# The South African Phytophthora trail and the way forward

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*In memory of Jan Toerien, Joe Darvas and Lindsay Milne*

**ABSTRACT**
The goal with this review is threefold: 1) Present a historical perspective of root rot control in South Africa; 2) Provide suggestions to widen the window of *Phytophthora* research; and 3) Highlight to farmers how they can assist in accelerating the avocado selection/breeding projects.

**INTRODUCTION**
Along the *Phytophthora* journey you will encounter the best of humankind – endurance, the advantage of experience, a keen sense of observation, leap frogging, creativity and cooperation. The research on root rot control placed South Africa on the avocado world map, which led to the First International Avocado World Symposium being held at Pretoria in 1987. However, you will also learn how the commercial interests of a company had a disruptive impact on progress, which eventually had a negative impact on their earnings.

Since the large-scale plantings of avocados with Californian varieties commenced at Tzaneen, Mataffin and Louis Trichardt in 1938, the industry experienced a honeymoon period. However, soon after the plantings, dying-back of avocado trees became eminent (Wager, 1941) which reached immense proportions in the late 1960s.

The first breakthrough came in 1972 when *Phytophthora cinnamomi* was identified as the main cause of avocado tree decline in South Africa (Milne, Brodrick & Hughes, 1975). A year later Broderick & Frean (1973) observed that more than 90% of avocado nurseries were infected with *Phytophthora* root rot.

When Jan Toerien was appointed at Westfalia, the company was in dire state. The estate lost 80 000 citrus trees between 1968 and 1971 due to the greening disease and 400 ha avocados were on its way to follow suite (Toerien, 2007). At that stage the view was that avocado tree decline was due to incorrect fertilising, poor soil management and associated with sunblotch (Anonymous, 1965). When Toerien was appointed in 1973 as Horticulturist and later Agricultural Manager, the directive message was clear: "Fix the problem and save the avocados".

**Nurseries**
Toerien took note of the work by Milne *et al.* (1972) and established the first avocado disease-free nursery in 1974 (Toerien, 1977 & 2010). Thereafter several other nurserymen followed his example. During the period, well defined protocols were formulated for nursery practices (Toerien, 1977; Mitchell, 1977). Allan *et al*. (1981) contributed to nursery management with their overview of sterilisation and pasteurisation of soil mixes.

Not all nurseries however followed these guidelines. When Westfalia purchased Everdon in 1984 from Koekie Leon, Toerien culled 35 000 trees in the nursery with root rot (Toerien, 2010).

The second development in the management of nurseries was the formation of the Avocado Nurserymens' Association under the auspices of SAAGA in 1983 to coordinate the avocado plant improvement scheme and monitor *Phytophthora* in nurseries.

Lucas McLean introduced the use of methyl bromide in nurseries (McLean & Kotzé, 1992a, b). With the regulatory limits on the use of methyl bromide, Ilze de Jager proposed the use of steam pasteurisation as an alternative to methyl bromide fumigation for disinfecting container medium (Jager & Kotzé, 1994).

**Metalaxyl**
Yet, despite nurseries supplying uninfected trees from 1974, more than 5 000 ha established orchards already had root rot.

**Jan Toerien, 1941-2010**

Jan Toerien, after graduating in 1964 at the University Stellenbosch, was appointed by Chesterford Park Research who did research for the ten largest chemical companies where he became well acquainted with various pesti- and fungi-herbicides and experimental formulations. Prior to his appointment at Westfalia in 1973, he had a two year stint at Ciba Geigy. Toerien was instrumental in organising the First International Avocado World Symposium. He received several awards, including the prestigious Golden Avocado Award by the South African Avocado Society.

In 1976 Toerien undertook a study tour to Israel and California where he was well received by a large number of researchers. A breakthrough came when Prof. George Zentmyer showed him some *Phytophthora* cultures. He observed a dramatic inhibition in one culture treated with a Ciba-Geigy product, CGA 48988 (Toerien, 2010).

Back home he perused this observation with Ciba-Geigy, who formulated a powder based on the codified fungicide in Prof. Zentmyer's collection. This resulted in the registration and commercial application of Ridomil. Jan stated "the trees recovered fantastically" (Toerien, 2010; Darvas, Kotzé & Toerien, 1978b; Darvas, Kotzé & Toerien 1979d; Darvas, Toerien & Kotzé 1979e).

Prior to the investigations into Metalaxyl, Joe Darvas developed an indispensible technique for quantitative and qualitative evaluations of avocado roots and *Phytophthora* spore populations where the New Zealand Blue Lupine seedlings were used as bait plants (Darvas, 1979c).

**Joe (Jozsef Mihaly) Darvas, 1945-2011**

Joe grew up and studied plant pathology in Hungary before he and his wife, Magdalena, at an international event defected to South Africa in 1974. There they were both employed as plant pathologists.

He earned a doctorate in Plant Pathology in 1982 at the University of Pretoria with his work on the control of avocado black spot, *Pseudocerospora purpurea*. His prediction model for black spot infection is still the basis of controlling this disease in South Africa. While working for May & Baker, Darvas began his work on the control of avocado root rot, which he continued after joining Westfalia Estate in 1976.

Darvas was honoured with an award for excellence at the World Avocado Congress II (1991) and the Golden Avocado Award by the South African Avocado Society.

In 1978, Moll *et al.,* concluded, with the Metalaxyl success and tolerant rootstocks ('Duke 6', 'Duke 7' and 'G6'), *Phytophthora* will be of relatively minor importance in the future. The situation changed significantly in a few years from John Moll's prediction.

Due to the success with Metalaxyl, the avocado production in South Africa increased more than 50% in 1980 from average of the previous five years (Lourens, 1981). However, six years after the then successful control of root rot, Toerien and his team observed a progressive *Phytophthora* resistance to Ridomil (Darvas & Becker,1984b). These results were confirmed by McKenzie (1984) and Snyman (1984).

**Rootstocks**

When in 1979, Dr. George Zentmyer visited South Africa, he also saw the first indications of reduced Ridomil efficacy. Dr. Zentmyer was of the opinion that resistant rootstocks were a better long term approach than chemical control (Zentmyer, 1979). This prompted Toerien and his team to introduce an integrated research system that included the search, selection and propagation of tolerant rootstocks. Soon there were a large number of rootstocks showing potential, at the same time Toerien exchanged ten of the most promising ones with Mike Coffey in California (Toerien, 2007).

Two years prior, André Ernst commenced with a comparison between 'Duke 6', 'Duke 7', 'Fuerte' and 'Edranol' seedlings resistant towards *Phytophthora* (Ernst & Holthause, 1977). However, no follow up reports were reported in SAAGA Yearbooks.

In 1986/87, Westfalia's management had an early and expensive lesson with rootstocks. 'Hass' orchards with alternate rows of 'Duke 6' and 'Duke 7' rootstocks were planted on a large commercial scale in 1983/84. Both rootstocks were tolerant to *Phytophthora*. However, after three years trees on 'Duke 6' started to die. The disease was never identified but the conclusion was that 'Duke 6' budwood was infected. About 100 ha of orchards were destroyed and replanted with 'Hass' on rootstocks other than 'Duke 6'.

The first success with the South African root selection programme was when Theuns Botha found that 'Dusa' (Merensky II), 'Latas' (both local selections) and 'Duke 7', in decreasing order, performed well against *Phytophthora* while 'G755' and 'Gordon' had almost no resistance to the pathogen (Botha, 1991). This success can entirely be ascribed to Joe Darvas, who was a passionate nature lover. While strolling along the water streams at Westfalia, he collected many seedlings. The three most promising towards *Phytophthora's* tolerance, he gave the Hungarian names 'Latas', 'Jovo' and 'Dusa'. The translated names are: Seeing, Future, Rich.

**To Joe**

*May you*
*while travelling alone*
*in tomorrow's universe*
*find a seedling,*
*called Peace.*

Other relevant papers are:

- In a comparison between 'Edranol' and 'Duke 7' against common root pathogens, it could not be confirmed that 'Duke 7' was superior to 'Edranol' (Snyman & Darvas, 1983; Snyman, Snyman & Kotzé, 1984).
- Durand (1986), with his survey on avocado rootstock/scion relationships, concluded a huge genetic variability in seedling rootstocks towards *Phytophthora.*
- Köhne (1991) in his investigation found 'Hass' on 'Duke 7' performed significantly better than the 'G6' and 'G755' rootstocks towards *Phytophthora*.
- Conradie *et al*. (1994) reported on the establishment of a project where 'Shepard', 'Pinkerton', 'Gwen',

'T142' and 'BL122' were to be evaluated as well as 'Hass' grafted on 'Barr Duke', 'Thomas' and 'D9'. Six-year old 'Fuerte' on 'D7' had a significant better yield efficiency than on 'G6' rootstocks (Kremer-Köhne & Köhne, 1994). In a comparison between the rootstocks 'Thomas', 'Barr Duke', 'D9', 'Duke 7', 'Wilg', 'Ryan' and 'Colin V-33', Roe & Köhne (1996) recommended the use of 'Duke 7' to be continued.

