Integrated use of yeast, *Bacillus*, hot water and potassium silicate treatments for the control of postharvest green mould of citrus and litchis

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With a growing recognition that many agrochemicals are hazardous to humans, animals and the environment, came the need to substitute these chemical products with biological and physical and/or change agronomic practices that are safe to the environment and human health. These practices have the potential to play an increasingly important role in plant disease control.

To achieve this objective on one specific disease, laboratory, field and commercial packhouses trials were conducted to develop effective alternative control measures against green mould (*Penicillium digitatum*) of citrus using multiple control measures, independently and in combination.

A South African isolate of *P. digitatum,* isolated from an infected orange fruit, was found to be resistant to imazalil (the standard postharvest fungicide). Alternative measures to control the disease are therefore essential for the citrus export industry. Biological control has been suggested as one of the alternatives. Sixty yeast and 92 *Bacillus* isolates were isolated and screened for their antagonistic activity against *P. digitatum*. None of the yeasts or *Bacillus* isolates produced a curative action against *P. digitatum* on oranges. However, yeast Isolate B13 provided excellent preventative control of *P. digitatum,* superior to all the *Bacillus* isolates, when it was applied to citrus fruit prior to artificial inoculation with *P. digitatum.* Electron microscopy showed that yeast Isolate B13 inhibited conidial germination of *P. digitatum*. For the control of *P. digitatum* pre-harvest, trees were sprayed with yeast, Isolate B13, a few months or a few days before harvest. However, this treatment alone proved to be ineffective in providing preventative control of green mould on Valencia oranges.

For the control of *P. digitatum* preharvest, trees were treated with potassium silicate for a full season. Regular potassium silicate treatments resulted in a significant preventative control of *P. digitatum* infection on both navel and Valencia oranges. Treatment of lemons with potassium silicate as a postharvest treatment for the control of *P. digitatum* resulted in reduced disease lesion diameters when applied preventatively or curatively. Electron microscopy showed that potassium silicate inhibited germination of *P. digitatum* conidia and growth of its mycelium.

Hot-water dip treatment at 54- 58℃ for 20-80 seconds (in increments of 15 seconds), significantly reduced infection development in inoculated wounds of Valencia oranges compared with control fruit treated with tap water, without causing any rind damage. Hot water treatment at 56℃ for 30- 90 seconds provided the best control of the pathogen.

The integration of the yeast, a hot water dip and potassium silicate pre-and postharvest applications provided control of *P. digitatum* control in multiple packhouse trials. The control achieved by the yeast Isolate B13 or hot-water, and K_2SiO_3 in the packhouse alone was superior or equivalent to that provided by imazalil.

A similar study was also carried out to determine possible control measures for *Penicillium* sp. on litchis. In this study, a total of 23 yeast and 13 *Bacillus* isolates were obtained from litchi fruit surfaces. Ten yeast and 10 *Bacillus* isolates that had showed good efficacy against *P. digitatum* of citrus were added to these for screening against *Penicillium* sp. of litchis. None of the yeasts or *Bacillus* isolates produced a curative action against *Penicillium* sp. infection on litchis. However, several yeast isolates (YL4, YL10, YLH and B13) resulted in reduced severity of the pathogen, when applied preventatively, compared with an untreated control. The yeast isolates were superior to all the *Bacillus* isolates, when applied to litchis prior to artificial inoculation by *Penicillium* infection on litchis. Potassium silicate as a postharvest treatment for the control of the pathogen caused reduced lesion diameters when applied preventatively or curatively to naturally infected litchis.

The results presented in this thesis highlight the use of biological, physical and agronomic practices singly or in combination as an alternative control strategy against citrus postharvest green mould. This thesis also provides an insight into expanding these strategies, partly or fully, for the control of other postharvest *Penicillium* infections using litchi as an example.

Biological control of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae)

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The two-spotted spider mite (TSM), Tetranychus urticae Koch, is an important pest of many greenhouse and field crops worldwide. The development of resistance in TSM populations to chemical acaricides, allied with public health concerns about pesticide residues, has motivated the search for alternative control measures to suppress the pest. Hyphomycetous fungi are promising agents for mite control and the fungus Beauveria bassiana (Bb) (Balsamo) Vuillemin was investigated in this study as a biocontrol agent. The principal objectives of this study comprised: a) screening Bb strains their pathogenicity against T. urticae; b) testing the effect of adjuvants on the efficacy efficiency of Bb; c) studding the effect of plant type on persistence and the efficient control of Bb against T. urticae; d) evaluating the field efficacy of Bb applications against T. urticae; e) testing the compatibility of Bb with selected fungicides; and f) assessing the synergy between Bb and soluble silicon for T. urticae control.

