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

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ABSTRACT

Avocado production is under severe pressure due to the impact of unfavourable environmental/abiotic factors, market-related aspects, pest impacts from various sources, and ever-limiting availability of chemical options due to fruit residue implications. Due to the world-wide 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 occurring 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 being present in nurseries and avocado orchard soils. A holistic root health approach, including biological and chemical options, might be the only sustainable way to address the replant problem. Results indicate that an Integrated Pest Management approach could reduce Phytophthora root rot and increase tree vigour.

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 causes 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 in the fruit always being present. Biological control has been advocated as a solution (Mamani and Aragon, 2018). However, the performance of biocontrol agents may be susceptible to a variety of factors (e.g., climate, soil characteristics, farming practice, etc.), 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 as a result of existing inoculum in the orchards. To address the challenges associated with transplanting a sapling, the use of soil moisture retention products may address climatic challenges related to water availability. Reducing moisture fluctuation extremes 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 sapling vigour and survival.

Several *Trichoderma* spp. including *Trichoderma harzianum*, have been identified as biocontrol agents. These have the ability to suppress *Phytophthora* spp. as well as other soilborne diseases and can serve as a bio-remedy to manage pathogen populations in the soil, promoting sapling growth (Azarmi *et al.*, 2011; Hermosa *et al.*, 2012). *Trichoderma harzianum* can outcompete pathogens for resources, such as root exudates, resulting in antagonistic behaviour (Azarmi *et al.*, 2011; Hermosa *et al.*, 2012). Growth promoting effects can also occur due to the fungus 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 to the tree establishment problem as it utilises the best aspects from both approaches (Coffey, 1987). Phosphorous acid-based products are well known for their multiple 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 registered in South Africa, either as foliar applications or stem injections. Soil 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 is that they can be integrated with the benefits of *Trichoderma* and *Bacillus* species, with which an IPM approach can be developed; whereby the chemical has 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 analogues 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 utilization, one of which is the use of microorganisms. *Bacillus subtilis* is well known for its solubilising efficacy of minerals and providing these 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 is 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. Although registered for its excellent properties in crop establishment on a variety of crops, this product has not been submitted for registration on avocado.

The abiotic stress mitigation of Zeba® during transplant has been established in a variety of crops including tree crops. Due to the nature of the product, water is absorbed and released, unlike other soil retention products on the market, as the plant requires, leading to reduced moisture fluctuation extremes 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 ammonium 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* diseases, however, not on avocado. This product is currently pending registration as a soil application in avocado.

Phosphite containing products have unique biocontrol properties specifically against the Oomycete group of fungi, the group that includes *Phytophthora* spp. 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 quicker response by the host plant against the pathogen. There is also a direct effect on the growth and sporulation of oomycete pathogens.

***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). Consisting of a South African strain specifically selected for its efficacy under local conditions, this liquid-based formulation is intended for application through drench and the irrigation systems.

From peer reviewed literature studies, it can be concluded that *Trichoderma*, depending on species and strain, can induce both plant growth promoting effects on host plants and 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 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 subtillis* HC8, *Bacillus amyloliquefa-

***ciens* subs. *plantarum* CH13** (Extrasol® product code UPL-EXS)
Specific strains of *B. subtillis* and *B. amyloliquefaciens* (UPL IP) were developed for plant growth promotion. This product is currently registered as a Group 3 fertilizer for application as a soil treatment. A liquid-based formulation intended for application through drench or irrigation systems, these bacteria interfere with *Phytophthora* growth and movement of spores, but also induced plant defence responses (Liu *et al.,* 2019).

AIMS AND OBJECTIVES

The aim of the project was to identify an integrated management programme for the establishment of avocado saplings, 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 needed to be achieved:

- Investigate the benefits to crop establishment using a super absorbent starch polymer as a soil moisture management product. Combination of *Trichoderma harzianum* and the starch polymer Zeba® to support sapling development and suppress *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 were applied in a programme. Soil applications of both products were investigated.
- Investigate the use of *Bacillus subtillis* and *Bacillus amyloliquefaciens* on sapling development and vigour stimulation by inducing benefits, as part of a soil/tree health approach.
- Trial protocol included two rootstock varieties, Duke 7 and Edranol.
- Assessment parameters for crop establishment trials included: disease incidence and severity throughout the trial period as well as sapling growth and vigour parameters; and for established orchard trials: disease incidence and severity throughout the trial period as well as tree vigour and yield.

MATERIALS AND METHODS

Established orchard trials

Trykosist® and Brilliant® were 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 in a total of five applications at a 14 to 28-day application interval, was assessed. This programme was compared to Brilliant® applied in a total of three applications at 28-day intervals. Also, Trykosist® in combination with Brilliant® was assessed for efficacy against Phytophthora root rot. The programme was set out with an initial Brilliant® application for its direct and indirect effects 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 water per tree. A total of 10 trials were conducted in four different climatic regions including Bsh, Cfb, Cwa, and Cfa (Pongola, Shakaskraal, Richmond, Nelspruit) on two different cultivars; Hass and Fuerte. Potassium phosphite (350 g/ℓ) was included as a standard.

Trials in established orchards were assessed visually for the percentage of Phytophthora root rot severity and incidence at five assessments during the trial period. The CIBA Geigy rating scale (Fig. 2) 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 respectively treated trees. Phytotoxicity and vigour were also assessed throughout comparing the treated to the untreated plots.

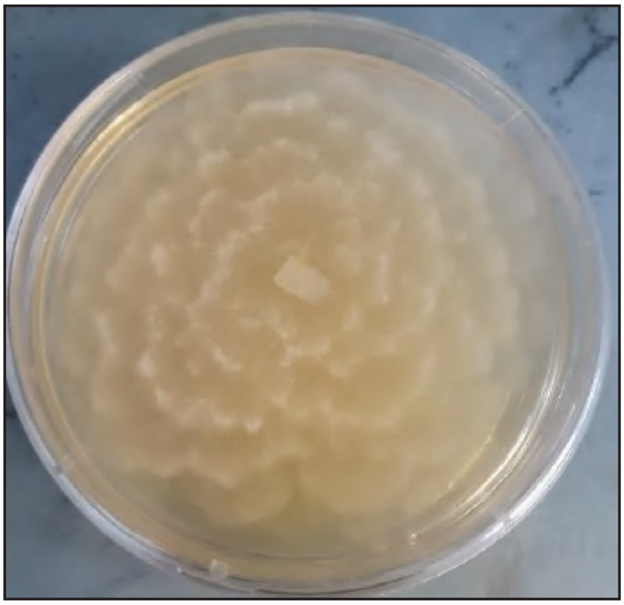

Figure 1: *Phytophthora cinnamomi* isolate obtained from the Agricultural Research Council of South Africa culture collection (PPRI number 4525), used in the glasshouse trials.