- 'Hass' on both 'Latas' and 'Dusa' rootstocks have out-yielded 'Hass' on 'Duke 7' for two seasons by at least 29%.
- Using 'Colin V-33' as an interstock had very little influence on tree vigor and yields, when compared to 'Hass' on 'Duke 7' (Roe & Morudu, 2000).

Kremer-Köhne & Duvenage (2000) reported in a four-year 'Hass' orchard the tree ratings with rootstocks from the healthiest to the poorest were: 'VC 256', 'VC 805', 'VC 801', 'VC 218', 'VC 207', 'VC 241', 'Duke 7', 'Edranol' seedlings and 'VC'. In a two-year 'Hass' orchard the trees were rated from the healthiest to the poorest: 'Velvick', 'Dusa', 'Merensky III', 'Duke 7', 'Merensky IV', 'Gordon', 'Edranol' seedlings and 'Jovo'.

The subsequent research by Kremer-Köhne *et al.* (2001 & 2002) is summarised in Figure 1.

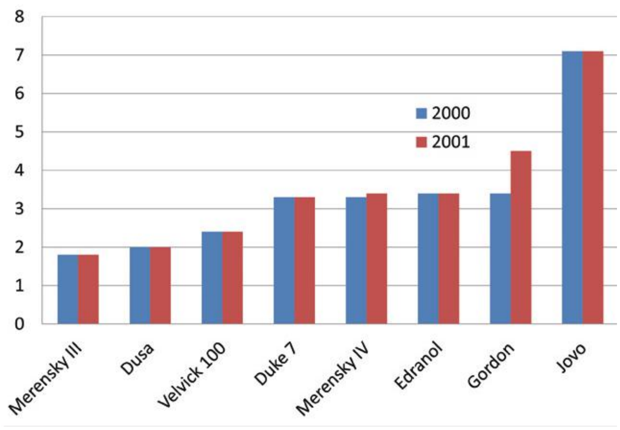



**Figure 1:** Tree rating on a scale of 0 (healthy) to 10 (dead)

After the success with 'Dusa', Stefan Köhne and his team trade marked 'Dusa'® and made a huge effort in promoting this rootstock, which is now grown worldwide. In meantime 'Latas'® was also trademarked by the company. We can expect more rootstocks from the Westfalia stable tolerant to *Phytophthora* and/or salt, lime.

The Agricultural Research Counsel's (ARC) ITSC at Nelspruit, now the ARC-TSC, embarked on an ambitious avocado rootstock breeding programme (Bijzet *et al.,* 1993; Koekemoer *et al.,* 1994; Bijzet *et al.,* 1996; Sippel *et al.,* 1997; Bijzet, 1998; Sippel *et al.,* 1998). Sadly the project, with the exception of 'Pinkerton' and 'Gwen' on 'Duke 7', never came any further than the planning stage.

**Phosphonate and phosphite**

Toerien was under no illusion that a *Phytophthora* tolerant rootstock would soon been discovered, and pursued with other fungicides. After three years, the experimental Aliette foliar and stem-painting applications started to show some promising results. A comprehensive experiment was initiated with aerial application of Aliette. After a year, it was noticed that Aliette disturbed the insect balance. The trees had increased populations of sucking insects and sooty mould (Toerien, 2007).

The experimental zinc injection treatment by Karel Buitendag from Outspan on citrus trees (Buitendag and Bronkhorst, 1980) made an unexpected major contribution to *Phytophthora* control for years to come. The injection technique gave Toerien and his team the idea to inject Aliette in avocados, as it has a systemic action. The available Aliette powder formulation was mixed with water and the precipitate-free liquid remaining above the solid or 'supernatant' was injected (Darvas, 1981, unpublished) with spectacular results.

Due to the complexity and intricacies of the situation during the period, the author wishes to quote a verbatim from the master:

"In 1981 Don Gustafson visited us and wrote: The amount and type of research being conducted by personnel at the Citrus and Subtropical Fruit Research Institute, Westfalia Estates and the University of Pretoria, was especially impressive. More avocado research is being conducted in South Africa at the present time than in most avocado production countries. This is commendable. The fungicidal work on avocado root rot is the best and most extensive anywhere (Gustafson, 1981).

"During 1981 we confirmed the potential of Aliette injections for control of *Phytophthora*. We decided to share the information with Rhone Poulenc, the manufacturer of Aliette, before we made the Aliette injection information public. Bruno Trepoz, who was the Aliette product manager in France, came to see us. We showed him the research work that we had done. Our results showed that one year of injections was superior to three years of foliar sprays or stem painting. We further proved that the injections would only use 3% of the volume of Aliette required for foliar applications and with injections there is no pollution. The injection technique had potential commercial implications for Rhone Poulenc.

"Bruno Trepoz warned us not to distribute or publish any information on Aliette injections. We were also threatened by court action if we continued with this research. He followed up this meeting with an attorney's letter forbidding us to publish our results or do further experimental work with Alliette [*Note by author: Westfalia at that stage used the same law firm in South Africa as the local branch of Rhone Poulenc. After this incident, Westfalia switched to other lawyers.*] We were shocked! The excitement of a scientific breakthrough made way for the difficult reality of the legal implications of which we were previously unaware. The prospect that our export avocado fruit could be banned in France was a very threatening possibility. To protect our avocado export to France, we decided not to publish the results of Aliette injections in the short term but continued with research.

Some confusion was experienced as some information leaked out about the injections without being accompanied by the scientific experimental data. It was very difficult to remain silent and read comments of scientists like Dr. Mike Coffee from UCR that 'there are rumors about injections but it is without any scientific basis of proof'.

"Publications only followed after a different injection formulation of Fosetyl-Al became available on the market (Darvas, 1983a; Darvas, 1983b; Darvas, Toerien & Milne, 1983c; Darvas, 1984; Toerien & Slabbert, 1984). The Westfalia research team became very lucky again when we read an article by Prof. Bompaix from the Sorbonne University in France. Prof. Bompaix described the mode of action of Aliette and phosphorous acid. We found that we could use the reformulated phosphorous acid with relative safety on avocados by changing the pH to 5.8 and by injecting instead of spraying it on the foliage. Experimental work with phosphorous acid progressed very well and we found that phosphorous acid was much better than Aliette for the control of *Phytophthora*. We were excited and could not wait to present and publish the information. Joe Darvas presented the results of *Phytophthora* control with phosphorous acid injection at the SAAGA Research Symposium in Magoebaskloof in 1984.

"Two representatives of Rhone Poulenc were in the audience and reported the information direct to Bruno Trepoz and the attorney's letter followed within days. They claimed an umbrella patent and the familiar threats were made to prevent us from publishing the results. This time they went further and forbade us to publish, but also threatened Joe Darvas in person with liability. We were obviously devastated by this news. The inability to publish the research results again caused frustration and created difficult situations for us and we could not respond to many rumours and speculations. Bruno Trepoz claimed that phosphorous acid was not permitted in food although it naturally occurs in plants and using it will disadvantage our exports to France. We worked closely with patent lawyers and it took more years of frustration and research. Dr. Jurg Bezuidenhout conducted many studies of residue analysis and finally proved that Aliette had a slow release of phosphorous acid (Bezuidenhout *et al*., 1985). Fresh Aliette contains a low concentration of phosphorous acid. Older stored Aliette contains more. This meant that the use of phosphorous acid for injection of avocados could not be stopped any longer (Toerien, 2007)."

Today there are a number of phosphite formulations competing with Aliette for the market share. An excellent formulation with boron, with a chelate, which is essential in preventing the formation of insoluble zinc phosphite, and phosphorous acid was described by Bezuidenhout & Darvas (1987).

After Mike Coffey's initial skepticism on phosphite he made a comment in Ramalea's Guestbook (1985) at Westfalia: "Phosphite is King!". Despite this, he was still unconvinced with phosphite (Coffey, 1985) and claimed that phosphite resistant *Phytophthora* isolates exist (Bower & Coffey, 1984). The claim of resistance by John Bower and Mike Coffey is yet to be validated.