Screening bioassays of six-two strains of Bb identified the two most effective strains, R289 (PPRI 7315) and R444 (PPRI 7861), that caused mortality levels of more than 80% of adult mites at 9d post-inoculation with 2×108 conidia ml-1. These strains performed significantly better than the Bb commercial strain PPRI 5339, in laboratory bioassays. The two strains also showed attacked mite eggs, causing 53.4% and 55.5% of reduction in egg hatchability at 2×108 conidia ml-1 respectively. However, R444 showed relatively higher production of conidia in culture and was therefore selected for further trials under greenhouse and field conditions.

Greenhouse evaluations of the effects of two adjuvants (Break-thru® and a paraffin oil based emulsion) on efficacy of Bb demonstrated a higher efficacy of the biocontrol agent (BCA) when it was applied with Break-thru® or the oil solutions than with water alone. Moreover, Bb conidia applied in Break-thru® solution resulted in greater control of TSM than conidia applied in the mineral oil. There was also a dose

response effect and the control of TSM by Bb increased when the concentration of conidia was increased.

The control of TSM by Bb in beans (Phaseolus vulgaris L), cucumber (Cucumis sativus L.), eggplant (Solanum melongena L.), maize (Zea mays L.) and tomato (Solanum lycopersicum L.) was tested in greenhouse trials. On these crops, the persistence of conidia declined over time. The rate of decline was significantly higher on maize. However, TSM mortality was positively correlated to the amount of conidia deposited on leaves immediately after spraying, rather than their persistence over time. Higher levels of mortality of TSM due to Bb application were observed on beans, cucumber and eggplants, suggesting that the type of crop must be taken into consideration when Bb is applied as a BCA.

Field efficacy of Bb against mites was evaluated in two trials on eggplants. Based on assessment of population densities of mites, and leaf damage assessments both trials showed that the strain R444 controls TSM in the field. Two commonly used fungicides, azoxystrobin and flutriafol, were investigated in vitro tests on culture medium and laboratory bioassay on detached bean leaves for their effects on Bb. Azoxystrobin (a strobilurin) was less harmful to Bb while flutriafol was found to be inhibitory.

Another important finding of this study was the substantial enhancement of Bb efficacy by soluble silicon. When Bb was combined with soluble Si control of TSM was better than when either of the two products was applied alone. Moreover, application of soluble Si as a plant fertilizer in hydroponic water nutrient increased accumulation of peroxidase, polyphenoloxidase, phenylalanine ammonia-lyase enzymes in leaves of infested plants by TSM. Increased activity of these defense enzymes in leaves deters feeding behavior of mites. We suggested that feeding stress renders them susceptible to Bb infection, which would explain the synergy observed between the two agents.

Studies on the use of biocontrol agents and soluble silicon against powdery mildew of zucchini and zinnia

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Powdery mildew (PM) is an important foliar disease of many crops, occurring under both greenhouse and field conditions. The application of biological control and soluble silicon (Si) against PM has received increasing acceptance as a result of increased environmental and public concern over the use of fungicides for disease management, and because many key fungicides are no longer effective because of resistance problems. However, success with these control options depends on the development of effective antagonists and understanding how best to use Si in agriculture.

Potential antagonists of PM were isolated from naturally infected leaves of different plants. A total of 2000 isolates were tested in a preliminary screening on detached leaves of zucchini. The best 30 isolates showing consistent results were further tested under greenhouse conditions for their efficacy against PM of zucchini. In a greenhouse trial, 23 isolates provided disease control to levels of 30 to 77%. Application of 29 isolates resulted in significant reductions in values of area under disease progress curve (AUDPC). The best five isolates were identified as *Clonostachys rosea* (Link) Schroers, Samuels, Seifert & Gams (syn. *Gliocladium roseum*) (Isolate EH), *Trichothecium roseum* (Pers.) Link (syn. *Cephalothecium roseum*) (Isolate H20) and Serratia marcescens (Bizio) (Isolates B15, Y15 and Y41).

