In vitro inhibition of several phytopathogenic fungi from avocado by soluble potassium silicate

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ABSTRACT

Silicon is a bioactive element only recently implicated as having fungicidal properties. The present study examined water soluble liquid potassium silicate for activity against several types of avocado phytopathogenic fungi. In vitro *dose-responses towards soluble potassium silicate (20.7% silicon dioxide) were determined for* Phytophthora cinnamomi, Phomopsis perniciosa, Pestalotiopsis maculans, Lasiodiplodia theobromae, Glomerella cingulata, Natrassia *sp., and* Collectotrichum gloeosporioides. *Inhibition of mycelial growth was dose-dependant with 100% inhibition observed at 80 ml (pH 11.7) and 40 ml (pH 11.5) soluble potassium silicate (20.7% silicon dioxide) per litre of agar, for all fungi tested in two of the replicate experiments with the exception of* Natrassia *sp.,* G. cingulata *and* C. gloeosporioides *at 40 ml in one replication. For both replicate experiments,* Phytophthora cinnamomi, Phomopsis perniciosa, Pestalotiopsis maculans, Lasiodiplodia theobromae, Glomerella cingulata, Natrassia *sp., and* Collectotrichum gloeosporioides *were only partially inhibited at 5, 10 and 20 ml soluble potassium silicate per litre of agar. Percentage inhibition was, however, positively correlated with soluble potassium silicate concentrations. Soluble potassium silicate raised the pH of the agar from 5.6 to between 10.3 and 11.7 at concentrations of 5 and 80 ml soluble potassium silicate per litre of agar respectively. The effect of pH on fungal growth does not follow a clear trend for all fungi tested. In the absence of soluble potassium silicate, high pH caused only partial inhibition of mycelial growth. Soluble potassium silicate therefore suppresses fungal growth effectively* in vitro *and this is largely a fungicidal effect.*

INTRODUCTION

It has long been known that silicon reduces the incidence of fungal diseases in various pathosystems (Fauteux, Rémus-Borel, Menzies & Bélanger, 2005). Numerous studies have shown increased resistance to fungal plant diseases as a response to silicon applications. These include increased resistance to powdery mildew (*Uncinula necator*) in grapes (Bowen, Menzies & Ehret, 1992), powdery mildew (*Sphaerotheca fuliginea*) in cucumbers (Adatia & Besford, 1986; Belanger, Bowen, Ehret, & Menzies, 1995), powdery mildew (*Erysiphe cichoracearum*) in muskmelons (Menzies, Bowen & Ehret, 1992) as well as *Cladosporium* spp. and *Pythium* spp. in cucumbers (Chérif, Asselin & Belanger, 1994). It was proposed that amorphous silica deposition in the leaf apoplast prevent penetration of fungal hyphae (Carver, Zeyen & Ahlstrand, 1987; Kunoh & Ishizaki, 1975). Although this in part explains the prophylactic properties of silicon, monomeric silicon is also implicated in activation of several plant natural defences (Fauteux *et al*., 2005), such as flavanoids phytoalexin mediated response to silicon applications (Fawe, Abou-Zaid, Menzies & Belanger, 1988) and prophylactic properties of silicon in plant defence reactions to fungal attack (Chérif *et al*., 1994).

Bekker, Kaiser, Van der Merwe & Labuschagne (2006) demonstrated for the first time that soluble potassium silicate has direct *in vitro* fungitoxic activity against a wide range of fungi. In the current study we are investigating the fungitoxic activity of soluble potassium silicate against phytopathogenic fungi affecting avocado.

Numerous avocado diseases are caused by pathogenic fungi present in the soil or air within avocado orchards. In many instances the world has become reliant on chemical control of these diseases, with the fear of fungicide resistance ever increasing (Hardy, Barrett & Shearer, 2001).

Development of fungicide resistant strains of phytopathogenic fungi has been amply demonstrated. It is therefore imperative that alternative control measures should be investigated.The current study was therefore initiated to assess the *in vitro* effect of soluble potassium silicate on *Phytophthora cinnamomi* and six other pathogenic fungi affecting avocado.