Subsequent publications, which confirmed the magnificent work by Toerien's team on the control of *Phytophthora cinnamomi* with phosphonate or phosphite are:

- Bezuidenhout, Darvas, & Kotzé (1987); Kotzé, Moll & Darvas, (1987).
- Schutte, Bezuidenhout & Kotzé (1991) found that trunk injections were more efficient than leaf spray. According to the author, applications should not exceed 42 for good control.
- The effect of phosphite on *Phytophthora* was confirmed by Van der Merwe & Kotzé (1992) with the zoospores of *Phytophthora cinnamomi* (Van der Merwe, 1993).
- Fungicidal action of phosphite in avocado root tips on *Phytophthora cinnamomi* (Duvenage, & Kotzé, 1994).
- Duvenage & Köhne (1995) claimed Dimethomorh (1 mg/l soil drench) was just as effective as Fosetyl-Al against *Phytophthora* root rot. The author could not find any follow up reports by the main author or other researchers.
- *In vitro* sensitivity of South African *Phytophthora* cinnamomi to phosphite and different phosphate concentrations (Ma & McLeod, 2014).
- Bezuidenhout & Toerien (1988) noticed that potassium borate improved *Phytophthora* infected tree health, compared to the control, although not nearly as effective as phosphite.

**Quantification**

***Fosetyl and derivatives***

Bezuidenhout *et al.* (1985) employed gas chromatography (GC), equipped with a phosphorous sensitive detector, to quantify phosphite after methylating the phosphite.

High performance ion chromatography introduced by Ma *et al.* (2014) to South Africans as the method to quantify phosphite which is superior to the GC method, and safe.

Bezuidenhout (2017) compiled a paper on the nomenclature and properties of phosphorus compounds which may assist phosphite researchers.

***Phytophthora***

The tree rating system developed by Ciba-Geigy was extensively used. The scale is based on a rating between 0 (complete healthy tree) and 10 (a dead tree). Rating 5 is where you can observe 50% of the background directly behind the tree.

The lupine bait technique for semi-quantative analysis of *Phytophthora cinnamomi* and other root pathogens in avocado soils is cheap and easy (Darvas, 1979c). Darvas (1982b) used this technique to recover *Phytophthora cinnamomi* and correlate the incidence of the pathogen with severity of avocado root rot.

Theuns Botha described the detached root technique in 1989 which was subsequently often employed in studies of root resistance and biocontrol (Botha, Wehner & Kotzé, 1989).

## *Phytophthora* in general

Lawrence Marais reported on the mechanisms of penetration by *Phytophthora* into young grapevine roots through light, scanning and transmission electron microscopy studies. They mentioned in the same article the zoospores were attracted by aspartic acid, glutamic acid and arginine and zoospores were eliminated with hot water at 50 °C within 5 minutes (Marais & De La Harpe, 1981).

James Lonsdale and colleagues found *Phytophthora cinnamomi* is the cause of crown and trunk canker of 'Duke 7' avocado rootstocks in South Africa (Lonsdale, Botha, Wehner & Kotzé, 1988) and not bacteria (Korsten & Kotzé, 1983, 1984 & 1985).

*Phytophthora cinnamomi* isolates from different avocado orchards differ *in vitro* toward phosphite, depending on the phosphate concentration (Ma & McLeod, 2014).

## Ecology, biocontrol and mulching

While in the quest to seek alternatives to conventional fungicides, researchers also ventured into biocontrol. Again, one of the first was Joe Darvas. In 1979 he isolated ten bacteria (pers. comm.) with antagonistic properties against *P. cinnamomi in vitro* but none were effective *in vivo* against the pathogen (Darvas & Toerien, 1979). In a publication in the same year he reported that apart from *P. cinnamomi*, fifteen other fungi which are known to be pathogenic on avocado and other host plants were isolated from avocado roots and root zones. In pathogenicity tests, *P. cinnamomi* and *Pythium* spp. were the most destructive organisms (Darvas, 1978a). The incidence of some of the organisms was recorded in commercial scale survey from avocado soils and their occurrence was analysed in soils treated with fungicides. It was found that Ridomil inhibits not only *P. cinnamomi* but also *Pythium* spp., but simultaneously increased the relative frequency *of Fusarium oxysporum* when tested with the lupine seedling bait technique (Darvas, 1979b).

In 1979 Nigel Wolstenholme reviewed the prospects for integrated and biological control of avocado root rot after an overseas visit. He concluded that climate and soil in current South African production areas make for a comparatively severe root rot problem. Careful soil selection, production of disease-free nursery trees, and increasing use of tolerant rootstocks, remain high priorities. In high risk areas, there is considerable evidence of the beneficial effects of organic amendments and high soil calcium content. Integration of all these control measures is recommended (Wolstenholme, 1979). Six years later Fritz Wehner introduced the concept of soil solarisation to reduce the inoculum of *P. cinnamomi* prior to replanting (Wehner, & Kotzé, 1985).

The following two decades saw a boom in research to combat *P. cinnamomi* by biocontrol and related soil practices. This mainly attributed towards drive of natural disease control at the University of Pretoria under the guidance of Prof. Kotzé.

In 1990 and 1991 Erna Maas with fellow colleagues reported the occurrence of soils suppressive where trees were grown with no visible symptoms of root rot (Duvenage & Maas, 1990). Of the nine-five bacteria isolated from these escape trees, 10% was antagonistic towards *P. cinnamomi* (Maas & Kotzé, 1990). Similar results were obtained by Duvenage *et al.* (1991) and Van der Merwe (1992).

In a glasshouse trial, Riaan Duvenage found the fungi, *Aspergillus candidus*, *Paeciliomyces lilacinus* and *Trichoderma hamatum*, and bacteria, *Bacillus azotoformans* and *B. megaterium*, reduced *P. cinnamomi* root rot on 'Edranol' seedlings grown over a three-month period (Duvenage & Kotzé, 1993). In a greenhouse trial Adéle Mcleod reported *Trichoderma hamatum* and *T. harzianum* isolates reduced root rot and stimulated root growth of Duke 7 seedlings (McLeod, Labuschagne & Kotzé, 1995). Millet seed as a carrier for *T. harzianum* outperformed citrus waste, peat or standard nursery mixes (Landman, Labuschagne & Kotzé, 1996). Continuous application in a greenhouse with *T. harzianum* eliminated the effect of *P. cinnamomi* on 'Duke 7' seedlings (Landman, Van Heerden, Kotzé, Labuschagne & Wehner, 1997).

An intensive study was conducted in the USA and Europe towards the attitude regarding biological control, legislation requirements and potential threats in commercialization (Bezuidenhout, 1995).

According to reports by Duvenage & Köhne (1997) and Duvenage & Kremer-Köhne (1998), trees treated with *Aspergillus candidus*, *Paeciliomyces lilacinus* and *Trichoderma hamatum*, or combination, in 1992 stayed healthy compared to the untreated control. In view of many other research papers, the author found this result somewhat surprising.

