SUPPORTING CROP ESTABLISHMENT AND TREES IN ESTABLISHED AVOCADO ORCHARDS, BY WAY OF COMBINED USE OF BIOLOGICAL AND CHEMICAL SOLUTIONS

Jordaan, E., Anelich, R. and Denner, F.

UPL OpenAg South Africa
PO Box 1726, Mount Edgecombe 4300, SOUTH AFRICA

Estiene.Jordaan@upl-ltd.com

Avocado production is under severe pressure due to the impact of unfavourable environmental/abiotic factors, market-related aspects, pest pressure from various sources and the ever limiting availability of chemical options due to residue implications. Due to the worldwide growing consumer market for avocados, statistics show a sharp increase in tree sales since 2018. In most cases, replanting of new trees occurring in old avocado orchards results in a replant problem, due to trees dying before reaching maturity. The reason for the die-back of trees is unknown, but the assumption is made that *Phytophthora cinnamomi* might play a role as the disease is often found present in nurseries and avocado orchards. A holistic root health approach, including biological and chemical options, might be the only sustainable way to address the replant problem. The project, therefore, focuses on the transplant of treelings during the crop establishment phase as well as addressing challenges in established orchards.

INTRODUCTION

Avocado is a crop of economic importance, with South Africa being an export country of the commodity (Imbert, 2008). Unfortunately, avocado is very susceptible to *Phytophthora* spp. that cause root rot, among other symptoms (Coffey, 1987; Mamani and Aragon, 2018). Infection, depending on severity, may lead to "die-back" of trees resulting in yield losses and, in worst case scenarios, death of host trees. Few chemical control measures provide effective and economically viable control of root rot infections with residue concerns always being present. Biological control has been advocated as a solution (Mamani and Aragon, 2018). However, biocontrol agents may be susceptible to a variety of factors (e.g., climate, soil characteristics, farming practice), potentially reducing their efficacy against pathogens. Crop establishment remains a challenge to avocado growers due to adverse environmental factors and plant pathogens, either originating from the nursery or existing inoculum in the orchards. To address the challenges associated with transplanting treelings, the use of soil retention products may address climatic challenges related to moisture availability. Reduced moisture fluctuations are essential in the initial stages of crop establishment following transplanting. This is usually a time when climatic stress conditions due to high temperatures may increase pressure on treeling vigour and survival.

Several *Trichoderma* spp. including *Trichoderma harzianum* have been identified as biocontrol agents. They have been identified as suppressing *Phytophthora* spp. as well as other soilborne diseases and can serve as a bioremedy to manage pathogen populations in the soil, promoting treeling growth (Azarmi *et al*., 2011; Hermosa *et al*., 2012). *Trichoderma harzianum* outcompetes pathogens for resources such as root exudates resulting in antagonistic behaviour (Azarmi *et al*., 2011; Hermosa *et al*., 2012). Growth promoting effects occur due to providing stimulating exudates to host plants (Azarmi *et al*., 2011; Hermosa *et al.*, 2012).

Related to established orchards, the use of biocontrol agents and chemical products as part of an Integrated Pest Management (IPM) strategy may be the answer due to utilising the best aspects from both disciplines (Coffey, 1987). Phosphorous acid-based products are well known for their modes of action, including the stimulation of root hairs for improved absorption, induced resistance and control of certain fungal pathogens including *Phytophthora* spp.. There are currently several phosphorous acid-based products

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registered in South Africa, either as foliar applications or stem injections. Drench applications are also coming of age, with products being developed and registered to be applied in this manner. In addition to applying phosphorous acid, the advantages of these chemical remedies can be integrated with the benefits of *Trichoderma* and *Bacillus* species, from which an IPM approach can be developed where the chemical serves as a "knock-down" effect on the pathogen population, while the biocontrol agents manage this reduced population below acceptable economic threshold levels.

Beneficial microorganisms play a vital role in soil and plant health. These organisms can be regarded as analogue to probiotics in humans, ensuring plants develop and prosper in nature. Whether it is during crop establishment or in established orchards, mineral utilisation remains a key objective during crop cultivation. There are several biostimulant methods in improving mineral utilisation, one of which is the use of microorganisms. *Bacillus subtilis* is well known for its solubilising efficacy of minerals and presenting it in an acceptable form to plants. In addition, *Bacillus amyloliquefaciens* is capable of releasing plant growth promoting exudates to improve plant vigour and growth. This may lead to improved resistance to abiotic stress factors and increase potential in yield development. Both *B. subtilis* and *B. amyloliquefaciens* also have biocontrol properties against several plant diseases.

Integrated methods to cultivate crops are becoming essential in ensuring sustainability towards future food production. This means utilising different methods, both in pest control and plant development, to realise full yield potential. To address the risk faced in establishing new avocado orchards or trees already in yield production, it is necessary to integrate chemical and biological methods described above. In addition, it is necessary to incorporate IPM pillars including host plant resistance (in this case rootstock varieties) and cultural practices. UPL South Africa (Pty) Ltd has access to several types of products described above and can contribute to developing them for optimal use on avocado. These proposed products include the following:

- **Starch based super absorbent polymer** (Zeba® product code UPL-ZB)
  A starch based super absorbent polymer for the regulation of soil moisture. The product is a granular based formulation to be applied during the transplanting process. This product is biodegradable with sustained water release efficacy. Although, registered for its excellent properties in crop establishment on a variety of crops, the product is not currently registered for use on avocado.
  The abiotic stress mitigation of Zeba® during transplanting has been established in a variety of crops, including tree crops. Due to the nature of the product water is absorbed and released as the plant requires, leading to reduced moisture fluctuation in the root zone. As *Phytophthora* requires wet and dry cycles for spore production, this product renders the environment for *Phytophthora* less ideal. Because the water is held by Zeba®, there is also less free water available for the movement of spores in the root environment and, as such, decreases the spread of *Phytophthora*. As a third benefit, Zeba® aids as a food source for the microbes present and added through the IPM strategy.

- **Ammonium phosphite 386 g/L** (Brilliant® product code UPLSA-F-002)
  A phosphorous acid product with a phosphorous acid equivalent of 300 g/L. This product contains phosphite as part of its active ingredient and is fully systemic in the plant. It is currently registered as a fungicide for use against *Phytophthora*, however, not on avocado. This SL formulation is currently pending registration as a soil application in avocado.
  Phosphite has unique biocontrol properties specifically against oomycetes, the group of fungi that includes *Phytophthora*. Applications of this product also lead to an upregulation of plant defences and pathogen recognition by the host upon infection (Dalio *et al*., 2014). This results in a quicker response by the host plant against the pathogen. There is also a direct effect on the growth and sporulation of oomycetes.

- ***Trichoderma harzianum*** **VBJ-16** (Trykosist® product code UPL-TH)
  Trykosist®, a proprietary bio-fungicide from UPL South Africa (Pty) Ltd and currently registered as a biostimulant on a variety of crops including avocado, harbours a specific strain of *Trichoderma harzianum* (UPL IP). It is a South African strain, specially selected for its performance in local conditions. The liquid-based formulation is intended for application through drench and an irrigation system.
  From peer reviewed literature studies, it can be concluded that *Trichoderma*, depending on species and strain, can induce not only plant growth promoting effects on host plants, but also biocontrol properties that include three different modes of action (Mohiddin *et al.*, 2010; Hoyous-Carvajal and Bisset, 2011; Da Silva *et al*., 2012; Hermosa *et al*., 2012). These include:
  - Activation of a host plant's immune response system
  - Direct mycoparasitism of certain pathogens
  - Antagonistic behaviour towards certain pathogens and outcompeting them for resources.