Glasshouse trial

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 saplings. Soil for the glasshouse trial was collected from a field with a previous history of Phytophthora root rot as well as 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 into sterilized wheat

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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 starting14 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 plant	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 plant	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 plant	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 plant	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 plant	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 plant	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

to colonize (incubation for three weeks). A total of 5 g of inoculated wheat was added to 10 ℓ soil and mixed before adding to the avocado sapling transplanted into a bigger 20 ℓ potting bag. Zeba® applications were also conducted during this stage, and were mixed with the soil before planting.

Thirteen treatments were included as well as an untreated control. Zeba®, Trykosist®, Extrasol®, and Brilliant® were 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 sapling. Trial protocol, layout, and statistical evaluation were managed using ARM software.

Saplings were transplanted following the initial applications as designated A in Table 1. Subsequent ap-

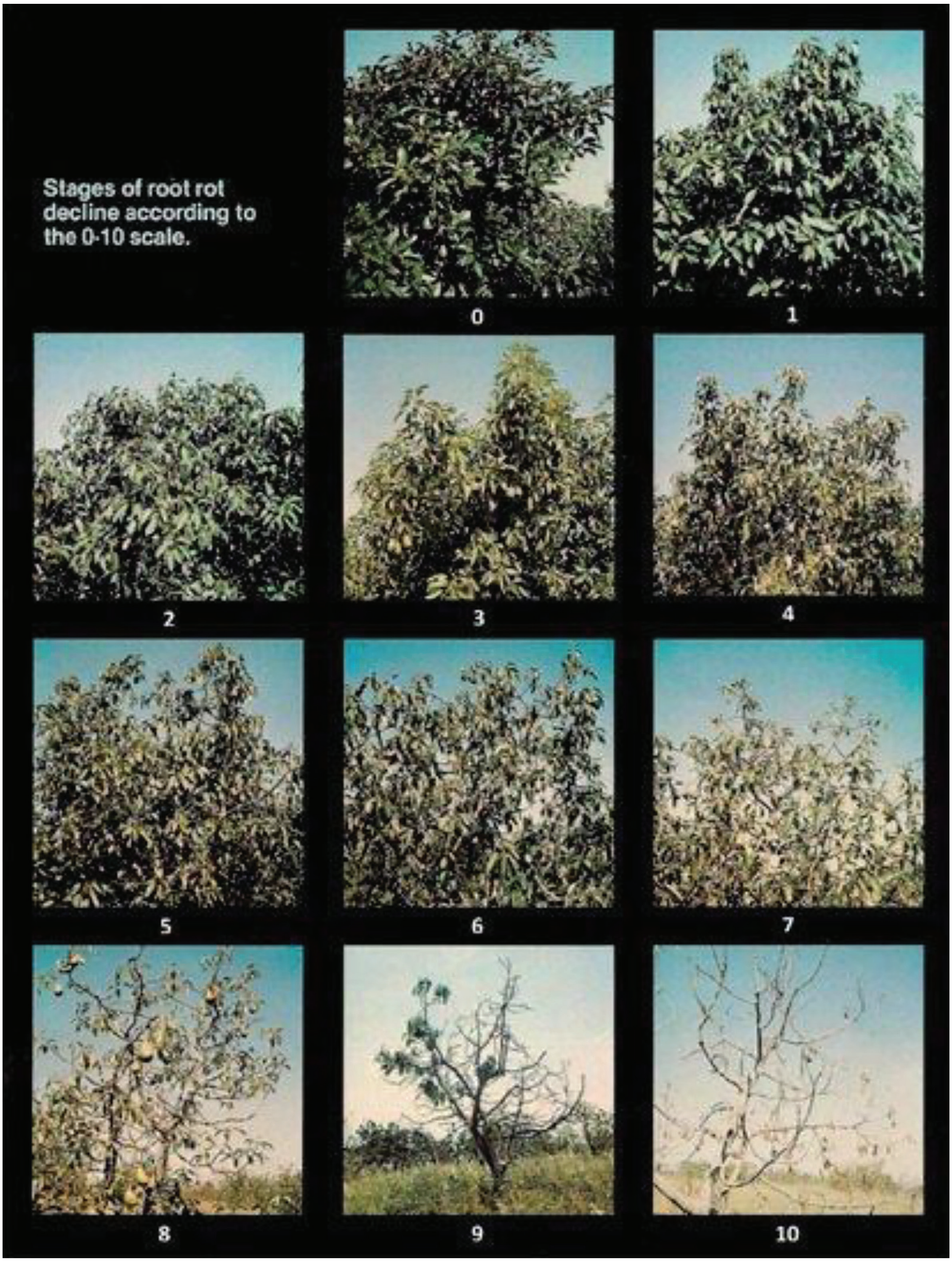

Figure 2: Severity key based on the CIBA Geigy ratings where Class 1 = Very healthy no decline; Class 2 = Healthy no decline; Class 3 = Early decline; Class 4 = Early decline to moderate; Class 5 = Moderate decline; Class 6 = Moderate to severe decline; Class 7 = Severe decline; Class 8 = Very severe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

plications (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 saplings were drenched with 1 ℓ water with or without Brilliant® as per respective treatments described in Table 1. Saplings 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 sapling.

Root rot foliar assessments for the glasshouse trial: The incidence and severity of Phytophthora 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, stag-horning, wilted leaves, brown tips, lack of new growth). Disease 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 transplant for the first three replicates and 124 days after transplant for the remaining six replicates.

Phytophthora 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 one to three where one is healthy with abundant feeder roots present and three is 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.

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 transplant for three and six replicates, respectively. Tree vigour was visually assessed compared to the untreated control on a scale from one (bad) to five (good) at the previously mentioned assessment dates.

Figure 3: The application of the drench treatment to the base of the plant in a 360° circle around the entire plant. (BioScience Research)

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

Transplant orchard trials

Three field trials were allocated to independent CROs in old avocado orchards confirmed to have *P. cinnamomi*. Soil samples were sent in for confirmation. The same treatments were included as allocated to the glasshouse trial (Table 1). 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 management, 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, each replicate represented by two saplings. Post-plant treatments were applied by mixing the products in a stock solution or tank-mix and applied directly to each sapling as indicated in Figure 3.

Field trials were set out in two different climatic regions, on Hass and Fuerte cultivars grafted onto Edranol and Duke 7 rootstocks, respectively. Table 2 indicates the field trials and particular information per trial location.