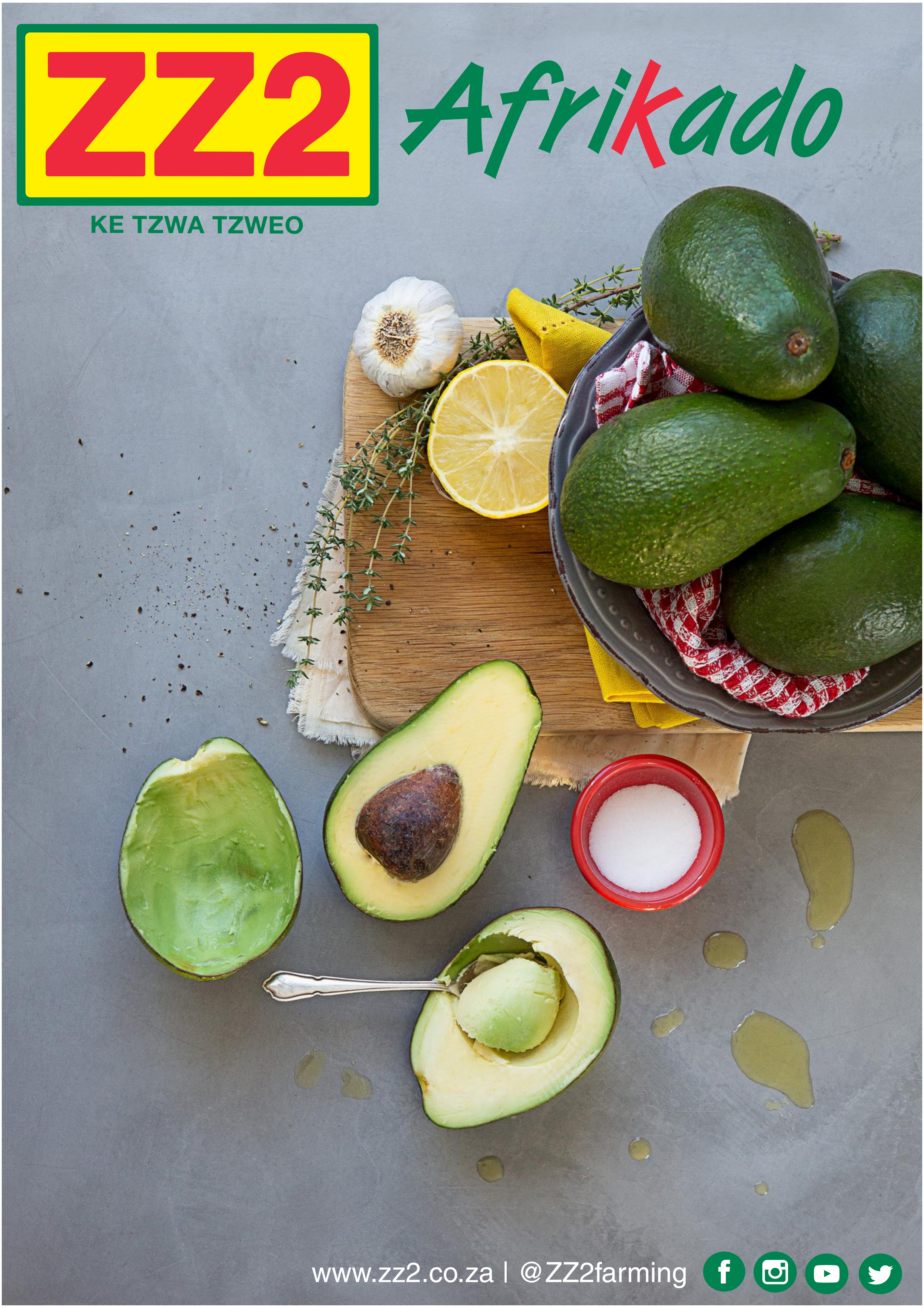
In healthy avocado orchards where chemical control of *P. cinnamomi* was discontinued for two years, the tree health did not decline significantly (Köhne & Kirkman, 1991). However, the next two years the condition decline significantly irrespective of mulching or cover crops (Duvenage, Köhne & Kirkman, 1993).

Lungi Mavuso reported mulch of partially decomposed avocado woodchips lessen the decline of tree health where chemical control of root was not done (Mavuso, 2008). With a groundcover, *Lippia canescens*, no build up of *P. cinnamomi* was noticed over a period of a year (Mans & Hattingh, 1992). Composted citrus waste inhibited *P. cinnamomi* in a glasshouse trial with lupine seedlings (Van Heerden, Wehner & Kotzé, 1995), however, the author is unaware if the research was extended to avocados.

## Inorganic soil amendments

Soil amendment with calcium, ammonium sulphate or soluble silicate assisted in *Phytophthora cinnamomi* control but cannot be solely used in the management of the diseases, according to the following reports. Indications are that scion/rootstock combination can modify the effect of calcium and nitrogen.

Although Fouche (1981) and Rowell (1981) did not explicitly considered *Phytophthora cinnamomi*, they published valuable information on the type Ca-source most suitable for a soil and amount to be applied; their recommendation was based on pH, and Ca, Mg and Al content of the soil.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Darvas (1977) summarised soil factors closely linked with avocado decline - high soil resistance, low Ca content, shallow topsoil. The role *of P. cinnamomi* was predominant in soils where resistance exceeded about 5 000 Ω-m, and Ca content was lower than approximately 150 ppm in acid soils, while the damage caused by the pathogen was negligible when resistance was as low as 3 000 Ω-m with over 600 ppm Ca in close to neutral clay soil.

Carel Snyman reported the influence of calcium on seedlings infected with *Phytophthora.* 'Duke 6' responded better to $CaSO_4$ and 'Guatemala' to $CaCO_3$ (Snyman & Darvas, 1982). In a subsequent article he found with inoculated *Phytophthora* 'Edranol', the mass lateral was significantly higher with 200 ppm Ca that with 1 ppm Ca (Snyman, 1984).

Riaan Duvenage also reported in several studies on the influence of N and Ca on *Phytophthora cinnamomi*:

- Significant difference was found between the control and Ca or $(NH_4)_2SO_4$, but none between $CaCO_3$ and $CaCl_2$ (Duvenage, 2000).
- The saprophytic growth of *Phytophthora cinnamomi* increased significantly by calcium. Ungrafted 'Duke 7', 'Edranol' and 'G755' trees showed a significant decrease in susceptibility to *Phytophthora cinnamomi* when treated with $CaSO_4$. This was however not found when these rootstocks were grafted with 'Hass' (Duvenage & Kotzé, 1991).
- When 'Hass' was grafted on 'Edranol', 'Duke 7' and 'G755', neither $CaSO_4$ nor $CaCO_3$ were significant effective in reducing root rot. In contrast, both $CaSO_4$ and $CaCO_3$ significantly reduced the disease on ungrafted 'Edranol', while $CaSO_4$ reduce root rot in ungrafted 'Duke 7' and 'G755' (Duvenage, Kotzé & Maas, 1992).

A number of articles reported the inhibition of *Phytophthora cinnamomi* by soluble silicate (Bekker *et al*., 2005, 6, 7a; Kaiser *et al*., 2005) or the accumulation of phenolics in roots by the compound (Bekker *et al*., 2007b).

### Application techniques

Initially fungicides were applied either as foliar sprays or soil drenches. After the success with tree injection, the concern was raised on tree injury with the technique. However, later, alternatives were investigated to injection mainly due to the rising labour cost.

### The path towards the future

#### *Trends in PC*

The trends in *Phytophthora* research is summarised in Figure 2. The research output declined significantly from the 1980s. The impression is that most issues with root rot are solved and only minor details need to be addressed.

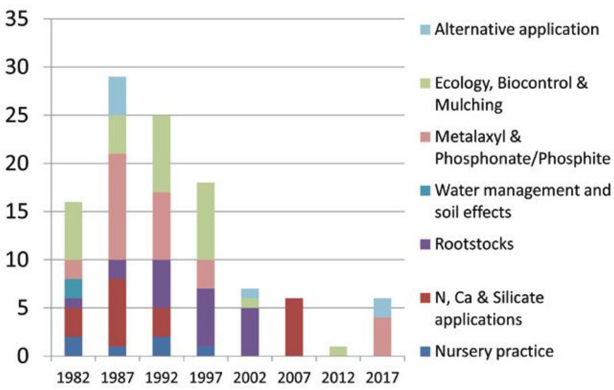


**Figure 2:** Number of publications in 5-year period

**Table 1:** Development of techniques for applying fungicides.

| Application(s) | Notes, author(s) |
|---|---|
| Foliar spray, soil drench | Monthly foliar sprays with Fosetyl-Al (0.3% active) were ineffective, while 2 x soil drenches with Metalaxyl (2.5 g active/$m^2$) improved health of mature trees within one year (Darvas, 1982b). Tree ratings ~5. |
| Trunk paint | Fosestyl improved health of mature trees within 2 years (Darvas, 1983a) with injections (0.4 g active/$m^2$). This occurred within a year (Darvas, Toerien & Milne, 1983c). Tree ratings ~5. |
| Sponge band and trunk paint | No difference was detected between control, Metalaxyl and Fosetyl-Al in the increase of trunk diameter 7 months after treating mature trees (Snyman & Kotzé, 1983a). Tree rating – not specified. |
| Foliar spray, trunk paint and sponge band | With 5-month old seedlings, Fosetyl-Al (1 g a.i./l) sponge band caused severe phytotoxicity. Fosetyl-Al (100 & 400 g a.i./l), stem paint and sponge band (0.5 g a.i./l) were effective (Snyman & Kotzé, 1983b, 1984). |
| Leaf spray and trunk injection | Although both neutralised 0.3% $H_3PO_3$ foliar sprays (3 x ) and 2 tree injections (0.4 g active/$m^2$) annually improved tree (rating 5-6) health, foliar spray was less effective than injections (Schutte, Bezuidenhout & Kotzé, 1991). |
| Leaf sprays and trunk injections | $H_3PO_3$ used – no statistical analysis given (Duvenage, 2001). |
| Bark spray, soil drench and trunk injection | Brilliant® and Avoguard® in conjunction with penetrants, enhancers or nutrients (Link®, Anngrow® and FoliarComplex®) were investigated on 11-year old 'Hass'. No statistical analysis given for 2014 (Serfontein, Liebenberg & Grobler, 2014). In next two years, two papers were published which reported that two bark sprays with Brilliant + Link or one soil drench with Brilliant + FoliarComplex improved tree health (Grobler, Meyer, Serfontein & Liebenberg, 2015 & 2016). |
| Foliar spray and trunk injection | Three foliar sprays (0.6% potasium phosphite) was not significantly different in root phosphite concentration compared to one potassium phosphite injection (0.5 g a.i./$m^2$) over a twelve-week period from April in mature 'Hass'/'Dusa' (McLeod, Novela, Pieterse, Beukes, Masikane & Wessels, 2016). |

In a sense this may be true, however, opportunities exist in *P. cinnamomi* research and the industry must take into account that:

- The increase in labour costs relative to net return will bring about mechanisation with corresponding alternative application methods.
- These methods, e.g. leaf spraying, will bring its own challenges (Toerien, 2007). The same applies to soil drenching (Bezuidenhout, publ. sub.).
- The South African industries do not make progress.
- Although *Phytophthora* tolerance/resistance to phosphite/phosphonate is not yet conclusive, signs are on the horizon (Ma & McLeod, 2014).