  Soil application of *T. harzianum* is selected as the primary application method due to optimal placement of *T. harzianum* in the root zone. This will not only provide plant development stimulation but induce host tree immune resistance as well as provide competition and antagonism towards *Phytophthora* spp.. In addition, *T. harzianum* is more suited to the soil environment, being less exposed to abiotic stress factors that may inhibit its viability.

- ***Bacillus subtilis* HC8, *Bacillus amyloliquefaciens* subsp. *plantarum* CH13** (Extrasol® product code UPL-EXS)
  Specific strains of *B. subtilis* and *B. amyloliquefaciens* (UPL IP) was developed for plant growth promotion. This product is currently registered as a Group 3 fertilizer for application to the soil. A liquid-based formulation is intended for application through drench or irrigation systems. These bacteria interfere with *Phytophthora* growth and movement of spores but also induce plant defence responses (Liu *et al*., 2019).

AIMS AND OBJECTIVES

The aim of the project is to identify an integrated management programme for the establishment of avocado treelings, transplanted to orchards with a history of Phytophthora root rot.

For the aim to be completed, the following objectives as part of a development plan need to be achieved:

- Investigate benefits to crop establishment using a super absorbent starch polymer as moisture retention product. A combination of *T. harzianum* and the starch polymer can support treeling development and suppression of *P. cinnamomi* causing root disease.
- Investigate the combined use of Brilliant® as a chemical fungicide and *T. harzianum* as a biocontrol agent to control *P. cinnamomi* as part of an IPM approach. Products will be applied in a programme at a specific phenological growth stage. Soil applications of both products to be investigated.
- Investigate the use of *B. subtilis* and *B. amyloliquefaciens* on tree development and vigour stimulation by inducing benefits as part of a soil/tree health approach.
- Trial protocols will include different rootstock varieties, e.g. Bounty, Dusa and/or Duke 7.
- Assessment parameters for crop establishment trials: disease incidence and severity throughout the trial period as well as treeling growth and vigour parameters; and for established orchard trials: disease incidence and severity throughout the trial period as well as tree vigour and yield.
- Trials must be completed over two seasons with results being submitted by August 2023.
- Data generated to be used in support of registration of products on avocado in accordance with the guidelines as published by the DALRRD.
- Registered solutions must be provided to the avocado industry, ensuring access to sustainable pest control and production tools.

MATERIALS AND METHODS

Trykosist® and Brilliant® was assessed against Phytophthora root rot in established orchards. Trials were conducted by an independent, PSCA registered Contract Research Organisation (CRO). The efficacy of Trykosist® alone, applied to a total of five applications at a 14- to 28-day application interval, was assessed. This programme was compared to Brilliant®, applied to a total of three applications at 28-day intervals. Also, Trykosist® in combination with Brilliant® were assessed against Phytophthora root rot. The programme was set out with an initial Brilliant® application for its direct and indirect effect on *Phytophthora*, followed by a Trykosist® application to ensure the establishment of *Trichoderma* within the root zone for competition against *Phytophthora* and induced resistance in the host. This action was repeated 14 days later, with a final Trykosist® application 28 days later. Trykosist® and Brilliant® were applied as a soil drench with 10 L of water per tree. A total of 10 trials were conducted in four climatic regions, including Bsh, Cfb, Cwa and Cfa on three different cultivars, Hass, Fuerte and Ryan. Potassium phosphite was included as a standard.

Trials in established orchards was assessed visually for the percentage Phytophthora root rot severity and incidence at five distinct assessments during the trial period. The CIBA Geigy rating scale was also used to assess Phytophthora root rot at the end of the trial period, two weeks after the final application. Yield was assessed by harvesting fruit from the respective treated trees. Phytotoxicity and vigour were also assessed throughout, comparing the treated to the untreated plots.

From the results obtained with the field trials in established orchards, the transplanting programme was created. Proof of concept data was generated for an avocado transplanting trial in the glasshouse. The glasshouse trial was conducted on Fuerte/Duke 7 avocado trees. Soil for the glasshouse trial was collected from a field with a previous history of Phytophthora root rot and artificially inoculated with *P. cinnamomi*. For inoculation, an isolate of *P. cinnamomi* was obtained from the Agricultural Research Council of South Africa culture collection (Fig. 1, PPRI 4525).

Inoculum was prepared according to Drenth and Sendall, 2001. In short, *P. cinnamomi* colonies were grown for seven days and placed onto sterilised wheat to colonise. A total of 5 g of inoculated wheat was added to 10 L soil and mixed before adding to

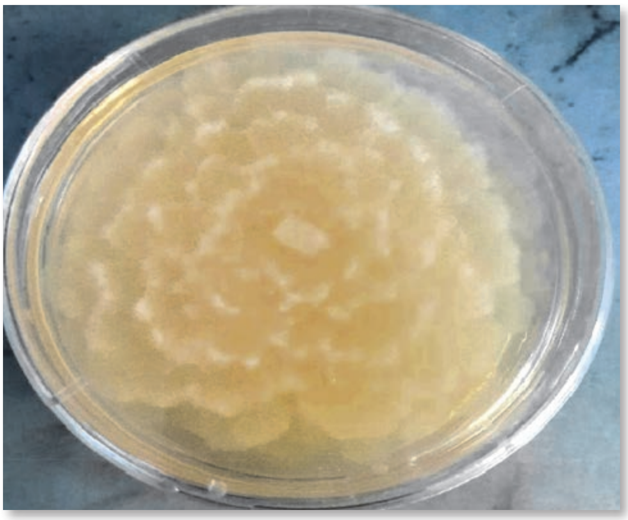


Figure 1: *Phytophthora cinnamomi* isolate from the Agricultural Research Council of South Africa culture collection (PPRI number 4525) used in the glasshouse trials.

