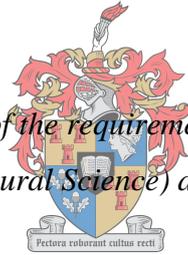

USE OF BIOSTIMULANTS AS AN ALTERNATE APPROACH TO ACHIEVE PLANT PERFORMANCE AND FRUIT QUALITY

By

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*Thesis presented in partial fulfilment of the requirements for the degree of Master of Science
in Agriculture (Horticultural Science) at Stellenbosch University*



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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

December 2018

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ACKNOWLEDGEMENTS

I am sincerely grateful to the following people and institutions:

- My supervisor: Dr Elmi Lötze, for her unwavering support, positivity, encouragement, guidance, insight and mentorship.
- My co-supervisors: Dr E.W. Hoffman and Mr T. Vahrmeijer, for their guidance and input.
- Mr G. Lötze and his staff in the Department of Horticulture Science, University of Stellenbosch for all their assistance in the field.
- My fellow buddy students (Anthony Mwije and Anouska Cameron) for their assistance with grammar, spelling and references.
- My friends and family for their support and encouragement.
- Citrus Research Institute[®], Karabos International[®] and MBFI[®] for funding the project.
- Almighty God for his enduring love that gave me strength, courage and hope to complete this study.

DEDICATION

The thesis is dedicated to my two heroes Mr. P and Mrs. H. Shereni for their love, support and courage throughout this study.

SUMMARY

Research in fruit crops using biostimulants as a strategy to improve tree performance by alleviating transplant shock and improving both yield and fruit quality under field conditions, is limited. The main aim of this study was thus to determine the efficacy of biostimulants to enhance plant performance and quality of fruit trees under South African conditions. The specific objectives were to determine: i) the effect of a combination of biostimulants on growth and fruit quality of bearing ‘Rubystar’ plum and ‘Forelle’ pear cultivars, ii) the efficacy of a combination of biostimulants to reduce transplant shock and promote establishment of non-bearing ‘Packham’s Triumph’ and ‘Celina’ pear trees and iii), the efficacy of the biostimulant application to improve transplanting and establishment success of ‘Nadorcott’ mandarin trees in the Western Cape, South Africa.

The first experiment was conducted on the commercial farms of ‘Bourgogne’ in Franschhoek and ‘Avontuur’ in Villiersdorp, Western Cape, whereas the second and third experiments were conducted at the Stellenbosch University Welgevallen Experimental Farm, Stellenbosch. The first two studies consisted of two treatments: the control (no application) and a soil-based biostimulant combination (Rizofos Fruit[®], Tri-Cure SP[®], Premax[®] and Technical[®]).

In the Franschhoek and Villiersdorp experiments, no significant differences were found between treatments, for either the plums or pears, with respect to vegetative growth and fruit quality parameters during the first season of application. However, a biological trend where the biostimulant treatment enhanced stem diameter, shoot length and yield efficiency, even though statistically non-significant compared to the control treatment in ‘Rubystar’ plums, warrants further investigation.

Similarly, the second experiment also did not realise significant differences between treatments with respect to vegetative growth for either ‘Celina’ or ‘Packham’s Triumph’ trees during the first season. When considering physiology however, the biostimulant treatment recorded a significantly higher stomatal conductance for ‘Packham’s Triumph’ and ‘Celina’ compared to that of the control treatment.

In the third experiment, five biostimulant treatments: compost (control), Terramax[®], Kelpak[®], RootAktiv[®] and Super Wortel[®] were evaluated. The RootAktiv[®] treatment had a significantly higher shoot growth than the Terramax[®] and Kelpak[®] treatments but was similar to the control. The Kelpak[®] treatment had a significantly higher root length and volume

compared to the RootAktiv[®] and Super Wortel[®] treatments but was similar to the control. The Terramax[®] and Kelpak[®] treatments had a high SWP than Super Wortel[®] and RootAktiv[®] in March, whereas in May, Terramax[®], Kelpak[®] and RootAktiv[®] treatments had a significantly higher SWP than the control and Super Wortel[®] treatments. The Kelpak[®] treatment showed significantly higher stomatal conductance than the control and Super Wortel[®] treatments, for the second evaluation date. Thus, contrary to expectations, significant differences and promising trends were already obtained after one season with young, non-bearing ‘Nadorcott’ mandarin trees. In addition, results suggest that the time of application and target organ (root or leaf) will be an important factor determining the efficacy of the applications.

From the study, it was concluded that certain biostimulants, under specific conditions, showed promising trends in enhancing performance of perennial crops under field conditions. The importance of selecting the correct product for a specific goal, based on mode of action, was emphasised. Similarly, the lack of significant differences between treatments despite evident trends confirmed the importance of the conduction of field experiments over at least two or more consecutive seasons before final conclusions are drawn.

OPSOMMING

Navorsing rakende die gebruik van biostimulante as strategie vir boom prestasie via bemiddeling van oorplantskok en verhoging van opbrengs en vrugkwaliteit onder veldtoestande, is beperk. Die hoof doel van die studie was om die effektiwiteit van biostimulante op verhoging van plant prestasie en vrugkwaliteit van vrugtebome onder Suid-Afrikaanse omstandighede te ondersoek. Die spesifieke doelwitte was die bepaling van i) die effek van 'n kombinasie van biostimulante op groei en vrugkwaliteit van draende 'Ruby Star' pruim- en 'Forelle' peer kultivars, ii) die effektiwiteit van 'n kombinasie van biostimulante op die verminderin van oorplantskok en bevordering van vestiging van nie-draende 'Packham's Triumph' en 'Celina' peerbome en iii), die effektiwiteit van biostimulant toedienings ter bevordering van oorplant en vestiging van 'Nadorcott' mandaryn bome in die Weskaap, Suid-Afrika.

Die eerste studie is uitgevoer op die kommersiële plase Bourgogne, in Franschhoek, en Avontuur, in Villiersdorp. Die tweede en derde eksperimente is uitgevoer op die Welgevallen Navorsingsplaas in Stellenbosch. Die eerste twee eksperimente het bestaan uit twee behandelings: die kontrole (geen toedienings) en 'n grondgebaseerde biostimulant kombinasie (Rizofos Fruit[®], Tri-Cure SP[®], Premax[®] and Technical[®]).

In die Franschhoek en Villiersdorp eksperimente, is geen betekenisvolle verskille in die pruime of pere tussen behandelings gevind ten opsigte van vegetatiewe groei en vrugkwaliteitsparameters gedurende die eerste seisoen van toediening nie. Desnieteenstaande was daar 'n tendens wat getoon het dat die biostimulant behandelings gelei het tot 'n toename in stamdeursnit, loot lengte en opbrengseffektiwiteit (nb) in vergelyking met die kontrole by die 'Ruby Star' pruime, wat 'n opvolg studie regverdig.

Soortgelyk, het die tweede studie ook nie betekenisvolle verskille tussen behandelings getoon vir die vegetatiewe groei vir 'Celina' of 'Packham's Triumph' pere gedurende die eerste seisoen nie. In soverre dit blaar fisiologie aangaan, het die biostimulant behandeling 'n betekenisvolle hoër huidmondjie geleiding getoon vir beide 'Celina' en Packham's Triumph', in vergelyking met die kontrole.

In die derde studie, is vyf biostimulant behandelings geëvalueer: kompos (kontrole), Terramax[®], Kelpak[®], RootAktiv[®] en Super Wortel[®]. Die RootAktiv[®] behandeling het 'n betekenisvolle hoër lootgroei getoon as die Terramax[®] en Kelpak[®] behandelings, maar was soortgelyk aan die res van die behandelings. Die Kelpak[®] behandeling het 'n betekenisvolle

hoër wortelvolumen getoon as die RootAktiv[®] en Super Wortel[®] behandelings, maar was soortgelyk aan die res van die behandelings. Die Terramax[®] en Kelpak[®] behandelings het 'n laer stamwaterpotensiaal (SWP) getoon as die RootAktiv[®] en Super Wortel[®] in Maart, teenoor die Terramax[®], Kelpak[®] en RootAktiv[®] behandelings se betekenisvolle laer SWP as die kontrole en Super Wortel[®] behandelings in Mei. Die Kelpak[®] behandeling het 'n betekenisvolle hoër huidmondjie geleiding getoon as die kompos en Super Wortel[®] behandelings tydens die tweede evaluasie datum. Dus, in teenstelling met verwagtings, is betekenisvolle verskille tussen behandelings reeds na een seisoen gekry in die jong, nie-draende 'Nadorcott' mandaryn bome. Bykomend, het resultate gedui daarop dat die tyd van toediening en teiken orgaan (lote of blare) belangrike faktore is wat die effektiwiteit van die goedienings sal beïnvloed.

Uit die studie kon afgelei word dat sekere biostimulante, onder spesifieke omstandighede, belowende tendense getoon het in die verhoging van prestasie van meerjarige gewasse onder veld toestande. Die belang van die keuse van die korrekte produk vir 'n spesifieke doelwit, gebaseer op die aksie van die produk, is beklemtoon. Soortgelyk, het die belowende tendense van die effekte van die biostimulante op boom prestasie parameters, maar gebrek aan betekenisvolle verskille tussen behandelings, die belang van die uitvoer van proewe in die veld oor 'n langer tydperk, bevestig voordat die finale gevolgtrekkings gemaak kan word.

This thesis is a compilation of chapters, starting with a literature review, followed by three research papers. Each paper is prepared as a scientific paper for submission to *Southern African Journal for Plant and Soil*. Repetition or duplication between papers are unavoidable.

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GENERAL INTRODUCTION

Tree and fruit quality can be defined by the degree of excellence or superiority which can either be intrinsic or extrinsic (Kader, 1999). Product quality determines marketable yield and price to growers (Radovich, 2010). An important aspect of achieving or maintaining fruit quality is provision of supplementary nutrition. One of the challenges in deciduous fruit production in South Africa, specifically in the Western Cape, is the characteristic, acidic, often shallow (30 – 50 cm) and inherently infertile soils (Kangueehi, 2008). Therefore, in order to achieve sustainable high yields and deliver top quality fruit, soil amelioration and proper soil management is often required.

The application of chemical fertilizers are generally considered as an effective and inexpensive method of applying mineral nutrients to fruit trees. However their extensive implicates a heavy carbon footprint and in addition, may lead to environmental pollution (Dong *et al.*, 2002). Furthermore, environmental pollution of the air and water may be caused by an oversupply of the fertilizers over many consecutive seasons to compensate for the depletion of nutrients by agriculture, particularly that of nitrogen (N) and phosphorous (P), despite high associated costs (Vance, 2001). Continual applications of N and P fertilisers from chemical source leads to disturbance in the soil microbial diversity (Bagyaraj & Revanna, 2016). Furthermore, substrate-induced respiration and the biomass carbon (C) production ability of the microbes has been inhibited by continuous use of chemical fertilizers from phosphate sources (Bolan *et al.*, 1996).

In addition to supplying optimal nutrition, another critical aspect of orchard management is to ensure successful tree establishment with high plant performance of most bare root nursery trees as is typical under South African conditions (personal communication with Dr. E. Lötze, Stellenbosch University). Trees are considered to be exposed to highly stressful conditions and also transplanting shock, when moved from one growing site to another, such as from the nursery to the orchard (Struve, 2009). Transplant shock makes plants more susceptible to injuries, depletion of reserves and often results in impaired physiological functions such as reduced photosynthesis, so that a process of adaptation and recovery to its new environment is almost always required (Struve, 2009). Root loss in particular is often associated with transplanting and has a negative impact by reducing the ability of the plant to absorb water and mineral nutrients, which then results in a loss of stored carbohydrates and

mineral nutrients, leading to poor plant performance or even death (Gauthier & Kaiser, 2014; Struve, 2009). Survival of newly planted trees is now widely recognised on extensive root extension subsequent to transplanting to ensure fast absorption of water thereby reducing drought-related water stress symptoms by replenishing transpirational water loss (Watson & Himelick, 2005). These challenges associated with transplanting has led to concern on agricultural practices that are organic sustainable and environmentally-friendly (Esitken *et al.*, 2010). They act as possible remedies for managing alleviation of environmental pollution (Vessey, 2003) and abiotic stress (Stirk & Van Staden, 2004; Khan *et al.*, 2009; Craigie, 2011) which may exist with transplanting. Such ameliorants may include the use of biofertilizers and biostimulants.

Biofertilizers are substances that contain living micro-organisms and are known to improve seed germination and promote expansion of the root system (Chen, 2006). Biofertilization of crops involves use of nitrogen-fixing and P-solubilizing bacteria which plays a significant role in N and P uptake by the plants (Alam *et al.*, 2002). The main micro-organisms used as biofertilizers include: vesicular arbuscular mycorrhiza (VAM), plant growth promoting rhizobacteria (PGPR), phosphate-solubilizing bacteria (PSB), rhizobia, azotobacters and azospirillum (Chen *et al.*, 2006; Igual *et al.*, 2001).

A biostimulant is considered as any substance applied to plants exogenously, thereby enhancing nutrition efficiency, promoting tolerance to abiotic stress, irrespective of its nutrient content (Du Jardin, 2015). Plant biostimulants contain substance(s) and/or microorganisms that stimulate natural processes when applied to the plants rhizosphere, enhancing nutrient uptake and efficiency, abiotic stress tolerance and crop quality improvement (Calvo *et al.*, 2014). In the regulatory framework of pesticides, biostimulants have been shown to have no direct action against pests (EBIC, 2012). Biostimulants are categorised into four main groups based on the effect on root growth and nutrient uptake. These include: (1) seaweed extract (SE), (2) protein hydrolysate and amino acid formulations (AA), (3) humic substances (HS), and (4) plant growth-promoting microorganisms (Calvo *et al.*, 2014; Du Jardin, 2015) which can also be classified in its own group as biofertilizers.

Several reviews on biofertilizers (Beneduzi *et al.*, 2012; Bi *et al.*, 2011; Vejan *et al.*, 2016) and biostimulants (Calvo *et al.*, 2014; Du Jardin, 2015; Khan *et al.*, 2009, 2011, 2012, 2013; Tandon & Dubey, 2015) have been published in recent years, but research pertaining to contribution of biostimulants products to fruit tree species on the establishment, yield and fruit quality under South African conditions remains extremely limited.

The main objective of this study was to determine the efficacy of a range of biostimulants to enhance plant performance and to promote fruit quality of trees under South African conditions. The specific objectives were to determine:

1. The effect of the use of biostimulants on yield and fruit quality: by evaluating the effect of a combination of Rizofos Fruit[®], Tri-Cure SP[®], Premax[®] and Technical[®] on plant performance and fruit quality of ‘Rubystar’ plum and ‘Forelle’ pear.
2. The effect of the use of biostimulants on promoting resistance to transplant shock and to enhance tree performance of a deciduous tree crop: by the application of a combination of Rizofos Fruit[®], Tri-Cure SP[®], Premax[®] and Technical[®] on non-bearing ‘Packham’s Triumph’ and ‘Celina’ pear trees.
3. The effect of the use of various biostimulants on reducing transplant shock and promoting tree performance on an evergreen tree crop ‘Nadorcott’ mandarin (*Citrus reticulata* Blanco).

References

- Alam, S., Khalil, S., Ayub, N. & Rashid, M. 2002. In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganisms (PSM) from maize rhizosphere. *International Journal of Agriculture and Biology* 4:454–458.
- Bagyaraj, D.J. & Revanna, A. 2016. Effect of chemical fertilizers on the beneficial soil microorganisms. *Fertilizers and Environment News* 2:10–11.
- Beneduzi, A., Ambrosini, A. & Passaglia, L.M.P. 2012. Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology* 35:1044–1051.
- Bi, F., Iqbal, S., Arman, M., Ali, A. & Hassan, M. 2011. Carrageenan as an elicitor of induced secondary metabolites and its effects on various growth characters of chickpea and maize plants. *Journal of Saudi Chemical Society* 15:269–273.
- Bolan, N.S., Currie, L.D. & Baskaran, S. 1996. Assessment of the influence of phosphate fertilizers on the microbial activity of pasture soils. *Biology and Fertility of Soils* 21:284–292.
- Calvo, P., Nelson, L. & Kloepper, J.W. 2014. Agricultural uses of plant biostimulants. *Plant Soil* 383:3–41.
- Chen, J.H. 2006. The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. *International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use* 16–20:1–11.
- Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A. & Young, C.C. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Applied Soil Ecology* 34:33–41.
- Craigie, J.S. 2011. Seaweed extract stimuli in plant science and agriculture. *Journal of Applied Phycology* 23:371–393.
- Dong, S., Cheng, L., Scagel, C.F. & Fuchigami, L.H. 2002. Nitrogen absorption, translocation and distribution from urea applied in autumn to leaves of young potted apple (*Malus domestica*) trees. *Tree physiology* 22:1305–1310.
- Du Jardin, P. 2015. Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae* 196:3–14.
- EBIC. 2012. Promoting the biostimulant industry and the role of plant biostimulants in making agriculture more sustainable. [Online], Available: <http://www.biostimulants.eu/>. Accessed on 12 June 2017].

- Esitken, A., Yildiz, H.E., Ercisli, S., Figen Donmez, M., Turan, M. & Gunes, A. 2010. Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. *Scientia Horticulturae* 124:62–66.
- Gauthier, N.W. & Kaiser, C. 2014. *Transplant shock: Disease or cultural problem?* Transplanting trees and shrubs, University of Kentucky College of Agriculture, Food and Environment, USA. 11 pp.
- Huysamer, M., 1997. Integrating cultivar, rootstock and environment in the export-driven South African deciduous fruit industry. *Acta Horticulturae* 451:755-760.
- Igual, J.M., Valverde, A., Cervantes, E. & Velazquez, E. 2001. Phosphate-solubilizing bacteria as inoculants for agriculture: use of updated molecular techniques in their study. *Agronomie* 21:561–568.
- Kader, A.A. 1999. Fruit maturity, ripening, and quality relationships. *Acta Horticulturae* 485:203–208.
- Kangueehi, G.N. 2008. Nutrient requirement and distribution of intensively grown 'Brookfield Gala' apple trees. MSc Thesis, Faculty of AgricSciences, Stellenbosch University.
- Khan, W., Rayirath, U.P., Sowmyalakshmi, S., Jithesh, M.N., Rayorath, P., Hodges, D.M., Critchley, A.T., Craigie, J.S., Norrie, J., Prithiviraj, B. 2009. Seaweed extracts as biostimulants of plant growth: Review. *Journal of Plant Growth Regulation* 28:386–399.
- Khan, W., Hiltz, D., Critchley, A.T. & Prithiviraj, B. 2011. Bioassay to detect *Ascophyllum nodosum* extract-induced cytokinin-like activity in *Arabidopsis thaliana*. *Journal of Applied Phycology* 23:409–414.
- Khan, W., Zhai, R., Souleimanov, A., Critchley, A.T., Smith, D.L. & Prithiviraj, B. 2012. Commercial extract of *Ascophyllum nodosum* improves root colonization of Alfalfa by its bacterial symbiont *Sinorhizobium meliloti*. *Communications in Soil Science and Plant Analysis* 43:2425–2436.
- Khan, W., Palanisamy, R., Critchley, A.T., Smith, D.L., Papadopoulos, Y. & Prithiviraj, B. 2013. *Ascophyllum nodosum* extract and its organic fractions stimulate rhizobium root nodulation and growth of *Medicago sativa* (Alfalfa). *Communications in Soil Science and Plant Analysis* 44:900–908.
- Radovich, T. 2010. Fruit and vegetable quality: It matters! Sustainable and organic agriculture program, Manoa, Hawaii. 1-3 pp.
- Stirk, W.A. 2006. World seaweed resources. *South African Journal of Botany* 72:666-680.
- Stirk, W. & Van Staden, J. 2004. Potential new applications for the southern African kelps. *South*

African Journal of Botany 70:145–151.

- Struve, D.K. 2009. Tree establishment: A review of some of the factors affecting transplant survival and establishment. *Arboriculture and Urban Forestry* 35:10–13.
- Tandon, S. & Dubey, A. 2015. Effects of Biozyme (*Ascophyllum nodosum*) biostimulant on growth and development of soybean [*Glycine Max* (L) Merrill]. *Communications in Soil Science and Plant Analysis* 46:845–858.
- Vance, C.P. 2001. Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. *Plant Physiology* 127:390–397.
- Vejan, P., Abdullah, R., Khadiran, T., Ismail, S. & Nasrulhaq Boyce, A. 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability-A review. *Molecules* 21:1–17.
- Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* 255:571–586.
- Watson, G. & Himelick, E.B. 2005. Best management practices: Tree planting. ANSI A300 Part 6: Tree, shrub and other woody plant maintenance-standard practices (transplanting). *International Society of Arboriculture*. Champaign, IL.

LITERATURE REVIEW: The role of mineral nutrient management by means of alternative approaches to promote plant growth and enhance fruit quality

1. INTRODUCTION

Deciduous fruit production in South Africa, specifically in the Western Cape region, is characterised by acidic, often shallow (30 - 50 cm) and inherently infertile soils (Kangueehi, 2008). These soils need to be rectified and managed properly to improve yield and quality of both the crop and its fruit. Mineral nutrition is one of the important factors in fruit tree production since minerals are responsible for several physiological functions such as enzyme activation, control of energy processes and osmotic regulation of the membranes (Kangueehi, 2008). Temperate fruit trees such as pears (*Pyrus communis* L.) and plums (*Prunus domestica* L.), but also subtropical crops such as *Citrus* spp. should be fertilized whenever soil of commercial orchards cannot provide sufficient amounts of nutrients to attain maximum yields. Therefore, it is important to determine the mineral nutrient requirements of the tree, as well as the appropriate phenological stage for additional application, to obtain optimum absorption of these elements in order to benefit the physiological processes and, ultimately, yield and fruit quality (Snijder, 2000). Of all considerations, product quality is most important as it determines marketable yield and its associated price (Radovich, 2010).

2. Role of mineral nutrition to growth and fruit quality of fruit trees

Plums, pears and citrus trees require 16 essential elements for the successful completion of their life cycle (Salisbury & Ross, 1992). Carbon, hydrogen and oxygen are derived from the atmosphere and soil water (Kangueehi, 2008). The remaining 13 essential elements: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), boron (B), molybdenum (Mo), and chlorine (Cl) are supplied, either from soil minerals and soil organic matter, or by organic or inorganic fertilizers (Uchida, 2000). Elements needed in relatively large amounts are termed macronutrients: nitrogen, phosphorus, potassium, calcium, magnesium and sulphur, whereas those required in smaller concentrations are known as micronutrients and include chlorine, iron, manganese, zinc, boron, copper and molybdenum (Taiz *et al.*, 2015).

Nutrient functions are well studied and reviewed (Marschner, 1993, 2011; Mengel & Kirkby, 1982; Mohr & Schopfer, 1994; Neilsen & Neilsen, 2003; Salisbury & Ross, 1992; Taiz *et al.*, 2015).

The specific nutrient requirements for optimal fruit production and quality are however crop specific and need to be determined. An effective method for determining tree nutrient requirements is one based on whole tree mineral analysis (Weibaum *et al.*, 2001). Although, fruit trees was reported to have in total requirement for 16 major nutrients, only six nutrients (N, P, K, Ca, Mg and S) were of importance in promoting the parameters that were quantified. These include growth, physiological activity and fruit quality.

2.1 Nitrogen

Nitrogen is a major constituent of amino acids, nucleic acids and other compounds (Kangueehi, 2008) and is available to plants as nitrate (NO_3^-) and ammonium (NH_4^+) ions (Uchida, 2000). Amino acids are central to plant growth and development as it is key in forming the protoplasm which is also the site for cell division. Nitrogen is furthermore a major structural component of the chlorophyll molecule and therefore supports photosynthesis by improving the quality and quantity of leaves (Uchida, 2000). Nitrogen is also required for growth, blossom formation, fruit set and to promote fruit size, all factors which collectively contribute to determine crop yield.

Nitrogen deficiency in pear, plum and citrus trees result in poor vegetative and reproductive growth, which lead to small fruit (Kangueehi, 2008), while it may reduce differentiation of reproductive buds and stunted growth may occur because of a reduction in cell division (Brunetto *et al.*, 2015).

2.2 Phosphorus

Phosphorus, as phosphate (PO_4^{3-}), next to N, is the most important mineral element with respect to nutrition of plants (Sharma *et al.*, 2013) and contributes to about 0.2 % of the plant's dry weight. Phosphorus plays a significant role in increasing root complexity and strength, thereby imparting both vitality and enhance disease resistance capacity to the plant (Taiz *et al.*, 2015). Characteristics symptoms of P deficiency include stunted growth of the entire plant, which is characterized by a dark green coloration of the leaves, where leaves may be malformed and contain small areas of dead tissue called necrotic spots (Sharma *et al.*, 2013; Taiz *et al.*, 2015; Uchida, 2000).

2.3 Potassium

Potassium is acquired by plants in the form of potassium ions (K^+) and exists in large quantities in both leaf and fruit tissues (Johnson & Uriu, 1989). This includes both young, actively growing cells and also guard cells, which is responsible for the opening and closing of stomata. Potassium is

essential for translocation of photosynthate (sugars) and formation of starch that is either required for plant growth or storage either in the fruit or roots. Although one of the functions of K^+ is to activate enzymes, most K ions are not tied up in complex molecules, but are used in the ionic form by cells as a solute, particular with respect to maintaining turgor (Zekri & Obreza, 2012). Important to note is that K is the most abundant nutrient in the fruit where it positively affects fruit size, firmness, skin colour, total soluble solids (TSS), acidity, juiciness and aroma (Brunetto *et al.*, 2015).

The most common symptom of K^+ deficiency is chlorosis along the edges of leaves (leaf margin scorching) (Taiz *et al.*, 2015). Excess K^+ can alternatively increase susceptibility to bitter pit (Bright, 2005; Brunetto *et al.*, 2015). Other symptoms of K^+ deficiency, especially in citrus, are reduced fruit size with very thin rind of a smooth texture and leaf scorching (Haifa, 2016; Tucker, 1999).

2.4 Calcium

Calcium is involved in cell physiology, integrity and stability of cell membranes, organization of the cell wall and tolerance against fungal and bacterial infections (Toselli *et al.*, 2012). Optimum fruit Ca concentration promotes fruit firmness, increases disease tolerance and reduces storage related disorders (Brunetto *et al.*, 2015). Characteristics symptoms of Ca deficiency include browning of root and leaf tips and symptoms appear first on younger leaves and leaf tips since Ca is immobile (Uchida, 2000).

2.5 Magnesium

Magnesium, similar to N, is a structural component of the chlorophyll molecule which is actively involved in the process of photosynthesis (Johnson & Uriu, 1989; Tucker, 1999). In addition, Mg is an activator of many important enzyme reactions and in particular, acts as a co-factor in several enzymatic reactions that activate phosphorylation processes, which is required to stabilize ribosome particles and also assist in stabilizing the structure of nucleic acids. Mg is also involved in promoting the movement of sugars within a plant (Uchida, 2000). The Mg deficiency symptom of interveinal chlorosis typically first appears in older leaves on plums and pears (Bright, 2005; Uchida, 2000).

2.6 Sulphur

Sulphur is utilized in the form of sulphate ions (SO_4^-). S aids in chlorophyll formation and is essential in forming plant proteins because it is a constituent of certain amino acids. In addition, S is also actively involved in metabolism of the B vitamins biotin and thiamine and co-enzyme A (Uchida,

2000), while S also affects carbohydrate metabolism (Zekri & Obreza, 2012). Most orchard soils are rarely deficient in S, partly as it is often added secondary in many different ways, including as fertilizers such as ammonium sulphate, gypsum, manure and other organic matter, as well as through atmospheric SO₂, which is carried into the soil by rain. S deficiency of younger leaves show up as being chlorotic with evenly, lightly coloured veins. In some plants, such as citrus, the older leaves may show symptoms first. Growth rate is retarded and maturity is delayed, plant stems are stiff, thin and woody. Symptoms may appear similar to N deficiency and are most often found in sandy soils that are low in organic matter and receive moderate to heavy rainfall (Brunnetto *et al.*, 2015; Tucker, 1999; Uchida, 2000).

3. Constraints in using mineral nutrients

In irrigated, commercial orchard systems, the magnitude and timing of plant demand for N and its retention in the root zone to allow root interception are important factors for efficient management of N fertilizer (Nielsen & Nielsen, 2002). The application of chemical fertilizers is an inexpensive and effective method of supplying crops with mineral nutrients (Chen, 2006). While N application can be sufficient to improve plant production, it also leads to a worldwide concern about environmental contamination resulting from excessive nitrate leaching (Dong *et al.*, 2005). In addition, fertilizers are often washed from the field in the runoff and can become unavailable to the crops through chemical, physical, or biological transformation (Halpern *et al.*, 2015). To compensate for these processes, farmers need to apply more chemical fertilizer than the plant actually needs and these large quantities of chemical fertilizers are used to replenish soil N and P, resulting in high costs and severe environmental contamination or the remainder is often released into the environment, polluting the air and water (Vance, 2016). Furthermore, the industrial production of chemical fertilizers is an energy-intensive process that is known to significantly contribute to global CO₂ emissions (Vance, 2016).

