STUDIES ON THE USE OF BIOCONTROL AGENTS AND SOLUBLE SILICON AGAINST POWDERY MILDEW OF ZUCCHINI AND ZINNIA

By

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Submitted in partial fulfilment of the
requirements for the degree of

Doctor of Philosophy

In the

Discipline of Plant Pathology
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Pietermaritzburg
Republic of South Africa

December   2008

ABSTRACT

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.

DECLARATION

I, Habtom Butsuamlak Tesfagiorgis, declare that

i. The research reported in this thesis, except where otherwise indicated, is my original work.

ii. This thesis has not been submitted for any degree or examination at any other university.

iii. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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Signed:…………………………….

H.B. Tesfagiorgis (Candidate)

Signed:…………………………….

Prof. M.D. Laing (Supervisor)

ACKNOWLEDGEMENTS

DEDICATION

To Bustuamlak Tesfagiorgis's family for the support,
understanding and spiritual encouragement
during my studies.

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TABLE OF ACRONYMS

ANOVA   =   Analysis of variance

ARC-PPRI   =   Agricultural Research Council - Plant Protection Research Institute

AUDPC   =   Area under disease progress curve

BCA(s)   =   Biological control agent

BK   =   Break-Thru$^{®}$

DDW   =   De-ionized distilled water

EDX   =   Energy dispersive X-ray fluorescence

ESEM   =   Environmental scanning electron microscopy

HDPE   =   High-density polyethylene () bottles

ICP-OES   =   Inductively coupled plasma-optical emission spectrometers

IPM   =   Integrated pest management

ISR   =   Induced systemic resistance

NA   =   Nutrient agar

NB   =   Nutrient broth

NDA   =   National Department of Agriculture

PDA   =   Potato dextrose agar

PM   =   Powdery mildew

PR   =   Partner$^{®}$ 650

RCBD   =   Randomized Block Design

SAR   =   Systemic acquired resistance

T-80   =   Tween-80$^{®}$

INTRODUCTION

In the Discipline of Plant Pathology, at the University of KwaZulu-Natal is a research team, called Biocontrol for Africa, which has been developing research momentum for the last 15 years. As a result, promising progress is being made in identifying beneficial microorganisms for disease control and growth stimulation. The development and successful commercialization of Eco-T®, a bio-fungicide product using a selected strain of *Trichoderma harzianum* Rifai. reflects the efforts being undertaken to achieve team goals, to deliver effective biocontrol products to farmers. The use of soluble silicon against biotic and abiotic stresses of plants has also shown promising results (Epstein, 1994, Bélanger *et al*., 1995). However, the relationships between Si and plants, Si and pathogens, and Si and the soil and other environmental factors, are not fully understood. In addition, Si is considered as safe and environmentally friendly. However, the option of using it integrated with application of BCAs for improved disease control, especially against PM, has not previously received much attention.

As an alternative approach for sustainable disease management programme, the application of Si in agriculture is a fast growing area of interest, and central to our research group. Several projects are in progress to uncover some of the myths of Si in agriculture in order to obtain acceptable level of disease control and optimum plant growth, without compromising the quality of crop produce, or harming the environment. The research contained in this thesis reflects part of the ongoing research of the Biocontrol for Africa team.

All the research was conducted at the University of KwaZulu-Natal, Pietermaritzburg, South Africa. The main emphasis of the research was to screen potential biocontrol agents and evaluate the efficacy of BCAs and silicon against PM of zucchini and zinnia caused by *Podosphaera xanthii* (Castagne) and *Glovinomyces cichoracearum* (DC) Gelyuta, V.P.*, respectively.

Research activities included isolation of potential antagonists from various plant species that were naturally infected with powdery mildew (PM), and *in vitro* screening of these isolates against PM of zucchini. Applications of Si as drenches, sprays and in hydroponic nutrient solution were investigated in glasshouse conditions on the severity of PM of zucchini and zinnia. The efficacy of the best BCAs and Si applications, plus the combined effects of these two treatments against PM, was further evaluated under glasshouse and field conditions. Finally, the impact of Si levels in a nutrient solution on elemental uptake by zucchini and zinnia, and the relationship between PM-infection and elemental uptake by both plants was assessed using EDX and ICP-OES analysis.

The objectives of this study were to:

1. Review available literature on the use of biocontrol agents and silicon against PM plant diseases;
2. Isolate potential biocontrol agents against PM, then evaluate their efficacy under greenhouse and field conditions;
3. Study the effects of Si levels on PM control applied as drench, foliar treatment and in nutrient solution;
4. Understand the mode of action of foliar-applied silicon, and to determine the effects of dosage, frequency and runoff of the spray on PM control;
5. Improve the spray efficiency of Si and BCAs by testing various adjuvants;
6. Evaluate the use of co-application of BCAs and silicon with Break-Thru® for integrated disease management of PM;
7. Evaluate the effects of Si levels in the nutrient solution on PM control, plant growth, quality of produce and nutrient uptake and accumulation by zucchini and zinnia;
8. Study the relationship between PM-infection and elemental uptake by zucchini and zinnia; and
9. Test the efficacy of the best BCAs plus Si on PM of zinnia grown hydroponically.

The scope of this thesis is broad, containing nine chapters, each chapter covering specific aspect of the research conducted on the use of biocontrol agents and silicon Si against PM. Each of these chapters is presented as discrete paper, resulting in repetition of some references

between chapters. This is the standard format for MSc and PhD theses adopted by the University of KwaZulu-Natal. References have been formatted in the style of Crop Science.

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CHAPTER ONE

LITERATURE REVIEW

APPLICATION OF BIOCONTROL AGENTS AND SOLUBLE SILICON FOR THE CONTROL OF POWDERY MILDEW

1.1 GENERAL INTRODUCTION

Production of enough palatable and safe food for the world's ever-increasing population presents a major challenge to the world's farmers and agricultural experts. In the last few decades, agricultural production has increased as a result of an increase in cultivated land and the use of agrochemicals for crop production and protection. However, the deterioration in productivity of arable lands, coupled with climatic changes is a strong indicator that such environmentally unfriendly and unsustainable agricultural systems cannot solve the problem of food shortage in the long term. Food security and agricultural sustainability require both development of new and appropriate technologies and an understanding of the ecosystems in which they are to be implemented. The need is for a new type of agriculture, based on sustainable production without intense use of fertilizers and pesticides. This would lead to the development of different production strategies (Campbell, 1989).

In the last 50 years attitudes toward the use of agrochemicals have changed, partly as a result of pressure from conservationists and consumers, and more recently from organic growers (Finch, 1992). Agricultural scientists are under pressure to find solutions for the needs presented by farmers who want to replace or reduce the use of agrochemicals for their organic production. Increased awareness of the impact of some agrochemicals to our health is also shifting consumers to demand products grown with a minimal use of chemicals. A demand for near zero residues in food crops is common in members of the Organizasion for Economic Co-operation and Develeopment (OECD).

The use of biological control agents (BCAs) to control powdery mildew (PM) has been studied extensively. For many years, potential BCAs have been isolated from the phylloplane and tested for their ability to control PM. The most promising BCAs have been further developed and marketed as alternatives to the traditional chemical-based fungicides. Jutsum (1988) predicted that the global market value of biocontrol products should have a significant proportion of the market of the total expenditure on crop protection and public health remedies by 2000. However, the development in the use of BCAs has been slower than predicted, largely due to inconsistent results in different growing environments, the challenge of making biological control of foliar pathogens competitive with agrochemicals (Schippers, 1988) and excessively difficult registration procedures in OECD countries. For example, it currently takes 5-10 years to get a BCA registered in the EU (M.D. Laing, 2008, pers. comm.). To improve this situation, more fundamental knowledge is needed on the biotic and abiotic factors affecting the population dynamics, survival and antagonistic activity of BCAs in the phylloplane (Campbell, 1989). The successful development of BCAs is likely to depend on a thorough understanding of the biology and ecology of each pathogen and its antagonists, and their interactions with other phylloplane inhabitants. Other biotic and abiotic factors such as the concentration of the antagonist applied, inoculum density of the pathogen, host genotype and conduciveness of the environment to the disease also affect the survival and activities of BCAs (Landa *et al.*, 2001).

Silicon (Si), being the second most abundant element on the earth's crust, is present in plants at 0.1-10% of their dry weight (Epstein, 1994, 1999). Research over the last 40-50 years has demonstrated that this element can benefit plants by ameliorating biotic and abiotic stresses. Significant control of PM, and a consequent increase in plant development and yield, has been demonstrated by a variety of plants as a result of the application of soluble Si, in both greenhouse and field trials (Bélanger *et al.*, 1995; Bowen *et al.*, 1992; Menzies *et al.*, 1991b; Remus-Borel *et al.*, 2005; Samuels *et al.*, 1991a & 1994). Silicon is safe and environmentally friendly. However, since the level of disease control is related to the amount of Si absorbed by the plant, more research is needed to determine the optimum concentraion and delivery methods that can provide acceptable levels of PM control, without compromising the quality and quantity of crop products.

1.2 POWDERY MILDEW

Powdery mildews are widespread plant diseases that are conspicuous by their superficial white mycelia and powder-like conidia (Kiss and Szentivanyi, 2001; Yarwood, 1957). They are mostly host-specific, ranging from a single species to a family of plants, and are obligate parasites that cannot survive without their host plant. Most PM fungi infect only one family of plants. For instance, the species *Podosphaera xanthii*, (syn. *Sphaerotheca fuliginea*) that causes PM on the Cucurbitaceae, usually does not attack plants in any other family. There is, however, one report, where a PM of cucumber (*P. xanthii*) infected a bean plant (*Phaseolus vulgaris*) (Kiss and Szentivanyi, 2001). As a group, PM fungi infect many species of plants including cereals and grasses, vegetables, ornamentals, weeds, shrubs and trees. It is a common disease of vegetables under both field and greenhouse conditions in most areas worldwide.

1.2.1   Taxonomy of powdery mildew pathogens

Powdery mildew fungi belong to the ascomycete fungi, in the order of Erysiphales with only one family, the Erysiphaceae (Huckelhoven, 2005). They are further subdivided into five tribes (Erysipheae, Golovinomycetinae, Cystotheceae, Phyllactinieae, Blumerieae) and further sub-tribes, making more than 10 genera in total (Braun *et al*., 2002; McGrath, 1996).

Classification of PM fungi is based on: the type of conidiophore, presence or absence of well-developed fibrisin bodies and mode of conidial germination (Sitterly, 1978). For instance, according to these criteria, this author classified major species of PM of Cucurbitaceae into three genera and six species. These include: *E. cichoracearum* (DC ex Mecat); *E. polyphaga* Hammarlund; *Leveillula taurica* (Lev) Arnaud; and *S. fuliginea* (Schlecht. ex Fr.) Poll. The genus *Leveillula* is considered as a synonym for *Erysiphe*. Among these, *E. cichoracearum* and *S. fuliginea* are the most common and important PM species on cucurbits. On the ornamental, zinnia, *E. cichoracearum* is the most common pathogen (Boyle and Wick, 1996; Kamp, 1985). *Sphaerotheca fuliginea* (syn. *Podosphaera fusca*) has been renamed recently as *Podosphaera xanthii* (Castagne) (Perez-Garcia *et al*., 2006; Shishkoff and McGrath, 2002),

and *E. cichoracearum* renamed as *Glovinomyces cichoracearum* (DC) Gelyuta, V.P. (Keinath and DuBose, 2004; Kobori *et al*., 2004)

1.2.2 Epidemiology of powdery mildew

The PM fungi overwinter on plant debris as colonies of mycelium or in minute brown to black sexual reproductive structure (cleistothecia) (McGrath and Thomas, 1996; Yarwood, 1957 & 1978). In the spring, the cleistothecia produce ascospores that can be transported onto susceptible host tissues by wind or rain splash (Iannotte, 2004). Once a spore (ascospore or conidium) lands on the host surface, it germinates within 2-3d and penetrates the host cell wall with its appressorium. Direct penetration of the host cell by PM fungi involves both enzymatic and mechanical power (Green *et al*., 2002 cited by Huckelhoven, 2005). Following penetration of the cell wall barrier, the fungus develops a haustorium (root-like structures) in the epidermal cells of the plant, from which it extracts nutrients through the plasma membrane of the host (Huckelhoven, 2005).

Severity of PM depends on: the genotype, age and condition of the host plant and prevalent weather conditions of the growing season. The most favourable environmental conditions for PM are dry atmospheric and soil conditions, moderate temperatures, reduced light intensity, fertile soil and succulent plant growth (Yarwood, 1957). According to McGrath (1996) and Yarwood (1978), importance of PM in different regions varies, based on the above-mentioned conditions. Generally, incidence of PM increases as rainfall decreases. Unlike most other fungi, conidia of PM can germinate in the absence of water at relative humidity below 20% (McGrath and Thomas, 1996). This is because conidia of PM fungi have high water content with an extremely efficient water conservation system. In contrast, rain and availability of free moisture on the surface of the leave are unfavourable conditions for the development of the disease because they favour the survival and establishment of other microbes that are antagonists to the pathogen (McGrath, 1996). Increased relative humidity of the air is also reported to enhance germination of PM conidia (Sitterly, 1978). According to the same author, optimum temperature for germination of conidia is 23-31°C, with a peak at 28°C. The fungus dies in few hours if the temperature rises above 27°C or drops below 1.1^{o}C.

Powdery mildew is usually more severe under glasshouse than under field conditions. This is because glasshouses provide reduced air circulation and light intensities as well as higher temperature and continuous cropping, the combination that often results in severe PM epidemics (Howard *et al*., 1994). Incidence of PM increases as relative humidity (RH) rises to 90 %, but it does not occur when leaf surfaces are wet (e.g., in a rain shower). This makes the disease common in crowded plantings, where air circulation is poor, and in damp, shaded areas. Howard *et al*. (1994) showed that young, succulent growth is usually more susceptible than older plant tissues. When the optimum environmental conditions are present, the disease spreads fast, resulting in production of large numbers of conidia for further cycles of infection (McGrath, 1996).

1.2.3   Symptoms caused by powdery mildew

The disease is noted with appearance of small, round, whitish, powder-like spots on leaf surfaces, petioles and stems (McGrath and Thomas, 1996). Symptoms appear first on crown leaves, on shaded lower leaves and on leaf undersurfaces. These white, powdery colonies grow in size and cover both sides of the leaf, petioles and young stems (Howard *et al*., 1994). Older leaves infected with *P. xanthii* turn a dirty-white with age; while those infected with *G. cichoracearum* remain white (Howard *et al*., 1994). Severely infected leaves become yellow, turn brown, and fall prematurely (Howard *et al*., 1994). Fruits rarely show visible symptoms of PM even in the presence of heavy infection.

1.2.4   Effects of powdery mildew in the plant

Although PM is rarely lethal, it is a major production problem, especially when the prevalent environmental conditions are ideal for its development (Choi *et al*., 2004). Severe infection of plants with PM can causes premature leaf senescence, resulting in reduced photosynthesis and transpiration efficiencies by the plant, leading to stunted and weakened plants (McGrath and Thomas, 1996). Loss of leaves and vigour in ornamental plants, as a consequence of severe PM infection, make the infected plants unsightly and of little commercial value as ornamentals (Gombert *et al*., 2001). The impact of PM on fruit is usually indirect because PM hardly ever infects fruit. However, it reduces the quality and quality of fruit by impairing the

photosynthetic rate of the plant. This often results in sunburned or prematurely-ripen fruits that have a low market value (Bélanger *et al*., 1997). Fruits produced from severely infected plants are known to have a poor appearance, inferior storage quality and diminished flavor due to reduced levels of soluble solids (Keinath *et al*., 2000; McGrath and Thomas, 1996). In South Africa, annual economic loss of R7-8milions was estimated on cucurbits as a result of infection by PM (Haupt, 2007), and the value of fungicides used to control the disease on various PM-susceptible crops is estimated to be more than R40million per annum (M.D. Laing, 2004, pers. comm.).

1.2.5   Control strategies

(a) Cultural practices

Any activity that alters the environmental conditions that favour germination of the conidia and survival of the PM fungi can reduce or prevent the disease. Howard *et al*. (1994) recommended that lowering plant populations in order to increase air circulation,reduce relative humidity and minimize shading. Avoiding excessive nitrogen fertilization can also reduce disease severity by avoiding succulent growth. In addition, removing infected leaves and destroying them, followed by cleaning the greenhouse thoroughly after each successive crop, can minimize spread of the disease and reduce survival of the fungus during winter and prevent a carry-over of PM from an infected crop to a new crop. Furthermore, since the fungus does not like high leaf wetness for its germination, spraying water onto infected leaves every 2-3d can reduce its establishment. However, since leaf wetness can cause infection of the plant by other foliar pathogens, spraying should be commenced early in the morning in order to allow the leaf to be dry within 2-3hr (Howard *et al*., 1994).

(b) Chemical control

If cultural practices fail to prevent or control the disease, application of contact or systemic fungicides is the most widely used option (McGrath, 1996). Some of the commonly used fungicides against PM of various crops are listed in Table 1.1. Some of these fungicides provide effective control of PM when applied properly. McGrath and Shishkoff (1999) recommended that once the disease is detected, fungicides should be applied every 7-10d.

Fungicide applications made to control PM should be effective against more than one disease since most crops suffer from several foliar diseases that are caused by more than one fungus (Keinath and Du Bose, 2004).

Since PM develops on the upper and lower surfaces of the leaf, the spray must cover both sides for effective control (McGrath, 2001, McGrath *et al*., 1996). However, these authors believe that even with modern techniques of spray, it is difficult to directly deliver fungicide to the lower surface. Hence, the use of translaminar or systemic fungicides is the ultimate choice for fungicide spray programmes. Unfortunately, because of their single-site modes of action, these types of fungicides have been prone to the development of resistance by the pathogen (McGrath, 2005).

In spite of some success in the use of fungicides for PM management, several researchers have reported on the development of resistance by PM fungi on multiple crops in different parts of the world (McGrath, 2001; McGrath *et al*., 1996). In addition, phytoxicity caused by fungicides may be a problem on some plants. For instance, during periods of intense solar radiation or high temperatures, application of chlorothalonil has been reported to injure mature watermelon fruit (Holmes *et al*., 2002). Similarly, Pasini *et al*. (1997) reported that repeated use of dodemorph resulted in the shortening of stems of roses and increased selection of resistant populations of PM. The rate at which populations of PM fungi became resistant to fungicides has been linked to repeated use of fungicides with one specific mode of action (Engels *et al*., 1996; O'Hara *et al*., 2000). Some of the PM species that have developed resistance to fungicides are presented in Table 1.2.

Table 1.1 Lists of some fungicides that have been commonly used for specific reference to target powdery mildew fungi.

Fungicide (active ingredient)	Crop	Target Pathogen	Reference(s)
azoxystrobin	melon	*Podosphaera xanthii*	Romero *et al*., 2007
azoxystrobin, benomyl, chlorothalonil, mancozeb, myclobutanil, pyraclostrobin	watermelon	*P. xanthii*	Keinath and DuBose, 2004
benomyl, bitertanol	apple	*P. leucotrica*	NDA, 1999
benomyl, bitertanol, bupirimate, carbendazim, fenarimol, pyrazophos, thiabendazole, triforine	tomato	*Oidium neolycopersici*	Jones *et al*., 2001
benomyl, fenarimol	zucchini	*P. xanthii*	Bettiol, 1999
benomyl, carbendazim, tebuconazole, triticonazole	wheat	*Blumeria graminis* f.sp. *tritici*	NDA, 1999
dodemorph	rose	*Sphaerotheca pannosa* var. *rosae*	Pasini *et al*., 1997
epoxiconazole	barley	*B. graminis* f.sp. *hordei*	Barber *et al*., 2003
fenarimol, polyoxin	cucumber	*P. xanthii*	Choi *et al*., 2004.
mancozeb, proquinazid, penconazole,	grape	*Uncinula necator*	Oliva *et al*., 199; Pianella *et al., 2006*
pyrazophos, triadimefon, tridemorph, triforine	long melon	*P. xanthii*	Jiskani *et al*., 2000
sulphur	papaya	*Ovulariopsis papayae*	NDA, 1999
triadimefon	pumpkin	*P. xanthii*	McGrath, 1996

NB: *Sphaerotheca fuliginea* and *P. fusca* are referred as *P. xanthii* (Perez-Garcia *et al*., 2006)

Table 1.2 Lists of powdery mildew species that have developed resistance to target fungicides

PM fungi	Fungicides	Host	Reference(s)
B. graminis f.sp. *tritici*	fenpropimorph, propiconazole, quinoxyfen	wheat	Bernhard *et al*., 2002; Engels *et al*., 1996
B. graminis f.sp. *hordei*	fenpropimorph	barley	O'Hara *et al*., 2000
P. xanthii	azoxystrobin, benomyl, kresoxim-methyl, mycobutanil, propiconazole, triadimefon	cucurbit	Ishii *et al*., 2001; McGrath, 2001; McGrath and Shishkoff, 2001; McGrath *et al*., 1996
U. necator	azoxystrobin, myclobutanil	grape	Northover and Homeyer, 2001; Wong and Wilcox, 2002

(c) Other control options

Pasini *et al*. (1997) showed that the use of several antifungal compounds such as salts, oils and plant extracts against PM of rose were as efficient as spraying the fungicide dodemorph. They also noted that spray applications of KH_2PO_4 and $NaHCO_3$ at 0.5-1% offered good control of PM. Wine vinegar, JMS Stylet Oil®, canola oil, synertrol and neem extract also provided satisfactory disease control. In addition, fatty acids formulated as potassium salts reduced the severity of the disease significantly. Partial control of PM was obtained from Milsana®, a concentrated extract from leaves of *Reynoutriu sachulinensis* (F. Schmidt) Nakai (Pasini *et al*., 1997). Bettiol *et al*. (1999) showed that foliar application of a mixture of cow's milk and water was effective in preventing infection of caused by *P. xanthii.* Similarly, spraying milk-based products onto pumpkin reduced PM and other foliar symptoms by 50-70% and post harvest fruit rot by 40-50% compared to the chemical control (Ferrandino and Smith, 2007). Bélanger *et al*. (1997) have reviewed a range of plant extracts and biocompatible products that have been used against PM.