## *Opportunities*

### Rootstocks

The author is of the opinion that the main impetus to root rot research should concentrate on the selection and genetic manipulation of rootstocks.

Rootstock selection can be a dumb and boring number game based on the variability of avocado population where thousands of seedlings are grown under root rot conditions and the few survivors selected for further testing. Or you may follow the path of natural selection, as in the case of Schieber in Chiepas, Guatemala (Schieber & Zentmyer, 1987), or Joe Darvas along the water streams at Westfalia, or explore your own farm.

Although today most of the trees sold are on clonal rootstocks, a substantial amount of avocado seedlings, with high genetic variability, is planted in Southern Africa. Even with clonal cultivars, the natural offspring, e.g. under the tree canopy, display a high degree of variability. The chance of finding two identical 'Hass' seedlings directly grown from seed is less than one in ten thousand (Bergh & Whitsell, 1973).

Seedlings sourced from under tree canopies, unless phosphite/phosphonate soil drenches or foliar sprays were applied, or in other habitat with high potential of *Phytophthora,* will be worthwhile to test for root rot tolerance without major financial outlay.

### Tissue culture

We travelled far since Dorothe Nel successful grew *Persea indica* seedlings from tissue culture (Nel, Kotzé, & Snyman, 1983) and *in vitro* clonal propagation of avocado rootstocks (Wessels, 1996) to the stage of 15 cm "intensive-care-unit" plants.

Yet even after these years, in an excellent review article by Chathurika *et al*. (2017) on the micro propagation of avocados, the authors concluded that we are far from the transfer from juvenile to mature plants. Rooting success is the missing step which is crucial to achieve an efficient and effective protocol to micro propagate avocados.

Avocados are one of the few hardwood horticultural crops difficult to propagate through tissue culture to a mature plant. Which at this stage prevent producing transgenic avocados with desirable traits, e.g. *Phytophthora* resistance, salt, lime or drought tolerance.

The same does not apply to other crops. Many transgenic grapevines exist in South Africa, although not on commercial scale (with resistance to powdery mildew). Many of these plants were obtained by Quantitative trait locus (QTL). Certain genetically modified (GM) orchard tree species have been deregulated for commercial use in the United States, including the papaya and plum, or in the process of deregulation – chestnut and citrus. The development, testing and use of GM trees remain at an early stage in comparison to GM crops.

> Quantitative trait locus (QTL) is a statistical method that links two types of information – phenotypic data (trait measurements) and genotypic data (usually molecular markers) – in an attempt to explain the genetic basis of variation in complex traits. QTL analysis allows researchers in fields as diverse as agriculture, evolution and medicine to link certain complex phenotypes to specific regions of chromosomes. The goal of this process is to identify the action, interaction, number and precise location of these regions.

### Quantification

Quantification of *Phytophthora* and fungicide is essential components of root rot research. The progress in determining phosphite by high performance ion chromatography is a great step forward (Ma *et al*., 2015) compared to previous methods.

Ma & McLeod (1994) used Polymerase chain reaction (PCR) to identify and distinguish *Phytophthora cinnamomi* phosphite tolerance isolates. A variation on PCR can also be used quantify the pathogen.

> Nucleic acid amplification and detection are among the most valuable techniques used in biological research today. Scientists in all areas of research – basic science, biotechnology, plant pathology, medicine, forensic science, agriculture, and more – rely on these methods for a wide range of applications. For some applications, qualitative nucleic acid detection is sufficient. Other applications include quantitative analysis.
>
> Polymerase chain reaction (PCR) is a laboratory technique used to make multiple copies of a segment of DNA. PCR is very precise and can be used to amplify or copy a specific DNA target from a mixture of DNA molecules. First, two short DNA sequences called primers are designed to bind to the start and end of the DNA target. Then, to perform PCR, the DNA template that contains the target is added to a tube that contains primers, free nucleotides, and an enzyme called DNA polymerase, and the mixture is placed in a PCR machine. The PCR machine increases and decreases the temperature of the sample in automatic, programmed steps. Initially the mixture is heated to denature, or separate, the double-stranded DNA template into single strands. The mixture

is then cooled so that the primers anneal, or bind, to the DNA template. At this point, the DNA polymerase begins to synthesize new strands of DNA starting from the primers. Following synthesis and at the end of the first cycle, each double-stranded DNA molecule consists of one new and one old DNA strand. PCR then continues with additional cycles that repeat the aforementioned steps. The newly synthesized DNA segments serve as templates in later cycles, which allow the DNA target to be exponentially amplified millions of times.

### Ecology and biocontrol

Although many of the organisms mentioned earlier have shown excellent efficacy in pure culture or in greenhouses against *Phytophthora*, they have not been commercially adopted. This has been mainly due to the problems associated with introducing and maintaining populations in the soil.

The report by Darvas (1979a) on the ecology of roots was on a limited scale and no comprehensive *Phytophthora*-avocado ecological study was done in South Africa. Only then can investigations provide insight how to establish a long lasting association between the beneficial microbial population and the avocado. Modern biotechnological techniques and tracers will greatly assist in these studies. With this approach our knowledge of the interactions between the avocado, *Phytophthora*, other microorganism and abiotic variables, will substantial improve, paving the way to successful use of antagonists and other organisms to control root rot on a commercial scale.

Ecological studies is not only applicable to avocado root environment (rhizosphere), but also to the leaf environment (phyllosphere) in light of the latest interest of phosphite foliar sprays.

## CONCLUSION

Although SAAGA and other South African researchers made huge contributions in the chemical control of root rot and root stock selections (where farmers can assist), new developments have occurred in the mean time. We must be cognizant of the *in vitro* phosphite/phosphate interaction on *P. cinnamomi*, and *in vitro* tolerance to phosphite of certain *P. cinnamomi* isolates. The success with micro propagation with hardwood tree crops may prompt a new interest into avocado tissue culture. Advances in biotechnology present opportunities for better understanding of ecology of avocado in relation to microorganisms which can lead to commercial biocontrol.

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## REFERENCES

ALLAN, P., LAMB, D. & CHALTON, D. 1981. Sterilization & pasteurization of soil mixes. *South African Avocado Growers' Association Yearbook* 14: 124-127.

ANONYMOUS, 1965. The avocado in South Africa. *California Avocado Society Yearbook* 49: 73-79.

BEKKER, T.F., LABUSCHAGNE, N. & KAISER, C. 2005. Effects of soluble silicon against *Phytophthora cinnamomi* root rot of avocado (*Persea americana* Mill.) nursery plants. *South African Avocado Growers' Association Yearbook* 29: 58-62.

BEKKER, T.F., KAISER, C. & LABUSCHAGNE, N. 2006. In vitro inhibition of several phytopathogenic fungi from avocado by soluble potassium silicate. *South African Avocado Growers' Association Yearbook* 29: 64-67.

BEKKER, T.F., LABUSCHAGNE, N., AVELING, T. & KAISER, C. 2007. Efficacy of water soluble potassium silicate against *Phytophthora cinnamomi* root rot of avocado under field conditions. *South African Avocado Growers' Association Yearbook* 30: 39-48.

BEKKER, T.F., LABUSCHAGNE, N., AVELING, T. & KAISER, C. 2007. The inhibition of *Phytophthora* root rot of avocado with potassium silicate applications under greenhouse conditions. *South African Avocado Growers' Association Yearbook* 30: 49-56.

BEKKER, T.F., LABUSCHAGNE, N., AVELING, T., KAISER, C. & REGNIER, T. 2007. Accumulation of total phenolics due to silicon application in roots of avocado trees infected with of *Phytophthora cinnamomi*. *South African Avocado Growers' Association Yearbook* 30: 57-63.

BERGH, B.O. & WHITSELL, R.H. 1993. Self-pollinated hass seedlings. *California Avocado Society Yearbook* 57: 118-126.

