In-vitro inhibition of mycelial growth of several phytopathogenic fungi, including *Phytophthora cinnamomi* by soluble silicon

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ABSTRACT

Currently, the world avocado industry relies almost solely on phosphorous acid to control Phytophthora cinnamomi *root rot. Alternative chemicals must be sought to prevent any potential resistance from developing. The present study examined the use of liquid potassium silicate for activity against several types of phytopathogenic fungi.* In-vitro *dose-responses towards soluble potassium silicate (20.7% silicon dioxide) were determined for* Phytophthora cinnamomi, P. capsici, Sclerotinia sclerotiorum, Sclerotium rolfsii, Pythium F-group, Mucor pusillus, Drechslera *spp,* Fusarium oxysporum, F. solani, Alternaria solani, Colletotrichum coccodes, Verticillium fungicola, Curvilaria lunata *and* Stemphylium herbarum*. The standard agar amended method was used for these tests. Inhibition of mycelial growth was dose-dependant with 100% inhibition at 80 ml (pH 11.7) and 40 ml (pH 11.5) soluble silicon (20.7% silicon dioxide) per litre of agar, for all fungi tested in two experiments with the exception of* Drechslera *at 40 ml in one experiment. In addition, for the first experiment,* Colletotrichum coccodes, Mucor pusillus, Sclerotium rolfsii, Sclerotinia sclerotiorum *and* Phytophthora cinnamomi *were completely inhibited at 5, 10 and 20 ml of soluble silicon per litre of agar. For the second experiment,* Sclerotinia sclerotiorum *and* Phytophthora cinnamomi *were completely inhibited at concentrations between 5 and 80 ml soluble silicon per litre of agar. For both experiments,* Phytophthora capsici, Pythium F-group, Drechslera *spp,* Fusarium oxysporum, F. solani, Alternaria solani, Verticillium fungicola, Curvularia lunata *and* Stemphylium herbarum *were partially inhibited at 5, 10 and 20 ml soluble silicon per litre of agar but percentage inhibition was positively correlated with dosage concentrations. Soluble silicon raised the pH of unameliorated agar from 5.6 to 10.3 and 11.7 at concentrations of 5 and 80 ml silicon per litre of agar respectively. Subsequent investigations into the effect of pH 10.3 and 11.7 in the absence of silicon, showed that* Phytophthora cinnamomi *was only partially inhibited (12.6%) at pH 10.3. Clearly, silicon had an inhibitory effect on fungal growth in vitro and this was mostly fungicidal. Pot and field trials are necessary to confirm efficacy of silicon against plant pathogenic fungi* in vivo.

INTRODUCTION

Avocado root rot, caused by *Phytophthora cinnamomi*, is a devastating disease affecting the entire world industry. Chemical control was developed in the 1980's and the world industry has come to rely heavily on the continued use of phosphorous acid. There is however, a danger of resistance in the long term and *in vitro* studies have already shown that streptomycin-resistant and chloramphenicol-resistant mutants of *P. cinnamomi* could easily be obtained from incubating culture blocks on medium containing these antibiotics (Ann & Ko, 1992). Furthermore, Zheng & Ko (1996) produced *P. cinnamomi* resistant to the fungicide chloroneb on agar medium within three weeks whereas, Gu & Ko (2000) produced mutants of *Phytophthora parasitica* on agar medium, which were resistant to Metalaxyl. Ideally, alternative non-chemical fungicides should be sought and to this end the role of silicon must be investigated.

Silicon is a major inorganic constituent of higher plants but has not been given much attention in commercial plant research as either a micro or macro-element. Historically, research from as early as 1860 maintained that Si is not necessary for plant growth.

This misconception has been perpetuated in modern science probably due to the inability to define "essentiality" satisfactorily (Epstein, 1999). According to the current accepted definition of essentiality, "an element is not considered essential unless (a) a deficiency of it makes it impossible for the plant to complete the vegetative or reproductive stages of its life cycle; (b) such deficiency is specific to the element in question, and can be prevented or corrected only by supplying this element; and (c) the element is directly involved in the nutrition of the plant quite apart from its possible effects in correcting some unfavourable microbiological or chemical condition of the soil or other cultural medium" (Arnon & Stout, 1939).

