Effects of soluble silicon against *Phytophthora cinnamomi* root rot of avocado (*Persea americana* Mill.) nursery plants

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ABSTRACT

The world avocado industry has become very reliant on the use of phosphorous acid for the control of root rot caused by Phytophthora cinnamomi. *The threat of resistance remains and every effort must be made to develop alternative control measures. In a greenhouse trial conducted during 2004 at the University of Pretoria, soluble silicon was applied as a soil drench to clonal 'Hass' on 'Edranol' seedling avocado rootstocks in pots either before or after inoculation with* P. cinnamomi*.*

Potting media were kept moist continuously for optimal P. cinnamomi *infection. Forty days after inoculation, trees were harvested, photographed and the root : shoot mass ratio on both a dry and a wet mass basis were determined. In terms of root regeneration, marked differences in trees treated with 20 ml.l-^{1} soluble silicon (20.7% silicon dioxide) before inoculation with* P. cinnamomi*, were observed when compared to all other treatments and untreated control trees. There were no significant differences in root : shoot mass ratios between any of the treatments on either a fresh or a dry mass basis. Trees grown in pine bark medium, however, had consistently higher root : shoot mass ratios on a dry mass basis than soil grown trees. Chlorophyll fluorescence ranged from 30 to 60 SPAD units over the duration of the trial but there were no significant differences between any of the treatments with the exception of the untreated control trees grown in soil on 16 August 2004, four days after inoculation. Leaf diffusive resistance ranged from 1.73 to 81.53 s.cm^{-1} but was only significantly higher in plants grown in soil and treated with silicon after inoculation with* P. cinnamomi.

INTRODUCTION

Phytophthora cinnamomi (*P.c.*) root rot is a plant pathogen of global significance as it affects wild and cultivated plants and is a serious threat to the diversity and structure of natural ecosystems (Wills and Keighery, 1994). It is a diploid oomycete for the majority of its life cycle and occurs only briefly as a haploid zoospore during sexual reproduction (Agrios, 1997). It causes root death in avocado (*Persea americana*) and on average leads to a 10% loss of the world avocado crop annually, which amounts to millions of US dollars worldwide (Zentmeyer & Schieber, 1991; Sheperd, 1991).

Control of *Phytophthora* root rot by chemical means has been explored for nearly half a century. The first significant field-control of root rot was achieved with the introduction of modern systemic fungicides, such as metalaxyl (Ridomil®) and Fosetyl-Al (Alliette®). Although metalaxyl was widely used in South Africa in the early 1980's on producing trees for a few years, resistance problems soon developed. Fosetyl-Al proved successful but was expensive.

Simultaneously, experimental trunk-injections of phosphorous acid undertaken at Westfalia Estate, South Africa in 1981 produced very encouraging results. Today, the South African avocado industry relies almost solely on trunk injections of phosphorous acid for the control of *P.c.* root rot. The danger however, exists for newly developed resistance to occur, which will destroy the industry unless new control measures are discovered and improved (Darvas, 1991).

It is commonly held that plants need 16 essential nutrient elements only to grow (Arnon and Stout, 1939). Epstein (1999), using this proviso, maintained that essentiality of an element depends on:

(a) deficiency of the element, where absence thereof makes it impossible for a plant to complete it's reproductive and/or vegetative cycles;

(b) the deficiency being specific to the element in question, can only be corrected or prevented if the element is supplied; and

(c) the element is directly involved in plant nutrition, apart from the possible correction of unfavourable microbiological or chemical condition of the growth medium.

In reality however, there have been many contradictions which suggest otherwise. Because silicon (Si) does not fit the above definition according to present knowledge, and no Si-O-C or Si-C bonds have been identified in plants, Si is generally considered a non-essential element (Meunier, 2003).

Silicon is the second most abundant molecule in the earth's crust, occurring in living organisms as amorphous silica (SiO_2 nH_2O) and to a lesser extent, soluble silicic acid ($Si(OH)_4$) (Fawe *et al*., 1998; Chen *et al*., 2000).

Although the physiological and nutritional roles of silicon appear to be limited, evidence is accumulating that silicon absorption has numerous benefits for the plant, and in particular plant protection. Inconsistent results have been found between different studies on different species where prophylactic properties are concerned. Cucumber (*Cucumic sativus* L.), rose (*Rosa spp.*) and rice (*Oryza spp.*) have however, received much attention and have been shown to benefit from the application of soluble Si, which leads to disease protection and consequent higher yields (Bowen *et al*., 1995).