Phytophthora root rot foliar assessment: The incidence and severity of Phytophthora root rot symptoms were determined, with the use of the severity key CIBA Geigy ratings on a scale of 1 to 10. Where possible, chlorophyll readings were taken on three

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Table 2: Information on field trial sites

Trial ID	B21SA-001-019-021	B21SA-001-019-022 (021-B)	B21SA-001-019-041
Area	eThekwini (KwaZulu-Natal)	Richards Bay (KwaZulu-Natal)	Hoedspruit (Limpopo)
Cultivar	Hass	Hass	Fuerte
Rootstock	Edranol	Edranol	Duke 7
Initiated	20/04/2022	25/04/2022	31/08/2022
Plot size	3 m x 3 m	3 m x 3 m	6 m x 1 m
Ambient temperature applications	16-27 °C	17-22 °C	27-35 °C
Soil temperature applications	23-24 °C	23 °C	18-25 °C
Mix size	2 ℓ	2 ℓ	10 ℓ
Sand	13	23	75
Silt	8	7	6
Clay	23	16	19
pH	5.21	5.1	5.1
CEC	12.86	12.01	5.84

Table 3: Root Damage Rating (1-5)

Rating	Description
1	Healthy roots
2	1 - 25% damage
3	26 - 50% damage
4	51 - 75% damage
5	76 - 100% damage

Table 4: Crop Vigour Rating (1-10)

Rating	Description
1	Crop dead
2	Crop nearly dead
3	Very bad crop condition
4	Bad crop condition
5	Unsatisfactory crop condition
6	Moderate crop condition
7	Reasonable crop condition
8	Good crop condition
9	Very good crop condition
10	Excellent crop condition

leaves per tree sampled at random on the same side of each tree and marked for future assessments; 2, 4, and 6 months after transplant. Foliar disease assessments were conducted 2, 4, and 6 months after transplant. At the final assessment, the number of leaves per tree was counted for each replicate and weighed without drying.

Phytophthora root rot root assessment: Four months after transplant, a single core of soil and root mass was removed from the top 20 cm and 20 cm from the stem to determine the volume of roots per replicate. After six months, one tree per replicate was removed and assessed for root damage on a scale from 1 to 5 (Table 3).

Plant vigour and growth: The stem circumference was measured 2 cm above the graft union, six months after transplant. Stem length from the graft union to the tallest growth point was also measured at six months. Tree vigour was determined on a scale from 1 to 10 at 2, 4, and 6 months after transplant (Table 4).

Phytotoxicity: Visual phytotoxicity was noted 14 days after each application.

RESULTS AND DISCUSSION

Established orchard trials

Excellent efficacy results were obtained against Phytophthora root rot in established orchards with the application of Trykosist® and Brilliant®, as indicated in Figure 4. 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. Very low residue levels for phosphonic acid (<4 ppm phosphonic acid) were obtained at harvest. Although not statistically different, a slight increase in yield was also observed with the Brilliant® followed by Trykosist® treatment (Fig. 5). The combined efficacy results and the low MRLs indicate that Brilliant® can be applied as a drench application during spring root flush.

Glasshouse trial

The glasshouse trial focused on establishing a good root system and tree vigour at transplanting before infection of *P. cinnamomi*. Similar efficacy was expected compared to the established orchard results

Table 5: Phytotoxicity rating (1-9)

Rating	Description	Plant Tolerance
1	0-10%	No effect
2	11-20%	Very slight effects: some stunting and yellowing just visible
3	21-30%	Slight effects: stunting and yellowing obvious; effects reversible
4	31-40%	Substantial chlorosis and/or stunting: most effects probably reversible
5	41-50%	Strong chlorosis/stunting; thinning of stand
6	51-60%	Increasing severity of damage
7	61-70%	Increasing severity of damage
8	71-80%	Increasing severity of damage
9	81-100%	All plants dead

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

Initial results were gathered. Three days after transplant, tree length was measured from graft union to growth tip. This was repeated one month after transplanting (Fig. 6). 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 7. During these measurements three of the treatment applications were 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, showed 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 observed on the trees from the untreated control pots of the glasshouse trial (Fig. 8), at the second assess-

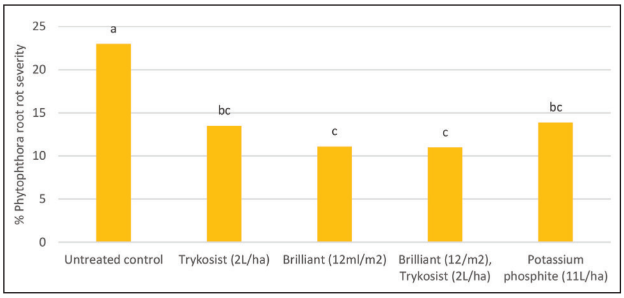

Figure 4: Percentage Phytophthora root rot severity observed on avocado trees in established orchards after a seven-month period. Trykosist® alone to a total of five applications at a 14 to 28-day application interval compared to Brilliant® applied to a total of three applications at 28-day intervals 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 different climatic regions including 3 different cultivars. Potassium phosphite was included as a standard. LSD 2.9 at 5% with 25.38% CV. Values with the same letter do not differ significantly.

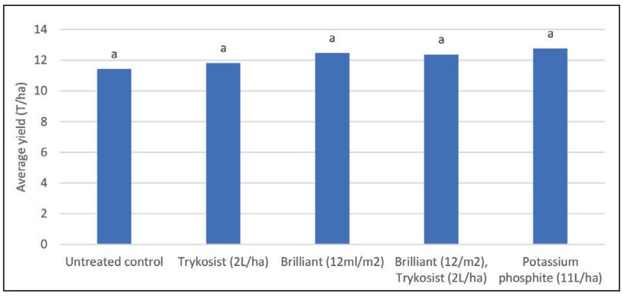

Figure 5: Average yield observed on avocado trees in established orchards after a seven-month period. Trykosist® alone to a total of five applications at a 14 to 28-day application interval compared to Brilliant® applied to a total of three applications at 28-day intervals and Trykosist® in combination 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 different climatic regions including 3 different cultivars. Potassium phosphite was included as a standard. LSD 0.6 at 5% with 5.39% CV. Values with the same letter do not differ significantly.

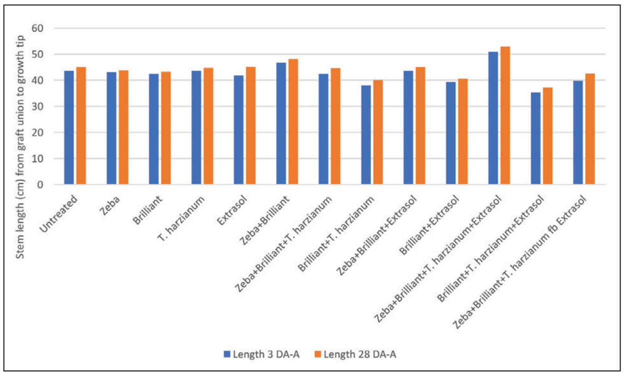

Figure 6: Stem length from graft union to growth tip of the avocado trees in glasshouse 3 and 28 days after transplant. These data represented after only applications A and B were completed (28 DA-A). No statistical differences at LSD 8.1 (5%) with 20.44% CV for 3 DA-A and LSD 8.89 (5%) with 21.45% CV for 28 DA-A.

ment conducted 124 days after planting. The presence of *P. cinnamomi* in these roots was confirmed with the use of Pocket diagnostics lateral flow devices specific to *Phytophthora* (Fig. 9). Thirty-three percent of the sampled roots in the untreated control were infected, while only 11% of the roots sampled in the treatment that only received Trykosist® were infected (Fig. 10). The varying results were related to the sampling method, although all treatments indicated some level of infection and as such indicated that inoculation was successful due to the presence of the pathogen in each of the treatments.