There are however, several flaws in this assumption viz. a) many plants may suffer deficiencies but complete their life cycle; b) some elements may substitute for others but as a result, plants will not yield optimally; and c) concentrations tested during previous investigations may not have been sufficiently low to achieve deficiencies.

Post 1950 research has shown a clear role for silicon in several instances including substantially increasing yields in sugarcane (Clements, 1965) and rice (Mitsui and Takatoh, 1963; Lian, 1976). There is also substantial evidence indicating a role in plant growth and development across a range of crops including loblolly pine, cotton and Poinsettia. Investigations into the effect of soluble silicon on cucumber, a C3 plant, found that plants grown in a recirculating nutrient solution with ten times ambient silicon concentrations had thicker, dark green lower leaves with noticeably rougher surfaces than control plants.

The petioles of the silicon treated leaves, had a greater dry weight per unit area, were less prone to wilting and senescing and had 50% higher Rubisco (Adatia and Besford, 1986). All this evidence points towards a structural role of silicon in cell walls,

where Si in the form of solid amorphous silica. $SiO_2.nH_2O$ is deposited into the cell walls as "opal phytoliths" (Perry, Williams & Fry, 1987; Samuels *et al*., 1993).

This in turn leads on to the role of silicon in plant diseases with numerous studies having shown increased resistance to plant fungal diseases as a response to silicon applications. These include increased resistance to powdery mildew (*Sphaerotheca fuliginea*) in cucumbers (Adatia and Besford, 1986; Belanger *et al*., 1995), powdery mildew (*Uncinula necator*) in grapes (Bowen, Menzies & Ehret, 1992), powdery mildew (*Erysiphe cichoracearum*) in muskmelons (Menzies, Bowen & Ehret, 1992) as well as *Pythium* spp. and *Cladosporium* spp. in cucumbers (Cherif, Asselin & Belanger, 1994).

In addition to the structural role that silicon is thought to play, other studies have shown a flavonoid phytoalexin mediated response to silicon applications (Fawe *et al*., 1988) and prophylactic properties of Si in plant defence reactions to fungal attack (Cherif, Asselin & Belanger, 1994).

In the light of the above evidence, preliminary research trials were laid out by the author while employed by the KZN Department of Agriculture and Environmental Affairs at Cedara in 2000/01. Here the effects of a once off application of potassium silicate solution to the root zone of *Phytophthora* infested avocados was compared with phosphorous acid treated control trees and untreated trees. Results were most promising as trees, which were treated with potassium silicate in summer showed the greatest recovery six months after application, during late winter when *Phytophthora* is most prevalent.

A recent study by Anderson *et al*. (2004) in Australia found that avocado trees, with a rating of 5.5 on the Ciba-Geigy 0 to 10 scale (Darvas, 1983), injected with 200 ppm silicon resulted in a 31.1% increase in tree vigour when compared to untreated control trees. Furthermore, vegetative vigour increases as a result of rapid growth of dormant epicormic buds. They suspected however, that the initial response was not solely due to the control of *Phytophthora*.

The current *in vitro* study was therefore initiated to assess the *in vitro* effect of soluble potassium silicate on *Phytophthora cinnamomi* and thirteen other plant pathogenic fungi from different groups, including the Ascomycota, Basidiomycota, Oomycota and the Zygomycota.

MATERIALS AND METHODS

Several fungal species, maintained on potato dextrose agar (PDA), were obtained from the University of Pretoria's culture collection. An isolate of *Phytophthora cinnamomi* was obtained from Merensky Technological Services, Tzaneen. Fungi were selected on the basis of their taxonomic diversity in order to compare the differences and similarities between different genera within six classes and four groups (Table 1).

Agar Preparation

Potato dextrose agar (PDA) was prepared and autoclaved. Soluble silicon was passed through a 0.45 µm Millipore filter and added to the PDA after autoclaving at a concentration of 5, 10, 20, 40 and 80 ml per litre of PDA after autoclaving. The Silicon-Agar solutions were mixed with magnetic stirrers to ensure even distribution of silicon and then decanted into Petri dishes and incubated for seven days to ensure no contamination had taken place.