According to Russell (1981), numerous physiological processes are affected by phytopathogens. Infected plants usually grow slower than corresponding healthy plants and internodes are generally shorter. Phytopathogens affect photosynthesis and chloroplast chlorophyll content either directly, or indirectly by affecting enzymes associated with photosynthesis. These effects are usually manifested in leaf chlorosis and sometimes altered starch metabolism. Infected plants have a high respiration rate, especially where hypersensitive host reactions are involved. Once infected, most plants are less vigorous, have smaller root and canopy systems than healthy plants and leaf development is usually delayed.

However, factors controlling carbon partitioning are independent of those that affect photosynthesis and diffusive resistance. Carbon partitioning between organs is scarcely understood and appreciable errors are made when estimating carbon partitioning as a result of photosynthesis alone. Clearly, this is due to the nutrient content of plant material that may range between 5-20% of the dry mass (Farrar, 1993).

Even more serious is the effect of respiration, as a growing organ respires roughly as much carbon as is contributed to its increased mass, while storage organs respire somewhat less (Farrar, 1993).

In general, there is an increase in specific respiration rate in infected plant tissue. A large portion of the increase in respiration rate is due to actual increases within the host, although respiration by the pathogen also contributes to the total.

Research aimed at understanding the rise in respiration, or decrease in diffusive resistance of infected tissue, is based on the proposal by Allen (1953) that pathogens are responsible for the production of a compound that uncouples ATP formation from mitochondrial electron transport.

Some evidence suggests that activity of the oxidative pentose phosphate pathway may increase relative to glycolysis in diseased tissues.

Plant tissue undergoing necrosis might also consume O_2 at accelerated rates due to peroxidase activity (Amthor, 1989). Daly (1976) and Kosuge & Kimpel (1981) proposed that a host-plant respiration interaction might be necessary for pathogen development and growth, and plants might therefore supply energy and carbon skeletons to the pathogen.

The overall aim of this study was to determine whether the addition of soluble silicon to *P.c.* inoculated avocado nursery trees would make them resistant to or more tolerant of the fungus. Simultaneously, the effects of soluble silicon on these infected trees in terms of changes in chlorophyll fluorescence and leaf diffusive resistance were also investigated.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Some one-year-old clonal 'Hass' on 'Edranol' seedling avocado rootstocks from Allesbeste Nursery, Duiwelskloof, grown in composted pine-bark medium, were replanted in steam-sterilized Hutton soil acquired from the University of Pretoria experimental farm and allowed to re-establish before the experiment was initiated.

Some of these trees were sprayed with 1% phosphorous acid (buffered to pH 7.2 using KOH) on 23 July 2004, three weeks before inoculation, and on 16 August 2004, four days after inoculation with *P.c.*.

Other trees were drenched with one litre 20 $ml.L^{-1}$ soluble silicon (20.7% silicon dioxide) after Kaiser *et al*. (2005) on both or the latter of two dates on which phosphorous acid foliar treatments were undertaken.

All these trees together with similar untreated controls were inoculated with *P.c.* (Table 1) and grown in a controlled environment glasshouse under growth conditions as follows: – diurnal fluctuations of 7ºC to 32ºC and relative humidity between 30 and 90%. Trees were irrigated twice daily with 100 ml using drippers, scheduled with a WaterMaster (Orbit®) irrigation control system to ensure adequate wetting of the media for *P.c.* survival. Trees were fertilized fortnightly using 10 g of Feed-All® per 2.5 l pot. The fertilizer contained all essential macro and micronutrients.

Table 1. Treatment layout for 20 ml soluble silicon (20.7% silicon dioxide), 1% phosphorous acid and *Phytophthora cinnamomi* (*P.c.*) inoculation.

Treatment	23/07/04	12/08/04	16/08/04
No silicon & no *P.c.*			
20 ml silicon only	A		A
20 ml silicon then *P.c.*	A	B	A
P.c. then 20 ml silicon		B	A
P.c. only		B	
1% phosphorous acid	C	B	C

A Application of 1 l of 20 $ml.l^{-1}$ soluble silicon/pot
B Inoculation with *P.c.*
C Foliar application of a 1% phosphorous acid

Table 2. Mean shoot and root dry mass (g) for avocado nursery trees grown in Hutton soil, inoculated with *Phytophthora cinnamomi* (*P.c.*) and harvested 40 days later (alphabetical letters denote significance at 5% confidence interval).

