexin-250-14 treatment had the lowest incidence of *Xanthomonas* infection, however this treatment did not differ significantly from Alexin-250-28 and Alexin-250-infection period treatments (Table 3). The Alexin-250-14 treatment had a significantly lower incidence of *Xanthomonas* infection compared to the Alexin-125-28, Xanbac-200-14, Xanbac-200-14+Alexin-250-14, Cu-300-14+Alexin-250-14, Alexiboost-250-14, Croplife-150-14, StCu-150-14 and Cu-300-14 treatments. Cu-300-14 + Alexin-250-14, Alexiboost-250-14, StCu-150-14 and Cu-300-14 treatments were not significantly different from the control. The Cu-30-14 treatment had the significantly highest incidence of *Xanthomonas* infection compared to all treatments except for the control which showed a similar infection rate.

Season 2: During the 2011/2012 season, none of the treatments had a significant effect on the *Xanthomonas* infection on leaves compared to the control and one another (Table 4). Little or no leaf loss was recorded due to *Xanthomonas* infection as the incidence of *Xanthomonas* in this season was much lower compared to the previous season. The lowest incidence in the first season (Table 3, Alexin-250-14) was equivalent to the highest percentage found in season 2 (Table 4, Control).

***Xanthomonas* infection on fruit**

Season 1: Control fruit and fruit that received the Xanbac-200-14 and Xanbac-200-14+Alexin-250-14 treatment had significantly higher *Xanthomonas* infection rates compared to the other treatments (Table 3). Fruit harvested from trees treated with Alexin-250-28 had the lowest infection rate, but was only significantly different from fruit treated with Xanbac-200-14, Xanbac-200-14+Alexin-250-14 and the control.

Season 2: At harvest, the CropbiolifeTM, K-MaxTM and XanbacTM, treatments resulted in a significantly lower percentage *Xanthomonas* infection on fruit compared to the control and the AlexinTM treatment. The XanbacTM treatment did not differ significantly from the CropbiolifeTM and K-MaxTM treatments, but resulted in a significantly lower percentage *Xanthomonas* infection on fruit than those that received the AlexinTM treatment evaluated at harvest. The K-MaxTM treatment resulted in the highest reduction of percentage *Xanthomonas* infection on sampled fruit, but this was only significantly different from the control (Table 4). No significant differences in *Xanthomonas* infection between treatments were observed on fruit evaluated after 28 days at -0.5 °C (Table 4). For stored fruit, the lowest incidence of infection recorded in season 2 (Table 4, XanbacTM) was more than double than that of the highest percentage found in season 1 (Table 3, Control).

Shoot length and fruit size

Season 1: Fruit mass showed a significant difference between the control and all other treatments, except the Cu-300-14 treatment (Table 5). Alexin-250-14 showed the highest fruit mass, but the fruit mass was not significantly different from that of fruit

which received treatments Alexin-250-infection period, Xanbac-200-14, Xanbac-200-14 + Alexin-250-14, Alexiboost-250-14, Croplife-150-14 and StCu-150-14 respectively. Fruit treated with Alexin-125-28, Alexin-250-28, Cu-300-14 + Alexin-250-14 and Cu-300-14 also did not differ from one another in terms of fruit mass. Fruit showed no significant difference in fruit height between that of the control and treatments Alexin-125-28, Alexin-250-28, Alexin-250-14, Alexin-250-infection period, Xanbac-200-14, Xanbac-200-14 + Alexin-250-14, Alexiboost-250-14, Croplife-150-14 and StCu-150-14 (Table 5). Fruit treated with Cu-300-14 was the only treatment where fruit height differed significantly (shorter) from that of the control. No significant differences were recorded for fruit diameter between fruit harvested from the control and treatments Alexin-250-28, Alexin-250-infection period, Xanbac-200-14, Cu-300-14 + Alexin-250-14 and StCu-150-14. Treatment Alexin-250-14 resulted in the largest fruit diameter, although it did not differ significantly from the fruit diameters resulting from treatments Alexin-125-28, Alexin-250-infection period, Xanbac-200-14 + Alexin-250-14, Alexiboost-250-14, Croplife-150-14 and Cu-300-14 (Table 5). Shoot length was not determined during season 1.

Season 2: The AlexinTM treatment resulted in fruit with the largest diameter, although not significantly different from the XanbacTM, K-MaxTM and CropbiolifeTM treatments. The fruit diameter from the XanbacTM and AlexinTM treatments did however differ significantly from those of the control at the 10% confidence level (Table 6, $p = 0.0845$). Fruit height, mass, yield and yield efficiency showed no significant differences between treatments.

Shoot length showed a significant difference between the control and the other treatments (Table 6), where shoots were significantly longer (18.3 mm) than the other

treatments. The AlexinTM treatment had the shortest shoots (9.42 mm), although not significantly different to that of the CropbiolifeTM, K-MaxTM and XanbacTM treatments (Table 6).

No significant difference was recorded for all parameters (fruit diameter, height and mass) measured after storage (Table 7). However, a general decline in fruit mass, diameter and height, was recorded in stored fruit when compared to fresh fruit. (Tables 6 and 7).

Fruit quality

Season 1: There was no significant treatment effect for TSS and back ground colour of the fruit (Table 8). However Cu-300-14 + Alexin-250-14 and Croplife-150-14 treatments had significantly higher TA compared to the control. Firmness showed no significant difference between the control and the treatments, however Cu-300-14 had a significantly lower firmness compared to the Alexin-125-28, Alexin-250-14, Xanbac-200-14 + Alexin-250-14 and StCu-150-14 treatments (Table 8).

Season 2: At harvest, treatments had no significant effects on the TSS; fruit back ground colour and firmness of the fruit. However, when fruit mass and diameter were used as covariates for fruit firmness, the foliar application AlexinTM significantly reduced the firmness (Table 9). Thus when compensating for difference in mass and diameter, only AlexinTM led to a significantly smaller fruit firmness compared to fruit of the control and other treatments which did not differ significantly from each other and from that of the control (Table 9).

There were no significant differences between treatments for the parameters TSS, fruit colour, shriveling, and firmness (with or without the use of a co-variate) for the stored fruit (Table 10).

DISCUSSION

Xanthomonas infection of leaves

The Cu-30-14 foliar application contains copper as an active ingredient, which in most situations, leads to a decreased disease severity (Datnoff *et al.*, 2007; Swietlik and Faust, 1984). The high incidence of *Xanthomonas* infection recorded when trees were sprayed with Cu-30-14 foliar application was unclear (Table 3). The significant lower levels of infection scored when trees were treated with an Alexin-related product (Alexin-250-14, Alexin-250-28 and Alexin-250-infection period) may be explained by a finding described by Durner *et al.* (1997). In this study the physical barrier of thicker cell walls together with the production of phytoalexins, when applied as AlexinTM, would assist in inhibiting or delaying the infection rate of *Xanthomonas*. In addition, nutrient elements, Ca, K, Mg and B which are included in the formulation of this product may have also contributed in improving the plant cell wall structure as well as supporting photosynthesis. This lowered rate of pathogen infection with Alexin-containing products concur with previous studies (Mathee & Daines, 1969); Sugawara *et al.*, 1981); Datnoff *et al.*, 2007).

The disease was more severe in season 1 than in season 2. The lowest incidence of *Xanthomonas* in season one (Table 3, Alexin-250-14 = 22.5) was equivalent to the highest incidence found in season 2 (Table 4, Control = 21.7). This may be due to the

environmental conditions being more conducive to infections of leaves in season 1, compared to season 2. According to Battilani *et al.* (1999) *Xanthomonas* is more severe under wind-driven rain conditions. Peach leaves are susceptible to *Xanthomonas* infection during the active vegetative growth phase (mid Jul to mid Jan) (personal communication E.L. Mansvelt). During the period before fruit set, season 1 had a higher rainfall associated with higher wind speeds compared to season 2 (Fig. 1 and 3). During season 2, rainfall was lower compared to that of season 1 and rain was not always associated with high wind speeds during the same period (Fig 1 and 3).. The average relative humidity and average temperature was also more conducive to leaf infection during season 1 compared to season 2. Season 1 had a high average relative humidity associated with high average temperature (Fig 5 and 6).

***Xanthomonas* infection of fruit**

The Alexin-250-28 foliar application led to the lowest *Xanthomonas* infection of fruit during season 1, but it did not differ significantly from the other AlexinTM treatments except for the Xanbac-200-14+Alexin-250-14 treatment. Fruit treated with Alexin-250-28 had however significant lower *Xanthomonas* infection rates than the control, Xanbac-200-14 and Xanbac-200-14+Alexin-250-14 treatments. Thus it can be assumed that the inclusion of SA in this foliar product led to the induction of SAR resistance in the fruit (Malamy *et al.*, 1990). The negative results obtained for the Xanbac-200-14 treatment where the highest infection rate recorded in fruit was associated with this treatment could not be explained. This trend was however not repeated in the next season where fruit treated with XanbacTM led to the lowest *Xanthomonas* infection recorded. The reason(s) for the high levels of pathogen

infection recorded on fruit at harvest and at sampling for fruit treated with Alexin™ is unclear. The failure of Alexin™ to provide similar protection against *Xanthomonas* as was observed in the previous season could possibly be attributed to insufficient establishment of SAR, an inadequate number of foliar applications or possibly insufficient uptake due to either environmental or tree physiological conditions. The Alexin™ treatment is a commercially available product, the guidelines used in its application complied with that of the manufacturer (Nulandis, Johannesburg, South Africa). The results can thus not be ascribed to the physiological time or concentration of application. The results obtained in season 2 for both harvest and sampled fruit, indicated K-Max™ and Cropbiolife™ led consistently to lower *Xanthomonas* infection on the fruit during this season, supporting results published by Mathee & Daines (1969) and Agati *et al.* (2012). These treatments managed to achieve similar results to that of the commercially used bactericide/fungicide Xanbac™. The higher *Xanthomonas* infection of harvested fruit compared to that of the sampled and stored fruit may be due to the method of sampling. Harvest fruit was randomly selected and is a more accurate representation of the whole tree, while the sampled and stored fruit was picked up to shoulder height. The efficacy of the foliar application of Alexin™ and Xanbac™ varied between seasons. This could be attributed to the variation in weather conditions, tree nutrient status, and pathogen pressure. The severity of *Xanthomonas* infection on fruit was higher during season 2 opposed to season 1; and was exactly the opposite for the *Xanthomonas* infection on the leaves. This phenomenon may be due to the weather conditions that were more conducive to infections during season 2. *Xanthomonas* infection in fruit is common when humid and warm weather conditions prevail (Battilani *et al.*, 1999). The fruit is susceptible from fruit set (Sep) until colour breaks (mid Jan) (personal communication E.L.

Mansvelt). During season 2 the high average relative humidity was more closely associated with high average temperature (Fig. 6). The rainfall was also higher from November onwards; associated with the moderate wind speeds (Fig. 2 and 4) during the period.

Therefore, the variation in climatic conditions between the two seasons during the critical phases when peach trees are susceptible to *Xanthomonas* may have contributed towards the difference in *Xanthomonas* infection found on the fruit and leaves during these seasons.

Fruit size and shoot length

In both seasons, AlexinTM containing treatments had a positive or non-significant effect on fruit mass, height and diameter measured at harvest. The increased fruit size and mass associated with the AlexinTM containing treatments could partly be ascribed to contribution of K as active ingredient in the formulation. K has been proven to increase fruit size and mass in fruit (Swietlik & Faust, 1984). Mg is also included in AlexinTM formulation and may have contributed to increased photosynthesis capacity, which in turn will facilitate increased fruit size and mass. The increase in fruit mass was not due to the increase in the height or diameter of the fruit as seen in Tables 5 and 6. SA (AlexinTM), K (K-MaxTM) and flavonoids (CropbiolifeTM) could lead to increased cell wall thickness which could explain the increases in fruit mass (Durner *et al.*, 1997; Datnoff *et al.*, 2007; Agati *et al.*, 2012). Both SA and flavonoids stimulates the production of cell wall polymers which causes thicker cell walls that contributed to fruit mass.

The shoot length during season 2 showed that the control had the longest shoots, which could indicate a higher vegetative vigour in these trees. All foliar applications used during this season including Cropbiolife™ and Alexin™ had significant shorter shoot lengths compared to the control treatment. This suggests that foliar application may have resulted in nutrient diversion away from vegetative growth in response to a plant defence response or a more reproductive strategy (Walters and Martin, 2007). This production of shorter shoots may have a negative effect on the subsequent year's yield, as less bearing positions, and subsequently photosynthetic leaves will be available. During storage fruit showed a general decrease in fruit mass, height and diameter, however no significant results between treatments were recorded. This suggests that the effect of the treatments may have dissipated.

Fruit quality

A study by Karlidag *et al.* (2009) found that foliar treatment of strawberries with SA led to an increase in TSS and fruit colour, however no significant results was recorded for the TA. Khan *et al.* (2003) found that exogenous application of SA on corn and soybean lead to an increase in photosynthesis. Hubbard *et al.* (1991) reported that SA as a post-harvest treatment inhibited ethylene production, which leads to the inhibition of the production of sucrose-phosphate synthase and ultimately lower TSS levels. This study however showed no significant results for TSS and fruit background colour for both seasons. The TA results from season 1 showed significant results, contradicting the findings by Karlidag *et al.* (2009), in which there was no significant effect on the TA. Compensating for difference in mass and diameter, Alexin™ led to the significantly smallest fruit firmness (covariate firmness). This finding contradicts

results obtained by Tareen *et al.* (2012). However the latter mentioned results were obtained from post-harvest treatments.

CONCLUSIONS

Results from this study show that AlexinTM-containing foliar applications were effective in controlling *Xanthomonas* infection on both fruit and leaves (tree), irrespective of date of application or concentration in the first experimental season. However this reported efficacy did vary between seasons as AlexinTM did not lead to significant reduction in *Xanthomonas* infection in season 2 compared to the control. The disease resistance mechanisms in plants is very complex (Durner *et al.*, 2007), and establishing systemic required resistance may require more than just the application of selected nutrients and SA. Additionally trade-offs may take place when SAR is established such as the production of physical barriers as opposed to the antimicrobial compounds. It is therefore advised that nutritional status of plants should be optimal. Also, when administering foliar applications, the time of day should be carefully considered as better uptake occurs in the early morning and late afternoon. The effect of both AlexinTM and CropbiolifeTM as a post-harvest treatment in addition to pre-harvest could be evaluated. Furthermore, it is important to establish the longevity of SAR in fruit trees and the significance of the correct timing of applications, synchronized with specific tree phenological stages. Interestingly, results from this study, both with K-MaxTM and AlexinTM applications, confirmed K as an element important for disease resistance. XanbacTM, as expected, also reduced the severity of *Xanthomonas* infections, but the efficacy can vary between seasons.

The fact that AlexinTM, CropbiolifeTM and K-MaxTM had positive effects on fruit size and mass, are additional motivation for their continued use in commercial orchards. A further two consecutive seasons would be required to confirm the effect of these foliar sprays on yield and fruit size as a reduction in growth vigour of the current season may influence the reproductive yield of the next season. A more clear understanding of the cost associated with SAR is needed as this will be an important factor to consider when tree nutrition strategies are established.

In conclusion the establishment of plant disease resistance can be induced with exogenous application of SA however the results may vary between seasons, thus the long term effects of SA on fruit quality and tree health still requires attention.

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Table 1: Rate, intervals and number of foliar applications on *Prunus persica* ‘Sandvliet’ during the 2008/2009 season.

Treatment	Rate (ml.100L ⁻¹)	Interval (days)	Number of applications
Control		14	4
Alexin-125-28	125	28	4
Alexin-250-28	250	28	4
Alexin-250-14	250	14	4
Alexin-250-infection period	250	Infection period	7
Xanbac-200-14	200	14	7
Xanbac-200-14+Alexin-250-14	200 + 250	Alternate every 14	7
Cu ²⁺ -300-14+Alexin-250-14	300 + 250	Alternate every 14	4
Alexiboost-250-14	250	14	6
Croplife-150-14	150	14	4
StCu ²⁺ -150-14	150	14	1
Cu ²⁺ -300-14	300	14	1

²StCu and Cu are coded due to trade sensitivity

Table 2: Rate, intervals and number of foliar applications on *Prunus persica* ‘Sandvliet’ during the 2011/2012 season.

Treatment	Rate (ml.100L ⁻¹)	Interval (days)	Number of applications
Control		28	3
Alexin TM	250	14	4
Xanbac TM	200	14	7
K-Max TM	500	14	3
CropbioLife TM	50	28	3

Table 3: The effect of foliar treatments on the incidence of *Xanthomonas* infection of ‘Sandvliet’ peaches in the 2008/2009 season.