(d) Integrated control

Integrated disease management is the practice of using a combination of control measures to prevent and manage diseases in crops. Strategies that integrate BCAs and chemical fungicides can overcome the lower efficacy of antagonists, while reducing the residues of chemical fungicides on the final products and the environment. The idea of IPM is based on good agricultural practices needed to produce profitable and productive crops in a sustainable way. Once the symptom is observed or a potential for infection is identified, different techniques can be used as preventative or curative measures to minimize the risk of disease infection and spread.

Combinations of different control strategies have provided promising disease control when tested against PM of various crops. For instance, application of *Ampelomyces quisqualis* Ces ex Schlect, *Trichoderma harzianum* Rifai and *Bacillus subtilis* (Ehrenberg) Cohn, with lower rates of fungicides provided the same level of control of PM of strawberry as fungicides applied at full rates (Pertot *et al*., 2008). Similarly, a mixture of BCAs (*B. subtilis*, *Tilletiopsis minor* Nyland and *Lecanicillium lecanii* (Zimm.) Viégas), mineral salts (KH_2PO_4), an

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antitranspirant (kaolin) and an antioxidant (ascorbic acid) controlled PM of mango effectively (Nofal and Haggag, 2006). A combination of BCAs and chemicals can give better results than when they are applied separately. Combination of these approaches can control the pathogen in climatic conditions beyond the effective range of the bio-protectant, minimize environmental pollution and the likelihood of the pathogen developing resistance, while providing localized and persistent control (Tronsmo and Hjeljord, 1998).

1.3 BIOLOGICAL CONTROL

Biological control, as a crop protection strategy system, emerged as a response to the search for a safe, effective and environmentally friendly approach to replace or supplement the use of chemical pesticides. Biological control of plant diseases involves the use of antagonistic microorganisms to control a pathogen. One form of biological control occurs if the activity of a microorganism, e.g., a plant pathogen, is controlled by another member of the community (Campbell, 1989).

Over the past three decades, research has repeatedly demonstrated that many microorganisms can act as natural antagonists to plant pathogens (Cook, 2000). Powdery mildew fungi are prime targets for biocontrol agents because of their superficial growth (Bélanger *et al*., 1997). This has attracted many researchers to conduct intensive investigations in order to find antagonists that can provide acceptable levels of disease control. The mostly studied microorganisms against PM of different species are listed in Table 1.3. Although consistency has been the major challenge in controlling PM with these antagonists, most of these BCAs have produced promising results in suppressing PM. The success and failure of biological control depends amongst other things, on the production and application of effective BCAs. Through research, some of the most successful BCAs have been processed into commercial products that are approved for the market. Table 1.4 is a summary of some of the promising biological products against foliar diseases that have been commercialized. However, it should be noted that there are many more which are not listed on Table 1.4.

Table 1.3 Lists of biocontrol agents that have been tested against different species of powdery mildew of various crops.

BCA	Target Pathogen	Crop	Reference(s)
Ampelomyces quisqualis	*E. cichoracearum*	cucumber	Sundheim, 1982
	P. aphanis	strawberry	Pertot *et al.*, 2008
	P. leucotricha	apple	Ozentivanyi and Kiss, 2003
	P. xanthii	cucumber, melon, pumpkin, squash, winter squash	Abo-Foul *et al.*, 1996; Dik *et al.*, 1998; Elad *et al.*, 1998; McGrath and Shishkoff, 1999; Romero *et al.*, 2003; Shishkoff and McGrath, 2002; Sundheim, 1982
	S. pannosa var. *rosae*	rose	Pasini *et al.*, 1997
	U. necator	grape	Falk *et al.*, 1995
	Various spp.	various crops	Sztejnberg *et al.*, 1989
Acremonium alternatum	*P. xanthii*	melon	Romero *et al.*, 2003
Bacillus sp.	*P. xanthii*	cucurbits	Romero *et al.*, 2004
B. subtilis	*P. xanthii*	squash zucchini	Bettiol, 1999
	P. aphanis	strawberry	Pertol *et al.*, 2008
Lecanicillium lecanii	*P. xanthii*	cucumber, melon	Askary *et al.*, 1998; Dik *et al.*, 1998; Romero *et al.*, 2003; Verhaar *et al.*, 1996
	S. maculares f.sp. *fragariae*	strawberry	Miller *et al.*, 2004
Meira geulakonigii	*P. xanthii*	cucumber	Sztejnberg *et al.*, 2004
Sporothrix flocculosa	*P. xanthii*	cucumber	Dik *et al.*, 1998
	S. pannosa var. *rosae*	rose	Bélanger *et al.*, 1994
	Several spp.	several plants	Neveu *et al.*, 2007
Sporothrix rugulosa	*P. xanthii*	cucumber	Verhaar *et al.*, 1996, 1998
Stephanoascus flocculosus	*P. xanthii*	cucumber	Jarvis *et al.*, 1989
	S. pannosa var. *rosae*	rose	Hajlaoui and Bélanger, 1991
Stephanoascus rugulosus	*P. xanthii*	cucumber	Jarvis *et al.*, 1989
	S. pannosa var. *rosae*	rose	Hajlaoui and Bélanger, 1991
Tilletiopsis albescens	*P. xanthii*	cucumber	Knudsen and Skou, 1993
	E. graminis f.sp. *hordei*	barley	Knudsen and Skou, 1993
T. minor	*P. xanthii*	cucumber	Hijwegen, 1992
T. washingtonensis	*S. pannosa* var. *rosae*	rose	Hajlaoui and Bélanger, 1991; Sztejnberg *et al.*, 1989
Trichoderma harzianum	*S. mors-uvae*	gooseberry	Picton and Hummer, 2003
	P. aphanis	strawberry	Pertot *et al.*, 2008
	P. xanthii	cucumber	Elad, 2000; Elad *et al.*, 1998

NB: *Sphaerotheca fuliginea* and *P. fusca* are referred as *P. xanthii* (Perez-Garcia *et al.*, 2006); *P. flocculosa* as *S. flocculosa* and *P. rugulosa* as *S. rugulosa* (Eken, 2005) and *Verticillium lecanii* as *Lecanicillium lecanii* (Verhaar *et al.*, 1998).

Table 1.4 List of biocontrol products registered and commercially available (developed or being developed) against foliar pathogens.

Product Name	BCA (s)	Target Pathogen	Producer	Reference(s)
AQ10 Biofungicide	*Ampelomyces quisqualis*	Powdery mildews	Ecogen Inc, USA	Hofstein and Chapple, 1998; McGrath and Shishkoff, 1999; Shishkoff and McGrath, 2002
Aspire	*Candida oleophila*	*Botrytis* and *Penicillium* spp.	Ecogen Inc, USA	Droby *et al*., 1998
BINAB-T®	*T. harzianum + T. polysporum*	Various fungal diseases	Bio-Innovation EFTR AB, Sweden	Moller *et al*., 2003; Mommaerts *et al*., 2008
Eco-77®	*T. harzianum*	*Botrytis cinerea, Eutypa lata, E. leptoplaca*	Plant Health Products, South Africa	Plant-Health, 2008
QST 713, Serenade™	*B. subtilis* strain QST 713	Powdery mildew, gray mold, downy mildew, early blight, late blight, bacterial spot, walnut blight	AgraQuest, Inc., USA	EPA, 2005; Marrone, 2002
Sporodox®	*P. flocculosa*	Powdery mildew	Plant Products Co. Ltd, Canada	Konstantinidou-Doltsinis *et al*., 2007
Trichodex	*T. harzianum*	Various fungal diseases	Makteshim Chemical Works Ltd, Israel.	Dik *et al*., 1999; Elad *et al*., 1998; Etebarian *et al*., 2000; Hofstein and Chapple, 1998
Trichodowels	*T. harzianum + T. viride*	Various fungal disease	Agrimm Technologies Ltd, New Zealand	Monte, 2001
TrichoFlow WP™	*T. harzianum*	Various fungal diseases	Agrimm Technologies Ltd, New Zealand	Bal and Altintas, 2006; Fourie and Halleen, 2006
Trichopel®	*T. harzianum*	Wide range of fungal diseases	Agrimm Technologies Ltd, New Zealand	Bhuiyan *et al*., 2003
Trichoseal	*T. viride*	Trunk and foliar pathogens	Agrimm Technologies Ltd, New Zealand	http://www.tricho.com/trichotech
YieldPlus	*Cryptococcus albidus*	*Botrytis* and *Penicillium* spp.	Anchor Yeast, South Africa	Droby *et al*., 2002

1.3.1 Characteristics of a successful biological control agent

A BCA must firstly be safe to humans, animals and the environment. It should have the ability to grow and colonize the phylloplane fast, produce large number of spores and survive with a minimal requirement for nutrients and favourable environmental conditions (Blakeman and Fokkema, 1982; Chao *et al.*, 1986). Ideally, a BCA should also have some means by which it can survive under unfavourable environmental conditions. For instance, *Bacillus* species produce spores which are resistant to UV light and desiccation and can be formulated easily (Raaijmakers *et al.*, 2002).

Active colonization of available substrates by BCAs can greatly reduce the success of pathogen inocula attempting to infect host plants. For instance, infection of host plants by PM usually occurs within 72h after the conidia of the pathogen land on a susceptible leaf (Huckelhoven, 2005). Therefore PM can only be controlled if there is active and rapid colonization of the phylloplane by the BCA after it is sprayed onto the leaves of the target crop. In addition, an ideal BCA should be competitive with agrochemicals in terms of cost and efficacy.

1.3.2 Mechanisms of action of biocontrol agents

During the development of beneficial microbes for the control of plant diseases, scientists recognized that an effective delivery system requires a thorough understanding of its biological relationship with its target (Bateman and Chapple, 2001). There are at least four mechanisms by which BCAs act against a target PM pathogen: competition for resources, antibiosis, mycoparasitism and induction of host resistance (Whipps, 1992).

Competition for soluble substrates occurs when two (or more) organisms require the same resource (Tronsmo and Hjeljord, 1998), and the use by one reduces the amount available to the other (Campbell, 1989). In such cases, one organism uses most of the nutrients and grows, while the other has insufficient nutrients for its growth and dies. This is typical for a fungus or bacterium that grows very fast and overwhelms the target organism. However, the use of competition for nutrients on the phylloplane as a biocontrol strategy seems unlikely against PM because they can germinate without exogenous nutrients and cover

relatively large areas of leaves within 1-2wk after infection (McGrath and Thomas, 1996). However, Elad *et al*. (1998) showed that competition is one of the mechanisms that is used by *T. harzianum* T39 during its establishment on the phylloplane.

Antibiosis occurs when the production of toxic metabolites or antibiotics of one organism has a direct negative effect on another organism. In pure culture, antibiotic production is common by many potential BCAs (Tronsmo and Hjeljord, 1998). It appears to be important to the survival of microorganisms through elimination of microbial competition for food sources, which are usually very limited in the phylloplane. Several species of *Bacillus* (Romero *et al*., 2004 & 2007) and yeast (Hajlaoui and Bélanger, 1991 & 1993; Urquhart and Punja, 2002) are known to use antibiotic production as their primary mode of action in suppressing PM. Production of ß-1,3-exo and endo-glucanase, chitinase and antifungal compounds by these antagonists was reported to inhibit germ tube development of *P. xanthii* and plasmolyse its spores (Urquhart and Punja, 2002). Many *Trichoderma* spp. are known to produce antibiotics that are is effective against a wide range of pathogens (Elad *et al*., 1998).

Another mechanism utilized by BCAs is mycoparasitism. This is a parasitism of PM fungus by the antagonist fungus. It involves direct contact between the fungi resulting in death of the host (PM pathogen), and nutrient absorption by the parasite (antagonist) (Whipps *et al*., 1988). To break down the walls of their host, mycoparasites possess various enzymes such as: cellulases, chitinases, β-1,3-glucanases and proteases (Elad, 1996 & 2000; Hijwegen, 1992; Patil *et al*., 2000; Urquhart and Punja, 2002)**.** The interaction between mycoparasites and their target fungi occurs in four sequential, but overlapping phases: target location, recognition, contact and penetration (Whipps *et al.,* 1988). In the first stage, a chemical stimulus from the pathogenic fungus attracts the parasite (the antagonist). The second step involves attack of the target pathogen by the mycoparasite with the help of enzymes. In the third step, the mycoparasitic antagonist attaches to the host (pathogenic fungi) either by coiling around or growing alongside it. In the final step, the mycoparasitic fungus degrades the pathogenic cell wall by producing various enzymes (Tronsmo and Hjeljord, 1998).

Some researchers have shown that BCAs may act against the pathogen by triggering host defense mechanisms. In induced systemic resistance (ISR), the BCA is not directly involved with the pathogen; instead, it activates the defense mechanisms of the host (Wei *et al*., 1996). Once ISR is expressed, it activates multiple potential defense mechanisms including increased production of antifungal compounds by the host (Elad, 2000; Wei *et al*., 1996). According to these authors, ISR has a wide range spectrum, protecting the plant from variety of pathogens. *Trichoderma harzianum* T39 (Elad, 2000; Elad *et al*., 1998) and some growth promoting bacteria such as *B. pumulus* Meyer and Gottheil, *Pseudomonas putida* (Trevisan) and *Serratia marcescens* (Bizio) (Wei *et al*., 1996) have been reported as BCAs that induced systemic resistance.

During screening, it is important to consider the different mechanisms of action, because inhibition of the pathogen by an introduced antagonist may involve different interactions. In addition, since the control system involves BCAs, pathogen, host and the environmental conditions, it is important to incorporate these components during the screening. Screening techniques often focus on detecting one or two modes of action and therefore fail to detect BCAs using other modes of action.

1.3.3   Challenges and opportunities in the development of biocontrol against foliar diseases

Biological control has been less successful in the phyllosphere than the rhizosphere (Andrews, 1992). This is partly due to extreme fluctuations in environmental conditions in the aerial part of the plant compared to the soil parts. Blakeman and Fokkema (1982) noted that the survival and activities of both saprophytes and pathogens on leaves is dependent on the microclimatological conditions and chemical properties of the leaf surface. Because of their requirements for specific environmental conditions, BCAs are usually effective within a limited range of temperature and RH. For instance, most antagonists of PM are more efficient when RH is maintained above 80% (Bélanger *et al*., 1994 & 1997; Hijwegen, 1992; Jarvis and Slingsby, 1977; Jarvis *et al*., 1989), which can be achieved by manipulating the greenhouse environment. However, this is not possible under field conditions. Hence, efficacy of most BCAs against PM and other foliar diseases has been inconsistent, especially under field conditions. In addition, BCAs rely on the availability of nutrients for their survival and production of antibiotics and siderophores. Unfortunately,

the amount of nutrients on the phylloplane is often limited and may be leached as a result of irrigation, rain, dew and fog (Andrews, 1992).

The rate of establishment and biocontrol activities of introduced antagonists is often slower than that of the pathogen. For instance, conidia of PM require 3-5d to germinate and infect the plant (Huckelhoven, 2005; McGrath and Thomas 1996). In contrast, except for a few antagonists such as *A. quisqualis* (Sundheim and Krekling, 1982), establishment of BCAs on the phylloplane may take 10-14d, by which time the disease is at a high level. Once the disease pressure is very high, efficacy of BCAs is limited (Elad *et al*., 1998; Pertot *et al*., 2008). Moreover, some introduced BCAs need the presence of their host on the phylloplane in order to establish themselves. For example, *T. minor* was more effective when applied 2d after the plant was infected with PM (Hijwegen, 1986).

In spite of these challenges, use of BCAs on the phylloplane does provide some opportunities. The method of application of BCAs is simple, and it is easy to see the effects of treatments because the disease is visible. Once applied and established, BCAs can move onto different parts of the foliage, and may provide long-term control without the risk of resistance.

1.3.4 Improvement of biocontrol efficacy

Since biological control is holistic in its approach, it is important to combine the manipulation of different aspects that are necessary to favour the antagonist and disadvantage the pathogen (Andrews, 1992). For instance, growth of introduced BCAs can be assisted by manipulation of the microclimate of greenhouses (Blakeman and Fokkema, 1982). Adjusting the RH to the level required by the antagonist or providing free water on the leaf surface can improve the efficacy of biological control. However, continuous monitoring is needed because increased leaf wetness can result in infection of the plant by other pathogens. In addition, the chemical properties of the leaf surface can also be modified to suit the demand of the antagonist by applying the right chemical. Growing BCAs in suitable media, applying correct dosage of BCA inoculum, using appropriate formulations and storage and placing inoculum in favourable positions can facilitate active colonization of the phylloplane by the antagonist. Application of BCAs before the disease reaches a certain level, and use of selected adjuvants and oils can improve efficacy of

biological control. Elad *et al*. (1996) noted that the efficacy of *Tilletiopsis* spp. was best when applied few days before or immediately after infection of the plant with PM. Furthermore, use of additives, such as sucrose, can increase the population of the antagonist on the phylloplane (Blakeman and Fokkema, 1982). However, growth of other pathogens must be managed carefully.

1.4 SOLUBLE SILICON

Silicon (Si) is the second most abundant element in the surface of the earth and can present in plants in amounts equivalent to those of macronutrients such as calcium, magnesium and phosphorus (Epstein, 1994). Although the level of Si in plant tissues ranges between 0.1-10%, sometimes exceeding that of nitrogen and potassium, its importance to agriculture has been underrated with the perception that most plants can grow in a nutrient solution without Si in their formulation (Epstein, 1999). However, when Si is readily available to plants, it plays a major role in their survival and growth. Some of the beneficial effects of Si to plants include increased growth and decreased susceptibility towards pathogens and insects, and amelioration of abiotic stresses (Epstein, 1994 & 1999).

1.4.1   Effects of silicon on powdery mildew control

The effects of Si on incidence and severity of PM on a range of crops has been studied by several researchers (Bélanger *et al*., 1995; Menzies *et al*., 1991b; Samuels *et al*., 1991a & 1994). Bélanger *et al*. (1995) noted that adding Si into nutrient solutions reduced incidence of PM in cucumber and delayed its rate of development. According to Menzies *et al*. (1991a), the reduction in the severity of PM was due to inhibition of germination of PM conidia by Si. Similarly, treatment with Si reduced PM of barley (Wiese *et al*., 2005), grape (Bowen *et al*., 1992; Reynolds *et al*., 1996), muskmelon (Menzies *et al*., 1992), wheat (Bélanger *et al*., 2003; Remus-Borel *et al*., 2005) and zucchini (Menzies *et al*., 1992). Whilst the use of Si for disease management is safe and effective, in most reports complete control of the disease was not obtained, with results differing among cultivars (Liang *et al*., 2005; Palmer *et al*., 2006), cropping seasons and temperature (Schuerger and Hammer, 2003).

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1.4.2 Effects of silicon on plant growth

Bélanger *et al*. (1997) noted that, in Europe, 60% of cucumber and 30% of rose growers were using soluble Si on a regular basis. Most of those users believed that they had benefited by reducing fungicide application and increasing yields. Even in the absence of disease, application of Si has been observed to increase yields of cucumber. However, it is not clear if this increase was as a result of disease control or the management of several biotic and abiotic stresses of the plants.

1.4.3 The role of silicon in amelioration of abiotic stresses

Silicon has been reported to ameliorate mineral toxicity effects in plants (Epstein, 1994; Marschner, 1995). For instance, Si can reduce or prevent toxicity effects caused by aluminum (Al), iron (Fe) and manganese (Mn) (Marschner, 1995; Tisdale *et al*., 1993). Equally, it can reduce the impact of nutrient deficiencies of phosphorus (P) and zinc (Zn) (Epstein, 1994; Marschner, 1995). In addition, it reduces stress of plants caused by soil salinity and excessive transpiration (Epstein, 1994; Marschner, 1995; Tisdale *et al*., 1993). Moreover, in sugar cane, Si protected leaves from ultraviolet radiation damage by filtering out harmful ultraviolet rays (Tisdale *et al*., 1993).