Table 1: Treatments applied to glasshouse and field trials

Treatment	Description	Timing	Application code	Application details
1	Untreated control	-		-
2	Zeba®	Single application at transplanting	A	Soil application Dosage rate: 5 g/tree
3	Brilliant®	Single application at transplanting	A	Soil application Dosage rate: 1 mL/tree
4	Trykosist®	Four applications in total at 14-day intervals starting 14 days after plant	BCDE	Soil application Dosage rate: 2.5 mL/tree
5	Extrasol®	Four applications in total at 14-day intervals starting 14 days after plant	BCDE	Soil application Dosage rate: 2.5 mL/tree
6	Zeba®	Single application at transplanting	A	Soil application Dosage rate: 5 g/tree
	Brilliant®	Single application at transplanting	A	Soil application Dosage rate: 1 mL/tree
7	Zeba®	Single application at transplanting	A	Soil application Dosage rate: 5 g/tree
	Brilliant®	Single application at transplanting	A	Soil application Dosage rate: 1 mL/tree
	Trykosist®	Four applications in total at 14-day intervals starting 14 days after planting	BCDE	Soil application Dosage rate: 2.5 mL/tree
8	Brilliant®	Single application at transplanting	A	Soil application Dosage rate: 1 mL/tree
	Trykosist®	Four applications in total at 14-day intervals starting 14 days after planting	BCDE	Soil application Dosage rate: 2.5 mL/tree
9	Zeba®	Single application at transplanting	A	Soil application Dosage rate: 5 g/tree
	Brilliant®	Single application at transplanting	A	Soil application Dosage rate: 1 mL/tree
	Extrasol®	Four applications in total at 14-day intervals starting 14 days after planting	BCDE	Soil application Dosage rate: 2.5 mL/tree
10	Brilliant®	Single application at transplanting	A	Soil application Dosage rate: 1 mL/tree
	Extrasol®	Four applications in total at 14-day intervals starting 14 days after planting	BCDE	Soil application Dosage rate: 2.5 mL/tree
11	Zeba®	Single application at transplanting	A	Soil application Dosage rate: 5 g/tree
	Brilliant®	Single application at transplanting	A	Soil application Dosage rate: 1 mL/tree
	Extrasol® and Trykosist®	Four applications in total at 14-day intervals starting 14 days after planting	BCDE	Soil application Dosage rate: 2.5 mL/tree
12	Brilliant®	Single application at transplanting	A	Soil application Dosage rate: 1 mL/tree
	Extrasol® and Trykosist®	Four applications in total at 14-day intervals starting 14 days after planting	BCDE	Soil application Dosage rate: 2.5 mL/tree
13	Zeba®	Single application at transplanting	A	Soil application Dosage rate: 5 g/tree
	Brilliant®	Single application at transplanting	A	Soil application Dosage rate: 1 mL/tree
	Trykosist®	First application 14 days after Brilliant®	B	Soil application Dosage rate: 2.5 mL/tree
	Extrasol®	Second application 14 days after Trykosist®	C	Soil application Dosage rate: 2.5 mL/tree
	Trykosist®	Third application 14 days after Extrasol®	D	Soil application Dosage rate: 2.5 mL/tree
	Extrasol®	Fourth application 14 days after second Trykosist®	E	Soil application Dosage rate: 2.5 mL/tree

the transplanted avocado treeling into a bigger 20 L potting bag. Zeba® applications were also conducted during this stage, and mixed with the soil before planting.

Thirteen treatments were considered and included the untreated control, Zeba®, Trykosist®, Extrasol® and Brilliant® applied on their own; as well as different combinations of these products as listed in Table 1. A maximum of six applications were made for the duration of the trial. The trial was laid out in a Randomized Complete Block Design (RCBD) with each replicate consisting of a single tree. Trial protocol, layout and statistical evaluation were managed using ARM software.

Treelings were transplanted following the initial applications as designated A in Table 1. Subsequent applications (B through E) were applied as designated in Table 1 after transplanting. A HOBO logger was installed in the glasshouse to monitor ambient temperatures and relative humidity throughout the trial period. Soil samples were assessed for the presence of *P. cinnamomi*. Transplanted trees were drenched with 1 L water with or without Brilliant® as per respective treatments described in Table 1. Trees were placed under drip irrigation delivering 2 L/h and watered as required for 5 min every second day. Wet and dry cycles were created during treatment application days where treatments were drenched into the soil with 1 L water per tree.

Root rot foliar assessments for the glasshouse trial: The incidence and severity of root rot symptoms on the foliage were determined with the use of a severity key (CIBA Geigy ratings, Fig. 2) and as a visual percentage rating of disease symptoms (small yellow leaves, staghorning, wilted leaves, brown tips, lack of new growth). Severity ratings were conducted on three replicates 70 days after planting as well as on six replicates 124 days after planting. Chlorophyll readings were taken from the 4th leaf from the top of each tree and marked for future assessments. A total of two chlorophyll readings were taken, 48 days and 102 days after planting. The total number of leaves per plant was counted 34 days after planting, as well as 70 days after planting for the first three replicates and 124 days after planting for the final six replicates. The percentage leaf area was assessed by harvesting all the leaves per tree and placing them on a 100% grid with 100 squares of 5 cm x 5 cm. These assessments on leaf area along with leaf fresh weight (g) were conducted 70 days after transplanting for the first three replicates and 124 days after transplanting for the remaining six replicates.

Root rot assessments on roots for the glasshouse trial: Roots (2 g randomly selected) from the trees in the glasshouse trial were assessed for the presence of *P. cinnamomi* with the use of Pocket diagnostics lateral flow devices (Forsite Diagnostics Ltd t/a Abingdon Health) at the 70-day and 124-day post-transplant assessments. Root rot severity was assessed on a scale from 1 to 3, where 1 is healthy with abundant feeder roots present and 3 very little feeder roots present with black roots more abundant. The fresh weight of the roots (g) was also measured after removal of the soil.

Figure 2: Severity key based on the CIBA Geigy ratings where Class 1 = Very healthy no decline; Class 2 = Healthy no decline; Class 3 = Early decline; Class 4 = Early decline to moderate; Class 5 = Moderate decline; Class 6 = Moderate to severe decline; Class 7 = Severe decline; Class 8 = Very severe decline; Class 9 = Almost denuded; Class 10 = Complete denuded (Salgadoe *et al*., 2018).

Plant vigour and growth assessments for the glasshouse trial: Stem length was measured from the graft union to the tip of the longest shoot, four days after transplanting. This measurement was repeated at the respective assessments 70 days after planting and 124 days after planting. The stem circumference was measured 2 cm above the graft union at 70 and 124 days after transplanting for three and six replicates respectively. Tree vigour was visually assessed compared to the untreated control on a scale from 1 (bad) to 5 (good) at the previously mentioned assessment dates.

Phytotoxicity assessments for the glasshouse trial: The presence of any visual phytotoxicity was assessed and assigned a 2 (two) for present and a 1 (one) for absent.

Three field trials were allocated to independent CRO's in old avocado orchards confirmed to have *P. cinnamomi*. The same treatments were included as allocated to the glasshouse trial. Trykosist® was integrated with chemical solutions to investigate the efficacy as part of an IPM strategy. Brilliant® was applied as a chemical partner for Trykosist® and Extrasol®, providing a "knock-down" of *Phytophthora* spp. populations. Zeba® was included to assist with soil moisture retention, while *T. harzianum* acts as a natural competitor and *Bacillus* species improve plant vigour and soil health. All trials were laid out in a RCBD that consisted of four replicates per treatment, with each replicate represented by two trees. Efficacy and selectivity assessments as set out for the glasshouse trials were also designated for field trial evaluations.

RESULTS AND DISCUSSION

Excellent efficacy results were obtained against Phytophthora root rot in established orchards with the application of Trykosist® and Brilliant®, as indicated in Figure 3. Similar levels of efficacy were observed compared to the application of potassium phosphite. Although resistance to phosphite has been reported for *P. cinnamomi*, good results were observed with the combination of Trykosist® and Brilliant®, resulting in fewer phosphite applications and the same level of control. A very low MRL for phosphonic acid (<4 ppm phosphonic acid) was obtained at harvest. Although not statistically different, a slight increase in yield was also observed with the Brilliant® followed by Trykosist® treatment (Fig. 4). The combined efficacy results and the low MRLs indicate that Brilliant® can be applied as a drench application during the spring root flush.