BEZUIDENHOUT, J.J., KORSTEN, L. & KOTZÉ, J.M. 1985. Monitoring phosphorus compounds in avocado tissues. *South African Avocado Growers' Association Yearbook* 8:100-102.

BEZUIDENHOUT, J.J., DARVAS, J.M. & KOTZÉ, J.M. 1987. Chemical control of *Phytophthora cinnamomi*. Proceedings of the World Avocado Congress: *South African Avocado Growers' Association Yearbook* 10: 106-108.

BEZUIDENHOUT, J.J., DARVAS, J.M. & KOTZÉ, J.M. 1987. The dynamics and distribution of phosphite in avocado trees treated with phosetyl-Al. Proceedings of the World Avocado Congress: *South African Avocado Growers' Association Yearbook* 10: 91-93.

BEZUIDENHOUT, J.J. & TOERIEN, J.C. 1988. Alternatiewe van fosetiel-Al vir die chemiese beheer van *Phytophthora* wortelvrot. *South African Avocado Growers' Association Yearbook* 11:100-102.

BEZUIDENHOUT, J.J. 1995. Evaluering van biologiese beheer in Europa en die VSA – 1994. *South African Avocado Growers' Association Yearbook* 18:118-120.

BEZUIDENHOUT, J.J. 2017. Phosphorus: Nomenclature and properties of phosphorus compounds. *Subtrop Quaterly Journal* 18: 26-28.

BIJZET, Z., SIPPEL, A.D. & KOEKEMOER, P.J.J. 1993. Avocado breeding: A progress report. *South African Avocado Growers' Association Yearbook* 16: 86-89.

BIJZET, Z., BREEDT, H.J., KOEKEMOER, P.J.J. & CILLIERS, B. 1996. Avocado rootstock breeding – new developments and intricacies. *South African Avocado Growers' Association Yearbook* 19: 14-15.

BIJZET, Z., VAN VUUREN, S.P. & SCHROEDER, L. 1997. Progress with local selection in quest for a more tolerant/resistant avocado rootstock than Duke 7. *South African Avocado Growers' Association Yearbook* 20: 18, 19, 21 & 23.

BIJZET, Z. 1998. Development of Avocado root-stocks with improved resistance/tolerance to *Phytophthora* root rot. *South African Avocado Growers' Association Yearbook* 21: 7, 8, 9 & 11.

BOTHA, T., WEHNER, F.C. & KOTZÉ, J.M. 1989. An evaluation of in vitro screening techniques for determining tolerance of avocado rootstocks to *Phytophthora cinnamomi. South African Avocado Growers' Association Yearbook* 12: 60-63.

BOTHA, T. 1991. Evaluasie van avokado-onderstamme – voorlopige verslag. *South African Avocado Growers' Association Yearbook* 14: 87-88.

BOWER, J.P. 1979. Water relations of *Phytophthora* infected fuerte trees and their influence on management. *South African Avocado Growers' Association Yearbook* 3: 25-27.

BOWER, L.A. & COFFEY, M.D. 1984. Development of resistance to phosphorous acid in *Phytophthora cinnamomi.* Annual Meeting American Phytopathological Society, Guelpp, Canada. *Phytopathology,* 811.

BRODERICK, H.T. & FREAN, R.T. 1973. Avocado growers beware of root rot! *The Citrus and Subtropical Fruit Journal* Jan: 6-8.

BUITENDAG, C.H. & BRONKHORST, G.J. 1980. Injection of Insecticides into Tree Trunks– a possible new method for the control of citrus pests. *Citrus and Subtropical Fruit Journal* No. 556: 5-7.

CHATHURIKA, A.H., JAYENI, HAYWARD, A. & MITTE, N. 2017. Micropropagation of Avocado (*Persea americana* Mill. *American Journal of Plant Sciences* 08(11): 2898-2921.

Coffey M.D. 1985. Chemical control of avocado root rot in California. *South African Avocado Growers' Association Yearbook* 8: 12.

CONRADIE, W., SMITH, D.G., KÖHNE, J.S. & KREMER-KÖHNE, S. 1994. Evaluation of New Avocado Scion and Rootstock Cultivars. *South African Avocado Growers' Association Yearbook* 17: 70-71.

DARVAS, J.M. 1977. Soil effects on *Phytophthora* of avocados. *Proceedings of the Technical Committee – South African Avocado Growers' Association* 1:17-20.

DARVAS, J.M. 1978a. Common root pathogens from avocados. *South African Avocado Growers' Association Yearbook* 2: 3-4.

DARVAS, J.M., KOTZÉ, J.M. & TOERIEN, J.C. 1978b. Preliminary results on chemical control of *Phytophthora* root rot in avocados. *South African Avocado Growers' Association Yearbook* 2: 5-6.

DARVAS, J.M., TOERIEN, J.C. & KOTZÉ, J.M. 1979a. Chemical control of *Phytophthora* root rot on fully grown trees. *South African Avocado Growers' Association Yearbook* 3: 23-24.

DARVAS, J.M. 1979b. Ecology of avocado root pathogen. *South African Avocado Growers' Association Yearbook* 3: 31-32.

DARVAS, J.M. 1979c. The lupine bait technique for semi-quantative analysis of *Phytophthora cinnamomi* and other root pathogens in avocado soils. *South African Avocado Growers' Association Yearbook* 3: 29-30.

DARVAS, J.M., TOERIEN, J.C. & KOTZÉ, J.M. 1979d. Control of *Phytophthora* root rot on young replanted avocado trees. *South African Avocado Growers' Association Yearbook* 3: 21-22.

DARVAS, J.M., KOTZÉ, J.M. & TOERIEN, J.C. 1979e. Chemical control of *Phytophthora* root rot on replanted avocado trees. *The Citrus and Subtropical Fruit Journal* 553: 4, 5 & 16.

DARVAS, J.M., 1982a. Chemical control of *Phytophthora* root rot on young replanted avocado trees. *South African Avocado Growers' Association Yearbook* 5: 94-95.

DARVAS, J.M. 1982b. Disease potential and recovery of *Phytophthora cinnamomi* in relation to severity of avocado root rot. *South African Avocado Growers' Association Yearbook* 5: 98-100.

DARVAS, J.M. 1983a. Systemic fungicides applied as trunk paint against root rot of avocados. *South African Avocado Growers' Association Yearbook* 6: 78.

DARVAS, J.M. 1983b. Five years of continued chemical control of Phytophthora root rot of avocados. *South African Avocado Growers' Association Yearbook* 6: 72-73.

DARVAS, J.M., TOERIEN, J.C. & MILNE, D.L. 1983c. Injection of established avocado trees for the effective control of *Phytophthora* root rot. *South African Avocado Growers' Association Yearbook* 6: 76-77.

DARVAS, J.M. 1984a. Zinc supplemented to avocado trees in conjunction with root rot injections. *South African Avocado Growers' Association Yearbook* 7: 79.

DARVAS, J.M. & BECKER, O. 1984b. Failure to control *Phytophthora cinnamomi* and *Pythium splendens* with metalaxyl after its prolonged use. *South African Avocado Growers' Association Yearbook* 7: 77-78.

DARVAS, J.M. & BEZUIDENHOUT, J.J. 1987. Control of *Phytophthora* root rot by trunk injections. Proceedings of the World Avocado Congress: *South African Avocado Growers' Association Yearbook* 10: 91-93.

DE JAGER, I. & KOTZÉ, J.M. 1994. Steam pasteurization as an alternative to Methyl Bromide fumigation for disinfecting container medium. *South African Avocado Growers' Association Yearbook* 17: 59-63.

DURAND, B.J. 1986. Avocado rootstock/scion relationships: A limited survey of the situation in South Africa. *South African Avocado Growers' Association Yearbook* 19: 83-86.

DUVENAGE, J.A. 1990. The influence of N- and Ca-sources on pathogenity of *Phytophthora cinnamomi* and of Ca-sources on resistance of avocado roots to the infection by the fungus – A preliminary report. *South African Avocado Growers' Association Yearbook* 13: 61-62.

DUVENAGE, J.A. & MAAS, E.M.C. 1990. The occurrence of soils suppressive to *Phytophthora cinnamomi*. *South African Avocado Growers' Association Yearbook* 13: 55.

DUVENAGE, J.A. & KOTZÉ, J.M. 1991. The influence of calcium on the saprophytic growth and pathogenicity of *Phytophthora cinnamomi* and on resistance of avocado root rot. *South African Avocado Growers' Association Yearbook* 14: 13-14.

DUVENAGE, J.A., KOTZÉ, J.M. & MAAS, E.M.C. 1991. Suppresive soils and biological control of *Phytophthora* root rot. *South African Avocado Growers' Association Yearbook* 14: 6-14.