1.4.4 Possible mechanisms of action of silicon

Although consistent results have been reported on the positive effects of Si in disease management, the mechanisms by which Si reduces severity of diseases are the subject of ongoing debate (Ghanmi *et al*., 2004). While several researchers believe that the possible mechanism of action of Si is purely mechanical, others claim that it is entirely based on the catalysis of induction of systemically acquired resistance (SAR) by the host plants. Zeyen *et al*. (1992) associated reduction of PM to be a result of increased in silification of the epidermal cells, which would impede the penetration by the pathogen germ tubes. Supportive reports by Carver *et al*. (1998) and Winslow (1992) showed that increased resistance against fungal pathogens in cereal species was correlated with the accumulation of Si in these plants. However, further investigations revealed that silification takes place at the trichome bases on the epidermis (Bélanger *et al*., 1995, Samuels *et al*., 1994), and around the fungal hyphae and infection pegs in infected cells of the host plant (Bélanger *et*

al., 1995; Datnoff *et al*., 1997). Microscopic studies by Menzies *et al*. (1991b) showed increased accumulation of phenolic compounds within cells of cucumber plants treated with Si. Similarly, Fawe *et al*. (1998) discovered that treatment with Si induced defense reactions in cucumber plants towards PM by promoting accumulation of antifungal flavonoids. Moreover, Bélanger *et al*. (2003) reported that treatment with Si induced defense mechanisms in wheat against *B. graminis* f.sp. *tritici* by promoting accumulation of electron-dense, phenolic compounds surrounding fungal haustoria within the epidermal cells of these plants. Using X-ray microanalysis and microscopic investigations, Ghanmi *et al*. (2004) found that treating *Arabidopsis thaliana* (L.) Heynh with Si induced the production of electron-dense, fungitoxic substance that accumulated within and around the collapsed haustoria of epidermal cells within the leaves of the disease-resistant plants. Furthermore, Si was shown to increase resistance against blast disease on rice caused by *Magnaporthe grisea* (T.T. Hebert) M.E. Barr. by catalysing the accelerated production of phenolic like compounds and antifungal momilactone phytoalexins (Rodrigues *et al*., 2004). Most of these conclusions were made based on applying Si in the nutrient solution. However, foliar application of Si has also provided effective control of PM in several crops (Bowen *et al*., 1992; Guével *et al*., 2007; Liang *et al*., 2005; Menzies *et al*., 1992; Palmer *et al*., 2006; Reynolds *et al*., 1996). What is not clear is whether similar mechanisms of action are involved when Si is used as a foliar treatment as when it is applied to plant roots as a fertilizer.

1.4.5   Methods of application of soluble silicon

Usually Si has been applied to plants by adding soluble forms of the element into a nutrient solution of a hydroponics system. This system ensures a continuity of supply of Si to the plants while the pH and concentration of each element in the nutrient solution can be balanced according to the requirements of the plants. With drip irrigation, Si may be added into the irrigation stream at the required level, in order to supply plants through the irrigation system. Under field conditions, Si is normally applied as drench or through the irrigation system (i.e. sprinkler or other techniques). Silicon can also be applied to plants as a foliar spray. Foliar application is commonly used to control foliar diseases directly rather than through amelioration of abiotic stresses of the plant related to the soil.

1.4.6   Challenges in using silicon

Although many studies have been made on Si in agriculture in the last 50 years in order to understand the role of Si in agriculture, its exploitation has been limited. This is partly due to inconsistency of the results under different conditions on different species. Susceptibility of plants to stress, and weather conditions and the characteristics of the soils such as soil physical properties, soil organic matter and soil chemistry have direct effects on the role of Si to plants. Bélanger (2008) noted that beneficial effects of Si are noticeable only when the plant is under stress. Unlike curative fungicides that can eradicate the disease completely, Si is more effective when applied as a protective treatment (Liang *et al*., 2005; Samuels *et al*., 1991b), and it is less effective under situations of high disease pressure (Kanto *et al*., 2007). Since Si is interactive with other elements, such as Ca, Al and Mn, its availability to plants is dependent on presence of other elements and the soil pH (Epstein, 1994 & 1999).

A lack of knowledge on optimum concentrations and application frequencies is another reason for incomplete control of diseases. For instance, application of high concentrations of Si to cucumber may provide effective control of PM, but may result in reduced quality of the fruit (Bélanger *et al*., 1997). Many researchers believe that disease resistance is linked with the amount of Si accumulated by the plant (Epstein, 1994; Jansen, 2004). However the level of Si that should be applied in order to give optimum disease control without compromising growth and quality of the produce is an issue of continuous research (Bélanger *et al*., 1995). A further challenge is that uptake of Si varies not only between crops (Mitani and Ma, 2005), but may also vary between cultivars within a single crop (Ago *et al*., 2008).

1.5 OPPORTUNITIES FOR THE USE OF BIOCONTROL AGENTS AND SILICON IN AN INTEGRATED DISEASE MANAGEMENT PROGRAMME

Each control option has its unique strengths and weaknesses relative to the other. Individual disease management options often result in incomplete disease control. This raises a question whether the use of biocontrol agents and Si can supplement each other for better disease control in a sustainable way.

An IPM programme may involves the combined use of chemical, biological, physical, biotechnical, genetic and agricultural techniques of disease control that offers the opportunity to growers to choose the most appropriate intervention once a threshold disease level is reached (Oliva *et al*., 1999). According to these researchers, unlike chemical control, where the main objective is to eradicate the disease, the aim of IPM is to keep the disease level below the tolerance threshold by intervening only when the severity of the pathogen exceeds a certain level.

The challenge to control PM with BCAs and Si is the extremely rapid development of the disease. Therefore, there is a need to find a means by which the rate of disease progress can be reduced so that the use of BCAs and Si can be more effective. If this challenge remains unsolved, farmers will continue to depend on using fungicides as their only effective tool to control PM. In South Africa, the proposed approach of using biological control and Si for disease management and plant growth promotion is getting encouraging acceptance by the farmers. Most soils in Africa are acidic and deficient in plant-available Si due to leaching. Therefore, application of Si fertilizer to crops should benefit most crops in most situations. Use of Si, combined with BCAs, by commercial farmers could replace the intensive use of agrochemicals in Africa.

Table 1.5 Comparison of the benefits and limitations in terms of fungicides, silicon and biocontrol agents against plant diseases.

Criteria	Fungicides	Silicon	BCAs
Efficacy			
• Short term	3	2	1
• Long term	1	2	3
• Rapidity of effect	3	2	1
• Effect under high disease pressure	3	1	1
• Curative control	3	1	1
• Preventative control	3	3	3
Reliability			
• Environmental influences	3	2	1
• Influence of plant and soil	2	1	1
• Shelf life	2	3	1
Reduction in risks			
• Risk of resistance	1	3	3
• Side effects to humans and animals	1	3	3
• Residues in food	1	3	3
• Persistence in the environment	1	3	3
Public acceptance	1	2	3
Profitability to producer	2	3	2
Cost effectiveness	1	2	3
Long-term benefit for society	1	3	3

1 (least), 2 (intermediate) and 3 (most) relative advantage of the control method. The table and criteria for comparison was adopted and modified from Yobo (2005).

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CHAPTER TWO

ISOLATION AND *IN VITRO* SCREENING OF POTENTIAL BIOCONTROL AGENTS AGAINST POWDERY MILDEW

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Abstract

Powdery mildew (PM) is an important disease of many plants worldwide. Potential antagonists of powdery mildew were isolated from naturally infected leaves of different plants. A total of 2000 isolates of bacteria, fungi and yeasts were tested in a preliminary screening on detached leaves. The best 30 isolates showing consistent results were further tested under glasshouse conditions for their efficacy against PM of zucchini. In a glasshouse trial, disease control of 30-77% was provided by 23 isolates, and significant reductions in AUDPC values were recorded as a result of 29 isolates being applied. The best five isolates, identified as *Gliocladium roseum* (syn. *Clonostachys rosea*) (Isolate EH), *Trichothecium roseum* (syn. *Cephalothecium roseum*) (Isolate H20) and *Serratia marcescens* (i.e., Isolates B15, Y15 and Y41), were selected for further studies.

2.1 INTRODUCTION

Powdery mildew (PM) is one of the most important foliar diseases of many plants worldwide, occurring both under greenhouse and field conditions (Kiss *et al*., 2004). Application of systemic fungicides has been the principal control option (McGrath, 1996; 2001). However, effective control of this disease with fungicides is becoming increasingly difficult due to concerns about health and environmental hazards, as well as the rapid development of fungicide resistance by these pathogenic fungi, rendering most fungicides ineffective against PM (McGrath, 2001). The possibility of using resistant cultivars towards this disease is also limited, especially in fruit and vegetable crops (Bélanger and

Benyagoub, 1997). Therefore, there is great research interest in identifying effective antagonists against PM that can supplement or replace conventional agrochemical fungicides.

Biocontrol of PM by various antagonists may involve competition for natural resources (Nofal and Haggag, 2006), antibiosis (Hajlaoui *et al*., 1992; 1994), mycoparasitism (Askary *et al*., 1997; Falk *et al*., 1995a; Kiss, 1998; Romero *et al*., 2003; Sundheim, 1982; Sztenjberg *et al*., 1989; 1995b; Verhaar *et al*., 1997) or induction of systemic resistance in plants against pathogens (Elad *et al*., 1998; Silva *et al*., 2004; Vogt and Buchenauer, 1997). The superficial growth of PM on leaves makes it an easy target for many antagonists that can be isolated and screened for their biocontrol efficacy.

The success of biological control depends on the initial screening because subsequent results depend on identifying the best isolates during the initial process (Chiou and Wu, 2003). Isolating potential antagonists from their natural hosts can make screening easier and more effective because they will be applied in the same environment, to which they should be well adapted.

There are several techniques of preliminary screening isolates for biological control agents, with the dual culture method being preferred by many researchers (Landa *et al*., 1997; Morton and Stroube, 1955; Paulitz *et al*., 1992). This technique involves culturing the target pathogen and the potential antagonist(s) together on the same artificial medium. Its simplicity and rapid results, with some level of reliability, makes it a good primary screening method when the antagonists and the target pathogens can be grown saprophytically. However, obligate pathogens such as PM cannot be cultured on artificial media, limiting the application of this approach. Moreover, some of the modes of action cannot be observed by dual culture, resulting in the loss of some effective isolates if their modes of action are not detected using this technique. Therefore, an alternative approach is needed for obligate parasites that require the screening of isolates in a natural way. The objectives of this study were to isolate and screen various potential antagonists of PM from naturally infected leaves of susceptible plants.

2.2 MATERIALS AND METHODS

2.2.1 Collection of samples

Samples of leaves of 16 plant species, naturally infected with PM, were collected from 8 sites in the province of KwaZulu-Natal (Table 2.1). Two leaf samples (one young and one old infected leaf) were taken from each plant species that were growing at least 1km apart, or after an interval of 10 d, if taken from the same plant.

2.2.2 Isolation and culturing of potential biocontrol agents

(a) Fungi

Mycelia of PM, and hence any associated fungi, were scratched from the plant with a sterilized scalpel, plated onto Potato Dextrose Agar (PDA) and incubated at 25°C. Plates were monitored for 1 wk, while developing colonies of fungi were sub-cultured. Each isolate was incubated for 1-2 wk depending on its growth and sporulation rates. Mycelia and spores were harvested by pouring 5ml of sterilized distilled water onto the plates of PDA for 5min and scratching the mycelial mat with a sterilized scalpel. The mycelial suspension was transferred into sterile bottles, shaken vigorously and spores were filtered using four layers of cheesecloth.

(b) *Bacillus*

Samples of infected leaves were placed into conical flasks containing sterilized distilled water and shaken vigorously. After removing the leaf materials from the flask, the suspension was serially diluted, then heated to 80°C for 15 min. Aliquots of 0.1 mℓ were plated on Tryptone Soy Agar (TSA) and incubated at 30°C overnight. Individual bacterial colonies were transferred onto clean PDA plates and incubated for 24 h at 30°C. For mass production, isolates were inoculated onto 100 mℓ flasks containing 50 mℓ of Tryptone Soya Broth (TSB) and incubated in a water bath operating at 30°C and 200-250 revolutions per minute (rpm). After an incubation period of 72 h, cultures were centrifuged at 9000 rpm for 15 min. The suspension was removed gently and the pellet was transferred into bottles containing sterilized distilled water.

(c) Yeasts

The same technique that was used for *Bacillus* isolates was adopted with slight modifications. Mycelial and spore suspensions of PM were prepared by putting infected leaves into conical flasks containing 100 mℓ of sterilized distilled water and shaken vigorously for few minutes. The suspensions were serially diluted in sterile distilled water and an aliquot of 0.1 mℓ of each dilution was plated onto a semi-selective media, prepared by adding Rose Bengal to PDA, with the objective of inhibiting bacterial growth. All colonies of yeast were re-streaked on Malt Extract Agar (MEA) to obtain pure cultures and incubated at 25°C. After 48 h of incubation, isolates were transferred into 100 mℓ flasks containing Malt Extract Broth (MEB) and fermented in a water bath for 72 h at 25°C with rotary agitation at 200-250 rpm.

2.2.3 Preparation of powdery mildew inoculum

Inoculum of PM was collected from naturally infected leaves of zucchini (*Cucurbita pepo,* F1-Hybrid Partenon) obtained from Starke Ayres[1], and maintained by inoculating conidia onto disease-free plants and keeping them in a separate glasshouse. Conidia of PM were harvested using the method of Askary *et al*. (1998) and Dik *et al*. (1998). To ensure a high level of conidial viability and to maintain the same age of conidia, dead conidia were removed by shaking the source leaves 24 h before inoculation. Infected leaves were then immersed in sterilized distilled water and shaken to remove PM conidia. Conidia were counted using a haemocytometer and the concentration was adjusted to 10^{3} conidia mℓ$^{-1}$. Inoculation was by spraying the conidial suspension onto leaves within two hours of counting.

2.2.4 Screening of isolates on detached leaves

Seeds of zucchini were planted in trays filled with a composted pine bark, and kept in a glasshouse (26-28°C, 75-85% RH). After 2 wk, fully developed leaves were cut off with their petioles and transferred into pairs of 90 mm diameter Petri dishes, as described by Shishkoff and McGrath (2002). Each leaf petiole was inserted through a hole that connected the bottom of the top Petri dish to the lid of the bottom Petri dish that contained a nutrient solution (Figure 2.1). Petri dishes were transferred into growth chambers set at a

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[1] Starke Ayres Seeds (Pty) Ltd., P. O. Box 304, Eppindust 7475, South Africa.

temperature of 25°C and 18°C (day and night), with a photoperiod of 16 h and an RH of 65-80%. The lid of the top Petri dish was removed to avoid fluctuations of humidity that might have affected antagonistic activity of potential antagonists. Potential antagonists were applied by putting 10-15 droplets of the microbial suspension onto the leaves using sterilized Pasteur pipettes. In order to give the antagonists a competitive advantage, they were applied 2 d before inoculation of the leaves with PM, and reapplied 4 d after inoculation with *Podosphaera xanthii.*

Ten days after inoculation, the percentage of leaf area covered with PM was recorded and transferred into a scale of 1-5 where: 1 = 0-20%, 2 = 20-40%, 3 = 40-60%, 4 = 60-80%, 5 = 80-100% of leaf area covered by PM. This was done in order to separate the most effective ones from the least based on a range. To avoid any possibility of cross contamination between treatments, each isolate was tested in two replicates, placed 150-200 mm from other isolates. Isolates that showed superior performance against PM were selected for further screenings that continued until the number of isolates was reduced to the best 30.

2.2.5   Screening of isolates on whole plants

Seedlings of zucchini were raised in the same way as described above and kept in the glasshouse until they produced two fully developed leaves. Seedlings were then transplanted into pots (180mm diameter) containing a composted pine bark medium and transferred into another glasshouse (24-30°C and 65-85% RH). The plants were supplied with a complete fertilizer [3:1:3 (38) from Ocean Agriculture[2] at 0.5g ℓ^{-1}] + [Ca(NO$_3$)$_2$ at 0.5 g ℓ^{-1}] by means of drip irrigation. Plant distance was 400mm within rows and 1.5-2m between rows (Figure 2.1). Once seedlings were well established, they were inoculated with approximately 3-5 mℓ of a conidial suspension of *P. xanthii* (10^3 conidia mℓ^{-1}) using a hand sprayer. Inoculation took place in the late afternoon to ensure sufficiently high relative humidity for germination of the conidia.

All BCAs were applied using hand sprayers 2d before inoculation of *P. xanthii* and reapplied 4 d later, when symptoms started to appear, and continued every week for 4 wk. The concentrations of propagules of BCAs applied were 10^6 mℓ^{-1} for fungi and 10^8 mℓ^{-1} for bacteria and yeasts, respectively. All BCAs were applied in the evenings to ensure

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[2] Ocean Agriculture (Pty) Ltd., P.O. Box 742, Muldersdrift, 1747, South Africa.

sufficient relative humidity for establishment of the antagonists. The best five isolates that controlled PM consistently were selected for further evaluations and sent for identification using DNA sequence. The bacterial and fungal BCAs were identified by Inqaba Biotechnical Industries (Pty) Ltd[3] and Agricultural Research Council (ARC-PPRI)[4], respectively.

Disease assessments were made weekly by estimating the percentage of leaf area infected for each treatment, immediately before the next spray application of the BCA. Values of area under disease progress curve (AUDPC) were calculated from the disease severity values using an AUDPC program (Shaner and Finney, 1977).

2.2.6   Data analysis

The experiment was arranged in a randomized complete block design with each treatment having three replications. Data was analysed using GenStat® Statistical Analysis Software (GenStat, 2006). Where the CV % was > 20%, the original data was transformed using a square root transformation and means of treatments were separated using Duncan's New Multiple Range Test. Effect of individual treatment (i.e. BCA) in controlling PM was assessed by calculating the percentages of final disease level and AUDPC value of the Untreated Control reduced by the biocontrol treatment.

2.3 RESULTS

2.3.1   Isolation and screening on detached leaves

When PDA was used as a growth media, many types of microbes grew from the scrapings of PM mycelium that were plated onto it. However, only fungal colonies were picked up using a sterilized scalpel. Rate of germination of germ-tubes as well as shape, size and colour of mycelia of these fungi were variable when observed with the naked eye. Microscopic examinations showed that the isolates differed widely in their spore structures and hyphae. When Rose Bengal was added to the PDA, growth of bacterial species was inhibited and yeasts, together with some fungi, grew on the medium. However, there were a

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[3] Inqaba Biotechnical Industries (Pty) Ltd., P.O. Box 14356, Hatfield 0028, South Africa.

[4] Agricultural Research Council (ARC-PPRI), P. Bag X134, Queenswood 0121, South Africa.

few Gram negative bacteria that grew on PDA containing Rose Bengal. The isolation technique used for *Bacillus* species provided many isolates, mostly Gram positive bacteria. Only colonies that showed some morphological resemblance to typical *Bacillus* colonies were chosen as potential isolates.

The isolation techniques were applied to leaves of 16 plant species infected with *P. xanthii* PM, collected from different sites. A total of 2,000 candidate microbes were selected for screening on detached leaves. Detached leaves started to show PM symptoms 3-4 d after inoculation and the disease could cover an entire leaf within 10 d because the environmental conditions were favourable for PM. Adventitious roots developed from the bottom of the petiole after 6-7 d. As long as the nutrient solution was available, the leaves could stay alive, in most cases, for more than one month.

During the evaluation, some leaves died due to high levels of PM and were considered as 100% diseased (i.e., 0% control). More than half of the potential BCA isolates reduced PM disease levels compared to the untreated control. From all the isolates tested, the best 100 isolates were selected for a second phase of screening, from which 70% of them were discarded, before a third phase of selection was conducted, because the efficacy of some of these isolates was less consistent than others. Some isolates were observed to produce an inhibition zone around them, restricting the expansion of the PM colony, while other isolates inhibited the establishment of the disease. After a series of screening on the detached leaves, a total of 30 isolates (11 bacteria, 12 fungi and 7 yeasts) were selected that reduced PM disease levels by more than 60%.