MATERIALS AND METHODS

Fungi tested

Isolates of seven fungal pathogens of avocado maintained on potato dextrose agar (PDA) were obtained from the Mycological herbarium of the Plant Protection Research Institute, Agricultural Research Council (ARC), Pretoria, and Westfalia Technological

Table 1. Fungal pathogens of avocado tested for their *in vitro* sensitivity to soluble potassium silicate (20.7% silicon dioxide).

Fungus	Disease	Plant part affected
Collectotrichum gloeosporioides	Anthracnose, Stem-end rot	Pre- and post-harvest fruit damage
Glomerella cingulata	Anthracnose	Pre- and post-harvest fruit damage
Lasiodiplodia theobromae	Stem-end rot	Pre- and post-harvest fruit damage
Dothiorella mangiferae (Natrassia mangiferae)	Fruit rot	Fruit
Pestalotiopsis maculans	Stem-end rot	Pre- and post-harvest fruit damage
Phomopsis perniciosa	Stem-end rot	Pre- and post-harvest fruit damage
Phytophthora cinnamomi	Root rot	Root rot

Services (WTS), Tzaneen, South Africa. Fungi were selected on the basis of availability of cultures as well as their importance as avocado pathogens (**Table 1**).

Agar preparation

Soluble potassium silicate solution was sterilized by passing through a 0.45 μm Millipore filter before adding it to autoclaved potato dextrose agar (PDA) to give final concentrations of 5, 10, 20, 40 and 80 ml soluble potassium silicate (20.7% silicon dioxide) per litre of PDA.

Soluble potassium silicate (20.7% silicon dioxide) has a pH of 12.7, which markedly increase the pH of PDA. A pilot study was thus conducted to determine the effect of various concentrations of soluble potassium silicate on the pH of PDA. It was found that unameliorated PDA has a pH of 5.6. Upon addition of 5, 10, 20, 40 and 80 ml of soluble potassium silicate, the pH of the PDA is raised to 10.3, 10.7, 11.2, 11.5 and 11.7 respectively.

The pH of PDA plates in the current study were therefore adjusted to pH 10.3, 10.7, 11.2, 11.5 and 11.7 using soluble potassium hydroxide. A non-ameliorated PDA (i.e. with no potassium silicate added) served as a control.

A 5 mm diam. mycelial disc taken from a 7 d old fungal culture on PDA was transferred to the centre of a Petri dish containing soluble potassium silicate amended PDA at the various concentrations, as well as unamended (control) plates respectively.

This was done for each fungus. Ten replicates were included for each treatment, with the entire experiment replicated twice. Plates were incubated at 25˚C in the dark and colony diameters were measured every second day for 8 consecutive days.

Percentage inhibition was calculated according to the formula:

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

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

This data was analysed with Genstat 4.23 DE (**Table 2, 3** and **4**).

Table 2. Mycelial growth of *Phytophthora cinnamomi* incubated for eight days at 25˚C on PDA. Agar was either amended with soluble potassium silicate (20.7% silicon dioxide) at various concentrations, or potassium hydroxide to increase pH value of the agar, after amendment with soluble potassium hydroxide mimicking the pH effect of soluble potassium silicate.

	Mean colony diameter (mm)[x]			
Treatment	Day 2	Day 4	Day 6	Day 8
0 ml/ℓ [y]	23.9 a	48.2 a	61.8 a	72.3 a
5 ml/ℓ	5.3 c	10.8 d	15.7 e	16.1 e
10 ml/ℓ	5.0 c	5.0 e	5.0 f	5.0 f
20 ml/ℓ	5.0 c	5.0 e	5.0 f	5.0 f
40 ml/ℓ	5.0 c	5.0 e	5.0 f	5.0 f
80 ml/ℓ	5.0 c	5.0 e	5.0 f	5.0 f
pH 10.3 [w]	17.5 b	28.1 b	45.9 b	55.6 b
pH 10.7	12.4 b	25.2 b	42.1 b	50.3 c
pH 11.3	15.5 b	30.7 b	46.1 b	56.9 b
pH 11.5	7.9 c	19.9 c	31.1 c	39.9 d
pH 11.7	7.9 c	18.68 c	25.6 d	38.2 d

[w] pH value of the agar after amendment with soluble potassium silicate.
[x] Means of 10 plates/ treatment. Measurements include initial 5 mm diam. mycelial disc.
[y] Concentration of the soluble potassium silicate per litre of PDA growth medium.
[z] In each column, values followed by the same letter do not differ significantly (F.Pr < 0.01).