DUVENAGE, J.A. 1992. The influence of nitrogen and calcium on *Phytophthora cinnamomi* and the effects on the resistance of avocado root rot. *South African Avocado Growers' Association Yearbook* 15: 12-14.

DUVENAGE, J.A. & KOTZÉ, J.M. 1993. Biocontrol of root rot of avocado seedlings. *South African Avocado Growers' Association Yearbook* 16: 70-72.

DUVENAGE, J.A., KÖHNE, J.S. & KIRKMAN, B. 1993. The influence of organic amendments and discontinuation of chemical root rot control on tree condition and yield of avocado. *South African Avocado Growers' Association Yearbook* 16: 75-76.

DUVENAGE, J.A. & KOTZÉ, J.M. 1994. Fungicidal action of phosphite in Avocado root tips on *Phytophthora cinnamomi*. *South African Avocado Growers' Association Yearbook* 17: 38-45.

DUVENAGE, J.A. 1994. Monitoring the resistance of *Phytophthora cinnamomi* to Fosetyl-Al and $H_3PO_3$. *South African Avocado Growers' Association Yearbook* 17: 35-37.

DUVENAGE, J.A. & KÖHNE, J.S. 1995. Efficacy of *Dimethomorh* against *Phytophthora* root rot of Avocado. *South African Avocado Growers' Association Yearbook* 18: 41-42.

DUVENAGE, J.A. & KÖHNE, J.S. 1997. Biocontrol of root rot in Avocado orchards and monitoring for resistance of *Phytophthora cinnamomi* to phosphites. *South African Avocado Growers' Association Yearbook* 20: 116-118.

DUVENAGE, J.A. & KREMER-KÖHNE, S. 1998. Breeding for resistance to and biocontrol of *Phytophthora cinnamomi* root rot – a progress report. *South African Avocado Growers' Association Yearbook* 21: 1-42.

DUVENAGE, J.A. 2001. Efficacy of $H_3PO_3$ leaf sprays and resistance of *Phytophthora cinnamomi* to $H_3PO_3$. *South African Avocado Growers' Association Yearbook* 24: 13-15.

ERNST, A.A. & HOLTHAUSEN, L.C. 1977. Tolerance of the three Avocado cultivars, Duke, Edranol and Fuerte against *Phytophthora cinnamomi*. Proceedings of the Technical Committee *South African Avocado Growers' Association* 1: 27.

LOURENS, F.J. 1981. Openingsrede. *South African Avocado Growers' Association Yearbook* 4: 14-15.

GROBLER, M., MEYER, J. & LIEBENBERG, J. 2015. Phosphonate treatment as part of early preventative control of *Phytophthora* root rot, pre- and post-planting. *South African Avocado Growers' Association Yearbook* 38: 88-91.

GROBLER, M., MEYER, J., SERFONTEIN, J.J. & LIEBENBERG, J. 2015. Evaluation of alternative methods to stem injections to apply phosphonate to avocado trees for *Phytophthora* control, i.e. bark and soil penetrants to enhance phophonate uptake – final report. *South African Avocado Growers' Association Yearbook* 38: 92-94, 96-97.

GUSTAFSON, D. 1981. Don Gustafson Visits South Africa. *South African Avocado Growers' Association Yearbook* 4: 11-12.

KAISER, C., VAN DER MERWE, R., BEKKER, T.F. & LABUSCHAGNE, N. 2005. *In vitro* inhibition of mycelial growth of several phytopathogenic fungi, including *Phytophthora cinnamomi* by soluble silicon. *South African Avocado Growers' Association Yearbook* 28: 70-74.

KOEKEMOER, P.J.J., BREEDT, H.J., MANICOM, B.Q. & BIJZET, I. 1994. Selection of *Phytophthora* tolerant Avocado rootstocks. *South African Avocado Growers' Association Yearbook* 17: 72-74.

KÖHNE, J.S. 1991. Performance of Hass on three clonal rootstocks. *South African Avocado Growers' Association Yearbook* 14: 39.

KÖHNE, J.S. & KIRKMAN, B. 1991. Observations of avocado orchards where chemical control of *Phytophthora cinnamomi* was discontinued for two years. *South African Avocado Growers' Association Yearbook* 14: 12.

KORSTEN, L. & KOTZÉ, J.M. 1983. Progess Report. Bacterial canker of avocado. *South African Avocado Growers' Association Yearbook* 6: 88-89.

KORSTEN, L. & KOTZÉ, J.M. 1984. Progess Report. Bacterial canker of avocado. *South African Avocado Growers' Association Yearbook* 7: 73-74.

KORSTEN, L. & KOTZÉ, J.M. 1985. Progess Report. Bacterial canker of avocado. *South African Avocado Growers' Association Yearbook* 8: 63-65.

KOTZÉ, J.M., MOLL, J.N. & DARVAS, J.M. 1987. Root rot in South Africa: Past, present and future. Proceedings of the World Avocado Congress: *South African Avocado Growers' Association Yearbook* 10: 89-91.

KREMER-KÖHNE, S. & KÖHNE, J.S. 1994. Horticultural performance of Fuerte avocado on Duke 7 and G6 rootstocks. *South African Avocado Growers' Association Yearbook* 17: 78-79.

KREMER-KÖHNE, S. & DUVENAGE, J.A. 2000. Field testing of new avocado rootstocks for tolerance to root rot. *South African Avocado Growers' Association Yearbook* 23: 70-71.

KREMER-KÖHNE, S., DUVENAGE, J.A. & MAILULA, S.M. 2001. Breeding and field testing of new avocado rootstocks for increased Hass yields and