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. 11). This disease 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 were just below 50%, the incidence (Fig. 12) and severity (Fig. 13) of Phytophthora root rot observed on the foliage were much lower compared to the untreated controls.

Although a similar percentage infection was observed in the sampled roots of the untreated control

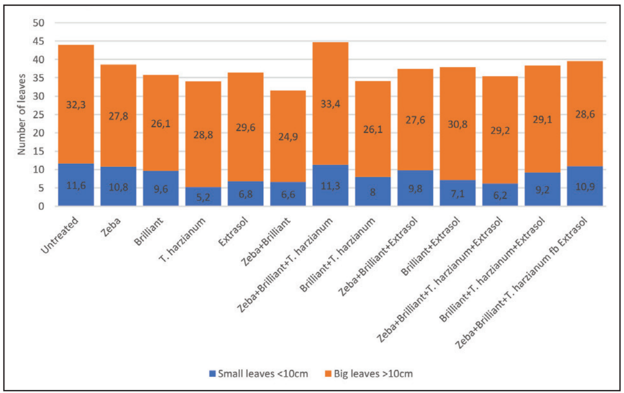

Figure 7: Number of leaves smaller than and bigger than 10 cm from petiole to leaf tip of the avocado trees in glasshouse 34 days after transplant. These data represented after applications A, B, and C were completed. No statistical differences between treatments at LSD 4.82 (5%) with 59.32% CV for small leaves and LSD 7.25 (5%) with 26.92% CV for big leaves.

Figure 8: Black avocado roots with few feeder roots, often indicative of Phytophthora root rot observed upon assessment of the glasshouse trial.

Figure 9: 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.

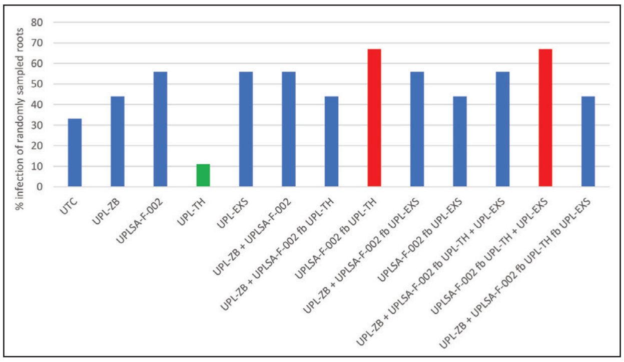

Figure 10: Percentage of randomly sampled roots infected with *Phytophthora* per treatment. Red bar indicates the highest infection, while the green bar indicates the lowest percentage infection. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist®, and UPL-EXS is Extrasol®. No statistical differences between treatments at LSD 47.79 (5%) with 104.84% CV.

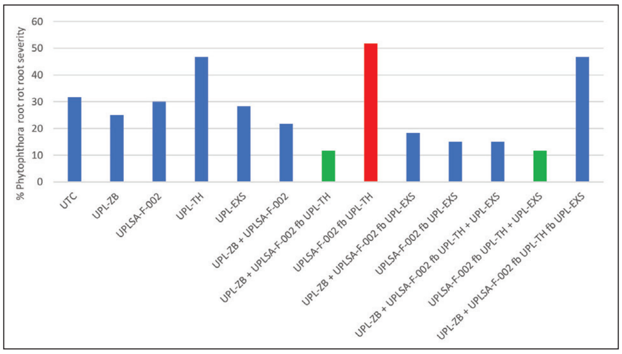

Figure 11: Percentage severity of Phytophthora root rot observed on the roots per treatment. Red bar indicates the highest severity while the green bars indicate the lowest severity observed in the Zeba® (UPL-ZB) plus Brilliant® (UPLSA-F-002) followed by Trykosist® (UPL-TH) treatment and Brilliant® followed by tank-mix applications of Extrasol® (UPL-EXS) and Trykosist®. No statistical differences at LSD 30.05 (5%) with 95.74% CV.

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and the combination treatments, Brilliant® followed by tank-mix applications of Extrasol® and Trykosist®, and Zeba® plus Brilliant® followed by alternating applications of Extrasol® and Trykosist®, lowered Phytophthora root rot severity and incidence observed in the combination treatments. These results showed that tree health improved when applying these irrespective of the primary infection.

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 in any of the treatments. A slight increase in chlorophyll content was observed throughout the plants in the glasshouse trial with no specific trend observed. Also achieving a noticeable effect on improving the chlorophyll content within three months of the glasshouse trial period might be too soon with respect to a data collection point.

The combination treatments where all four products were included resulted in an increased number of leaves and stem growth (Figs. 14 and 15). In the combination treatment where Zeba® plus Brilliant® followed by tank-mix applications of Extrasol® and Trykosist® was applied, a two-fold increase was observed in the number of leaves compared to the untreated control. Similarly, the 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 or combinations of 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. 16). This indicated a bio-stimulating effect from the products on plant development even when confronted with *P. cinnamomi.* In conclusion, 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 (biotic and abiotic) environment.

Based on the good trend observed in favour of the

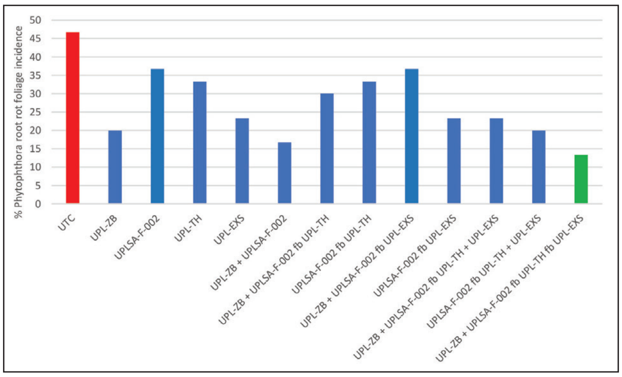

Figure 12: Percentage incidence of Phytophthora root rot observed on the foliage per treatment. Red bar indicates the highest severity while the green bar indicates the lowest severity observed in the Zeba® (UPL-ZB) plus Brilliant® (UPLSA-F-002) followed by alternating applications of Extrasol® (UPL-EXS) and Trykosist® (UPL-TH). No statistical differences at LSD 20.91 (5%) with 65.98% CV.

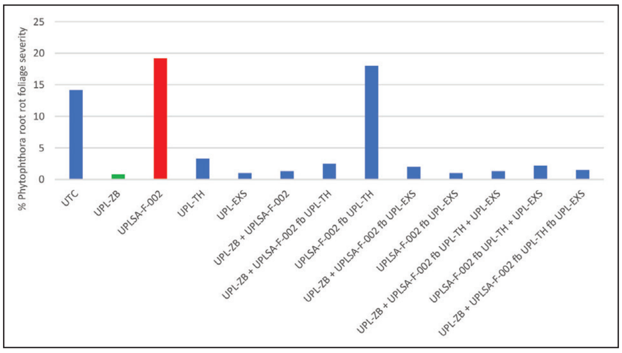

Figure 13: Percentage Phytophthora root rot severity observed on the foliage per treatment. Red bar indicates the highest severity while the green bar indicates the lowest severity. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist®, and UPL-EXS is Extrasol®. No statistical differences at LSD 18.29 (5%) with 301.35% CV.