RESULTS

Soluble potassium silicate (20.7% silicon dioxide) completely suppressed mycelial growth of all fungi (**Table 3**) at concentrations of 40 and 80 ml.l^{-1} PDA, with the exception of *Natrassia* sp., *G. cingulata* and *C. gloeosporioides* (mean colony diam. of 5.4, 11.5 and 13.1 mm respectively) in one experimental repetition.

A soluble potassium silicate concentration of 5-10 ml.ℓ$^{-1}$ PDA inhibited mycelial growth of *P. cinnamomi* (**Figure 1**; **Table 2**), but in contrast increased fungal growth of *P. perniciosa* (**Figure 2**). Suppression of mycelial growth at concentrations of 5 to 20 ml.ℓ$^{-1}$ PDA varied between all fungi tested and an effective suppressive concentration are to be determined for each fungus.

Although PDA amended with soluble potassium hydroxide (pH range 10.3 to 11.7) did inhibit mycelial growth of most fungi to some degree (**Table 3**), the effect of added soluble potassium silicate over and above the pH effect is apparent. The effect of pH on mycelial growth as exemplified by soluble potassium hydroxide amended PDA plates seems to be species-specific, and is not correlated to the inhibition due to soluble potassium silicate presence.

DISCUSSION

The results of this study clearly indicate that soluble potassium silicate (20.7% silicon dioxide) has fungicidal activity *in vitro*. Seebold (1998) and Seebold, Datnoff, Correa-Victoria & Snyder (1995) confirmed this when soluble potassium silicate tested as a fungicide resulted in a 40% suppression of rice neck blast. Suppression of fungal growth in the current study was shown to be dose dependent. However, growth of some fungi such as *P. perniciosa* was actually stimulated at a concentration of 5 ml.ℓ$^{-1}$ soluble potassium silicate. This phenomenon has been noted in numerous fungi with silicon by Wainwright, Al-Wajeeh & Gray-

Table 3. Mean % inhibition of different fungi at different soluble potassium silicate (20.7% SiO_2) concentrations and different pH values in ameliorated potato dextrose agar.

Treatment	*C. gleosporioides*	*G. cingulata*	*L. theobromae*	*Natrassia* sp.	*P. maculans*	*P. perniciosa*	*P. cinnamomi*
5 ml Si (pH 10.3)	10.2 a	5.4 a	5.7 ab	28.0 b	14.8 a	19.6 ab	84.0 c
10 ml Si (pH 10.7)	48.8 b	45.0 b	59.5 c	60.8 c	74.2 c	70.6 c	100.0 c
20 ml Si (pH 11.2)	77.6 c	79.5 c	96.0 d	91.1 d	100.0 d	93.3 d	100.0 c
40 ml (pH 11.5)	94.6 d	95.6 c	100.0 d	99.7 d	100.0 d	100.0 d	100.0 c
80 ml Si (pH 11.7)	100.0 d	100.0 c	100.0 d	100 d	100.0 d	100.0 d	100.0 c
pH 10.3	10.2 ab	0.0 a	0.0 a	4.2 a	5.2 a	1.1 a	24.4 a
pH 10.7	8.6 a	1.9 a	0.0 a	12.8 ab	1.9 a	5.6 a	33.9 ab
pH 11.2	8.1 a	4.7 a	0.0 a	0.0 a	6.4 a	8.2 a	21.9 a
pH 11.5	13.2 ab	7.8 a	0.0 a	9.5 ab	21.5 ab	16.6 ab	49.3 b
pH 11.7	30.2 b	18.1 a	21.5 b	17.0 ab	40.3 b	32.5 b	51.4 b

Table 4. Mean colony diameter (minus 5 mm diam. initial disc) of different fungi at different soluble potassium silicate concentrations and different pH values in ameliorated PDA on the day when the first concentration reached maximal 80 mm Petri dish diameter.