Table 2. 1 Sites where naturally infected leaves of host plants were collected

Site	Host plants
Albert Falls	weed 1
Durban, KwaMashu	cucumber, grape, pumpkin
Pietermaritzburg, Agriculture Campus	cabbage, cucumber, papaya, pepper, pumpkin, rose, tomato, weed 2, strawberry, zinnia, zucchini
Pietermaritzburg, Cedara	cucumber, pumpkin
Pietermaritzburg, Scottsville	grape, mulberry, pumpkin, weed 3, zucchini
Pietermaritzburg, Ukulinga Farm	beans, pepper, tomato, weed 4, zucchini
Tala Valley	pumpkin
Tongaat	pumpkin
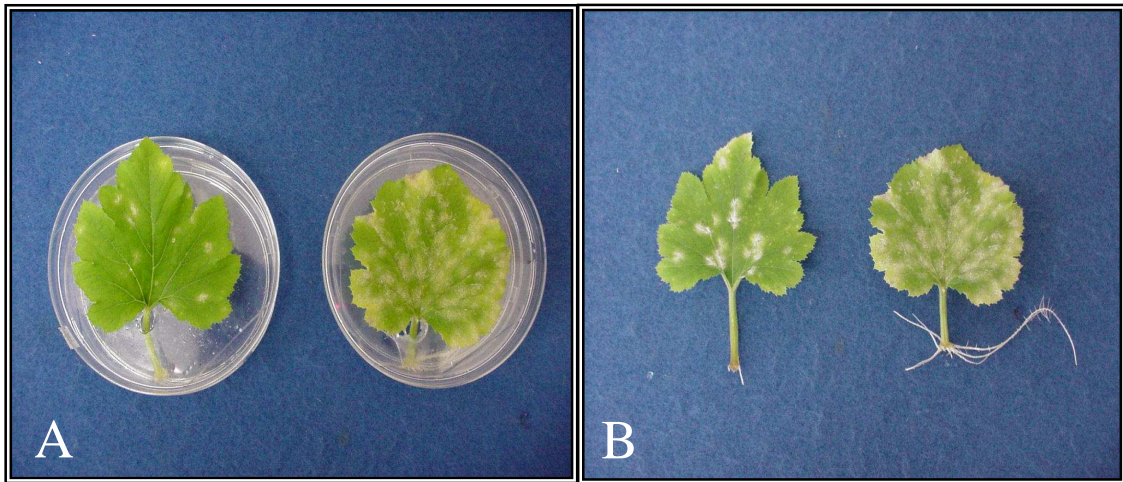
Figure 2.1 Plates showing preliminary screening of potential isolates using detached leaves inoculated with *Podosphaera xanthii* and treated with biocontrol agents (A). Note the formation of adventitious roots from the petioles, and the development of powdery mildew (B).

Figure 2.2 Zucchini plants growing in greenhouse.

2.3.2 Screening on whole plants

Under glasshouse condition, plants started to show symptoms of PM 4d after inoculation and the disease level could reach up to > 80% within 2 wk, if not treated. Application of BCAs had a significant effect on the severity of PM (P < 0.001). Compared to the treatment containing only water, 23 isolates significantly reduced the final disease level by 30-77%. Some isolates that showed promising results on detached leaves (e.g., Isolates CR, CK, CFC, CE, C, B14, E-77) were less efficient in controlling the disease when tested under glasshouse condition, although they caused significant control (Table 2.2).

The AUDPC value of the Untreated Control, was significantly reduced (P < 0.001) by 29 isolates (Figure 2.3). There was a direct relationship between final disease level and AUDPC values. Whenever the final disease was high, the corresponding value of AUDPC was high.

The best five biocontrol 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 3 isolates *Serratia marcescens* (Bizio) (i.e., Isolates B15, Y15 and Y41).

Table 2.2 Effect of selected biocontrol agents on final disease level (FDL) and AUDPC of powdery mildew of zucchini plans after 10 d and 5 wk of growth in a growth chamber and greenhouse, respectively.

Isolates (BCAs)	Type	Disease rating on detached leaves	Greenhouse assessment	
			FDL	AUDPC
Control	0	5	88.3^i	1152.7 (33.9)^k
Y41	B	1	22.3^a	204.2 (14.3)^a
Y15	B	1	24.0^a	194.8 (13.8)^a
B-52	B	2	52.3^{def}	613.7(24.6)^{efghi}
B-29	B	1	70.7^{fghi}	855.2 (29.2)^{ij}
B-21	B	1	64.3^{defg}	740.8 (27.2)^{fghij}
B-15	B	1	20.3^a	379.2 (19.4)^{bcd}
B-14	B	1	75.3^{ghi}	653.3 (25.4)^{efghi}
B-1272	B	2	62.3^{defg}	624.3 (24.6)^{efghi}
ML	F	1	50.0^{cdef}	519.2 (22.5)^{defg}
EH	F	1	21.7^a	220.5 (14.8)^a
E-77	F	2	74.7^{ghi}	808.5 (28.3)^{hij}
DY	F	1	68.3^{efghi}	730.3 (26.9)^{fghij}
DS	F	1	62.3^{defgh}	663.8 (25.7)^{efghij}
CR	F	2	74.3^{ghi}	696.5 (26.4)^{efghij}
CK	F	1	81.0^{hi}	947.3 (30.7)^{jk}
CFC	F	2	81.0^{hi}	807.3 (28.4)^{hij}
CE	F	2	80.7^{hi}	730.3 (26.9)^{fghij}
C218	F	2	28.3^{ab}	259.0 (16.1)^{ab}
H20	F	2	21.0^a	192.5 (13.9)^a
C12	F	1	47.7^{cde}	456.2 (21.3)^{cde}
C	F	1	74.3^{ghi}	758.3 (27.5)^{ghij}
BK	F	1	45.7^{bcd}	530.5 (22.5)^{defg}
Y9	Y	1	24.0^a	200.7 (14.1)^a
Y38	Y	2	56.0^{defg}	574.0 (23.9)^{defgh}
Y31	Y	1	31.3^{abc}	310.3 (17.6)^{abc}
Y30	Y	1	52.0^{def}	527.3 (22.9)^{defg}
Y27	Y	2	51.7^{def}	499.3 (22.3)^{cdef}
Y26	Y	2	48.0^{cde}	493.5 (22.0)^{cdef}
Y20	Y	2	55.3^{defg}	567.0 (23.8)^{defghi}
Y18	Y	2	57.0^{defg}	610.2 (24.5)^{defghi}
Effects			**P-values**	**P-values**
P-value			< 0.001	< 0.001
MSE			112.5	15263.34
CV (%)			19.74	11.4

F= fungus, B= bacterium, Y= yeast

Values within column followed by a common letter were not significantly different according to Duncan's New Multiple Range Test at $\alpha = 0.05$.

Values in brackets are means of data transformed using square root transformations.

Ratings: 1 disease < 20%; 2 = < 40%, 3 = < 60%; 4 = <80%, 5 = totally susceptible.
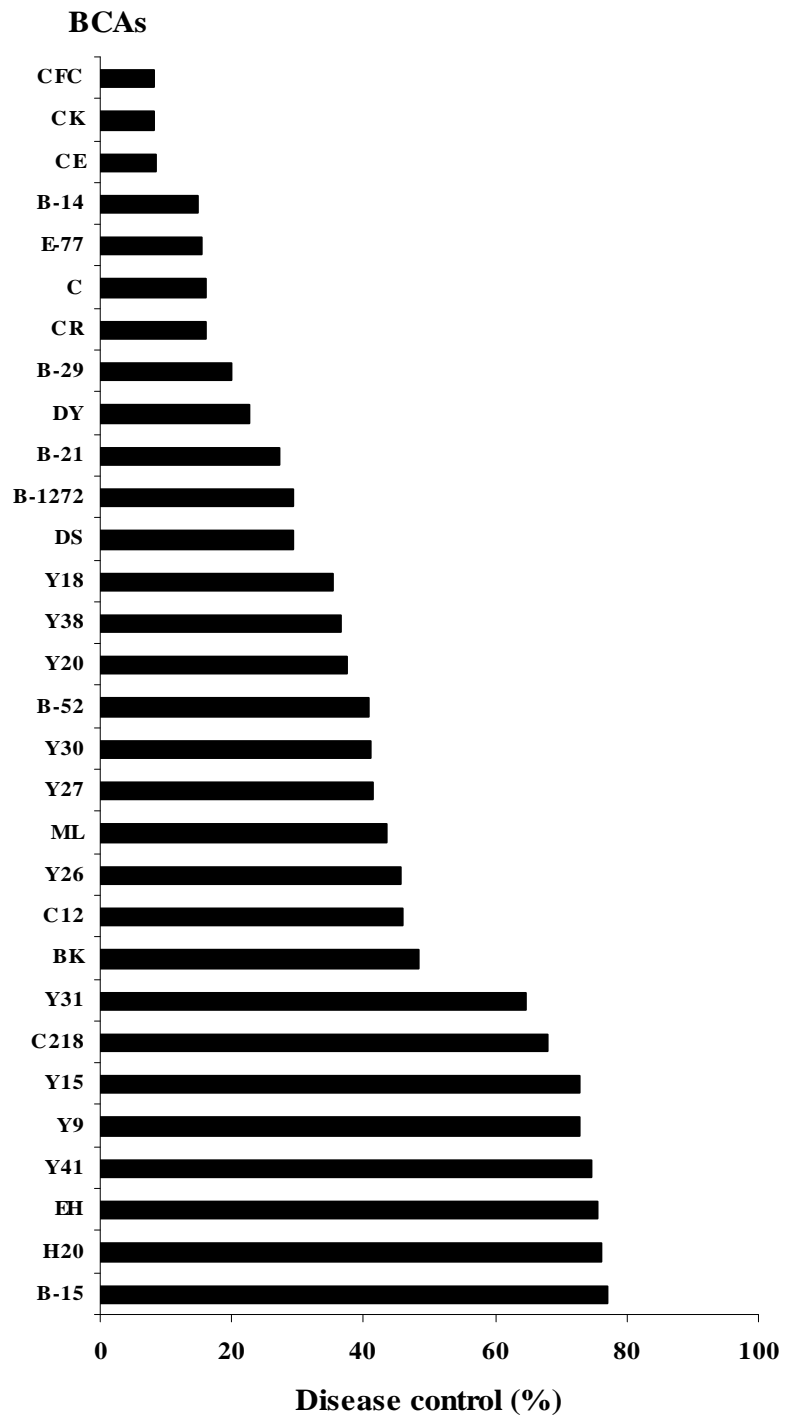
Figure 2.3 Efficacy of potential BCAs in controlling powdery mildew of zucchini after five weeks of treatment under greenhouse conditions.

BCAs
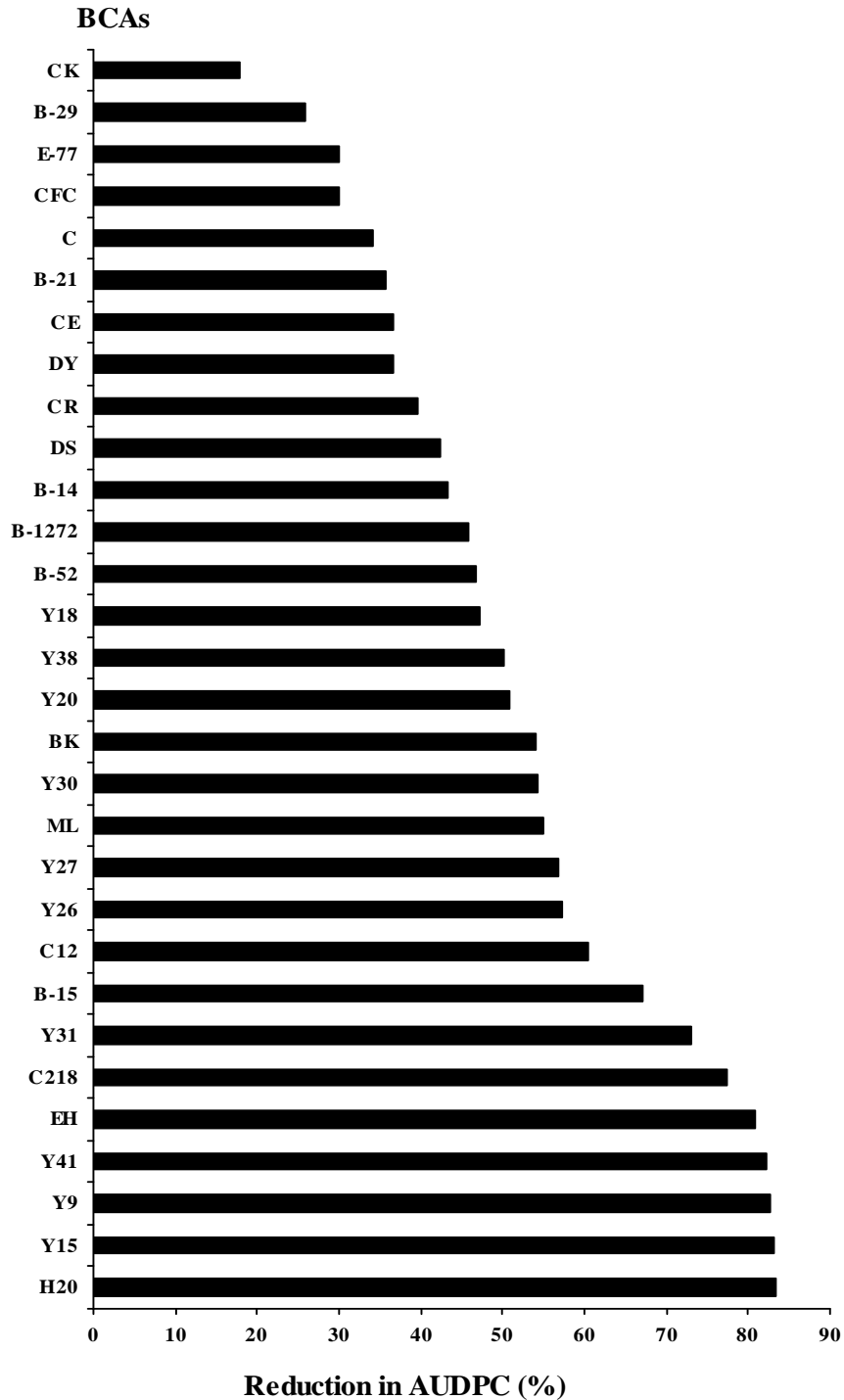
Figure 2.4 Effects of selected isolates in development of powdery mildew of zucchini represented as area under disease progress curve (AUDPC) after five weeks of treatment under greenhouse conditions.

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2.4 DISCUSSION

The development of a proper isolation and *in vitro* screening protocol that provides rapid, repeatable and reliable results is an important initial step in screening efficient antagonists for biocontrol of plant diseases. This is because the success of all subsequent stages depends on the ability of the initial screening procedure to identify appropriate candidates (Whipps *et al*., 1988). Biological control of plant diseases typically involves three components: the biocontrol agent (BCA), the pathogen and the plant itself (Nevew *et al*., 2007). If one of these components is missing, the screening process will be incomplete.

In order to be an appropriate candidate, an isolate should consistently produce a promising result under various environmental conditions. It is a common challenge during the screening process that some potential candidates show good efficacy initially, but fail to perform at later stages, or when they are tested in different environments.

During the screening process, BCAs were applied before and after inoculation of the pathogen. Application before infection was aimed at including competition as a possible mechanism of action in controlling the disease by allowing the antagonists to establish themselves before the disease. When spores or conidia of a pathogen require exogenous nutrients for germination and germ tube elongation, they are subjected to competition for these nutrients with the indigenous saprophytic microbial community (Brodie and Blackeman, 1975). However, Wilson (1997) determined that conidia of PM fungi do not require exogenous nutrients during germination, and host penetration occurs within a short period following germination, limiting the value of competition as biocontrol strategy. He concluded that suppressing the sporulation and dissemination of the pathogen using mycoparasites is the only viable approach to control PM fungi. However, other mechanisms of action such as antibiosis and induction of host resistance are important mechanisms for many antagonists. Hence, initial screening should minimize loss of potential antagonists by including every possible opportunity for all antagonists to display their potential biocontrol activity.

In most cases, the performance of biocontrol isolates on agar has less predictive value than *in vivo* tests (Verhaar *et al*., 1998). This is because biocontrol activity of all antagonists is dependant on several factors that determine the survival of the introduced microbe. For

their survival and establishment, BCAs may require the presence of their host on the phylloplane. For instance, growth of *Pseudozyma flocculosa* (Traquair, Shaw and Jarvis) Boekhout and Traquair is dependent on the presence of PM (Nevew *et al.*, 2007). This may be related to availability of nutrients from the target pathogen.

Many isolates of yeasts suppressed PM development. Often there was a clear zone around the antagonists. Yeasts are known to produce several inhibitory products (Hajilaoui *et al.*, 1994, Benyagoub *et al.*, 1996a; Urquhart and Punja, 2002). For instance, several species of *Tilletiopsis* produce ß-1,3 exo- and endo-glucanase, chitinase and antifungal compounds (Urquhart and Punja, 2002). An active fraction of ester fatty acids produced by species of this genus was reported to inhibit germ tube development of *P. xanthii* and plasmolyse its spores (Urquhart and Punja, 2002). *Pseudozyma flocculosa* secretes modified long-chain fatty acids that caused cytoplasmic granulation and collapse of PM hyphae (Hajilaoui *et al.*, 1994, Benyagoub *et al.*, 1996a). This might be the reason for the clear zone around many of the yeast isolates tested.

The formation of an inhibition zone by bacterial isolates species is believed to be the result of antibiosis or competition, through which the antagonists control the disease. A report by Romero *et al.* (2004) indicated that these two mechanisms of action are common in bacteria. Further work by these researchers showed that production of lipopeptide antibiotics such as surfactin, fengycin, and iturin A or bacillomycin, and butanolic by *Bacillis subtilis* (Ehrenberg) Cohn showed that antibiosis is the main factor in PM suppression by *Bacillus* (Romero *et al.*, 2007). Few candidates of fungal isolates showed hyperparasitism in addition to the modes of actions demonstrated by yeasts and bacterial isolates.

The screening was performed using one species of plant, zucchini (F1 hybrid), infected with a single PM fungus (*P. xanthii*). The hybrid used for this experiment is very susceptible to PM and grows upward, making it an ideal choice for this research. Although all cucurbit plants are known to be susceptible to PM (McGrath and Thomas, 1996), most of them grow horizontally which requires large growing areas and it also increases the possibility of inter-plot interference between treatments.

The approach adopted was based on the hypothesis that if an isolate can antagonize PM of this plant (i.e. zucchini), then it will probably be effective against other PM species of different plants. This approach has been used by several authors (Sztejnburg *et al*., 1989; Szentivanyi and Kiss, 2003). These groups of authors showed that *Ampelomyces quisqualis* Cesati ex Schlechtendahl isolated from a specific PM provided effective control against other PM species on different crops.

The efficacy of some of the potential antagonists used was reduced when they were tested under greenhouse conditions. This could be due to greater fluctuations of the environmental conditions of the glasshouse compared to the growth chamber, where temperature, light intensity and relative humidity were all stable. However, these factors are more variable under glasshouse conditions subjected to fluctuating daily weather conditions. In spite of such fluctuations, some isolates gave consistent results, probably due to their adaptability to environmental fluctuations.

Under both growing conditions, PM covered the whole leaf within 2wk after inoculation. This demonstrated the potential for the disease to destroy the whole plant if the environmental conditions were favourable for its development, and there were no effective control measures.

The screening procedure followed in this experiment yielded potential antagonists that can be used under glasshouse conditions where PM is prevalent, with environmental conditions that favour both the antagonists and the pathogen. Paulitz and Bélanger (2001) described glasshouses as providing conducive environmental conditions under which BCAs can operate at their best. However, to be effective, the potential BCAs must be able to establish themselves soon after their application because the development of PM is so fast in such environments. The control efficacy of these potential isolates is promising in controlling the disease. However, the level of control provided by all BCAs tested was not complete. Therefore, there is a need to enhance their efficacy with a better understanding of these BCAs, the pathogen and the host plant. The efficacy of these antagonists can also be enhanced by co-application of compatible products or adjuvants. There is also a need to test these selected isolates under field conditions, where environmental conditions are not controlled, in order to develop biofungicides that can be used for integrated disease management purposes for field crops.

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CHAPTER THREE

EFFECTS OF ADJUVANTS ON THE CONTROL OF POWDERY MILDEW OF ZUCCHINI WHEN USING FOLIAR APPLICATIONS OF SOLUBLE SILICON AND SELECTED BIOCONTROL AGENTS

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Abstract

Three adjuvants (Break-Thru® (BK), Partner® (PR) and Tween-80® (T-80)) were compared for their ability to improve the efficacy of soluble silicon (Si) applied as a foliar spray against powdery mildew of zucchini (PM). The best adjuvant was applied alone to evaluate its direct effect on the pathogen. Among the adjuvants tested, BK followed by PR, improved efficacy of Si significantly ($P < 0.05$). However, T-80 did not affect the efficacy of Si. Microscopic studies showed that BK inhibited germination of conidia, and caused collapse and disintegration of both conidia and mycelia of the pathogen. It also enhanced the deposition of BCAs on the pathogen.

The compatibility of BK was tested with selected biocontrol agents (BCAs) and zucchini at various concentrations. Biocompatibility tests showed that mixing BK (0.2-1 mℓ ℓ$^{-1}$) to nutrient broth improved the c.f.u. of Isolates Y15 and Y41 while not affecting that of Isolate B15. However, mycelial growth of fungal Isolates EH and H20 was inhibited when BK was mixed into PDA agar at concentrations of more than 0.2 mℓ ℓ$^{-1}$. Spraying BK at 0.25 mℓ ℓ$^{-1}$ was compatible with zucchini plants, but showed phytotoxic effect at more than 0.5 mℓ ℓ$^{-1}$. In the presence of BK, the efficacy of foliar spray of Si was improved by increasing the Si concentration to an optimum concentration of 750 mg ℓ$^{-1}$. Break-Thru® at concentraions of 0.2-0.4 mℓ ℓ$^{-1}$ enhanced the activity of Si sprays and three BCAs in controlling PM.