resistance to root rot. *South African Avocado Growers' Association Yearbook* 24: 33-34.
KREMER-KÖHNE, S., DUVENAGE, J.A. & MAILULA, S.M. 2002. Breeding and field testing of new avocado rootstocks for increased Hass yields and resistance to root rot – a progress report. *South African Avocado Growers' Association Yearbook* 25: 17-19.
LANDMAN, W.P., LABUSCHAGNE, N. & KOTZÉ, J.M. 1996. Optimization of biological control of *Phytophthora* root rot. *South African Avocado Growers' Association Yearbook* 19: 52-53.
LANDMAN, W.P., VAN HEERDEN, I., KOTZÉ, J.M, LABUSCHAGNE, N. & WEHNER, F.C. 1997. Evaluation of *Trichodesma harzianum*, *Bacillus megaterium*, composted Citrus waste and soil solarization on Avocados with and without *Phytophthora cinnamomi* – preliminary results. *South African Avocado Growers' Association Yearbook* 20: 113-115.
LONSDALE, J.H., BOTHA, T., WEHNER, F.C. & KOTZÉ, J.M. 1988. *Phytophthora cinnamomi*, the cause of crown and trunk canker of duke 7 avocado rootstocks in South Africa. *South African Avocado Growers' Association Yearbook* 11: 27-28.
MA, J. & MCLEOD, A. 2014. In vitro sensitivity of South African *Phytophthora cinnamomi* to phosphite and different phosphate concentrations. *South African Avocado Growers' Association Yearbook* 37: 79-84.
MAAS, ERNA M.C. & KOTZÉ, J.M. 1990. The effect of bacteria on root rot caused by *Phytophthora cinnamomi*. *South African Avocado Growers' Association Yearbook* 13: 65-66.
MANS, C.C. & HATTINGH, D.A. 1992. Evaluering van permanente grondbedekkers te Everdon Landgoed, Natal. *South African Avocado Growers' Association Yearbook* 15: 94-96.
MARAIS, P.G. & DE LA HARPE, A.C. 1981. Some aspects of *Phytophthora cinnamomi* rands infection of grapevines. *South African Avocado Growers' Association Yearbook* 4: 109-115.
MAVUSO, Z. 2008. Effect of different mulch materials, compost and organic treatments on tree condition and root health. *South African Avocado Growers' Association Yearbook* 31: 30-33.
MCKENZIE, D. 1984. The long term implications of the use of Ridomil 5G for the control of avocado root rot in South Africa. *South African Avocado Growers' Association Yearbook* 7: 84-88.
MCLEAN, L.C. & KOTZÉ, J.M. 1992a. Efficacy of methyl bromide in nurseries. *South African Avocado Growers' Association Yearbook* 15: 15-17.
MCLEAN, L.C. & KOTZÉ, J.M. 1992b. Effect of different dosages of methyl bromide on nursery pathogens. *South African Avocado Growers' Association Yearbook* 15: 18-23.
MCLEOD, A., LABUSCHAGNE, N. & KOTZÉ, J.M. 1995. Evaluation of *Trichoderma* for biological control of Avocado root rot in bark medium artificially infected with *Phytophthora cinnamomi*. *South African Avocado Growers' Association Yearbook* 18: 32-37.
MCLEOD, A., MA, J., NOVELA, P., NYONI, M., NZANZ, B., STANDER, M. & PIETERSE, P. 2015. Investigating root phosphite quantification methods for optimizing foliar spray application in avocado – a preliminary report. *South African Avocado Growers' Association Yearbook* 38: 80-82, 84-87.
MCLEOD, A., NOVELA, P., PIETERSE, P., BEUKES, I., MASIKANE, S. & WESSELS, J.P.B. 2016. Evaluating foliar phosphonate sprays as an alternative to trunk injections for controlling avocado rot preventatively. *South African Avocado Growers' Association Yearbook* 39: 60-63.
MILNE, D.L., BRODRICK, H.T. & HUGHES, J.P. 1975. Progress in solving the *Phytophthora* problem when replanting avocados. *Citrus Sub-Trop. Fruit J.* 502: 22-24.
MITCHELL, C.F. 1977. Treatment of irrigation water for avocado nurseries. Proceedings of the Technical Committee *South African Avocado Growers' Association* 1: 23.
MOLL, J.N., WOOD, R., FREAN, R.T. & MATARÉ R. 1978. *Phytophthora* Root Rot. *South African Avocado Growers' Association Yearbook* 2: 10-11.
NEL, D.D., KOTZÉ, J.M. & SNYMAN, C.P. 1983. Progress in tissue culture of avocado. *South African Avocado Growers' Association Yearbook* 4: 90-91.
ROE, D. & KÖHNE, J.S. 1996. A progress report of Avocado rootstock research at Merensky Technological Services. *South African Avocado Growers' Association Yearbook* 19: 26-27.
ROE, D., MORUDU, T.M. & KÖHNE J.S. 1998. Commercial avocado rootstock evaluation at Merensky Technological Services: Further progress in 1997. *South African Avocado Growers' Association Yearbook* 21: 3 & 5.
SCHUTTE, G.C., BOTHA T., BEZUIDENHOUT, J.J. & KOTZÉ, J.M. 1988. Distribution of phosphite in avocado trees after trunk injection with phosphorous acid and its possible response to *Phytophthora cinnamomi*. *South African Avocado Growers' Association Yearbook* 11: 32-34.
SCHUTTE, G.C., BEZUIDENHOUT, J.J. & KOTZÉ, J.M. 1991. Timing of application of phosphonate fungicides using different application methods as determined by means of gas-liquid-chromatography for *Phytophthora* root rot control of citrus. *Phytophylactica* 23: 69-71.
SCHIEBER, E. & ZENTMYER, G.A. 1987. Exploring for sources of resistance among *Persea americana* variety Guatamalensis and *Persea schiedeana* in Middle America. *South African Avocado Growers' Association Yearbook* 10: 20-21.
SERFONTEIN, J.J., LIEBENBERG, J. & GROBLER, M. 2014. Evaluation of alternative methods to stem injection to apply phosphonate to avocado trees for *Phytophthora* control, i.e. bark and soil penetrants to enhance phosphonate uptake – preliminary report. *South African Avocado Growers' Association Yearbook* 37: 75-78.
SIPPEL, A.D., SNIJDER, B., WERKSMAN, J. & BIJZET, Z. 1997. Performance of rootstock cultivars in the Avocado evaluation programme: 1997. *South African Avocado Growers' Association Yearbook* 5: 28-29.

SIPPEL, A.D., MATASHA, C.W. & BIJZET, Z. 1998. Performance of rootstock cultivars in the Avocado phase II evaluation programme: 1997. *South African Avocado Growers' Association Yearbook* 21: 13, 15, & 17.

SNYMAN, C.P. 1982. Die evaluasie van swammiddels teen avokadowortelvrot. *South African Avocado Growers' Association Yearbook* 5: 85-93.

SNYMAN, C.P. & DARVAS, J.M. 1982. Die uitwerking van kalsium op die wortelvrot by avokado. *South African Avocado Growers' Association Yearbook* 5: 80-84.

SNYMAN, C.P. & KOTZÉ, J.M. 1983. Evaluation of application techniques of four fungicides for the control of *Phytophthora* root rot on avocado seedlings. *South African Avocado Growers' Association Yearbook* 6: 79-81.

SNYMAN, C.P. & DARVAS, J.M. 1983. Pathogenicity of avocado root isolates to Edranol seedlings and Duke 7 rooted cuttings. *South African Avocado Growers' Association Yearbook* 6: 74-75.

SNYMAN, C.P. & KOTZÉ, J.M. 1983. Efficacy of Systemic fungicides applied as a trunk paint and a sponge band for the control of root rot on five year old avocado trees. *South African Avocado Growers' Association Yearbook* 6: 70-71.

SNYMAN, A., SNYMAN, C.P. & KOTZÉ, J.M. 1984. Pathogenicity of avocado root rot fungi to Edranol and Duke 7 rooted cuttings. *South African Avocado Growers' Association Yearbook* 7: 80-81.

SNYMAN, C.P. 1984a. Loss of control of avocado root rot by metalaxyl. *South African Avocado Growers' Association Yearbook* 7: 89-90.

SNYMAN, C.P. 1984b. The effect of calcium on avocado root growth and on avocado root rot caused by *Phytophthora cinnamomi*. *South African Avocado Growers' Association Yearbook* 7: 91-92.

SNYMAN, C.P. & KOTZÉ, J.M. 1984. The evaluation of new application methods for the control of root rot in field experiments. *South African Avocado Growers' Association Yearbook* 7: 82-83.

SNYMAN, C.P. & KOTZÉ, J.M. 1984. Efficacy of fungicides application for the control of *Phytophthora cinnamomi* root rot on avocado seedlings in the glasshouse. *South African Avocado Growers' Association Yearbook* 7: 75-76.

TOERIEN, J.C. 1977. Production of avocado trees in the nursery. Proceedings of the Technical Committee *South African Avocado Growers' Association* 1: 21-22.

TOERIEN, J.C. & SLABBERT, M.J. 1984. Phosphorus nutrition of Avocado through trunk injection – A preliminary report. *South African Avocado Growers' Association Yearbook* 7: 96.

TOERIEN, J. 2007. The Phytophthora Challenge. *California Avocado Society Yearbook* 90: 89-101.

TOERIEN, J. 2010, 20 May. Westfalia Navorsingsnotas. *Letter to Jurg Bezuidenhout*.

VAN DER MERWE, D.M., MAAS, E.M.C. & KOTZÉ, J.M. 1990. Evaluation of field trees for the resistance to *Phytophthora cinnamomi* by means of the detached root technique. *South African Avocado Growers' Association Yearbook* 13: 63-64.

VAN DER MERWE, D.M. 1990. An improved method to evaluate rootstocks for resistance to *Phytophthora cinnamomi* by means of the detached root technique. *South African Avocado Growers' Association Yearbook* 13: 67-68.

VAN DER MERWE, D. & KOTZÉ, J.M. 1992. Effect of phosphite in avocado roots on the zoospores of *Phytophthora cinnamomi*. *South African Avocado Growers' Association Yearbook* 15: 24-26.

VAN DER MERWE, D.M. 1992. Biological control of *Phytophthora* root rot *in vitro* and *in vivo*. *South African Avocado Growers' Association Yearbook* 15: 27-28.

VAN DER MERWE, DE V.M. 1993. The effect of phosphite in rooted cuttings of Duke 7 avokado on *Phytophthora cinnamomi*. *South African Avocado Growers' Association Yearbook* 16: 72-74.

VAN HEERDEN, I., WEHNER, F.C. & KOTZÉ J.M. 1995. Citrus waste compost for control root-infecting *Phytophthora* species. *South African Avocado Growers' Association Yearbook* 18: 38-40.

WAGER, V.A. 1941. The dying-back or decline disease of avocado trees. *Farming in South Africa* 16: 185-186.

WEHNER, F.C. & KOTZÉ, J.M. 1985. Soil solarisation: A brief introduction. *South African Avocado Growers' Association Yearbook* 8: 75.

WESSELS, H. 1996. *In vitro* clonal propagation of avocado rootstocks. *South African Avocado Growers' Association Yearbook* 19: 59-60.

WOLSTENHOLME, B.N. 1979. Prospects for integrated and biological control of avocado root rot – some overseas impressions. *South African Avocado Growers' Association Yearbook* 3: 17-20.

WOOD, R. & MOLL, J.N. 1981. Results obtained in 1980 from avocado root rot field trials. *South African Avocado Growers' Association Yearbook* 4: 105-108.

ZENTMYER, G.A. 1979. Report on *Phytophthora* root rot of avocado in South Africa. *South African Avocado Growers' Association Yearbook* 3: 7-9.