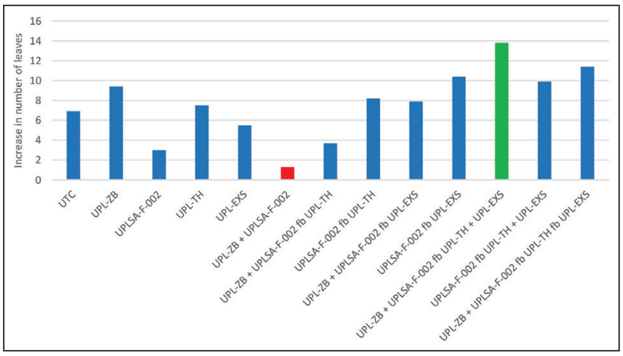

Figure 14: Increase in average number of leaves per treatment from planting to final harvest. The red bar indicates the least amount of increased growth while the green bar indicates the most increase in growth when considering the number of leaves per tree. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist®, and UPL-EXS is Extrasol®. No statistical differences at LSD 14.86 (5%) with 119.88% CV.

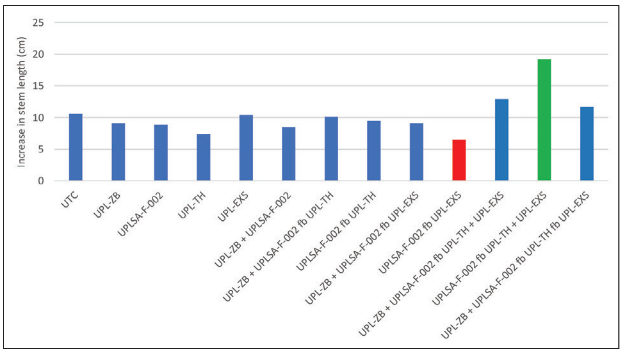

Figure 15: Increase in average stem length per treatment from planting to final assessment. The red bar indicates the least amount of increased growth while the green bar indicates the most increase in growth when considering stem length from graft union to longest shoot tip. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist®, and UPL-EXS is Extrasol®. No statistical differences at LSD 14.39 (5%) with 103.65% CV.

Figure 16: Overall increased vigour observed in the combination treatments compared to the untreated control treatments where, from left to right, 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.

three combination treatments, the statistical analysis was repeated only including the untreated control and the three combination treatments, Zeba® plus Brilliant® followed by four applications of Trykosist® 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 with a total of 15 degrees of freedom considering a CRBD. The results for the analysis indicated that all three combinations significantly decreased the Phytophthora root rot incidence compared to the untreated control and resulted in considerably lower severity observed on the foliage (Fig. 17).

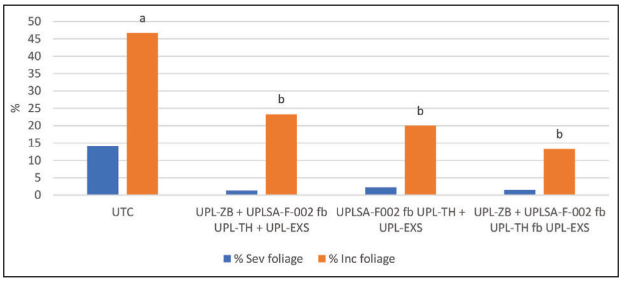

Figure 17: Percentage incidence (Inc foliage with LSD of 19.37 at α=5%, CV: 15.74%) and severity (Sev foliage with LSD of 24.33 at α=5%, CV: 19.77%) observed on the foliage in the combination treatments, Zeba® (UPL-ZB) plus Brilliant® (UPLSA-F-002) followed by four applications of Trykosist® (UPL-TH) 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.

Transplant orchard trials

The most common symptoms associated with Phytophthora root rot include small, pale green to yellow leaves, sparse foliage due to leaf drop, necrotic leaf tips, and limited new growth (Fig. 18). Compared to the glasshouse trial, there were some similarities and some differences observed in the various parameters measured to assess the symptoms associated with Phytophthora root rot in the three field trials. However, all three field trials indicated similar trends with all observations made which indicated that extending the trial period from four to six months allowed for better separation of significant effects.

The field trial conducted in Hoedspruit on 'Fuerte'/Duke 7 showed a higher incidence and severity compared to the KwaZulu-Natal trials conducted on 'Hass'/'Edranol'. This could be due to higher inoculum levels present or a more susceptible rootstock. Additionally soil conditions could be more conducive for disease development bearing in mind that Hoedspruit had higher temperatures and a higher sand compo-

Figure 18: Actively growing healthy tree (Brilliant® followed by four applications of Trykosist® and Extrasol® in tank-mix treatment) on the left vs. a diseased plant from the Untreated on right. (BioScience Research)

nent in the soil. Six months after transplant, results indicated that the untreated trees showed a decline of foliage based on the CIBA Geigy rating scale compared to the trees that received any of the treatments (Fig. 19). A similar trend was observed among all three field trials conducted.

This reduced foliage severity was also observed on the roots at the six-month assessment where all the treatments applied significantly reduced the Phytophthora root rot severity compared to the untreated controls (Fig. 20 and Fig. 21). Root weight, measured at four months, provided a good correlation compared to the root severity assessment (Fig. 22). Numerically higher root fresh weight was observed in the treatments where Zeba® was applied alone, Zeba® plus Brilliant®, and all three combination treatments including Zeba® plus Brilliant® followed by four applications of Trykosist® 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.

There was no clear trend observed with regards to the number of leaves per tree counted at the final assessment, six months after transplanting (Fig. 23). This could be attributed to a large variation in different sizes of the leaves and was taken to account with the leaf fresh weight measurement. With the leaf fresh weight, although not significantly different and only measured in one trial, good correlation to foliage severity was observed (Fig. 24). In addition, there was a significant increase in chlorophyll content in all the treatments accept in the ones receiving Brilliant® alone and Trykosist® alone, across two trials in which chlorophyll content could be measured (Fig. 25).