3.1 INTRODUCTION

Adjuvants are widely used with agrochemicals, with the objective of enhancing spray performance by improving the coverage, absorption and efficacy of the spray treatment (Gent *et al*., 2003). Amer *et al*. (1993) reported a 30% increase in control of powdery mildew (PM) of wheat (*Erysiphe graminis* f.sp. *tritici* Marchal) when adjuvants were mixed with fungicides. Several reports have indicated that adjuvants may have beneficial effects when applied with some BCAs (Bélanger *et al.,* 1997; Phillip *et al*., 1990; Picton and Hummer, 2003). Enhanced survival as a result of homogenous distribution of BCAs on leaves was reported when Sigma Oil, Aqua Aid and Tween-80 were mixed with a suspension of BCAs for control of PM of cucumber (Dik *et al*., 1998). Efficacy of *Sporothrix flocculosa* Traquir, Shaw and Jarvis against PM of rose was also improved when surfactants were added into the spray mix (Bélanger *et al.,* 1994). Moreover, the biocontrol activity of several yeasts against postharvest diseases of fruits and vegetables was improved by adding various adjuvants into the spray suspension (Lima *et al*., 2005)

Foliar applications of silicon (Si) to control PM have been moderately successful on a few crops (Bowen *et al*., 1992; Guével *et al*., 2007; Liang *et al*., 2006; Menzies *et al*., 1992; Palmer *et al*., 2006; Reynolds *et al*., 1996). However, there is little information about the optimum concentration of Si that can provide acceptable levels of control, or on efforts to improve its efficacy. As with agrochemicals, the efficacy of foliar sprays of Si may be improved by adding adjuvants to enhance their coverage, and retention of Si on the phylloplane, and therefore reducing the concentrations of Si required for good control of PM. Similarly, adjuvants may enhance the even distribution of BCAs on leaves, and therefore improve the level of PM control that they can provide.

Before any adjuvant may be recommended for large-scale usage, a thorough assessment on its potential risks is essential. Assessment of its direct impact on the plant, the BCA and the pathogen are needed. The use of adjuvants alone can limit development of PM by inhibiting germination of conidia and/or inhibiting its mycelial growth. For instance, mineral oil, Tween-80 and some surfactants were reported to have inhibitory effects on PM of cucumber (Dik *et al*., 1998), rose (Bélanger *et al*., 1994) and wheat (Ziv and Frederiksen, 1987). Therefore, applying such adjuvants with BCAs can inflate the perceived activity of the antagonists (Bélanger *et al.*, 1997).

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The objectives of this study were to compare three adjuvants for their capacity to improve foliar-applied soluble Si; to study the direct effect of the best adjuvant on the pathogen; to test its compatibility with the plant and selected BCAs; and to determine the optimum concentration of Si that can give acceptable level of control, when combined with the best adjuvant.

3.2 MATERIALS AND METHODS

3.2.1   Effects of adjuvants and silicon on powdery mildew of zucchini

Seedlings of zucchini were grown and inoculated with PM as described in Chapter 2. Three adjuvants were tested: Break-Thru[®] (polyether-polymethylsiloxane-copolymer), Partner 650[®] (alkoxylated fatty alkylamine polymer/ethoxylated sorbitane ester) and Tween-80[®] (polyoxyethylene 20 sorbitan monooleate). These were obtained from Universal Crop Protection[1], Degussa Africa[2] and Merck Chemicals[3], respectively.

As a source of soluble silicon (Si), potassium silicate (K_2SiO_3) (a product K2550 containig 20.5-20.9% SiO_2 and 8.0-8.15% K_2O) was obtained from PQ Silicas SA[4] and concentrations of Si was calculated according to the product's information. Break-Thru[®], Partner, and Tween-80 at a rate of 0.5, 0.5, and 0.2 mℓ ℓ$^{-1}$, respectively, were mixed with Si (at 250, 500, 750 and 1000 mg ℓ$^{-1}$) and sprayed onto leaves of infected zucchini plants until runoff. Water was used as a control. Treatments were applied once a week in the late afternoon to avoid the hottest period of the day. Percentage of leaf area covered by PM was recorded weekly for five weeks and the area under disease progress curve (AUDPC) was calculated from the disease data using an AUDPC Program (Shaner *et al.*, 1977). Analysis of variance (ANOVA) analysis was performed with GenStat[®] Statistical Analysis Software (GenStat, 2006). Comparisons between means of treatments was made using Fisher's protected least significant difference (FLSD) and effect of adjuvants on percentage reductions of final diseae level and AUDPC values were calculated by comparing the values of each treatment against that of the Untreated Control (only water). Polynomial

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[1] Universal Crop Protection (Pty) Ltd., P.O. Box 801, Kempton Park 1620, South Africa.
[2] Degussa Africa (Pty) Ltd., P.O. Box 261, Somerset West 7129, South Africa.
[3] Merck Chemicals (Pty) Ltd., 259 Davidson Rd. Wadeville 1422, Gauteng, South Africa.
[4] PQ Silicas South Africa (Pty) Ltd, 169 Tedstone Rd, P.O. Box 14016 ,Wadeville 1422, Gauteng, South Africa.

regression analysis was also performed to determine the effects of concentration of Si applied with different adjuvants at various concentrations. The adjuvant that provided best result in suppressing PM with Si was selected for further investigations.

3.2.2 Scanning electron microscop studies on the effects of Break-Thru[®] on *Podosphaera xanthii* and biocontrol agents

Two fungal (*Clonostachys rosea* (Link) Schroers, Samuels, Seifert & Gams, Isolate EH) and *Trichothecium roseum* (Pers.) Link*,* Isolate H20) and 3 isolates of *Serratia marcescens* (Bizio) (i.e., Isolates B15, Y15 and Y41) were prepared, as described in Chapter 2, and BK was added into the microbial suspension at a rate of 0.25 mℓ ℓ^{-1}. All treatments were sprayed onto diseased plants that were growing in a greenhouse, with the environmental conditions set at $25^{o}C$ and $18^{o}C$ (day and night), a photoperiod of 16 h and an RH of 65-80%. Treatments were applied weekly during the late afternoon to ensure enough humidity and a favourable temperature for the establishment of the BCAs. After 5 wk, samples were taken from the infected leaves to investigate the direct impact of BK on the pathogen and BCAs using scanning electron microscopy.

Samples of the infected leaves were cut into circles of approximately 10mm diameter and fixed overnight in 3% glutaraldehyde in cacodylate buffer (0.1 M; pH 7.0), and then dehydrated in a graded alcohol series. Specimens were critical point dried in a Hitachi HCP-2 using CO_2 as a transfusion fluid. Dried specimens were mounted on copper stubs using double-sided carbon tape. All stubs were then coated with gold-palladium in a Polaron E500 Sputter Coater and viewed with a Philips XL30 environmental scanning electron microscope (ESEM) operating at 12 KV.

3.2.3 Biocompatibility test of Break-Thru[®]

Microbial media containing BK were prepared for fungal and bacterial BCAs. A solution of BK was prepared by mixing 10 mℓ ℓ^{-1} of the adjuvant with 90mℓ of sterilized distilled water and filtered through a filter paper. Full strength Potato Dextrose Agar (PDA) was autoclaved for 15min at $121^{o}C$ and left at room temperature to cool down. When the temperature of the flask was approximately $40^{o}C$, the solution of BK was added to the

medium at concentrations of 0.02-1 mℓ ℓ$^{-1}$. The PDA-BK medium was mixed thoroughly using a magnetic stirrer, poured to Petri dishes and kept in a laminar flow for 24 h. Approximately 3-5 mm-diameter agar plugs of Isolates EH and H20, taken from the leading edge of 2wk old cultures, were plated on the medium and incubated at 25°C. After 2 wk mycelial growth of Isolate EH was measured using a ruler. However, growth of Isolate H20 was patchy when BK was added to the agar and mycelial growth was measured by estimating the percentage of the plate covered by the fungus and converting the value into a diameter. Nutrient Broth (NB) was prepared in 100 mℓ conical flasks and autoclaved at 121°C for 15 min. After the broth was cold, BK was added into the flasks at concentrations of 0.01-0.1 mℓ ℓ$^{-1}$. The three isolates of *S. marcescens* (i.e., Isolates B15, Y15 and Y41) were inoculated into the broth using a sterile loop and fermented in a shaker, set at 150 rpm at 28°C. After 48 h of incubation, cultures were serial-diluted in McCarthy bottles containing sterile distilled water and plated onto PDA. Finally, plates were incubated for 24 h at 30°C and colony-forming units (c.f.u.) were counted. Data of each BCA was analysed as Randomized Block Design using GenStat$^{®}$ Statistical Analysis Software separately (GenStat, 2006) and relationships between concentrations of BK and microbial growth were confirmed using polynomial regression analysis.

3.2.4 Phytotoxicity test of Break-Thru$^{®}$ on zucchini plants

Break-Thru$^{®}$ (0.25-2 mℓ ℓ$^{-1}$) was sprayed onto zucchini plants infected with PM once a week until runoff. After 3wk, visual assessments were made of the toxicity of BK to the plants.

3.3 RESULTS

3.3.1 Effects of three adjuvants on powdery mildew control of with silicon

All adjuvants used in this study improved spray coverage and retention of Si on zucchini leaves. When leaves were sprayed with Si that contained any of these three adjuvants, they remained wet for longer periods than leaves sprayed with Si without an adjuvant. Adding BK and PR to Si sprays gave better results, reducing disease levels by an average of 18-20%. However, T-80 did not improve the efficacy of Si sprays significantly. Application of BK and PR alone significantly reduced disease severity levels by 20% and 18%, respectively. However, spraying T-80 alone exacerbated the disease level (Table 3.1).

The concentration of Si in the spray solution had a significant impact on PM control ($P < 0.001$). Regardless of the adjuvant used, the efficacy of Si was improved as the spray concentration was increased, with 750 mg ℓ^{-1} being the optimum dosage. The effect of adjuvants and Si applied at various concentrations on severity of PM of zucchini after 5 wk of infection and the level of disease control obtained by these treatments are presented in Table 3.1 and Figure 3.1, respectively.

The effects of adjuvants on AUDPC values were highly significant ($P < 0.001$). Without Si, BK and PR reduced the AUDPC values significantly (Table 3.2). AUDPC values of Si plus BK or PR were also significantly low. Spraying Si containing BK and PR reduced the AUDPC value by 26 and 23%, respectively. Even without Si, these two adjuvants reduced AUDPC values by 20-25%. However, application of T-80 did not affect the AUDPC value significantly and the efficacy of Si was unaffected by the presence of T-80. When comparisons were made among concentrations, the lowest AUDPC values were obtained when Si was applied at 750 mg ℓ^{-1}. The AUDPC values of PM as affected by three adjuvants, applied with Si at various concentrations, are presented in Figures 3.1 & 3.2.

Table 3.1 Effects of different adjuvants sprayed with soluble silicon (Si) at various concentrations on final disease level of powdery mildew of greenhouse grown zucchini after five weeks of infection with *Podosphaera xanthii*.

Adjuvants	Si concentration (mg ℓ^{-1})				
	0	**250**	**500**	**750**	**1000**
Break-Thru®	73.3[b]	66.7[ab]	63.7[ab]	60.0[a]	61.7[ab]
Partner®	75.0[b]	65.0[ab]	70.0[ab]	58.3[a]	60.0[a]
Tween-80	92.3[c]	79.0[b]	73.3[b]	63.3[ab]	75.0[b]
Water	91.7[c]	78.3[b]	76.7[b]	76.7[b]	75.0[b]

Effects	P-Values
Adjuvants	< 0.001
Concentration	< 0.001
Adjuvants*Concentration	0.704
FLSD	11.686
CV (%)	9.9

Means followed by a common letter were not significantly different according to Fisher's protected least significant difference ($P < 0.05$).

Table 3.2 Effects of different adjuvants and soluble silicon, sprayed at various concentrations, on AUDPC values of powdery mildew of zucchini grown under glasshouse conditions after 5 weeks of infection with *Podosphaera xanthii*

Adjuvants	Si concentration (mg ℓ^{-1})				
	0	250	500	750	1000
Break-Thru®	1724[b]	1392[ab]	1416[ab]	1031[a]	1169[ab]
Partner®	1636[b]	1524[b]	1456[b]	1229[ab]	1180[ab]
Tween-80	2164[c]	1871[bc]	1787[bc]	1289[ab]	1599[b]
Water	2168[c]	1851[bc]	1735[bc]	1649[b]	1712[b]

Effects	P-Values
Adjuvants	< 0.001
Concentration	< 0.001
Adjuvants*Concentration	0.955
FLSD	420.9
CV (%)	16.2

Means followed by a common letter were not significantly different according to Fisher's protected least significant difference (P<0.05).

3.3.2 Visual and scanning electron microscopic observations on direct impact of Break-Thru® on powdery mildew

BK-treated PM colonies were small, flat and dry. Environmental scanning electron microscopic examinations revealed that the hyphae and conidia of untreated PM were turgid and smooth (Figure 3.5 A). However, these structures collapsed, disintegrated, or were bound together when treated with BK (Figure 3.5 B-D). Deposition of BCAs onto powdery mildew mycelium was also enhanced by the presence of BK in the spray (Figure 3.5 E). Enhanced germination and establishment of Isolate H20 on the pathogen was also observed (Figure 3.5 F). Propagules of all BCAs seemed to be unaffected by the adjuvants.
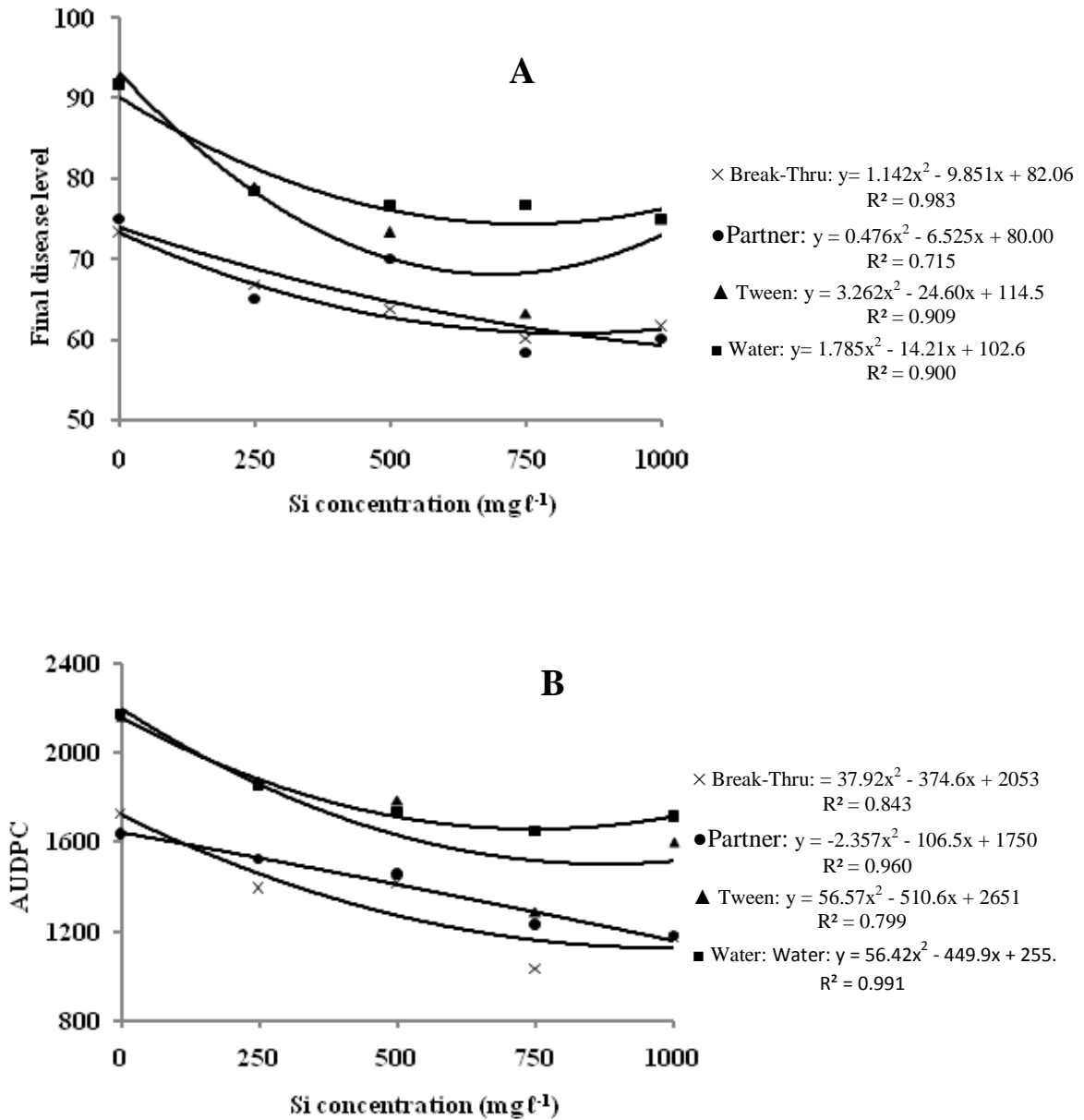
Figure 3.1 Relationships between adjuvants and Si applied at differing concentrations on severity (A) and AUDPC values (B) of powdery mildew of zucchini after five weeks of infection with *Podosphaera xanthii*.

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× Break-Thru: y = 9.308ln(x) + 20.48

R² = 0.913

● Partner: y = 10.09ln(x) + 18.53

R² = 0.766

▲ Tween 80: y = 15.54ln(x) + 1.519

R² = 0.726

■ Water: y = 10.45ln(x) + 2.990

R² = 0.817
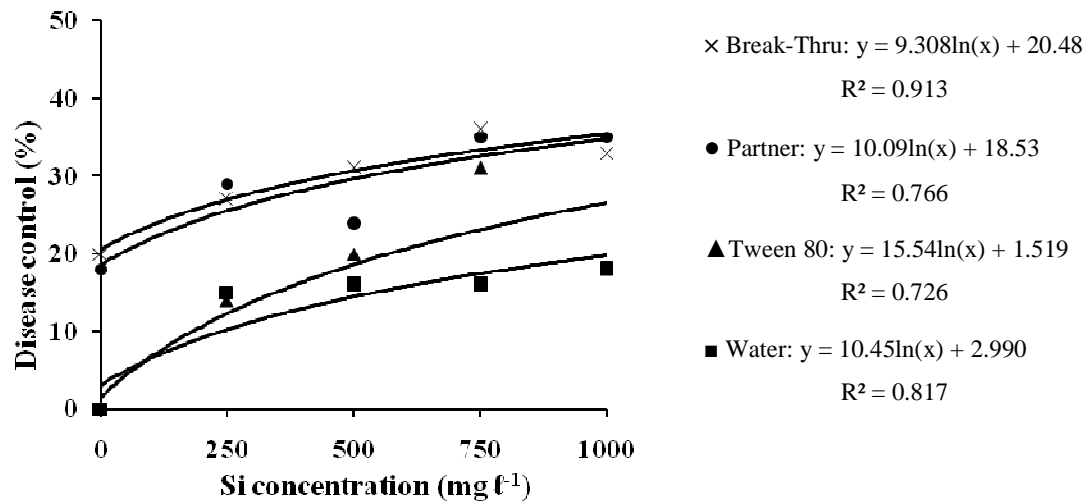
Figure 3.2 Effects of different adjuvants in controlling powdery mildew of zucchini in combination with foliar-applied Si at various concentrations.

× Break-Thru: y = 17.55ln(x) + 21.09

R² = 0.833

● Partner: y = 13.35ln(x) + 22.41

R² = 0.892

▲ Tween 80: y = 20.53ln(x) - 0.014

R² = 0.758

■ Water: y = 14.19ln(x) + 2.322

R² = 0.899
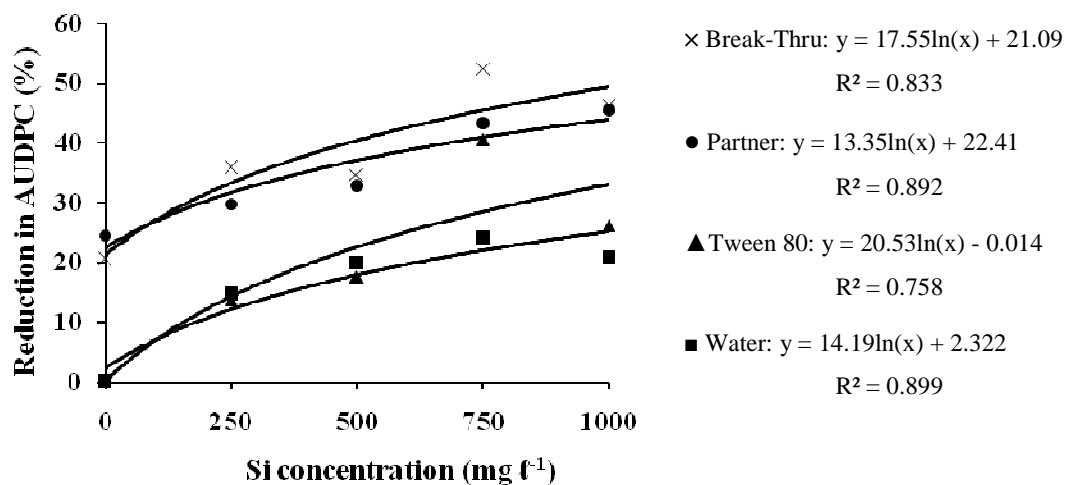
Figure 3.3 Effects of different adjuvants in suppressing development of PM of zucchini with foliar-applied Si at various concentrations after five weeks of inoculation. AUDPC values are as a % of the untreated control AUDPC value.