Treatments	<i>Xanthomonas</i> on leaves (%)	<i>Xanthomonas</i> on fruit at harvest (%) ^x
Control	47.5 ab ^z	16.0 a
Alexin-125-28	35.0 de	4.00 bc
Alexin-250-28	29.2 ef	0.50 c
Alexin-250-14	22.5 f	3.00 bc
Alexin-250-infection period	23.3 f	2.00 bc
Xanbac-200-14	34.2 de	13.5 ab
Xanbac-200-14+Alexin-250-14	34.2 de	6.00 ab
Cu-300-14+Alexin-250-14	45.0 bc	3.00 bc
Alexiboost-250-14	44.2 bc	1.50 bc
Croplife-150-14	37.5 cde	5.00 bc
StCu-150-14 ^y	39.2 bcd	4.50 bc
Cu-300-14 ^y	54.2 a	2.00 bc
LSD	8.6760	
p-value	<.0001	0.0473

^xLog transformation was carried out on data, the % values shown are the untransformed data

^yStCu and Cu are coded due to trade sensitivity

^zLetters different within a column indicate significance at the 5% level

Table 4: The effect of foliar treatments on the incidence of *Xanthomonas* infection of ‘Sandvliet’ peaches in the 2011/2012 season.

Treatments	<i>Xanthomonas</i> on leaves (%)	<i>Xanthomonas</i> on fruit at harvest (%)	<i>Xanthomonas</i> on fruit at sampling (%)	<i>Xanthomonas</i> on fruit after 28 days cold storage at -0.5°C (%)
Control	21.7 ns ^z	53.0 a ^x	52.0 a	11.7 ns
Cropbiolife™	15.0	40.3 bc	32.4 b	15.0
K-Max™	11.7	40.0 bc	18.3 b	6.67
Xanbac™	18.3	35.2 c	25.0 b	13.3
Alexin™	15.0	50.7 ab	36.7 ab	15.0
LSD	8.9045	12.566	18.554	0.1317
p-value	0.2169	0.0839 ^y	0.0133	0.6677

^x Letters different within a column indicate significance at the 5% level

^y Significant at $p \leq 0.10$; ^z ns non-significant at the 5% level

Table 5: The effect of foliar treatments on the fruit size characteristics of ‘Sandvliet’ peaches in the 2008/2009 season at harvest.

Treatments	Mass (g)	Height (mm)	Diameter (mm)
Control	98.4 e ^z	64.9 abc	56.8 d
Alexin-125-28	111.1 d	65.2 abc	59.5 ab
Alexin-250-28	118.5 bcd	65.6 ab	57.4 cd
Alexin-250-14	132.2 a	65.7 ab	60.0 a
Alexin-250-infection period	125.3 abc	65.9 a	58.5 abcd
Xanbac-200-14	123.7 abc	65.6 ab	58.0 bcd
Xanbac-200-14+Alexin-250-14	125.0 abc	65.8 a	58.5 abc
Cu-300-14+Alexin-250-14	114.5 cd	64.3 cd	56.9 cd
Alexiboost-250-14	123.7 abc	65.2 abc	58.6 abc
Croplife-150-14	128.4 ab	64.9 abc	59.4 ab
StCu-150-14 ^y	123.2 abc	64.6 bcd	56.9 cd
Cu-300-14 ^y	107.1 ed	63.6 d	58.5 abc
LSD	11.554	1.1568	1.7923
p-value	<.0001	0.0092	0.0060

^yStCu and Cu are coded due to trade sensitivity^zLetters different within a column indicate significance at the 5% level

Table 6: The effect of foliar treatments on shoot length, yield, yield efficiency and the fruit size characteristics of ‘Sandvliet’ peaches in the 2011/2012 season at harvest.

Treatments	Shoot Length (cm)	Diameter (mm)	Height (mm)	Mass (g)	Yield (kg)	Yield Efficiency (kg.cm ⁻¹ trunk)
Control	18.3 a ^x	58.2 b	66.4 ns ^z	135.4 ns	46.3 ns	1.21 ns
Cropbiolife™	9.88 b	59.6 ab	66.9	141.8	45.3	1.15
K-Max™	11.2 b	60.3 ab	67.8	145.1	46.8	1.26
Xanbac™	10.2 b	60.8 a	68.3	142.2	42.9	1.24
Alexin™	9.42 b	61.1 a	68.9	150.5	53.9	1.41
LSD	3.6249	2.2212	2.2692	13.621	11.004	0.2483
p-value	0.0003	0.0845 ^y	0.1751	0.2667	0.3402	0.3261

^xLetters different within a column indicate significance at the 5% level

^ySignificant at $p \leq 0.10$; ^zns non-significant at the 5% level

Table 7: The effect of foliar treatments on the fruit size characteristics of ‘Sandvliet’ peaches in the 2011/2012 season after 28 days storage at -0.5 ° C.

Treatments	Diameter (mm)	Height (mm)	Mass (g)
Control	56.2 ns ^z	64.4 ns	126.1 ns
Cropbiolife™	57.0	64.4	128.0
K-Max™	57.8	65.0	133.2
Xanbac™	57.0	64.9	128.0
Alexin™	58.4	65.8	133.8
LSD	2.3085	2.0575	10.749
p-value	0.3322	0.6065	0.4837

^zns non-significant at the 5% level

Table 8: The effect of foliar treatments on the fruit quality characteristics of ‘Sandvliet’ peaches in the 2008/2009 season at harvest.

Treatments	TSS ^v (Brix ^o)	TA ^w (%)	Back ground Colour	Firmness (kg)
Control	10.9 ns ^z	0.52 cd ^x	2.75 ns	6.91 abc
Alexin-125-28	12.4	0.57 bc	3.00	7.35 a
Alexin-250-28	11.9	0.56 bcd	2.50	6.98 abc
Alexin-250-14	11.7	0.54 bcd	3.00	7.30 a
Alexin-250-infection period	11.1	0.52 d	3.00	6.69 bc
Xanbac-200-14	10.8	0.54 bcd	2.75	6.70 bc
Xanbac-200-14 +Alexin-250-14	12.2	0.57 bc	2.75	7.32 a
Cu-300-14 +Alexin-250-14	11.6	0.62 a	2.50	6.87 abc
Alexiboost-250-14	11.4	0.54 bcd	3.00	6.85 abc
Croplife-150-14	12.0	0.58 ab	3.00	7.01 abc
StCu-150-14 ^y	11.7	0.55 bcd	3.00	7.15 ba
Cu-300-14 ^y	11.1	0.55 bcd	3.00	6.52 c
LSD	1.1694	0.0439	0.5022	0.5022
p-value	0.1523	0.0103	0.2730	0.0276

^vTSS is total soluble solutes; ^wTA is titratable acids

^xLetters different within a column indicate significance at the 5% level

^yStCu and Cu are coded due to trade sensitivity; ^zns non-significant at the 5% level

Table 9: The effect of foliar treatments on the fruit quality characteristics of ‘Sandvliet’ peaches in the 2011/2012 season at harvest.

Treatments	TSS ^w (Brix°)	Back ground Colour	Firmness (kg)	Firmness (kg) ^y Covariate
Control	11.9 ns ^z	2.00 ns	6.82 ns	6.82 a ^x
Cropbiolife TM	11.6	2.00	6.77	6.77 a
K-Max TM	11.4	1.83	6.70	6.70 a
Xanbac TM	11.4	2.00	6.66	6.66 a
Alexin TM	11.5	1.83	5.68	5.68 b
LSD	0.9612	0.3186	0.9729	0.8776
p-value	0.8247	0.5919	0.1206	0.0407

^wTSS is total soluble solutes^xLetters different within a column indicate significance at the 5% level^yMass and diameter was used as covariates; ^z ns non-significant at the 5% level**Table 10:** The effect of foliar treatments on the fruit quality characteristics of ‘Sandvliet’ peaches in the 2011/2012 season after 28 days storage at -0.5 ° C.

Treatments	TSS ^x (Brix°)	Back ground Colour	Shrivelling	Firmness (kg)	Firmness (kg) ^y Covariate
Control	12.5 ns ^z	1.67 ns	20.0 ns	6.16 ns	6.16 ns
Cropbiolife TM	12.3	1.67	18.3	6.33	6.33
K-Max TM	12.1	1.83	15.0	6.19	6.19
Xanbac TM	10.9	2.00	31.7	6.57	6.57
Alexin TM	11.7	1.83	18.3	5.65	5.65
LSD	1.2889	0.656	22.745	0.8587	0.8886
p-value	0.1452	0.8110	0.6064	0.2867	0.3473

^xTSS is total soluble solutes^yMass and diameter used as covariate; ^z ns non-significant at the 5% level

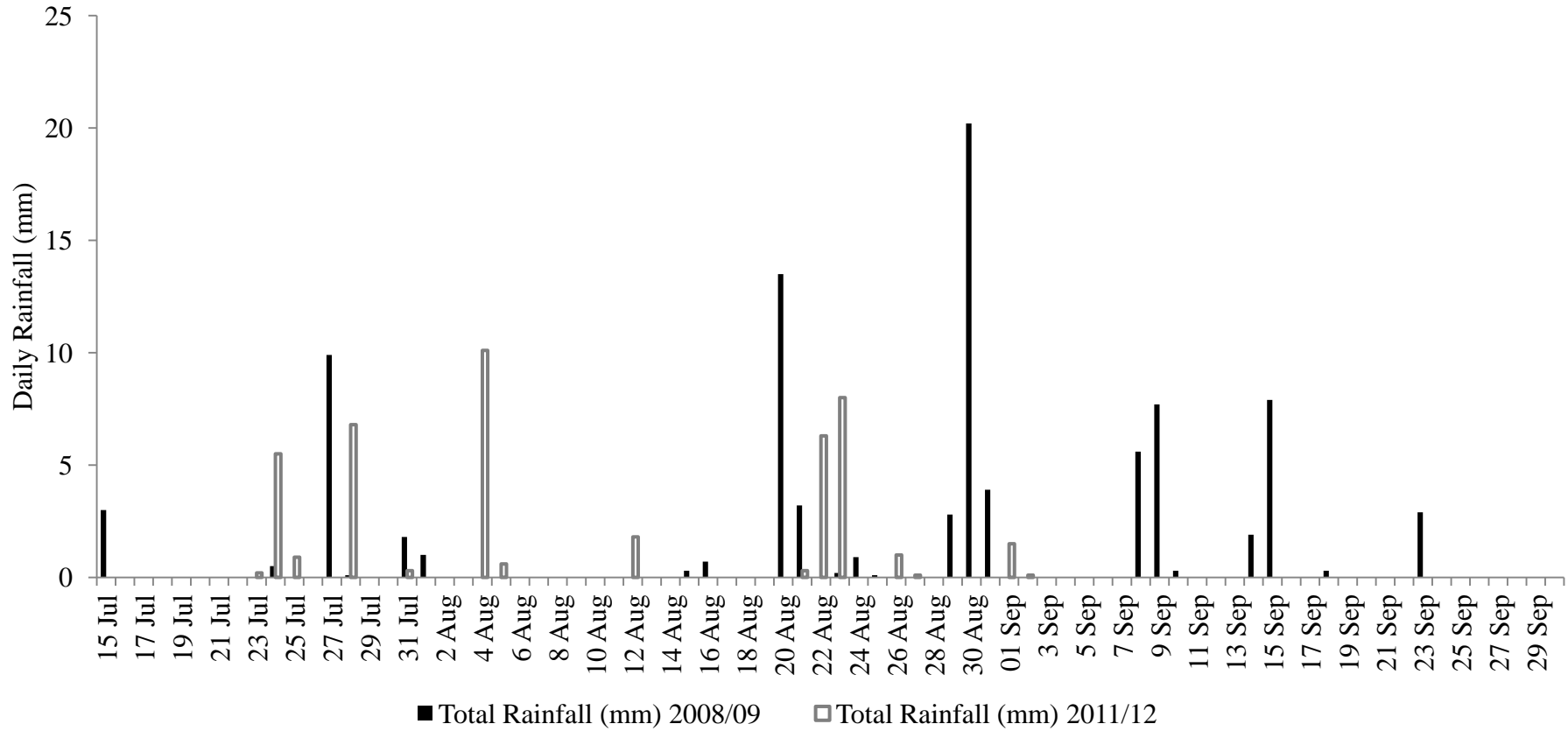


Fig. 1. Total rainfall (mm) for the leaf *Xanthomonas* susceptibility period of the 2008/09 and 2011/12 seasons as recorded by the Nuy weather station, Worcester.

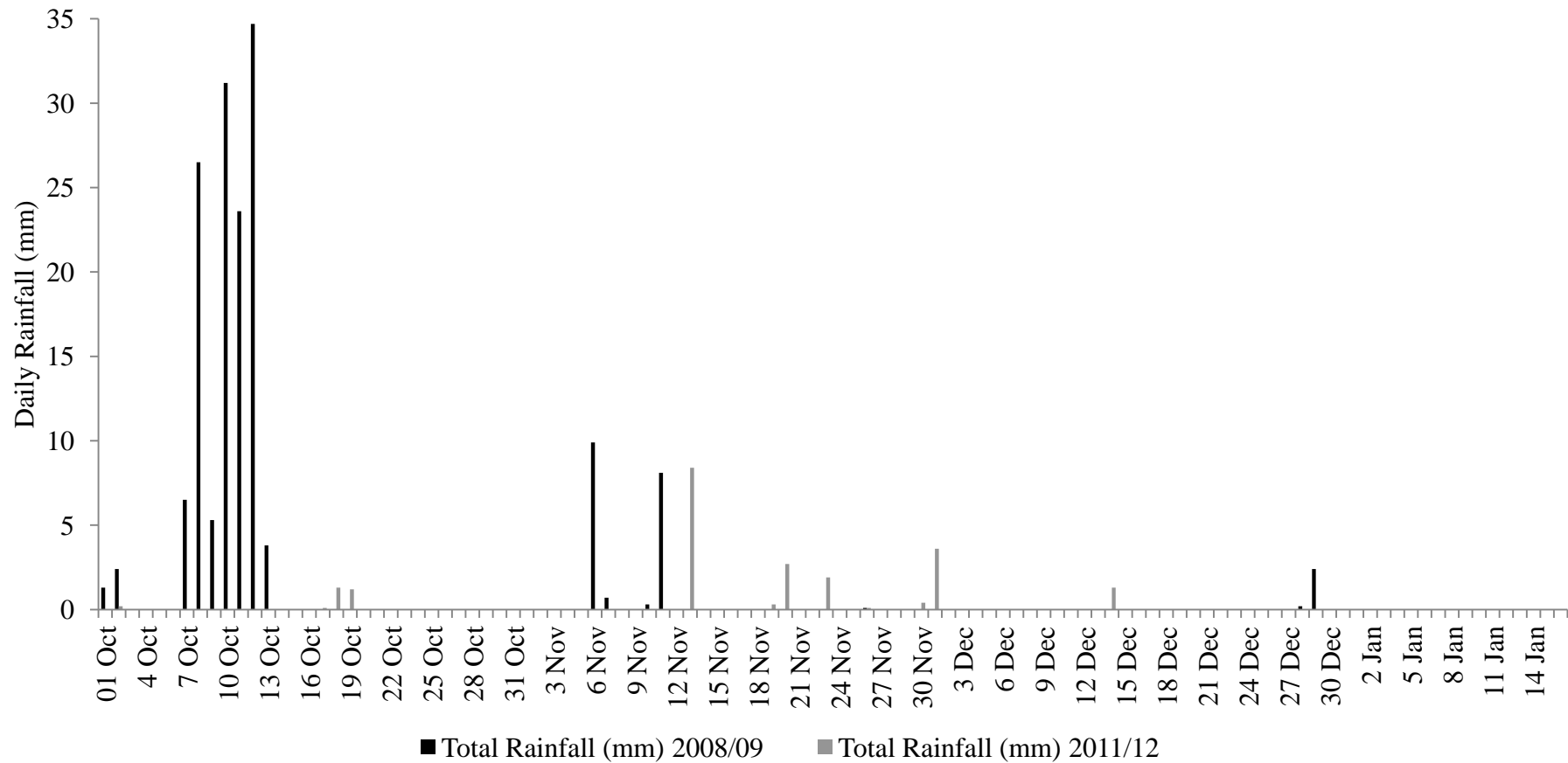


Fig. 2. Total rainfall (mm) for the leaf and fruit *Xanthomonas* susceptibility period of the 2008/09 and 2011/12 seasons as recorded by the Nuy weather station, Worcester.