Antifungal Activity Assay

Silicon ameliorated agar plates were inoculated with each fungus by placing a 5 mm diameter disc from an actively growing culture in the centre of each plate. Six replicate plates were used per treatment. Fungi were also grown on non-ameliorated PDA (i.e. with no silicon) as a control. All fungi were incubated at 25°C for seven days in the dark. Fungal growth (colony diameter) was measured and percentage inhibition calculated according to the formula:

$$\text{Percentage inhibition} = \frac{(C-T) \times 100}{C}$$

Where, C = colony diameter (mm) of the control
T = colony diameter (mm) of the test plate.

Percentage inhibition was calculated and an analysis of variance for the different treatments was conducted using Genstat 4.23 DE (Tables 2 & 3).

pH determination

Soluble silicon (20.7% silicon dioxide) has a pH of 12.7, which markedly increased the pH of PDA solutions. Unameliorated PDA has a pH of 5.6 but upon addition of 5, 10, 20, 40 and 80 ml of soluble silicon, the pH of the PDA was raised to 10.3, 10.7, 11.2, 11.5 and 11.7 respectively. High pH is known to suppress fungal growth and the effects of elevated pH in the absence of silicon were investigated. The pH of PDA plates were adjusted to pH 10.3 and 11.5, using potassium hydroxide. Six replicate plates were used per treatment and colony growth of all fungi were recorded after seven days at 25ºC in the dark. An analysis of variance was performed using Genstat 4.23 DE (Table 4).

Table 1. Different fungal species within different classes and groups tested.

Fungal group	Class	Genus & species	Disease name	Host plant
Ascomycota	Loculoascomycetes	*Alternaria solani*	Early blight	Tomato
		Curvularia lunata	Leaf spot	Lettuce
		Stemphylium herbarum	Leaf spot	Lettuce
		Dreschlera spp.	Leaf spot	Tomato
	Perithecial Ascomycetes	*Colletotrichum coccodes*	Anthracnose	Tomato
		Fusarium oxysporum	Wilt	Banana
		Fusarium solani	Wilt	Cucumber
		Verticillium fungicola	Wilt	Tomato
	Discomycetes	*Sclerotinia sclerotiorum*	Rot	Lettuce
Basidiomycota	Basidiomycetes	*Sclerotium rolfsii*	Wilt	Tomato
Oomycota	Oomycetes	*Phytophthora capsici*	Root rot	Tomato
		Phytophthora cinnamomi	Root rot	Avocado
		Pythium F-group	Root rot	Lettuce
Zygomycota	Zygomycetes	*Mucor pusillus*	Postharvest rot	Various hosts

RESULTS AND DISCUSSION

At concentrations of 40 and 80 ml.l $^{-1}$ PDA, soluble silicon (20.7% silicon dioxide) had completely suppressed all fungi tested (Tables 2 & 3), with the exception of *Drechslera* spp. (96.7% inhibition) in the first experiment (Tables 2 & 3). In both instances, 5 ml or more of soluble sili-

Table 2. Mean % inhibition of different fungi at different silicon (20.7% SiO_2) concentrations (Experiment 1) in ameliorated potato dextrose agar.

PATHOGEN	PERCENTAGE INHIBITION					
	5 ml Si/l (pH 10.3)	10 ml Si/l (pH 10.7)	20 ml Si/l (pH 11.2)	40 ml Si/l (pH 11.5)	80 ml Si/l (pH 11.7)	F Pr.
Alternaria solani	15	52	80	100	100	<0.001
Curvularia lunata	6.3	25	98	100	100	<0.001
Stemphylium herbarum	8.2	24	97	100	100	<0.001
Drechslera spp.	25	42	82	96.7	100	<0.001
Colletotrichum coccodes	100	100	100	100	100	<0.001
Fusarium oxysporum	-26	58	61	100	100	<0.001
Fusarium solani	5.8	0.8	59	100	100	<0.001
Verticillium fungicola	-2	44	87	100	100	<0.001
Sclerotinia sclerotiorum	100	100	100	100	100	<0.001
Sclerotium rolfsii	100	100	100	100	100	<0.001
Phytophthora capsici	23	97	100	100	100	<0.001
Phytophthora cinnamomi	100	100	100	100	100	<0.001
Pythium F-group	0	100	100	100	100	<0.001
Mucor pusillus	100	100	100	100	100	<0.001

Table 3. Mean % inhibition of different fungi at different silicon (SiO_2) concentrations (Experiment 2) in ameliorated potato dextrose agar.