Three adjuvants (Break-Thru® (BK), Partner® (PR) and Tween-80® (T-80)) were compared for their ability to improve efficacy of spray application of silicon (Si) and biocontrol agents (BCAs) against PM. Both BK and PR improved the efficacy of Si significantly ($P < 0.05$). Microscopic studies showed that BK affected PM fungi directly and enhanced the deposition of BCAs on the pathogen. Break-Thru® was only toxic to the pathogen mycelia when used at > 0.25 mℓ ℓ-1, but phytotoxic to zucchini plants when used at > 0.45mℓ ℓ-1. However, it did not affect the c.f.u. of bacterial BCAs. Use of BK at 0.2-0.4 mℓ ℓ^{-1} can be recommended to assist spray application of Si (at 750 mg ℓ-1) or BCAs for improved control of PM.

The effect of concentration, frequency of application and runoff of Si sprays applied to the foliage was evaluated for control of PM of zucchini. Silicon (250-1000 mg ℓ-1) + BK (0.25 mℓ $ℓ^{-1}$), was sprayed onto zucchini plants at frequencies of 1-3 wk-1. Spraying Si reduced the severity of PM significantly ($P < 0.05$). Regardless of the concentration of Si, the best results were obtained when the frequency of the treatment was increased, and when spray drift or spray runoff were allowed to reach the rhizosphere of the plants. When Si was applied onto leaves, direct contact between the spray and the pathogen resulted in mycelial death. Part of the spray (i.e., drift and runoff) was absorbed by plant roots, and subsequently played an important role in the health of the plants. If affordable, soluble Si should be included in nutrient solutions of hydroponics or supplied with overhead irrigation schemes when PM susceptible crops are grown.

Under greenhouse conditions, application of BCAs, with or without Si, reduced the severity and development of PM significantly ($P < 0.001$). Application of Si significantly reduced the severity and AUDPC values of PM ($P < 0.05$ for both parameters). Silicon alone reduced the final disease level and AUDPC values of PM by 23-32%, and improved the efficacy of most BCAs. In the course of the investigation, antagonistic fungi consistently provided superior performances to bacterial isolates, providing disease control levels of up to 90%. Higher overall disease levels reduced the efficacy of Si against PM, but did not affect the efficacy of BCAs.

Under field conditions, Si alone reduced disease by 32-70%, Isolate B15 reduced disease by 30-53% and Isolate B15 + Si reduced disease by 33-65%. Other BCAs applied alone or together with Si reduced the disease level by 9-68%. Most BCAs reduced AUDPC values of PM significantly. For most antagonists, better efficacy was obtained when Si was drenched into the rhizosphere of the plant. However, efficacy of some of the BCAs and Si were affected by environmental conditions in the field. Repeated trials and better understanding of how to use Si and the BCAs, in terms of their concentration and application frequency, and their interactions with the plant and the environment, are needed before they can be used for the commercial control of PM.

Elemental analysis was conducted to determine the impact of differing application levels of silicon (Si) in a form of potassium silicate (KSi) in solution in terms of Si accumulation and selected elements in different tissues of zucchini and zinnia and growth of these plants, and to study the effect of PM on the levels of selected elements in these two plant species. Plants were grown in re-circulating nutrient solutions supplied with Si at different concentrations and elemental composition in different parts were analysed using EDX and ICP-OES. Increased levels of Si in the solution increased the levels of Si in leaves and roots of both plants without affecting its distribution to other plant parts. In zucchini, the roots accumulated the highest levels of Si, substantially more than in the shoots. In contrast with zinnia, accumulation of Si was highest in the leaves. Accumulation of potassium (K) in shoots of both plants increased with increased levels of KSi in the nutrient solution. However, K levels in flower of zinnia, fruits of zucchini and roots of both plants remained unaffected. Increased level of Si reduced accumulation of calcium (Ca) in both plants.

Adding Si into the nutrient solution at 50 mg ℓ-1 resulted in increased growth of zucchini and increased uptake of P, Ca, and Mg by both plant species. However, application of higher levels of Si did not result in any further biomass increase in zucchini. Levels of Si in the nutrient solution had no effects on elemental composition and characteristics of the fruits of zucchini. In both plant species, the presence of PM on the leaves of plants resulted in these leaves accumulating higher levels of Si and Ca, but less P, than leaves of uninfected plants exposed to the same levels of soluble Si. The highest concentrations of Si were observed in leaf areas infected with PM, and around the bases of trichomes. For optimum disease control and maximum accumulation of different elements in these two plants, hydroponic applications of Si at 50-150 mg ℓ-1 is recommended.