3.3.3 Biocompatibility of Break-Thru® with biocontrol agents

Analysis of ANOVA on the effects of BK on colony forming units of Isolates B15, Y15 and Y41 were not significant at $P < 0.05$. However, regression analysis showed that there was relationship between concentrations of BK in the medium and c.f.u. of Isolate B15, with increasing levels of BK inhibiting the c.f.u. of this isolate slightly. Adding BK at a concentration of 0.4 mℓ ℓ$^{-1}$ was optimum for growth of Isolates Y15 and Y41. The effect of BK on mycelial growth of Isolates EH and H20 was significant ($P < 0.001$) (Figure 3.4 B). Growth of these two fungal BCAs was significantly inhibited when BK was added into the medium at a concentration of 0.4 mℓ ℓ$^{-1}$. Mycelial growth of Isolates EH and H20 were reduced by 19 and 49%, respectively, when BK was mixed to PDA at 1 mℓ ℓ$^{-1}$ (Figures 3.4 and 3.5). Result of an in vitro bioassay on the effects of BK on growth of all BCAs is presented in Figure 3.5.
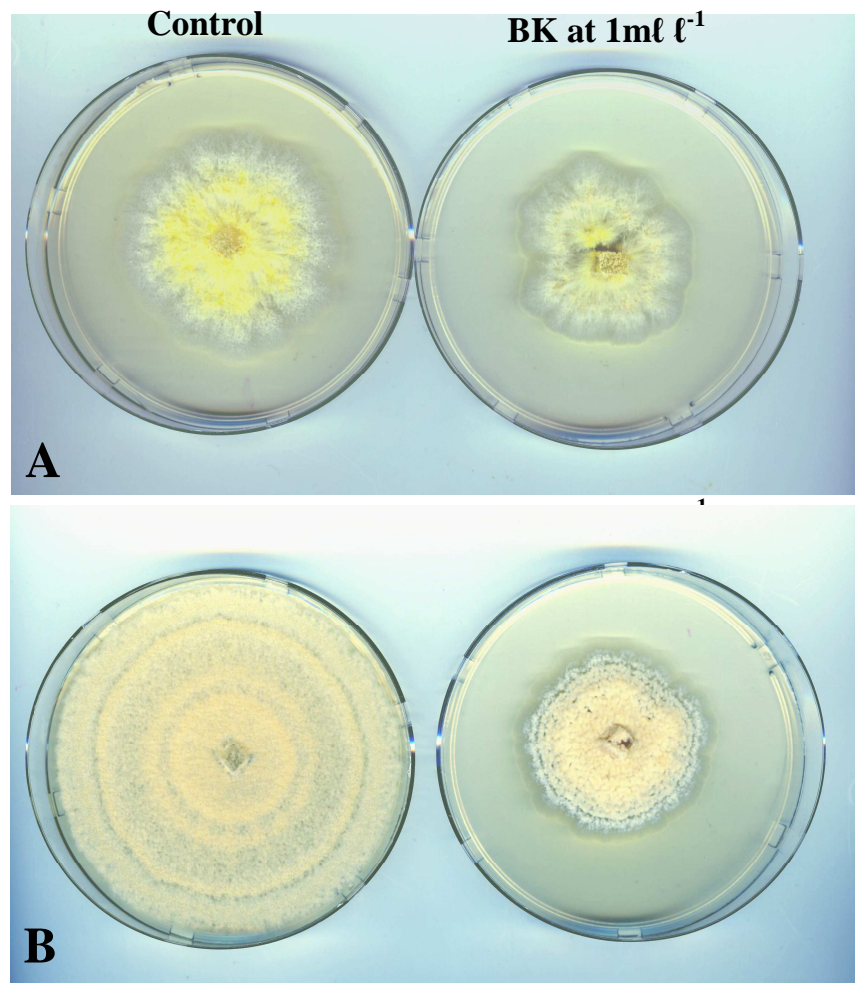
Figure 3.4 Plates of PDA showing the effects of Break-Thru® on mycelial growth of Isolates EH (A) and H20 (B).
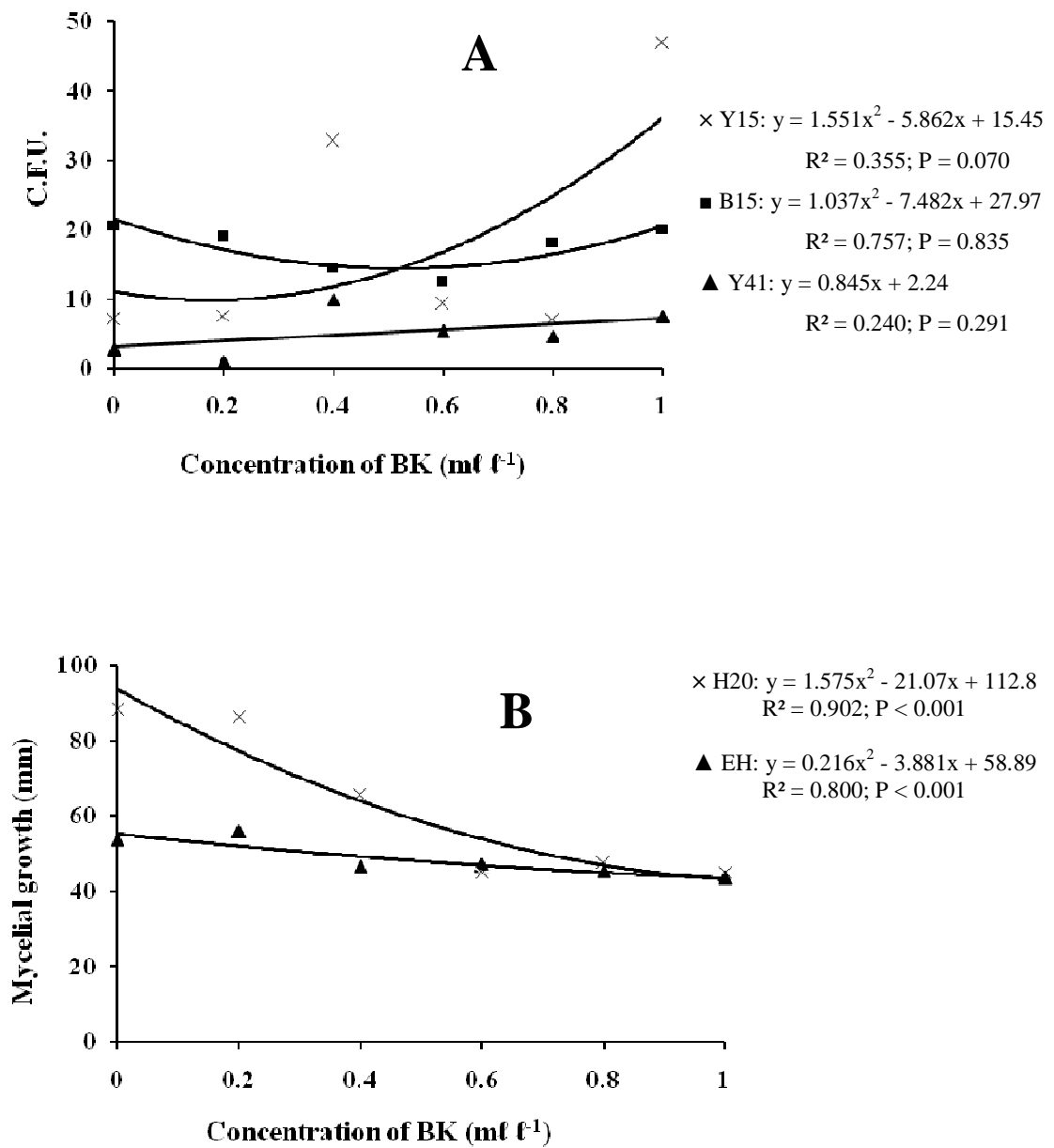
Figure 3.5 *In vitro* bioassay on the effects of Break-Thru® mixed with growth medium on growth of BCAs. A: colony forming units of Isolates B15, Y15 and Y41 after two days of incubation at 30°C. C.F.U. of Isolates B15 and Y15 were diluted by 10^{10} and Isolate Y41 by 10^{9}; B: mycelial growth of Isolates EH and H20 after two weeks of inoculation at 25°C.

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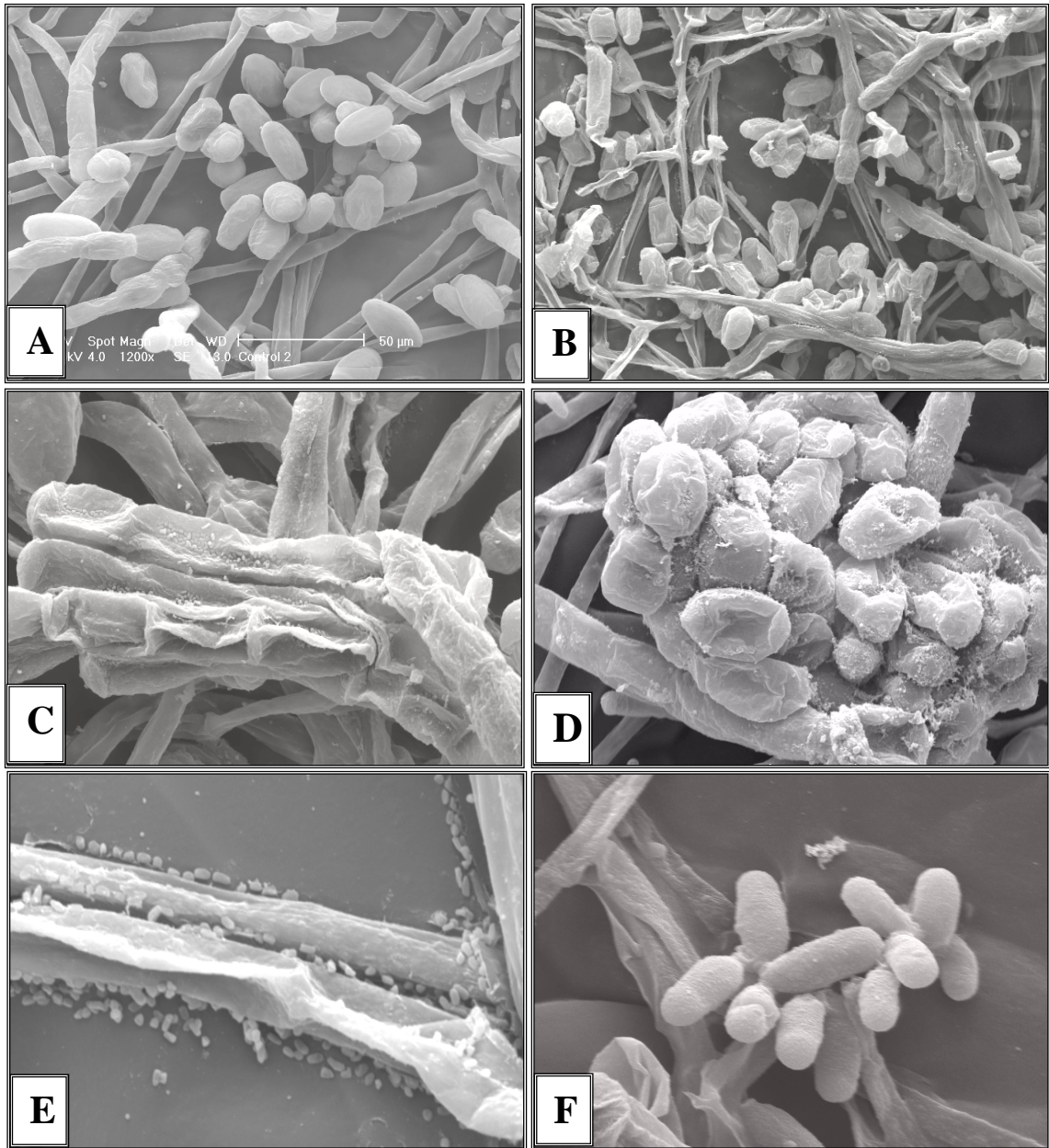
Figure 3.6 Environmental Scanning electron microscopic observations of the effects of Break-Thru® on *Podosphaera xanthii* and selected BCAs. A: Conidia and hyphae of the control; Plate 2: Collapsed and disintegrated hyphae and conidia of the fungus; C: Hyphae of the fungus sticking to each other; D: Conidia bond each other and BCAs deposited on top of the fungal structures; E: Enhanced deposition of BCAs on the surface of the pathogen; F: *Trichothecium roseum* (Isolate H20) establishing on the hyphae of the pathogen.

3.3.4 Phytotoxicity test of Break-Thru[®]

Application of BK at 0.25 mℓ ℓ$^{-1}$ provided moderate disease control with no phytotoxic effects to the plant. When BK was applied at 0.5 mℓ ℓ$^{-1}$, PM was reduced by more than 60%. At 1 mℓ ℓ$^{-1}$, BK provided complete control of PM. However, leaves of the plants showed symptoms of shrinking followed by burning when BK was applied at higher levels. Repeated application of BK, even at 0.5 mℓ ℓ$^{-1}$, caused stunting of the plant, with young leaves being malformed and reduced in size.

3.4 DISCUSSION

Selection of an effective adjuvant for an integrated disease management programme can reduce the impact of agrochemicals on the environment by reducing the quantity of active ingredient required to deliver disease control without compromising yield or quality (Kirkwood, 1993). When used properly, adjuvants are expected to have no toxic effect to the plants or the environment. However, recent studies have indicated that some adjuvants, primarily surfactants, can have direct impact on the BCAs and the target pest (Dr A. Charudattan, 2007[5], personal communication).

Although many available adjuvants provide application information on the label, each plant management project is unique and requires an assessment before the appropriate adjuvant can be selected and utilized. Adjuvants can increase the efficacy of foliar treatments by altering spray droplet sizes, distribution of sprays on the plant, viscosity (stickiness) of the sprays, their evaporation rate, the rate of uptake of agrochemicals by the target plant, and the solubility of agrochemicals in solution (Young, 2004).

In this study, some of the above properties were shown by all adjuvants used. For instance, better coverage of zucchini leaves was achieved with the same amount of Si solution or BCA suspension when BK, PR and T-80 were added into the spray mixture. Prokop and Kejklícek (2002) noted that most water soluble adjuvants improve spray coverage by reducing the percentage of small droplets (i.e., < 75 μm) which are the major contributors to off-target drift. Longer retention of the spray on the surface of the leaves was assumed

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to be the result of increase in viscosity of the spray solution by these adjuvants. When any of these three adjuvants were mixed in the spray solution, they formed foam. Formation of foam is reported to control drifts, making the spray application more effective and economical (Young, 2004).

At higher dosages (> 0.5 mℓ ℓ^{-1}), BK was shown to control PM effectively on its own, but it had phytotoxic effects on zucchini. Plants that were sprayed with BK at higher levels frequently showed stunted growth with malformed leaves. Young leaves were more sensitive to this adjuvant. Spraying BK at a concentration of 0.25 mℓ ℓ^{-1} provided moderate disease control without affecting the plant negatively. Both BK and PR showed promising results in reducing PM of zucchini when they were sprayed alone or together with Si. Addition of BK and PR improved the efficacy of Si by improving distribution of the Si sprays, and by suppressing the pathogen directly. Foliar application of Si without adjuvants also resulted in significantly lower disease values, especially when used at higher concentrations. Regardless of the adjuvant used, the efficacy of Si was increased as concentration increased up to 750 mg ℓ^{-1}.

The addition of T-80 into spray mixtures was reported to have an inhibitory effect on PM fungi (Bélanger *et al*., 1994; Dik *et al*., 1998; Ziv and Frederiksen, 1987) and to have enhanced the efficacy of *B. amyloliquefaciens* Ribonuclease (Barnase) as a BCA by improving the distribution of the antagonist on the surface of the plant (Chiou and Wu, 2003). Dik *et al*. (1998) also reported increased control of PM by *A. quisqualis* Ces ex Schlect when T-80 was mixed in the spray suspension. In this study, T-80 improved spray efficiency, but did not affect the disease directly. The size and shape of the PM colonies were not apparently affected when sprayed with T-80. Even when applied together with Si, the colonies of PM were large and fluffy, similar to colonies of the control treatment. In some cases, the disease severity and AUDPC value of treatments containing T-80 were even higher than treatments without any adjuvant. The reason for the failure of T-80 to have any impact on the disease is not known. Although each adjuvant was applied based on recommendations from previous studies and their registration labels, the concentration of T-80 was much less than that of both BK and PR. Therefore, the concentration of T-80

used might not have been strong enough to affect the disease or the pathogen might have been resistant towards this adjuvant.

Colonies of PM treated with BK were small, flat and dry. Further investigations with ESEM revealed that the disease suppression shown by BK resulted from the direct impact of the adjuvant on the pathogen. Conidia of PM were less in number and mycelial growth of the PM fungus was limited after treatment with BK. In addition, conidia and hyphae of the pathogen collapsed and disintegrated as a result of direct contact with BK. It is probable that this impact is related to the chemical properties of the adjuvant. The adhesive properties of BK also forced conidia and hyphae of the PM fungus to stick to each other, restricting spread of conidia and expansion of its colonies Moreover, mixing BK with suspensions of BCAs resulted in increased deposition of these antagonists on the pathogen, resulting in improved control of PM. When used at an appropriate rate, BK has the potential to improve the efficacy of BCA antagonists significantly.

A major limitation to the effective use of BCAs and Si against PM has been due to the rate of disease development and spread. The direct impact of BK on the pathogen therefore gives a novel opportunity to enhance the management of PM with BCAs and Si. It is conceivable that BK would provide a short-term control function, followed by the combined effects of Si and a BCA to provide medium to long-term disease control.

Growth of Isolate EH and Isolate H20 was inhibited when BK was added to PDA at concentration of 0.4 mℓ ℓ^{-1}. In contrast, adding BK into NB at 0.2-1 mℓ ℓ^{-1} had little effect on the c.f.u. of isolates of *S. marcescens*. Based on the biocompatibility and phyto-toxicity tests, it is recommended that for effective control of PM combined with BCAs or Si, BK must be used at a concentration range of 0.2-0.4 mℓ ℓ^{-1}, depending on the sensitivity of the plant towards the adjuvant. During the course of disease control, repeated application may result in an accumulation of BK on the phylloplane, which may have adverse effect on the plant or the BCAs. To avoid such risks, further investigations on the impacts of BK deposition on the plant and antagonists is needed before the recommended rate is adopted for implementation. In addition, direct contact between the adjuvant and the BCA in target may affect the antagonistic properties of the BCAs. Therefore, more *in vitro* research is

needed to determine effects of BK on the metabolic properties of the BCA isolates. Moreover, although most adjuvants are considered to have no pesticidal properties and are exempted from regulations of U.S. Environmental Protection Agency (Hock, 1998), investigation is needed on the hazards that BK may pose to consumers and beneficial microbial inhabitants of the phylloplane.

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CHAPTER FOUR

EFFECTS OF CONCENTRATION, FREQUENCY OF APPLICATION AND RUNOFF OF FOLIAR-APPLIED SILICON ON POWDERY MILDEW OF ZUCCHINI

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Abstract

The effect of concentration, frequency of application and runoff of foliar-applied soluble silicon (Si) on the severity of powdery mildew (PM) (*Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*) of zucchini (*Cucurbita pepo* L.) was evaluated. Soluble Si (250-1000 mg ℓ^{-1}), together with Break-Thru® (0.25 mℓ ℓ^{-1}), was sprayed onto zucchini plants at frequencies of 1-3 wk^{-1}. The leaves of all plants were inoculated with a known concentration of conidia of *P. xanthii* 2 d after the sprays were applied. The effect of runoff was determined by covering some of the pots with polyethylene sheets, while others were left open.

Spraying Si onto zucchini crops reduced the severity of PM significantly. The efficacy of Si was improved by increasing the spray frequency. However, concentration of Si did not have a major impact on the efficacy of Si. Regardless of the concentration of Si, the best results were obtained when the frequency of the treatment was increased, and when Si was allowed to reach the root zone of the plants.

When Si was applied onto leaves, direct contact between the spray and the pathogen seemed to be the main mechanism of action involved in disease control, and part of the spray (i.e., the runoff) was absorbed by the 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 hydroponic crops or supplied from overhead irrigation schemes.