Some of the major constraints in using chemical fertilizers include: (i) leaching and pollution of water resources, destruction of micro-organisms and beneficial insects, crop susceptibility to disease attack, acidification, alkalization of the soil or reduction in soil fertility, thus causing irreparable damage to the overall system; (ii) oversupply of N leads to softening of plant tissue resulting in plants that are more sensitive to diseases and pests; (iii) reduction of the colonization of plant roots with mycorrhizae and inhibit symbiotic N fixation by rhizobia due to high N fertilization; (iv) enhancing the decomposition of soil organic matter, which leads to degradation of soil structure

and (v), easy loss of nutrients from soils through fixation, leaching or gas emission that can lead to reduced fertilizer efficiency (Chen, 2006; Kanguuehi, 2008; Van Schoor, 2009; Taiz *et al.*, 2015).

4. Alternative approaches in promoting growth and fruit quality.

Chen (2006) suggested the use of biofertilizers and organic farming practices as good alternative approaches in promoting growth and fruit quality, whilst maintaining the fertility of the soil. Introduction of biostimulants to crops via leaves, seeds or soil can serve as a means of stimulating growth and maintaining fruit quality (Halpern *et al.*, 2015). Current research indicates biofertilization and the use of biostimulants to be the best alternative approach for increased production and to obtain top fruit quality (Bashan *et al.*, 2014; Calvo *et al.*, 2014; Du Jardin, 2015).

4.1 Biofertilization

Biofertilizer is defined as a substance which contains living micro-organisms and is known to promote the expansion of the root system and promote seed germination (Chen, 2006). N₂-fixing and P-solubilizing bacteria may be important for plant nutrition by increasing N and P uptake by the plants in addition to playing a significant role as plant growth promoting rhizobacteria (PGPR) in the bio-fertilization of crops (Alam *et al.*, 2002). Increasing and extending the role of bio-fertilizers would reduce the need for chemical fertilizers and decrease the associated adverse environmental effects (Karlidag *et al.*, 2007).

Biofertilizers differ from chemical and organic fertilizers in the sense that they do not directly supply any nutrients to crops, being mostly cultures of special bacteria and fungi (Igual *et al.*, 2001). The main micro-organisms used as biofertilizers include: rhizobia, azotobacters and azospirillum, phosphate-solubilizing bacteria (PSB), vesicular arbuscular mycorrhiza (VAM) and plant growth promoting rhizobacteria (PGPR) (Chen, 2006).

4.1.1 Phosphate-solubilizing bacteria (PSB),

Under acidic or calcareous soil conditions, large amounts of P are fixed in the soil and is therefore unavailable to the plants (Bashan & Holguin, 1998; Bashan *et al.*, 2014). Phosphobacterins, mainly bacteria and fungi, can however make insoluble P available to the plant. The solubilization effect of phosphobacterins is generally due to the production of organic acids that lower the soil pH and bring about the dissolution of bound forms of P (Igual *et al.*, 2001; Whitelaw, 2000). It is reported that PSB culture increased yield up to between 200 - 500 kg.ha⁻¹ and thus, 30 to 50 kg of superphosphate can be saved (Kloepper *et al.*, 1989; Vessey, 2003).

4.1.2 Plant growth promoting rhizobacteria (PGPR)

PGPR represent a wide variety of soil bacteria which, when grown in association with a host plant, result in stimulation of host growth (Karakurt & Aslantas, 2010). PGPR modes include fixing N₂, increasing the availability of nutrients in the rhizosphere, positively influencing root growth and morphology and promoting other beneficial plant-microbe symbioses (Hayat *et al.*, 2010). Some researchers indicated that PGPR will often have multiple modes of action. Ratti *et al.* (2001) found that a combination of the arbuscular mycorrhizal fungi *Glomus aggregatum*, the PGPR *Bacillus polymyxa* and *Azospirillum brasilense* maximized biomass and P content of the aromatic grass palmarosa (*Cymbopogon martinii*) when grown with an insoluble inorganic phosphate.

4.2 Biostimulants

Plant biostimulants include diverse substances and microorganisms that enhance plant growth. By definition, a plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content (Du Jardin, 2015). Plant biostimulants contain substance(s) and/or microorganisms whose function when applied to plants or rhizosphere is to stimulate natural processes to enhance nutrient uptake, nutrient efficiency, tolerance to abiotic stress and crop quality (Calvo *et al.*, 2014). Biostimulants have no direct action against pests and therefore, do not fall within the regulatory framework of pesticides (EBIC, 2012).

According to Calvo *et al.* (2014), biostimulant effects on plants are amongst other, to foster plant growth and development throughout the crop life cycle from seed germination to plant maturity; to improve efficiency of the plant's metabolism to induce yield increases and enhancing crop quality; to increase plant tolerance to and recovery from abiotic stresses; to facilitate nutrient assimilation, translocation and use; to enhance quality attributes of produce, including sugar content, colour, fruit seeding, rendering water use more efficient; to enhance certain physicochemical properties of the soil and foster the development of complementary soil micro-organisms. In addition, biostimulants also enhance root and foliage development, improve soil texture and structure, increase availability of micro and macro nutrients, improve plant's ability to recover from disease and insect damage, enhance plant resistance to environmental stresses, improve the efficiency of any fertility program and reduce the effects of pH and soil imbalance (EBIC, 2012).

Biostimulants are available in a variety of formulations and with varying components, but are generally classified into four major groups on the basis of their source and content. These groups

include: humic substances (HS) , hormone containing products (HCP) such as seaweed extracts (SE), amino acid containing products (AACP) and plant growth promoting microorganisms such as phosphorous solubilizing bacteria (PSB) (Du Jardin, 2015), which can also be categorised on its own as biofertilizers (mentioned above section). This research focused on diverse types of commercial biostimulant products that are currently in the South African market and fall under these four categories mentioned by Calvo *et al.* (2014).

4.2.1 Compost (humic acid, humins or fulvic acids)

Compost (humic acid, humins or fulvic acids) substances are natural constituents of the soil organic matter resulting from the decomposition of plant, animal and microbial residues (Du Jardin, 2015). Compost substances are also end products of microbial decomposition and chemical degradation of dead biota in soils and are considered to be the most abundant naturally occurring organic molecules on earth and the major components of soil (Calvo *et al.*, 2014). Compost substances are collections of heterogeneous compounds, originally categorized according to their molecular weights and solubility into humins, humic acids and fulvic acids.

Humic substances (HS) can be extracted from many different sources, including soils, municipal waste, vermicomposts and earthworm casts, but also from various coal deposits peat. It can be applied to the plant in a number of ways, including foliar applications in the irrigation water and as direct applications to the soil (Halpern *et al.*, 2015). HS has been shown to increase the number of fruits and flowers and improve fruit quality (Arancon *et al.*, 2006). It also improves soil structure along with the micronutrient solubility in the soil, it alters root morphology, increase root function with regard to ATPase activity and increase the action of nitrate assimilation enzymes (Halpern *et al.*, 2015).

Fulvic acid (FA) is considered to be the soil organic fraction that is soluble in both alkali and acids (Stevenson, 1994) and have greater total acidity, greater numbers of carboxyl groups and higher adsorption and cation exchange capacities than humic acid (Bocanegra *et al.*, 2006). Fulvic acids are responsible for chelation and mobilization of metal ions, including that of Fe and Al (Esteves da Silva *et al.*, 1998; Lobartini *et al.*, 1998). Given their small molecular size, fulvic acids can pass through micro pores of biological or artificial membrane systems, while humic acids cannot. The combined capacity of fulvic acids both to chelate nutrients, such as Fe, and move through membranes, has suggested the fulvic acids may play similar roles as natural chelators in the mobilization and transport of Fe and other micronutrients (Bocanegra *et al.*, 2006). It has also been suggested that, since they have smaller molecular weights, FAs can be remained in soil solution even at high salt concentrations

and at a wide range of pH (Zhang *et al.*, 2003; Zhang & Ervin, 2004, 2008; Zimmerli *et al.*, 2008). FAs thus have long-lasting potential to interact with plant roots (Varanini & Pinton, 2001).

Many compost substances were shown to elicit diverse morphological changes in plants, leading to changes in plant growth (Trevisan *et al.*, 2010). They have been reported to enhance some aspect of growth within a number of species of plants, including important agronomic crops such as soybean, wheat, rice and maize; vegetable crops such as potato, tomato, cucumber and pepper and also in fruit crops such as citrus (*Citrus limon*) and grape (*Vitis vinifera*) (Calvo *et al.*, 2014). Enhancement of lateral roots or general increased seedling root growth has been reported with tomato (Canellas *et al.*, 2011), whereas shoot growth promotion by compost substances has also been reported with cucumber, wheat, maize and pepper (Trevisan *et al.*, 2010; Canellas *et al.*, 2011; Calvo *et al.*, 2014). In a hydroponic study conducted in a growth chamber where multiple soil applications of humic substance and fulvic were made, inhibition of shoot growth of maize was reported (Asli & Neumann, 2010), suggesting that it is possible, at least under experimental conditions, to over apply humic acid.

The effect of humic- and fulvic acid in compost on plant growth has been studied extensively. A number of researchers reported humic/fulvic-like substances to increase root growth (Canellas *et al.*, 2011; Calvo *et al.*, 2014; Trevisan *et al.*, 2010). Their research showed that humic/fulvic acids primarily increased root growth by increasing root cell elongation. Canellas *et al.* (2011) found that humic/fulvic acids can produce root systems with increased branching and numbers of fine roots and as a result, potentially increase nutrient uptake by way of more root surface area.

4.2.1.1 Compost extracts

Van Schoor (2009) investigated the effect of organic material (straw mulch, compost) and biological amendments (compost extract, *Bacillus* inoculants, Effective Microorganisms) on pear tree performance, nutrient availability and soil biological properties. The effect of these biological management practices was studied with the aim of assisting interpretation of tree performance effects. Results showed that annual compost applications improved soil microbial, as well as chemical properties. However, tree performance, in terms of vigour and yield, was only improved when compost was used in addition to the compost extract, applied on a monthly basis. It was suggested that monthly compost extract applications resulted in maximum efficiency of nutrient utilisation through synchronisation of nutrient release with plant demand (Van Schoor *et al.*, 2012).

Over the six year trial period (Van Schoor, 2009), regular application of compost extract in addition to annual compost applications significantly improved shoot growth, as well as trunk

circumference. After the third growing season, the addition of compost extract resulted in an increase of 46 % total growth compared to the control. This was in agreement with the tendency of increased cumulative yield (51%) noted with the application of compost extract in addition to compost. However, in terms of fruit quality, no significant differences were found between any of the treatments during the trial period. In literature, compost application on its own has shown few significant effects on fruit quality (Kotzé & Joubert, 1992; Neilsen *et al.*, 2003, 2007; Pinamonti *et al.*, 1995). However, in studies comparing different management systems, the majority of results show higher fruit firmness for apples produced in organic systems, along with higher TSS and lower acidity (Andrews *et al.*, 2001; Peck *et al.*, 2006; Reganold *et al.*, 2001).

4.2.1.2 Slow release of nitrogen using coated fertilizers

Different ‘organic’ nitrogen fertilizer formulations using coating technology were introduced recently to add value by increasing the efficiency of N (Nyati, 2004). A subsequent study by Janse van Vuuren (2018) addressed the efficiency of these formulations (Black Urea™ and Black di-ammonium-phosphate (Black DAP™) on tree physiology and fruit quality and yield of ‘Rosy Glow’ (*Malus domestica* Borkh.).

The term Black™ is derived from an organic compound mixture used to coat the urea and DAP granule, drenching the granules to the point where it is totally covered by the humic and fulvic rich coating (Janse van Vuuren, 2018). The coating is reported to consist of a unique combination of biostimulants and nutrient biocatalysts, derived from humic acid, plant hormones, vitamins and minerals, creating a biological management system within the soil. Black Urea™ consists of granular urea high biuret (HB) coated with enriched coating. This nutrient enhanced coating was assumed to improve the biological release of available N to the plant, while minimizing the losses often associated with urea-based fertilizers (leaching and volatilisation) (Nyati, 2004). However, Janse van Vuuren (2018) reported no significant differences in yield or fruit quality after two years of application. Yet, treatments differed with regard to white root numbers. In addition, there was an indication of higher yields in the Black Urea™ treatment compared to the Urea HB. Whether this was due to slower release of N was not established in this study.

4.2.2 Seaweed extracts (SE)

Seaweed extracts (SE) are heterogeneous substances that can be characterized by its parent material, pH of the extraction solution and ¹H-NMR spectroscopy (Halpern *et al.*, 2015). SE have been shown to contain plant hormones such as auxins, cytokinin, abscisic acid and also amino acids (Khan *et al.*,

2009; Lötze & Hoffman, 2016). Other constituents contributing to the plant growth promotion include micro- and macronutrients, sterols, N-containing compounds like betaines and hormones, all which is known to act on soils as well as on the plants (Halpern *et al.*, 2015; Lötze & Hoffman, 2016). SE have been shown to increase plant growth, chlorophyll levels, flowering and yield and seed germination. These extracts promote the success of in vitro propagation and enhance plant protection against pathogens and pests (Khan *et al.*, 2009).

Seaweed and seaweed-derived products have been widely used as amendments in crop production systems due to the presence of a number of plant-growth-stimulating compounds (Tandon & Dubey, 2015). Plant algal residues such as seaweed (*Ascophyllum nodosum*), kudzu (*Pueraria montana var. lobata*) and sea kelp (*Yucca schidigera*) are considered to be good sources of biostimulants (Khan *et al.*, 2009). The commercial products RootAktiv[®] and Super Wortel[®] contains extract from *Ascophyllum nodosum* (L.), whilst Kelpak[®] is obtained from *Ecklonia maxima*. These are seaweed algae known to be rich in cytokinin and auxin precursors, enzymes, some chelating agents, minerals, betaines, polyamines, organic acids, oligosaccharides, amino acids and hydrolysed proteins (Khan *et al.*, 2009; Lötze & Hoffman, 2016; Tandon & Dubey, 2015). *A. nodosum* is the most widely researched seaweed species for agricultural purposes (Tandon & Dubey, 2015), with evidence of plant growth hormones as active ingredients in this seaweed being reported as early as the 1950s.

Seaweed is now widely recognized as an excellent source of natural plant growth regulators with demonstrated activity (Crouch *et al.*, 1992; Khan *et al.*, 2009). Seaweed products promote root growth and development (Khan *et al.*, 2009). The root growth stimulatory effect was more pronounced when extracts were applied at an early growth stage in maize and the response was similar to that of auxin, an important root growth- promoting hormone (Calvo *et al.*, 2014). Wheat plants treated with seaweed Kelpak[®] exhibited an increase in root:shoot dry mass ratio, indicating that the components in the seaweed had a considerable effect on root development (Van Staden, 2015). The root growth-promoting activity was observed when the seaweed extracts were applied either to the roots or as a foliar spray. The concentration of kelp extract is a critical factor in its effectiveness, as Van Staden *et al.* (2015) showed that, when tomato plants was exposed to high concentrations (1:100 seaweed extract: water), root growth was inhibited, whereas stimulatory effects were found at a lower concentration (1:600). Biostimulants, in general, are capable of affecting root development by both improving lateral root formation and increasing total volume of the root system (Khan *et al.*, 2009; Mancuso *et al.*, 2006).

5. Justification to use alternative approaches to promote growth and fruit quality of fruit trees

Research by various authors (Aslantaş *et al.*, 2007; Karlidag *et al.*, 2007; Orhan *et al.*, 2006;) reported that PGPRs, in particular *Pseudomonas fluorescens*, stimulate plant growth and showed a significant yield increase on raspberry and young apple trees. Plant growth promoting effects of two *Bacillus* strains OSU-142 (N₂-fixing) and M3 (N₂-fixing and phosphate solubilizing) were tested alone and/or in combinations on organically grown primo cane fruiting raspberry (CV. Heritage) plants in terms of yield, growth, nutrient composition of leaves and variation of soil nutrient element composition. Results showed that the *Bacillus* M3 treatment stimulated plant growth and resulted in a significant increase in yield, cane length, number of cluster per cane and number of berries per cane compared to the control (Orhan *et al.*, 2006).

Plant growth promoting effects of *Bacillus* M3, *Bacillus* OSU-142 and micro-bacterium FS01 were also tested alone or in combination on apple (*Malus domestica* L.) CV. Granny Smith in terms of yield, growth and nutrient composition of leaves. The presence of M3 and/or OSU-142 and/or FS01 combinations stimulated plant growth and resulted in significant yield increases in ‘Granny Smith’. Root inoculation of PGPR strains significantly increased cumulative yield, fruit weight, shoot length and shoot diameter compared to the control (Karlidag *et al.*, 2007). It was concluded that bacterial elicitors such as treatment with *Pseudomonas fluorescens* improved number of leaves, plant height and fresh and dry weights.

Several reviews focussing on the impact of PGPRs in initiating growth at a molecular level have been published (Beneduzi *et al.*, 2012; Bi *et al.*, 2011; Vejan *et al.*, 2016), however field research reporting on the use of PGPR in promoting growth and establishment, whilst enhancing physiological parameters and fruit quality is however limited.

Studies on the efficacy of biostimulants for improved plant growth have not always yielded positive results. A study by Csizinszky (2000), using two cultivars of the two bell peppers ‘Early Calwonder’ and ‘Jupiter’, demonstrated that six granular and liquid biostimulants, applied according to the manufacturer’s recommendations, had no influence on yields or nutrient content of the peppers. Even though some of the biostimulant treatments increased the fruit yield in earlier harvests, marketable yields of among the biostimulant treatments were not significantly different. ‘Early Calwonder’ peppers treated with biostimulants produced similar fruit yields to control treatments, despite that the nutrient levels for all the peppers were at or above those needed for plant growth for all treatments. For the ‘Jupiter’ peppers, those treated with biostimulants recorded lower yields

compared to the control. Of interest is that, even though some of the biostimulant treatments increased the levels of some of the nutrients, these increases did not have any effect on the marketable yield of the peppers. Higher than normal yields were attributed to differences in the production system and cultivar used, fertilizer treatments and number of harvests, rather than to the use of the biostimulants. It was however stated that biostimulants containing cytokine may be beneficial during periods of plant stress as internal cytokine production may be limited under such conditions.

In a study by Kelting *et al.* (1997) evaluating the benefits of biostimulants and its interaction with fertilizers on the overall growth of Turkish hazelnut when transplanting bare-root to containers under greenhouse conditions, no significant interactions between fertilizer rates and biostimulant treatments for any parameter of top growth could be demonstrated. It was in fact concluded that the particular seaweed-based biostimulants and its associated humic acid used in the experiment were detrimental to the growth of several container-grown woody plant species. As root lengths were not significantly different between treatments, it was suggested that root damage may have occurred due to high salt concentrations associated with liquid humate treatments.

6. Conclusions

Farmers have been using chemical fertilizers since the manufacturing of nitrates after World War II to promote growth and fruit quality of fruit trees. Although these fertilizers have shown significant advances in promoting growth, plant performance, fruit quality and yield, detrimental effects to the environment such as land degradation, deforestation, environmental pollution, depletion of biodiversity, pest resurgence and lowering of ground water table has been well documented. A shift from fertilizer and pesticides-based conventional agriculture practices to natural and renewable resource-based sustainable agriculture is justified since the latter type of approach is generally cheap, environment friendly and emphasizes on the conservation of natural resources. Such an alternative sustainable approach include use of biofertilizers, biostimulants and organic farming.

Organic farming is being conducted by creating community seed banks to conserve biodiversity hence, increasing food production, without depleting the soil fertility. In addition, biofertilizers and biostimulants have been developed as a better and alternative approach to improve soil fertility and maintaining biodiversity.

Within the South African context, Janse van Vuuren (2018) and Van Schoor *et al.* (2012) evaluated possible reduction of volatilization and leaching of N using different and more alternative, sustainable approaches. However, both authors cautioned that longer observation periods are required to attain maximum results in tree performance and fruit quality. The impact of the use of biostimulants

in promoting growth, fruit quality and yield have mostly been studied using a molecular level approach. Extrapolating results obtained from laboratory-based research is a cause of concern, as these results often contradicts those observed in field experiments. In addition, the impact of biostimulants has often proved to be more effective on annual crops than on perennial crops such as deciduous and citrus trees. More in depth studies are thus required to validate the efficacy of biostimulants under field conditions, particular to promote growth and development of woody, perennial crops and their ability to increase the yield of quality fruit in order to confirm the applicability of this alternative approach to the broader agricultural sector.

7. References

- Alam, S., Khalil, S., Ayub, N. & Rashid, M. 2002. In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganisms (PSM) from maize rhizosphere. *International Journal of Agriculture and Biology* 4:454–458.
- Andrews, P., Fellman, J., Glover, J. & Reganold, J. 2001. Soil and plant mineral nutrition and fruit quality under organic, conventional, and integrated apple production systems in Washington. *Acta Horticulturae* 564:291–298.
- Arancon, N.Q., Edwards, C.A., Lee, S. & Byrne, R. 2006. Effects of humic acids from vermicomposts on plant growth. *European Journal of Soil Biology* 42:65–69.
- Aslantaş, R., Çakmakçı, R. & Şahin, F. 2007. Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. *Scientia Horticulturae* 111:371–377.
- Asli, S. & Neumann, P.M. 2010. Rhizosphere humic acid interacts with root cell walls to reduce hydraulic conductivity and plant development. *Plant and Soil* 336:313–322.
- Bashan, Y. & Holguin, G. 1998. Proposal for the division of plant growth-promoting rhizobacteria into two classifications: Biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. *Soil Biology and Biochemistry* 30:1225–1228.
- Bashan, Y., de-Bashan, L.E., Prabhu, S.R. & Hernandez, J.P. 2014. Advances in plant growth-promoting bacterial inoculant technology: Formulations and practical perspectives (1998-2013). *Plant and Soil* 378:1–33.
- Beneduzi, A., Ambrosini, A. & Passaglia, L.M.P. 2012. Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology* 3:1044–1051.
- Bi, F., Iqbal, S., Arman, M., Ali, A. & Hassan, M. 2011. Carrageenan as an elicitor of induced secondary metabolites and its effects on various growth characters of chickpea and maize plants. *Journal of Saudi Chemical Society* 15:269–273.
- Bocanegra, M.P., Lobartini, J.C. & Orioli, G.A. 2006. Plant uptake of iron chelated by humic acids of different molecular weights. *Communications in Soil Science and Plant Analysis* 37:239–248.
- Bright, J. 2005. Apple and pear nutrition. *Primefact* 85:1–12.

- Brunnetto, G., Wellington, G., De Melo, B., Toselli, M., Quartieri, M. & Tagliavini, M. 2015. The role of mineral nutrition on yields and fruit quality in grapevine, pear and apple. *Revista Brasileira de Fruticultura* 37:1089–1104.
- Calvo, P., Nelson, L. & Kloepper, J.W. 2014. Agricultural uses of plant biostimulants. *Plant and Soil* 383:3–41.
- Canellas, L.P., Dantas, D.J., Aguiar, N.O., Peres, L.E.P., Zsögön, A., Olivares, F.L., Dobbss, L.B., Façanha, A.R., Nebbioso. & Piccolo, A. 2011. Probing the hormonal activity of fractionated molecule humic components in tomato auxin mutants. *Annals of Applied Biology* 159:202–211.
- Chen, J.H. 2006. The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. *International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use* 16–20:1–11.
- Crouch, I.J. 1990. The effect of seaweed concentrate on plant growth. Ph.D Thesis, Faculty of Science, University of Natal, Pietermaritzburg.
- Crouch, I. J., Smith, M. T., Van Staden, J., Lewis, M. J., Hoad, G. V. 1992. Identification of auxins in a commercial seaweed concentrate. *Journal of Plant Physiology* 139:590-594.
- Csizinszky, A.A. 2000. Yield response of bell pepper cultivars to foliar-applied 'Atonik' biostimulant. University of Florida Extension, USA. 8 pp.
- Dong, S., Neilsen, D., Neilsen, G.H. & Fuchigami, L.H. 2005. Foliar N application reduces soil NO₃ N leaching loss in apple orchards. *Plant and Soil* 268:357–366.
- Du Jardin, P. 2015. Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae* 196:3–14.
- EBIC. 2012. Promoting the biostimulant industry and the role of plant biostimulants in making agriculture more sustainable. [Online], Available: <http://www.biostimulants.eu/>. Accessed on 12 June, 2017.
- Esteves da Silva, J.C., Machado, A.A.S. & Oliveira, C.J. 1998. Effect of pH on complexation of Fe (III) with fulvic acids. *Environmental Toxicology and Chemistry* 17:1268–1273.
- Haifa. 2016. Nutritional recommendations for *Citrus*. Haifa Group: Pioneering the future, Brackenfell Industrial, Cape Town. 56 pp.
- Halpern, M., Bar-Tal, A., Ofek, M., Minz, D., Muller, T. & Yermiyahu, U. 2015. The use of biostimulants for enhancing nutrient uptake. *Advances in Agronomy* 130:141–174.