Treatment	Shoot Dry Mass (g)	Root Dry Mass (g)
No Silicon + no *P.c.*	11.61a	8.96 bc
20 ml silicon only	12.83a	13.46 ab
20 ml Silicon then *P.c.*	11.50a	15.28 a
P.c. then 20 ml silicon	12.10a	11.26 abc
P.c. only	9.78a	8.53 c
1% phosphorous acid	11.85a	12.61 abc

Inoculation Procedure

P.c., obtained from a recent field isolate at Merensky Technological Services, Tzaneen was grown for two weeks on 1 kg millet seed to ensure sufficient inoculation material. On 12 August 2004, three equidistant cylindrical holes 10 mm in diameter were made in the soil medium of each pot, at a distance of 50 mm from the stem of each tree. Thereafter, 20 ml of *P.c.* inoculated millet seed were placed in each hole, which was then sealed and wet thoroughly. Six trees were assigned to each treatment and pots were placed randomly on the glasshouse worktop benches to ensure even growth.

Diffusive Resistance and Chlorophyll Fluorescence

Leaf diffusive resistance was measured using a LI-1600 portable steady-state porometer (Li-Cor®, Lincoln, NE). Diffusive resistance was recorded fortnightly between on 2 August and 13 September 2004 (Fig. 1). All measurements were taken in the upper third of the canopy on the youngest most fully expanded leaf. Leaf temperatures for all treatments were regulated by the porometer cuvette, so that they were constant for the day of measurement. Simultaneously, chlorophyll fluorescence was recorded using a Minolta® SPAD-502 chlorophyll meter (Fig. 2).

Harvesting

On 21 September 2004, forty days after inoculation with *P.c.*, trees were destructively harvested and

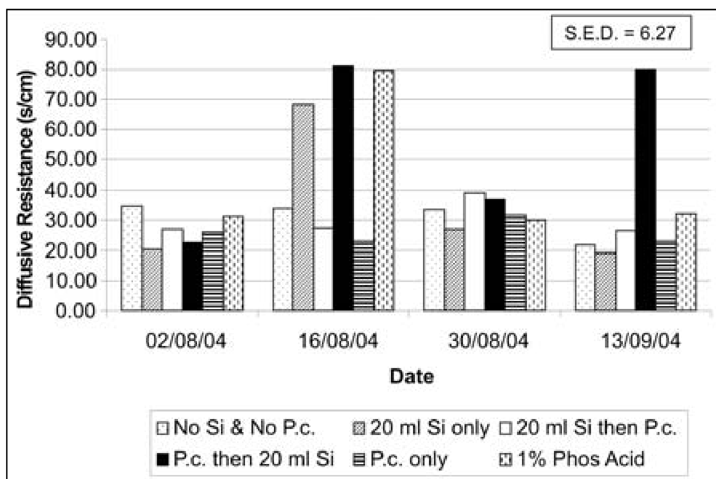

Figure 1. Diffusive resistance (s.cm^{-1}) of avocado seedling trees before and after inoculation with *Phytophthora cinnamomi* (*P.c.*).

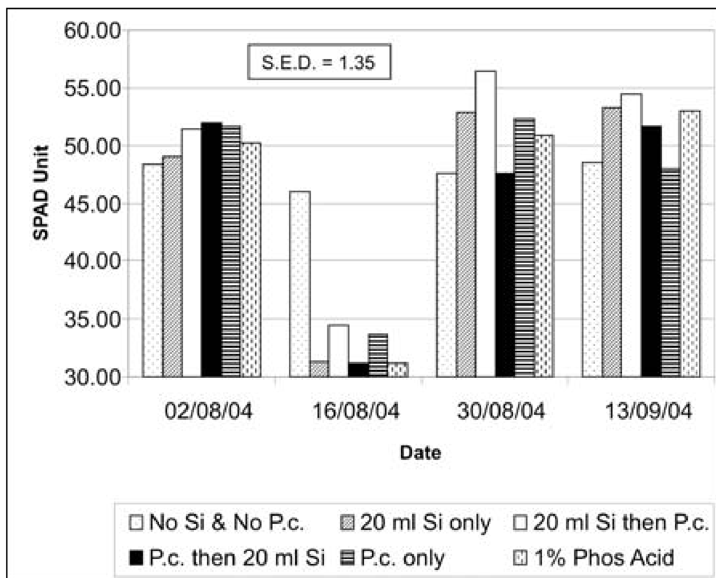

Figure 2. Chlorophyll fluorescence (SPAD units) of avocado seedling trees before and after inoculation with *Phytophthora cinnamomi* (*P.c.*).