	C. gleosporioides	*G. cingulata*	*L. theobromae*	*Natrassia* sp.	*P. maculans*	*P. perniciosa*	*P. cinnamomi*
Treatment	**Day 8**	**Day 8**	**Day 4**	**Day 4**	**Day 8**	**Day 8**	**Day 8**
0 ml Si (pH 5.6)	74.9 a	75 a	75 a	75 a	66.9 a	74.1a	67.3a
5 ml Si (pH 10.3)	67.2 b	71 b	70.8 b	54 d	57.3 b	59.3c	11.1e
10 ml Si (pH 10.7)	38.8 d	41.3 d	30.4 d	29.4 e	17.9 d	21.5e	0f
20 ml Si (pH 11.2)	16.8 e	15.4 e	3.1 e	6.7 f	0 e	4.9f	0f
40 ml (pH 11.5)	4.05 f	3.3 f	0 e	0.2 g	0 e	0f	0f
80 ml Si (pH 11.7)	0 g	0 f	0 e	0 g	0 e	0f	0f
pH 10.3	67.3 b	75 a	75 a	71.9 b	63.8 a	73.3a	50.8b
pH 10.7	68.5 b	73.6 a	75 a	65.4 c	65.8 a	69.9a	44.4c
pH 11.2	68.9 b	71.5 b	75 a	75 a	62.9 a	68b	52.5b
pH 11.5	65 b	69.2 b	75 a	67.9 c	53.2 b	61.6c	34d
pH 11.7	52.3 c	61.4 c	58.9 c	62.3 c	40.9 c	49.7d	32.6d

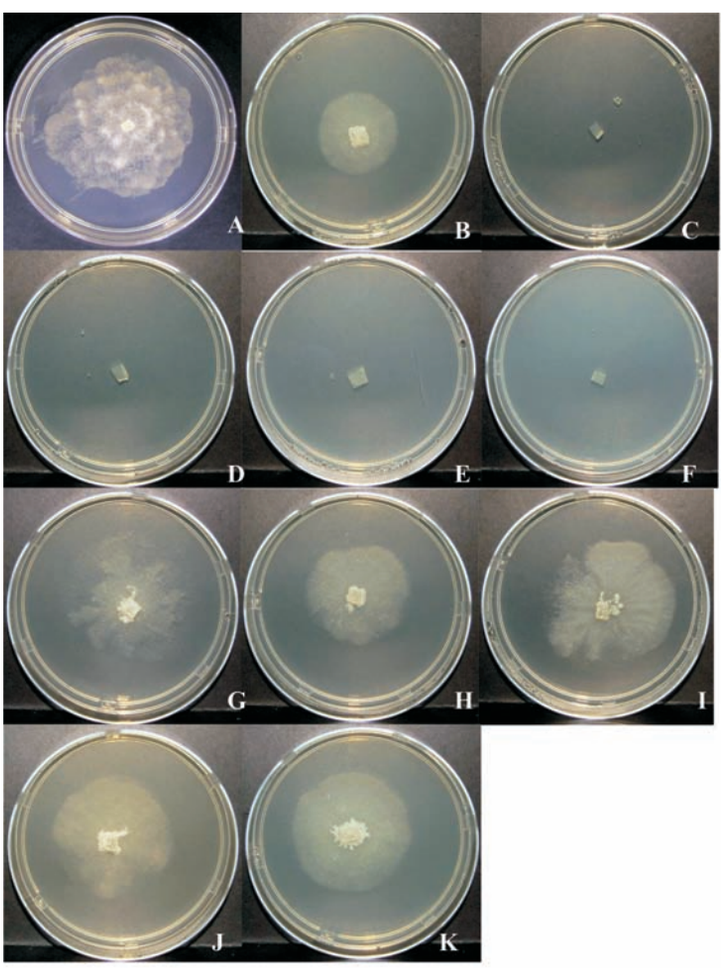

Figure 1. Mycelial growth of *Phytophthora cinnamomi* in response to 0 ml (pH 5.6), 5 ml (pH 10.3) (B), 10 ml (pH 10.7) (C), 20 ml (pH 11.2) (D), 40 ml (pH 11.5) (E), and 80 ml (pH 11.7) (F) soluble potassium silicate (20.7% silicon dioxide) per litre of potato dextrose agar (PDA) compared to a potassium hydroxide control group including pH 10.3 (G), pH 10.7 (H), pH 11.2 (I), pH 11.5 (J), and pH 11.7 (K).