The glasshouse trial focussed on establishing a good root system and tree vigour at transplanting before infection of *Phytophthora*. Similar efficacy was expected compared to the established orchard results which addressed an established *Phytophthora* infection. The addition of Zeba® was to improve the expected outcome as it aids in regulating the soil moisture in the root zone, essentially decreasing the wet-dry cycles within the root zone that are conducive to zoospore development. The glasshouse trial for proof of concept was planted on 8 October 2021.

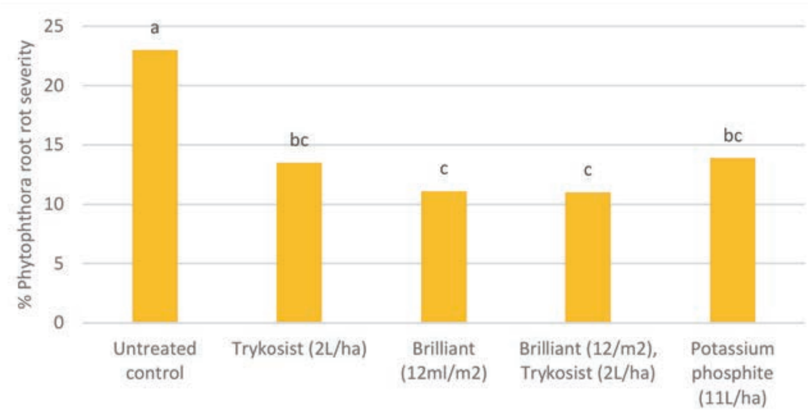


Figure 3: Percentage Phytophthora root rot severity observed on avocado trees in established orchards after a seven-month period. Trykosist® alone to a total of five applications at a 14- to 28-day application interval compared to Brilliant® applied to a total of three applications at 28-day intervals and Trykosist® in combination with Brilliant® set out as an initial Brilliant® application followed by a Trykosist® application, both repeated 14 days later with a final Trykosist® application after 28 days. Data represented here include a summary across trials of 10 trials conducted in 4 climatic regions, including 3 cultivars. Potassium phosphite included as a standard. LSD 2.9 at 5% with 25.38% CV.

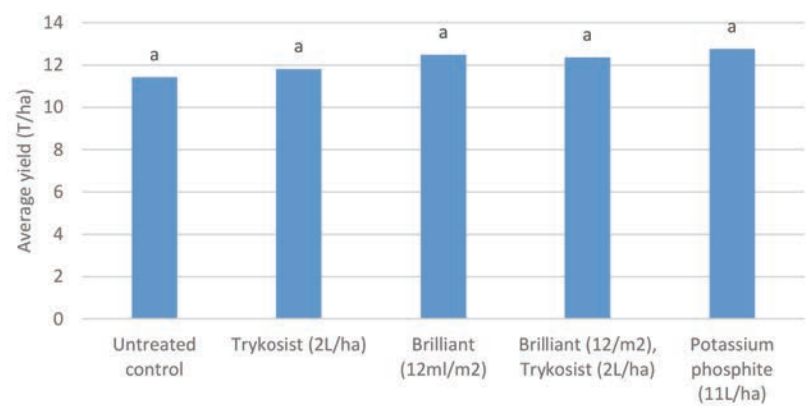
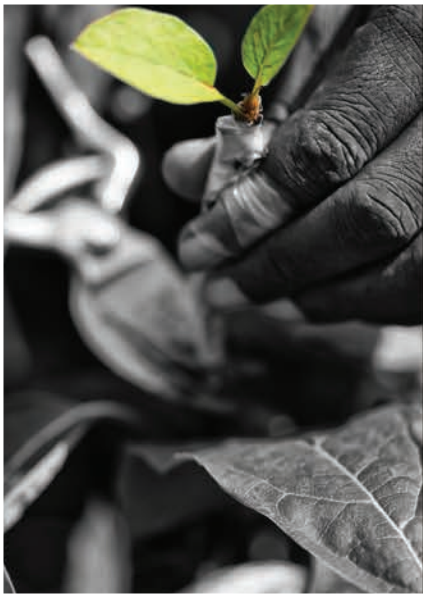


Figure 4: Average yield observed on avocado trees in established orchards after a seven-month period. Trykosist® alone to a total of five applications at a 14- to 28-day application interval compared to Brilliant® applied to a total of three applications at 28-day intervals and Trykosist® in combination with Brilliant® set out as an initial Brilliant® application followed by a Trykosist® application, both repeated 14 days later with a final Trykosist® application after 28 days. Data represented here include a summary across trials of 10 trials conducted in 4 climatic regions, including 3 cultivars. Potassium phosphite included as a standard. LSD 0.6 at 5% with 5.39% CV.



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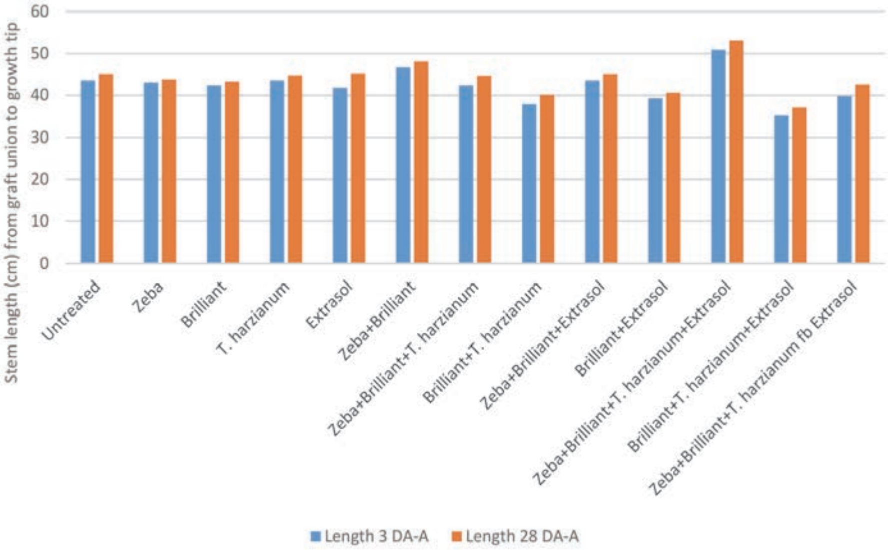

Figure 5: Stem length from graft union to growth tip of the avocado trees in glasshouse 3 and 28 days after transplanting. These data are presented after only applications A and B were completed (28 DA-A). No statistical differences at LSD 8.1 (5%) with 20.44% CV for 3 DA-A and LSD 8.89 (5%) with 21.45% CV for 28 DA-A.