- Hayat, R., Ali, S., Amara, U., Khalid, R. & Ahmed, I. 2010. Soil beneficial bacteria and their role in plant growth promotion: A review. *Annals of Microbiology* 60:579–598.
- Huysamer, M., 1997. Integrating cultivar, rootstock and environment in the export-driven South African deciduous fruit industry. *Acta Horticulturae* 451: 755-760.
- Igual, J.M., Valverde, A., Cervantes, E. & Velazquez, E. 2001. Phosphate-solubilizing bacteria as inoculants for agriculture: use of updated molecular techniques in their study. *Agronomie* 21:561–568.
- Janse van Vuuren, L. 2018. Quantifying the effect of commercial nitrogen formulations BlackUrea™/BlackDAP™ as alternative nitrogen source on fruit tree physiology, yield and fruit quality of two apple cultivars. MSc Thesis. Faculty of Agrisciences, Stellenbosch University.
- Johnson, R.S. & Uriu, K. 1989. Mineral nutrition. In: J.H. LaRue & R.S. Johnson (eds.) Peaches, Plums, and Nectarines: Growing and Handling for Fresh Market, 68–82 pp.
- Kader, A.A. 2000. Quality of horticultural products. *Acta Horticulturae* 518:15–16.
- Kangueehi, G.N. 2008. Nutrient requirement and distribution of intensively grown ‘Brookfield Gala’ apple trees. MSc Thesis, Faculty of AgricSciences, Stellenbosch University.
- Karakurt, H. & Aslantas, R. 2010. Effects of some plant growth promoting rhizobacteria (PGPR) strains on plant growth and leaf nutrient content of apple. *Journal of Fruit and Ornamental Plant Research* 18:101–110.
- Karlidag, H., Esitken, A., Turan, M. & Sahin, F. 2007. Effects of root inoculation of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient element contents of leaves of apple. *Scientia Horticulturae* 114:16–20.
- Kelting, M.P., Harris, J.R., Appleton, B.L. & Niemiera, A.X. 1997. Effects of soil amendments and biostimulants on the post-transplant growth of landscape trees. MSc Thesis, Department of Horticulture, Virginia Polytechnic Institute and State University.
- Khan, W., Rayirath, U.P., Subramanian, Sowmyalakshmi, Jithesh, M.N., Rayorath, P., Hodges, D.M., Critchley, A.T., Craigie, J.S., Norrie, J. & Prithiviraj, B. 2009. Seaweed extracts as biostimulants of plant growth: Review. *Journal of Plant Growth Regulation* 28:386–399.
- Kloepper, J., Lifshitz, R. & Zablotowicz, R. 1989. Free-living bacteria inocula for enhancing crop productivity. *TibTec* 7:39–45.
- Kotzé, W.A. & Joubert, M. 1992. Effect of different organic materials for soil improvement,

- mulching and fertilization on the performance of apricot trees. *Journal of South African Society for Horticultural Science* 2:31–35.
- Lobartini, J.C., Tan, K.H. & Pape, C. 1998. Dissolution of aluminum and iron phosphate by humic acids. *Communications in Soil Science and Plant Analysis* 29:535–544.
- Lötze, E. & Hoffman, E.W. 2016. Nutrient composition and content of various biological active compounds of three South African-based commercial seaweed biostimulants. *Journal of Applied Phycology* 28:1379–1386.
- Mancuso, S., Azzarello, E., Mugnai, S. & Briand, X. 2006. Marine bioactive substances (IPA extract) improve foliar ion uptake and water stress tolerance in potted *Vitis vinifera* plants. *Advances in Horticultural Science* 20:156–161.
- Marschner, H. 1993. Mineral nutrition of higher plants, 2nd edition. Elsevier Science, London. 672 pp.
- Marschner, H. 2011. Mineral nutrition of higher plants, 3rd edition. Elsevier Science, London. 672 pp.
- Mengel, K. & Kirkby, E.A. 1982. Principles of plant nutrition, 3rd edition. International Potash Institute, Worblaufen-Bern, Switzerland. 446 pp.
- Mohr, H. & Schopfer, M. 1994. Plant physiology, 4th edition. Springer-Verlag, Berlin-Heidelberg. 629 pp.
- Neilsen, D. & Neilsen, G.H. 2002. Efficient use of nitrogen and water in high-density apple orchards. *Horticulture Technology* 12:19–25.
- Neilsen, G., Hogue, E., Forge, T. & Neilsen, D. 2003. Mulches and biosolids affect vigor, yield and leaf nutrition of fertigated high density apple. *Horticulture Science* 38:41–45.
- Neilsen, G.H. & Neilsen, D. 2003. Nutritional requirements of apple. In: D.C. Feree and I.J. Warrington (eds.). Apples: Botany, production and uses. CAB International, Cambridge. 267–302 pp.
- Neilsen, G., Hogue, E., Forge, T., Neilsen, D. & Kuchta, S. 2007. Nutritional implications of biosolids and paper mulch applications in high density apple orchards. *Canadian Journal of Plant Science* 87:551–558.
- Nyati, C. 2004. Preliminary results of the BlackUrea™ trials. Agricultural Research Trust, Harare.
- Orhan, E., Esitken, A., Ercisli, S., Turan, M. & Sahin, F. 2006. Effects of plant growth

- promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. *Scientia Horticulturae* 111:38–43.
- Peck, G.M., Andrews, P.K., Reganold, J.P. & Fellman, J.K. 2006. Apple orchard productivity and food quality under organic, conventional, and integrated management. *Horticulture Science* 41:99–107.
- Pinamonti, F., Zorzi, G., Gasperi, F., Silvestri, S. & Stringari, G. 1995. Nutritional status of grapevines and apple trees grown under various soil management techniques in the plant row. *Acta Horticulturae* 383:313–321.
- Radovich, T. 2010. Fruit and vegetable quality: It matters! sustainable and organic agriculture program, Manoa, Hawaii. 3 pp.
- Ratti, N., Kumar, S., Verma, H.N. & Gautams, S.P. 2001. Improvement in bioavailability of tricalcium phosphate to *Cymbopogon martini* var. motia by rhizobacteria, AMF and azospirillum inoculation. *Microbiology Research* 156: 145-149.
- Reganold, J.P., Glover, J.D., Andrews, P.K. & Hinman, H.R. 2001. Sustainability of three apple production systems. *Nature* 410:927–929.
- Salisbury, F.B. & Ross, C.W. 1992. Plant physiology. Wadsworth Publishing Company, Belmont California. 682 pp.
- Sharma, S.B., Sayyed, R.Z., Trivedi, M.H. & Gobi, T.A. 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus* 2:1-14.
- Snijder, B. 2000. Macro nutrient accumulation and requirement of ‘Hass’ avocado trees. *South African Avocado Growers Association* 23:56–62.
- Stevenson, F. 1994. Humus chemistry: genesis, composition, reactions. New York: John Wiley & Sons. 496 pp.
- Taiz, L., Zeiger, E., Moller, I.M. & Murphy, A. 2015. Plant physiology and development 6th edition. Vol. 117. I.M.M: L.In: E. Taiz, A. Zeiger & A. Murphy (eds.). Sinauer Associates, Inc. 855 pp.
- Tandon, S. & Dubey, A. 2015. Effects of biozyme (*Ascophyllum nodosum*) biostimulant on growth and development of soybean [*Glycine Max(L.) Merrill*]. *Communications in Soil Science and Plant Analysis* 46:845–858.
- Toselli, M., Sorrenti, G., Quartieri, M., Baldi, E., Marcolini, G., Solieri, D., Marangoni, B. &

- Collina, M. 2012. Use of soil and foliar applied calcium chloride to reduce pear susceptibility to brown spot (*Stemphylium Vesicarium*). *Journal of Plant Nutrition* 35:1819–1829.
- Trevisan, S., Francioso, O., Quaggiotti, S. & Nardi, S. 2010. Humic substances biological activity at the plant-soil interface: from environmental aspects to molecular factors. *Plant Signaling & Behavior* 5:635–43.
- Tucker, M.R. 1999. Essential plant nutrients: Their presence in North Carolina soils and role in plant nutrition. University of Agronomic Division, NCDA and CS, North Carolina, USA. 1-9 pp.
- Uchida, R. 2000. Essential nutrients for plant growth : In: J.A. Silva & R. Uchida (eds.). University of Hawaii at Manoa: College of Tropical Agriculture and Human Resources. 35 pp.
- Vance, C.P. 2016. Update on the state of nitrogen and phosphorus nutrition symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. *Plant Physiology* 127:390–397.
- Varanini, Z. & Pinton, R. 2001. Direct versus indirect effects of soil humic substances on plant growth and nutrition. The rhizosphere. In: Z. Varanini, R. Pinton, & P. Nannipieri (eds.). Marcel Dekker, Basel. 141–158 pp.
- Van Schoor, L. 2009. Effect of biological amendments on soil microbial properties and performance of pome fruit trees. Ph.D Thesis, Faculty of Agrisciences, University of Stellenbosch.
- Van Schoor, L., Stassen, P.J.C. & Botha, A. 2012. Effect of organic material and biological amendments on pear tree performance and soil microbial and chemical properties. *Acta Horticulturae* 933:205–214.
- Van Staden, J. 2015. Kelp products international (Pty) Ltd. Simon’s Town, South Africa. 4 pp.
- Vejan, P., Abdullah, R., Khadiran, T., Ismail, S. & Nasrulhaq Boyce, A. 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability-A review. *Molecules* 21:1–17.
- Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* 255:571–586.
- Weinbaum, S.A., Brown, P.H., Rosecrance, R.C., Picchioni, G.A., Niederholzer, F.J.A.,

- Youseffi, F. & Muraoka, T.T. 2001. Necessity for whole tree excavations in determining patterns and magnitude of macronutrient uptake by mature deciduous fruit trees. *Acta Horticulturae* 564:41-49.
- Whitelaw, M.A. 2000. Growth promotion of plants inoculated with phosphate solubilizing fungi. *Advances in Agronomy* 69:99–151.
- Zekri, M. & Obreza, T.A. 2012. Plant nutrients for citrus trees. University of Florida-IFAS Extension. 5 pp.
- Zhang, X. & Ervin, E.H. 2004. Cytokinin-containing seaweed and humic acid extracts associated with creeping bentgrass leaf cytokinins and drought resistance. *Crop Science* 44:1737–1745.
- Zhang, X. & Ervin, E.H. 2008. Impact of seaweed extract-based cytokinins and zeatin riboside on creeping bentgrass heat tolerance. *Crop Science* 48:364–370.
- Zhang, X., Ervin, E. & Schmidt, R. 2003. Physiological effects of liquid applications of a seaweed extract and a humic acid on creeping bentgrass. *Journal of the American Society for Horticultural Science* 128:492–496.
- Zimmerli, L., Hou, B.H., Tsai, C.H., Jakab, G., Mauch-Mani, B. & Somerville, S. 2008. The xenobiotic β -aminobutyric acid enhances *Arabidopsis* thermotolerance. *Plant Journal* 53:144–156.

PAPER 1: THE EFFECT OF A COMBINATION OF BIOSTIMULANTS (RIZOFOS FRUIT[®], TRI-CURE SP[®], Premax[®] and Technical[®]) ON PLANT PERFORMANCE AND FRUIT QUALITY OF ‘RUBYSTAR’ PLUM AND ‘FORELLE’ PEAR

Abstract

Biofertilizers have been recognized as an alternative approach to chemical fertilizers to increase crop production and soil fertility within the context of sustainable farming. Increased usage of plant growth promoting rhizobacteria (PGPR) is gaining acceptance, because of their efficacy in promoting growth and enhancing quality of crops. The aim of the study was to investigate the efficacy of a biostimulant combination (‘Rizofos plus’) Rizofos Fruit[®], Tri-Cure SP[®], Premax[®] and Technical[®] in promoting plant growth and fruit quality at orchard level, on ‘Rubystar’ plum and ‘Forelle’ pear. The experimental design consisted of two treatments: the control (untreated) and a soil-based biostimulant combination (Rizofos Fruit[®], Tri-Cure SP[®], Premax[®] and Technical[®]). The biostimulants were applied twice, during fruit development and two weeks after initial application. Results indicated no significant differences between treatments with respect to growth and fruit quality parameters quantified. However, a trend in the biostimulant combination treatment that enhanced yield efficiency compared to the control treatment, warrants further investigation.

Key words: PGPR, sustainability, yield efficiency

1. INTRODUCTION

The plum fruit industry in South Africa is well established and predominantly produces fruit destined for the export market. The majority of South African plums are exported to northern hemisphere countries during winter and spring seasons. During the 2015/16 production season, plums contributed R1.2 billion in terms of gross value of production (DAFF, 2017a). The main plum producing areas in South Africa, which account for over half of all the plums produced, are all situated in the Western Cape, with its Mediterranean climate of hot dry summer and cold winters. These areas include the Little Karoo, Paarl, Wolseley/Tulbagh and Stellenbosch.

Pear production is even more important as a deciduous fruit crop for South Africa than plums, taking into consideration their foreign exchange earnings, employment creation and linkages with support institutions (DAFF, 2017b). During the 2015/16 season, pears contributed approximately 15.5 % (R3.9 billion) of the total gross value of all deciduous fruits (R20 billion) in South Africa. The main pear producing areas in the Western Cape are Ceres, Groenland, Wolseley/Tulbagh with the Western Cape Province accounting for more than half of all the pears produced in South Africa (Tshabalala, 2015).

One of the challenges in deciduous fruit production in South Africa, specifically in Western Cape, is the characteristic, acidic and often shallow (30-50cm) soils (Kangueehi, 2008). Therefore, in order to achieve high yields and quality fruit each season, soil amelioration and proper soil management is required. The application of chemical fertilizers is generally considered an inexpensive and effective method of supplying mineral nutrients to crops. Yet, the application of inorganic fertilizer has some serious associated detrimental effects such as possible contamination of the environment as a result of excessive nitrate leaching (Dong *et al.*, 2002). Environmental pollution of the air and water may be caused by an oversupply of the fertilizers over many consecutive seasons to compensate for the depletion of nutrients by agriculture, particularly that of nitrogen (N) and phosphorous (P) (Vance, 2001).

Microbial inoculants such as PGPR and P-solubilizing bacteria has prominently increased in the past decade in agriculture (Hayat *et al.*, 2010). In this present review, microbial inoculants that act as biofertilizers are considered as biostimulants. Biostimulants can be applied to either crops to stimulate growth and maintain fruit quality (Halpern *et al.*, 2015). The application of biofertilizers comprising beneficial microorganisms instead of synthetic chemicals is known to improve plant growth through the supply of plant nutrients (Calvo *et al.*, 2014). Plant growth promoting rhizobacteria (PGPR) (Kloepper *et al.*, 1989) and plant growth promoting bacteria (PGPB) are amongst the biofertilizers are both free living bacteria mainly isolated from the rhizosphere (Bashan

et al., 2014). Several papers regarding PGPR and PGPB have been published in the past few years (Antoun & Kloepper, 2001; Bhattacharyya & Jha, 2012; Spaepen *et al.* 2009) in promoting growth, yield and fruit quality of crops.

Plant growth promoting rhizobacteria have been associated with improving plant growth and yield, nutrient status and enhancing plant nutrient uptake. Wu *et al.* (2005) reported that stimulation of plant growth after inoculation of maize (*Zea mays*) with strains of *Bacillus megaterium* and *Bacillus muciaraglaginous* in combination with arbuscular mycorrhizal fungi (AMF), improved nutritional assimilation of plant total N, P and K. Application of PGPR resulted in a significant increase in N, P, and K uptake as well as root and shoot dry weight in cotton (*Gossypium hirsutum*) (Egamberdiyeva & Höflich, 2004) and wheat (*Triticum aestivum*) (Shaharoon *et al.*, 2008). Adesemoye *et al.* (2008) reported that a three-strain combination of *Bacillus* spp. Plant growth promoting rhizobacteria promoted the growth of tomato (*Solanum lycopersicon* L) and increased plant uptake of N-depleted soils. Additionally, a field study with maize, PGPR, AMF and a combination of the two treatments increased yield and enhanced the total nutrient content of grain per plot during a three year period (Adesemoye *et al.*, 2008). Several reviews on PGPR have been published in the past few years (Beneduzi *et al.*, 2012; Bi *et al.*, 2011; Vejan *et al.*, 2016), but research pertaining to contributions of soil applied phosphorous solubilising bacteria (PSB) and biocontrol fungi such as *Trichoderma* spp. products to fruit tree species on the establishment, yield and fruit quality under local conditions are limited, despite its application being widely practised.

The use of a range of biostimulant products have been adopted recently in many crops with reported beneficial effects in growth (Fraser & Percival, 2003; Rathore *et al.*, 2009; Vernieri *et al.*, 2005). A trial on the use of different biostimulants on apple by Dominguez (2015) also showed no significant differences in the first season, however differences were observed by the second and third season.

Osman & Salem (2015) postulated that biostimulants containing minerals are unable to supply all the essential nutrients in the quantities required by plants, but operate instead by promoting root growth of plants subjected to stress, possibly by increasing the antioxidant defence system. Besides several positive reports of biostimulants on plant production, other studies reported the opposite where the applications of commercial protein hydrolysate products of animal origin caused a phytotoxicity and a decline in plant growth (Cerdán *et al.*, 2009; Lisiecka *et al.*, 2011; Ruiz *et al.*, 2000). Alternatively, incorrect product concentrations or unfavourable environmental condition such as adverse field conditions may contribute to non-responsiveness of crops to biostimulants (Nardi *et al.*, 2016). For example, excess application of biostimulants induced a negative response in crops. A

report by Asli & Neumann (2010) showed that humic acid application inhibited growth of maize grown within a hydroponic system. Kirn *et al.* (2010) also reported lack of positive effects on okra (*Abelmoschus esculentus*) grown in field soil experiments, when no significant yield were observed when it was deviated from the recommended dose. It was therefore concluded that, although biostimulants play a promotive role as a supplement to facilitate plant growth stimulation, it is not formulated as a substitute for fertilizers as it was not able to sustain the expected yield when the fertilizer was reduced below recommended levels. Calvo *et al.* (2014) recommended the use of a combination of some of the different classifications of biostimulants, for example, microbial inoculants in combination with humic substances or seaweed extracts to enhance the positive impacts as such combinations could potentially provide more reproducible benefits to crop production systems.

A combination of a seaweed extract (Stimplex), vitamins and enzymes (Vitazyme), phosphite and organic acids (Nutriphite Magnum), together with foliar Ca fertilizer (System-Ca), was applied on spindle apples and significant differences could not be achieved for most growth parameters studied including yield per tree, stem diameter and yield efficiency (Dominguez & Robinson, 2015). Also, in earlier work by Kelting *et al.* (1998) on the use of humate- based biostimulants, little increase in top growth resulting from biostimulant application was observed. In addition, Fraser & Percival (2003) found no significant differences based on the impact of biostimulants on the growth of three urban tree species following transplanting, although our research was based on the field conditions.

Furthermore, Results by Karakurt & Aslantas (2010) indicated that leaf area was consistent and not significantly affected by the diverse rootstocks under observation. In their experiment, the inoculation approaches with bacteria strains and the respective rootstocks performed an important role in showing no significant differences at growth and plant performance of evaluated apple cultivars. Consequently, PGPR may have interaction with the selected rootstock and cultivar. Cultivar interactions and rootstock were observed for fruit yield, as bacterial competence of rootstock 'M9' was higher compared to 'MM 106', yet the growth, performance and yield were not significantly affected as the rootstock genotype in addition also affected bacterial rhizosphere community composition and tree growth.

This paper aimed towards quantifying vegetative changes, yield and fruit quality in plum and pear trees during the first year after application of these biostimulant products.

2. MATERIALS AND METHODS

2.1 Sites, plant material, experimental design and treatments

Study sites and plant material included ‘Rubystar’ plums, as established on Marianna rootstock, at Bourgogne in Franschhoek (33°55’S; 19°08’E) and ‘Forelle’ pear, on BP3 rootstock, at Avontuur farm in Villiersdorp (S34°15’5.29”; E19°16’5.05”). The Bourgogne farm is characterised by high weathered yellow- to reddish-brown soils with very low phosphorous levels and pH of 5.5 and use a micro sprinkler irrigation system. The Avontuur farm is characterised with the stony, clay-rich soils and pH range from 5.3-5.5, also using micro sprinkler irrigation system (5ML/Ha/season). The experimental design for the study on both farms consisted of a randomised complete block design with two treatments and ten blocks, using single trees as experimental units. Buffer trees were included between every tree to reduce any possible contamination crossover between experimental units.

The treatment consisted of a control (untreated) and “Rizofos Plus”, a combined application of Rizofos Fruit[®], containing the live bacteria strain *Pseudomonas fluorescens*, and Tri-Cure SP[®] (MBFI International, Delmas, SA), which is a soluble powder formulation of a biological fungicide containing *Trichoderma harzianum* for the control of pathogenic root diseases (Harman *et al.*, 2008). The Rizofos Fruit[®] and Tri-Cure SP[®] was applied in combination with additional biostimulants and hormones: Premax[®] and Technical[®]. Recommended application concentrations and application rates were followed (Table 1).

Application of the biostimulant combination was only done during the 2015/16 season. Rizofos Fruit[®] plus Tri-Cure SP[®] was applied according to protocol which required an application twice during the growing season following the initial application. The first application to ‘Rubystar’ was on 18 November 2015, with a next application on 17 December 2015 during fruit development phase. For ‘Forelle’, the first application was on 19 November 2015, with a next application on 14 December 2015. Standard disease, insect and weed control, irrigation schedule and fertiliser practices were carried out on both farms throughout the growing season.

2.2 Data collection

2.2.1 ‘Rubystar’: Plant growth, maturity indices and yield

Initial trunk circumference (cm) for ‘Rubystar’ was recorded approximately 30 cm above the graft union and at harvest using a measuring tape. All fruit from each replicate was harvested on 3 March

2016. From each replicate, 10 fruit were harvested for quality measurements. Fruit size (diameter in mm) and firmness (kg) were determined at the Department of Horticultural Science at Stellenbosch University using a fruit texture analyser (FTA, Guss instruments, CA, USA), fitted with 10 mm tip that was applied on a 2 cm area of peeled fruit cheeks (both sides) for approximately 2 seconds. Fruit were harvested at commercial maturity. Yield per tree (kg.tree^{-1}) was determined by harvesting all fruit per tree. Yield efficiency (kg.cm^{-1}) was calculated by dividing the yield per tree by the trunk circumference.

2.2.2 ‘Forelle’: Plant growth, maturity indices and yield

As in ‘Rubystar’, the trunk circumference (cm) of the ‘Forelle’ trees was recorded 30 cm above the graft at harvest on 3 March 2016 using a measuring tape. On the day of harvest, 20 fruit per replication were collected for fruit quality indices which were determined at the Department of Horticultural Science, Stellenbosch University. Fruit quality assessment implied the determination of the physical properties of fruit mass (g), fruit diameter (mm), fruit firmness (mm) as estimated by a fruit texture analyser (FTA, Guss instruments, CA, USA) fitted with an 11 mm tip and applied on both peeled cheeks. Total soluble solids (TSS) were determined and expressed as % Brix on a pooled juice sample with a temperature-controlled digital refractometer (Pallete, PR-32 ATAGO, and Bellevue, USA). Yield per tree (kg.tree^{-1}) was recorded using the same protocol as with ‘Rubystar’.

2.3 Statistical Analysis

Data were analysed by a two way analysis of variance (ANOVA) using the Generalised Linear Model (GLM) procedures with Statistical Analysis System (SAS) programme (SAS Institute Inc., Cary, NC) version 9.1. Separation of means was done using the Fisher’s least significant difference (LSD) test. Treatment effects were considered significant at 5% confidence level ($P \leq 0.05$).

3. RESULTS

3.1 ‘Ruby star’ and ‘Forelle’

For the plum cultivar ‘Rubystar’, no growth parameter showed any significant differences between treatments at harvest (Table 2). Neither yield nor yield efficiency differed significantly between treatments. The quality parameters fruit diameter and firmness did not result in any significant differences between treatments (Table 4).

As reported for ‘Rubystar’, no significant differences were obtained between treatments for plant performance (yield, stem circumference and yield efficiency) for the ‘Forelle’ pear cultivar (Table 3). Fruit firmness, diameter, weight and TSS showed no significant differences between treatments (Table 4).

4. DISCUSSION

4.1 Plant performance and fruit quality of ‘Rubystar’

A combined application of “Rizofos Plus” did not improve tree growth and performance in our study with ‘Rubystar’. Individual positive effects on growth were reported by other authors in different crops (Malboobi *et al.*, 2009; Park *et al.*, 2009; Rodríguez & Fraga, 1999) and it was hypothesized that their combined use would provide an improved, synergistic effect. However, this was not prominent during the first season of evaluation.

The biostimulant combination “Rizofos Plus” in our study did not significantly improve any fruit quality parameter during the first growing season. This finding is in line with results reported by Thalheimer & Paoli (2002) on the efficiency of different foliar applied biostimulants on the productivity and fruit quality on apple trees. This lack of response could have been partly due to satisfactory foliar nutrient concentrations for the orchard for all elements (data not presented).

Adams *et al.* (2007) suggested that the nature of the soil is more important to promote mycorrhiza colonization other than the presence of soil fungi. Growth promotion by means of mycorrhiza is known to be suppressed under environments of high phosphate availability (Harman *et al.*, 2008). Microbe competition in a limited nutrient environment suppress the growth and development of soil microorganisms such as *Pseudomonas* and *Trichoderma*, preventing or downscaling any arbuscular mycorrhiza fungal (AMF) attack (Whipps, 2001). In our study, competition between the *Pseudomonas fluorescens* and *Trichoderma harzianum* and other soil microorganisms for suitable colonization sites on the root surfaces were not quantified and may have reduced the potential of colonization by *T. harzianum* (Adams *et al.*, 2007), resulting in less efficient increases in P uptake in the biostimulant treatment.

4.2 Plant performance, fruit quality and maturity indices of ‘Forelle’

The application of the biostimulant combination “Rizofos Plus” did not produce any significant differences in ‘Forelle’ growth or fruit quality compared to control. Leaf and soil samples were not

collected and quantified in this study due to funding constraints and it was assumed all trees that was included in the experimental design had similar N and P content.

Dominguez & Robinson (2015) conducted a similar study, under very favourable, and not stressful, or harsh climatic conditions. In addition, trees in their study received sufficient irrigation, with standard cultural and disease management regimes followed, to ensure that healthy orchard practices prevailed at all time. They reported that the use of biostimulants products is recommended in a more stressful environment, where it could potentially induce a greater, promotive effect on yield. The same principle is also applicable to our study that was conducted on a productive orchard under favourable production conditions for 'Forelle', resulting in no significant differences between treatments. Of interest is that when Sahain *et al.* (2007) evaluated the use of two biostimulant products with different concentrations in a more adverse environment, which include hot and dry climate, calcareous soil and the use of flood irrigation, biostimulants was found to significantly improved growth and yield of the apple cultivar 'Anna' compared to the untreated control treatment.

In research by Karakurt & Aslantas (2010), interactions were recorded between cultivars and rootstocks on their ability to use plant promoting rhizobacteria. They concluded that increases in vegetative growth and nutrient content of apple fruits in comparison to the control treatment were initiated by the inoculation with PGPR with results being strongly depended on the cultivars and bacterial strain tested. Castle (1995) and Al-Hinai & Roper (2004) reported similar trends. In contrast Motosugi *et al.* (2002) performed a study on grape vine (*Vitis vinifera* L.) rootstocks inoculated with *Gigaspora margarita*, and reported a response to nitrate nitrogen fertilization on total soluble solids, fruit surface colour and acidity. Although PGPR possibly interact synergistically with rootstocks and scions, this was not quantified in the scope of our study and can thus not be discussed.

5. CONCLUSION

This study aimed to provide fruit growers with management strategies to improve plant performance, growth and fruit quality on plums and pears by using sustainable and environmentally friendly organic mineralisation options. Microbial inoculants such as PGPR and P-solubilizing bacteria have been recommended by many studies as effective in improving growth and fruit quality of crops. However, in our evaluation of the biostimulant combination (Rizofos Fruit[®], Premax[®], Technical and Tri-Cure SP[®]), no significant effect on tree growth was recorded, yet a trend indicating possible benefits of the application of the biostimulant combination was noted where a small improvement in yield for both fruit crops could be observed. Fruit quality was also not significantly improved, especially on 'Forelle' where the control produced better results. Although, leaf and soil mineral

analyses were not quantified due to financial limitations, no visual deficient symptoms on leaves were noticed and thus the overall nutritional status of the orchard indicated a satisfactory nutrient condition. Under these circumstances, it is always more difficult to show a small increase in nutrient uptake with biostimulants.

Inconclusive results from this study warrants further research, using the same protocol over an extended period of two or three growing seasons. In addition, in deciduous crops, buds are initiated in the previous season, thus any manipulation later during the following season may only have limited results during the first season. This confirms results from Adams *et al.* (2007) who stated that the efficiency of PGPR on tree growth and yield is best observed long-term. It has also been suggested that primary and secondary metabolic pathways root tissues and leaves are the prime targets of PGPR as biostimulants, thus requiring further investigation on root growth parameters and the physiological responsive active with stress amelioration following biostimulant application.

6. Acknowledgements

Microbial Biological Fertilizers International[®] (MBFI[®]) Company partly contributed towards funding for this trial.