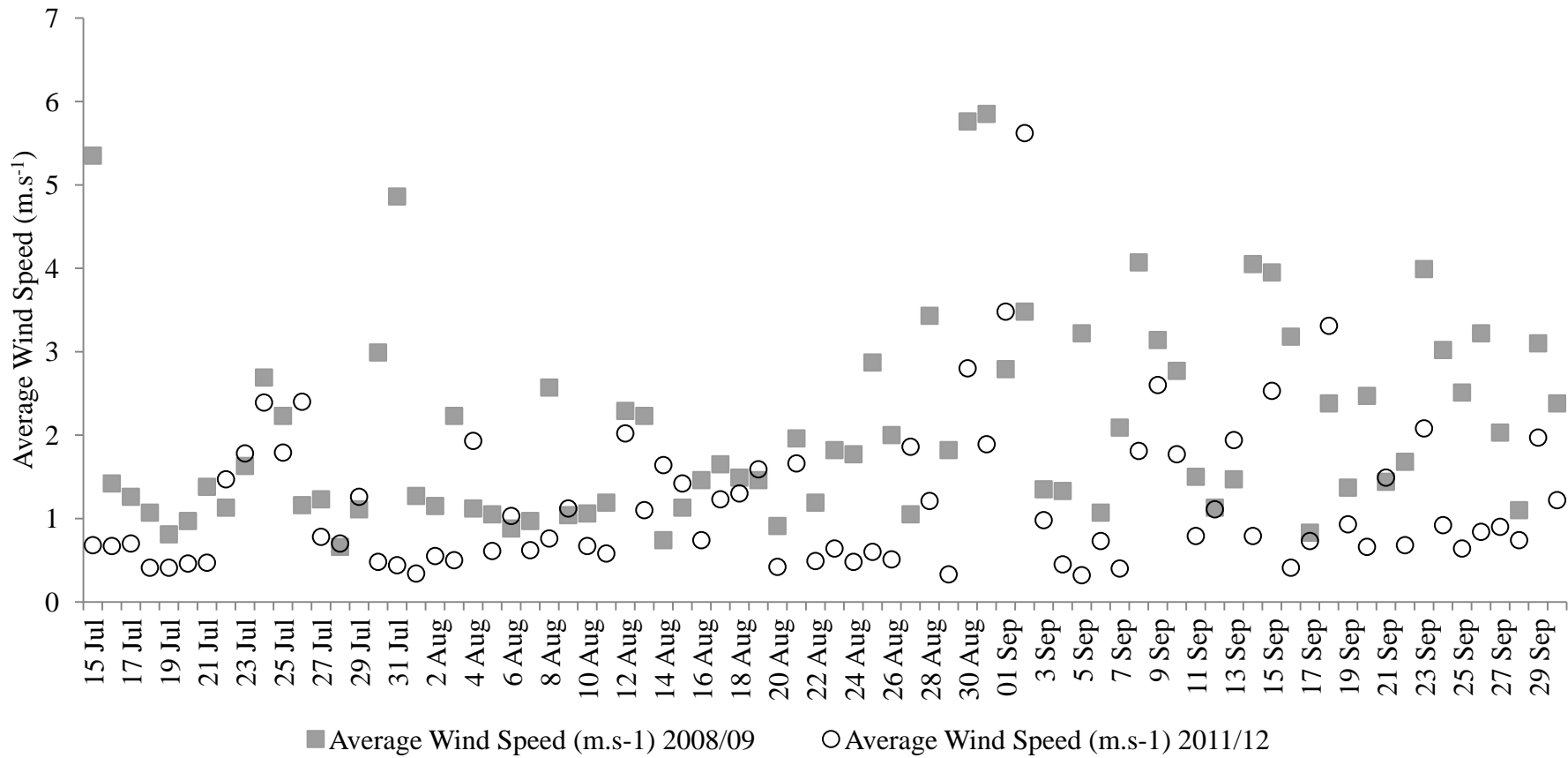


Fig. 3. Average wind speed (m.s⁻¹) for the leaf *Xanthomonas* susceptibility period of the 2008/09 and 2011/12 seasons as recorded by the Nuy weather station, Worcester.

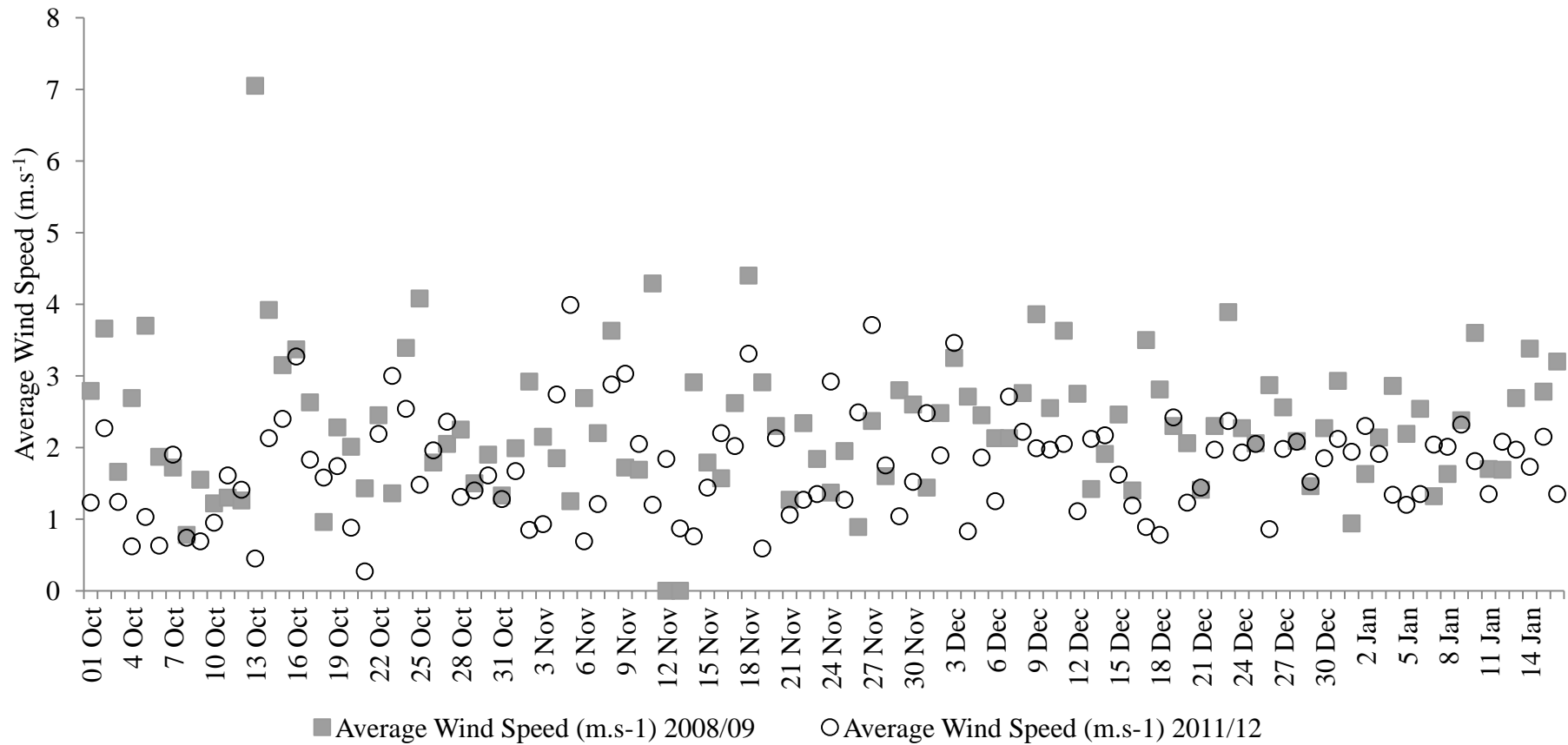


Fig. 4. Average wind speed (m.s⁻¹) for the leaf and fruit *Xanthomonas* susceptibility period of the 2008/09 and 2011/12 seasons as recorded by the Nuy weather station, Worcester.

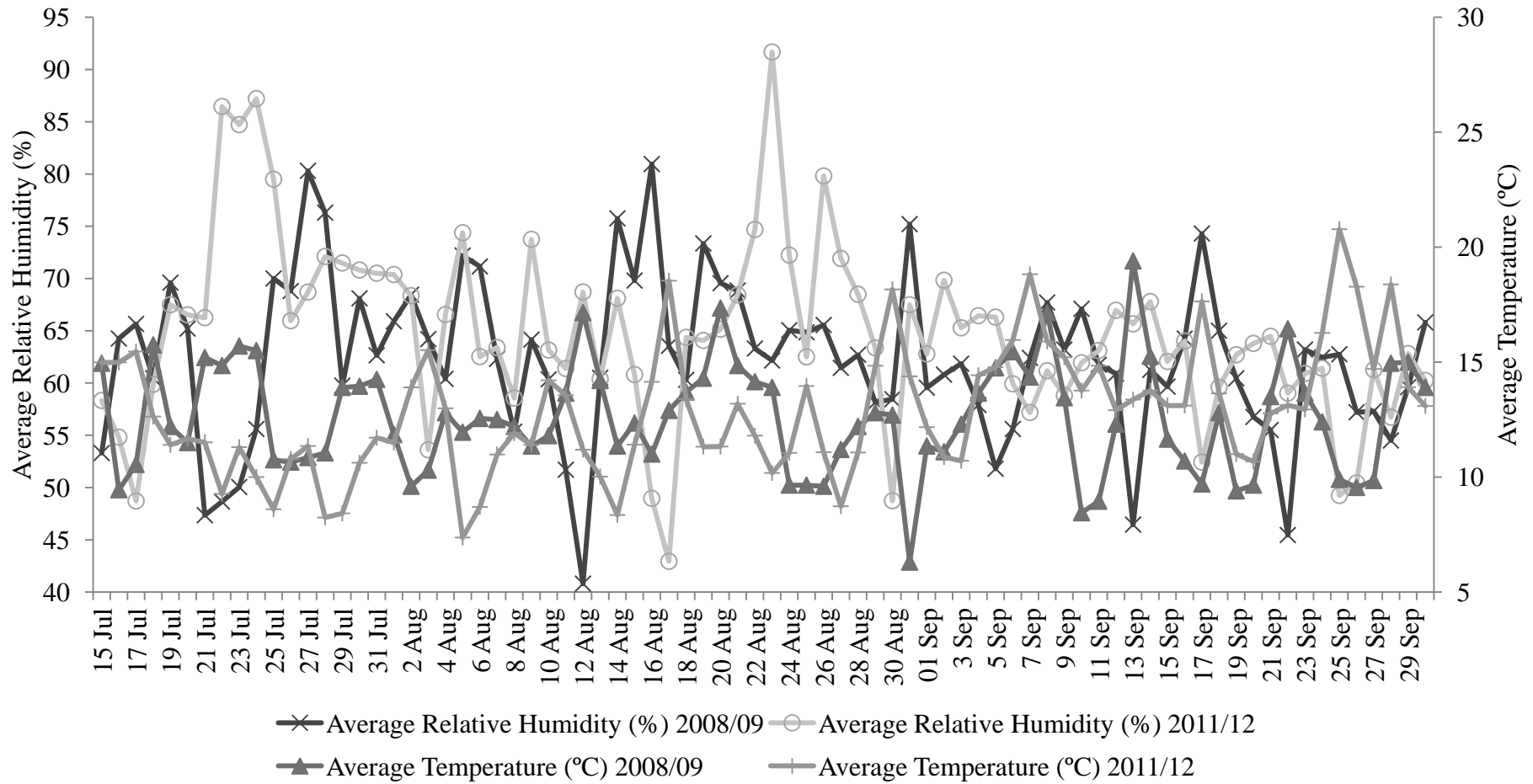


Fig. 5. Average relative humidity (%) and temperature (°C) for the leaf *Xanthomonas* susceptibility period of the 2008/09 and 2011/12 seasons as recorded by the Nuy weather station, Worcester.

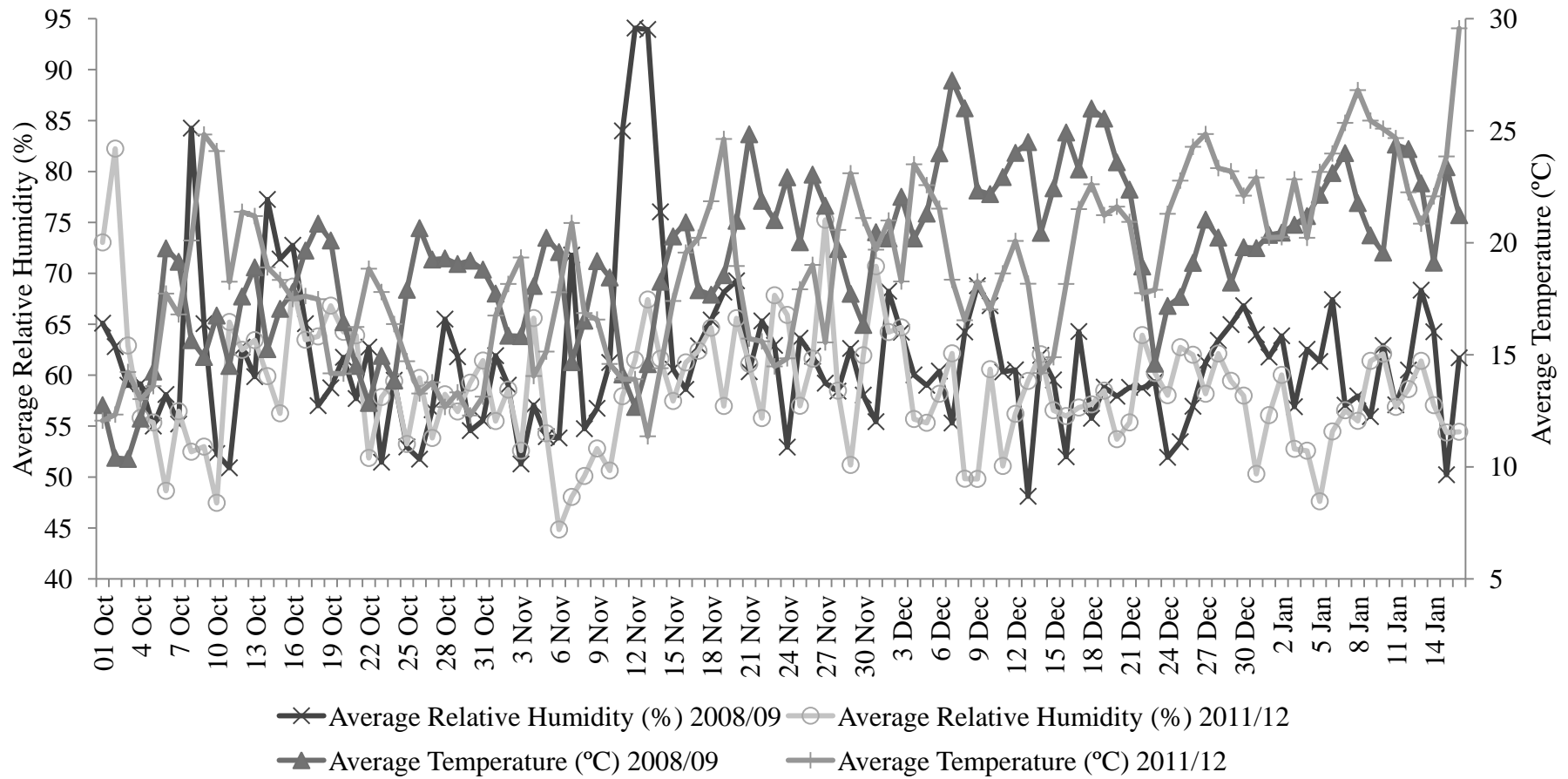


Fig. 6. Average relative humidity (%) and temperature (°C) for the leaf and fruit *Xanthomonas* susceptibility period of the 2008/09 and 2011/12 seasons as recorded by the Nuy weather station, Worcester.

**ENHANCING TREE AND FRUIT QUALITY OF STRESS INDUCED
PRUNUS SALICINA (LINDL.) CV. 'LAETITIA' AND 'SONGOLD'
THROUGH SALICYLIC ACID CONTAINING FOLIAR APPLICATIONS**

INTRODUCTION

Fruit trees are exposed to different environmental stress conditions during their life cycles, which can adversely affect the production of quality fruit. Abiotic stresses such as drought, high temperatures and radiation are just a few that will affect fruit production through a reduction in photosynthesis and increased oxidative stress (Lawlor, 2002). Biotic stresses such as insects, bacteria, fungi and viruses are always present in fruit production. The one factor that is and will be very evident in future in South Africa is drought, which has been attributed as a major limiting factor in agriculture (Reddy *et al.*, 2004). Photosynthesis decreases in drought conditions as the relative water content and leaf water potential decreases (Lawlor & Cornic, 2002). Under these stress conditions, plant cells usually produce reactive oxygen species (ROS), causing oxidative stress (Agati *et al.*, 2012). These ROS are believed to act as messengers to activate defense gene expression (Durner *et al.*, 1997). To produce high quality fruit for the export market, these stresses should be minimized. Some of the options include production practices, such as foliar application with formulations that include nutrients and salicylic acid (SA).

Salicylic acid (SA) is a natural elicitor of plants defence mechanisms. It also causes various physiological effects in plants (Raskin, 1992; Barkosky & Einhellig, 1992). SA may affect the uptake of ions, ethylene biosynthesis, activity of nitrate reductase, membrane permeability, level of cellular ATP and mitochondrial respiration (Glass,

1973; Harper & Balke, 1981; Jain & Srivastava, 1981; Balke, 1985; Leslie & Romani, 1988; Quah, 1992). Additionally Khan *et al.* (2003) found that pre-harvest exogenous application of SA on corn and soybean lead to an increase in photosynthesis. As a postharvest treatment of peaches, SA caused a reduction in fruit weight loss, softening and pH (Tareen *et al.*, 2012). Additionally they found an increase in the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) in peaches. SA has also increased the total soluble solids (TSS), fruit colour and yield on strawberries (Karlidag *et al.*, 2009).