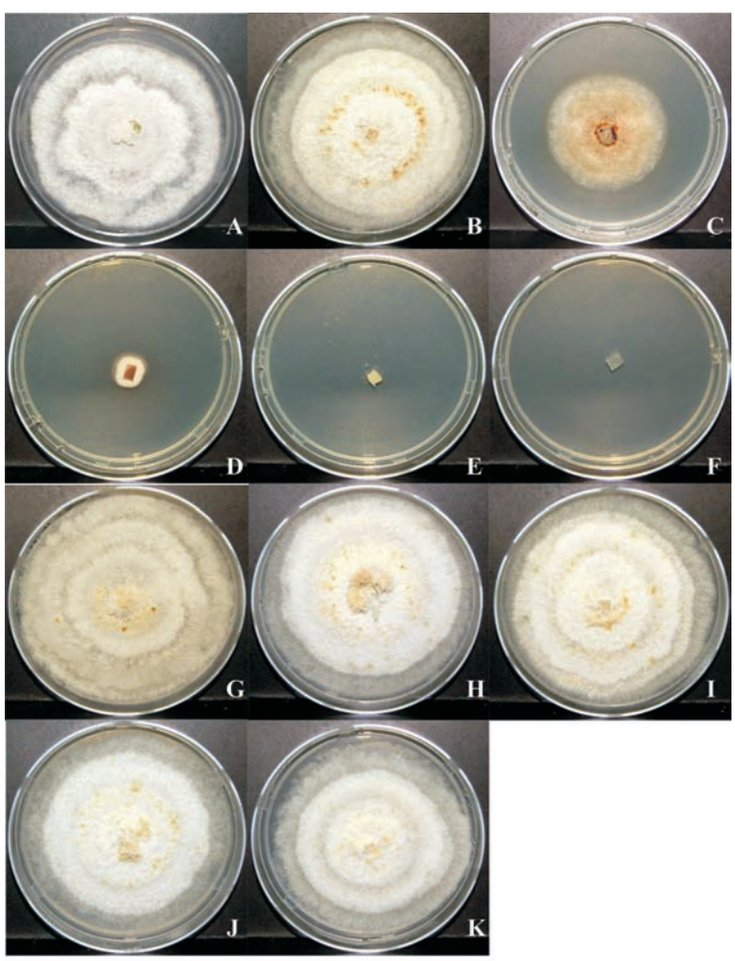

Figure 2. Mycelial growth of *Phomopsis perniciosa* in response to 0 ml (pH 5.6), 5 ml (pH 10.3) (B), 10 ml (pH 10.7) (C), 20 ml (pH 11.2) (D), 40 ml (pH 11.5) (E), and 80 ml (pH 11.7) (F) soluble potassium silicate (20.7% silicon dioxide) per litre of potato dextrose agar (PDA) compared to a potassium hydroxide control group including pH 10.3 (G), pH 10.7 (H), pH 11.2 (I), pH 11.5 (J), and pH 11.7 (K).

ston (1997).

This has implications if soluble silicon is to be used in disease control, and it should therefore be ensured that silicon addition does not increase ambient silicon concentrations in soil to a level conducive to the growth of these fungi. Especially at soluble potassium silicate concentrations between 5 and 20 ml, suppression of fungi was variable and at these low concentrations results were not consistent between replications.

Phytophthora cinnamomi was however suppressed even at concentrations of 5 ml.ℓ^{-1} soluble potassium silicate and completely inhibited at 10 ml.ℓ^{-1} and higher concentrations. Based on the current results and that of previous studies conducted (Kaiser, Van der Merwe, Bekker & Labuschagne, 2005), a minimum dosage of 20 ml per litre of soluble potassium silicate solution per litre of water as a soil drench is recommended to ensure adequate control of *Phytophthora cinnamomi* in the field and pot trials.

The effect of pH on mycelial growth was inconsistent. In all fungi tested, mycelial growth continued at high pH in the absence of soluble potassium silicate, even though at a slower rate.

It can therefore be concluded that soluble potassium silicate does have a direct fungitoxic activity. Soluble potassium silicate's fungicidal effect was thus the overriding factor especially for fungi such as *P. cinnamomi*, *P. perniciosa* and *P. maculans* (**Table 4**).

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