7. References

- Adams, S.W. 2016. The effects of rootstock, scion, grafting method and plant growth regulators on flexural strength and hydraulic resistance of apple. MSc Thesis, Faculty of Science, Utah State University.
- Adams, P., De-Leij, F.A.A.M. & Lynch, J.M. 2007. *Trichoderma harzianum* rifai 1295-22 mediates growth promotion of crack willow (*Salix fragilis*) saplings in both clean and metal-contaminated soil. *Microbial Ecology* 54:306–313.
- Adesemoye, A.O., Torbert, H.A. & Kloepper, J.W. 2008. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. *Canadian Journal of Microbiology* 54:876–886.
- Al-Hinai, Y.K. & Roper, T.R. 2004. Rootstock effects on growth and quality of “Gala” apples. *Horticulture Science* 39:1231–1233.
- Antoun, H & Kloepper J. 2001. Plant growth promoting rhizobacteria (PGPR). In: S. Brenner & J.H Miller (eds) *Encyclopedia of genetics*. Academic Press, New York, 1477–1480 pp.
- Asli, S. & Neumann, P.M. 2010. Rhizosphere humic acid interacts with root cell walls to reduce hydraulic conductivity and plant development. *Plant and Soil* 336:313–322.
- Bashan, Y., De-Bashan, L.E., Prabhu, S.R. & Hernandez, J.P. 2014. Advances in plant growth-promoting bacterial inoculant technology: Formulations and practical perspectives (1998-2013). *Plant Soil* 378:1–33.
- Beneduzi, A., Ambrosini, A. & Passaglia, L.M.P. 2012. Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology* 35:1044–1051.
- Bhattacharyya, P.N. & Jha, D.K. 2012. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World Journal of Microbiology and Biotechnology* 28:1327–1350.
- Bi, F., Iqbal, S., Arman, M., Ali, A. & Hassan, M. 2011. Carrageenan as an elicitor of induced secondary metabolites and its effects on various growth characters of chickpea and maize plants. *Journal of Saudi Chemical Society* 15:269–273.
- Calvo, P., Nelson, L. & Kloepper, J. 2014. Agricultural uses of plant biostimulants. *Plant Soil* 383:3–41.
- Castle, W.S. 1995. Rootstock as a fruit quality factor in citrus and deciduous tree crops. *New Zealand Journal of Crop and Horticultural Science* 23:383–394.
- Cerdán, M., Sánchez-Sánchez, A., Oliver, M., Juárez, M. & Sánchez-Andreu, J.J. 2009. Effect of

- foliar and root applications of amino acids on iron uptake by tomato plants. *Acta Horticulturae* 830:481–488.
- DAFF. 2017a. A profile of the South African plum market value chain 2017. Directorate Marketing, Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa. 57 pp.
- DAFF. 2017b. A profile of the South African pear market value chain 2017. Directorate Marketing, Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa. 51 pp.
- Dominguez, L.I. 2015. Strategies to improve growth and yield in the early life of a tall spindle apple planting. MSc.Thesis in Horticulture, Cornell University.
- Dominguez, L.I. & Robinson, T.L. 2015. Strategies to improve early growth and yield of tall spindle apple plantings. *New York State Horticultural Society* 23:5–10.
- Dong, S., Cheng, L., Scagel, C.F. & Fuchigami, L.H. 2002. Nitrogen absorption, translocation and distribution from urea applied in autumn to leaves of young potted apple (*Malus domestica*) trees. *Tree physiology* 22:1305–1310.
- Egamberdiyeva, D & Höflich, G. 2004. Effect of plant growth promoting bacteria on growth and nutrient uptake of cotton and pea in a semi-arid region of Uzbekistan. *Journal of Arid Environment* 56:293–301.
- Fraser, G.A. & Percival, G.C. 2003. The influence of biostimulants on growth and vitality of three urban tree species following transplanting. *Arboricultural Journal* 27:43–57.
- Halpern, M., Bar-Tal, A., Ofek, M., Minz, D., Muller, T. & Yermiyahu, U. 2015. The use of biostimulants for enhancing nutrient uptake. *Advances in Agronomy* 130:141–174.
- Harman, G.E., Björkman, T., Ondik, K. & Shores, M. 2008. Changing paradigms on the mode of action and uses of *Trichoderma spp.* for biocontrol. *Outlooks on Pest Management* 19:24–29.
- Hayat, R., Ali, S., Amara, U., Khalid, R. & Ahmed, I. 2010. Soil beneficial bacteria and their role in plant growth promotion: A review. *Annals of Microbiology* 60:579–598.
- Kangueehi, G.N. 2008. Nutrient requirement and distribution of intensively grown “ Brookfield Gala ” apple trees. MSc Thesis, Faculty of AgricSciences, Stellenbosch University.
- Karakurt, H. & Aslantas, R. 2010. Effects of some plant growth promoting rhizobacteria (PGPR) strains on plant growth and leaf nutrient content of apple. *Journal of Fruit and Ornamental Plant Research* 18:101–110.
- Karakurt, H., Kotan, R., Dadaşoğlu, F., Aslantaş, R. & ŞahİN, F. 2011. Effects of plant growth promoting rhizobacteria on fruit set, pomological and chemical characteristics, color values, and vegetative growth of sour cherry (*Prunus cerasus* cv. Kütahya). *Turkish Journal of Biology* 35:283–291.

- Kelting, M., Roger Harris, J., Fanelli, J. & Appleton, B. 1998. Humate-based biostimulants affect early post-transplant root growth and sapflow of balled and burlapped red maple. *Horticulture Science* 33:342-344.
- Khan, Z.U., McNeil, D.L. & Samad, A. 1998. Root pruning reduces the vegetative and reproductive growth of apple trees growing under an ultra high density planting system. *New Zealand Journal of Crop and Horticultural Science* 26:291–297.
- Kirn, A., Kashif, S.R. & Yaseen, M. 2010. Using indigenous humic acid from lignite to increase growth and yield of okra (*Abelmoschus esculentus* L.). *Soil and Environment* 29:187–191.
- Kloepper, J., Lifshitz, R. & Zablotowicz, R. 1989. Free-living bacteria inocula for enhancing crop productivity. *Trends in biotechnology* 7:39–45.
- Lisiecka, J., Knaflowski, M., Spizewski, T., Fraszczak, B., Kałużewicz, A. & Krzesiński, W. 2011. The effect of animal protein hydrolysate on quantity and quality of strawberry daughter plants Cv. “Elsanta”. *Acta Scientiarum Polonorum Hortorum Cultus* 10:31–40.
- Lo Bianco, R. L., Policarpo, M. & Scariano, L. 2003. Effects of rootstock vigour and in-row spacing on stem and root growth, conformation and dry-matter distribution of young apple trees. *The Journal of Horticultural Science and Biotechnology* 78:828–836.
- Malboobi, M.A., Behbahani, M., Madani, H., Owlia, P., Deljou, A., Yakhchali, B., Moradi, M. & Hassanabadi, H. 2009. Performance evaluation of potent phosphate solubilizing bacteria in potato rhizosphere. *World Journal of Microbiology and Biotechnology* 25:1479–1484.
- Motosugi, H., Yamamoto, Y., Naruo, T., Kitabyashi, H. & Ishi, T. 2002. Comparison of the growth and leaf mineral concentrations between three grapevine rootstocks and their corresponding tetraploids inoculated with arbuscular mycorrhizal fungus *Gigaspora margarita*. *Vitis* 41:21-25
- Muscolo, A. & Sidari, M. 2009. Carboxyl and phenolic humic fractions affect callus growth and metabolism. *Soil Science Society of America Journal* 73:1119–1129.
- Nardi, S., Pizzeghello, D., Schiavon, M. & Ertani, A. 2016. Plant biostimulants: physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism. *Scientia Agricola* 73:18–23.
- Osman, H.E. & Salem, O.M.A. 2015. Effects of seaweed extracts as foliar spray on sunflower yield and oil content. *Egyptian Journal of Phycology* 12:59–72.
- Park, K.H., Lee, C.Y. & Son, H.J. 2009. Mechanism of insoluble phosphate solubilization by *Pseudomonas fluorescens* RAF15 isolated from ginseng rhizosphere and its plant growth-promoting activities. *Letters in Applied Microbiology* 49:222–228.
- Piccolo, A., Nardi, S. & Concheri, G. 1996. Macromolecular changes of humic substances induced

- by interaction with organic acids. *European Journal of Soil Science* 47:319–328.
- Rathore, S.S., Chaudhary, D.R., Boricha, G.N., Ghosh, A., Bhatt, B.P., Zodape, S.T. & Patolia, J.S. 2009. Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (*Glycine max*) under rainfed conditions. *South African Journal of Botany* 75:351–355.
- Rodríguez, H. & Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances* 17:319–339.
- Ruiz, J.M., Castilla, N. & Romero, L. 2000. Nitrogen metabolism in pepper plants applied with different bioregulators. *Journal of Agricultural and Food Chemistry* 48:2925–2929.
- Sahain, M.F.M., Abd, E.Z., Motty, E., Shiekh, M.H.E. & Hagagg, L.F. 2007. Effect of some biostimulant on growth and fruiting of ‘Anna’; apple trees in newly reclaimed areas. *Research Journal of Agriculture and Biological Sciences* 3:422–429.
- Shaharoon, B., Naveed, M., Arshad, M. & Zahir, Z.A. 2008. Fertilizer-dependent efficiency of Pseudomonads for improving growth, yield, and nutrient use efficiency of wheat (*Triticum aestivum* L.). *Applied Microbiology and Biotechnology* 79:147–155.
- Spaepen, S., Vanderleyden, J. & Okon, Y. 2009. Plant growth promoting actions of rhizobacteria. *Advances of Botanical Research* 51:283–320.
- Thalheimer, M. & Paoli, N. 2002. Effectiveness of various leaf-applied biostimulants on productivity and fruit quality of apple. *Acta Horticulturae* 594:335–339.
- Tshabalala, P.M. 2015. Estimating the economic rate of return to plum research investments in South Africa, South Africa. 67 pp.
- Vance, C.P. 2001. Symbiotic nitrogen fixation and phosphorus acquisition. plant nutrition in a world of declining renewable resources. *Plant Physiology* 127:390–397.
- Vejan, P., Abdullah, R., Khadiran, T., Ismail, S. & Nasrulhaq Boyce, A. 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability: A review. *Molecules* 21:1–17.
- Vernieri, P., Borghesi, E., Ferrante, A. & Magnani, G. 2005. Application of biostimulants in floating system for improving rocket quality. *Journal of Food, Agriculture & Environment* 3:86–88.
- Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* 255:571–586.
- Whipps, J.M. 2001. Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany* 52:487–511.
- Wu, S.C., Cao, Z.H., Li, Z.G., Cheung, K.C. & Wong, M.H. 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: A greenhouse trial. *Geoderma* 125:155–166.

8. Tables

Table 1: The effect of biostimulant combinations with their application rates and active components on ‘Rubystar’ plums and ‘Forelle’ pears in Franschhoek, and Villiersdorp respectively during 2015/16.

Treatments	Application rate	Active component
Untreated	No application	No application
Rizofos Fruit [®]	12 ml in 5L water (1.2 ml.tree ⁻¹)	<i>Pseudomonas fluorescens</i>
Tricure SP [®]	30 g in 5L water (3 g. tree ⁻¹)	<i>Trichoderma harzianum</i>
Premax [®]	30 ml in 5L water (0.3 ml.tree ⁻¹)	Rizobacter and <i>Pseudomonas fluorescens</i>
Technical [®]	8 ml in 5L water (0.8 ml.tree ⁻¹)	Calcium (49.2 g.kg ⁻¹) and cytokinins + auxins

Table 2: Effect of a biostimulant combination (Rizofos Fruit[®], Premax[®], Technical and Tri-Cure SP[®]) on plant performance and growth of ‘Rubystar’ plums in Franschhoek during 2015/16.

Treatment	Yield efficiency (kg.cm ⁻¹)	Yield.tree ⁻¹ (kg)	Number of fruit.tree ⁻¹	Stem Circumference (cm)
Untreated (control)	0.808 ^{ns}	17.79 ^{ns}	171.7 ^{ns}	22.05 ^{ns}
“Rizofos Plus”	0.865	18.91	190.5	21.85
LSD	0.14	3.48	42.01	1.93
<i>P</i>	0.4157	0.5073	0.3595	0.8304

Table 3: Effect of biostimulant combination (Rizofos Fruit[®], Premax[®], Technical and Tri-Cure SP[®]) on plant performance and growth of ‘Forelle’ pears in Villiersdorp during 2015/16.

Treatment	Stem Circumference (cm)	Yield.tree ⁻¹ (kg)	Yield efficiency (kg.cm ⁻¹)
Untreated (control)	78.1 ^{ns}	42.11 ^{ns}	0.547 ^{ns}
“Rizofos Plus”	70.6	46.74	0.663
LSD	7.83	11.51	0.1805
<i>P</i>	0.0584	0.3865	0.1801

Table 4: Effect of biostimulant combination (Rizofos Fruit[®], Premax[®], Technical and Tri-Cure SP[®]) on maturity indices of ‘Ruby’ plum in Franschhoek and ‘Forelle’ pear in Villiersdorp during 2015/16.

Treatment	‘Rubystar’		‘Forelle’			
	Firmness (kg)	Fruit diameter (mm)	Fruit diameter (mm)	Firmness (kg)	Fruit weight (g)	TSS (%)
Untreated (control)	5.61 ^{ns}	57.58 ^{ns}	67.86 ^{ns}	6.65 ^{ns}	172.39 ^{ns}	15.6 ^{ns}
Rizofos Plus [®]	5.57	57.72	67.05	6.66	168.66	15.4
LSD	0.75	3.15	1.77	0.37	14.2	0.76
<i>P</i>	0.9123	0.9267	0.3268	0.953	0.5671	0.4600

PAPER 2: EFFECT OF A BIOSTIMULANT COMBINATION (RIZOFOS FRUIT[®], TRI-CURE SP[®], Premax[®] and Technical[®]) ON GROWTH AND DEVELOPMENT OF NON-BEARING ‘PACKHAM’S TRIUMPH’ AND ‘CELINA’ PEAR TREES

Abstract

The application of plant growth promoting rhizobacteria (PGPR) are increasingly gaining acceptance for its efficacy in promoting growth of crops through its impact on the rhizosphere. The main aim of this study was to investigate the efficacy of a biostimulant combination (‘Rizofos plus’) composing of Rizofos Fruit[®], Tri-Cure SP[®], Premax[®] and Technical[®] in promoting plant performance at orchard level, using ‘Packham’s Triumph’ and ‘Celina’ pear cultivars. The experimental design consisted of two treatments: the untreated (control) and a soil-based biostimulant application of ‘Rizofos Plus’. The biostimulants were applied twice (at transplanting and two weeks after transplanting). Results indicated that application of the biostimulant combination ‘Rizofos Plus’ did not produce significant differences in comparison to the untreated with respect to any of the growth parameters on either of the two cultivars. However, the treatment resulted in a significantly higher stomatal conductance for Packham’s Triumph ($307 \text{ mmol.m}^{-2}.\text{s}^{-1}$) and Celina ($315 \text{ mmol.m}^{-2}.\text{s}^{-1}$) compared to that of the untreated treatment ($222 \text{ mmol.m}^{-2}.\text{s}^{-1}$; $213 \text{ mmol.m}^{-2}.\text{s}^{-1}$) respectively. Thus results confirmed the importance of long term studies to quantify potential beneficial contributions of biostimulants in perennial crops.

Keywords: leaf total soluble solids, PGPR, stomatal conductance.

1. INTRODUCTION

Specialized organic molecules to activate and regulate plant metabolism for enhanced growth performance may be considered as an alternative to achieve more cost effective improvement of growth and performance of plants (Esitken *et al.*, 2010). Biofertilization were reported to be effective in promoting growth of certain pome fruit species and are considered to have a significant possibility as a renewable, supplementary and environmental friendly source of plant nutrients (Mosa *et al.*, 2016). Furthermore, it is a vital aspect of assimilated plant nutrient management (Bhattacharjee & Dey, 2014).

The use of biofertilizers, as an alternative to artificial chemicals, has been shown to improve plant performance by supplying plant nutrients and thus assist soil productivity (Mosa *et al.*, 2016). Soil microorganisms can contribute to crop nutrition through a number of mechanisms, including direct effects on the availability of nutrients or plant growth-promoting substances, or by facilitating the absorption of certain nutrients from the environment (Farina, 2012).

Pear trees endure transplanting shock when transplanted from the nursery to the orchard (Arnold, 2005). Transplant shock is a condition of stress from injuries, depletion and impaired functions of trees, during adaptation to a new environment (Struve, 2009). Transplant shock is often associated with loss of root mass associated with harvesting and up to 95% of the original root volume is lost when field grown trees are harvested (Watson & Sydnor, 1987). Roots are critical in many physiological processes of a fruit tree. The critical roles include initial uptake and subsequent transport of water and nutrients, the production of stress response signals, the secretion of bioactive molecules, and the establishment of microbiological populations in the rhizosphere (Cui *et al.*, 2016). Therefore, if the root systems of different rootstocks vary significantly, it will impact on tree growth due to these diverse associated roles.

The arbuscular mycorrhiza (AM) fungal inoculation played a significant positive role on plant growth via improved acquisition of nutrients of low mobility, especially phosphorous (P) in low-nutrient and constrained soils (Egamberdieva *et al.*, 2015). arbuscular mycorrhiza (AM) fungi-colonised plants showed greater tolerance over non-mycorrhizal plants to several biotic and abiotic stresses such as toxic metals, root pathogens, drought, high soil temperature, saline soils (Khaliel *et al.*, 2011), adverse soil pH and transplanting shock (Evelin *et al.*, 2009; Lu *et al.*, 2003; Tang *et al.*, 1999).

Kloepper *et al.* (2004) showed that PGPR elicited growth promotion, reduced transplant shock and stimulated rooting with several vegetable transplant system. Another field evaluation showed that PGPR enhanced transplant growth, vigour and survival in both tomato and pepper (*Piper nigrum*. L) transplants (Kokalis-Burelle *et al.*, 2002). The effect of PGPR on tomato growth, yield and fruit quality was conducted under field stresses in northeast Alabama. Commercially available PGPR products lessened the stress of transplant shock and nitrogen stress resulting from organic fertilizer (Hu, 2005).

Further studies on PGPR, in particular *Pseudomonas fluorescens*, application to young apple trees, improved shoot length and increase in yield (Orhan *et al.*, 2006; Aslantaş *et al.*, 2007; Karlidag *et al.*, 2007). Karlidag *et al.* (2007) showed that that bacterial elicitor treatments such as those containing *Pseudomonas fluorescens* exhibited a greater number of leaves and increased plant height in ‘Granny Smith’ apple tree. Ferree *et al.* (1990) did not find the beneficial effects of PGPR which contradicted Ogrodnictwa (2014) who reported that the application of Biofeed ‘Amin’ stimulated performance of ‘Ariwa’ apple trees. In addition, growth was significantly improved compared to a standard NPK treatment by an application, both July and November, of a product ‘Florovit Eko’ that was enriched with beneficial bacteria. These observations agreed with the reports by Grz *et al.* (2012) where ‘Florovit Eko’, with the addition of mycorrhiza fungi, were found to improve the circumference of apple trees cv. ‘Topaz’ and of sour cherry cv. ‘Debreceni Bötermö’. However, an increase in stem and shoot lengths on ‘Crispin’, ‘Honeycrisp’, ‘Jonagold’ and ‘Macoun’ apple cultivars were only observed by Dominguez & Robinson (2015) from the third up to the fifth season following biostimulant treatments when compared to the control treatment.

Phosphorus is widely recognized as a key element for tree growth, performance and development, and, yet, it is often a severely deficient nutrient, particularly in soils with high P fixation capacity (Sharma *et al.*, 2013). A considerable number of bacterial species, are able to exert a beneficial effect upon plant growth, by initiating high stomatal conductance on leaves. Phosphate solubilizing microorganisms convert insoluble phosphates into soluble forms through the process of acidification, chelation and exchange reactions (Jiang & Sato, 1994).

The choice of cultivar may result in differences when biostimulants are applied. This view was supported by Khan *et al.* (1998) and Lo Bianco *et al.* (2003) who reported no direct effect of biostimulants on growth and yield, but rather found an interaction among rootstock, cultivar and treatments. A study of twelve rootstocks and four apple cultivars, provided insights into relative tree growth performance when cultivated under conventional, non-organic conditions.

This paper aimed towards quantifying plant performance in non-bearing pear trees i) first at establishment of a Packham's Triumph and ii) one year after establishment of a 'Celina' orchard, following a soil application of a locally manufactured (MBFI International) biostimulant combination.

2. MATERIALS AND METHODS

2.1 Site

The experiment was performed at Welgevallen experimental farm in Stellenbosch (33°56'33"S; 18°51'56"E). The experimental site, characterised by clay soils with a pH range from 5.3 to 5.5, is classified according to the South African soil classification system as Kroonstad ('Packham's Triumph') and Tukulu ('Celina') soil classifications. Summers are dry and warm to hot, as some days in February and March may rise to over 40 °C. Winters are cool and rainy, with daytime temperatures averaging 16 °C and an average yearly rainfall of 800-1000 mm (Midgley *et al.*, 2016). 'Celina' and 'Packham's Triumph' pear cultivars, both obtained from Stargrow Nursery (Stellenbosch), were established on 'BP3' rootstock and planted 16 October 2015 and 16 August 2016 respectively. Trees for both cultivars were planted at a row spacing of 1.75 x 4.75 m with 1270 trees. ha⁻¹. Irrigation was supplied by means of a micro sprinkler irrigation system adjacent to the tree, with an irrigation scheduling of 65 % field capacity.

2.2 Experimental design and treatment application

The experiments were set up in a randomized complete block design with two treatments and ten blocks, where single tree replications was used as experimental units. Treatment one was an untreated (control), whereas the second treatment was biostimulant formulation "Rizofos Plus" which comprised combination of Rizofos Fruit[®], Tri-Cure SP[®], Premax[®] and Technical[®] (MBFI International, Delmas, SA). **Bemlab analysis for foliar mineral application were taken on 2 February 2017 (Table 4).** Standard disease, insect and weed control practices were carried out on both cultivars throughout the growing season. Trees were planted on soil previously planted to plums and no fumigation was used. Standard procedures with regard to soil amelioration according to soil analyses were performed before planting. Recommended application concentrations and application rates were followed (Table 1).

One application of 30 ml “Rizofos Plus” was done on 26 August 2016, to coincide with the planting of the ‘Packham’s Triumph’ trees. The liquid biostimulant was applied on the open roots of the trees before planting. The application on ‘Celina’ was performed one year after planting, on 26 August 2016, using the localised placement method on the soil and a similar formulation that used with the ‘Packham’s Triumph’ cultivar.

2.3 Data collection

2.3.1 Vegetative production parameters

At planting, trees of both cultivars were marked 20 cm above the graft union as the position where tree diameter (mm) was recorded. Tree diameter was measured at the first application of the treatments and then again at the end of the study on 10 May 2017, using a digital Vernier calliper (ATAGO, PR, USA). Two representative shoots per tree were identified at the end of the growing season (10 May 2017) and the total length was determined (mm) using a measuring tape.

2.3.2 Leaf total soluble solids (TSS)

Leaf TSS measurements for both cultivars was done on 17 March 2017, during a period of active vegetative growth. Measurements were carried out in the morning, were temperate and humidity were conducive for active photosynthesis. Three healthy, newly mature leaves per tree were randomly selected from the outer, 45° sun-exposed canopy of the tree canopy for all replications, approximately 1 m from soil level. The use of newly mature leaves, which were less prone to fluctuations in % Brix, also promoted the collection of leaf sap, when leaf samples were inserted into a leaf press. Leaf TSS was then determined using a calibrated handheld refractometer (ATAGO, PR 32, USA). After each reading, the refractometer glass was rinsed with distilled water to avoid contamination of the following sap sample.

2.3.3 Stomatal conductance

Stomatal conductance was recorded three times per leaf, per replication, on 25 May 2017 by means of a porometer (AP4 Delta-T Devices, Cambridge, England), using an average of three leaves located on the outer, 45° sun-exposed canopy of each tree.

2.4 Statistical Analysis

Data was analysed with a two-way analysis of variance (ANOVA) using the generalised linear model (GLM) in Statistical Analysis System (SAS) programme (SAS Institute Inc., Cary, NC, USA) version 7.1. Separation of means was done using the Fisher's least significant difference (LSD) test. Treatment effects were considered significant at the 5% confidence level ($P \leq 0.05$). Stem diameters was analysed by means of covariance (ANCOVA), where the final diameter represented the dependant variable with the initial diameter as the covariate.

3. RESULTS AND DISCUSSION

3.1 Vegetative growth parameters

The application of "Rizofos Plus" had no significant effect on stem diameter and shoot length of 'Packham's Triumph' trees following the first year of application (Table 2). No significant effect on shoot length or stem diameter was detected for 'Celina' (Table 3).

Due to limitations of the experiment trial, below soil growth of roots and microbial dynamics were not quantified which might have impacted growth of stems and shoots. The general lack of improved vegetative growth for both cultivars following the first season of application is in contrast with reports by Karlidag *et al.* (2007) on bearing apple trees, where they investigated root inoculations of the PGPR strains, *Bacillus* 'M3', 'OSU-142' and microbacterium 'FS01, which were tested alone or in combination on apple (*Malus domestica* L.) cv. 'Granny Smith', the presence of these microbes was shown to initiate growth. This then resulted in significant increase in shoot length and diameter, but it also promoted cumulative yield and fruit weight in 'Granny Smith' (Karlidag *et al.*, 2007). In addition, a minimum of two growing seasons are usually required to observe the effects of PGPR on tree growth (Karakurt & Aslantas, 2010). Results from our study present data following only one season, which may be insufficient to allow for any significant differences to emerge.

The mode of action of Rhizobacteria is amongst other on promoting P uptake by the tree, thus the efficacy of the treatment is dependant both on available P in the soil and root growth. Soil amelioration for both cultivars was performed prior to planting, with mineral corrections based on soil analysis results. Mineral composition of soils could thus be considered optimum, even for 'Celina' which was planted in 2016, as deficiencies occurring within two years of planting in a heavy soil type, is highly unlikely. Leaf analyses in February 2017 confirmed no deficiencies, supporting

the assumption that no soil nutrient deficiency occurred during the early stages of establishment. In both cultivars P leaf levels were high in the control, which suggest that the initial sufficient to high P-soil levels would have reduced the possible beneficial and promotive effect of P uptake in the “Rizofos Plus” treatments (data not shown). Previous studies confirmed that the effectiveness of biofertilizers is influenced by the available plant mineral nutrient status and P solubilisation (Mosa *et al.*, 2016). Finally, Bradshaw (2015) and Dominguez & Robinson (2015) showed that tree growth was not significantly increased by biostimulants, as the primary contribution to plant growth is through soil mineral nutrition, thus the contribution of biostimulants should be viewed in this context.

3.2 Leaf Total Soluble Solids and stomatal conductance

The “Rizofos Plus” treatment had no significant effect on the leaf TSS of either ‘Packham’s Triumph’ (Table 1) or ‘Celina’ during the season after application (Table 2). However, the biostimulant treatment had significantly higher stomatal conductance ($307.6 \text{ mmol.m}^{-2}.\text{s}^{-1}$; $315 \text{ mmol.m}^{-2}.\text{s}^{-1}$) compared to the control treatment ($222.5 \text{ mmol.m}^{-2}.\text{s}^{-1}$; $213.4 \text{ mmol.m}^{-2}.\text{s}^{-1}$) as recorded on both ‘Packham’s Triumph’ (Table 2) and ‘Celina’ leaves (Table 3).

Leaf total soluble solids provides an estimate of the sugar content of a plant as a higher refractive index (% Brix) and maturity will generally have a higher sugar, mineral and protein content, as well as a greater specific gravity of density along with a lower water content. Plants with brix readings within a range of 3 to 5 (higher than average) are considered to improve resistance to environmental stress (Farm and Garden Supply, 2017). Several studies have shown *Pseudomonas*, *Rhizobium*, *Klebsiella* and *Pseudomonas* bacteria to possess the potential in solubilizing insoluble inorganic phosphates, making them accessible to plants (Richardson *et al.* 2000, 2001; Vyas & Gulati, 2009; Ahemad & Khan, 2012). Furthermore, Khan *et al.* (2009) linked the important role of P with overall better physiological performance in the plant. In accordance, Apastambh *et al.* (2014) showed P deficiency to significant impact on leaf photosynthesis and other carbon metabolic pathway in plants (Shah *et al.*, 2013), which resulted in smaller size of stomatal opening, thus lowering stomatal conductance (Apastambh *et al.*, 2014).

Stomatal conductance is a measure of the resistance to water vapour loss to the atmosphere and is considered a reliable indicator of the physiological state of the plant (Bragg *et al.*, 1998) and an important indicator in the comparison of the performance of different crops in response to environmental variations and stresses (Bragg *et al.*, 1998). Similar to our findings on both pear

cultivars, Kelting (1997) indicated a high stomatal conductance on landscape trees following application of rhizobacteria, although tree growth was not significantly affected. In their study, high stomatal conductance was associated with higher P content of leaves of plants subjected to ‘M3’ inoculations, known for their high phosphate solubilizing capacity.

PGPR has been associated with the production of signal molecules that directly adjust the gene expression and protein synthesis involved in photosynthesis, growth and metabolism (Mu *et al.*, 2015). In addition to a higher available P level, the presence of signal molecules and their associated impact may partly explain the higher stomatal conductance as was measured in the “Rizofos Plus” treatment compared to the control.

4. CONCLUSION

Findings of this study indicate that soil application of a combination of biostimulants on non-bearing ‘Packham’s Triumph’ and ‘Celina’ pear cultivars had no significant impact on stem diameter and shoot growth during the first season.

A possible reason for the lack of increase in stem diameter (‘Celina’) or shoot growth (both cultivars) calls of the duration of the observation period to be extended as this study was carried out over one season whereas previous researchers confirmed that a longer period of data collection is required to be able obtain conclusive results. Furthermore, the use of this novel approach in tree establishment under orchard conditions, especially using the plant growth promoting rhizobacteria (PGPR), is in its initial stages. Most papers pertaining the use of *Pseudomonas fluorescens* refer to efficacy more that at molecular level rather than at field level, making comparison with our study difficult.

Applying “Rizofos Plus” did not result in significant changes in leaf TSS compared to the control. However, on a physiological level, significant differences were observed in stomatal conductance for both cultivars. This significant higher stomatal conductance of the “Rizofos Plus” treatment may indicate as state of reduced stress (Bragg *et al.*, 1998). Increased photosynthesis as a result of higher stomatal conductance was not translated into an increase in TSS or vegetative growth, following the “Rizofos Plus” treatment. However, as these features would be as a result of a cascade effect set in motion by higher available P and the presence of signalling molecules, measureable outputs may thus be premature after only one season, as it also proposed by Kelting (1997).