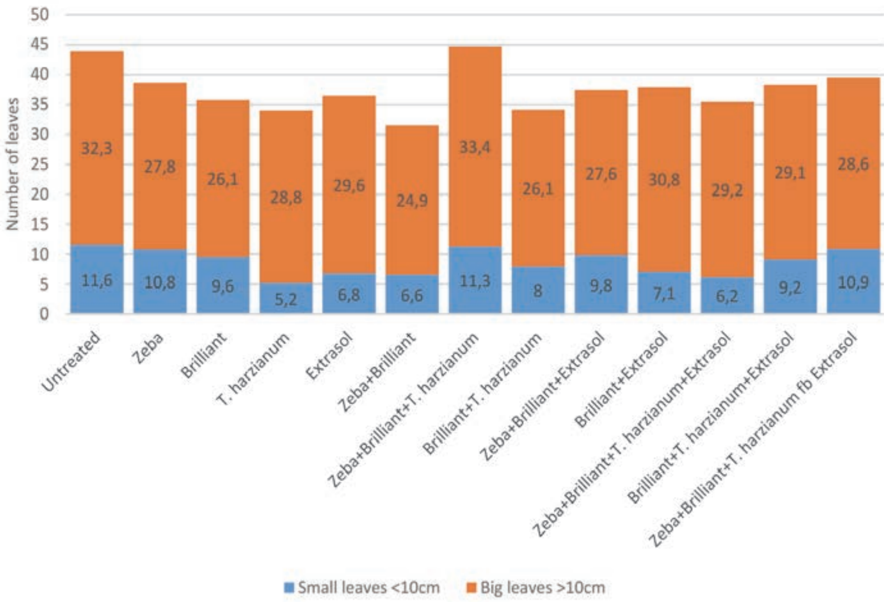

Figure 6: Number of leaves smaller than and bigger than 10 cm from petiole to leaf tip of the avocado trees in the glasshouse 34 days after transplanting. These data are presented after only applications A, B and C were completed. No statistical differences at LSD 4.82 (5%) with 59.32% CV for small leaves and LSD 7.25 (5%) with 26.92% CV for big leaves.

Soil from an avocado orchard in Nelspruit with a history of *P. cinnamomi* was obtained for transplanting.

Initial results were gathered. Three days after transplanting, tree length was measured from graft union to growth tip. This was repeated one month after transplanting (Fig. 5). The number of leaves per tree was also counted 34 days after transplant and designated as small (<10 cm from petiole to tip) and large (>10 cm from petiole to tip) as can be seen in Figure 6. During these measurements three of the treatment applications have been made as per protocol. Although no statistical differences were observed at this early evaluation, it appeared that the IPM strategy including all four products Zeba® plus Brilliant® followed by four applications of Trykosist® and Extrasol® applied as tank-mix, shows the most promise in terms of growth, while in the Zeba® plus Brilliant® followed by four applications of Trykosist® treatment the most total leaves were counted.

Typical black roots with little feeder roots were

Figure 7: Black avocado roots with few feeder roots often indicative of Phytophthora root rot observed upon assessment of the glasshouse trial.

Figure 8: Positive (two vertical stripes) and negative (one vertical stripe at C) reactions observed with the pocket diagnostic lateral flow device for the presence of *Phytophthora* in roots displaying symptoms of Phytophthora root rot.

observed on the trees from the untreated control pots of the glasshouse trial (Fig. 7) at the second assessment conducted 124 days after planting. The presence of *P. cinnamomi* in these roots were confirmed with the use of Pocket diagnostics lateral flow devices specific to *Phytophthora* (Fig. 8). Seventy percent of the sampled roots in the untreated control were infected, while 100% of the sampled roots in the treatment only receiving Extrasol® were infected (Fig. 9).

The lowest Phytophthora root rot severity observed on the roots in the glasshouse trial was in the Zeba® plus Brilliant® followed by Trykosist® treatment and the Brilliant® followed by tank-mix applications of Extrasol® and Trykosist® treatment (Fig. 10). This severity was almost three times less compared to that observed in the untreated controls. Although the severity observed on the roots in the Zeba® plus Brilliant® followed by alternating applications of Extrasol® and Trykosist® treatment was just below 50%, the incidence (Fig. 11) and severity (Fig. 12) of Phytophthora root rot observed on the foliage were much lower compared to the untreated controls.

Although the same percentage infection was observed in the sampled roots of the untreated control and the combination treatments of Brilliant® followed by tank-mix applications of Extrasol® and Trykosist®, and Zeba® plus Brilliant® followed by alternating applications of Extrasol® and Trykosist®, lower Phytophthora root rot severity and incidence were observed in the combination treatments.

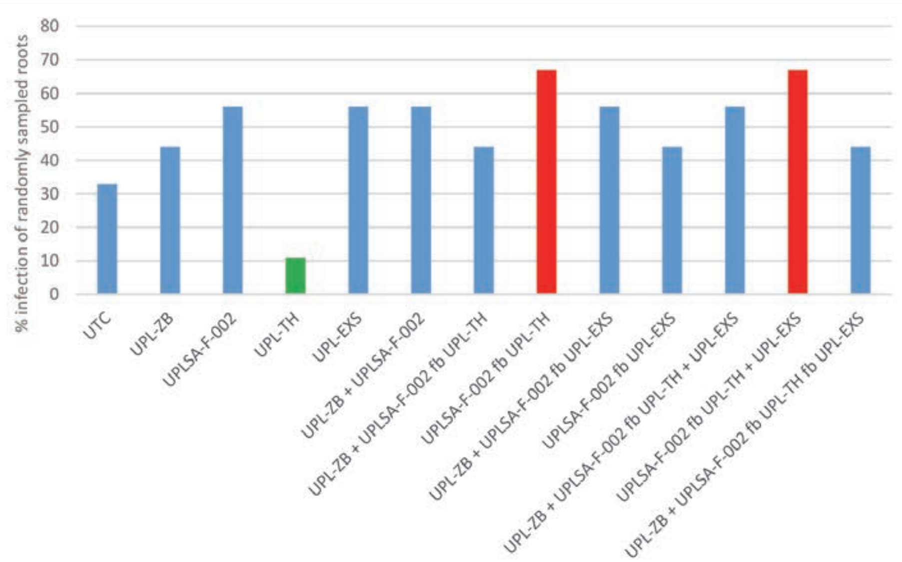

Figure 9: Percentage of randomly sampled roots infected with *Phytophthora* per treatment. Red bars indicate the highest infection, while the green bar indicates the lowest infection. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist® and UPL-EXS is Extrasol®. No statistical differences at LSD 47.79 (5%) with 104.84% CV.