Furthermore, mineral nutrition of plants has shown to be critical in alleviating drought stress (Waraich *et al.*, 2011). Mineral elements that often occur in foliar applied formulations include potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), boron (B), manganese (Mn) and copper (Cu). Potassium is an essential element in plant nutrition and plays a key role in many processes such as the activation of enzymes, protein synthesis, photosynthesis, osmoregulation, cell extension, stomatal movement and also reduces the uptake of Na and Fe under saline and flooded conditions (Marschner, 1995). Calcium has been shown to play an important role in cell wall stabilization, cell extension and secretory processes (Marschner, 1995). Waraich *et al.* (2011) pointed out that Ca plays a role in metabolic activities of plants under stress conditions such as drought through calmodulin. Magnesium is well known as a constituent of the chlorophyll, but it also activates many important enzymes such as glutathione synthase, ATPases and phosphoenolpyruvate (PEP) carboxylase to name just a few (Marschner, 1995). Fisher & Bussler (1988) found that, under Mg deficiency, there was a decrease in the export of carbohydrates from source organs as the sucrose formation was inhibited and starch formation promoted. This accumulation led to a decrease in CO₂ fixation, which caused a buildup of excess

electrons and absorbed energy and ultimately led to the production of ROS and thus oxidative stress. This carbohydrate buildup in source organs causes a decrease in available resources for growth, as Mg deficiency also causes reduced root growth (Marschner, 1995). Zinc was found to increase root growth by increasing auxin levels in the plant (Waraich *et al.*, 2011). The application of Zn also leads to a reduction of ROS, through inhibiting membrane bound NADPH oxidase and increasing superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activity (Waraich *et al.*, 2011). Manganese is required in many physiological processes such as photosynthesis, lipid metabolism, protein synthesis and root growth due to its role in cell division and elongation (Marschner, 1995). Copper is required for lignin formation in cell walls, carbohydrates and nitrogen metabolism (Marschner, 1995; Waraich *et al.*, 2011). Boron is essential for root growth, cell wall formation, pollen tube growth as well as sugar transport through the formation of borate-sugar complexes (Marschner, 1995; Waraich *et al.*, 2011). As water becomes a scarcer commodity around the world and drought more evident (Pimentel *et al.*, 1997), the adequate mineral nutrition and elicitation of resistance to these stress conditions may become more important.

The purpose of this study was to determine the effect of specific foliar applications, namely formulations with SA, nutrients or both, under drought induced stress on the fruit size, quality and tree health of *Prunus salicina* (Lindl.) cultivars ‘Laetitia’ and ‘Songold’.

MATERIAL AND METHODS

Plant material

The trials were conducted during the 2011/2012 season on the Stellenbosch University experimental farm, Welgevallen, in the Stellenbosch area in the Western Cape Province, South Africa. 'Laetitia' and 'Songold' plum trees on Marianna rootstock were used. Both orchards are planted in a Kroonstad soil type (Macvicar *et al.*, 1991) and the trees are planted on a ridge due to shallow depths and potential water logging. The 'Laetitia' trees were planted in 1992 with a tree spacing of 4.5 m x 1.5 m with 'Songold' plum trees as cross pollinator. The 'Songold' plum trees were planted in 1998 with a tree spacing of 4.5 m x 1.5 m, with every second row planted with 'Laetitia' trees as cross pollinator. Standard orchard management continued except for any applications that were similar to the treatments.

Experimental design and treatments

A randomized complete block design was used as an experimental lay out for both trials. Five different treatments were carried out in the 'Laetitia' trial with six replicates (Table 1). Four different treatments were used in the 'Songold' trial with five replicates (Table 2). Standard irrigation was supplied with two micro-jets per tree, with two irrigation cycles of 2.5 h per week. The irrigation was decreased from 60 L.h⁻¹ to 28 L.h⁻¹ from 25th of October 2011 to induce stress in the tree. The different treatments were applied with a Stihl mist blower (SR420, Germany), on the same day and the trees were sprayed until the point of runoff. The first spray was

applied at 75% petal drop on the 11th of October 2011 and the second spray on the 24th of October 2011 with the final spray applied on the 7th of November 2011.

AlexinTM (Nulandis, Johannesburg, South Africa) is an organic foliar nutrient complex which contains salicylic acid, Ca, Mg, B and K. AlexSal (Nulandis, Johannesburg, South Africa) contains only salicylic acid as an active ingredient, while AlexMax (Nulandis, Johannesburg, South Africa) contains Ca, Mg, B and K, with no SA included in this treatment. ReZistTM (Stoller, Houston, USA) is an organic foliar nutrient complex which contains salicylic acid and chelated Cu, Mn and Zn. The applications were applied according to the manufacturer's guidelines, and all formulations were applied in the phenological stages as suggested for fruit trees by Stassen and Stadler (1987).

Data collection and sampling

Eco-physiological data: A DFM (DFM, Pniel, South Africa) continuous logging probe was installed in the tree row, next to a stress induced treatment, to quantify the relative water content and temperature of the soil at depths of 0 – 60 cm. Logging started from the 1st of October 2011 until the end of March 2012. IRGA (Infra-Red Gas Analysis) readings were carried out with a Li-Cor IRGA (LX-6400XT, Nebraska, USA) on both the 'Songold' and 'Laetitia' plum trees to determine rate of CO₂ assimilation. The chamber temperature was kept at 25 °C, with an air flow rate of 300 $\mu\text{mol}\cdot\text{s}^{-1}$, the PAR set at 1500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and the CO₂ set at 350 $\mu\text{mol}\cdot\text{mol}^{-1}$. IRGA readings were taken on the 'Laetitia' just after harvest on the 6th of February 2012, and on 'Songold' on the 14th of February 2012 – where dates resembled mid-summer temperatures. IRGA measurements were carried out to establish if the induced stress

was achieved. Pressure bomb measurements were done on both the ‘Laetitia’ (8th of February 2012) and the ‘Songold’ (13th of February 2012) trees. Pressure bomb measurements were carried out to determine the stem water potential of the trees, as a measure of water stress of the trees. Stem diameters were also determined half way between the orchard floor and first branches, coinciding with the dates for the pressure bomb measurements.

Growth and mineral status: Four randomly selected shoots per tree were measured to determine average shoot length (two on each side of the tree), and coincided with the dates for the IRGA measurements. Leaf samples, 10 leaves per experimental unit, were taken for mineral analyses just at harvest from both cultivars. Mineral analyses for macro and micro elements were performed by a commercial analytical laboratory (Bemlab Pty. Ltd, Strand, South Africa).

Yield and fruit quality characteristics: Fruit samples from both ‘Laetitia’ and ‘Songold’ (1st of February and 9th of February 2012 respectively) were harvested according to industry recommended firmness and total soluble solids (TSS) from every experimental unit (85 fruit per plot), and transported to the Department of Horticultural Sciences at Stellenbosch University. A randomly selected branch from the top third of every experimental tree was harvested to determine sunburn fruit percentage per treatment. All the fruit was harvested and weighed to determine the yield and yield efficiency per treatment. Five fruit from each experimental unit was randomly selected and analyzed for macro and micro nutrient content at a commercial analytical laboratory (Bemlab Pty. Ltd, Strand, South Africa). Maturity and quality indexing for the ‘Laetitia’ and ‘Songold’ cultivars was done on the 2nd of February 2012 and the 9th of February 2012, respectively, on 35 fruit per experimental unit. The following fruit quality parameters were determined: fruit mass, diameter, fruit

firmness and back ground colour, as well as, total soluble solids (TSS) and titratable acids (TA). The ground color was determined with a Unifruco Research Service (Pty.) Ltd colour chart for ‘Songold’ (Set PL.25) and ‘Laetitia’ (Set PL.19) plums. The fruit diameter (across the seam), mass and firmness (after fruit was peeled on both sides) were determined with a GÜSS fruit texture analyzer (FTA-409, Switzerland). The fruit was then juiced in an AEG electric juicer (DE-107, Germany) and the TSS were determined with an Atago Paletta Refractometer (PR-32, Japan) on the pooled juice sample per experimental unit. TA was determined with a Metrohm electronic titrator (719, Switzerland) on a sub sample of the pooled juice sample per experimental unit. Additionally 35 fruit per experimental unit was stored for 42 days at -0.5 ° C regular atmosphere and quality indexing was performed on the 16th of March (‘Laetitia’) and the 23rd of March 2012 (‘Songold’). The same protocol was performed as for fruit evaluated at harvest with the addition of visual examination for internal browning (IB) and shriveling. The IB and shriveling was determined as present or not.

Glutathione and ascorbic acid: Glutathione and ascorbic acid concentration of the fruit flesh was simultaneously determined using High Performance Liquid Chromatography (HPLC) based on the method of Davey *et al.* (2003) with minor modifications (personal communication, M. Jooste). The oxidised and reduced forms of ascorbic acid and glutathione are present in plant tissue. Only the reduced forms of ascorbic acid (L-AA) and glutathione (GSH) can directly be determined on the HPLC. Dehydroascorbic acid (DHA) the oxidised form of ascorbic acid and glutathione disulphide (GSSG) the oxidised form of glutathione needs to be reduced before HPLC analysis can be performed. The total ascorbic acid is thus equal to the sum of L-AA and DHA, while the total glutathione is the sum of GSH and GSSG. The sample extract (Ex) represents the reduced ascorbic acid and the reduced glutathione, while

the reduced sample extract (REx) represents the total ascorbic acid and total glutathione which are analysed in separate HPLC runs. The analysis of the REx thus represents the total ascorbic acid (L-AA + DHA) and total glutathione (GSH + GSSG). The oxidised forms DHA and GSSG are determined by using the following formulas:

$$\text{DHA} = (\text{L-AA} + \text{DHA})_{\text{REx}} - (\text{L-AA})_{\text{Ex}} \quad (\text{Equation 1})$$

$$\text{GSSG} = (\text{GSH} + \text{GSSG})_{\text{REx}} - (\text{GSH})_{\text{Ex}} \quad (\text{Equation 2})$$

During sampling 10 fruit was taken for each cultivar from the 85 sampled fruit. Five fruit for each cultivar was stored for 42 days at -0.5°C , after which it was kept at room temperature (25°C) for 3 days to simulate shelf life and used as evaluation 2. Evaluation 1 refers to the HPLC evaluation carried out at harvest, while evaluation 2 refers to the HPLC evaluation carried out after 42 days storage at -0.5°C and three days of shelf life. During evaluation 1, five fruit per cultivar was peeled using a potato peeler, and flesh cut in to small pieces. The fruit flesh was immediately frozen in liquid nitrogen and stored at -80°C . After storage, the samples were grounded in liquid nitrogen. Once ground, two plastic vials was filled and stored at -80°C until analysis. The same procedure was performed on the evaluation 2 samples. Five grams of the sample was weighed and added to 10 ml of extraction buffer. The extraction buffer was made up of 3% metaphosphoric acid (MPA), 1 mM ethylenediaminetetracetic acid (EDTA) and 2% insoluble polyvinylpolypyrrolidone (PVPP) in MQ H_2O . The extraction buffer was chilled (4°C) and stirred before adding it to the sample. Afterwards 1.8 ml of Ex was centrifuged at 20 000 rcf for 15 min at 4°C in a 2 ml plastic micro centrifuge tube. Subsequently 1 ml of supernatant

was centrifuged again at 20 000 ref for 15 min at 4 °C in a 1.5 mL micro centrifuge tube and 600 µL of the supernatant was pipetted into marked HPLC vials for analysis.

Additionally the reduction of the subsample known as the reduced sample extract (REx) was done by adding 40 µL of Ex with 20 µL of a stock solution of 400 mM DL-dithiothreitol (DTT) in 400 mM Tris base (pH ~ 6 to 6.8) at room temperature and left for 20 min. The reduction reaction was stopped 20 µL of stop solution (8.5% *o*-phosphoric acid). A double volume of this sample was placed in marked HPLC vials to compensate for the dilution of the original sample.

Reverse phase high-performance liquid chromatography (RP-HPLC) analysis was performed by injecting 10 µL of the sample extract (Ex) and 20 µL of the REx immediately after preparation into an Agilent HPLC system (Series 1100, Germany). A photodiode array detector was used and the auto sampler was kept at 4 °C and the column at 16 °C. The system was controlled and data were collected and integrated using the Agilent Chemstation for LC 3D systems software (Rev. B.10.03 (204), Germany). Separations were performed on a 250 x 4.6 mm CapcellPak HPLC column packed with C₁₈ 5-µm stationary phase protected by a 4.6 mm x 12.5 mm Agilent Zorbax guard cartridge (SB-C18, USA). The elution solvents used were: (A) 6 mM *o*-phosphoric acid, 0.1 mM EDTA (to complex metal ions which might promote oxidation of L-AA) in MQ H₂O (pH ~ 2.5) and (B) 6 mM *o*-phosphoric acid, 0.1 mM EDTA in MQ H₂O: acetonitrile (70:30, v/v). The flow rate was 1.0 ml. min⁻¹ and the mobile phase was 100% A (isocratic from 0 to 7 min), 70% A and 30% B (gradient from 7 to 10 min), 100% B (isocratic from 10 to 13 min) and 100% A (isocratic from 13 to 22 min). L-AA was quantified at its UV absorption maximum of 243 nm, while GSH was quantified at 197 nm. The identities of the L-AA and GSH peaks were confirmed by co-elution with authentic standards and by the characteristic absorption

spectra recorded over the range of 195 nm to 300 nm. Concentrations of L-AA and GSH were calculated from standard curves.

The detector was calibrated daily with stock solutions of 0.05 mg mL⁻¹ (w/v) L-AA and GSH that was prepared in a solution of 1 mM EDTA and 2.5 mM dithiothreitol DTT in 3% MPA. Aliquots of 450 µL of the stock solutions were stored at -20 °C.

The concentration of DHA and GSSG were calculated using equations 1 and 2 respectfully after determination of the total L-AA (L-AA + DHA) and total GSH (GSH + GSSG). Results were expressed as µg per g FW.

Statistical analysis

Data were analyzed using the General Linear Means Procedure (GLM) of the Statistical Analysis Systems (SAS) (SAS Institute Inc., Cary, NC, 2004). Means were separated with the least significant difference (LSD) test at $p \leq 0.05$.

RESULTS

Yield and eco-physiological status

‘Laetitia’ trial: None of the eco-physiological or yield-based results showed significant differences between treatments (Table 3). Fig. 1 indicates that the average soil moisture was above the % readily available water limit of 45 % soil moisture, indicating that the plants did not experience water stress during the trial period, in spite of reducing irrigation volumes and the relative high maximum temperatures experienced above 25 °C during January 2012. Water stress would have been

experienced below 25 % soil moisture under these conditions (communication, DFM, Pniel, South Africa). The soil temperature followed the trend of the air temperature.

‘Songold’ trial: This trial recorded no significant differences between treatments (Table 4). Again, the soil moisture in Fig. 1 indicated that the trees experienced waterlogged conditions, as the soil moisture was above the 45 % which is the upper limit for % readily available water.

Fruit size and quality

‘Laetitia’ trial: Data recorded for diameter, mass, firmness, fruit colour and TSS at harvest showed no significant differences between treatments and the control, except for TA (Table 5). None TA results of the treatments differed significantly from the control. The TA differed significantly between the AlexinTM and the AlexSal and AlexMax treatments, with the AlexinTM recording a higher TA compared to the other treatments. After storage, there were no significant differences between treatments for any of the quality parameters, except for TA (Table 7). Again none of the treatment differed significantly from the control. The AlexinTM treatment again recording a higher TA than the AlexSal and AlexMax treatments.

‘Songold’ trial: There were no significant differences between treatments at harvest (Table 6). After storage there were no significant differences between treatments, except for TA (Table 8). The TA was significantly higher in the AlexinTM and the control compared to the AlexMax treatments. TA in the AlexSal and AlexMax treatment did not differ significantly from each other.

Leaf nutrition

‘Laetitia’ trial: Leaf analyses recorded no significant differences between treatments except for nitrogen (N) levels. Only the AlexSal treatment differed significantly from the control, whilst the ReziTM treatment was significantly higher than the AlexSal treatment (Table 9).

‘Songold’ trial: The mineral analysis of the ‘Songold’ leaves as shown in Table 10 reported no significant differences between treatments for any nutrient except iron (Fe). The AlexMax and Control treatments recorded significantly higher Fe concentrations compared to the AlexinTM en AlexSal treatments

Fruit nutrition

‘Laetitia’ trial: Significant differences between treatments were found in fruit for Ca, Mg, Cu and Zn concentrations (Table 11). The control and AlexinTM treatment had significantly higher Ca, Mg, Cu and Zn concentrations, when compared to the AlexMax treatment (Table 11).