Due to limitations of the experiment below soil growth and microbial dynamics were not quantified. It is possible that the growth promotive effects of the “Rizofos Plus” treatment may have occurred in the root zone and thus will only be visible during the following spring of the following season, after root volume was expanded, as would be followed by the inherent increase in cytokinin production and carbohydrate export to the top growth. The lowered stress induced by the “Rizofos Plus” treatment as can be deduced from the higher stomatal conductance recorded in the leaves of both cultivars, testifies of the possible beneficial effect the biostimulant may have offered the ‘Packham’s Triumph’ trees during transplanting, a process which poses severe stress to nursery trees which are provided as bare rooted saplings for the establishment of orchards. It is also noteworthy that the adjusted stomatal conductance trend continued to be measurable in the two year old ‘Celina’ trees, retaining thus the possible positive effect experienced with establishment and transferring it to the established trees.

Promising results urge an extension of the study in order to continue observations of growth and development over several seasons as is suggested throughout the literature in similar studies. The inclusion of root observations and the following season’s leaf mineral analysis would provide significant insights to unravelling the role of PGPR’s to enhance orchard productivity in a sustainable manner.

5. Acknowledgements

MBFI International[®] provided partial funding for this trial.

6. References

- Ahemad, M. & Khan, M.S. 2012. Effects of pesticides on plant growth promoting traits of *Mesorhizobium* strain MRC4. *Journal of the Saudi Society of Agricultural Sciences* 11:63–71.
- Apastambh, A.R., Taneever, K. & Baig, M.M.V. 2014. Isolation and of plant growth promoting rhizobacteria and their application in plant growth. characterization *International Journal of Current Microbiology and Applied Sciences* 37:72–78.
- Arnold, M.A. 2005. Challenges and benefits of transplanting large trees: An introduction to the workshop. *Horticulture Technology* 15:115-117.
- Aslantaş, R., Çakmakçı, R. & Şahin, F. 2007. Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. *Scientia Horticulturae* 111:371–377.
- Bhattacharjee, R. & Dey, U. 2014. Biofertilizer, a way towards organic agriculture: A review. *African Journal of Microbiology Research* 8:2332–2342.
- Bradshaw, T.L. 2015. Comprehensive assessment of organic apple production in vermont: Experience from two orchard systems 206-2013 . PhD Thesis, Faculty of the Graduate College, University of Vermont.
- Bragg, T., Webb, N., Spencer, R., Wood, J., Nicholl, C. & Potter, E. 1998. User Manual for the porometer type AP4. Vol. 35. N. Webb (ed.), Delta-T Devices Ltd, Cambridge, England. 184 pp.
- Cui, X., Dong, Y., Gi, P., Wang, H., Xu, K. & Zhang, Z. 2016. Relationship between root vigour, photosynthesis and biomass in soybean cultivars during 87 years of genetic improvement in the northern China. *Photosynthetica* 54:81–86.
- Dominguez, L.I. & Robinson, T.L. 2015. Strategies to improve early growth and yield of tall spindle apple plantings. *New York State Horticultural Society* 23:5–10.
- Egamberdieva, D., Shrivastava, S & Varma, A. 2015. Plant growth- promoting phizobacteria (PGPR) and medicinal plants. Vol. 2. D. In: S. Egamberdieva, S. Shrivastava & A. Varma (eds.). *Soil Biology*. Springer International, Switzerland 2015. 444 pp.
- Esitken, A., Yildiz, H.E., Ercisli, S., Figen Donmez, M., Turan, M. & Gunes, A. 2010. Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. *Scientia Horticulturae* 124:62–66.
- Evelin, H., Kapoor, R & Giri, B. 2009. Arbuscular mycorrhizal fungi in alleviation of salt stress: A review. *Annals of Botany* 104:1263–1280.

- Farina, A. 2012. A biosemiotic perspective of the resource criterion: toward a general theory of resources. *Biosemiotics* 5:17–32.
- Farm and Garden Supply. 2017. Refractometer product guide. Peaceful valley farm & garden supply grass valley, CA, USA. 2 pp.
- Ferree, D.C., Gaus, A., Hirst, P.M., Mullins, C.A. & Schupp, J.R. 1990. Performance of ‘Golden Delicious,’ ‘Jonagold,’ ‘Empire,’ and ‘Rome Beauty’ apple trees on five rootstocks over ten years in the 1990 NC-140 Cultivar/Rootstock Trial 1. *Journal of American Pome Society* 12:1–12.
- Grz, Z.S., Piotrowski, W., Bielicki, P., Paszt, L.S. & Malusà, E. 2012. Effect of different fertilizers and amendments on the growth of apple and sour cherry rootstocks in an organic nursery. *Journal of Life Sciences* 200:43–53.
- Hu, C.H. 2005. Induction of growth promotion and stress tolerance in arabidopsis and tomato by plant growth promoting rhizobacteria. PhD Thesis, The Graduate Faculty of Auburn University, Alabama.
- Jiang, H. & Sato, K. 1994. Soil science and plant nutrition interrelationships between bacterial populations on the root surface of wheat and growth of plant. *Soil Science and Plant Nutrition* 40:683–689.
- Karakurt, H. & Aslantas, R. 2010. Effects of some plant growth promoting rhizobacteria (PGPR) strains on plant growth and leaf nutrient content of apple. *Journal of Fruit and Ornamental Plant Research* 18:101–110.
- Karlidag, H., Esitken, A., Turan, M. & Sahin, F. 2007. Effects of root inoculation of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient element contents of leaves of apple. *Scientia Horticulturae* 114:16–20.
- Kelting, M.P. 1997. Effects of soil amendments and biostimulants on the post-transplant growth of landscape trees. MSc Thesis, Department of Horticulture, Virginia Polytechnic Institute and State University.
- Khaliel, A.S., Shine, K & Vijayakumar, K. 2011. Salt tolerance and mycorrhization of *Bacopa monneiri* grown under sodium chloride saline conditions. *African Journal of Microbiology Research* 5:2034–2040.
- Khan, A.A., Jilani, G., Akhtar, M.S., Saqlan, S.M. & Rasheed, M. 2009. Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. *Journal of Agriculture and Biological Sciences* 1:48–58.
- Kloepper, J.W., Reddy, M.S., Kenney, D.S., Vavrina, C.S., Kokalis-Burelle, N & Martinez-Ochoa,

- N. 2004. Theory and applications of rhizobacteria for transplant production and yield enhancement. Proc. XXVI IHC – Transplant production and stand establishment, 2004. In: S. Nicola, J. Nowak & C.S. Vavrina (eds.). *Acta Horticulturae* 631:217-219.
- Kokalis-Burelle, N., Vavrina, C.S., Roskopf, E.N & Shelby, R.A. 2002. Field evaluation of plant growth-promoting rhizobacteria amended transplant mixes and soil solarization for tomato and pepper production in Florida. *Plant and Soil* 238: 257-266.
- Laiche, A. J., Jr. 1991. Evaluation of humic acid and slow release fertilizers on container-grown landscape plants. Research Report, *South Mississippi Branch Experiment Station* 16:1-3.
- Lo Bianco, R., Policarpo, M. & Scariano, L. 2003. Effects of rootstock vigour and in-row spacing on stem and root growth, conformation and dry-matter distribution of young apple trees. *Journal of Horticultural Science and Biotechnology* 78:828–836.
- Lu, J.Y., Mao, Y.M., Shen, L.Y., Peng, S.Q & Li, X.L. .2003. Effects of VA mycorrhizal fungi inoculated on drought tolerance of wild Jujube (*Zizyphus spinosus* Hu). *Acta Horticulturae* 30:29–33.
- Midgley, G., Makholela, T. & Mantlana, B. 2016. South Africa’s 1st Annual climate change report : Climate change trends, risks, impacts and vulnerabilities. Department: Environmental Affairs, Pretoria, South Africa. 60 pp.
- Mosa, W.F.A.E-G., Paszt, L.S., Frac, M., Trzcinski, P., Przybyl, M., Treder, W. & Klamkowski, K. 2016. The influence of biofertilization on the growth, yield and fruit quality of cv. ‘Topaz’ apple trees. *Horticultural Science* 43:105–111.
- Mu, X., Chen, F., Wu, Q., Chen, Q., Wang, J., Yuan, L. & Mi, G. 2015. Genetic improvement of root growth increases maize yield via enhanced post-silking nitrogen uptake. *European Journal of Agronomy* 63:55–61.
- Ogrodnictwa, I. 2014. Influence of various biofertilizers on the growth and fruiting of ‘Ariwa’ apple trees growing in an organic orchard. *Journal of Research and Applications in Agricultural Engineering* 59:96–99.
- Orhan, E., Esitken, A., Ercisli, S., Turan, M. & Sahin, F. 2006. Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. *Scientia Horticulturae* 111:38–43.
- Richardson, A.E., Hadobas, P.A. & Hayes, J.E. 2000. Acid phosphomonoesterase and phytase activities of wheat (*Triticum aestivum* L.) roots and utilization of organic phosphorus substrates by seedlings grown in sterile culture. *Plant, Cell and Environment* 23:397–405.
- Richardson, A.E., Hadobas, P.A., Hayes, J.E., O’hara, C.P. & Simpson, R.J. 2001. Utilization of

phosphorus by pasture plants supplied with myo-inositol hexaphosphate is enhanced by the presence of soil micro-organisms. *Plant and Soil* 229:47–56.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H. & Gobi, T.A. 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus* 2:1-14.

Struve, D.K. 2009. Tree establishment: A review of some of the factors affecting transplant survival and establishment. *Arboriculture and Urban Forestry* 35:10–13.

Tang, M., Chen, H & Shang, H.S. 1999. Effects of arbuscular mycorrhizal fungi (AMF) on *Hippophae rhamnoides* drought-resistance. *Scientia Silvae Sinica* 35:48–52.

Vyas, P. & Gulati, A. 2009. Organic acid production in vitro and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*. *BMC Microbiology* 9:1-15.

Watson, G.W. & Sydnor, T.D. 1987. The effect of root pruning on the root system of nursery trees. *Journal of Arboriculture* 13:126–130.

7. Tables

Table 1: The effect of biostimulant combinations with their application rates and active components on ‘Packham’s and ‘Celina’ pears at Welgevallen experimental farm, Stellenbosch in August 2016.

Treatments	Application rate	Active component
Untreated	No application	No application
Rizofos Fruit [®]	12 ml in 5L water (1.2 ml.tree ⁻¹)	<i>Pseudomonas fluorescens</i>
Tricure SP [®]	30 g in 5L water (3g. tree ⁻¹)	<i>Trichoderma harzianum</i>
Premax [®]	30 ml in 5L water (0.3 ml.tree ⁻¹)	Rizobacter and <i>Pseudomonas fluorescens</i>
Technical [®]	8 ml in 5L water (0.8 ml.tree ⁻¹)	Calcium (49.2 g.kg ⁻¹) and cytokinins + auxins

Table 2: Stem growth parameters and leaf characteristics as recorded in 2017 on ‘Packham’s Triumph’ following a “Rizofos Plus” soil application at planting in August 2016.

Treatments	Stem diameter	Shoot length	*Leaf TSS	Stomatal conductance
	(mm) 8 May	(mm) 22 March	(%) 17 March	(mmol.m ⁻² .s ⁻¹) 6 June
Untreated (control)	13.52	224.65 ^{ns}	1.72 ^{ns}	222 ^b
“Rizofos Plus”	13.04	200.15	1.62	307 ^a
LSD	1.52	62.13	1.07	74.87
<i>P</i>	0.5497	0.4599	0.8625	0.0301

*TSS-Total soluble solids

Table 3: Stem growth parameters and leaf characteristics as recorded in 2017 on ‘Celina’ following a “Rizofos Plus” soil application one year after at planting in August 2016.

Treatments	Stem diameter	Shoot length	*Leaf TSS	Stomatal conductance
	(mm) 8 May	(mm) 22 March	(%) 17 March	(mmol.m ⁻² .s ⁻¹) 6 June
Untreated (control)	17.60 ^{ns}	328.35 ^{ns}	2.62 ^{ns}	213 ^b
“Rizofos Plus”	17.06	265.40	3.86	315 ^a
LSD	1.57	206.71	1.46	47.15
<i>P</i>	0.9058	0.553	0.1313	0.0009

*TSS-Total soluble solids

Table 4: Bemblab analysis for foliar mineral application taken on 2 February 2017 on ‘Celina’ and ‘Packham’s Triumph’ pears at Welgevallen experimental farm, Stellenbosch in August 2016.

Orchard	Lab. No.	Fruit type	Cultivar	N %	P	K	Ca	Mg	Na mg/kg	Mn	Fe	Cu	Zn	B
‘Celina’	16840	Pear		2.79 B	0.29 B	1.35	2.02	0.28	341	191 H	278	14	101 T	39 H
‘Packham’s Triumph’	16842	Pear		2.84 B	0.34 B	1.15	1.76	0.27	891 H	120 H	387	13	99 H	28 H

D = Deficiency; L = Low; H = High; B = Very high; T = Toxics

Standard	Low	Pears		1.90	0.09	0.98	0.86	0.25		26	63	3	20	23
	High			2.66	0.17	1.97	1.73	0.40	500	106	252	19	50	32

Standards for deciduous fruit are corrected for the date of the sample (31 January)

PAPER 3: THE POTENTIAL FOR IMPROVED ESTABLISHMENT OF 'NADORCOTT' MANDARIN TREES WITH BIOSTIMULANT APPLICATIONS

Abstract

Citrus spp establishment is often poor or unsuccessful, due to a delay in root development and growth, resulting from transplant shock when trees are transferred from the nursery to the orchard. The aim of this study was to assess whether transplant and establishment of young citrus trees, in particular 'Nadorcott' mandarins, can be improved through the use of biostimulants during the transplanting process. The study was conducted at Welgevallen research farm in Stellenbosch, Western Cape, South Africa using 'Nadorcott' mandarin nursery trees. Treatments consisted of five biostimulant treatments, applied twice at planting and two weeks thereafter: compost (control), Terramax[®], Kelpak[®], RootAktiv[®] and Super Wortel[®] (foliar). Results showed that RootAktiv[®] (265.7 mm) improved shoot growth ($P = 0.0111$) relative to other treatments but was similar to the control (191.9 mm). Terramax[®] and Kelpak[®] improved root length (310.8 and 331.8 mm) and root volume (189.0 and 253.4 mm³) respectively compared to Super Wortel[®] treatments (103.07 mm and 60.23 mm) and RootAktiv[®] (120.03 mm and 75.25 mm). With respect to physiological growth, Terramax[®] and Kelpak[®] (-0.53 MPa and -0.83 MPa) showed high stem water potential than the control (-2.2 MPa), stomatal conductance (257.7 mmol.m⁻².s⁻¹ and 228.4 mmol.m⁻².s⁻¹) and photosynthesis (7.96 μmol m⁻².s⁻¹ and 9.215 μmol m⁻².s⁻¹) compared to the control (145.6 mmol.m⁻².s⁻¹ and 4.785 μmol m⁻².s⁻¹) respectively. From the results obtained in this study, application of biostimulants had positive effects on both plant growth and physiology. Results varied depending on the active component of the different biostimulant products.

Key words: transplant shock, root growth, plant physiology.

1. INTRODUCTION

Woody perennials endure a stress condition, known as transplant shock, when being transferred from the nursery to the orchard (Struve, 2009). Transplant shock makes plants more susceptible to injuries, depletion of reserves and often results in impaired physiological functions such as reduced photosynthesis, so that a process of adaptation and recovery to its new environment is almost always required (Struve, 2009). Root loss in particular is often associated with transplanting and has a negative impact by reducing the ability of the plant to absorb water and mineral nutrients, which then results in a loss of stored carbohydrates and mineral nutrients, leading to poor plant performance or even death (Gauthier & Kaiser, 2014; Struve, 2009). Although plant diseases are often implicated, transplant stresses are most often the primary cause of decline or death of newly planted trees and shrubs (Gauthier & Kaiser, 2014). It is therefore now widely recognised that the survival of newly established trees is largely dependent on the rapid extension of root growth following transplanting, to ensure sufficient absorption of water to replenish transpirational water loss and reduce drought-related water stress symptoms (Watson & Himelick, 2005).

Recent developments in fertiliser technology have led to the formulation of a range of products broadly termed to be 'biostimulants'. Biostimulants can be defined as any substance or micro-organism applied exogenously to plants with the aim to enhance nutrient use efficiency and to promote abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content (Du Jardin, 2015). These biostimulants can be introduced to crop leaves, seeds, or soil with the objectives to stimulate root growth (Canellas *et al.*, 2011; Khan *et al.*, 2009), facilitate effective root uptake of minerals (Pinton *et al.*, 1999) and to promote beneficial microbial populations (Chen, 2006; Vessey, 2002).

Four major groups of biostimulants have been shown to affect root growth and nutrient uptake: (1) humic substances (HS), (2) protein hydrolysate and amino acid formulations (AA), (3) seaweed extract (SE), and (4) plant growth promoting microorganisms (Calvo *et al.*, 2014; Du Jardin, 2015). Biostimulants are mostly used complementary to strategies designed specifically for crop nutrition and crop protection. Furthermore, recent developments in fertiliser technology now include biofertilizers, as a recognized subdivision of biostimulants. Biofertilisers mainly differ from the conventional nitrogen, phosphorous and potassium (NPK) fertilisers in that their active ingredients tend to consist of a range of organic compounds such as humic acids, marine algae extracts, vitamins and other chemicals that can vary according to

the formulation used by a particular company (Ferrini & Nicese, 2002; Fraser & Percival, 2003).

Reported benefits of biostimulants as ameliorants for transplanting have been diverse (Struve, 2009). In a study on Goldenraintree (*Koelreuteria paniculata* Laxm.), field-grown trees were treated with Bioplex™ (organic compounds) (Mt. Joy, PA), either as a foliar spray, a soil drench, or in combination as a foliar spray and soil drench three days before being harvested (Sammons & Struve, 2005). Results from this study indicated that the Bioplex™ treatments reduced transpiration up to three days after application, when compared to untreated plants. Following the Bioplex™ treatments, some of the plants were then transplanted, whereas the control were left intact. On evaluation one year after transplanting, the only treatment difference noted was that transplanted trees had a relatively smaller leaf canopy, with slightly reduced shoot length compared to the control (Sammons & Struve, 2004).

Compost (humic, humins or fulvic acids) and organic compounds (oligopeptides and polysaccharides) substances are the final products of microbial decomposition and chemical degradation of dead biota in soils (Calvo *et al.*, 2014). These metabolites, which are the major components of soil organic matter, are also considered to be the most abundant naturally occurring organic molecules on earth (Calvo *et al.*, 2014; Du Jardin, 2015). Numerous studies have shown that the amphiphilic properties of organic acids in root exudates can dissociate humic substance (HS) into low molecular and high molecular size groups (Nardi *et al.*, 1997, 2000, 2002, 2016; Piccolo *et al.*, 2002; Piccolo & Spiteller, 2003). These findings support the hypothesis that the conformational behaviour of dissolved humus in the rhizosphere and therefore also the interaction of humic metabolites with plant-root cells, may be controlled by the presence of root-exuded or microbe-release organic acids in the soil solution (Piccolo *et al.*, 2003). Studies indicating the potential for these substances to improve abiotic stress tolerance in plants include those where the addition of seaweed extract and humic acid to fescue (*Festuca arundinacea* Schreb.) and creeping bentgrass (*Agrostis palustris* Huds. A.) increased leaf hydration under dry soil conditions as well as root- and shoot growth, in addition to an elevated antioxidant capacity status (Park *et al.*, 2004; Zhang *et al.*, 2002). Further studies with bent grass reported these cytokinin-rich extracts, when used in combination with humic acid, to increase drought tolerance as well as promote the endogenous cytokinin content (Zhang & Ervin, 2004).

Vermicomposting, which is promoted as an alternative media component for the horticultural industry (Bachman & Metzger, 2008), is produced by the non-thermophilic

biodegradation of organic materials through interactions between earthworms and microorganisms (Bhagat *et al.*, 2013). In pistachio, the addition of 10 % vermicomposting has been reported to improve seedling growth (Golchin *et al.*, 2006). Alternatively, Sorrenti *et al.* (2008) found the growth of non-bearing citrus trees to be positively affected by vermicomposting when combined with blood meal, in comparison with other treatments. This unexpected finding was reported despite the total amount of nitrogen supplied by this treatment 40% lower than that of the nitrogen supplied by the mineral fertilizer treatments. In addition, vermicomposting application has also been reported to have a positive effect on growth of crops such as strawberry (Singh *et al.*, 2008), ornamental plants (Atiyeh *et al.*, 2000), vegetables (Kashem *et al.*, 2015) and pepper (Narkhede *et al.*, 2011).

Terramax[®] is a known product formulation of biological vermicomposting extracts, which is recommended for the amelioration of depleted soils. In addition, Terramax[®] can also act as an effective plant growth stimulant by positively affecting initial root and plant development as well as alleviating abiotic stress through plant growth regulators which is extracted from vermicomposting (Lazcano *et al.*, 2010; Pant *et al.*, 2009). Terramax[®] have been reported to have high microbial activity as inoculated with various *Bacillus* and *Trichoderma spp.*, thus containing more than a million protozoa per litre, with a bacteria:fungal ratio of 67:33 (Victus Biology, 2017). These properties render it effective to reduce soil compaction and to provide soils with a more desired, crumbly structure. Being classified as vermicompost, Terramax[®] is effective in transforming and stabilising organic wastes into humus through the joint action of earthworms and microorganisms (Yuan, 2016).

Seaweed, in particular *Ascophyllum nodosum* (Tandon & Dubey, 2015), is considered the single most researched and widely used type of biostimulant for agricultural purposes (Du Jardin, 2015), with the global seaweed processing industries utilizing an estimated 10 to 12 million tons biomass annually (Nayar & Bott, 2014). Seaweed extracts have been documented to enhance seed germination, improve plant growth, yield, flower set and fruit production, whilst also promoting postharvest shelf life (Mancuso *et al.*, 2006; Norrie & Keathley, 2006; Hong *et al.*, 2007; Khan *et al.*, 2009; Craigie, 2011; Mattner *et al.*, 2013). In terms of metabolism and physiology, seaweed extracts have been shown to improve nutrient partitioning and mobilisation, whilst promoting chlorophyll content and leaf area, along with stimulating the development of a vigorous root system and the retardation of leaf senescence (Stirk & Van Staden, 2004). Furthermore, positive results on plant-growth-enhancing properties together with increased disease resistance and tolerance to climatic stresses such as

cold or drought following application of seaweed extracts have been reported (Arioli *et al.*, 2015; Briceño-Domínguez *et al.*, 2014; Colavita *et al.*, 2011).

A range of biostimulants have been developed to be marketed as root growth stimulators, primarily for the agricultural sector, with the aim to increase yields of root- and tuberous crops such as leek, carrots and potatoes in particular (Woods, 2005). As in these vegetable crops, it is hypothesized that these biostimulants may also provide a means of significantly reducing transplant shock in woody perennials by promoting new root growth and increasing water uptake post planting. However, the efficacy of these products in enhancing root vigour of *Citrus* trees in particular to ameliorate transplanting shock has to date received little attention in South Africa. Studying the efficacy of biostimulants on *Citrus* species is important in the South African context since it is the third largest horticultural industry after deciduous fruits and vegetables, representing 20.6 % of the total gross value (R59.9 billion) of horticulture during the 2014/15 production season (DAFF, 2016).

The aim of this study was thus to assess whether transplant shock could be reduced and if establishment of Citrus, in particular ‘Nadorcott mandarin’ (*C. reticulata* Blanco), could be promoted through the use of biostimulants applications at planting, within the Mediterranean climate of the Western Cape, South Africa.

Five biostimulants, obtained from South African (SA) manufacturers, comprising of seaweed extracts and humic substances were evaluated. These biostimulants included: Terramax[®] (Victus Bio, Kyalami, Gauteng, SA), the *Ecklonia maxima*-based Kelpak[®] (Kelpak[®], Simon’s Town, SA), the *Ascophyllum nodosum*-based RootAktiv[®] (Karabos[®], Augrabies Northern Cape, SA), and organic mineral based compound-Super Wortel[®] (Karabos[®], Augrabies Northern Cape, SA), as well as compost (crop residues, unused, bedding materials, silage and manures), prepared at Welgevallen research farm, Stellenbosch University, as the control treatment. If effective, the use of one or more of these biostimulants would provide a mechanism to promote young tree establishment and to reduce losses associated with root damage during transplanting, which in turn will enhance the profitability of South African citrus growers.

2. MATERIALS AND METHODS

2.1 Trial site

The study was performed at Welgevallen experimental farm, Stellenbosch University (33°56'33"S; 18°51'56"E). The experimental site, characterised by clay soils with a pH range of between 5.3 to 5.5, is classified according to the South African soil classification system as Kroonstad and Tukulu soil types (Ellis, 2006). Summers are dry and warm to hot, as some days in February and March may rise to over 40 °C. Winters are cool and rainy, with daytime temperatures averaging 16 °C and an average yearly rainfall of 800-1000 mm (Midgley *et al.*, 2016). During the study period, temperature range was around 30-36°C with a reduced average yearly rainfall of approximately 400-500 mm.

‘Nadorcott’ mandarin (*Citrus reticulata*) on ‘Carrizo citrange’ rootstock, planted in November 2016 at 4.5 m x 7.5 m row spacing and in a north/south orientation, was used. Irrigation was supplied by means of a micro sprinkler irrigation system adjacent to the tree at 6.9 ML/ha/season. Due to erratic rainfall which necessitated water saving strategies, irrigation was reduced to 45-50 % field capacity from its normal irrigation scheduling of 65 % field capacity.

2.2 Experiment design and treatment application

A randomized complete block design with five treatments and ten blocks (n=10) composing of single tree replications as experimental units was used. The treatments consisted of compost (crop residues, unused bedding materials, silage and manures), as a control treatment; Terramax[®] (Victus Bio, Kyalami, Gauteng, SA), Kelpak[®] (Simon’s Town, SA); RootAktiv[®] (Karabos[®], Augrabies Northern Cape, SA) and Super Wortel[®] (Karabos[®], Augrabies Northern Cape, SA). The four products were applied in combination with compost material (Table 1). Recommended application concentrations and application rates were followed (Table 1). Super Wortel[®] was the only foliar-based treatment, whereas the rest of the treatments were soil applied. The biostimulants were applied twice: at planting on 9 November 2016 and a follow up application 12 days later on 23 November 2016. Standard disease, insect and weed control and irrigation schedule practices were carried out throughout the growing season.

2.3 Data collection

2.3.1. Above-ground growth

At planting, trees were marked at 30 cm above the graft union. Tree diameter (mm) was measured to coincide with the first application of treatments and again at the end of the study (4 May 2017), using a digital Vernier calliper (ATAGO, PR, USA).. Two shoots per tree were identified to be of nearly the same size and were then marked for repeated measurements. Length measurements (mm) were taken with a measuring tape from the main trunk to the upper most leaf petiole. Recordings commenced on 20 January 2017 during vegetative growth after initial application, where after it was collected at two monthly intervals up to the termination of the trial on 5 May 2017.

2.3.2 Root growth

In-situ root growth was determined using a mini-rhizotron root scanner (CID-600, Camas, WA, USA) where transparent underground polytubes facilitated plant roots to be studied non-destructively. Three trees per treatment were dug with a hand auger, approximately 30 cm from the tree trunk, at a 45° angle and approx. 60 cm deep (Fig. 1). The transparent tubes, equipped with watertight bottom plug and insulated top caps, were inserted into the holes. Three images were obtained per tube, documenting root growth up to approximately 70 cm of soil depth (Fig. 2). Once the desired images were captured, Root Snap Analysis Software (CID-600, Camas, WA, USA) was used to quantify various root growth parameters including root length (mm), volume (mm³) and diameter (mm) (Fig. 3). At the start of the study, initial root images were taken to be used for comparison to images that were captured on a monthly basis from 21 March to 6 September 2017.

2.3.3 Physiological parameters

2.3.3.1 Stomatal conductance

Stomatal conductance (mmol. m⁻².s⁻¹) was measured 3 times per leaf per replication on 25 May 2017 by means of a porometer (AP4 Delta-T Devices, Cambridge, England), using the average of 2 mature leaves located on the outer, 45° sun-exposed canopy of each tree. Gas exchange data was recorded on late summer/early autumn 16 March 2017 and 31 May 2017 during rapid leaf flush.

2.3.3.2 Photosynthesis

The rate of photosynthesis was measured using a field-portable, and closed system LI-COR Infra-Red Gas Analyser (IRGA), (LI-6400; LI-COR Inc.). Calibration was done one hour before the measuring time. The internal light source was maintained at the ambient photosynthetically active radiation (PAR) as determined with a LI-6400 light sensor. Chamber air carbon dioxide concentration was set to 200 $\mu\text{mol}\cdot\text{mol}^{-1}$. Irradiance was aligned to track ambient photosynthetically active radiation of between 1200 and 1500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, whilst a flow rate of 500 $\mu\text{mol}\cdot\text{s}^{-1}$ was maintained. Leaf temperature ranged from 16 to 24°C. Measurements were conducted from 09:00 to 11:00. Photosynthesis was recorded twice, on 10 March and 13 May 2017. Three large healthy leaves located on the outer sun-exposed canopy of each tree were selected for measurements.