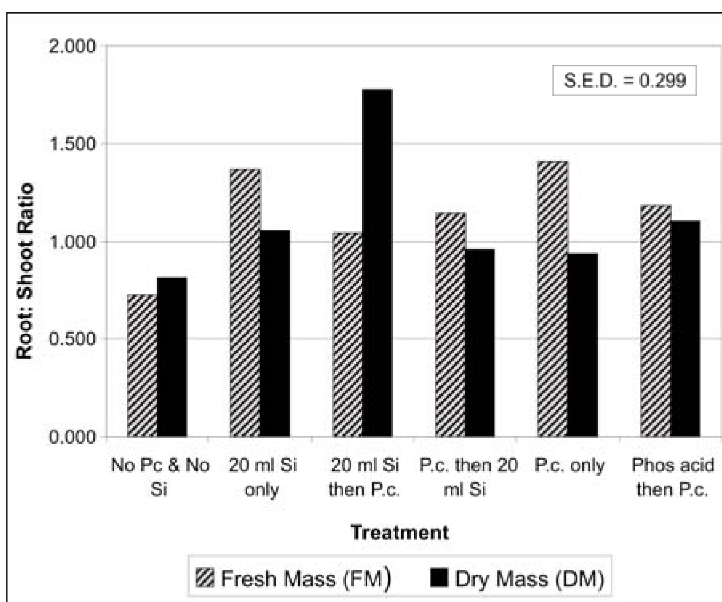

Figure 3. Root : shoot mass ratio (fresh mass and dry mass basis) of avocado nursery trees in Hutton soil before and after inoculation with *Phytophthora cinnamomi* (*P.c.*).

intact roots and shoots were photographed for each replicate. With the use of these photographs, an infection-key was compiled with ratings from 1 to 5, where 1 = all roots dead, and 5 = persistent old roots, as well as copious new root-growth. Fresh mass was determined gravimetrically for both roots and shoots of each plant. All plant material was dried in a forced draught oven at 65ºC and final dry mass recorded for roots and shoots of each plant and root : shoot mass ratios on a fresh and a dry mass basis were graphed (Fig. 3).

Data analysis

All data were analysed using Genstat® 4.23 DE for Windows®. Means, standard errors and LSDs were calculated for diffusive resistance, chlorophyll fluorescence and root : shoot mass-ratios.

RESULTS AND DISCUSSION

Diffusive resistance was fairly constant throughout the experiment (Fig. 1) with no significant differences detected on 2 August 2004, ten days before inoculation, nor on 30 August 2004, eighteen days after inoculation. However, on 16 August 2004, four days after inoculation with *P.c.*, untreated controls, trees drenched with silicon then inoculated with *P.c.* or trees inoculated with *P.c.* only had significantly lower diffusive resistance than trees inoculated with *P.c.* followed by silicon, trees sprayed with 1% phosphorous acid treatments, and trees drenched with 20 ml silicon only. On 13 September 2004, 32 days after inoculation, trees inoculated with *P.c.* and then drenched with silicon had significantly higher diffusive resistance than any of the other treatments, which were all similar.

Where chlorophyll fluorescence was concerned, there were no significant differences between any of the treatments on 2 August 2004 before inoculation with *P.c.*, nor after inoculation on 30 August and 13 September 2004 (Fig. 2). On 16 August 2004, four days after inoculation, chlorophyll fluorescence was significantly lower than on any other sampling date (F Pr. <0.001). Furthermore, all treatments which were inoculated with *P.c.* had significantly lower chlorophyll fluorescence than those trees which were not drenched with soluble silicon nor inoculated with *P.c.* (F Pr. = 0.016). Interestingly, trees which were drenched with soluble silicon but not inoculated with *P.c.*, also had low chlorophyll fluorescence but the exact reason for this is unclear.