‘Songold’ trial: Fruit analyses only recorded significant differences between treatments for Mg (Table 12). There was no significant difference between the control and the AlexinTM and AlexMax treatments. All of the above mentioned treatments had a significantly higher Mg concentration compared to the AlexSal treatment.

Glutathione and Ascorbic acid

‘Laetitia’ trial: There were no significant results recorded for the HPLC evaluation at harvest (evaluation 1) (Table 13) and after 42 days storage at -0.5 ° C and three days shelf life (evaluation 2) (Table 15).

‘Songold’ trial: No significant results were recorded for the HPLC evaluation at harvest (evaluation 1) between treatments (Table 14). After storage and shelf life (evaluation 2), no significant results were recorded except for glutathione disulfide values (Table 16). None of the treatments except the AlexMax treatment differ significantly from the control. The AlexinTM treatment differed significantly from AlexSal and AlexMax treatments.

DISCUSSION

Yield and eco-physiological status

‘Laetitia’ trial: However, none of the treatment in this study resulted in a significant increase in yield and yield efficiency (Table 3) in comparison to each other. This contradicted findings of Karlidag *et al.* (2009) that the exogenous application of SA lead to a significant increase in yield in strawberries. Similarly, Khan *et al.* (2003) which reported an increase in photosynthesis after SA treatment, implying an increase in yield. The longer shoot lengths found in the control (though not significantly different at the 5% level) was not unexpected, as the other treatments may have involved a physiological cost associated with SAR (Walters & Heil, 2007).

The stem water potential values obtained for all treatments can be classified as an indication of stress when using the study of Intrigliolo & Castel (2004) as a criteria

where values between -1.3 to -1.5 at the similar time of the growing season was obtained in plums. The purpose was to induce drought stress, but Fig. 1 clearly shows that the trees experienced waterlogged conditions. Under these conditions the normal transpiration of the trees would be effected. The oxygen in the soil decreases and the CO₂ concentration increases causing a reduction in passive root absorption (Kozlowski, 1984). Kozlowski (1984) also reported that there is an associated decrease in stem water potential under flooded conditions. This would explain the low stem water potential values obtained for all the treatments (Table 3). A CO₂ assimilation value of less than 10 $\mu\text{mol. m}^{-2}.\text{s}^{-1}$ has been reported as an indication of stress in plum trees as these trees usually have a rate of CO₂ assimilation between 14 and 20 $\mu\text{mol. m}^{-2}.\text{s}^{-1}$ (personal communication, S. Midgley). The rate of CO₂ assimilation for all treatments indicated a value lower than 10 $\mu\text{mol. m}^{-2}.\text{s}^{-1}$ implicating that the trees were under stress. Results from this study indicated that none of the treatments was effective to overcome the stress. The treatment have reported of having a positive effect of disease resistance, and assisted in overcoming drought stress (Durner *et al.*, 1997). These treatments have not been evaluated under South African conditions, and may have a effect if applied at higher concentrations or for a longer period.

‘Songold’ trial: Similarly as observed in the ‘Laetitia’ trial none of the treatments led to a significant increase in yield and yield efficiency (Table 4). The treatments were expected to significantly increase both the yield and yield efficiency (Karlidag *et al.*, 2009). Similarly the low stem water potential and CO₂ assimilation rates can ascribed to stress, as Fig. 1 also indicates waterlogged conditions for this trial.

Fruit size and quality

‘Laetitia’ trial: AlexSal contains SA which Khan *et al.*, (2003) found increased photosynthesis in corn and soybean. Karlidag *et al.* (2009) found that in strawberries, SA significantly increased the TSS, however no significant results was recorded for the TA. In this study the TSS was not significantly affected, however the TA was significantly affected although not significantly different from the control, in fruit both at harvest and after storage (Tables 5, 7). Values for TA however varied inconsistently between the various SA-containing treatments formulations, with AlexSal showing lower TA values than the AlexinTM formulation. The SA containing treatments was not able to significantly increase the TA compared to the control. The stored fruit showed a similar trend for the TA, indicating that the nutrients in combination with SA (AlexinTM) led to a positive increase in TA opposed to a treatment which only consists of SA (AlexSal) or nutrients (AlexMax) respectively.

‘Songold’ trial: ‘Songold’ plums generally have a higher TSS than the ‘Laetitia’ plum; however there were no significant results between treatments for the TSS values (Table 6). In fact, none of the measured parameters was significantly affected by the treatments. As observed in the ‘Laetitia’ trial, the stored fruit showed significant differences between treatments for TA (Table 8). Again, the nutrients and SA (AlexinTM) combination had significantly increased the TA as opposed to the nutrients treatment only (AlexMax). Not supporting the findings by Karlidag *et al.* (2009), which found no significant effect of exogenous SA on TA.

Leaf nutrition

‘Laetitia’ trial: RezistTM, AlexSal and AlexinTM contain SA, which as Barkosky & Einhellig (1992) pointed out, assists with ion uptake, cellular ATP and membrane permeability. The effect of uptake on nutrients may explain the mineral analysis from the leaves where RezistTM had significantly higher N values than that measured in AlexSal, Alexin and AlexinMax (Table 9). This may be due to the formulation of the SA that is contained in RezistTM, which is unknown. This result may also be due to the interaction with other nutrients; however none of the other nutrients showed a significant difference between treatments. The nutrients measured were however at industry acceptable levels, as compared by Kotzé (2001).

‘Songold’ trial: The result of high Fe was recorded was probably not due direct supply to the leaves by foliar application as none of the treatments contained Fe (Table 10). Fageria & Baligar (1999) reported that high Ca led to the reduced uptake of Fe. However none of the treatments showed significant results for the Ca concentration. The comparison of the Fe concentration obtained with that of industry norms as reported by Kotzé (2001) shows that the Ca- concentration to be well above the maximum (140 mg.kg⁻¹) concentration.

Fruit nutrition

‘Laetitia’ trial: Fruit analysis showed that the control contained the highest content of Ca, Mg, Cu and Zn (Table 11). The AlexinTM and AlexMax formulations contain both Ca and Mg, with the only difference that AlexinTM also have SA included in the formulation. Thus, under these conditions, the SA in AlexinTM may have assisted to

achieve the significant increase in the Ca and Mg concentrations recorded in Alexin™ opposed to that recorded in the leaves of the AlexMax treatment.

‘Songold’ trial: The Alexin™ and AlexMax formulations contain Mg which may account for significantly higher Mg recorded for those treatments compared to the AlexSal treatment (Table 12). This explanation however does not address the higher Mg recorded in the control compared to the AlexSal treatment.

Glutathion and Ascorbic acid

‘Laetitia’ trial: Durner *et al.* 1997 suggested that SA inhibits certain antioxidant enzymes namely catalase, leading to an increase in H₂O₂ which is used as secondary messenger to stimulate the production of antioxidants such as glutathione and ascorbic acid. In this study the application of SA was ineffective to induce higher levels of the studied antioxidants. There was no significant increase in the glutathione and ascorbic acid concentrations, irrespective whether the fruit was evaluated fresh or stored (Table 13).

‘Songold’ trial: The treatments was expected to increase the concentrations of both the ascorbic acid and glutathione, however no significant difference was recorded at harvest (evaluation 1; Table 14). Only the glutathione disulfide concentration, the oxidized form of glutathione, was recorded at significantly higher concentrations in the Alexin™ treatment than the AlexSal and AlexMax treatments. This increase may be ascribed to the interaction between the nutrients and SA contained in Alexin™, but offers no explanation for the corresponding high glutathione disulfide levels observed in the control.

CONCLUSIONS

Soil moisture data showed that the trees were most likely under waterlogged conditions, even though the goal was to induce drought stress. However, similar to drought stress these conditions may act as an equivalent stressor as Wooldridge (1994) found that the growth and reproduction of trees decrease when grown in waterlogged soils due to sub-optimal levels of available oxygen in the soil to sustain root growth and mineral uptake. None of the treatments was however able to alleviate the stress induced by the waterlogged stress. However the stress may not have been sufficiently severe as no visual symptoms typical to that of waterlogging were observed.

The stress response mechanism of plants is very complex and may consist of many signals and substances. The results showed that the expected significant effect of SA (Karlidag *et al.*, 2009) on the fruit size and quality could not be achieved under these stress conditions. Both the induced stress conditions as well as the resources allocation cost ascribed to exogenous SA induction of SAR and stress resistance may have prevented the increase in fruit size and quality with SA, as reported in other studies.

The effect of SA as a post-harvest treatment in addition to its application as a pre-harvest treatment should also be evaluated. In addition, a more clear understanding of how long SA and the effects of SA remains in the tree after application are required. The AlexinTM treatment increased the TA in the ‘Laetitia’ plums both at harvest and after storage, while it did not have a significant effect on the TA of the ‘Songold’ plums at harvest. These results obtained suggest that in ‘Laetitia’ plums, a generally

more acidic fruit that ‘Songold’, SA may have amplified the acidity of the ‘Laetitia’ plums.

In conclusion: A better understanding of the cost and tradeoff associated with exogenous application of SA is required to improve application strategies for the use of SA in fruit production. Furthermore, more research is required to establish the various mechanisms plants use to alleviate stress under different stress conditions, together with the active role SA and SAR may play, within each system.

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Table 1: Treatment rates and intervals of foliar applications for the ‘Laetitia’ trial.

Treatment	Rate (ml.100L ⁻¹)	Intervals (days)
Control	0	14
Alexin TM (nutrients + salicylic acid)	250	14
AlexMax (Ca, Mg, B, K only)	250	14
AlexSal (salicylic acid only)	250	14
Rezist TM (nutrients + salicylic acid)	250	14

Table 2: Treatment rates and intervals of foliar applications for the ‘Songold’ trial.

Treatment	Rate (ml.100L ⁻¹)	Interval (days)
Control	0	14
Alexin TM (nutrients + salicylic acid)	250	14
AlexMax (Ca, Mg, B, K only)	250	14
AlexSal (salicylic acid only)	250	14

Table 3: The effects of foliar treatments on the yield, yield efficiency, shoot length, stem water potential and the rate of CO₂ assimilation of water-stressed ‘Laetitia’ trees, following a range of foliar applications.

Treatment	Yield (kg)	Yield Efficiency (kg.cm ⁻¹ trunk)	Shoot Length (cm)	Stem Water potential (MPa)	Rate of CO ₂ assimilation (μmol. m ⁻² .s ⁻¹)
Control	50.6ns ^z	1.37ns	74.9ns	-1.95ns	8.57ns
AlexSal	59.4	1.53	64.5	-1.82	8.68
Rezist TM	61.7	1.66	65.2	-1.86	9.53
Alexin TM	48.2	1.43	68.0	-1.85	9.23
AlexMax	55.7	1.50	62.6	-1.89	9.73
LSD	18.0930	0.4089	11.2130	0.2593	2.7512
p-value	0.5002	0.6510	0.2149	0.8625	0.8553

^z ns non-significant at the 5% level**Table 4:** The effects of foliar treatments on the yield, yield efficiency, shoot length, stem water potential and the rate of CO₂ assimilation of water-stressed ‘Songold’ trees, following a range of foliar applications.

Treatment	Yield (kg)	Yield Efficiency (kg.cm ⁻¹ trunk)	Shoot Length (cm)	Stem Water Potential (MPa)	Rate of CO ₂ assimilation (μmol. m ⁻² .s ⁻¹)
Control	49.1ns ^z	1.43ns	99.4ns	-1.74ns	9.77ns
AlexSal	42.0	1.22	98.0	-1.76	9.21
Alexin TM	42.0	1.26	102.8	-1.86	7.78
AlexMax	48.0	1.35	99.0	-1.74	10.5
LSD	15.3030	0.4112	10.9250	0.2056	2.2515
p-value	0.6302	0.6988	0.7897	0.5145	0.1105

^z ns non-significant at the 5% level

Table 5: The effect of foliar treatments on fruit size and quality characteristics of the 'Laetitia' trial at harvest.

Treatment	Diameter (mm)	Mass (g)	Firmness (kg)	Colour	TSS ^v (Brix°)	TA ^w (%)
Control	47.6ns ^z	64.7ns	8.50ns	11.0ns	11.3ns	1.75ab ^x
AlexSal	48.7	69.2	7.94	11.0	11.4	1.65b
Rezist TM	47.4	63.4	7.77	11.0	11.3	1.75ab
Alexin TM	46.6	60.2	8.66	11.0	11.2	1.83a
AlexMax	47.7	64.6	7.75	11.0	11.2	1.67b
LSD	1.6102	6.3413	0.7924	1.3138	0.4903	0.1008
p-value	0.1603	0.0944	0.0733	0.9298	0.8519	0.0129

^vTSS is total soluble solutes; ^wTA is titratable acids

^x Letters different within a column indicate significance at the 5% level

^z ns non-significant at the 5% level

Table 6: The effect of foliar treatments on fruit size and quality characteristics of the 'Songold' trial at harvest.

Treatment	Diameter (mm)	Mass (g)	Firmness (kg)	Colour	TSS ^v (Brix°)	TA ^w (%)
Control	56.4ns ^z	96.6ns	7.67ns	2.0ns	13.0ns	1.56ns
AlexSal	56.9	101.4	7.91	2.0	13.1	1.56
Alexin TM	56.6	99.6	7.65	3.0	13.3	1.57
AlexMax	56.8	100.2	7.81	2.0	13.4	1.60
LSD	1.344	7.6477	0.4021	0.6536	0.4582	0.1099
p-value	0.8164	0.8764	0.4842	0.5100	0.2738	0.8260

^vTSS is total soluble solutes; ^wTA is titratable acids

^z ns non-significant at the 5% level

Table 7: The effect of foliar treatments on the fruit size and quality characteristics of the ‘Laetitia’ trial after 42 days storage at -0.5 °C.

Treatment	Diameter (mm)	Mass (g)	Firmness (kg)	Colour	TSS ^v (Brix°)	TA ^w (%)	IB ^y (%)
Control	46.9ns ^z	62.8ns	5.41ns	7.0ns	10.9ns	1.21ab ^x	46.7ns
AlexSal	47.7	65.7	5.10	7.0	10.9	1.16b	28.6
Rezist TM	45.9	58.4	4.82	7.0	10.6	1.24ab	21.4
Alexin TM	45.9	58.2	5.40	7.0	10.7	1.29a	30.5
AlexMax	46.7	62.1	4.96	7.0	10.8	1.16b	30.0
LSD	2.0655	7.9704	0.5916	0.885	0.5068	0.0914	0.2145
p-value	0.3769	0.2709	0.1521	0.8269	0.5300	0.0354	0.2069

^vTSS is total soluble solutes; ^wTA is titratable acids; ^yIB is internal browning

^x Letters different within a column indicate significance at the 5% level

^z ns non-significant at the 5% level

Table 8: The effect of foliar treatments on the fruit size and quality characteristics of the ‘Songold’ trial after 42 days storage at -0.5 °C.

Treatment	Diameter (mm)	Mass (g)	Firm (kg)	Colour	TSS ^v (Brix°)	TA ^w (%)	IB ^y (%)
Control	81.6ns ^z	97.8ns	2.90ns	5.0ns	12.4ns	1.06a ^x	98.0ns
AlexSal	81.9	97.5	2.99	5.0	12.9	1.02ab	92.0
Alexin TM	81.4	96.2	2.88	5.0	12.6	1.07a	94.7
AlexMax	82.1	101.2	3.07	5.0	13.0	0.94b	98.0
LSD	2.5018	6.3258	0.4214	0.6536	0.8257	0.0981	0.0872
p-value	0.9291	0.3990	0.7433	0.5100	0.4369	0.0574	0.4043

^vTSS is total soluble solutes; ^wTA is titratable acids; ^yIB is internal browning

^x Letters different within a column indicate significance at the 5% level

^z ns non-significant at the 5% level

Table 9: The effect of foliar treatments on the leaf mineral content of the ‘Laetitia’ trial as sampled on the 1st of February 2012 at harvest.

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Cu (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)	B (mg.kg ⁻¹)
Control	3.14ab ^x	0.168ns ^z	2.09ns	2.09ns	0.620ns	201.3ns	78.8 ns	230.0ns	10.5ns	57.3ns	34.8ns
AlexSal	2.97c	0.167	2.38	2.17	0.625	185.83	77.0	197.0	10.2	69.7	37.7
Rezist TM	3.18a	0.185	2.10	1.98	0.620	177.67	65.8	220.3	11.3	48.7	33.7
Alexin TM	3.01bc	0.172	1.84	2.30	0.663	169.50	76.3	150.7	11.3	58.2	35.2
AlexMax	3.04bc	0.165	1.93	2.35	0.703	180.5	72.8	530.3	10.7	70.3	31.7
LSD	0.13	0.01	0.43	0.29	0.08	28.02	24.61	468.95	1.84	17.84	7.49
p-value	0.0135	0.0671	0.1267	0.0984	0.2022	0.2251	0.8204	0.4807	0.6062	0.0948	0.5726

^x Letters different within a column indicate significance at the 5% level^z ns non-significant at the 5% level**Table 10:** The effect of foliar treatments on the leaf mineral content of the ‘Songold’ trial as sampled on the 9th of February 2012 at harvest.