2.3.3 3 Stem water potential (SWP)

Midday SWP was recorded between 12:30 and 13:30 on leaves exposed to direct solar radiation, adjacent to the tree trunk. Two mature leaves per tree per treatment were bagged for at least two hours prior to measurements, to reach equilibrium with the stem. Stem water potential was measured on 16 March 2017 and 13 May 2017 with a Scholander pressure chamber (PMS Instruments, USA).

2.4 Statistical analysis

Where applicable, data were analysed by means of an analysis of variance (ANOVA) using the generalised linear model (GLM) in Statistical Analysis System (SAS) programme (SAS Institute Inc., Cary, NC) version 7.1. Separation of means was done using the Bonferroni test. Treatment effects were considered significant at 5 % confidence level ($P \leq 0.05$). Since initial stem diameters of citrus trees were not the same, the stem diameter was calculated using analysis of covariance (ANCOVA) with the final diameter as the dependant variable and initial diameter as the covariate. Regression analysis was also applied to root growth parameters using Excel in Microsoft Office.

3. RESULTS

3.1 Above-ground growth

The application of the various biostimulant treatments had no significant effect on stem diameter of ‘Nadorcott’ mandarin, on the final day of measurements in May 2017). Shoot length was however significantly affected, with trees treated with RootAktiv[®] exhibiting the longest shoot length at 266 mm, compared to shoots from the Terramax[®] (178 mm) and Kelpak[®] (181 mm) treatments which did not differ significantly from one another, having the shortest shoots (Table 2). The remainder of the treatments did not differ significantly from each other, or from that of RootAktiv[®], Terramax[®] and Kelpak[®] (Table 2).

3.2 Root growth

Root length showed little initial root growth following transplanting in November 2016 (Fig. 4). No roots were observed for the compost treatment in March 2017, with resumption of growth only first recorded from April 2017 onwards. No root length increase was observed in May for all treatments except for Terramax[®] only. This phase was followed by a further increase from July to September 2017, in particular for roots from the compost, Terramax[®] and Kelpak[®] treatments. During June 2017, the scanner was not available, thus no root data was collected for this period. For roots treated with RootAktiv[®] and Super Wortel[®], a slow increase in root growth was documented until July, where after the growth rate remained stable until September 2017. On the final day of measurement (6 September 2017), there was a significant difference in root length between treatments (Fig. 5). Roots from the Kelpak[®] treatment (332 mm) vs Super Wortel[®] treatment (103 mm) did not differ from that of the compost, Terramax[®] and RootAktiv[®] treatments. These treatments also did not differ significantly from each other, or the Kelpak[®] and Super Wortel[®] treatments (Fig. 5).

Low root volume was noted for all treatments (from March to April 2017), similar to observations for root length, (Fig. 6). However, with the May observation date, a different trend was observed when the compost, RootAktiv[®] and Super Wortel[®] treatments showed a decline in root volume, whilst in contrast, the Kelpak[®] and Terramax[®] treatments displayed a sharp increase in root volume (Fig. 6). Root volume remained stable with the July monitoring date, but then either declined slightly towards September in the RootAktiv[®] and Super Wortel[®] treatments, but with moderate to steady increases for roots associated with the compost,

Kelpak[®] and Terramax[®] treatments. Root volume between treatments were significantly different on the final measurement day (6 November 2017) (Fig. 7). Kelpak[®] had the highest root volume (253 mm³) and differed significantly from RootAktiv[®] (75 mm³) and Super Wortel[®] (69 mm³), with lowest root volume. The other treatments did not differ from one another, or from the Kelpak[®], RootAktiv[®] and Super Wortel[®] treatments (Fig. 7).

Root diameter showed a similar pattern for all the treatments, where an increase in diameter from March to April and then a lag phase until May was observed (Fig. 8). Thereafter root diameter again increased for all treatments towards July, with the exception of roots that was developed subjected to the Super Wortel[®] treatment. In the final observation phase, all treatments showed an increase in root diameter towards September 2017, except for the RootAktiv[®] treatment where a slight decline was detected (Fig. 8). Root diameter however did not differ significantly between treatments ($P = 0.4031$) on the final day of measurement (6 September 2017) (Fig. 9).

Root diameter and root volume on the final date of measurement on 6 September 2017 was well correlated ($r = 0.76$), as well as root length and root volume ($r = 0.85$) and that of root diameter and root length ($r = 0.83$) (Fig. 10).

3.3 Physiological parameters

3.3.1 Stem water potential (SWP)

Treatments had a significant effect on SWP for both measurement dates in March and May 2017 (Fig. 11). In March 2017, the RootAktiv[®] treatment had the most negative SWP value (-2.65 MPa) which was followed by a similar SWP value for the Super Wortel[®] treatment (-2.4 MPa). These values did not differ significantly from each other, or from that obtained from the compost treatment (-1.4 MPa). However, these treatments differed significantly from the SWP values obtained from the Kelpak[®] (-0.9 MPa) and Terramax[®] treatment (-0.52 MPa).

In May, the compost treatment was reported with the most negative SWP value (-2.2 MPa), followed by SWP values obtained from the Super Wortel[®] treatment (-1.98 MPa), although these two treatments did not differ significantly from one another, they were significantly more negative than that reported for the rest of the treatments (Fig. 11). The RootAktiv[®] (-1.05 MPa), Kelpak[®] (-0.83 MPa) and Terramax[®] (-0.53 MPa) treatments all reported more positive SWP values and did not differ significantly from one another.

3.3.2 Stomatal conductance

The application of biostimulant treatments had no significant effect on stomatal conductance earlier in the season, in March, whereas significant differences were recorded between treatments later in the season during autumn on May 2017 (Fig. 12). The Kelpak[®] treatment had the highest stomatal conductance (257 mmol. m⁻². s⁻¹), which was found to be significantly higher than of leaves obtained from the compost (145 mmol m⁻² s⁻¹) and Super Wortel[®] (152 mmol. m⁻². s⁻¹) treatments. However, the stomatal conductance reported for the Kelpak[®] treatment was similar to that of the Terramax[®] and RootAktiv[®] treatments.

3.3.3 Photosynthesis

Photosynthesis was not differently affected by treatments in March, but significant differences were reported during May (Fig. 13). The RootAktiv[®] treatment had the highest leaf photosynthesis rate (10.57 μmol m⁻².s⁻¹), but was not significantly different from that reported for the Kelpak[®] (9.21 μmol m⁻².s⁻¹) and Terramax[®] (7.96 μmol m⁻².s⁻¹) treatments. Photosynthesis was however significantly higher in the RootAktiv[®] compared to that recorded for the Super Wortel[®] (6.20 μmol m⁻².s⁻¹) and compost (4.79 μmol m⁻².s⁻¹) treatments.

4. DISCUSSION

4.1 Above-ground growth characteristics

The lack of significant differences between treatments with regard to stem diameter was unexpected. However, as evaluation of stem diameter was conducted over only one season, differences in stem diameter between treatments could have emerged when observed over a longer study period. In addition, the significant increase observed in both shoot length and root growth between treatments might have created strong competition for photosynthates, thus impacting directly on stem growth (Halpern *et al.*, 2015; Khan *et al.*, 2012). The physiological parameters of SWP and photosynthesis also differed significantly between treatments and could thus have similarly influenced stem diameter growth (Calvo *et al.*, 2014; Du Jardin, 2015).

The impact of water stress on vegetative growth with regard to stem growth was similarly reported by Ginestar & Castel (1996) for a study in Spain on eight-year-old ‘Clementina de Nules’ mandarin trees (*C. clementina* grafted on ‘Carrizo citrange’ rootstock). When irrigation was withheld on ‘Clementina de Nules’ in the spring it had no effect on stem

of the first (spring) flush, however water stress in summer reduced shoot growth severely in the second (summer) flush, even to the point where no flushing occurred. Water stress in the late summer/early autumn had a similar effect than reported for summer, however when irrigation was supplied at this time, a vigorous second (summer) flush with abundant flowering was reported. A delay in stem growth in citrus subjected to conditions of water stress was also confirmed by Kriedemann & Barrs (1981) and Levy *et al.* (1999) where reduced canopy development and stem growth were reported. Doorenbos & Kassam (1979) emphasized that water stress during expected periods of vigorous vegetative growth should be avoided as it may impact negatively on the initiation of both summer and autumn flushes.

Stem and shoot growth of citrus spp. occurs in a series of discrete flushes that are initiated by a rise in air temperature (>12.5 °C) or in the tropics, where the flushes are less discrete, by the availability of water (Davies & Albrigo, 1994). In this study, average daily temperatures in 2016 (data not shown) reported a relatively high average temperature of 24°C and a declared period of drought where rainfall that has been reduced by more than a third of that of the normal rainfall pattern for the experimental site over the evaluation period (Rustenburg climate, 2016). It is thus likely that the combination of high temperatures and suboptimal available soil water (average yearly rainfall of 400-500 mm), due to drought-enforced cuts in the irrigation scheduling, could have affected stem diameter growth negatively.

Significantly higher shoot growth was recorded for trees that received the soil-applied RootAktiv[®] treatment, a product containing *Ascophyllum nodosum*, than that achieved in trees treated with both Terramax[®] (vermicompost) and Kelpak[®] (*Ecklonia maxima*). The observed increase in shoot growth in our study which was limited to non-bearing trees only is supported by numerous studies (Stirk & Van Staden, 2006; Khan *et al.*, 2009; Craigie, 2011) where beneficial effects of seaweed extract from *A. nodosum* on shoot growth and then later crop yield was also reported.

In a study by Van Staden, (2015), the increase in shoot growth by RootAktiv[®] and Super Wortel[®] was assumed to be influenced by high level of cytokinins and cytokinin-like compounds in the roots which in turn stimulated an increase in shoot growth. Yet, Kelpak[®] which also claims to contain seaweed extract of *Ecklonia maxima* in the formulation proved to be less effective than RootAktiv[®] to stimulate shoot growth. *Ecklonia maxima*-based Kelpak[®] is also assumed to contain high levels of auxin and auxin-like compounds that preferentially stimulates root production over shoot growth (Papenfus *et al.*, 2013). The site of seaweed

application has been reported as important for the efficacy of the product on *Pinus pinea* seedlings (Atzmon & Van Staden (1994). However, in the *Pinus* study shoot application of seaweed was found to increase plant weight, whereas root drenches did not bring about any change in total plant weight, which is in contrast to our study where root applications of seaweed RootAktiv[®] was more effective to promote shoot growth.

4.2 Root growth parameters

Root growth involves complex physiological processes, combining the direct effects of environmental factors and the endogenous mechanisms of the plant (Castle, 1978). The periodicity of *Citrus* root growth, particularly in relation to shoot growth and the effects of soil temperature and water content, have received considerable attention (Castle, 1995). In addition, root growth is known to be cyclic because of a consistent alternation with periods of shoot growth (Castle, 1978). This dynamics between shoot and root growth was evident in our study without exception, as the rate of total shoot extension growth increased, a decrease in the rate of total visible root growth extension was reported. This phenomenon was evident in September 2017, compared to the previous measurement of shoot growth in June.

The pattern of root growth observed in young trees in this study differed distinctly from that reported for older trees where root growth was reported to occur in distinct cycles spaced 3 to 10 weeks apart (Crider, 1927; Hatton, 1949; Reed & MacDougal, 1937; Waynick & Walker, 1930). In this study, visible root growth only commenced approximately 19 weeks after transplanting. Furthermore, no periods of inactivity were noted during the growing season, though fluctuations in growth were observed. In deciduous fruit trees, the pattern of root growth is greatly affected by two factors, namely crop load and soil climate (Atkinson, 1983; Psarras *et al.*, 2000). However, in this study that was conducted on non-bearing mandarin trees, no cropping load occurred, thus only shoot growth could have influenced root growth.

Peak shoot extension growth in our study occurred during times coinciding with the lowest rate of root extension for all treatments. This observation was confirmed by reports from Bevington & Castle (1985) and Castle (1978, 1995) and is consistent with the concept of a functional relationship inherent to competition between roots and shoots for available assimilates, with developing shoots being the stronger sink, resulting in the inhibition of root growth by auxin as produced during periods of shoot extension (Bevington & Castle, 1985). Some root growth occurred throughout the seven month observation period of our study, although the annual root growth pattern of young, non-bearing citrus could be described as one

of continuous growth, with a favourable soil environment, being periodically interrupted by cycles of shoot growth which then temporarily depress root extension (Bevington & Castle, 1985; Castle, 1995).

Root length increase was shown to be well correlated with increases in root volume and root diameter. This demonstrated that the periodic fluctuations in total root growth were primarily the result of changes in the number of growing roots along with the elongation rate of individual roots. Although the latter is influenced by shoot growth and soil environment, the growth response of the root system to these factors was mediated largely through the number of roots initiating growth.

Root length and volume were significantly different between treatments. The results showed that soil-applied treatments containing vermicompost and *Ecklonia maxima*-based seaweed extract (Terramax[®] and Kelpak[®]) respectively increased root growth compared to other treatments, confirming previous findings (Crouch, 1990; Jeannin *et al.*, 1991; Mancuso *et al.*, 2006; Papenfus *et al.*, 2013; Slávik, 2005; Van Staden, 2015; Vernieri *et al.*, 2005). The effect of the Terramax[®] (vermicompost) treatment in promoting root growth was also confirmed in a study by Muchena (2017) on hydroponically grown rough lemon (*Citrus jambhiri lush*) seedlings.

The application of seaweed extracts to reduce transplant shock in seedlings of marigold, cabbage (Aldworth & Van Staden, 1987) and tomato (Crouch & Van Staden, 1992) by increasing root size and vigour, has been reported before, but not for transplant shock in citrus. The root-growth stimulatory effect was more pronounced when extracts were applied at an early growth stage in maize, and the response was similar to that of auxin, an important root growth-promoting hormone (Jeannin *et al.*, 1991), which was in line with our findings.

Various researchers reported a significant increase in root growth and overall plant size when seedlings from different plant species received an early application of 10% seaweed extract Kelpak[®] (*Ecklonia maxima*) solution compared to the control (Atzmon & Van Staden, 1994; Van Staden *et al.*, 1994; 1995; Jones & Van Staden, 1997). Seaweed extract treatments enhanced both root:shoot ratios and biomass accumulation (Nelson & Van Staden, 1984; Crouch & Van Staden, 1992). However, in this trial that was conducted over only one season, biomass accumulation was not evident after the application of the two seaweed-based biostimulant treatments RootAktiv[®] and Super Wortel[®] compared with other treatments.

Indirect stimulation of root growth in the compost and Super Wortel[®] treatments, when estimated through root diameter increase, might have also occurred via enhancement of their

associated soil microorganisms, similar to that previously reported by Alam *et al.* (2013), Kuwada *et al.* (2006) and Khan *et al.* (2012, 2013), as none of the treatments significantly affected this root growth parameter. Although, the elongation rate of individual roots was influenced by shoot growth and soil environment, the growth response of the root system to these factors was mediated largely through the number of roots that initiated growth. This was evident from the biostimulant treatments Terramax[®] and Kelpak[®] which played a major role in initiating root growth, whereas other treatments such as RootAktiv[®] and Super Wortel[®] prioritized the initiation of vigorous shoot growth hence, causing fewer root flushes.

4.3 Physiological parameters

4.3.1 Stem water potential (SWP)

Terramax[®] and Kelpak[®] treatments resulted in significantly more positive SWP values with the earlier observation date in March, compared to that recorded for the Super Wortel[®] and RootAktiv[®] treatments, but was comparable to values obtained from the compost treatment for this evaluation date. This lower SWP value may indicate an advantage gained and a possible reduction of transplant shock, which relates to the accompanying higher root volume in the Terramax[®] (ns) and Kelpak[®] (significant) treatments compared to the compost, Super Wortel[®] and RootAktiv[®] treatments. The advantage provided earlier in the season by the compost however did not extend to later in the season when the most negative SWP values were again recorded for the Super Wortel[®] treatment, but this time also for the compost treatment. Differences in SWP has been reported to be partly influenced by root distribution (Barry *et al.*, 2004). In addition, amelioration capacity could also have been extended to roots of the Kelpak[®] and Terramax[®] treatments by their known, associated plant growth regulating compounds which then would have alleviated water stress to some extent as was detected with the less negative SWP values of these treatments (Craigie, 2011; Khan *et al.*, 2009; Mancuso *et al.*, 2006; Zhang *et al.*, 2003).

4.3.2 Stomatal conductance

Williams & Araujo (2002) stated that leaf water potential and predawn leaf water potential were highly correlated with stomatal conductance and that the correlation for stomatal conductance with stem water potential was higher than that with leaf water potential. Naor (1998) argued that stomatal conductivity is regulated to control leaf water deficit, and thus

maintains midday SWP at a constant value. Spann & Little (2011) reported that ‘Hamlin’ sweet orange trees exposed to a commercial extract of brown seaweed displayed a level of tolerance toward drought as citrus plants sprayed with seaweed extract, under drought conditions, showed intermediate water use efficiency (Spann & Little, 2010). It was suggested that seaweed extract promotes stem water potential in citrus rootstocks, whether under full irrigation or under conditions of drought, in addition to affecting photosynthesis, stomatal conductance and water use efficiency in a cultivar-dependent manner (Spann & Little, 2010).

The Kelpak[®] treatment had a significantly higher stomatal conductance in May 2017 compared to the compost and Super Wortel[®] treatments. Changes in stomatal conductance of the treatments in March and May 2017 also seemed to be influenced by SWP. Stomatal conductance decrease in the compost treatment appeared to be related to the low SWP (more negative) in the same treatment, confirming the relationship between stomatal conductance and SWP reported before (Mata *et al.*, 1999; Williams & Trout, 2005). Likewise, increases in stomatal conductance of the Terramax[®], Kelpak[®] and RootAktiv[®] treatments was influenced by high SWP (less negative) in May for the same treatments. Hence, these treatments proved to be influential in reducing transplanting shock. Flexas & Medrano (2002) suggested that stomatal closure is one of the earliest responses of plants to drought and the dominant limitation to photosynthesis at mild to moderate drought, confirming subsequent results that stomata are sensitive indicators of water deficits, resulting in stomatal closure and in higher SWPs (Jones, 2004; 1998). Stomata close at SWPs below -0.7 MPa at low saturation density (SD), and at less than -1.2 MPa at high SD (Levy *et al.*, 1999) which was also in line with our work.

The formulations (Terramax[®], Kelpak[®] and RootAktiv[®]) containing seaweed extracts and their associated plant growth regulators, promoted stomatal conductance, confirming reports on these products in other studies (Papenfus *et al.*, 2013; Rengasamy *et al.*, 2014; Stirk *et al.*, 2014; Stirk & Van Staden, 2004). In addition, extracts of *Ascophyllum nodosum*, which was included in the RootAktiv[®] formulation, has been shown to contain betaines (Tyihák *et al.*, 1994). In plants, betaines is a recognized compatible metabolite that alleviates osmotic stress, induced by salinity and drought stress, by amongst other affecting stomatal conductance. In addition, seaweed extract application have also been associated with enhanced leaf chlorophyll content of treated plants (Blunden *et al.*, 1996), probably through decreased chlorophyll degradation (Whapham *et al.*, 1993), which likewise affects stomatal conductance. However, in this trial, chlorophyll was not quantified.

4.3.3 Photosynthesis

Increase in shoot length has been associated with a reduction in chlorophyll degradation (Tyihák *et al.*, 1994) and a delay in senescence, rather than a net increase in photosynthesis rate (Jannin *et al.*, 2013).

The RootAktiv[®] and Kelpak[®] treatment were found to promote photosynthesis of ‘Nadorcott’ mandarins when compared to the compost and Super Wortel[®] treatments in May. Trends in photosynthesis are considered to be directly linked to trends in SWP where a more negative SWP indicate water stress that would initiate stomatal closure to reduce water loss and thus indirectly result in reduced CO₂ uptake and consequently photosynthesis. Corresponding with this hypothesis the compost treatment displayed in May 2017 a SWP of -2.2 MPa which testifies of a significant environmental stress that would likely result in reduced stomatal conductance (145 mmol m⁻².s⁻¹) and photosynthesis (4.78 μmol m⁻².s⁻¹). Likewise, in Kelpak[®] and RootAktiv[®] treatments, a trend of less negative SWP (-0.83 and -1.05 MPa) respectively in May 2017 which provides evidence of lower environment stress resulted in high stomatal conductance (257 and 211 mmol m⁻².s⁻¹) respectively and photosynthesis (9.21 and 10.67 μmol m⁻².s⁻¹) respectively.

Ecklonia maxima (present in Kelpak[®]) and *Ascophyllum nodosum*-based (represented in RootAktiv[®]) seaweed extracts have been reported to increase photosynthesis in crops (Blunden *et al.*, 1996). Featonby-Smith & Van Staden (1983) also reported significant increases in yield of K⁺-stressed and nutrient-stressed wheat, with an application to stimulate incidences of root flush development with ‘Kelpak’ treatments, similar to findings in our study.

Stomatal conductance also has an important and direct effect on photosynthesis (Brakke & Allen Jr, 1995). Midday depressions in stomatal conductance regularly observed in citrus are well associated with high temperatures, thus rendering photosynthesis sensitive through a reduced carbon dioxide uptake to high temperatures and saturation deficits. The mode of action of the biostimulants evaluated in this study on photosynthesis may be related to the natural plant hormones (betaine) known to be present in seaweed extracts and their ability to stabilize chlorophyll content (Blunden *et al.* 1996; Khan *et al.* 2009).

5. CONCLUSION

A number of studies on a wide range of agricultural crops have reported the ability of biostimulants in alleviating transplanting shock and improving establishment, yet very few

studies on citrus under field conditions have been conducted to date. From the results obtained in this study, the application of a range of biostimulants evaluated in general had a promotive effect on the physiology of ‘Nadorcott’ mandarin, which was translated into enhanced plant growth, despite different modes of action. RootAktiv[®] treatment appeared to enhance above-ground growth (shoot length) following transplanting compared to the other treatments. Alternatively, Terramax[®] and Kelpak[®] treatments acted in being more effective in enhancing root development as could be observed in favourable SWP and photosynthetic parameters. Enhancement of rapid shoot elongation by RootAktiv[®] caused slow and fluctuating root development, resulting in reduced root length, volume and diameter. Alternatively, Terramax[®] and Kelpak[®] stimulated rapid root development, because of the high plant growth regulator’s, which in turn resulted in relatively low shoot growth.

Surprisingly, the RootAktiv[®] treatment was similarly effective in promoting above-ground performance (shoot length), despite apparent low root development. When SWP decreased, stomatal conductance increased, thereby improving the photosynthesis of the trees. Despite less observed root development in the RootAktiv[®] treatment within the limitations of the study, this biostimulant proved to be effective advancing shoot growth of ‘Nadorcott’ mandarin. However, Terramax[®] and Kelpak[®] treatments improved root growth and physiological growth of ‘Nadorcott’ mandarin. This suggests RootAktiv[®], Terramax[®] and Kelpak[®] could be considered as ameliorant to reduce transplant stress and improve the successful establishment of citrus cultivars. In addition, this study highlighted the regulating role of shoot growth in root growth dynamics. Hence, close consideration to the influence of external factors such as soil climate is essential when biostimulants are applied to reduce stress, rather than relying on the efficacy of the treatment alone.

6. Acknowledgments

Profound acknowledgements goes to Karabos International and the Citrus Research International (CRI) for funding this study.

7. References

- Alam, M.Z., Braun, G., Norrie, J. & Hodges, D.M. 2013. Effect of *Ascophyllum* extract application on plant growth, fruit yield and soil microbial communities of strawberry. *Canadian Journal of Plant Science* 93:23–36.
- Aldworth, S.J. & Van Staden, J. 1987. The effect of seaweed concentrate on seedling transplants. *South African Journal of Botany* 53:187–189.
- Arioli, T., Mattner, S.W. & Winberg, P.C. 2015. Applications of seaweed extracts in Australian agriculture: past, present and future. *Journal of Applied Phycology* 27:2007–2015.
- Atiyeh, R.M., Arancon, N., Edwards, C.A. & Metzger, J.D. 2000. Influence of earthworm-processed pig manure on the growth and yield of greenhouse tomatoes. *Science* 75:175–180.
- Atkinson, D. 1983. The growth, activity and distribution of the fruit tree root system. *Plant and Soil* 71:23–35.
- Atzmon, N. & Van Staden, J. 1994. The effect of seaweed concentrate on the growth of *Pinus pinea* seedlings. *Horticultural Science* 8:279–288.
- Bachman, G.R. & Metzger, J.D. 2008. Growth of bedding plants in commercial potting substrate amended with vermicompost. *Bioresource Technology* 99:3155–3161.
- Barry, G., Castle, W. & Davies, F. 2004. Rootstocks and plant water relations affect sugar accumulation of citrus fruit via osmotic adjustment. *Journal of the American Society for Horticultural Science* 129:881–889.
- Bevington, K.B. & Castle, W.S. 1985. Annual root growth pattern of young citrus trees in relation to shoot growth, soil temperature, and soil water content. *Journal of the American Society for Horticultural Science* 110:840–845.
- Bhagat, S., Thakur, A. & Dhaliwal, H.S. 2013. Organic amendments influence growth, budding ability and budding success in rough lemon (*Citrus jambhiri* Lush.). *Biological Agriculture and Horticulture* 29:46–57.
- Blunden, G., Jenkins, T. & Liu, Y.W.Y. 1996. Enhanced leaf chlorophyll levels in plants treated with seaweed extract. *Journal of Applied Phycology* 8:535–543.
- Brakke, M. & Allen Jr, L.H. 1995. Gas exchange of citrus seedlings at different temperatures, vapor-pressure deficits, and soil water contents. *Journal of the American Society for Horticultural Science* 120:497–504.

- Briceño-Domínguez, D., Hernández-Carmona, G., Moyo, M., Stirk, W. & Van Staden, J. 2014. Plant growth promoting activity of seaweed liquid extracts produced from *Macrocystis pyrifera* under different pH and temperature conditions. *Journal of Applied Phycology* 26:2203–2210.
- Calvo, P., Nelson, L. & Kloepper, J.W. 2014. Agricultural uses of plant biostimulants. *Plant Soil* 383:3–41.
- Canellas, L.P., Dantas, D.J., Aguiar, N.O., Peres, L.E.P., Zsögön, A., Olivares, F.L., Dobbss, L.B., Façanha, A.R., Nebbioso, A & Piccolo, A. 2011. Probing the hormonal activity of fractionated molecular humic components in tomato auxin mutants. *Annals of Applied Biology* 159:202–211.
- Castle, W.S. 1978. Citrus root systems: their structure, function, growth, and relationship to tree performance. *Proceedings of the International Society of Citriculture* 62–69 pp.
- Castle, W.S. 1995. Rootstock as a fruit quality factor in citrus and deciduous tree crops. *New Zealand Journal of Crop and Horticultural Science*. 23:383–394.
- Chen, J.H. 2006. The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. *International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use* 16–20:1–11.
- CID Bio-Science. 2016. *Minirhizotron Systems*. CID Bio-Science, Camas, WA, USA. 2 pp.
- Colavita, G.M., Spera, N., Blackhall, V. & Sepulveda, G.M. 2011. Effect of seaweed extract on pear fruit quality and yield. *Acta Horticulturae* 909:601–608.
- Craigie, J.S. 2011. Seaweed extract stimuli in plant science and agriculture. *Journal of Applied Phycology* 23:371–393.
- Crider, F.J. 1927. Root studies of citrus trees with practical applications. *Citrus Leaves* 7:1-3,27-30.
- Crouch, I.J. 1990. The effect of seaweed concentrate on plant growth. PhD Thesis, Faculty of Science, University of Natal, Pietermaritzburg.
- Crouch, I.J. & Van Staden, J. 1992. Effect of seaweed concentrate on the establishment and yield of greenhouse tomato plants. *Journal of Applied Phycology* 4:291–296.
- DAFF. 2016. A profile of the South African Citrus market value chain. Directorate Marketing, Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa. 111 pp.
- Davies, F.S. & Albrigo, L.G. 1994. *Citrus*. CAB International, Wallingford, 254 pp.
- Doorenbos, J., & Kassam, A.H. 1979. Yield response to water. FAO irrigation and drainage. FAO, Rome, Italy. 193 pp.