There were no significant differences between treatments with regard to leaf mass on both a fresh and a dry mass basis, nor root mass on a fresh mass basis. There were however, significant differences in dry mass of the roots between treatments (F Pr. = 0.047). Here, 20 ml.l^{-1} soluble silicon followed by inoculation with *P.c.* had the highest mean dry root mass at 15.28 g compared to untreated control trees, which had the lowest mean dry root mass of 8.53 g (Table 2). Regeneration of roots followed a similar trend but the results were not significantly different and it was mostly likely due to the experiment being terminated prematurely. Despite the fact that differences in mean root mass on a dry mass basis were significant, there were no significant differences in root : shoot mass ratios on a fresh (0.724 to 1.369) or a dry mass (0.815 to 1.774) basis. This was surprising, given the fact that root masses were greater than the shoot mass in more instances (Fig. 3).

Where root regeneration was concerned, only roots of trees treated with either 1% phosphorous acid (Fig. 4A) or drenched with soluble silicon and then inoculated with *P.c.* (Fig. 4B) showed major signs of regrowth when compared to those inoculated with *P.c.* only (Fig. 4C). Statistically however, there were no significant differences between any of the different treatments and this was most likely because the trial was terminated prematurely where root regeneration was concerned. Field trials investigating root regeneration of established trees suffering from *P.c.* are implicated.

Figure 4. Digital images of tree roots treated with 1% phosphorous acid (A); with soluble silicon and then inoculated with *Phytophthora cinnamomi* (B); and with *Phytophthora cinnamomi only* (C). New root growth can only be seen on trees sprayed with 1% phosphorous acid or drenched with 20 ml soluble silicon (20.7% silicon dioxide) per litre of water.

CONCLUSIONS

Diffusive resistance for trees from all treatments was relatively constant before inoculation with *P.c.* but increased in those trees which were sprayed with phosphorous acid or drenched with soluble silicon four days after inoculation. Farrar (1993) provided supporting evidence for this when he found that *P.c.* infection decreased diffusive resistance, thus increasing respiration of avocado plants. Furthermore, respiratory increases due to fungal infection have been attributed to a decline in photosynthesis and increased activity of the pentose phosphate pathway (Daly, 1976; Scott & Smillie, 1963; Scott & Smillie, 1966). Scholes and Farrar (1986) suggested that respiratory increases might have been due to a loss in chlorophyll.

After inoculation with *P.c.*, the increase in diffusive resistance effect was not carried over except for trees drenched with soluble silicon after inoculation on 13 September 2004, some thirty-two days after inoculation. Clearly, phosphorous acid foliar sprays or drenching of soil with soluble silicon after inoculation had a beneficial effect on diffusive resistance of avocado trees in this study and it is imperative that this be confirmed for trees growing in the field.

Before inoculation with *P.c.* there were no significant differences in chlorophyll fluorescence between different treatments on 2 August 2004, which implies that silicon applications did not have any effect on photosynthesis (Fig. 2). Chlorophyll fluorescence was, however, significantly lower in all plants inoculated with *P.c.* four days after inoculation on 16 August 2004. On 30 August and 13 September 2004, chlorophyll fluorescence in all treatments had recovered from the effects of *P.c.* infection as there were no significant differences observed on these dates. Clearly, *P.c.* infection has an initial effect on photosynthesis but fortunately is not perpetuated.

Indeed, Bishop *et al.* (2002) found an increase in cellular carbohydrates of *P.c.* infected tissues viz. the roots. Furthermore, this was accompanied by a depletion of stored carbohydrates in the leaves and the decrease in chlorophyll fluorescence observed in the current study is supporting evidence for a decrease in photosynthesis of infected plants.

No significant differences were found in the root : shoot mass ratio on either a fresh or a dry mass basis but is it possible that attrition of roots may have been responsible for some of these differences. There were however, highly significant differences in root dry mass between different treatments. Trees which were drenched with 1 l of 20 ml.l^{-1} soluble silicon, ten days before inoculation with *P.c.* had the highest root mass when compared to all other treatments. This implies that silicon imparts some form of protection to avocado roots if applied prior to *P.c.* inoculation. Furthermore, it may be possible to exploit this protection if soluble silicon is applied even earlier than ten days before inoculation. Drawing on this knowledge, where *P.c.* infection is already prevalent in the field, it is expected that protection of large trees, as a result of drenching the soil with soluble silicon, would be incremental and future research is paramount. Finally, root regeneration studies in the field in response to drenching trees with soluble silicon are also critical.

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