Five selected biocontrol agents and potassium silicate, used as source of soluble Si, were tested under hydroponic conditions at various concentrations against PM of zinnia (*Glovinomyces cichoracearum* (DC) Gelyuta, V.P.). Application of BCAs resulted in reductions in final disease level and AUDPC values of PM by 38-68% and 30-65%, respectively. Both severity and AUDPC values of PM were reduced by 87-95% when plants were supplied with Si (50-200 mg ℓ-1). It is proposed that the provision of a continuous supply of Si and the ability of this plant species to accumulate high levels of Si in its leaves were the major reasons for the good response of zinnia to Si treatments against PM. Silicon played a protective role before infection and suppressed development of PM after infection. The combination of the best selected BCAs and Si can be used as an effective control option against PM of zinnia when grown in hydroponic system.

Management of Fusarium wilt diseases using non-pathogenic Fusarium oxysporum and silicon

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In the genus Fusarium are many important plant pathogens. The diversity of hosts the genus attacks, the number of pathogenic taxa and the range of habitats in which they cause disease are the greatest in plant pathology. One important species complex within the genus *Fusarium* is *Fusarium oxysporum* Schlecht. This species complex consists of more than 80 pathogenic *forma specialis* and is particularly difficult to control. The fungi can survive in soil for decades as specialized spores, known as chlamydospores. Interestingly, however, this species complex also contains beneficial non-pathogenic forms that can be exploited to manage Fusarium wilt diseases.

In this study, the ability of non-pathogenic *F. oxysporum* strains, *Trichoderma harzianum* Rifai Eco-T®, soluble silicon (potassium silicate), and their combination was evaluated on two important crops, banana (*Musa* sp. L.) and beans (*Phaseolus vulgaris* L.), for their potential to suppress pathogenic strains of *F. oxysporum*. The ability of these crops to take up and accumulate silicon in their organs, and the element's effect on low temperature stress was also investigated.

Several endophytic fungi, mainly *Fusarium* spp. and bacteria, were isolated from healthy mature banana plants. After preliminary and secondary *in vivo* screening tests against *F. oxysporum* f.sp. *phaseoli* on beans (*Phaseolus vulgaris* L.) cv. Outeniqua, two non-pathogenic *F. oxysporum* strains were selected for further testing. These two non-pathogenic *F. oxysporum* strains were found to colonize banana (*Musa* sp.) cv. Cavendish Williams and bean plants, and to suppress Fusarium wilt of these crops. In order to improve the efficacy of these biocontrol fungi, soluble silicon was introduced.

The relationship between plant mineral nutrition and plant diseases have been reported by several authors. Plants take up silicon equivalent to some macronutrients, although it is not widely recognized as an essential element. In this study, we established that roots, the target plant organ for soilborne plant pathogens, accumulated the greatest quantity of silicon of any plant organs when fertilized with high concentrations of silicon. On the other hand, the corm and stem accumulated the least silicon. Such observations contradict the concept of passive uptake of silicon via the transpiration stream in these plants as the only uptake mechanism.

The prophylactic properties of silicon have been documented for many crops against a variety of diseases. *In vitro* bioassay tests of silicon against these wilt pathogens showed that potassium silicate can be toxic to Fusarium wilt fungi at high concentrations (>7840 mg ℓ^{-1}), resulting in complete inhibition of hyphal growth, spore germination and sporulation. However, low concentrations of silicon (<490 mg ℓ^{-1}) encouraged hyphal growth. Silicon fertilization of banana and beans significantly reduced disease severity of these crops by reducing the impact of the Fusarium wilt pathogens on these crops. However, it could not prevent infection of plants from the wilt pathogens on its own. Synergistic responses were obtained from combined applications of silicon and non-pathogenic *F. oxysporum* strains against Fusarium wilt of banana. Combinations of silicon with the non-pathogenic *F. oxysporum* strains significantly suppressed disease severity of Fusarium wilt of banana, caused by *F. oxysporum* f.sp. *cubense* (E.F. Smith) Snyder & Hansen, better than applications of either control measure on their own.

Banana production in the subtropical regions frequently suffer from chilling injury, and from extreme variations between night and day temperatures. Such stress predisposes banana plants to Fusarium wilt disease. Silicon, on the other hand, is emerging as important mineral in the physiology of many plants, ameliorating a variety of biotic and abiotic stress factors. We established that silicon fertilization of banana plants significantly reduced chilling injury of banana plants. Membrane permeability, lipid peroxidation (MDA level) and proline levels were higher in silicon-untreated plants than the treated ones, all of which demonstrated the stress alleviating effect of silicon. Low temperatures damage the cell membrane of susceptible plants and cause desiccation or dehydration of these cells. Levels of sucrose and raffinose, recognized as cryoprotectants, were significantly higher in silicon-amended banana plants than unamended plants.