- Du Jardin, P. 2015. Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae* 196:3–14.
- Ellis, F. 2006. Report on a soil survey and agricultural suitability investigation of the property known as Barclay Property (Farm 792 Worcester). Worcester, South Africa. 1-14 pp.
- Featonby-Smith, B.C. & Van Staden, J. 1983. The effect of seaweed concentrate on the growth of tomatoes in nematode infested soil. *Scientia Horticulturae* 20:137–146.
- Ferrini, F. & Nicese, F.P. 2002. Response of english oak (*Quercus robur* L.) trees to biostimulants application in the urban environment trees to biostimulants application in the urban environment. *Journal of Arboriculture* 28:70–75.
- Flexas, J. & Medrano, H. 2002. Drought-inhibition of photosynthesis in C₃ plants: Stomatal and non-stomatal limitations revisited. *Annals of Botany* 89:183–189.
- Fraser, G.A. & Percival, G.C. 2003. The influence of biostimulants on growth and vitality of three urban tree species following transplanting. *Arboricultural Journal* 27:43–57.
- Gauthier, N.W. & Kaiser, C. 2014. Transplant shock : Disease or cultural problem? Transplanting trees and shrubs, University of Kentucky College of Agriculture Food and Environment, USA. 11 pp.
- Ginestar, C. & Castel, J. 1996. Responses of young clementine citrus trees to water stress during different phenological periods. *Journal of Horticultural Science* 71:551–559.
- Golchin, A., Nadi, M. & Mozaffari, V. 2006. The effects of vermicomposts produced from various organic solid wastes on growth of pistachio seedlings. *Acta Horticulturae* 726:301–305.
- Halpern, M., Bar-Taly, A., Ofeky, M., Minzy, D., Mullerx, T. & Yermiyahu, U. 2015. The use of biostimulants for enhancing nutrient uptake. *Advances in Agronomy* 103:141–171.
- Hatton, T.T. 1949. Studies on the periodic development of roots and shoots in certain citrus species. MSc Thesis, University of Florida, Gainesville.
- Hong, D.D., Hien, H.M. & Son, P.N. 2007. Seaweeds from Vietnam used for functional food, medicine and biofertilizer. *Journal of Applied Phycology* 19:817–826.
- Jannin, L., Arkoun, M., Etienne, P., Laine, P., Goux, D., Garnica, M., Fuentes, M., Francisco, S.S., Baigorri, R., Cruz, F., Houdusse, F., Garcia-Mina, M., Yvin, J & Ourry, A. 2013. *Brassica napus* growth is promoted by *Ascophyllum nodosum* (L.) Le Jol. seaweed extract: Microarray analysis and physiological characterization of N, C, and S metabolisms. *Journal of Plant Growth Regulation* 32:31–52.
- Jeannin I., Lescure, J & Morot-Gaudry, J. 1991. The effects of aqueous seaweed sprays on the

- growth of maize. *Botanica Marina* 34: 469-473
- Jones, H.G. 1998. Stomatal control of photosynthesis and transpiration. *Journal of Experimental Botany* 49:387–398.
- Jones, H.G. 2004. Irrigation scheduling: Advantages and pitfalls of plant-based methods. In Vol. 55 *Journal of Experimental Botany* 2427–2436.
- Jones, N.B. & Van Staden, J. 1997. The effect of a seaweed application on the rooting of pine cuttings. *South African Journal of Botany* 63:141–145.
- Karabos. 2016. Products. [Online], Available: www.karabos.co.za/en/products.php. Accessed on 20 February 2017.
- Kashem, M. A., Sarker, A., Hossain, I. & Islam, M.S. 2015. Comparison of the effect of vermicompost and inorganic fertilizers on vegetative growth and fruit production of tomato (*Solanum lycopersicum* L.). *Open Journal of Soil Science* 5:53–58.
- Khan, W., Rayirath, U.P., Subramanian, Sowmyalakshmi, Jithesh, M.N., Rayorath, P., Hodges, D.M., Critchley, A.T., Craigie, J.S., Norrie, J & Prithiviraj, B. 2009. Seaweed extracts as biostimulants of plant growth: Review. *Journal of Plant Growth Regulation* 28:386–399.
- Khan, W., Zhai, R., Souleimanov, A., Critchley, A.T., Smith, D.L. & Prithiviraj, B. 2012. Commercial extract of *Ascophyllum nodosum* improves root colonization of Alfalfa by its bacterial symbiont *Sinorhizobium meliloti*. *Communications in Soil Science and Plant Analysis* 43:2425–2436.
- Khan, W., Palanisamy, R., Critchley, A.T., Smith, D.L., Papadopoulos, Y. & Prithiviraj, B. 2013. *Ascophyllum nodosum* extract and its organic fractions stimulate rhizobium root nodulation and growth of *Medicago sativa* (Alfalfa). *Communications in Soil Science and Plant Analysis* 44:900–908.
- Kriedemann, P.E. & Barrs, H.D. 1981. Citrus orchards. Water deficits and plant growth, Chapter 5, Vol. VI. In: T.T. Kozlowski. (ed.). Academic Press, New York, NY. 325–416 pp.
- Kuwada, K., Wamocho, L.S., Utamura, M., Matsushita, I. & Ishii, T. 2006. Effect of red and green algal extracts on hyphal growth of arbuscular mycorrhizal fungi, and on mycorrhizal development and growth of papaya and passion fruit. *Agronomy Journal* 98:1340–1344.
- Lazcano, C., Sampedro, L., Zas, R. & Domínguez, J. 2010. Assessment of plant growth promotion by vermicompost in different progenies of maritime pine (*Pinus pinaster* Ait.). *Compost Science & Utilization* 48:111–118.
- Levy, Y., Lifshitz, J. & De Malach, Y. 1999. The response of several *Citrus* genotypes to high

- salinity irrigation water. *Horticulture Science* 34:878–881.
- Mancuso, S., Azzarello, E., Mugnai, S. & Briand, X. 2006. Marine bioactive substances (IPA extract) improve foliar ion uptake and water stress tolerance in potted *Vitis vinifera* plants. *Advances in Horticultural Science* 20:156–161.
- Mata, M., Girona, J., Goldhamer, D., Fereres, E., Cohen, M. & Johnson, S. 1999. Water relations of lysimeter-grown peach trees are sensitive to deficit irrigation. *California Agriculture* 53:17–21.
- Mattner, S.W., Wite, D., Riches, D.A., Porter, I.J. & Arioli, T. 2013. The effect of kelp extract on seedling establishment of broccoli on contrasting soil types in southern Victoria, Australia. *Biological Agriculture and Horticulture* 29:258–270.
- Midgley, G., Makholela, T. & Mantlana, B. 2016. South Africa's 1st Annual climate change report: Climate change trends, risks, impacts and vulnerabilities. Department: Environmental Affairs, Pretoria, South Africa. 60 pp.
- Muchena, L. 2017. Improved growth of hydroponically grown rough lemon (*Citrus jambhiri* lush) seedlings treated with kelp and vermicast extracts. MSc Thesis, Department of Plant Production and Soil Sciences, University of Pretoria.
- Naor, A. 1998. Relations between leaf and stem water potentials and stomatal conductance in three field-grown woody species. *Journal of Horticultural Science and Biotechnology* 73:431–436.
- Nardi, S., Reniero, F. & Concheri, G. 1997. Soil organic matter mobilization by root exudates of three maize hybrids. *Chemosphere* 35:2237–2244.
- Nardi, S., Pizzeghello, D., Reniero, F. & Rascio, N. 2000. Chemical and biochemical properties of humic substances isolated from forest soils and plant growth. *Soil Science of America Journal* 64:639–645.
- Nardi, S., Pizzeghello, D., Muscolo, A. & Vianello, A. 2002. Physiological effects of humic substances on higher plants. *Soil Biology and Biochemistry* 34:1527–1536.
- Nardi, S., Pizzeghello, D., Schiavon, M. & Ertani, A. 2016. Plant biostimulants: physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism. *Scientia Agricola* 73:18–23.
- Narkhede, S., Attarde, S. & Ingle, S. 2011. Study on effect of chemical fertilizer and vermicompost on growth of chilli pepper plant (*Capsicum annum*). *Journal of Applied Sciences in Environmental Sanitation* 6:327–332.
- Nayar, S. & Bott, K. 2014. Current status of global cultivated seaweed production and markets.

World Aquaculture 45:32–37.

- Nelson, W.R. & Van Staden, J. 1984. The Effect of seaweed concentrate on seedling transplants. *Journal of Plant Physiology* 115:433–437.
- Norrie, J. & Keathley, J.P. 2006. Benefits of *Ascophyllum nodosum* marine-plant extract applications to “Thompson seedless” grape production. *Acta Horticulturae* 727:243–247.
- Pant, A.P., Radovich, T.J.K., Hue, N. V., Talcott, S.T. & Krenek, K.A. 2009. Vermicompost extracts influence growth, mineral nutrients, phytonutrients and antioxidant activity in pak choi (*Brassica rapa* cv. Bonsai, Chinensis group) grown under vermicompost and chemical fertiliser. *Journal of the Science of Food and Agriculture* 89:2383–2392.
- Papenfus, H.B., Kulkarni, M.G., Stirk, W.A., Finnie, J.F. & Van Staden, J. 2013. Effect of a commercial seaweed extract (Kelpak[®]) and polyamines on nutrient-deprived (N, P and K) okra seedlings. *Scientia Horticulturae* 151:142–146.
- Park, E.J., Jeknić, Z., Sakamoto, A., DeNoma, J., Yuwansiri, R., Murata, N. & Chen, T.H.H. 2004. Genetic engineering of glycinebetaine synthesis in tomato protects seeds, plants, and flowers from chilling damage. *Plant Journal* 40:474–487.
- Piccolo, A. & Spiteller, M. 2003. Electrospray ionization mass spectrometry of terrestrial humic substances and their size fractions. *Analytical and Bioanalytical Chemistry* 377:1047–1059.
- Piccolo, A., Conte, P., Trivellone, E., Van Lagen, B. & Buurman, P. 2002. Reduced heterogeneity of a lignite humic acid by preparative HPSEC following interaction with an organic acid. Characterization of size-separates by Pyr-GC-MS and ¹H-NMR spectroscopy. *Environmental Science and Technology* 36:76–84.
- Piccolo, A., Conte, P., Spaccini, R. & Chiarella, M. 2003. Effects of some dicarboxylic acids on the association of dissolved humic substances. *Biology and Fertility of Soils* 37:255–259.
- Pinton, R., Cesco, S., Iacoletting, G., Astolfi, S. & Varanini, Z. 1999. Modulation of NO₃⁻ uptake by water- extractable humic substances: Involvement of root plasma membrane H⁺ ATPase. *Plant and Soil* 215:155–161.
- Psarras, G., Merwin, I.A., Lakso, A.N. & Ray, J.A. 2000. Root growth phenology, root longevity, and rhizosphere respiration of field grown “Mutsu” apple trees on “Malling 9” rootstock. *Journal of the American Society for Horticultural Science* 125:596–602.
- Reed, H.S & MacDougal, D.T. 1937. Periodicity in the growth of the orange tree. *Growth* 1:371-373.

- Rengasamy, K.R.R., Kulkarni, M.G., Stirk, W.A. & Van Staden, J. 2014. Eckol - a new plant growth stimulant from the brown seaweed *Ecklonia maxima*. *Journal of Applied Phycology* 27:581–587.
- Rustenburg climate. 2016. *Cape Town climate* @ www.saexplorer.co.za. [Online], Available: http://www.saexplorer.co.za/south-africa/climate/cape_town_climate.asp. Accessed on 10 July 2017.
- Sammons, J.D., & Struve, D.K. 2004. Effect of Bioplex™ on transplant success of non-dormant red oak (*Quercus rubra* L.). *Journal of Environmental Horticulture* 22:197–201.
- Sammons, J.D., & Struve, D.K. 2005. Effect of Bioplex™ on transplant success and recovery of summer-dug Goldenraintree. *Journal of Environmental Horticulture* 23:59–62.
- Singh, R., Sharma, R.R., Kumar, S., Gupta, R.K. & Patil, R.T. 2008. Vermicompost substitution influences growth, physiological disorders, fruit yield and quality of strawberry (*Fragaria x ananassa* Duch.). *Bioresource Technology* 99:8507–8511.
- Slávik, M. 2005. Production of Norway spruce (*Picea abies* [L.] Karst.) seedlings on substrate mixes using growth stimulants. *Journal of Forest Science* 51:15–23.
- Sorrenti, G.B., Fachinello, J.C., Castilhos, D.D., Bianchi, V.J & Marangoni, B. 2008. Effect of different organic fertilization strategies on vegetative growth and soil properties of non bearing citrus trees (cv Clemenules). *Revista Brasileira de Fruticultura* 30:1129–1135.
- Spann, T.M. & Little, H.A. 2010. Effect of Stimplex® crop biostimulant on drought tolerance of “Hamlin” sweet orange. *Proceedings of the Florida State Horticulture Society* 123:100–104.
- Spann, T.M. & Little, H.A. 2011. Applications of a commercial extract of the brown seaweed *Ascophyllum nodosum* increases drought tolerance in container-grown “Hamlin” sweet orange nursery trees. *Horticulture Science* 46:577–582.
- Stirk, W. & Van Staden, J. 2004. Potential new applications for the southern African kelps. *South African Journal of Botany* 70:145–151.
- Stirk, W.A., Tarkowská, D., Turečová, V., Strnad, M. & Van Staden, J. 2014. Abscisic acid, gibberellins and brassinosteroids in Kelpak®, a commercial seaweed extract made from *Ecklonia maxima*. *Journal of Applied Phycology* 26:561–567.
- Struve, D.K. 2009. Tree establishment: A review of some of the factors affecting transplant survival and establishment. *Arboriculture and Urban Forestry* 35:10–13.
- Tandon, S. & Dubey, A. 2015. Effects of Biozyme (*Ascophyllum nodosum*) biostimulant on growth and development of soybean [*Glycine Max* (L.) Merrill]. *Communications in Soil*

- Science and Plant Analysis* 46:845–858.
- Tyihák, E., Blunden, G. & Yuan, M. 1994. Quantitative estimation of betaines in commercial seaweed extracts using overpressured layer chromatography. *Journal of Applied Phycology* 6:469–473.
- Van Staden, J. *Kelp Products International (Pty) Ltd.* Simon's Town, South Africa. 4 pp.
- Van Staden, J., Upfold, S.J & Drewes, F.E. 1994. Effect of seaweed concentrate on growth and development of the marigold *Tagetes patula*. *Journal of Applied Phycology* 6:427-428.
- Van Staden, J., Beckett, R.P & Rijkenberg, M.J. 1995. Effect of seaweed concentrate on the growth of the seedlings of three species of *Eucalyptus*. *South African Journal of Botany* 61:169-172.
- Vernieri, V., Borghesi, E., Ferrante, A. & Magnani, G. 2005. Application of biostimulants in floating system for improving rocket quality. *Journal of Food, Agriculture & Environment* 3:86–88.
- Vessey, J.K. 2002. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* 255:571–586.
- Victus Biology. 2017. Victus Banking on Biology: Healthy soil; healthy plant; healthy crop; healthy profit. [Online], Available:<http://www.victus/biology/healthysoil/healthy crop/healthy profit>. Accessed on 25 February 2017.
- Watson, G., & Himelick, E.B. 2005. Best management practices: Tree planting. ANSI A300 Part 6: Tree, shrub and other woody plant maintenance—standard practices (transplanting). *International Society of Arboriculture*, Champaign, IL.
- Waynick, D.O. & Walker, S.J. 1930. Rooting habits of citrus trees. *California Citrog.* 15:201, 238-239.
- Whapham, C.A., Blunden, G., Jenkins, T & Hankins, S.D. 1993. Significance of betaines in the increased chlorophyll content of plants treated with seaweed extract. *Journal of Applied Phycology* 5: 231-234
- Williams, L.E. & Araujo, F.J. 2002. Correlations among predawn leaf , midday leaf , and midday stem water potential and their correlations with other measures of soil and plant water status in *Vitis vinifera*. *Journal of American Society Horticultural Science* 127:448–454.
- Williams, L.E. & Trout, T.J. 2005. Relationships among vine- and soil-based measures of water status in a Thompson seedless vineyard in response to high-frequency drip irrigation. *American Journal of Enology and Viticulture* 56:357–366.

- Woods, N. 2005. Agricultural chemical users manual. In: G. Cowles, J. Crome, R. Lambourne, P. Simpson, & R. Webster (eds.). DPI & F. Gatton, Australia. 145 pp.
- Yuan, Y. 2016. Vermicomposting, waste recycling and plant growth. MSc Thesis, Horticultural Science, Lincoln University.
- Zhang, X. & Ervin, E.H. 2004. Cytokinin-containing seaweed and humic acid extracts associated with creeping bentgrass leaf cytokinins and drought resistance. *Crop Science* 44:1737–1745.
- Zhang, X., Ervin, E.. & Schmidt, R. 2003. Physiological effects of liquid applications of a seaweed extract and a humic acid on creeping bentgrass. *Journal of the American Society for Horticultural Science* 128:492–496.
- Zhang, X., Schmidt, R., Ervin, E. & Doak, S. 2002. Creeping bentgrass physiological responses to natural plant growth regulators and iron under two regimes. *Horticulture Science* 37:898–902.

8. Tables and Figures

Table 1: The effect of a range of biostimulants with their application rates and active components on ‘Nadorcott’ mandarin’ at Welgevallen Experimental Farm, Stellenbosch in November 2016.

Treatment	Application rate	Active component
Compost (<i>Control</i>)	44t.ha ⁻¹	Crop residues, unused bedding materials, silage and manures
Terramax [®]	200ml in 5L water (200ml.tree ⁻¹)	Vermicomposting (<i>Bacillus</i> and <i>Trichoderma spp</i>)
Kelp (<i>Kelpak</i> [®])	1ml in 1L water (4L. tree ⁻¹)	<i>Ecklonia maxima</i>
Kelp (<i>RootAktiv</i> [®])	40ml in 20L water (2L.plant ⁻¹)	<i>Ascophyllum nodosum</i> and biofulvinate based organic mineral composite
Kelp (<i>Super Wortel</i> [®])	2ml in 1L water	Oligopeptides and polysaccharides-organic mineral based composite

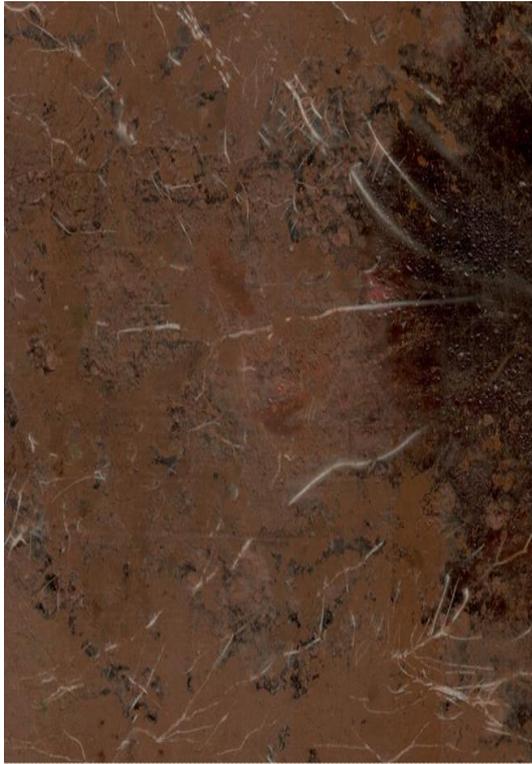
Table 2: The effect of a range of biostimulants on the vegetative growth of ‘Nadorcott’ mandarin at Welgevallen Experimental Farm, Stellenbosch, as recorded at the termination of experiment in May 2017, following application at transplant in November 2016.

Treatments	Stem diameter (mm)	Shoot length (mm)
Compost	12.1	191.9 ^{ab}
Terramax [®]	11.1	177.8 ^b
Kelpak [®]	11.5	181.0 ^b
RootAktiv [®]	12.2	265.7 ^a
Super Wortel [®]	12.3	188.7 ^{ab}
<i>LSD</i>	2.36	79.33
<i>P</i>	0.6351	0.0111



Figure 1: Transparent mini-rhizotron tubes installed with watertight bottom plugs and insulated top caps into holes, inserted approx. 70 cm deep, in a parallel position to ‘Nadorcott’ mandarin roots, to track root growth and development following biostimulant treatments to reduce transplant shock.

A



B



Figure 2: A nearly 360-degree image of the Kelpak[®] treatment roots at 40 cm (A) and 20 cm soil depth of Terramax[®] treatment roots (B), both captured on 6 September 2017 at Welgevallen Experimental Farm, Stellenbosch, on ‘Nadorcott’ mandarin roots, to track root growth and development following biostimulant treatments to reduce transplant shock.



Figure 3: Root Snap Analysis Software images indicating root growth measurements using different colour lines on the image to calculate parameters such as root length (mm), volume (mm^3), diameter (mm) and number of roots from the images.

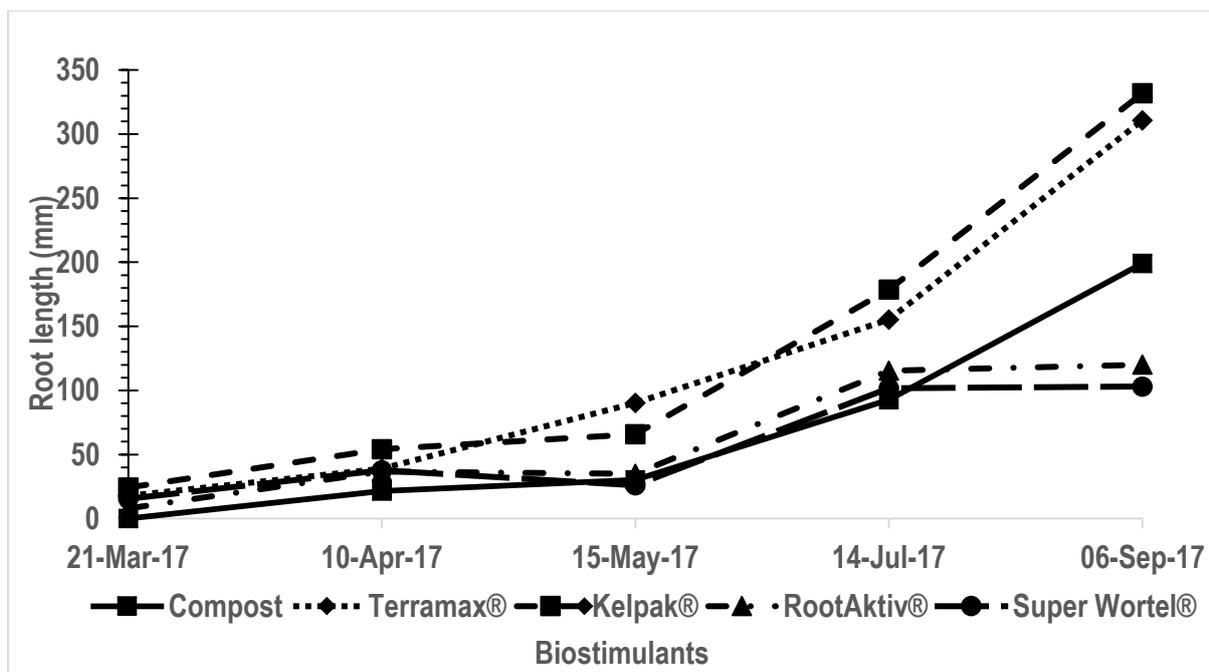


Figure 4: Root length (mm) of young, non-bearing ‘Nadorcott’ mandarin trees (n=15) as recorded from March to September 2017 at Welgevallen Experimental Farm, Stellenbosch, following the application of a range of biostimulants in November 2016 at transplanting.

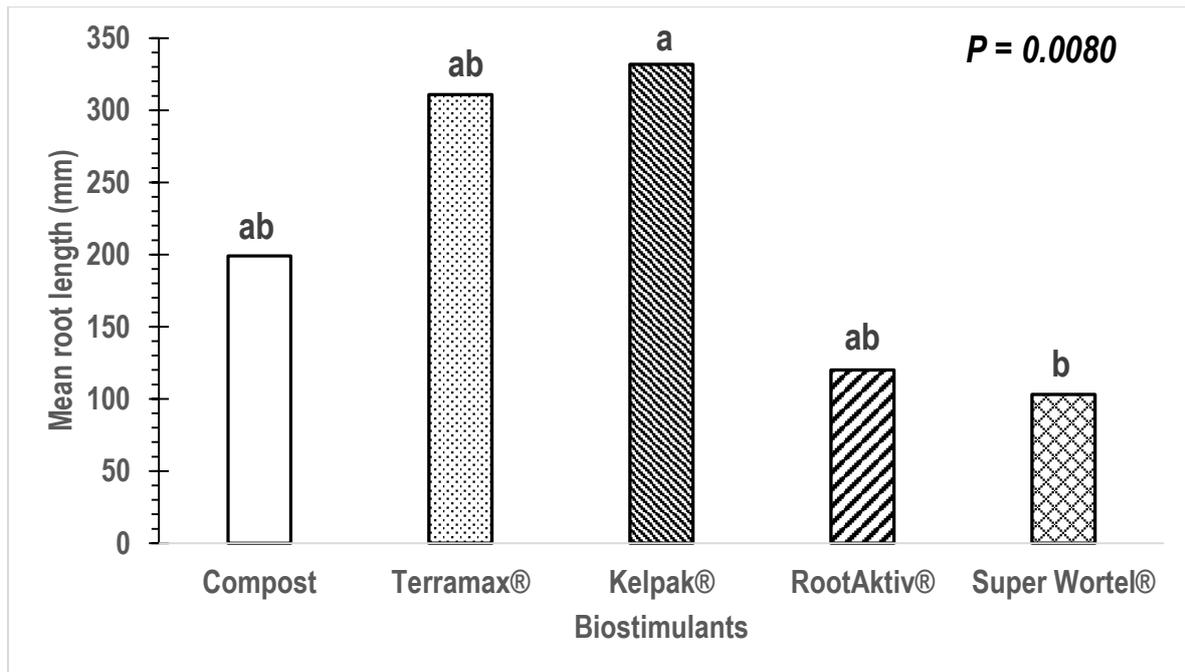


Figure 5: Effect of a range of biostimulants on the final mean root length (mm) of young, non-bearing 'Nadorcott' mandarin trees (n=15) as recorded on 6 September 2017, one year following treatment application at transplanting in November 2016 at Welgevallen Experimental Farm, Stellenbosch.

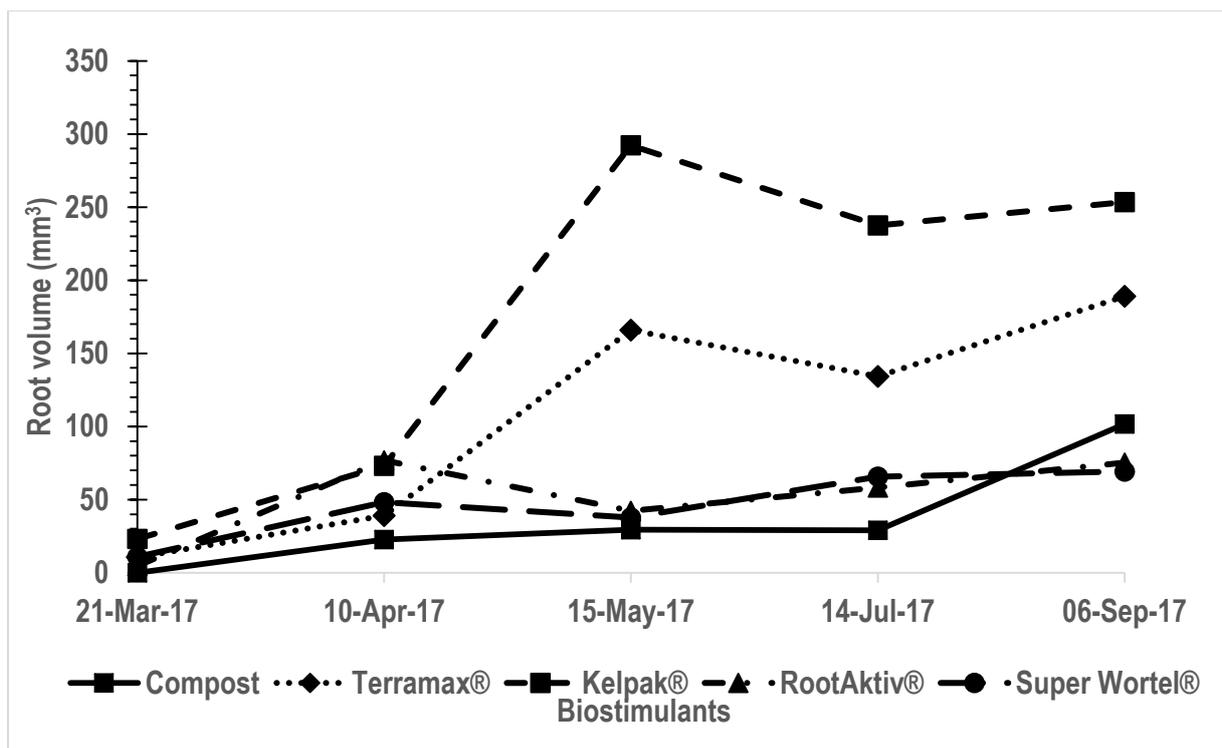


Figure 6: Root volume (mm^3) increase on young, non-bearing 'Nadorcott' mandarin trees ($n=15$) as recorded from March to September 2017 at Welgevallen Experimental Farm, Stellenbosch following the application of a range of biostimulants at transplant in November 2016.