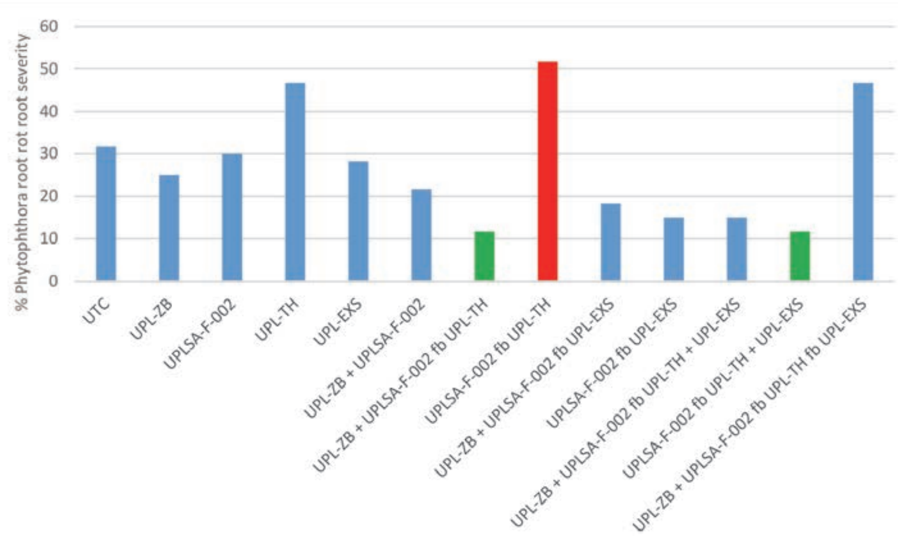

Figure 10: Percentage severity of Phytophthora root rot observed on the roots per treatment. The red bar indicates the highest severity while the green bars indicate the lowest severity observed in the Zeba® (UPL-ZB) plus Brilliant® (UPLSA-F-002) followed by Trykosist® (UPL-TH) treatment and Brilliant® followed by tank-mix applications of Extrasol® (UPL-EXS) and Trykosist®. No statistical differences at LSD 30.05 (5%) with 95.74% CV.

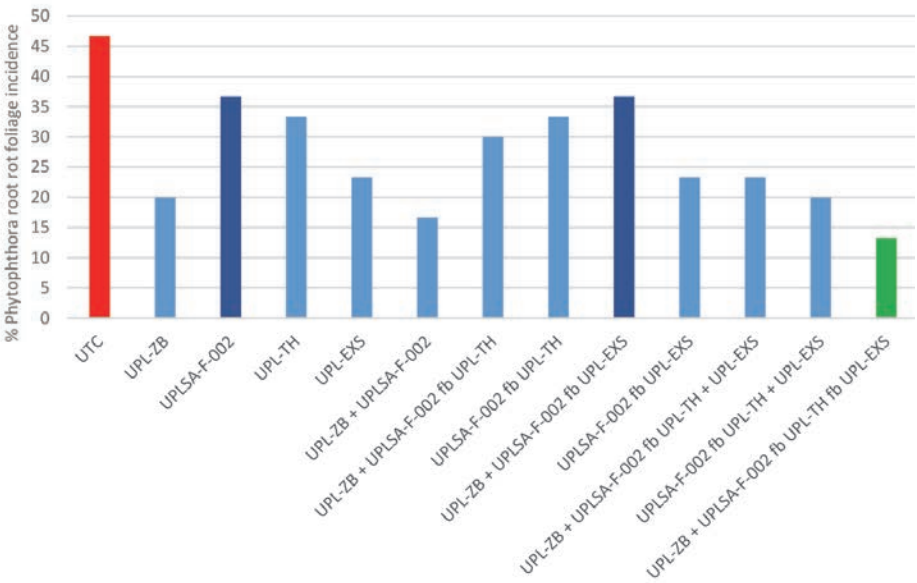

Figure 11: Percentage incidence of Phytophthora root rot observed on the foliage per treatment. The red bar indicates the highest severity while the green bar indicates the lowest severity observed in the Zeba® (UPL-ZB) plus Brilliant® (UPLSA-F-002) followed by alternating applications of Extrasol® (UPL-EXS) and Trykosist® (UPL-TH) treatment. No statistical differences at LSD 20.91 (5%) with 65.98% CV.

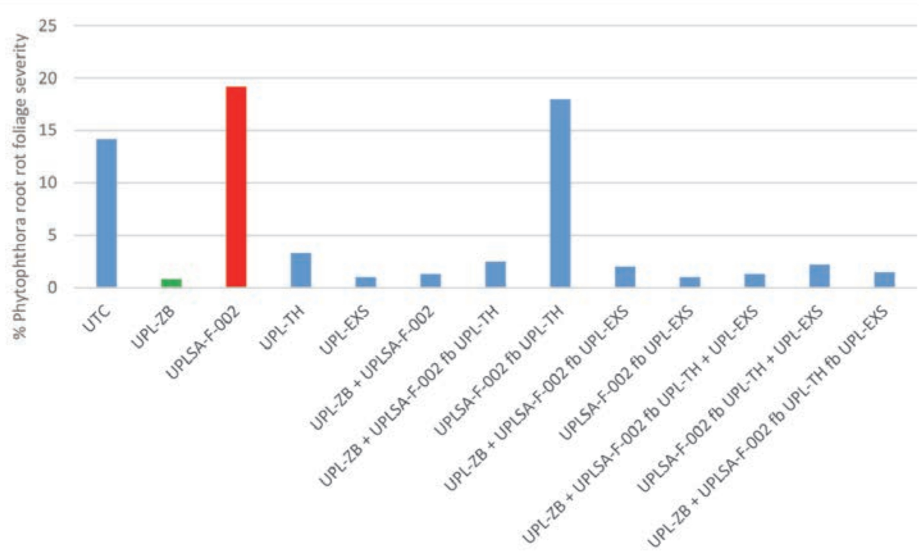

Figure 12: Percentage Phytophthora root rot severity observed on the foliage per treatment. The red bar indicates the highest severity while the green bar indicates the lowest severity. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist® and UPL-EXS is Extrasol®. No statistical differences at LSD 18.29 (5%) with 301.35% CV.

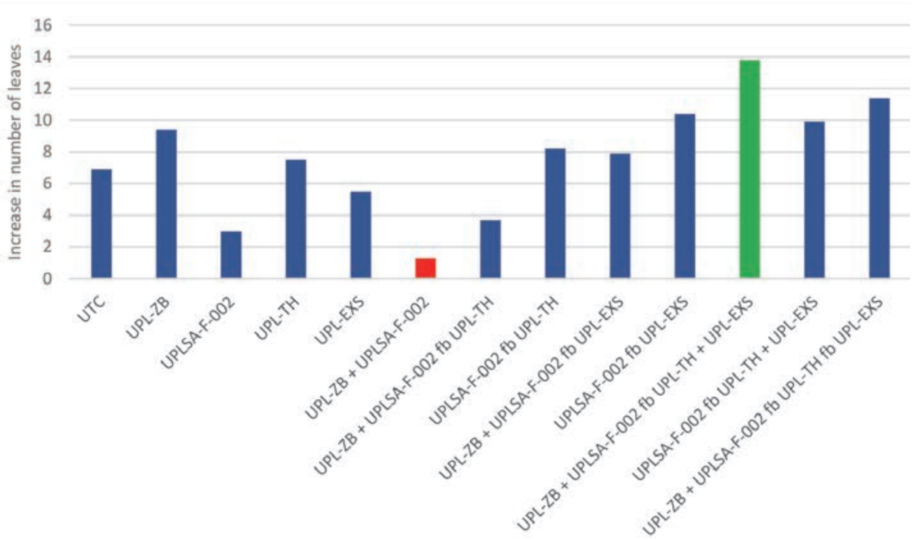

Figure 13: Increase in average number of leaves per treatment from planting to final harvest. The red bar indicates the least amount of increased growth while the green bar indicates the most increase in growth when considering the number of leaves per tree. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist® and UPL-EXS is Extrasol®. No statistical differences at LSD 14.86 (5%) with 119.88% CV.