PATHOGEN	PERCENTAGE INHIBITION					
	5 ml Si/l (pH 10.3)	10 ml Si/l (pH 10.7)	20 ml Si/l (pH 11.2)	40 ml Si/l (pH 11.5)	80 ml Si/l (pH 11.7)	F Pr.
Alternaria solani	23	19	100	100	100	<0.001
Curvilaria lunata	0	59	100	100	100	<0.001
Stemphylium herbarum	2.1	7.3	82	100	100	<0.001
Drechslera spp.	1.8	1.4	44	100	100	<0.001
Colletotrichum coccodes	41	56	90	100	100	<0.001
Fusarium oxysporum	0	0	99	100	100	<0.001
Fusarium solani	-27	-20	100	100	100	<0.001
Verticillium fungicola	-18	6.5	23	100	100	<0.001
Sclerotinia sclerotiorum	100	100	100	100	100	<0.001
Phytophthora cinnamomi	100	100	100	100	100	<0.001
Pythium F-group	53	72	100	100	100	<0.001
Mucor pusillus	0	100	100	100	100	<0.001

Table 4. Mean colony diameters and percentage inhibition as compared to pH 5.6 of different fungal mycelia on potato dextrose agar at different pH values, adjusted using potassium hydroxide.

Fungal species	pH 5.6	pH 10.3		pH 11.7		F.Pr.
	Colony diameter	Colony diameter	% Inhibition	Colony diameter	% Inhibition	
Alternaria solani	8.17	4.67	42.8	4.12	49.5	<0.01
Stemphylium herbarum	6.58	6.86	-0.04	6.48	1.5	0.21
Fusarium solani	13.85	14.83	-0.07	9.67	30.1	<0.01
Verticillium fungicola	5.35	5.62	-0.05	4.75	11.2	<0.01
Phytophthora cinnamomi	37.5	32.75	12.6	0	100	<0.01
Phytophthora capsici	15.75	13.00	17.4	0	100	<0.01
Pythium F-group	37.5	37.5	0	2.83	92.4	<0.01

con suppressed fungal growth of *Phytophthora cinnamomi* (Fig. 1), *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*. In contrast, 5 ml of soluble silicon actually increased fungal growth of *Fusarium oxysporum* (Fig. 2) and *Verticillium fungicola* and up to 10 ml of soluble silicon increased fungal growth of *Fusarium solani*. This has implications for the control of these diseases using soluble silicon and researchers and growers alike must ensure that ambient soil concentrations of silicon are not such that addition of soluble silicon will be conducive to the growth of these fungi. All the other fungi tested in this investigation showed various degrees of suppression of growth at silicon concentrations between 5 and 20 ml. Furthermore, at these low concentrations, results were not consistent between the two replicate experiments.

Where the effect of pH only on fungal growth was concerned (Table 4), at pH 10.3, mycelial growth of *Pythium* F-Group was unchanged when compared to unameliorated PDA at pH 5.6. At pH 11.7 *Pythium* F-Group was inhibited by 92.4%. Indeed, at pH 10.3, *Stemphylium herbarum*, *Fusarium solani* and *Verticillium fungicola* fungal growth was actually accelerated by 0.04, 0.07 and 0.05% respectively. At pH 11.7 however, this trend was reversed and growth of these three fungi was inhibited by 1.5, 30.1 and 11.2% respectively. In contrast, at pH 10.3 fungal growth of *Alternaria solani*, *Phytophthora capsici* and *Phytophthora cinnamomi* were inhibited by 42.8, 17.4 and 12.6% respectively. At pH 11.7 however, fungal growth was completely inhibited in both *Phytophthora capsici* and *P. cinnamomi* whereas *Alternaria solani* was only inhibited by 49.5%.

Complete inhibition of *P. cinnamomi* was found at the lowest silicon concentration of 5 ml soluble silicon per litre of agar (Tables 2 & 3). This was however, not due to a pH effect because the pH value of 10.3 (which prevails at this silicon concentration) only caused a 12.6% reduction in growth of the fungus during the pH investigation (Table 4).