4.1 INTRODUCTION

Powdery mildew (PM) of many crops causes significant losses in yield if not managed properly (Romero *et al*., 2003 & 2004). Soluble silicon (Si) has been investigated by many authors (Bélanger *et al*., 1995 & 1997; Epstein, 1994 & 1999; Liang *et al*., 2005) as an environmentally safe option for the management of this disease. These researchers have demonstrated that effective control of PM can be achieved by the application of soluble Si at appropriate dosages and frequencies.

Foliar applications of Si have shown promising results in controlling PM of several crops (Bowen *et al*., 1992; Guével *et al*., 2007; Liang *et al*., 2005; Menzies *et al*., 1992; Palmer *et al*., 2006; Reynolds *et al*., 1996). These authors have showed that it is possible to replace or supplement the fungicides being used against PM with soluble Si sprays. The level of disease control that can be achieved using this approach is, however, limited due to a lack of information on the optimum concentration. Efficacy of Si can also be improved by increasing the frequency of application as this increases the deposition of the element on the phylloplane.

Furthermore, when a foliar spray is used, there is always runoff which is intercepted by the soil. If the Si treatment is absorbed by the roots, as a quasi-essential element, it may play a role in the health of the plant. Therefore, to assess the direct effect of foliar application of Si on PM, it is important to differentiate the impact of runoff from the total effect of the treatment. Understanding the effect of such components can determine whether foliar applications of Si should be more or less effective than root drenches.

The objectives of this study were to determine the effects of foliar applied Si on the severity of PM, determine the optimum concentration and application frequencies of Si on PM, and study the impact of runoff on the efficacy of foliar sprays of soluble Si.

4.2 MATERIALS AND METHODS

4.2.1   Preparation of plants

Seeds of zucchini (*Cucurbita pepo*, F1-Hybrid Partenon) were planted in seedling trays and kept in a greenhouse at a temperature of 26-28°C and relative humidity of 75-85%. Trays were irrigated with a complete fertilizer [Ocean Agriculture[1] 3:1:3 (38) at 0.5 g ℓ^{-1}] + [Ca(NO$_3$)$_2$ at 0.5 g ℓ^{-1}] for 2 wk using overhead irrigation. Once the germinated seedlings had fully developed a second leaf, they were transplanted into pots filled with composted pine bark and transferred to another glasshouse set at 26-28°C, 75-85% RH. The plants were irrigated with the same nutrient solution using drip irrigation until the end of the experiment.

4.2.2   Preparation of *Podosphaera xanthii* for inoculation

Inoculum of PM was obtained from plants that were inoculated with previously collected samples of natural inoculum. Conidia of PM were harvested using the same technique, as described in Chapters 2-3, and counted using a haemocytometer. Finally, seedlings were inoculated by spraying 3-5 mℓ of conidial suspensions of PM (10^3 conidia mℓ^{-1}) onto the leaves of each seedling using a hand sprayer within two hours of counting. Inoculation was done 2 d after Si was sprayed onto plants.

4.2.3   Application of soluble silicon

Soluble Si was sprayed onto zucchini plants in the form of KSi at four different concentrations (250, 500, 750 and 1000 mg ℓ^{-1}) until runoff. Treatments were applied at a frequency of 1-3 wk^{-1}. Impact of Si spray that is absorbed by the roots on disease level was assessed by controlling movement of Si onto the roots. The lower part of the plants and their pots were sealed with polyethylene sheets cut and taped into place to provide a waterproof barrier to stop any Si spray from reaching the root zone of the plants, in the form of runoff and drift,. The aerial parts of the plants were left open for spray. Other pots were left uncovered, allowing the drift and runoff of spray applications to reach to the rhizosphere and to be taken up by the roots.

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[1] Ocean Agriculture (Pty) Ltd., P. O. Box 742, Muldersdrift, 1747, South Africa.

For all treatments, Break-Thru® (BK) was used as a wetter at concentration of 0.25 mℓ ℓ$^{-1}$. Distilled water containing only BK was sprayed at the same frequencies as the control treatment. All the treatments were applied in the late afternoon in order to minimize dryness of the leaves due to heat. Disease levels, as percentage of leaf area infected, were evaluated 3 wk after inoculation.

4.2.4   Disease assessment and statistical analysis

The experiment was conducted twice, with each treatment having three replications. The treatments were arranged in a factorial randomized complete block design. Analysis of Variance (ANOVA) were performed using a factorial treatment structure. Where the CV % was > 20%, the original data was transformed using a square root transformation. Interactive effects of application concentration, frequency and runoff of Si on the severity of PM was analysed using GenStat® Statistical Analysis Software (GenStat, 2006). Treatment means were separated using Fisher's protected least significant difference (LSD). The percentage of disease reduction by each treatment was calculated by taking the final disease of the Untreated Contarol as a reference. Finally, polynomial regression analysis was performed on the percentage of disease reduced by different levels of Si applied on open or covered pots at 3 different frequencies.

4.3 RESULTS

In both experiments, application of Si reduced the disease severity significantly. The effect of concentration on the efficacy of soluble Si sprays was not significant (Table 4.1 and 4.2). After spraying the plants with Si at various concentrations (250-1000 mg ℓ$^{-1}$) for 3 wk, disease severity of the control treatment was reduced by 42-61% and 75-79% in the first and second trials, respectively. In both experiment, extra Si had little or no added effect on the efficacy of the spray. When the effect of Si at different concentrations on severity of PM was compared, there was no significant difference between the values.

In both trials, spray frequency had a significant effect on the efficacy of Si in controlling PM. Using the same concentration of Si, efficacy of the treatment was increased initially by 30% and almost doubled in the subsequent experiment when the spray frequency was

tripled. Even in the control treatment which contained only BK, the disease level was reduced when the treatment was sprayed at higher frequency (Figures 4.1 & 4.2).

A comparison of PM severity between open and covered pots showed that drift and runoff of foliar-applied Si had a significant impact on disease control (Tables 4.1). In both trials, efficacy was improved significantly when Si was sprayed onto plants in open pots. An overall increase of 17% (Trial 2) and 18% (Trial 1) in disease reduction occurred on plants in open pots, where Si was allowed to reach the rhizosphere, compared to the sealed pots where the spray was restricted to the phylloplane (Figure 4.2).

In both trials, the interaction of Si concentration and frequency was significant. Interactive effects of concentration and frequency on the efficacy of Si was significant in the second experiment but not in the first experiment. However, trends of these two experiments showed that the best results were obtained when Si was used at higher concentrations with higher frequencies (i.e., 3 times per week). For instance, when Si was applied 3 times a week at 1000 mg ℓ^{-1}, then its efficacy was increased by more than two - three times (Figures 4.1 & 4.2).

Interactive effects of Si concentration and application method were not significant on the efficacy of Si. However, improved efficacy was obtained when treatments with higher levels of Si were sprayed on plants in open pots. Interaction of frequency and runoff on the efficacy of Si was not significant. In both trials, efficacy of Si was improved when sprayed onto plants in open pots at higher frequencies. Increasing the frequency from 1 to 3 times per week increased the efficacy of the treatment by a mean of 35% in the first experiment and nearly 100% in the second experiment. Trends of the two trials also showed that the increase in efficacy was slightly higher when sprays were applied to plants in the open pots.

Interaction of concentration, frequencies and application method was only significant on the second experiment. However, individual observations showed that the best results were obtained when Si was sprayed at higher frequencies to plants in open pots. Effects of Si at various concentrations sprayed onto plants in open and sealed pots, at different frequencies, in controlling PM are presented on Figures 4.1 & 4.2.

Trial 1

Trial 2
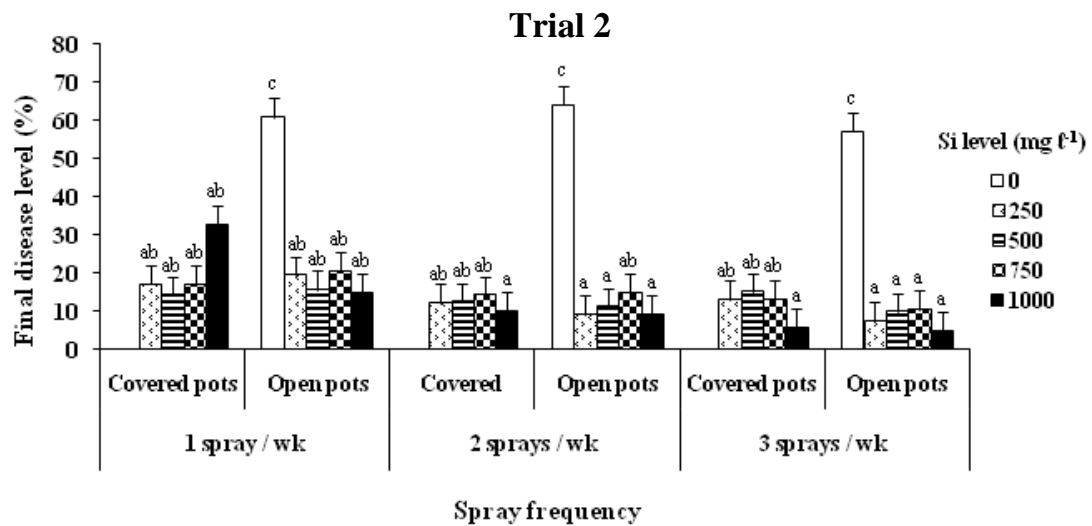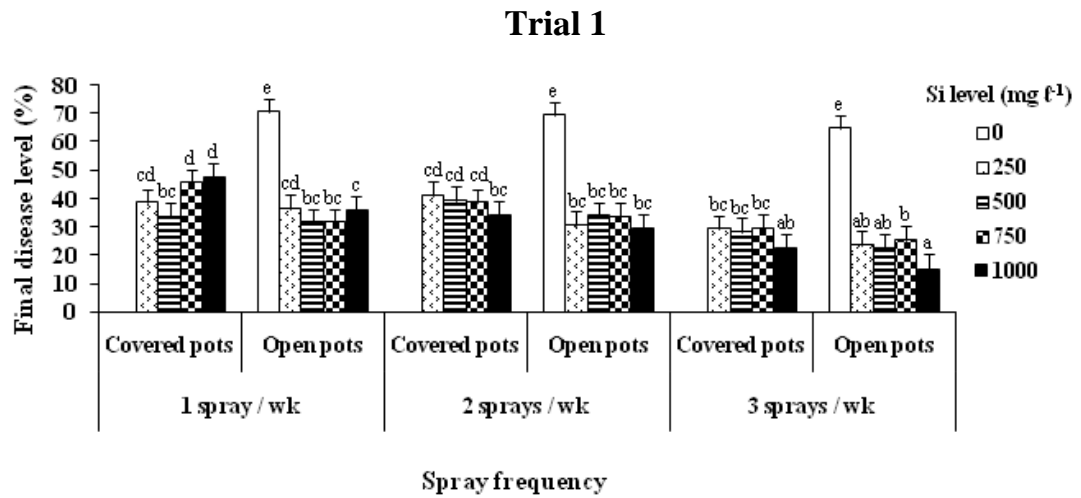
Figure 4.1 Histograms showing the effects of spraying Si on the severity of powdery mildew of zucchini, when applied at various concentration and frequencies on plants grown under greenhouse conditions in open or covered pots in two different trials.

Covered pots

Open pots

Trial 1

Spray frequencies

3 / wk (▲): $y = 2.199x^2 - 8.252x + 61.77$
$R^2 = 0.815$

2/wk (×): $y = 0.844x^2 - 1.176x + 41.17$
$R^2 = 0.972$

1 / wk (■): $y = -2.488x^2 + 6.896x + 43.01$
$R^2 = 0.707$

Spray frequencies

3 / wk (▲): $y = 3.497x^2 - 14.22x + 75.78$
$R^2 = 0.745$

2 / wk (×): $y = 2.536x^2 - 12.34x + 65.82$
$R^2 = 0.999$

1 / wk (■): $y = -3.320x^2 + 16.88x + 34.82$
$R^2 = 0.999$

Trial 2

Spray frequencies

3 / wk (▲): $y = 4.092x^2 - 16.25x + 89.46$
$R^2 = 0.998$

2 / wk (×): $y = 1.691x^2 - 7.780x + 87.88$
$R^2 = 0.696$

1 / wk (■): $y = -7.689x^2 + 30.20x + 48.92$
$R^2 = 0.989$

Spray frequencies

3 / wk (▲): $y = 3.508x^2 - 16.26x + 100.3$
$R^2 = 0.945$

2 / wk (×): $y = 2.867x^2 - 15.06x + 98.96$
$R^2 = 0.718$

1 / wk (■): $y = 0.823x^2 - 2.583x + 71.45$
$R^2 = 0.235$

Figure 4.2 Relationship between Si concentrations and frequency of sprays applied in open or covered pots on PM control of zucchini plants grown under glasshouse conditions in two different trials.

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Table 6.1 Analysis of variance showing factorial interactions between concentration, frequency and runoff of foliar-applied silicon on severity of powdery mildew of zucchini.

Source of variation	Trial 1		Trial 2	
	F-value	P-value	F-value	P-value
Concentration	1.03	0.389	0.84	0.481
Frequency	32.56	< 0.001***	25.89	< 0.001***
Runoff	21.86	< 0.001***	5.19	0.027*
Concentration*Frequency	2.26	0.053	3.54	0.006**
Concentration * Runoff	0.33	0.805	1.84	0.153
Frequency*Runoff	0.13	0.877	0.48	0.621
Concentration * Frequency * Runoff	0.90	0.503	3.06	0.013*
CV (%)	18.4		24.42	

Values with *= significant at $P < 0.05$; **= significant at $P < 0.01$; *** = significant at $P < 0.001$

4.4 DISCUSSION

In this study, spraying soluble Si onto the leaves of zucchini reduced the severity of PM significantly. This confirmed the findings of previous research on the use of Si sprays for the control of PM of cucumber (Menzies *et al*., 1992; Liang *et al*., 2005), grape (Bowen *et al*., 1992; Reynolds *et al*., 1996), muskmelon (Menzies *et al*., 1992), strawberry (Kanto *et al*., 2004; Palmer *et al*., 2006), wheat (Guével *et al*., 2007) and zucchini (Menzies *et al*., 1992).

In most cases, foliar applications of Si provides less disease control compared to when the element is fed to the plant in a nutrient solution or as a soil amendment (Guével *et al*., 2007; Liang *et al*., 2005). However, in some plants foliar application provides better efficacy than supplying the element to the roots. For instance, Bowen *et al*. (1992) found that spraying Si at 1000mg ℓ^{-1} onto grape leaves reduced the severity of PM significantly; whereas treating the plant with a Si-amended solution did not. This was considered to be because foliar application allows Si to be applied to non-hydroponic crops or crops that are unable to transport Si from their root to shoots through their vascular system, (Bélanger *et al*., 1995).

When Si is applied as a foliar treatment, its mode of action in reducing severity of PM may be different from when it is fed through the roots (Wang and Galletta, 1998). Spraying Si on cucumber can result in the formation of a coating (film) on the leaves (Menzies *et al.*, 1992), which would act as a physical barrier, preventing the penetration of fungal hyphae into the host (Bowen *et al.*, 1992). Guével *et al.* (2007) showed that foliar application of Si has a direct effect on PM over and above any effects mediated by the plant, as results from root amendments which may lead to induced resistance. A direct action by Si spays on PM was confirmed by our results when Si was sprayed onto plants where the element was restricted from reaching the roots of the plants. When KSi is used against PM, the active ingredient of the spray appears to be Si (Menzies *et al.*, 1992).

Results of this study showed that in the range of 250-1000 mg ℓ^{-1}, the level of Si in the spray solution had little impact on the efficacy of Si in PM control. Severity of PM was significantly reduced by all treatments, indicating that application of Si at lower concentrations could provide the optimum disease control while minimizing the cost of control. Observations by Menzies *et al.* (1992) on the use of Si against PM of different hosts showed that the efficacy of a foliar spray of Si at 1000 mg ℓ^{-1} was equivalent to a solution of 100 mg ℓ^{-1} applied as a soil amendment.

In this study, the efficacy of Si was improved by increasing the spray frequency. When the application frequency was increased from 1 to 3 times per week, efficacy of Si was doubled. This agrees with a report of Reynolds *et al.* (1996), who increased the efficacy of Si sprays against PM of grape (*Uncinula necator* (Schwein.) Burrill) by increasing spray frequency. Increasing application frequencies could increase the total amount of Si deposited on the surface of the leaf, resulting in better efficacy. Spraying Si at a concentration of 250 mg ℓ^{-1} with a frequency of 3 times per week could be expected to result in a lower amount of Si being deposited on the leaf than if it was applied once a week at a concentration of 1000 mg ℓ^{-1}. However, this study showed that spraying Si at 250 mg ℓ^{-1} x 3 x wk^{-1} was more effective than spraying 1000 mg ℓ^{-1} x 1 x wk^{-1}. Even for the adjacent treatment, which did not contain Si, disease severity was reduced by increasing the frequency of spray treatment. Therefore, when spray frequency is increased, there must be other factors that are involved in improving the efficacy of Si other than the total amount of the element deposited on the phylloplane. The wetting agent (BK) and increased leaf wetness could have their own impact on the pathogen. Break-Thru [®] was

shown to have a direct effect on PM restricting expansion of its colonies and collapsing conidia and hyphae of the pathogen (Chapter 3). Although extended periods of leaf wetness can favour infection of zucchini by several foliar pathogens, it has a negative effect on the development of PM by inhibiting germination of the conidia (Bushnell and Rowell, 1967; Quinn and Powell, 1982; Sakurai and Hirata, 1959). Most importantly, increasing the frequency of applications could improve the continuity of Si supply to plants roots through runoff. Even though the major factors remain unknown, this study demonstrated that better disease control could be achieved by spraying Si at lower concentrations with increased spray frequencies instead of applying higher concentrations of Si at lower frequencies.

When the same concentration of Si was sprayed at the same frequencies per week, the severity of PM recorded from plants grown in uncovered pots was less than that of plants grown in covered pots. This was because, in open pots, part of the spray solution was intercepted by the soil as a result of drift and runoff. Once the Si solution was in the rhizosphere of the plant, it could be absorbed by the roots and translocated to different parts of the plant. Reviews by Epstein (1994, 1995 & 2001) showed that adding Si into the nutrient solution or adding it to the soil benefits plants by providing protection against pathogens through physical barriers and triggering plant resistance against pathogens, and ameliorating other biotic and abiotic stresses of the plant. However, when KSi is sprayed onto the leaf, it may be deposited on the surface of the leaf without penetrating into the plant (Buck *et al*., 2008). Therefore, the possible mechanisms of protection it provides might have been through direct contact with the pathogen and alteration of the chemical properties of the leaf such as pH, which could lead to changes in osmotic properties of the leaf surface (Liang *et al*., 2005). Using scanning electron micrographs, Bowen *et al*. (1992) observed an active accumulation of Si around the appressoria of the PM fungi when Si was sprayed onto leaves of cucumber plants. However, Reynolds *et al*. (1996) suggested that exogenously applied silicates act to augment the activity of their endogenous counterparts, resulting in the accumulation of the element around the pathogen.

In most research conducted on the management of PM using foliar-applied Si, the effects of the drift or runoff of soluble Si on the disease has not been considered. However, as seen in this study, such drift and runoff can have a significant impact on the health of the plant and give a confounded conclusion because different modes of action can be involved, once the element is absorbed by the plant via its roots. For instance, foliar application of Si did not reduce severity of PM of strawberry (Palmer *et al*., 2006), but enhanced growth of the plant by increasing its chlorophyll content (Wang and Galletta, 1998). Therefore, such metabolic changes of the plant might be related to the absorption of Si through the roots as a result of drift and runoff of foliar-applied Si.

In this study, the plants were irrigated using drip irrigation, avoiding any wash-off of Si from leaves to the soil. However, due to size of the growing area and other technical limitations, most growers prefer overhead irrigation than drip irrigation. In such cases, Si can be mixed with the nutrient solution and supplied to the plant as part of the irrigation. Using that technique could improve the efficacy and minimize/avoid the costs of labour that would be involved in spraying of the element three times a week to control the disease. This technique may improve the efficacy of the treatment by increasing the spray coverage, and ensuring a better contact between the spray and the pathogen. If proven economical, the foliar use of Si at appropriate application intervals and concentrations could replace fungicides for the management of PM on zucchini, a welcome development for organic farmers.

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CHAPTER FIVE

STUDIES ON THE EFFECTS OF SELECTED BIOCONTROL AGENTS AND SOLUBLE SILICON ON THE DEVELOPMENT OF POWDERY MILDEW OF ZUCCHINI, UNDER GREENHOUSE CONDITIONS

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Abstract

Duplicate trials were conducted under greenhouse conditions to evaluate the effects of five potential biocontrol agents and soluble silicon (Si) for the control of powdery mildew of zucchini caused by *Podosphaera xanthii*. Biocontrol agents were sprayed onto leaves with a wetter (Break-Thru®), and Si was drenched onto zucchini roots (250mℓ of K_2SiO_3 at 100mg ℓ^{-1}, applied weekly). All five BCAs provided significant control of PM, whether Si was drenched to the roots or not. The effects of Si applied alone on disease severity and AUDPC were significant, reducing them by 23% and 32%, respectively. Application of Si improved the efficacy of most BCAs significantly. Of the five BCAs, the fungi provided better control of PM than the bacterial isolates, reducing disease levels by up to 90%. Higher disease pressure reduced the efficacy of Si against PM but, did not affect the performance of BCAs.