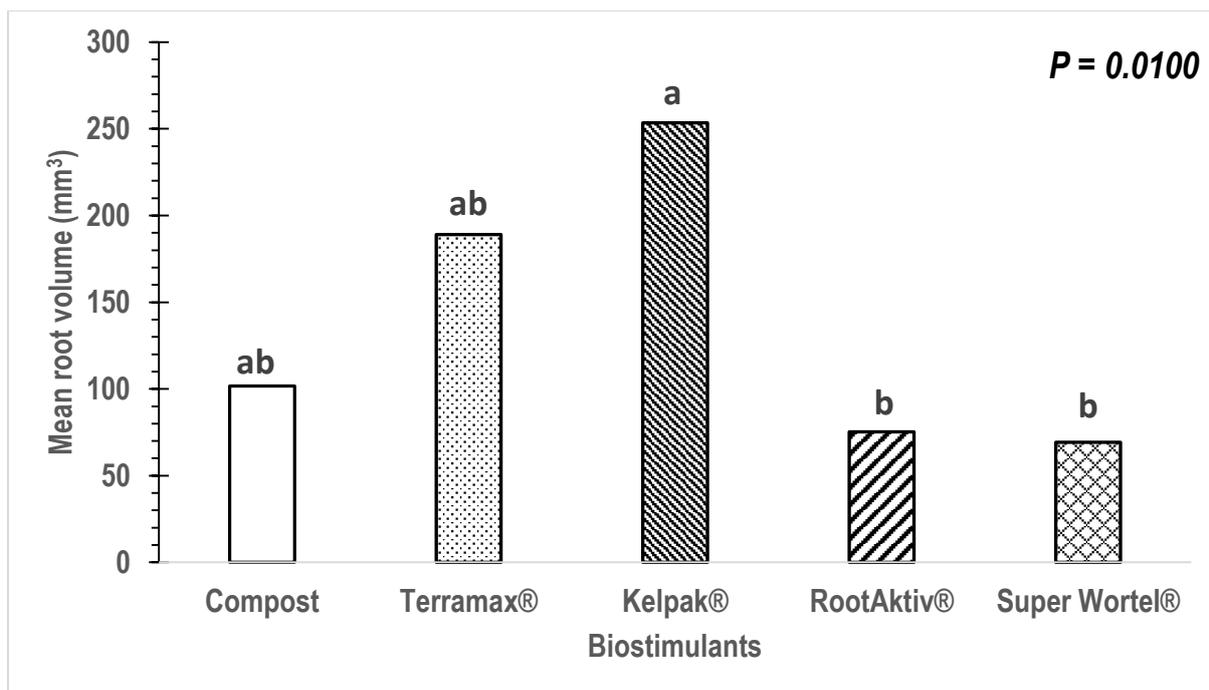


Figure 7: Effect of a range of biostimulants on the final mean root volume (mm³) of young, non-bearing ‘Nadorcott’ mandarin trees (n=15) as recorded on 6 September 2017, one year following treatment application at transplanting in November 2016 at Welgevallen Experimental Farm, Stellenbosch.

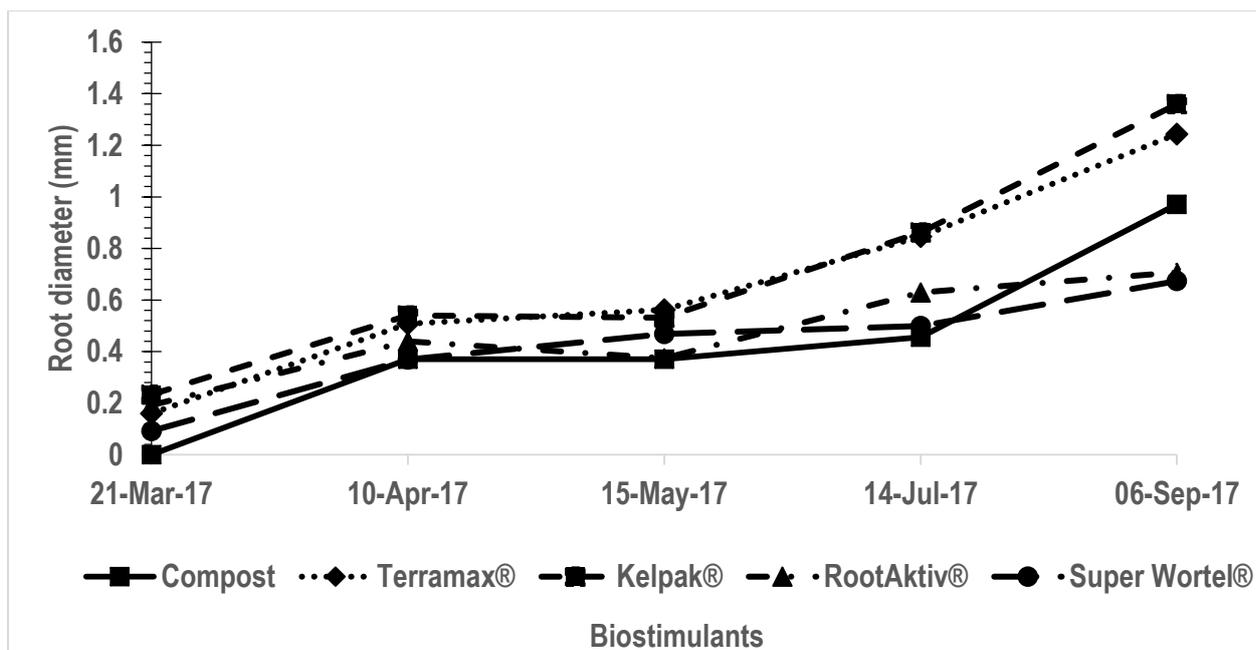


Figure 8: Root diameter (mm) increase on young, non-bearing 'Nadorcott' mandarin trees (n=15) as recorded from March to September 2017 at Welgevallen Experimental Farm, Stellenbosch following the application of a range of biostimulants at transplant in November 2016.

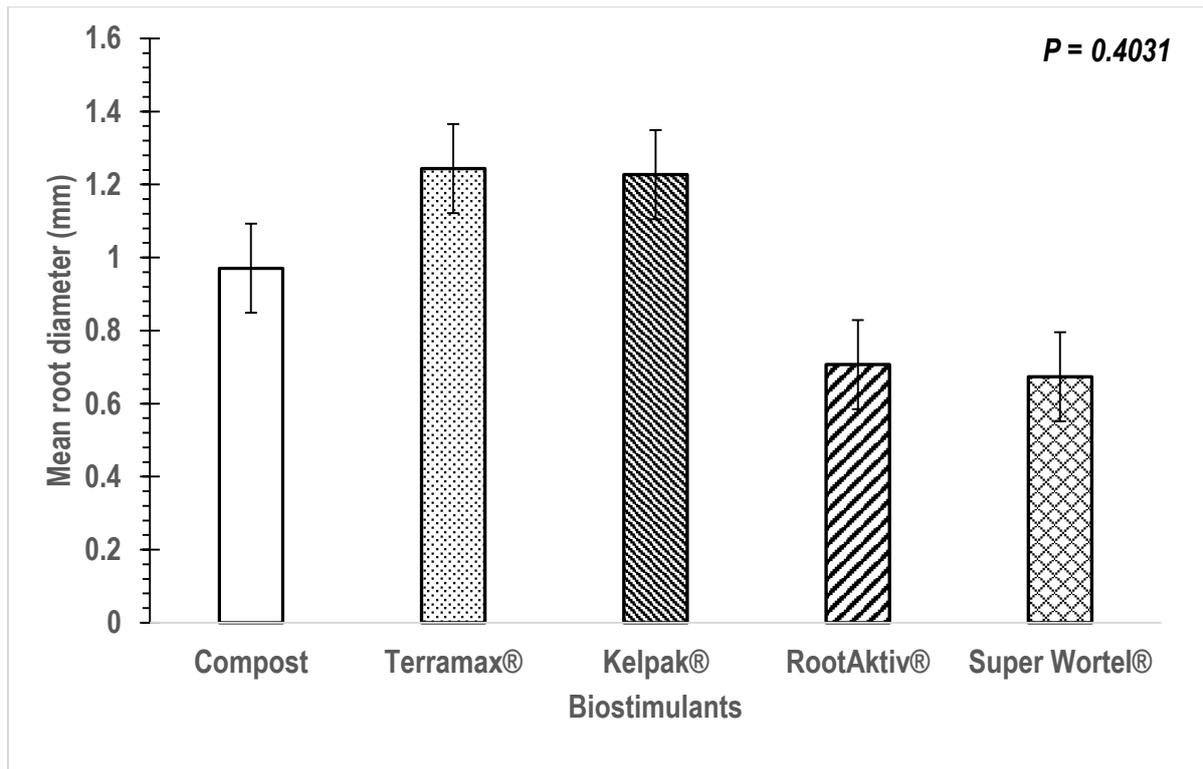


Figure 9: Effect of a range of biostimulants on the final mean root diameter (mm) of young, non-bearing ‘Nadorcott’ mandarin trees (n=15) as recorded on 6 September 2017, one year following treatment application at transplanting in November 2016 at Welgevallen Experimental Farm, Stellenbosch.

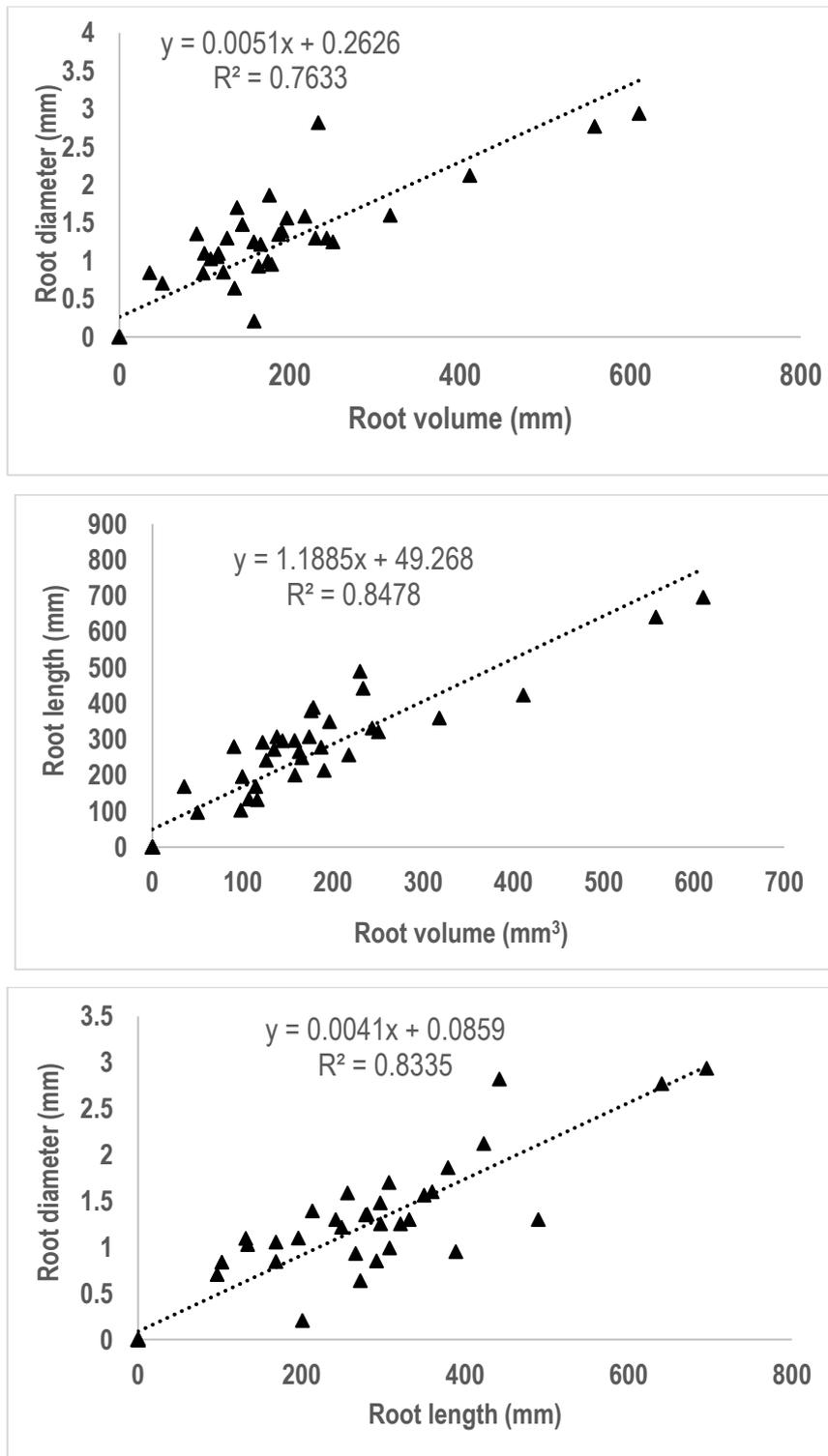


Figure 10: Correlations between the root growth parameters of root length, volume and diameter as recorded on the final day of measurement on 6 September 2017, following the application of a range of biostimulants at transplanting in November 2016. The regression functions showing the respective correlations between the respective tree root growth parameters are provided.

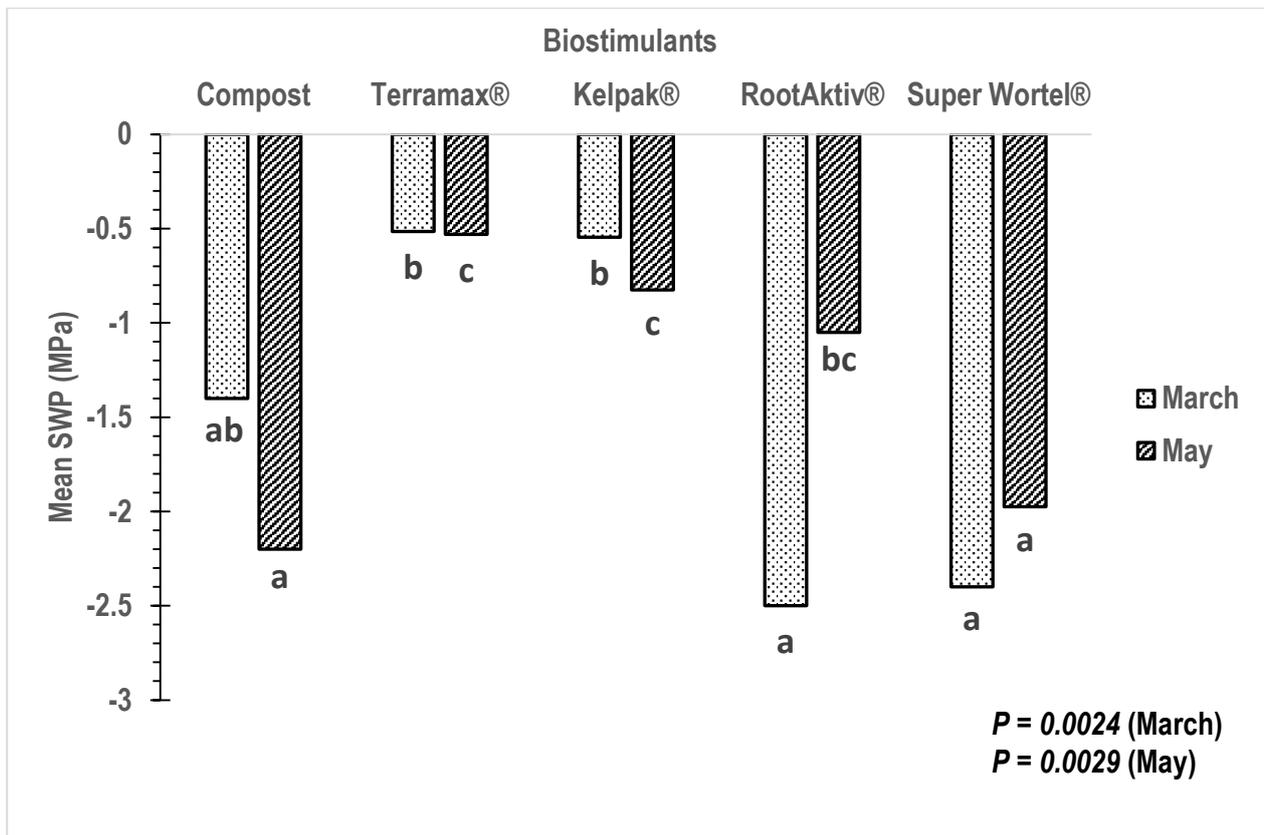


Figure 11: Effect of a range of biostimulants on the final mean stem water potential (SWP) (MPa) of young, non-bearing ‘Nadorcott’ mandarin trees (n=50) as recorded on 16 March and 16 May 2017, following treatment application at transplanting in November 2016 at Welgevallen Experimental Farm, Stellenbosch.

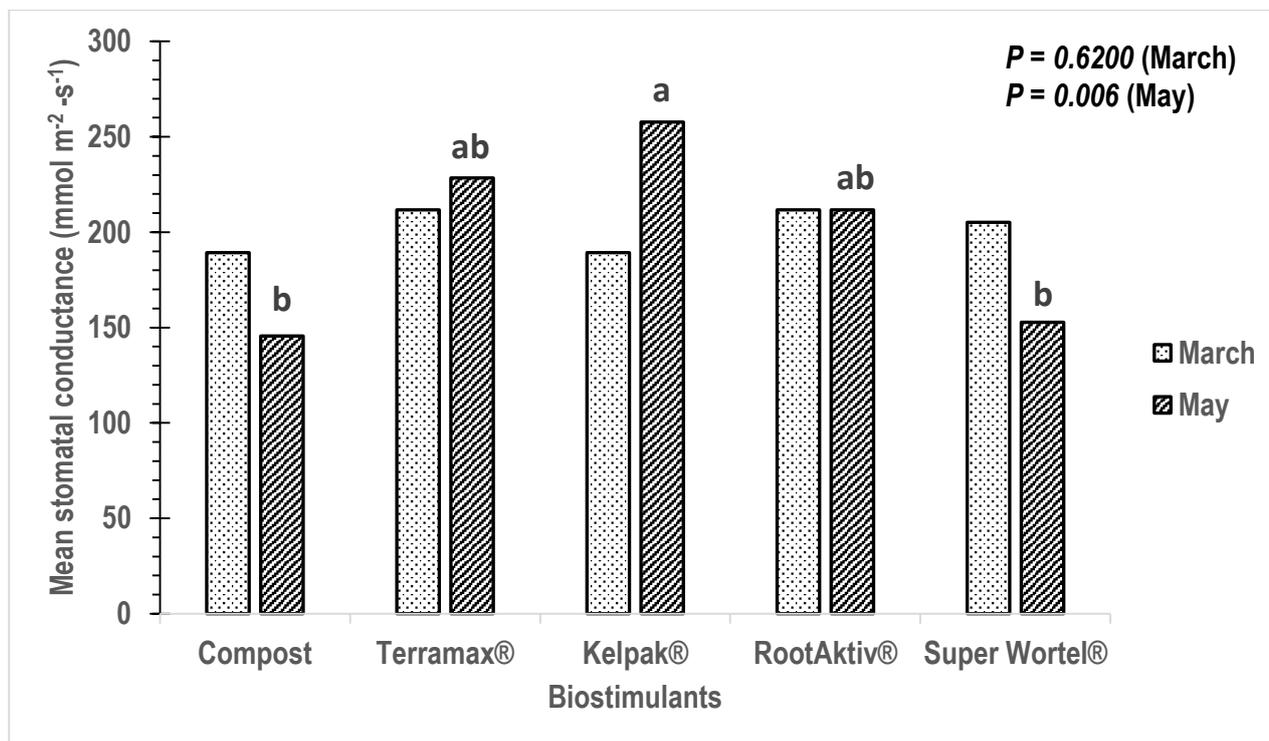


Figure 12: Effect of a range of biostimulants on the final mean stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) of young, non-bearing ‘Nadorcott’ mandarin trees ($n=50$) as recorded on 16 March and 31 May 2017, following treatment application at transplanting in November 2016 at Welgevallen Experimental Farm, Stellenbosch.

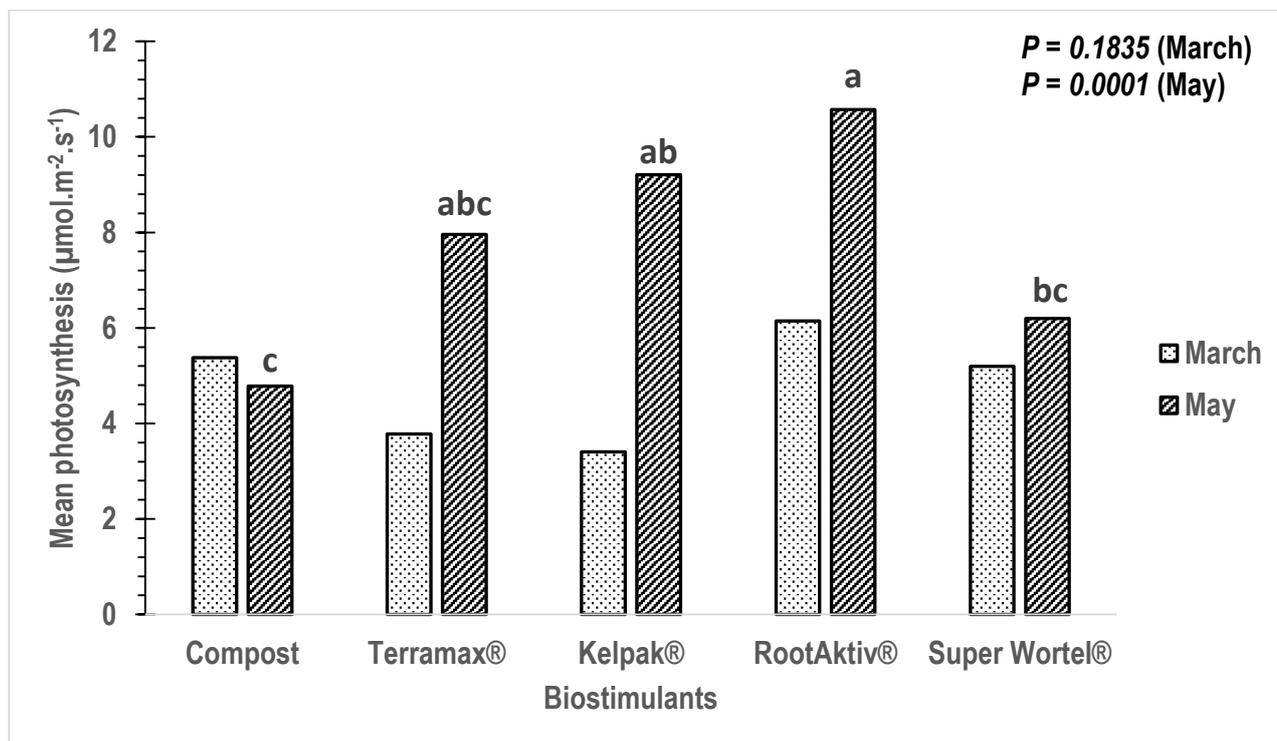


Figure 13: Effect of a range of biostimulants on the final mean photosynthesis ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) of young, non-bearing ‘Nadorcott’ mandarin trees ($n=50$) as recorded on 10 March and 13 May 2017, following treatment application at transplanting in November 2016 at Welgevallen Experimental farm, Stellenbosch.

GENERAL DISCUSSION AND CONCLUSION

A number of reports showed positive effects of biostimulants on plant growth of different crops at molecular level, but few studies have been performed on promoting growth and fruit quality at orchard or field level. Therefore, the effect of biostimulants was investigated in promoting growth and fruit quality of different commercial fruit crops under local conditions.

In paper 1 and 2 results are only presented for one season due to the untimely withdrawal of funding from the company. Results obtained from paper 1 and paper 2 did not show any significant differences between treatments with regard to vegetative growth and fruit quality parameters of bearing plums and pears. A possible reason for lack of significant differences is based on the duration of the observation period (only one season). However, a trend was observed where the biostimulant treatment (paper 1) had slightly higher growth and quality parameters, though not significant, which gives optimism that if the study is carried out for a longer period, there may be conclusive results.

Results from paper 2 showed that the same biostimulant combination improved selected parameters significantly for non-bearing pear trees (paper 2). On plant physiological level, significant differences were observed in stomatal conductance for ‘Packham’s Triumph’ and ‘Celina’ pear cultivars. This significant higher stomatal conductance of the biostimulant treatment may indicate a reduction of stress after transplant. In spite the difference, a longer duration is required to produce more conclusive results for other physiological parameters such as stem water potential and photosynthesis. In addition, below soil growth and microbial dynamics were not quantified due to logistics and this may partly explain the lack of growth results on vegetative growth. This was confirmed by results from paper 3 where root growth was quantified.

In paper 3, different biostimulant products were applied on ‘Nadorcott’ citrus trees: compost (control), Terramax[®], Kelpak[®], RootAktiv[®] and Super Wortel[®]. The RootAktiv[®] treatment had a significantly higher shoot growth than the Terramax[®] and Kelpak[®] treatments but was similar to the control. The Kelpak[®] treatment had a significantly higher root length and volume compared to the RootAktiv[®] and Super Wortel[®] treatments but was similar to the control. The Terramax[®] and Kelpak[®] treatments had a high SWP than Super Wortel[®] and RootAktiv[®] in March, whereas in May, Terramax[®], Kelpak[®] and RootAktiv[®] treatments had a significantly higher SWP than the control and Super Wortel[®] treatments. The Kelpak[®] treatment showed significantly higher stomatal conductance than the control and Super Wortel[®] treatments, for the second evaluation date. Variation in results of these biostimulants depended on their active components.

Furthermore, enhancement of rapid shoot elongation by RootAktiv[®] treatment resulted in more fluctuations and slow root development with low root length, volume and diameter compared to compost, Terramax[®] and Kelpak[®]. Likewise, Terramax[®] and Kelpak[®], had rapid root development, probably due to the prominent PGR contribution of these products and this resulted in lower shoot growth. Surprisingly, the RootAktiv[®] treatment was also effective in improving stem water potential (sign.), stomatal conductance (ns) and photosynthesis (sign) compared to some of the treatments, despite low root development. Physiologically, in the RootAktiv[®] treatment, stem water potential decreased, stomatal conductance increased and this resulted in increased photosynthesis of trees.

Apart from the effect of biostimulants on root growth, we also observed an effect of climate and shoot growth on root growth dynamics. This was more prominent during winter when root growth dropped drastically in some treatments (compost, RootAktiv[®] and Super Wortel[®]). Rapid root growth was observed in spring up to summer. On shoot growth, this was observed when rapid shoot growth initiated, there was a slow root growth vice versa despite the influence from the treatments (data not present). Hence, it's advisable to analyse the influence of external factors rather than the treatment alone. Despite less root development in the RootAktiv[®] treatment, it proved to be effective in improving shoot growth of citrus cultivars. This suggests that RootAktiv[®] can be applied to improve vegetative growth of citrus cultivars. However, Terramax[®] and Kelpak[®] can be applied to improve root growth and physiological development of citrus cultivars compared to the rest of treatments (paper 3), possibly because of growth stimulating hormones (auxins and cytokinins) that activate root development from a dormant phase to initial growth and alleviating abiotic stress.

Further studies can be implemented in using these biostimulants in alleviating biotic factors such as controlling pests and disease attack. The most common are the nematodes and *Phytophthora spp.* which affects the citrus roots. Common practise is use of fumigants which have negative effects on the environment. Hence, sustainable and organic methods can be applied such as these biostimulants improving root vigour of tree crops.

However, as the active component of the selected biostimulant products differed, the importance of selecting the correct product for a specific goal, was emphasised. This will allow for effective evaluation of the different products in the different areas of producing field crops.