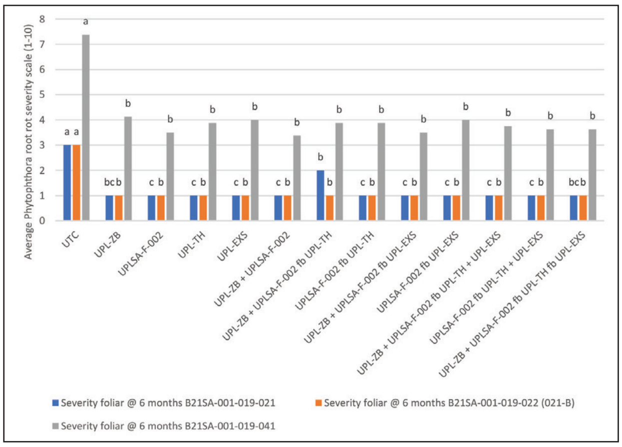

Figure 19: Phytophthora root rot severity according to the CIBA Geigy rating scale observed on avocado trees six months after planting. UPL-ZB (Zeba® applied at planting), UPLSA-F-002 (Brilliant® applied at transplant), UPL-TH (Trykosist® applied 14 days after planting to a total of 4 applications at 14-day intervals), UPL-EXS (Extrasol® applied 14 days after planting to a total of 4 applications at 14-day intervals). Data represented here include three trials. B21SA-001-019-021: LSD 0.5 at 5% with 26.16 % CV; B21SA-001-019-022 (021-B): LSD 0.3 at 5% with 19.63% CV; B21SA-001-019-041: LSD 1.1 at 5% with 19.55% CV. Significance is indicated within each trial and does not indicate differences among the trials.

Good growth was observed in all three of the field trials in the Brilliant® alone, Trykosist® alone, Brilliant® followed by Trykosist®, and Zeba® plus Brilliant® followed by four applications of Trykosist® and Extrasol® in tank-mix treatments (Fig. 26 and Fig. 27). Similar to the glasshouse trial, no phytotoxicity was observed, with increased vigour observed in all of the tree received treatments. In particular, the Zeba® plus Brilliant® treatment as well as the two combination treatments, 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, showed the highest vigour.

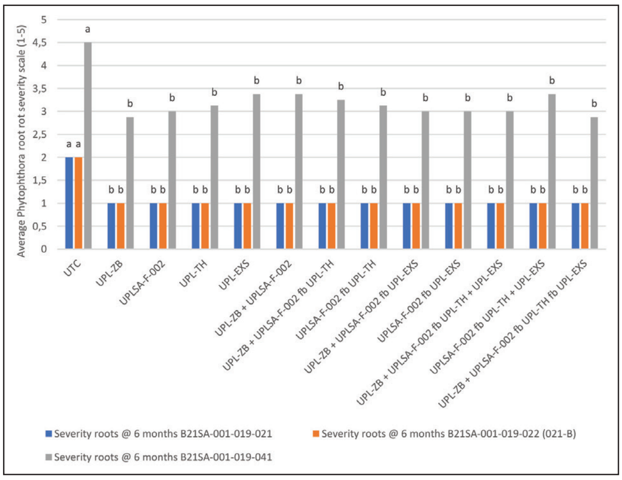

Figure 20: Percentage severity of Phytophthora root rot observed on the roots per treatment, six months after planting. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist®, and UPL-EXS is Extrasol®. Data represented here include three trials. B21SA-001-019-021: LSD 0.4 at 5% with 25.11% CV; B21SA-001-019-022 (021-B): LSD 0.4 at 5% with 24.44% CV; B21SA-001-019-041: LSD 0.6 at 5% with 13.89% CV. Significance is indicated within each trial and does not indicate differences among the trials.

Figure 21: The photo left shows roots from the Untreated plots. Note the presence of root decay. The photo right shows roots from the Brilliant® followed by four applications of Trykosist® and Extrasol® in tank-mix treatment. Note the presence of healthy actively growing roots. (BioScience Research)

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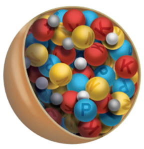
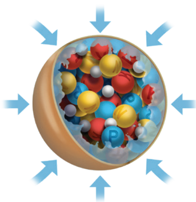
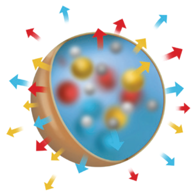


1 Multicote® products are based on fertilizer granules encapsulated in patented polymer coating.



2 Following to application, soil moisture penetrates the polymer capsule and dissolves the nutrients



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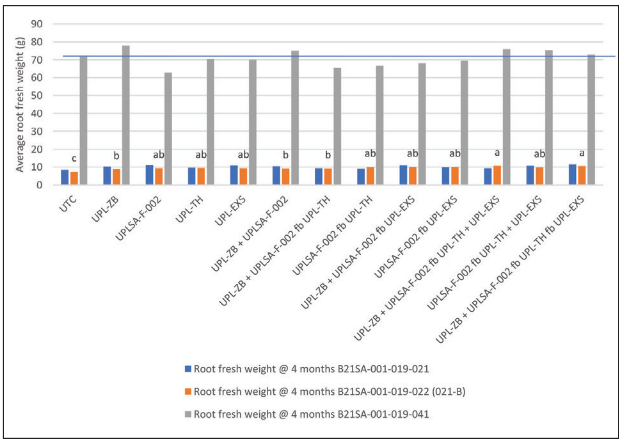

Figure 22: Root fresh weight per treatment, four months after planting. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist®, and UPL-EXS is Extrasol®. Data represented here include three trials. B21SA-001-019-021: LSD 2.04 at 5% with 14.01% CV; B21SA-001-019-022 (021-B): LSD 1.4 at 5% with 10.15% CV; B21SA-001-019-041: LSD 26.8 at 5% with 26.35% CV. Significance is indicated within each trial and does not indicate differences among the trials.

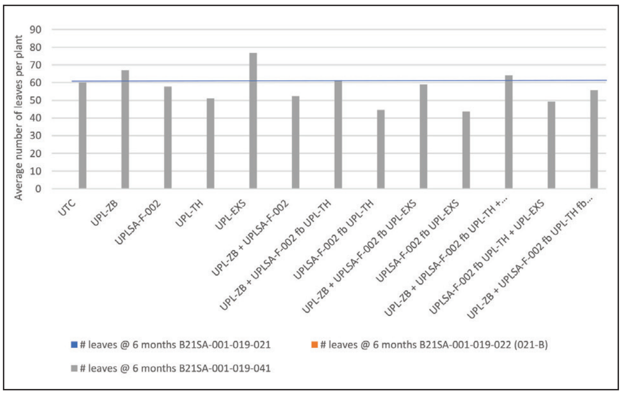

Figure 23: Number of leaves per treatment, six months after planting. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist®, and UPL-EXS is Extrasol®. Data represented here include one trial B21SA-001-019-041. No statistical differences at LSD 24.8 (5%) with 30.26% CV.

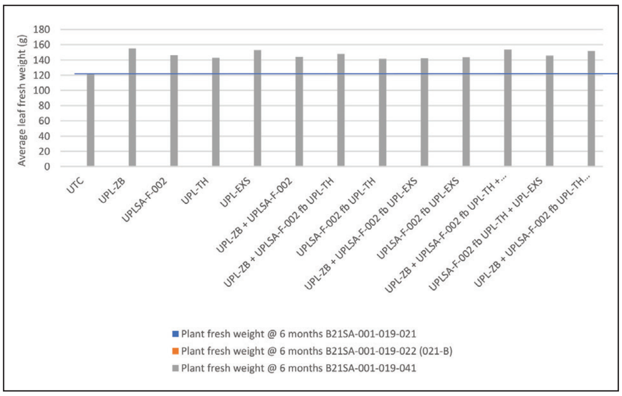

Figure 24: Plant fresh weight per treatment, six months after planting. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist®, and UPL-EXS is Extrasol®. Data represented here include one trial B21SA-001-019-041. No statistical differences at LSD 17.3 (5%) with 8.31% CV.