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Cu (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)	B (mg.kg ⁻¹)
Control	2.88ns ^z	0.176ns	2.65ns	1.79ns	0.520ns	182.4ns	61.0ns	257.6a ^x	10.0ns	77.2ns	43.2ns
AlexSal	2.77	0.186	2.59	1.96	0.564	174.0	67.6	178.2b	9.8	71.6	40.6
Alexin TM	2.75	0.180	2.65	1.92	0.558	173.4	63.0	168.8b	10.2	74.4	43.2
AlexMax	2.86	0.172	2.74	1.86	0.548	181.0	56.6	329.0a	9.8	79.4	45.0
LSD	0.17	0.04	0.45	0.29	0.08	35.18	22.62	76.18	1.30	20.65	4.86
p-value	0.3021	0.8485	0.9181	0.6283	0.6472	0.9168	0.746	0.0019	0.8905	0.8560	0.3140

^x Letters different within a column indicate significance at the 5% level^z ns non-significant at the 5% level

Table 11: The effect of foliar treatments on the fruit mineral content of the ‘Laetitia’ trial as sampled on the 1st of February 2012 at harvest.

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Cu (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)	B (mg.kg ⁻¹)
Control	101.5ns ^z	15.1ns	167.3ns	5.78a ^x	8.03a	22.8ns	1.17ns	3.53ns	0.500a	0.883a	2.63ns
AlexSal	90.0	13.7	150.7	5.67ab	7.22bc	21.5	1.03	2.10	0.383bc	0.700b	2.37
Rezist TM	96.8	12.3	150.7	5.58 ab	7.27bc	23.0	1.05	2.23	0.433abc	0.767ab	2.40
Alexin TM	98.0	13.2	157.5	6.23a	7.40ab	24.0	1.17	2.42	0.467ab	0.767ab	2.50
AlexMax	89.2	12.5	144.8	5.10b	6.72c	21.7	0.98	2.43	0.367c	0.683b	2.33
LSD	18.23	2.61	15.00	0.668	0.637	4.08	0.151	1.62	0.091	0.126	0.455
p-value	0.5734	0.2234	0.0502	0.0337	0.0071	0.7024	0.0663	0.3910	0.0315	0.0285	0.4598

^x Letters different within a column indicate significance at the 5% level

^z ns non-significant at the 5% level

Table 12: The effect of foliar treatments on the fruit mineral content of the ‘Songold’ trial as sampled on the 9th of February 2012 at harvest.

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Cu (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)	B (mg.kg ⁻¹)
Control	113.4ns ^z	18.0ns	157.4ns	8.36ns	15.8a ^x	20.5ns	0.92ns	3.42ns	0.28ns	0.82ns	3.18ns
AlexSal	115.8	15.0	142.8	7.94	13.9b	19.2	0.92	1.54	0.22	0.68	3.06
Alexin TM	109.2	18.3	167.2	9.10	16.3a	21.0	0.88	2.26	0.34	0.72	3.84
AlexMax	114.2	19.5	164.8	9.70	16.9a	20.1	0.92	2.58	0.28	0.88	3.76
LSD	25.65	4.28	23.98	1.39	1.62	3.59	0.275	2.34	0.105	0.249	0.943
p-value	0.9500	0.1880	0.1243	0.0725	0.0102	0.7305	0.9844	0.4060	0.1555	0.3255	0.2232

^x Letters different within a column indicate significance at the 5% level

^z ns non-significant at the 5% level

Table 13: Glutathione and Ascorbic acid concentrations of the ‘Laetitia’ trial at harvest (Evaluation 1).

Treatment	Glutathione $\mu\text{g}\cdot\text{g}^{-1}$ FW	Total glutathione $\mu\text{g}\cdot\text{g}^{-1}$ FW	Glutathione disulfide $\mu\text{g}\cdot\text{g}^{-1}$ FW	Ascorbic acid $\mu\text{g}\cdot\text{g}^{-1}$ FW	Total ascorbic acid $\mu\text{g}\cdot\text{g}^{-1}$ FW	Dehydroascorbic acid $\mu\text{g}\cdot\text{g}^{-1}$ FW
Control	23.0ns ^z	23.8ns	0.09ns	64.5ns	82.0ns	17.5ns
AlexSal	29.0	33.7	4.77	59.0	79.3	20.3
Rezist TM	24.3	26.9	2.53	63.6	80.3	16.6
Alexin TM	33.8	39.8	6.08	69.0	86.7	17.7
AlexMax	27.0	29.2	2.29	67.7	83.2	15.5
LSD	0.0117	0.0162	0.0047	0.0096	0.0076	0.0054
p-value	0.3653	0.3031	0.1931	0.2599	0.3244	0.4478

^z ns non-significant at the 5% level**Table 14:** Glutathione and Ascorbic acid concentrations of the ‘Songold’ trial at harvest (Evaluation 1).

Treatment	Glutathione $\mu\text{g}\cdot\text{g}^{-1}$ FW	Total glutathione $\mu\text{g}\cdot\text{g}^{-1}$ FW	Glutathione disulfide $\mu\text{g}\cdot\text{g}^{-1}$ FW	Ascorbic acid $\mu\text{g}\cdot\text{g}^{-1}$ FW	Total ascorbic acid $\mu\text{g}\cdot\text{g}^{-1}$ FW	Dehydroascorbic acid $\mu\text{g}\cdot\text{g}^{-1}$ FW
Control	24.3ns ^z	25.8ns	15.8ns	103ns	122ns	19.6ns
AlexSal	26.0	27.5	14.7	108	126	18.3
Alexin TM	25.2	27.1	18.8	104	123	19.1
AlexMax	26.3	28.0	17.1	108	128	20.4
LSD	0.0030	0.0033	0.0011	0.0121	0.0146	0.0061
p-value	0.5007	0.5602	0.8667	0.7028	0.7968	0.9005

^z ns non-significant at the 5% level

Table 15: Glutathione and Ascorbic acid concentrations of the ‘Laetitia’ trial after 42 days storage at -5 °C plus three days at room temperature of 25 °C (Evaluation 2).

Treatment	Glutathione $\mu\text{g}\cdot\text{g}^{-1}$ FW	Total glutathione $\mu\text{g}\cdot\text{g}^{-1}$ FW	Glutathione disulfide $\mu\text{g}\cdot\text{g}^{-1}$ FW	Ascorbic acid $\mu\text{g}\cdot\text{g}^{-1}$ FW	Total ascorbic acid $\mu\text{g}\cdot\text{g}^{-1}$ FW	Dehydroascorbic acid $\mu\text{g}\cdot\text{g}^{-1}$ FW
Control	22.7ns ^z	23.4ns	0.90ns	108ns	119ns	11.1ns
AlexSal	22.4	22.4	0.50	113	121	8.30
Rezist TM	23.7	23.3	0.30	108	115	6.90
Alexin TM	22.6	22.5	0.50	108	117	8.80
AlexMax	22.2	23.0	0.80	107	113	5.90
LSD	0.0033	0.0036	0.0007	0.0098	0.0065	0.0060
p-value	0.8916	0.9676	0.3463	0.7049	0.0971	0.4512

^z ns non-significant at the 5% level**Table 16:** Glutathione and Ascorbic acid concentrations of the ‘Songold’ trial after 42 days storage at -5 °C plus three days at room temperature of 25 °C (Evaluation 2).

Treatment	Glutathione $\mu\text{g}\cdot\text{g}^{-1}$ FW	Total glutathione $\mu\text{g}\cdot\text{g}^{-1}$ FW	Glutathione disulfide $\mu\text{g}\cdot\text{g}^{-1}$ FW	Ascorbic acid $\mu\text{g}\cdot\text{g}^{-1}$ FW	Total ascorbic acid $\mu\text{g}\cdot\text{g}^{-1}$ FW	Dehydroascorbic acid $\mu\text{g}\cdot\text{g}^{-1}$ FW
Control	29.2ns ^z	34.8ns	5.60ab ^x	41.6ns	68.8ns	27.2ns
AlexSal	24.9	28.6	3.60bc	38.1	61.7	23.7
Alexin TM	28.4	35.1	6.70a	31.7	62.5	30.7
AlexMax	26.6	29.2	2.60c	23.0	57.8	27.8
LSD	0.0051	0.0066	0.0024	0.0132	0.0095	0.0079
p-value	0.3128	0.0932	0.0131	0.2413	0.1381	0.3234

^x Letters different within a column indicate significance at the 5% level^z ns non-significant at the 5% level

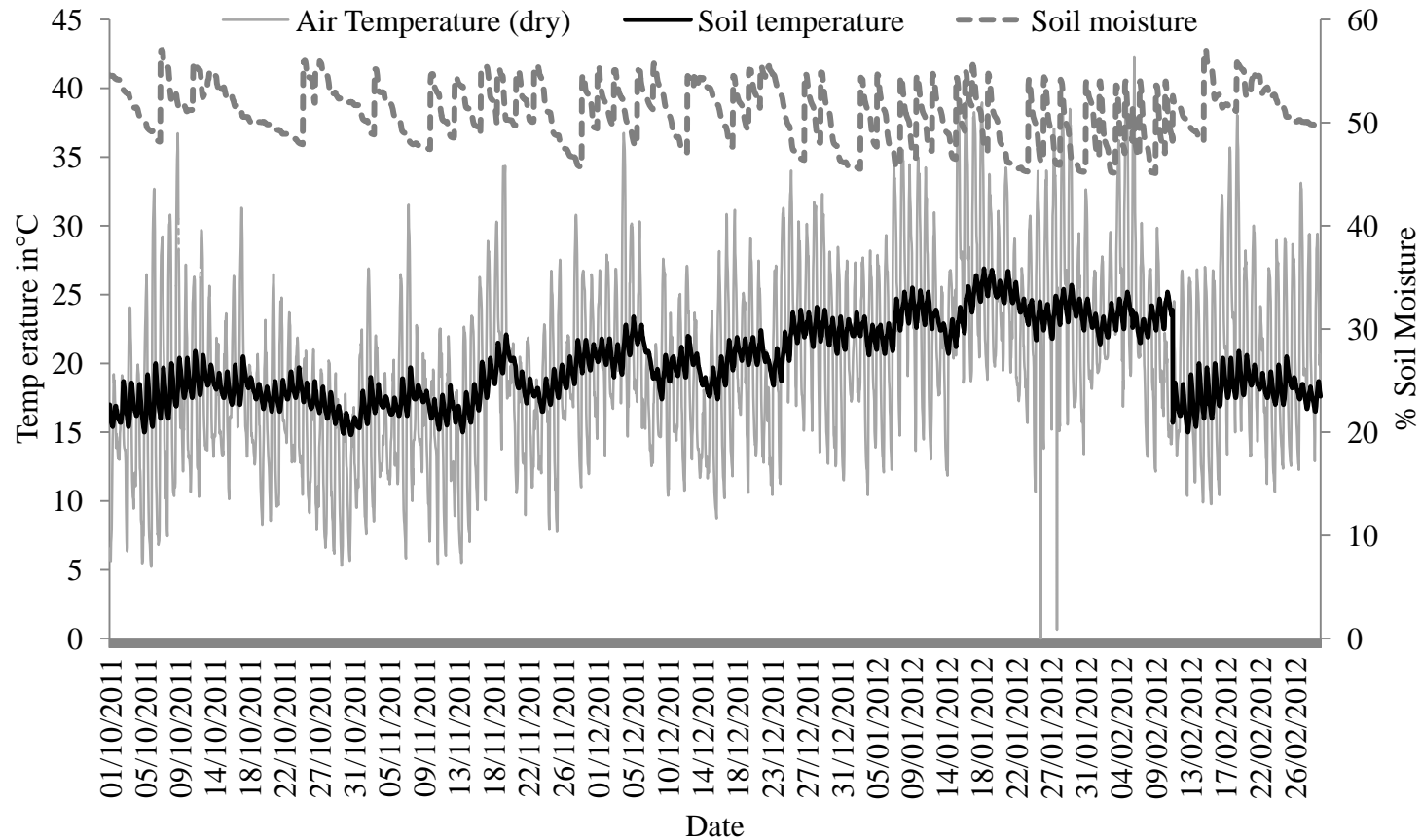


Fig. 1. Soil temperature (°C), and the percentage (%) soil moisture of the ‘Laetitia’ and ‘Songold’ trials as measured by the DFM probe between 0 and 30 cm and air temperature (°C) as recorded by the Helderfontein weather station, Stellenbosch (33°55'21.64" S, 18°52'05.03" E ca. 127 m a.s.l).

NON-BEARING DECIDUOUS FRUIT TREE RESPONSE TO FOLIAR APPLICATIONS TO ALLEVIATE INDUCED MAGNESIUM AND MANGANESE DEFICIENCY SYMPTOMS IN LEAVES

INTRODUCTION

The beneficial effect of mineral nutrients in agriculture has been known for more than 2000 years (Marschner, 1995) however the growing cost of applying mineral nutrients to the soil is increasing (Swietlik & Faust, 1984). Foliar nutrient application has been shown to be an effective method of rectifying nutritional imbalances in plants (Swietlik & Faust, 1984). The limitation of rectifying a nutritional imbalance in fruit trees through soil application throughout the growing season is that this method is not always sufficient to maintain optimum growth and yield. However, various reports showed that magnesium (Mg) and manganese (Mn) deficiencies can be corrected successfully with foliar applications (Labanauskas *et al.*, 1962; Alcaraz-Lopez *et al.*, 2003).

Magnesium is a structural component of chlorophyll, and also activates key enzymes such as glutathione synthase, ATPases and phosphoenolpyruvate (PEP) carboxylase (Marschner, 1995). Fisher & Bussler (1988) found a decrease in the export of carbohydrates from source organs under conditions of Mg deficiency for *Phaseolus vulgaris*, the common bean. This resulted in accumulation of assimilates which in turn led to a decrease in CO₂ fixation and subsequently, a buildup of excess electrons and absorbed energy. Ultimately this leads to the production of reactive oxygen species (ROS) and thus oxidative stress. The carbohydrate accumulation in source organs causes a decrease in available resources for growth, as Mg deficiency is associated

with reduced root growth (Marschner, 1995). Manganese is required in the many primary physiological processes such as photosynthesis, lipid metabolism, protein synthesis and root growth due to its role in cell division and elongation (Marschner, 1995).

Symptoms of Mg deficiency in plants usually occur first in older leaves, due to the mobility of this element in plants. Symptoms are expressed as interveinal chlorosis, development of smaller leaves, necrosis and premature abscission of leaves. (Marschner, 1995; Datnoff *et al.*, 2007; Taiz & Zeiger, 2010). The deficiency symptoms of Mn are very similar to that of Mg (Marschner, 1995; Taiz & Zeiger 2010), but, Mn deficiency symptoms appear firstly on younger leaves as interveinal chlorosis and are accompanied by the small necrotic spots due to its poor mobility in plants.

There are many factors that affect the efficacy of foliar application of nutrients - including the interaction of both Mg and Mn with other nutrients, the chemical formulation of the foliar nutrient and various environmental factors (Swietlik & Faust, 1984). Forshey (1959) found that apple trees with an adequate nitrogen (N) status increase the foliar absorption of Mg, to alleviate the Mg deficiency in apple trees.

The purpose of the study was to determine if a foliar application of Mg and Mn can overcome induced deficiency of these nutrients in non-bearing, *Malus x domestica* cv 'Royal Beaut' apple and *Prunus* cv 'Laetitia' plum trees, after visual symptoms were detected on the leaves.

MATERIAL AND METHODS

Plant material and treatments

One year old ‘Laetitia’ plum trees on Marianna rootstock and ‘Royal Beaut’ apple trees on M7 rootstock (open roots) were sourced from commercial nurseries in Prins Alfred’s Hamlet (Stemmet Nursery) and Ceres (Koue Bokkeveld Nursery) respectively. Twenty large size trees per cultivar were planted out in 10 liter planting bags and filled with 9 liters of filter sand on the 2nd of August 2011. The trial was conducted in an open roofed tunnel at Welgevallen Experimental Farm, Stellenbosch University. At planting, both the apple and plum trees were headed back to 100 cm to allow for subsequent growth in the tunnel. The trial was laid out in a randomized complete block design with five treatments and four blocks for each fruit species (Table 1). Individual trees served as both experimental and sampling units. Drip irrigation was installed and maintained with an automated MiraclePlus (Netafim) irrigation system and irrigated until water drained. The plants were irrigated twice a day for 10 minutes from planting (1st of August 2011) until November 2011. The irrigation pulses were subsequently raised to three times a day according to irrigation demand and this schedule was maintained until the end of the trial in March 2012. The average monthly temperatures were 12.49 °C (August), 13.85 °C (September 2011), 16.29 °C (October), 17.35 °C (November), 18.11 °C (December) during 2011. During 2012 the average monthly temperatures were 24.35 °C (January), 21.83 °C (February) and 21.41 °C (March).