No clear correlation could be observed in the root or foliage fresh weight in relation to Phytophthora root rot symptoms in the various treatments. During the trial there was no visual phytotoxicity observed on any of the trees. A slight increase in chlorophyll content was observed throughout the plants in the glasshouse trial with no specific trend observed. A noticeable effect on chlorophyll content within three months of the glasshouse trial period might be too soon.

The combination treatments where all four products were included resulted in increased numbers of leaves and stem growth (Figs. 13 and 14). In the combination treatment receiving Zeba® plus Brilliant® followed by tank-mix applications of Extrasol® and Trykosist®, a two-fold increase was observed in the number of leaves compared to the untreated control. Similarly, a bigger percentage leaf area was observed in the combination treatments compared to the untreated controls. A higher increase in stem length was observed in the combination treatments including all four products. The overall vigour of the trees also increased as more products were added into the programme with the most vigour observed in the Brilliant® followed by tank-mix applications of Extrasol® and Trykosist® and Zeba® plus Brilliant® followed by alternating applications of Extrasol® and Trykosist® combination treatments (Fig. 15). This indicated a bio-stimulating effect from the products on plant development even when confronted with *P. cinnamomi*. This suggests that Zeba®, Brilliant®, Trykosist® and Extrasol® used in a programme as set out in the glasshouse will result in a stronger plant grown within a stressful environment.

Based on the good trend observed in favour of the three combination treatments, the statistical analyses were repeated only including the untreated control and the three combination treatments, Zeba® plus Brilliant® followed by four applications of Trykosist®

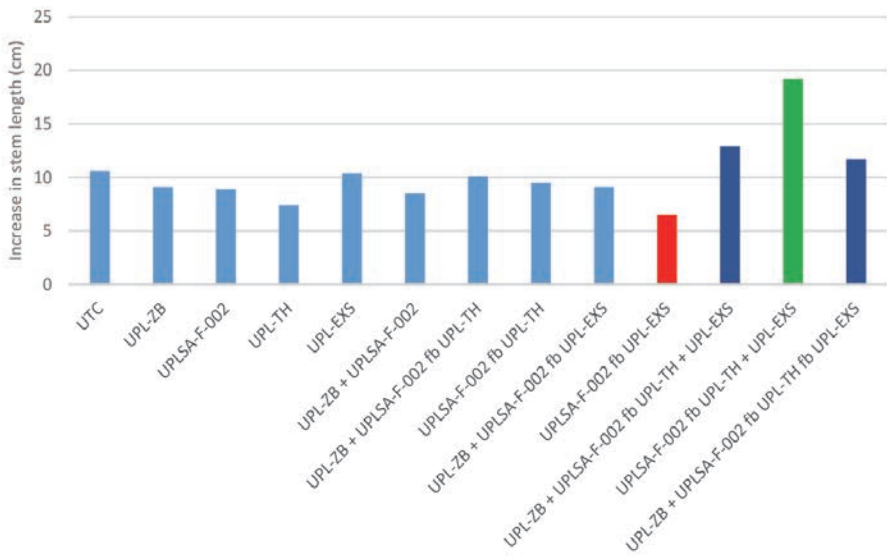

Figure 14: Increase in average stem length per treatment from planting to final assessment. The red bar indicates the least amount of increased growth while the green bar indicates the most increase in growth when considering stem length from graft union to longest shoot tip. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist® and UPL-EXS is Extrasol®. No statistical differences at LSD 14.39 (5%) with 103.65% CV.

Figure 15: Overall increased vigour observed in the combination treatments compared to the untreated control treatments where T1 is the untreated control, T2 is Zeba® applied alone, T3 is Brilliant® applied alone, T4 is Trykosist® applied alone, T5 is Extrasol® applied alone, T6 is Zeba® plus Brilliant®, T7 is Zeba® plus Brilliant® followed by four applications of Trykosist®, T8 is Brilliant® followed by four applications of Trykosist®, T9 is Zeba® plus Brilliant® followed by four applications of Extrasol®, T10 is Brilliant® followed by four applications of Extrasol®, T11 is Zeba® plus Brilliant® followed by four applications of Trykosist® and Extrasol® in tank-mix, T12 is Brilliant® followed by four applications of Trykosist® and Extrasol® in tank-mix, and T13 is Zeba® plus Brilliant® followed by alternating applications of Trykosist® and Extrasol® to a total of two applications Trykosist® and Extrasol® each.

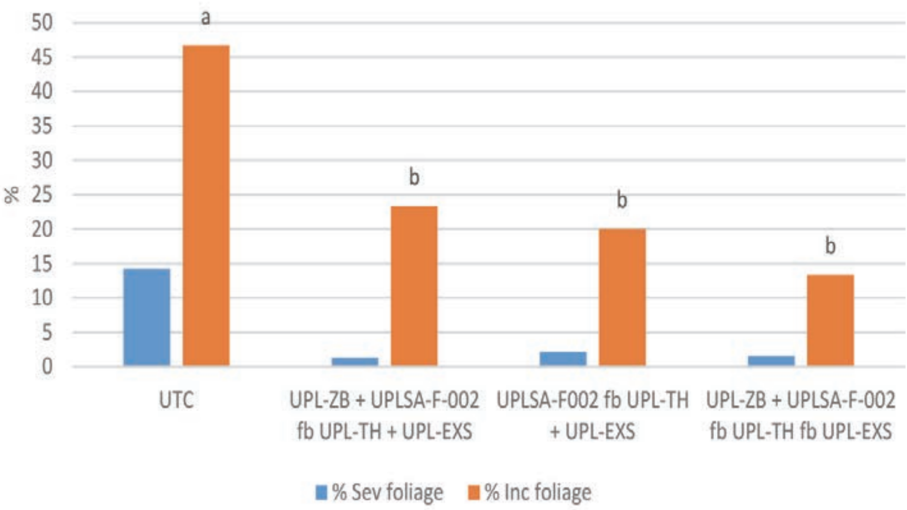

Figure 16: Percentage incidence (Inc foliage with LSD of 19.37 at α=5%, CV: 15.74%) and severity (Sev foliage with LSD of 24.33 at α=5%, CV: 19.77%) observed on the foliage in the combination treatments, Zeba® (UPL-ZB) plus Brilliant® (UPLSA-F-002) followed by four applications of Trykosist® (UPL-TH) and Extrasol® (UPL-EXS) in tank-mix, Brilliant® followed by four applications of Trykosist® and Extrasol® in tank-mix, and Zeba® plus Brilliant® followed by alternating applications of Trykosist® and Extrasol® to a total of two applications Trykosist® and Extrasol® each, compared to the untreated control.