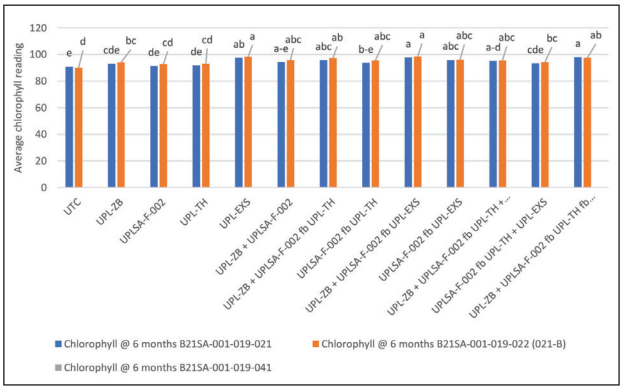

Figure 25: Average chlorophyll content per treatment, six months after planting. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist®, and UPL-EXS is Extrasol®. Data represented here include two trials. B21SA-001-019-021: LSD 4 at 5% with 2.93% CV and B21SA-001-019-022 (021-B): LSD 3.7 at 5% with 2.7% CV. Significance is indicated within each trial and does not indicate differences among the trials.

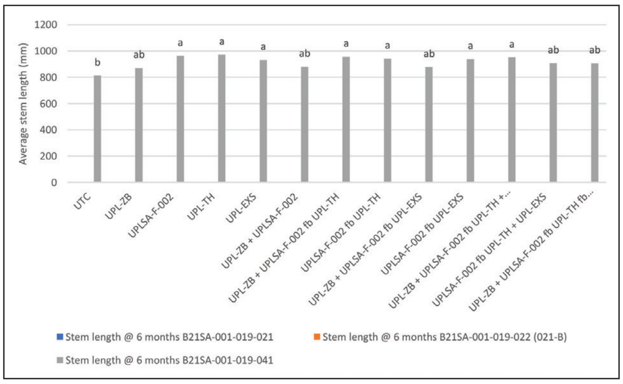

Figure 26: Average stem length per treatment, six months after planting. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist®, and UPL-EXS is Extrasol®. Data represented here include one trial B21SA-001-019-041. Statistical differences at LSD 72.7 (5%) with 5.54% CV.

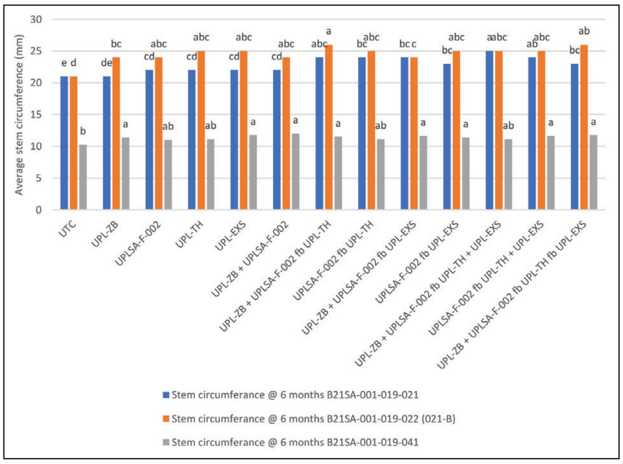

Figure 27: Average stem circumference per treatment, six months after planting. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist®, and UPL-EXS is Extrasol®. Data represented here include three trials. B21SA-001-019-021: LSD 1.5 at 5% with 4.74% CV; B21SA-001-019-022 (021-B): LSD 2.1 at 5% with 5.89% CV; B21SA-001-019-041: LSD 0.7 at 5% with 4.26% CV. Significance is indicated within each trial and does not indicate differences among the trials.

When disease progress over time in all treatments, for a single trial, was plotted graphically, it was clear that the disease intensity increased over time (R^2 value of 0.98) from first evaluation at two months after transplanting to six months after transplanting in the untreated control (Fig. 28). All the treatments applied ensured that the disease severity did not go over a score of 5 on the CIBA Geigy rating scale, which equates to a moderate decline. Upon further investigation, it was clear that the treatment of Zeba® plus Brilliant® followed by alternating applications of Trykosist® and Extrasol® to a total of two applications of Trykosist® and Extrasol® each showed a significant decrease (R^2 value of 0.85) in disease severity over the trial period, compared to the other treatments (Figs. 29 to 31).

CONCLUSION AND PROSPECTS

It appeared that extending the trials over one year would have been beneficial and possibly achieved stronger results, allowing for better establishment of the disease and more prominent symptoms to be observed. Zeba®, as a soil conditioner, applied alone showed good results compared to the untreated control. This strengthens the hypothesis that Zeba® decreases the wet and dry fluctuations in the soil that leads to increased spore production and decrease of zoospore movement. Further investigation is warranted to establish the exact mode of action with regards to Zeba® to manage soil conditions so as to be more unfavourable for the development of Phytophthora root rot.

The results also showed that other biotic or abiotic factors besides *P. cinnamomi* might play a bigger role than was originally suspected in the transplanting of saplings, and that *Phytophthora* is possibly not the major contributing factor as originally suspected. Based on this assumption, the addition of biostimulants alone also seems to have a positive effect and gives the trees more resilience under pressure due to abiotic and biotic factors associated with transplanting saplings into the orchard. The results also indicate that including too many biostimulants at once may become antagonistic and not synergistic as would be expected. Alternating the products in a programme yielded a more favourable response.

The combination treatment of Zeba® plus Brilliant® applied at transplant and followed by alternating applications of Trykosist® and Extrasol® resulting in a total of two applications of Trykosist® and Extrasol® each, resulted in the best overall outcome in terms of improving tree vigour and growth, while also decreasing the development of Phytophthora root rot over a period of six months from transplanting. These trial results, although over a six-month period, already proved that where producers rely heavily on chemical control measures such as the phosphonates, there is value in combining chemical and biological control measures to control not only *P. cinnamoni* but also to mitigate abiotic stress conditions affecting the replanting problem on avocados.