The trees were fertigated with a Long Ashton solution (pH 6.5) (Hewitt, 1966) at an initial rate of 200 ml (11th of August 2011-7th of November 2011), twice a week, which was raised to three times a week (7th of November 2011- 2nd of February 2012).

Treatments ‘-Mg’ and ‘MagMaxTM’, and ‘-Mn’ and ‘ManMaxTM’ received a full strength Long Ashton solution, but where the Mg and Mn was omitted respectively to induce the required deficiency. The control received the complete Long Ashton solution at full strength. When visual deficiency symptoms were detected, the ‘MagMaxTM’ treatment received a commercial Mg containing foliar application ‘MagMaxTM’ at a rate of 5 ml.L⁻¹ (Nulandis, Johannesburg, South Africa), whilst likewise the ‘ManMaxTM’ treatment received a commercial Mn containing foliar application ‘ManMaxTM’ (Nulandis, Johannesburg, South Africa). ‘MagMaxTM’, which was applied at a rate of 1 ml. L⁻¹, both was applied by hand, until the point of run-off. The application of these treatments was carried out in the mornings between 8:30 am and 10 am. Applications of ‘MagMaxTM’ and ‘ManMaxTM’ was done on 12th and 20th of December 2011 and on 6th, 13th and 19th of January 2012 for the ‘Laetitia’ plum trees, both applied on the same day. Applications of ‘Royal Beaut’ apple trees was done on 14th, 17th, 20th, 24th and the 27th of February 2012 and on the 2nd, 5th, 9th, 19th, 22nd and the 27th of March 2012. The control, ‘-Mg’ and ‘-Mn’ treatments received a foliar application of tap water which coincided with the foliar applications of treatments ‘MagMaxTM’ and ‘ManMaxTM’.

Data collection and sampling

The visual expression of Mg and Mn deficiencies was monitored throughout the season. Preliminary leaf (10 leaves per tree) samples were collected on the 12th of December 2011 for mineral nutrient analysis at Bemblab Pty. Ltd laboratories (Strand, South Africa). The leaves were randomly sampled in the middle of the shoots. Subsequent leaf (10 leaves per tree) samples were collected for analyses on the 13th of

February 2012 for the ‘Laetitia’ trial and 30th of March 2012 for the ‘Royal Beaut’ trial. Digital photographs were also taken during the season to qualify visual signs of the deficiencies.

Statistical analysis

Data were analyzed using the General Linear Means Procedure (GLM) of the Statistical Analysis Systems (SAS) (SAS Institute Inc., Cary, NC, 2004). Means were separated with the least significant difference (LSD) test at $p \leq 0.05$.

RESULTS AND DISCUSSION

‘Laetitia’ trial: The preliminary mineral analysis conducted in December 2011 showed that the general nutritional status of the plants was not satisfactory (Table 2). This was correlated with the visual appearance of the plants, which indicated visual symptoms of deficiencies in the control (Plate 1 (a)). On investigation an error in the formulation of the nutrient concentrations of the Long Ashton solution was detected. Macro-nutrients were supplied at only 10 percent of the required strength, whilst the micro nutrients were administered at a dosage 100 percent higher than recommended. The formulation was adjusted immediately and application there of commenced in January 2012. Additionally six Urea sprays was at 0.005 g. L^{-1} applied to rectify the suboptimal N concentration.

Mineral analysis of leaves done in February 2012 showed significant differences between treatments for nitrogen (N), potassium (K), calcium (Ca), Mg, Mn and copper (Cu). The control showed a significantly higher Mg concentration compared to

all other treatments. The ‘-Mg’ treatment had a significant lower Mg concentration in comparison to the control, but did not differ significantly from the other treatments (Table 3). Nevertheless, the Mg concentration for the ‘MagMaxTM’ treatments was above the minimum concentration recommended by industry (0.300 %) (Fig. 1.) (Kotzé, 2001), while that of the ‘-Mg’ treatments was below the minimum threshold value and approached concentration values considered deficient (0.200 %) for Mg (Kotzé, 2001). The ‘MagMaxTM’ treatment succeeded to increase the Mg concentration to the same level as treatments that received Mg in the Long Ashton solution such as the ‘ManMaxTM’ treatment.

Leaf analysis for manganese reported the control treatment to have significantly higher Mn concentration than leaves of trees that received the ‘MagMaxTM’, ‘-Mg’ and ‘-Mn’ treatments. However, there was no significant difference between the control and the ‘ManMaxTM’ treatment (Table 3), suggesting that the ‘ManMaxTM’ treatment effectively increased the Mn concentration compared to the ‘-Mn’ treatment (Table 3). The Mn concentration of the control and ‘ManMaxTM’ treatment was above the maximum threshold (85 mg.kg⁻¹) and below toxicity value recommended by industry (600 mg.kg⁻¹). The ‘MagMaxTM’, ‘-Mg’ and ‘-Mn’ treatments was above the minimum (80 mg.kg⁻¹), but below the maximum for Mn (Fig. 3) (Kotzé, 2001).

The control had the highest N concentration which was significantly different from all other treatments. The lowest N concentration was observed for the ‘-Mg’ treatment which was significantly lower than the other treatments (Table 3). Forshey (1959) found a positive relationship between N and Mg. This relationship between N and Mg as reported by Forshey (1959) was confirmed in our study. The lack of Mg absorption in the ‘MagMaxTM’ treatment (Table 3) may thus be linked to the generally low N

levels measured in the leaves, where concentration levels well below the deficiency levels were recorded. The control showed significantly higher K, Ca and Cu concentrations than the other treatments, although Cu concentrations of the control did not differ significantly from the ‘-Mn’ treatment (Table 3). Figs. 1 and 2 compare the results obtained during this trial (13th February 2012) to industry leaf nutrients norms (typically analyzed on 31st of January) for plum trees grown under similar climatic conditions (ARC Infruitec-Nietvoorbij) than was experienced in our trials (Kotzé, 2001). A comparison of macro nutrient levels indicated that all treatments except the control, suffered from N and K deficiencies (Fig. 1). Only the ‘MagMaxTM’ and ‘-Mg’ treatments showed deficiencies for P and Ca respectively. None of the treatments showed Mg deficiencies (Fig. 1). When the micro nutrients levels between treatments were compared, Zinc (Zn) deficiencies were noticeable in all treatments except the control (Fig. 2) (Kotzé, 2001). In plates 2 and 3, the typical visual symptoms of Mg deficiency (Plate 2 (b)) and Mn deficiency (Plate 3 (b)) are illustrated – note the pale green colour suggests the presence of an N deficiency in plates 2 (c) and 3 (c).

‘Royal Beaut’ trial: The same experimental error was extended into this trial regarding the incorrect Long Ashton solution formulation that was applied before January 2012 (Plate 1 (b)). Significant differences were obtained between treatments for the elements N, K, Mn and B (Table 4). The ‘ManMaxTM’ treatment showed the highest Mn concentration, which differed significantly from all other treatments, indicating that ‘ManMaxTM’ assisted in increasing the Mn concentration to a level similar or above that of treatments that received Mn. The K concentration was significantly higher in the ‘-Mg’ treatment and B concentrations, significantly higher in the ‘MagMaxTM’ treatment, compared to the rest of the treatments (Table 4).

Ramani & Kannan (1974) reported that K, Ca and Mg play a role in regulating the uptake of Mn by promoting Mn uptake under low Mn conditions and depressing uptake under excess Mn conditions. Thus, low levels of K, Ca and Mg was insufficient to suppress the uptake of Mn under excess Mn as occurred prior to the rectification of the nutrient solution, partly explaining the high concentrations of Mn in the ‘-Mn’ treatment.

In the apple trial, leaf samples for mineral analysis were collected much later (30th of March 2012) than the recommended date (30th of January), to allow for clear visual leaf deficiency symptoms to develop and also to have sufficient time to apply the treatments afterwards. The micro nutrient analysis of the apple leaves showed a Cu deficiency in all treatments, and sufficient levels for Mn (Fig. 4) – based on the industry norms as valid for the month of January (Kotzé, 2001). This further explains the high concentration of Mn as Cu has an antagonistic effect on Mn (Fagaria, 2001). Plates 4 and 5 also showed typical Mg deficiencies (Plate 4 (b)) and Mn deficiencies (Plate 5 (b)).

CONCLUSION

The results show that the ‘ManMaxTM’ treatment can successfully increase the Mn concentration after deficiency symptoms for Mn was observed, to similar levels or higher than the control in young, one-year-old plum and apple trees respectively. The ‘MagMaxTM’ treatment was not able to correct the Mg deficiency, possibly as result of the interaction between Mg and the deficient N concentration (Forshey, 1959), causing insufficient uptake of Mg supplied with the ‘MagMaxTM’ treatment.

The imbalanced initial nutrition resulted in stunted vegetative growth, which in turn resulted in a delayed expression of Mg and Mn symptoms, and subsequently late foliar treatment applications.

This study shows that there are strong interactions between mineral elements. Furthermore also indicates that ‘MagMaxTM’ and ‘ManMaxTM’ foliar applications may not be successful in overcoming deficiencies of Mg and Mn if other minerals imbalances occur under stress factors that enforces those imbalances or causes it. These foliar applications can therefore be used as part of a management strategy but should not be used in isolation.

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Table 1: Soil nutrition and foliar applications for the ‘Laetitia’ and ‘Royal Beaut’ trials at Welgevallen Experimental farm, Stellenbosch University during 2011/12.

Treatment	Nutrition	Foliar Treatment	Rate (ml. L ⁻¹)
Control	Long Ashton solution	Tap Water	Full strength
-Mg	Long Ashton solution minus Magnesium	Tap Water	Full strength
-Mn	Long Ashton solution minus Manganese	Tap Water	Full strength
MagMax TM	Long Ashton solution minus Magnesium	MagMax TM	5
ManMax TM	Long Ashton solution minus Manganese	ManMax TM	1

Table 2: The preliminary leaf mineral composition of the ‘Laetitia’ trial for 12th of December 2011 as an average of four leaf samples per treatment, after Long Ashton solution nutrition.

TRT	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Cu (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)	B (mg.kg ⁻¹)
ManMax TM	1.45 c ^x	0.128 ns ^z	0.580 ns	0.465 b	0.153 b	130.8 ns	26.0 ns	197.0 ns	4.25 ns	10.0 ns	24.3 ns
MagMax TM	1.64 b	0.128	0.670	0.455 b	0.155 b	165.0	33.0	1909.5	9.50	24.3	26.8
-Mg	1.63 b	0.135	0.640	0.553 b	0.210 b	126.3	34.3	496.5	4.75	13.0	25.3
-Mn	1.52 bc	0.135	0.640	0.465 b	0.160 b	136.3	25.8	230.3	4.75	10.5	25.8
Control	1.81 a	0.150	0.748	0.745 a	0.323 a	109.0	26.5	172.8	4.25	10.0	24.3
LSD	0.12	0.03	0.21	0.14	0.07	38.70	16.27	1576.60	5.78	11.57	2.62
p-value	0.0003	0.3756	0.5653	0.0330	0.0019	0.0871	0.6559	0.1427	0.2819	0.0859	0.2496

^x Letters different within a column indicate significance at the 5% level

^z ns non-significant at the 5% level

Table 3: The effect of treatments on the leaf mineral concentration of the ‘Laetitia’ trial on the 13th of February 2012 as an average of four leaf samples per treatment, after adjustment of Long Ashton solution nutrition and the additional foliar application of Urea.

TRT	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Cu (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)	B (mg.kg ⁻¹)
ManMax TM	1.59 b ^x	0.10 ns ^z	0.53 b	1.42 b	0.475 b	210.5 ns	140.5 ab	124.3 ns	6.00 b	12.3 ns	21.5 ns
MagMax TM	1.57 b	0.08	0.42 b	1.15 b	0.358 b	191.5	66.0 b	106.3	5.00 b	7.75	28.5
-Mg	1.24 c	0.10	0.49 b	0.85 b	0.245 b	285.0	49.3 b	151.3	7.50 b	11.3	22.0
-Mn	1.31 b	0.16	0.83 b	1.19 b	0.333 b	325.8	61.0 b	263.5	10.0 ab	15.3	29.8
Control	2.05 a	0.23	1.68 a	2.78 a	1.01 a	397.0	187.0 a	308.8	13.5 a	19.8	39.3
LSD	0.12	0.10	0.75	1.06	0.37	175.31	95.71	162.28	5.14	9.71	20.36
p-value	<.0001	0.0517	0.0181	0.0154	0.0066	0.1308	0.0335	0.0674	0.0236	0.1508	0.3641

^x Letters different within a column indicate significance at the 5% level

^z ns non-significant at the 5% level

Table 4: The effect of treatments on the leaf mineral concentration of the ‘Royal Beaut’ trial on the 30th of March 2012 as an average of four leaf samples per treatment, after adjustment of Long Ashton solution nutrition and the additional foliar application of Urea.

TRT	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Cu (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)	B (mg.kg ⁻¹)
ManMax TM	1.16 b ^x	0.070 ns ^z	0.770 b	0.843 ns	0.273 ns	174.5 ns	106.5 a	103.0 ns	3.25 ns	11.0 ns	28.5 b
MagMax TM	1.18 b	0.073	0.855 b	0.950	0.300	203.3	64.8 b	115.0	3.50	10.3	43.8 a
-Mg	1.21 ab	0.093	1.090 a	1.100	0.268	171.3	65.3 b	144.3	3.50	11.5	27.8 b
-Mn	1.09 b	0.075	0.845 b	0.880	0.288	187.8	61.8 b	120.8	3.25	10.5	28.0 b
Control	1.35 a	0.080	0.823 b	0.948	0.315	173.5	72.3 b	143.3	3.75	11.8	25.0 b
LSD	0.15	0.01	0.15	0.19	0.04	26.98	27.64	52.15	0.85	1.79	4.44
p-value	0.0360	0.0651	0.0093	0.1156	0.2557	0.1104	0.0225	0.3843	0.6911	0.3619	<.0001

^x Letters different within a column indicate significance at the 5% level

^z ns non-significant at the 5% level

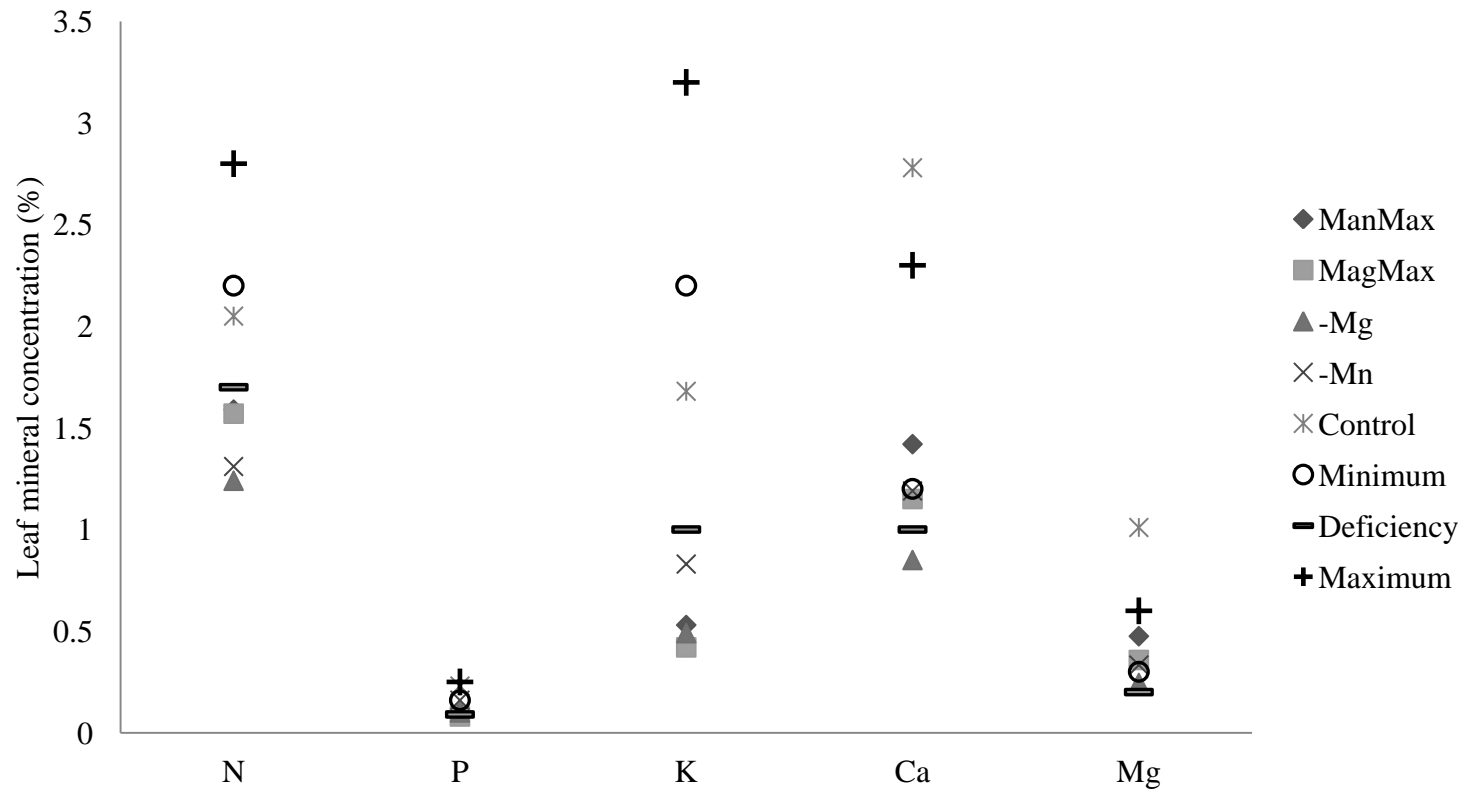


Fig. 1. Comparison of foliar treatments to overcome Mg and Mn deficiency with industry plum norms published by Kotzé (2001) for macro nutrients in the 'Laetitia' trial .