Project timeline

Month	Mar-22	Apr-22	May-22	Jun-22	Jul-22	Aug-22	Sep-22	Oct-22	Nov-22	Dec-22
Field trials										
Initiation of field trials season 1										
First evaluation										
Second evaluation										
Final evaluation										
Initiation of field trials season 2										
First evaluation										
Second evaluation										
Final evaluation										

and Extrasol® in tank-mix; Brilliant® followed by four applications of Trykosist® and Extrasol® in tank-mix; and Zeba® plus Brilliant® followed by alternating applications of Trykosist® and Extrasol® to a total of two applications Trykosist® and Extrasol® each. This resulted in a total of four treatments with six replicates each and so a total of 15 degrees of freedom considering a CRBD. The results for the analyses indicated that all three combinations significantly decreased the Phytophthora root rot incidence compared to the untreated control and resulted in lower severity observed on the foliage (Fig. 16).

Varying results emphasise that trials should be repeated under field conditions. A trend is observed in favour of the IPM treatments in the established orchards trials as well as in the greenhouse and should give rise to clear results following field trials. The biological remedy registration for Trykosist® is currently pending, as is the label extension registration for the use of Brilliant® in avocado against Phytophthora root rot.

The results obtained from the greenhouse study give a good trend observed in a short amount of time that suggest the IPM programme as proposed will aid in supporting new trees planted into old avocado orchards. The information obtained is critical for the second phase of the project where the IPM strategy is assessed under field conditions. Sites have been identified in different climatic regions and trials are already underway. The first evaluations were expected in June 2022 and trials will be assessed at 4, 6 and 12 months after planting. Trials are set out as to generate data for registration.

Good levels of efficacy were observed with the combination of Ammonium phosphite and Trykosist®, a proprietary bio-fungicide from UPL, in a programme against Phytophthora root rot in established orchards. Promising results were also obtained from the holistic root health approach in the greenhouse transplanting trial. Ongoing field efficacy trials would inform on the optimal programme for the management of Phytophthora root rot at transplanting.

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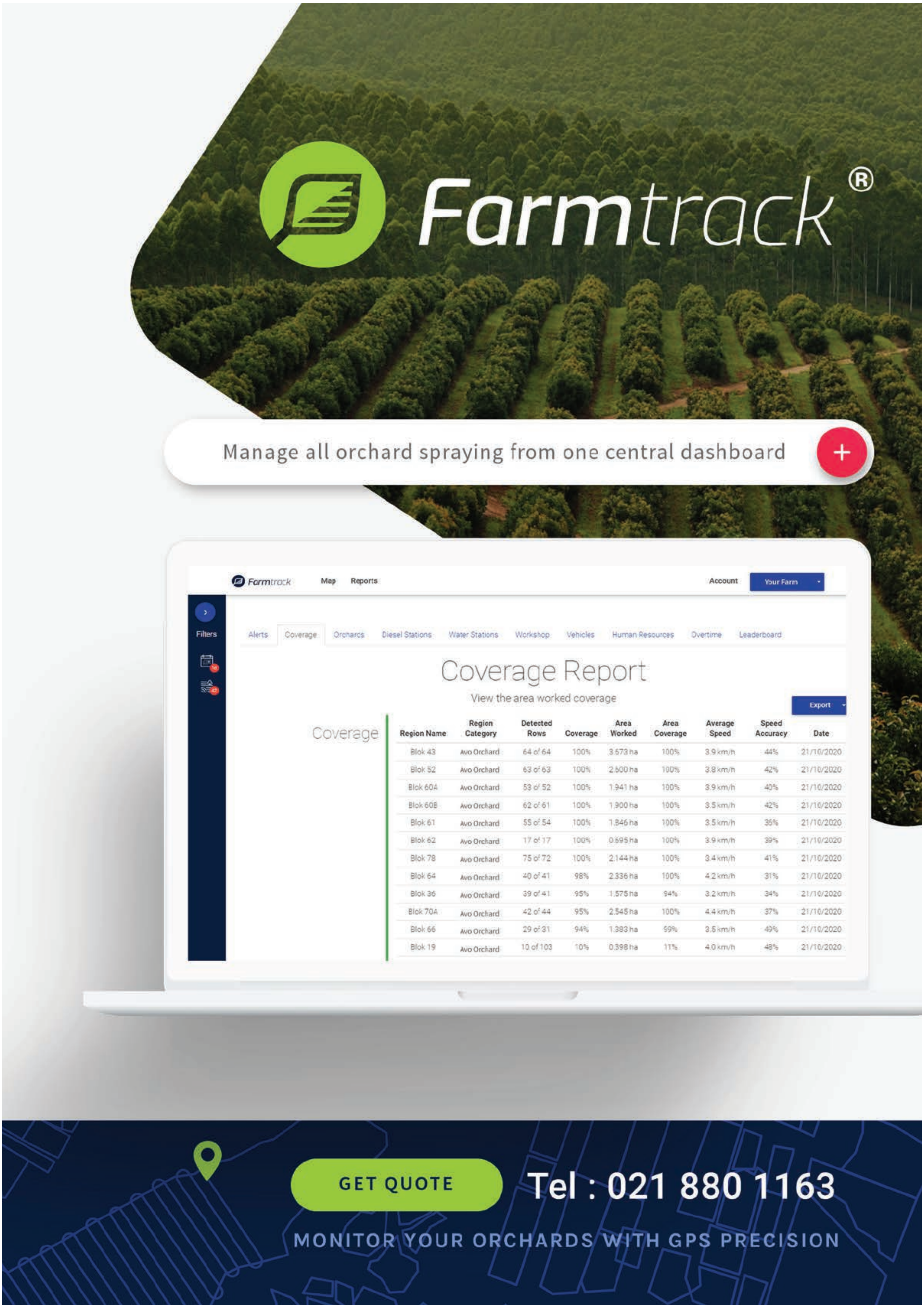

Region Name	Region Category	Detected Rows	Coverage	Area Worked	Area Coverage	Average Speed	Speed Accuracy	Date
Blok 43	Avo Orchard	64 of 64	100%	3.573 ha	100%	3.9 km/h	44%	21/10/2020
Blok 52	Avo Orchard	63 of 63	100%	2.600 ha	100%	3.8 km/h	42%	21/10/2020
Blok 60A	Avo Orchard	53 of 52	100%	1.941 ha	100%	3.9 km/h	40%	21/10/2020
Blok 60B	Avo Orchard	62 of 61	100%	1.900 ha	100%	3.5 km/h	42%	21/10/2020
Blok 61	Avo Orchard	55 of 54	100%	1.846 ha	100%	3.5 km/h	36%	21/10/2020
Blok 62	Avo Orchard	17 of 17	100%	0.695 ha	100%	3.9 km/h	39%	21/10/2020
Blok 78	Avo Orchard	75 of 72	100%	2.144 ha	100%	3.4 km/h	41%	21/10/2020
Blok 64	Avo Orchard	40 of 41	98%	2.336 ha	100%	4.2 km/h	31%	21/10/2020
Blok 36	Avo Orchard	39 of 41	95%	1.575 ha	94%	3.2 km/h	34%	21/10/2020
Blok 70A	Avo Orchard	42 of 44	95%	2.545 ha	100%	4.4 km/h	37%	21/10/2020
Blok 66	Avo Orchard	29 of 31	94%	1.383 ha	99%	3.5 km/h	49%	21/10/2020
Blok 19	Avo Orchard	10 of 103	10%	0.398 ha	11%	4.0 km/h	48%	21/10/2020