Future action includes the registration of Trykosist®

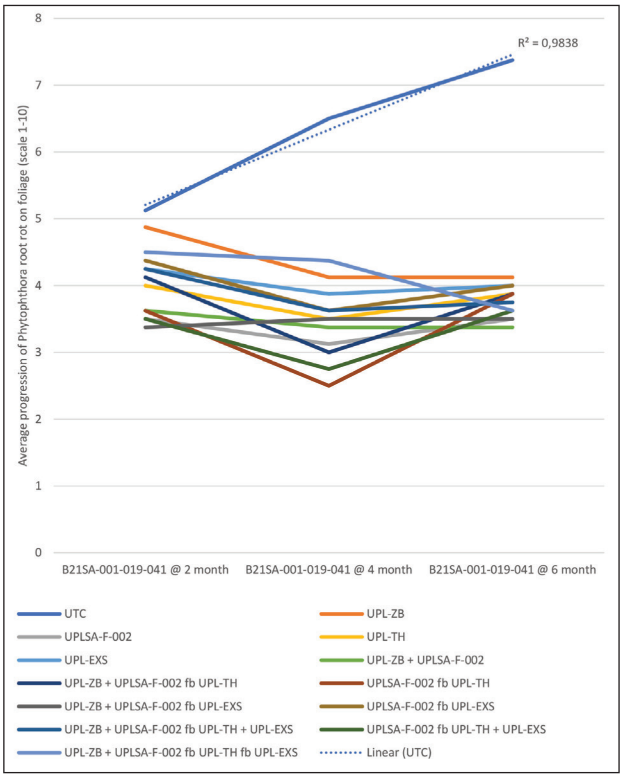

Figure 28: Phytophthora root rot disease progress over trial period. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist®, and UPL-EXS is Extrasol®. Only data from trial B21SA-001-019-041 are presented in this graph.

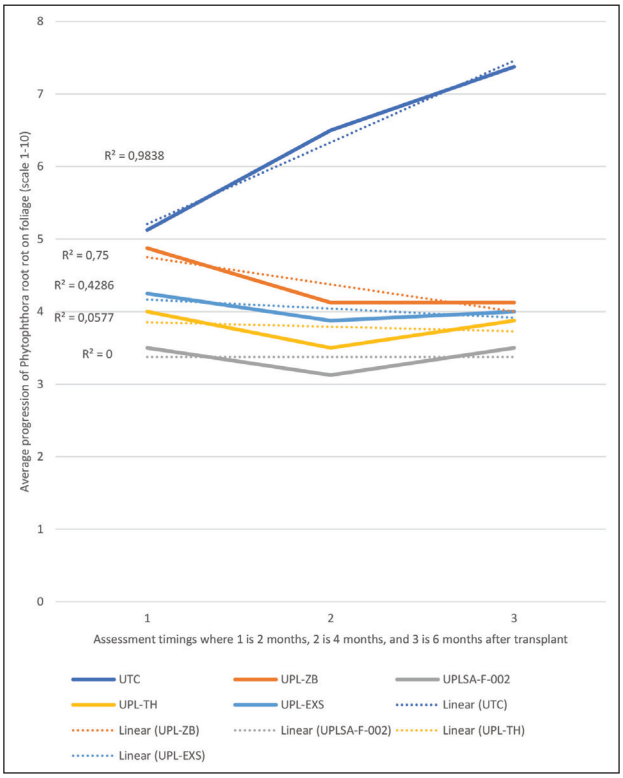

Figure 29: Phytophthora root rot disease progress over trial period. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist®, and UPL-EXS is Extrasol®. Only data from trial B21SA-001-019-041 are presented in this graph.

as a biological remedy that is currently pending, as well as the label extension registration for the use of Brilliant® in avocado against Phytophthora root rot. The registration for use of Zeba® on tree crops will be submitted within the coming months and Extrasol® is currently registered for its biostimulant properties.

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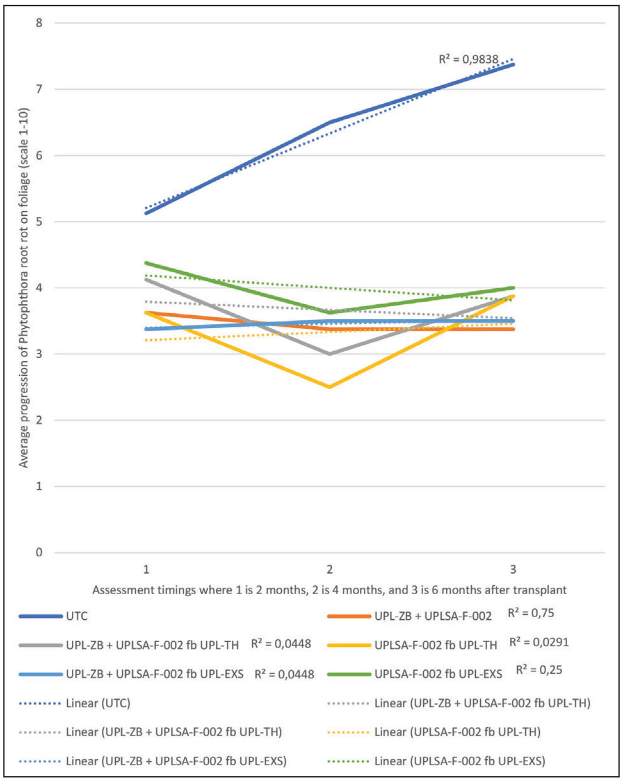

Figure 30: Phytophthora root rot disease progress over trial period. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist®, and UPL-EXS is Extrasol®. Only data from trial B21SA-001-019-041 are presented in this graph.

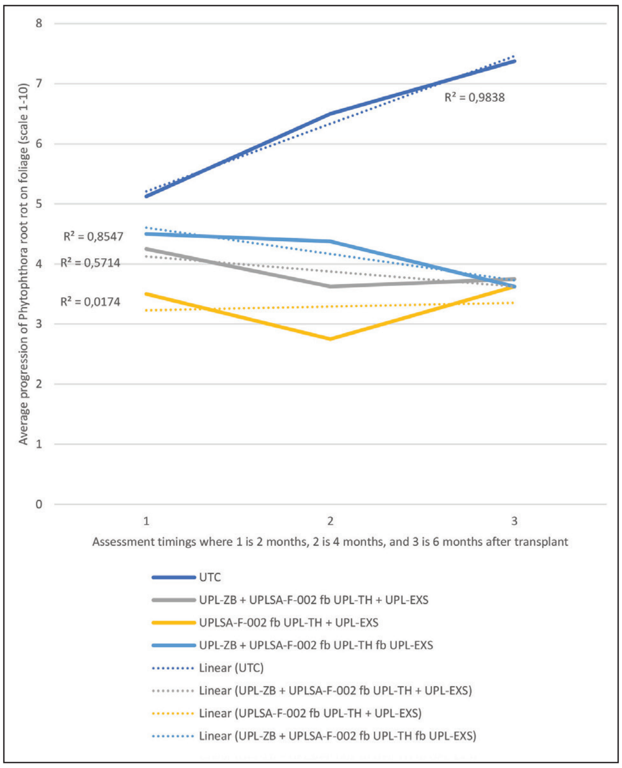

Figure 31: Phytophthora root rot disease progress over trial period. UPL-ZB is Zeba®, UPLSA-F-002 is Brilliant®, UPL-TH is Trykosist®, and UPL-EXS is Extrasol®. Only data from trial B21SA-001-019-041 are presented in this graph.

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