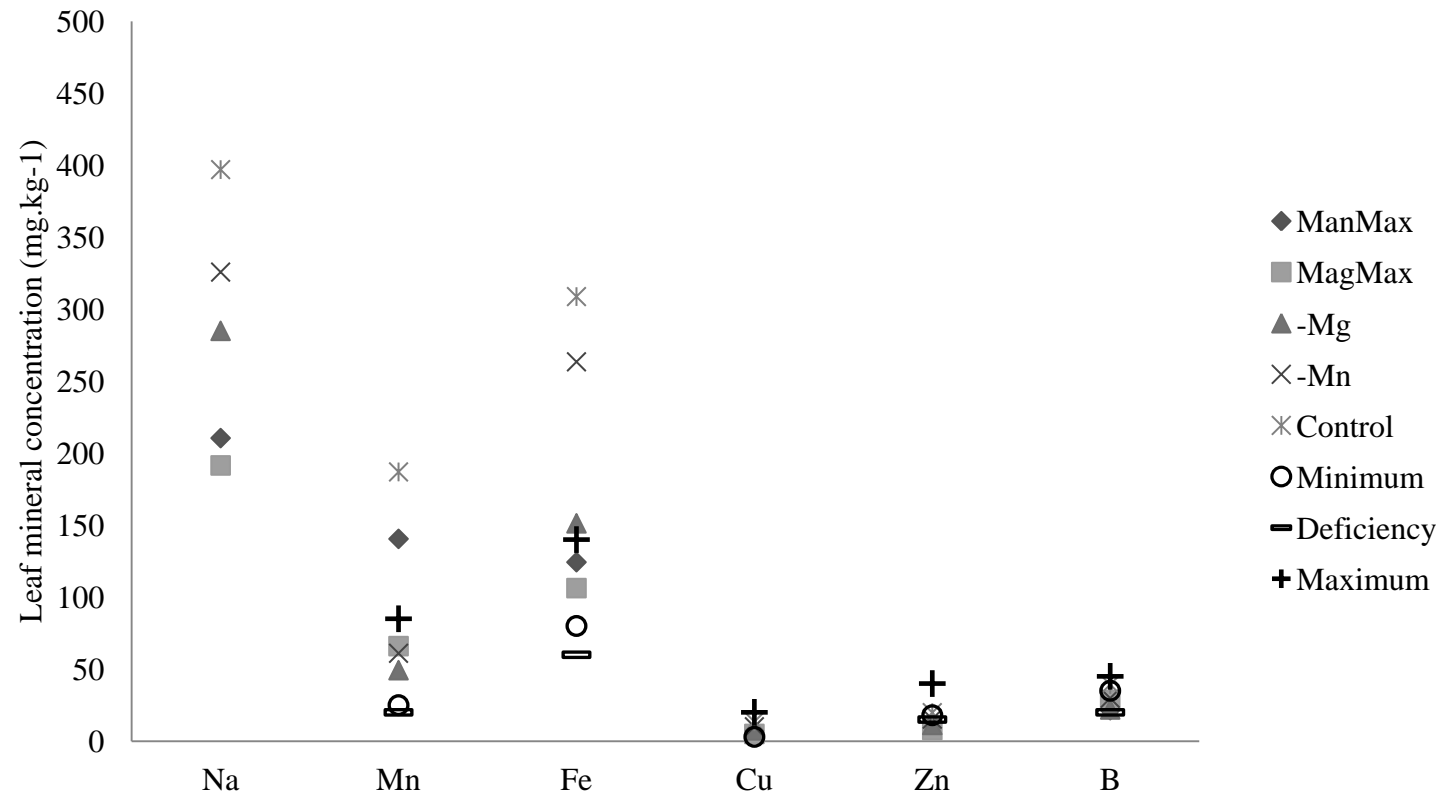


Fig. 2. Comparison of foliar treatments to overcome Mg and Mn deficiencies with industry plum norms published by Kotzé (2001) for micro nutrients of the 'Laetitita' trial.

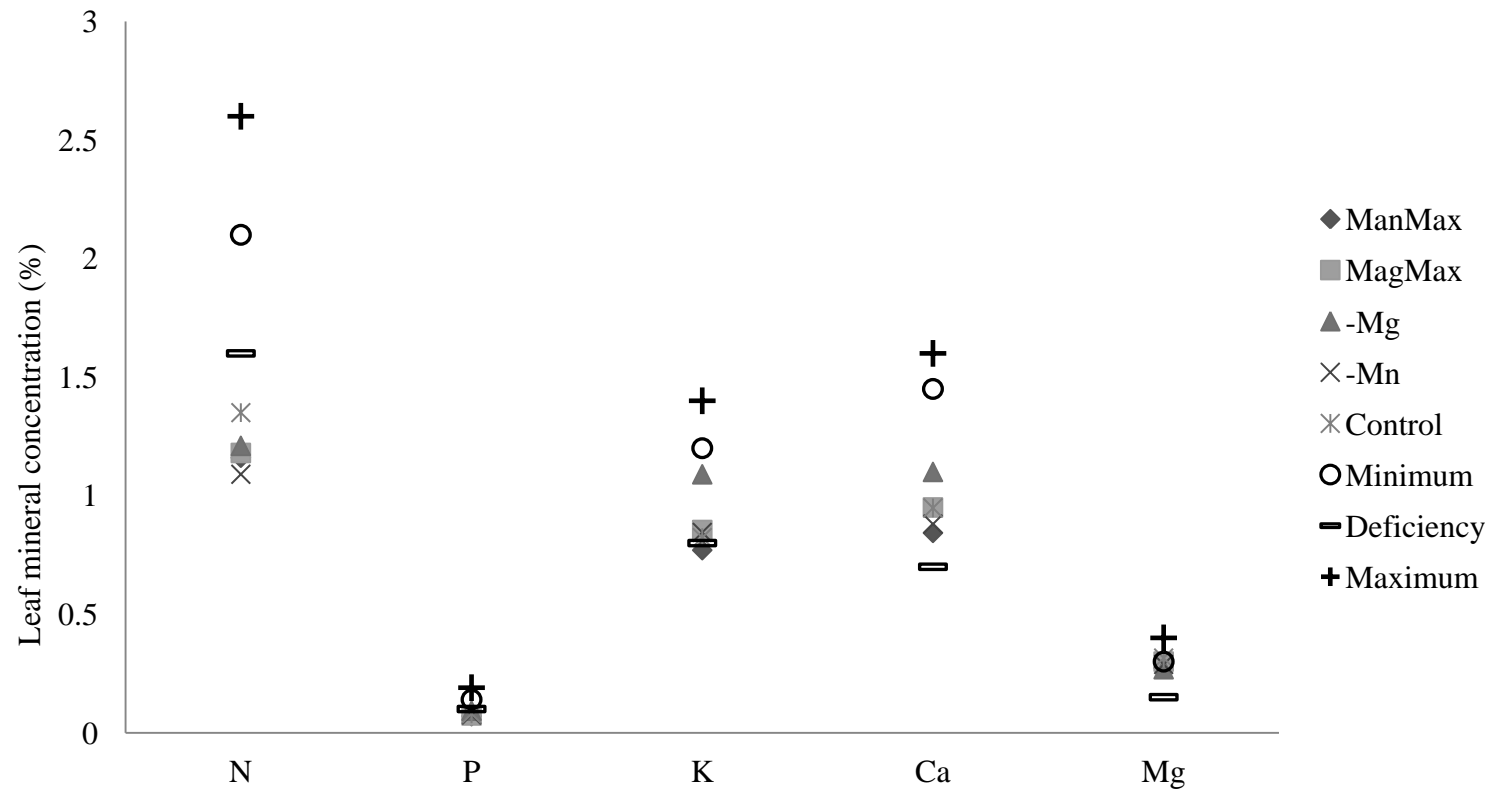


Fig. 3. Comparison of foliar treatments to overcome Mg and Mn deficiencies with industry apple norms published by Kotzé (2001) for macro nutrients in the ‘Royal Beaut’ trial.

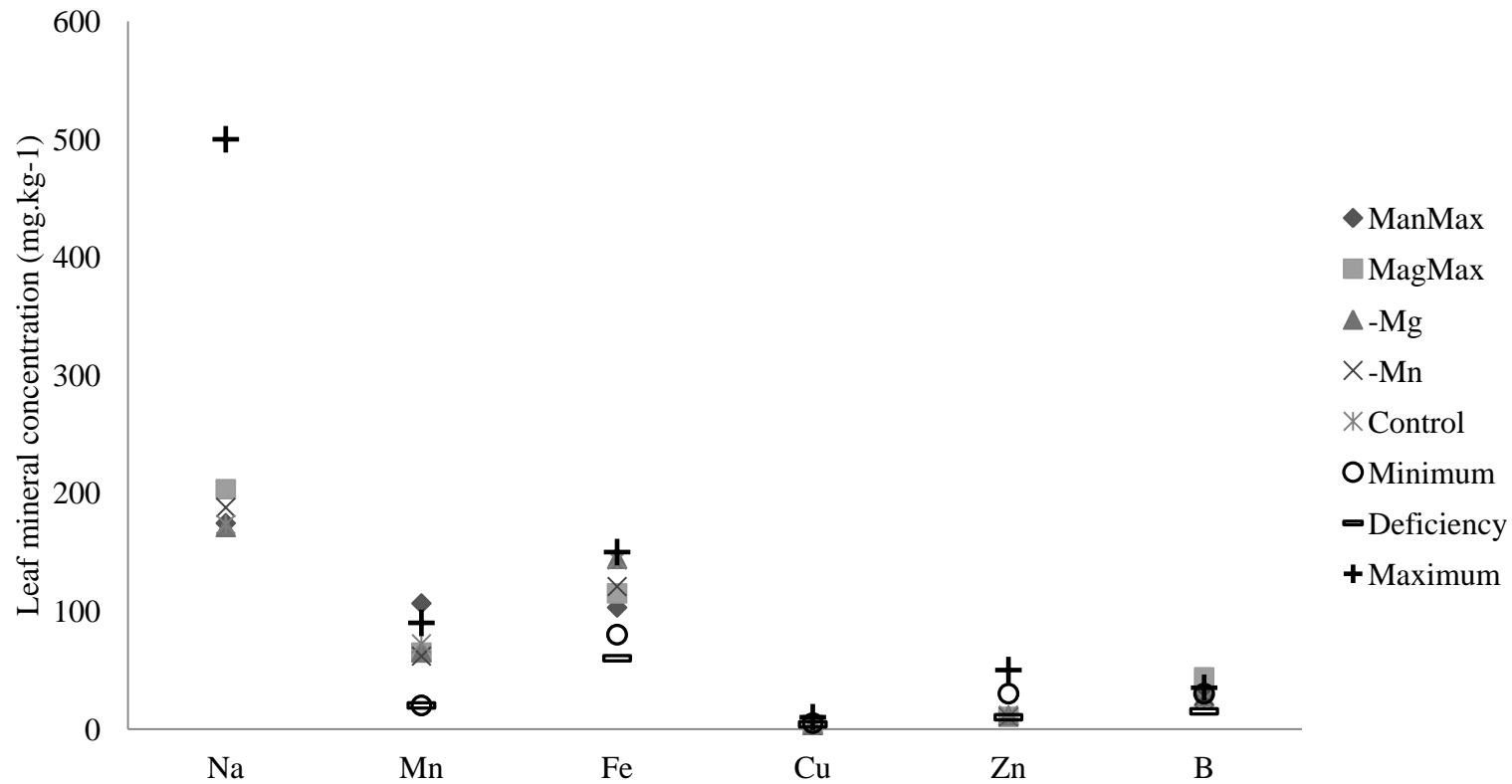


Fig. 4. Comparison of foliar treatments to overcome Mg and Mn deficiencies with industry apple norms published by Kotzé (2001) for micro nutrients in the ‘Royal Beaut’ trial.



a)



b)

Plate 1. a) 'Laetitia' and b) 'Royal Beaut' leaves from the control with nitrogen deficiency symptoms in December 2011 after feeding with an incorrect Long Ashton solution formulation.



a)

b)

c)

Plate 2. ‘Laetitia’ plum leaf samples of a) the control with no indications of magnesium deficiency symptoms b) ‘-Mg’ treatment with typical magnesium deficiencies and c) ‘MagMaxTM’ treatment with a pale green colour indicating possible nitrogen deficiency, taken on the 13th of February 2012.



a)

b)

c)

Plate 3. 'Laetitia' plum leaf samples of a) the control showing no visual symptoms of manganese deficiency b) '-Mn' indicating a typical nitrogen deficiency and c) 'ManMax™' treatment indicating no visual manganese deficiency taken on the 13th of February 2012.



a)

b)

c)

Plate 4. 'Royal Beaut' apple leaf samples for a) the control with no visible of magnesium deficiency b) '-Mg' treatment with typical symptoms of magnesium deficiency and c) 'MagMax™' treatment with no visible symptoms of magnesium deficiency taken on the 30th of March 2012.



a)

b)

c)

Plate 5. ‘Royal Beaut’ apple leaf samples of a) the control with possible symptoms of a deficiency b) ‘-Mn’ treatment with visible symptoms of deficiency, possibly nitrogen and c) ‘ManMaxTM’ treatment with possible visible symptoms of nitrogen deficiency taken on 30th of March 2012.

GENERAL CONCLUSION

Results showed that AlexinTM and CropbiolifeTM are effective in controlling *Xanthomonas* infection on both fruit and leaves of ‘Sandvliet’ peaches. Disease resistance mechanisms in plants are very complex (Durner *et al.*, 2007) and establishing systemic acquired resistance (SAR) in plants may require more than just the application of certain nutrients and salicylic acid (SA). The effect of both AlexinTM and CropbiolifeTM as a post-harvest treatment in addition to the pre-harvest treatment should also be further evaluated. More information is required to establish the longevity of SAR in fruit trees, to develop a more effect strategies to use these treatments in a commercial orchard.

The fact that AlexinTM, CropbiolifeTM and K-MaxTM had positive effects on fruit size and mass as well as increased disease resistance, strengthening recommendations to apply these products in commercial orchards. However, the increases in fruit mass and size have come at a cost as results for shoot length indicated. As the lack of vegetative growth may have an effect on the consecutive year’s reproductive growth in peaches, thus the effect of these commercial formulations on yield need to be followed up. A better understanding of the physiological cost associated with SAR is needed as this may lead to better tree nutrition strategies, in conjunction with the artificial establishment of SAR.

Potassium presented itself as a mineral element important for disease resistance, confirming reports from Matthee and Daines (1969), with both K-MaxTM and AlexinTM (contains K) showing a significant increase in disease resistance in ‘Sandvliet’ peaches over the control.

AlexMax, AlexSal, AlexinTM and ResistTM were unsuccessful in overcoming induced stress in ‘Laetitia’ and ‘Songold’ plum trees. SA contained in the AlexinTM, ResistTM and AlexSal treatments also showed that it assists with the absorption of nutrients. The ‘ManMaxTM,

treatment was successful in increasing the Mn concentration of both ‘Laetita’ plum and ‘Royal Beaut’ apple trees to levels similar to that of treatments that received Mn. The study indicates that ‘MagMaxTM’ and ‘ManMaxTM’ foliar applications may not be successful in overcoming deficiencies of Mg and Mn if other mineral imbalances are present in the trees. These foliar applications can therefore be used as part of a management strategy adopted, but cannot be used in isolation as there are strong interactions between mineral elements as shown in this study (Fageria, 2001).

In conclusion, the establishment of plant disease resistance can successfully be established with the exogenous application of SA in ‘Sandvliet’ peaches. In spite the fact that the treatments were not successful to overcome the waterlogged stress in the plum trees, it generally had a positive effect on the fruit size and quality. However, more research is required on the long term effects on both fruit quality and tree health to fully exploit the benefits of SA in fruit production.

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