CONCLUSIONS

Clearly, soluble silicon (20.7% silicon dioxide) has fungicidal activity. The concentration at which complete suppression of a particular fungus occurs is variable and must be determined *in vitro* before *in vivo* investigations are initiated. Growth of some fungi such as *Fusarium* spp. and *Verticillium* spp. was actually enhanced at concentrations of 5 and 10 ml of soluble silicon. *Phytophthora cinnamomi* was however, suppressed at concentrations of ≥5 ml soluble silicon per litre of agar. Consequently, future research into the effects of soluble silicon on *Phytophthora cinnamomi*, must include a safety margin where *in vivo* investigations are concerned. Based on the current results a minimum dosage of 20

ml per litre of soluble silicon solution per litre of water is recommended to ensure adequate control of *Phytophthora cinnamomi* in the field and pot trials. To this effect subsequent research by co-workers is reported on elsewhere in this volume (Bekker *et al*., 2005).

Furthermore, given the fact that fungal growth continued, albeit at a slower rate, at high pH (in the absence of silicon), the implication is that soluble silicon has a two-fold effect on *Phytophthora*. The fungicidal effect was evidently the overriding factor. This was especially so for other fungi such as *Fusarium* spp., *Stemphylium herbarum* and *Verticillium fungicola*, where fungal growth was enhanced at pH 10.3 in the absence of silicon.

On this aspect, it would be interesting to determine whether the release of dormant epicormic buds of avocado trees, suffering from *Phytophthora* root rot, in response to stem injections by Anderson *et al*. (2004) was perhaps a pH effect rather than a fungicidal effect. Future investigations on this aspect are currently underway.

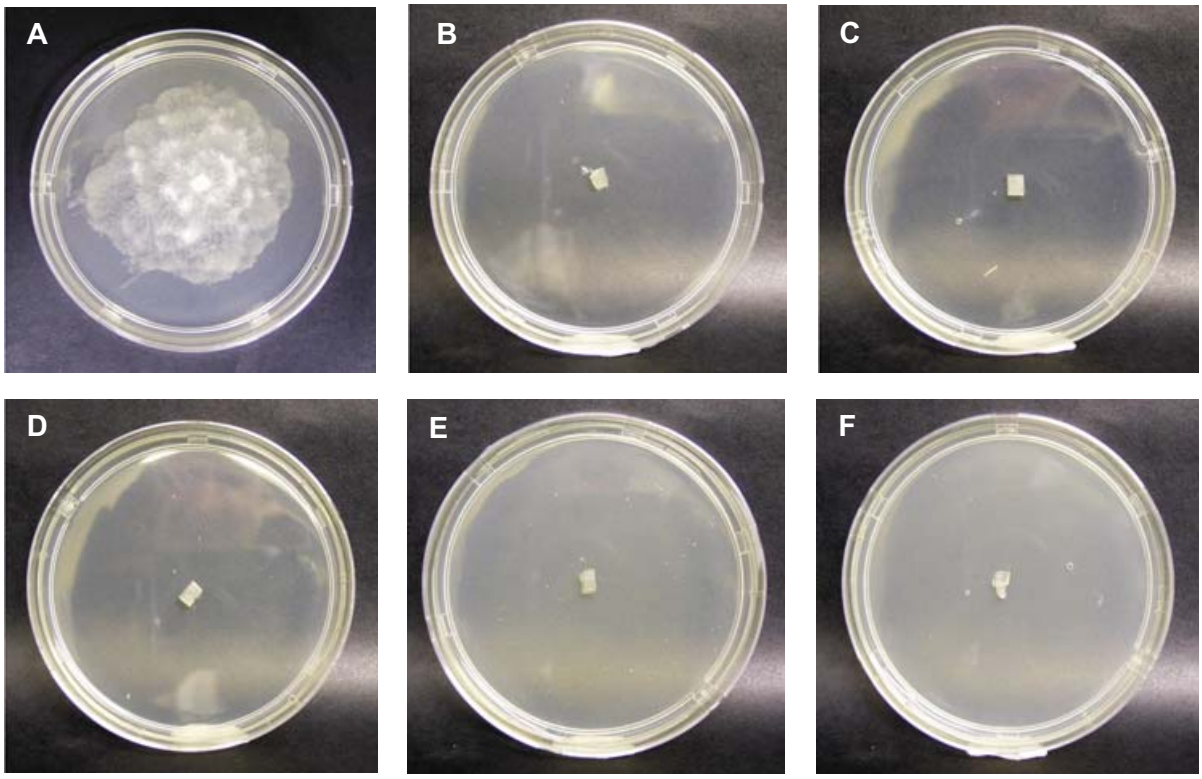

Figure 1. Mycelial growth of *Phytophthora cinnamomi* in response to 0 ml (A), 5 ml (B), 10 ml (C), 20 ml (D), 40 ml (E) and 80 ml (F) soluble silicon (20.7% silicon dioxide) per litre of potato dextrose agar (PDA).

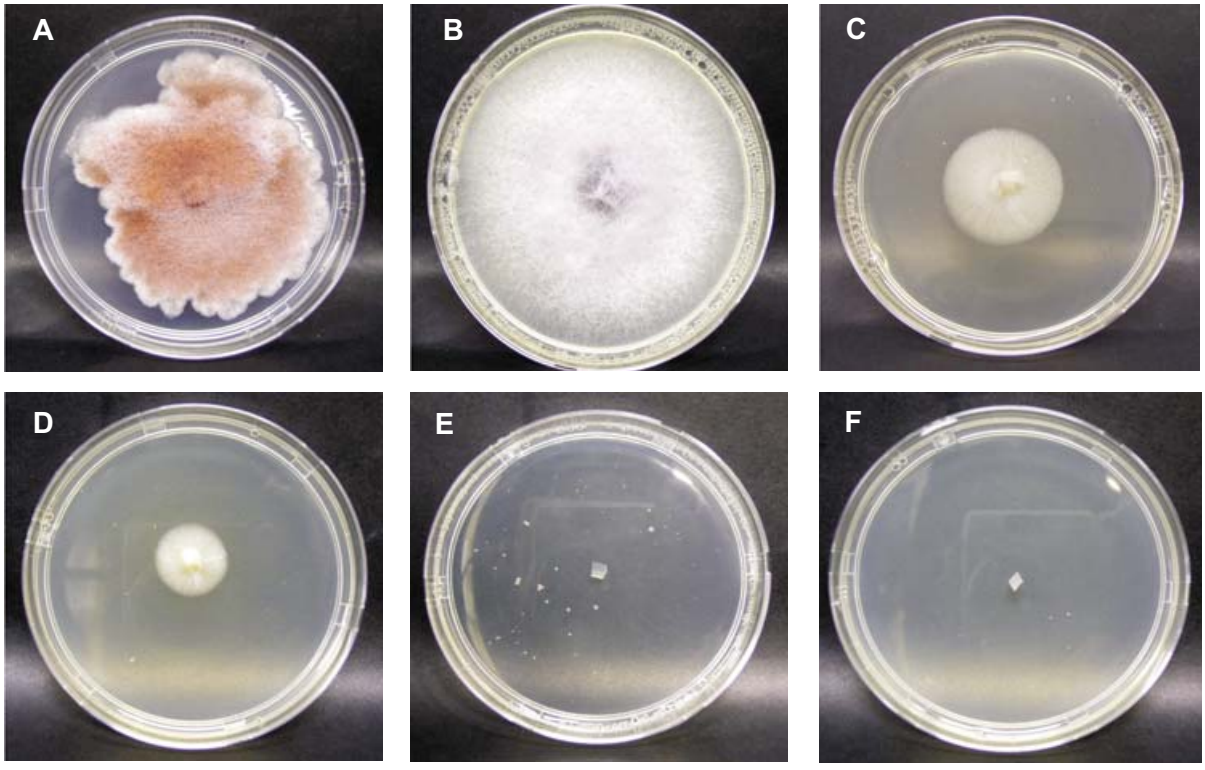

Figure 2. Mycelial growth of *Fusarium oxysporum* in response to 0 ml (A), 5 ml (B), 10 ml (C), 20 ml (D), 40 ml (E) and 80 ml (F) soluble silicon (20.7% silicon dioxide) per litre of potato dextrose agar (PDA).

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