5.1 INTRODUCTION

Powdery mildews (PM) are prime targets for biocontrol agents (BCAs) because they are superficial pathogens, and are therefore accessible to external agencies. As a result, several biocontrol studies have been conducted to control PM under greenhouse conditions (Dik *et al*., 1998; Elad *et al*., 1998; Hijwegen, 1992; Verhaar *et al*., 1993, 1996). Controlled environmental variables of greenhouses provide optimum environmental conditions for the establishment and survival of antagonists. Most antagonists of PM require high humidity, making greenhouses an ideal environment for implementation of biocontrol.

Ampelomyces quisqualis Ces. (Pertot *et al*., 2008; Sundheim, 1982), *Bacillus subtilis* (Ehrenberg) Cohn (Keinath and DuBose, 2004), *Lecanicillium lecanii* (Zimm.) Zare & W. Gams (syn. *Verticillium lecanii* (Zimm.) Viegas) (Askary *et al*., 1997; Miller *et al*., 2004; Verhaar *et al*., 1993; 1996), *Sporothrix flocculosa* Traquir, Shaw & Jarvis (syn. *Pseudozyma flocculosa*) (Bélanger *et al*., 1994; Jarvis *et al*., 1989; Hajlaoui and Bélanger, 1991), *Tilletiopsis* spp. (Urquhart *et al*., 1994; Hijwegen, 1992), and *Trichoderma harzianum* Rifai (Elad, 2000; Elad *et al*., 1998; Pertot *et al*., 2008) have all shown moderate to good disease control of PM when tested in greenhouses. Some of these antagonists have been commercialized. For instance, *A. quisqualis* strain AQ10 was marketed as AQBiofungicide (Elad *et al*., 1998), *B. subtilis* Strain QST 713 as Serenade[TM] (Ngugi *et al*., 2005) and *T. harzianum* strain T39 as TRIXODEX[TM] (Elad *et al*., 1998).

Low humidity levels have been the major factor inhibiting the use of BCAs against PM on field crops. As a result, biocontrol of this disease has been limited to greenhouse conditions, where humidity is not a limiting factor. To overcome this limit under field conditions, scientists have tried amending the BCAs with adjuvants or oils (Bélanger *et al*., 1994; Philipp *et al*., 1990).

The efficacy of BCAs depends on the climatic conditions of the crop. Powdery mildew can thrive under dry conditions, whereas most BCAs need relative humidity above 70% (Hajlaoui and Bélanger, 1991). Furthermore, the rate of development of PM may influence the level of control of PM by BCAs, especially in the case of hyperparasites. This means that the efficacy of BCAs may differ from season to season, from cultivar to cultivar, and may be influenced by other control measures such as fungicides.

Use of soluble silicon (Si) in PM management has been studied under controlled conditions with some degree of success (Bélanger *et al*., 1995; Menzies *et al*., 1991b; Samuels *et al*., 1991a & 1994). Both BCAs and Si have drawn considerable attention because they are considered as environmentally friendly. However, the level of disease control obtained from each control options is often incomplete, raising the possibility of using them together in order to supplement each other.

The objective of this study was to compare the efficacy of five selected BCAs against PM of zucchini (caused by *Podosphaera xanthii* (Castagne)), to determine the efficacy of Si in controlling PM and improving efficacy of BCAs by combining them with this element.

5.2 MATERIALS AND METHODS

5.2.1 Preparion of plants and inoculation with *Podosphaera xanthii*

Seedlings of zucchini (*Cucurbita pepo* L., F1-Hybrid Partenon), were raised in a greenhouse operating at a temperature of 26-28°C and relative humidity (RH) of 75-85%. After producing two fully developed leaves, they were transplanted into pots (180 mm in diameter) containing composted pine bark and transferred into another greenhouse (24-30°C and 65-85% RH) permanently. The plants were irrigated with complete fertilizer [Ocean Agriculture[1] 3:1:3(38) at 0.5 g ℓ^{-1}] + [Ca(NO$_3$)$_2$ at 0.5 g ℓ^{-1}] by means of drip irrigation. Pots were kept at a distance of 40 cm within rows and 150-200 cm between rows. After 3 d of transplanting, seedlings were inoculated by spraying 3-5 mℓ of conidial suspensions of *P. xanthii* (10^3 conidia mℓ^{-1}) onto the leaves of each seedling using a hand sprayer. First, the source plants that were growing in a separated greenhouse were shaken 24 h before spores harvested for inoculation. This was done to ensure that all conidia in the suspension were fresh and of the same age. Once the conidia had been counted, inoculation commenced immediately, within 2 h during late afternoon to ensure a sufficiently high temperature and relative humidity for infection.

5.2.2 Application of biocontrol agents and silicon

Clonostachys rosea (Link) Schroers, Samuels, Seifert & Gams (Isolate EH), *Trichothecium roseum* (Pers.) Link (Isolate H20) and 3 isolates of *Serratia marcescens* (Bizio) (i.e.,

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[1] Ocean Agriculture (Pty) Ltd., P. O. Box 742, Muldersdrift, 1747, South Africa.

Isolates B15, Y15 and Y41) were used as biocontrol agents against PM. All BCAs were produced according to their requirements as described in Chapter 2 and applied until runoff using a hand sprayer 2d before inoculation of the seedlings with PM and repeated after 4d when PM symptom started to appear and then continued weekly for 5 wk. The concentration of propagules per $m\ell$ of each BCA was 10^6 for Isolates EH and H20 and 10^8 for isolates of *S. marcescens*. As a wetter, Break-Thru® (at 0.25 $m\ell$ ℓ^{-1}) was added to each microbial suspension and mixed thoroughly. All BCAs were applied in the evenings to improve establishment of the antagonists by providing sufficient relative humidity. Si, in the form of KSi, was drenched onto the roots of each plant 3 d before inoculation and weekly thereafter (250 $m\ell$ at 100 mg ℓ^{-1}).

5.2.3   Disease assessment

Assessment of the percentage of leaf area infected was recorded for each treatment weekly before the next application. Ratings on percentage of the leaf covered by PM was recorded for 3 leaves every week to represent disease severity and averaged to give the percentage of disease. The development of disease was very high, especially for the Untreated Control. This created a problem because the rated leaves soon developed 100% disease. To deal with this problem, ratings of the severity level of PM of the control plants were made on new leaves that were not fully covered by the PM colonies. Then the leaves that corresponded to the leaves rated for the Untreated Control plants were assessed to get a severity rating for the treatments. Finally, AUDPC was calculated from PM levels using an AUDPC Program (Shaner and Finney, 1977).

5.2.4   Data analysis

Two trials were conducted, with each treatment having three replications, arranged in a randomized complete block design. Analysis of variance and contrast analysis between treatments that contained Si and without Si were performed using GenStat® Statistical Analysis Software (GenStat, 2006). Comparison between means of treatments was performed using Fisher's Protected LSD and efficacy of each treatment on percentage reductions of FDL and AUDPC values were calculated by comparing the values of each treatment with that of the Untreated Control.

5.3 RESULTS

One week after inoculation, the mean leaf area infected of 3% was recorded on the first three leaves of the Untreated Control. The level of disease increased exponentially and at the end of the second week, these leaves were 100% diseased, and the newly emerging leaves were infected. In the second trial, the mean disease level after one week was 18% and subsequently followed a similar pattern. At the end of the month, the level of leaf area infected of of plants from the Untreated Control was 61% and 89% for the first and second trials, respectively. In both trials, the effects of treatments on the severity and AUDPC values were highly significant (F < 0.001).

In the first week of the first trial, the severity levels of PM recorded for all treatments were similar, ranging from 0-5%. However, severity levels of PM started to differ after the second week of treatments. In the second trial, the severity levels of PM were obvious after only one week of infection, with the antagonistic fungi Isolates EH and H20, with and without Si, reducing disease severity to a level of < 3% versus a level of 18% recorded for the Untreated Control. The levels of PM of plants treated by bacterial BCAs alone, or with Si, were within the range of 2-7%; while plants treated with Si alone developed an initial infection level of 10%. In both trials, all BCAs reduced PM level significantly (P < 0.001). The ranking order of treatments and the percentage of reduction obtained by all treatments are presented in Table 5.1 and Figures 5.1 and 5.2, respectively.

Application of Si had a significant effect on the severity of PM (P < 0.05), and the contrast in severity levels of PM between plants treated with Si and those that were not treated with Si was highly significant (P < 0.05). Furthermore, most BCAs provided improved control of PM when they were applied to plants that were being treated with Si.

The effects of treatments on AUDPC were significant (P < 0.001) for both trials. All BCAs reduced PM development, as reflected by significantly lower AUDPC values. Similarly, application of Si had a significant impact on AUDPC values of the second trial, but not in the first trial. In both trials, Si alone significantly reduced the AUDPC values. Integrated applications of Si and BCAs resulted in significantly lower AUDPC values, with most BCAs treatments showing an improved efficacy, compared to their respective values without Si (Table 5.1). Overall, application of Si provided a mean reduction of 13 % in disease severity and AUDPC values.

Table 7.1 Effects of selected biocontrol agents and soluble silicon (Si) on final disease level (FDL) and area under disease progress curve (AUDPC) of powdery mildew of greenhouse-grown zucchini five weeks after inoculation with *Podosphaera xanthii*.

Treatments	Trial 1				Trial 2			
	FDL		AUDPC		FDL		AUDPC	
	-Si	+Si	-Si	+Si	-Si	+Si	-Si	+Si
Control	61.3^c	41.7^b	688.8^c	470.2^b	89.3^e	68.7^d	965.0^e	758.2^d
B15	43.3^b	37.7^b	448.2^{bc}	396.7^b	39.0^c	35.0^{bc}	379.2^c	221.8^b
EH	32.0^{ab}	30.0^{ab}	361.7^{ab}	331.3^{ab}	11.0^a	6.3^a	145.8^{ab}	126.0^a
H20	36.0^b	30.0^{ab}	374.5^{ab}	309.2^{ab}	5.3^a	9.0^a	71.2^a	128.3^a
Y15	25.3^a	34.0^{ab}	249.7^a	361.7^{ab}	28.0^b	26.3^b	275.3^b	271.8^b
Y41	36.0^b	30.3^{ab}	399.0^b	337.2^{ab}	29.0^b	31.3^{bc}	255.5^b	292.8^{bc}
Average	39.0	33.9	420.3	367.7	33.6	29.4	348.7	299.8

Effects	F-values		F-values		F-values		F-values	
BCAs	< 0.001		< 0.001		< 0.001		< 0 .001	
Si	0.020		0.052		0.045		0.009	
BCAs * Si	0.020		0.041		0.022		< 0.001	
FLSD	10.25		130.14		9.95		87.08	
CV (%)	16.6		19.5		18.6		15.9	

- Si = treatments without Si, + Si= treatments with Si

Means within column followed by a common letter were not significantly different according to Fisher's protected least significant difference (P<0.05).
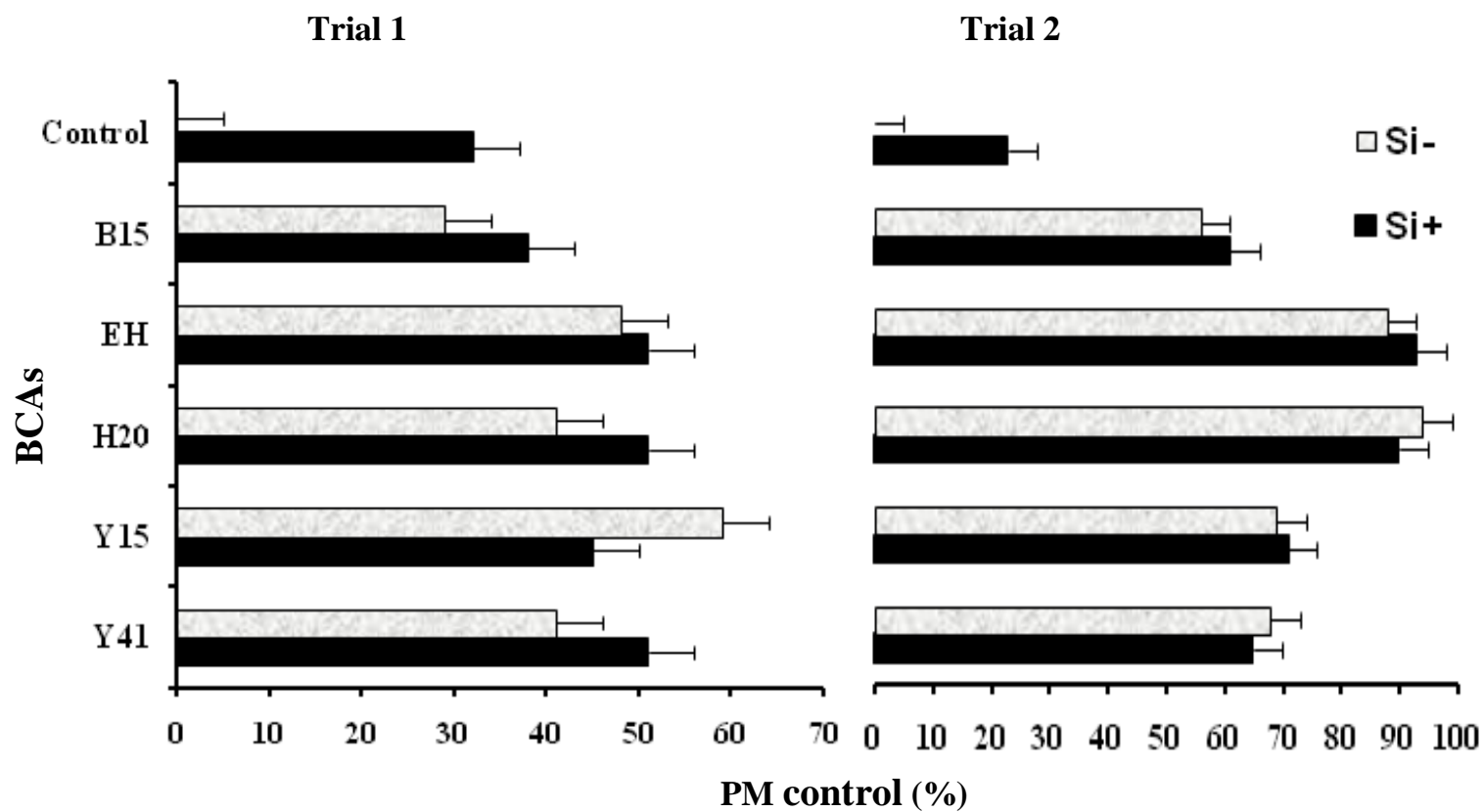
Figure 5.1 Percentage reduction in final disease levels of powdery mildew of greenhouse-grown zucchini by five biocontrol agents and soluble silicon compared to an Untreated Control treatment five weeks after inoculation with *Podosphaera xanthii*.
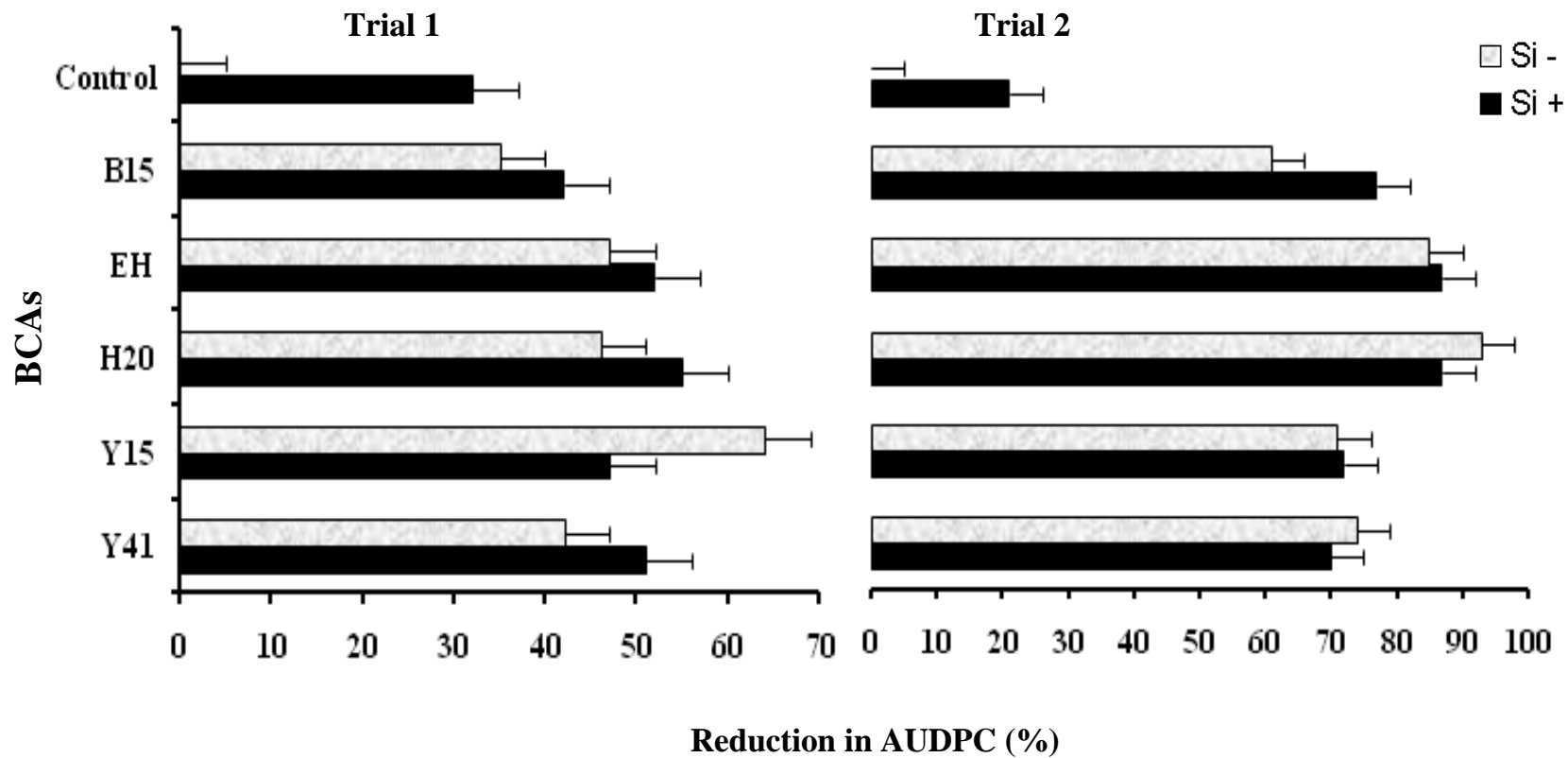
Figure 5.2 Percentage reduction of the Area under disease progress curve (AUDPC) values of powdery mildew of greenhouse-grown zucchini by five biocontrol agents and soluble silicon compared to an Untreated Control treatment, five weeks after inoculation with *Podosphaera xanthii*.

5.4   DISCUSSION

Infection by PM and consequently, development of PM, was very rapid in both trials, partly due to the environmental conditions of the greenhouse that favoured PM. Powdery mildew progress on the Untreated Control plants demonstrated the ideal conductions of the greenhouse to the development of PM. Powdery mildew is a very common disease of crops that are grown under such conditions. This is because its high RH, high temperature and restricted air circulation provide an ideal environment for germination of conidia of PM fungi and their establishment (Howard *et al*., 1994). Once infection starts in such environment, the disease spreads throughout the greenhouse at a fast rate and causes a massive impact on the yield and quality of the fruit, if not managed properly (Dik *et al*., 1998).

Restrictions on the use of fungicides have intensified the use of biological control as an alternative approach in controlling PM. As a result, some promising results have been reported by several researchers (Dik *et al*., 1998; Elad *et al*., 1998; Hijwegen, 1992; Miller *et al*., 2004; Verhaar *et al*., 1993 & 1996). This is because PM grows superficially on the leaf and its exposure to the external environment makes it vulnerable to attack by many microbes (Bélanger *et al*., 1998). However, due to the dependency of antagonists on specific environmental conditions for establishment and survival, most of them operate more effectively within a limited range of temperature and RH (Bélanger *et al*., 1998; Jarvis and Slingsby, 1977; Jarvis *et al*., 1989). When BCAs are tested against PM, the most limiting factor has been their requirements for a high RH. In this regard, greenhouses are ideal sites for exploitation of biological control because they provide the best environmental conditions for the development and activity of BCAs. This was demonstrated in our trials where the severity levels of PM were very high in the Untreated Control.

In both trials, all BCAs reduced disease severity by suppressing the infection rate and development of PM. After 4wk of treatments with these BCAs, promising levels of disease reduction (40-90%) were obtained. Reductions in the levels of PM can increase the amount and quality of yields by zucchini. We did not find a publication on the impact of PM on yields of zucchini. However, in cucumber, a level of infection as little as 5% for 60d will translate into a yield loss of 6% (Bélanger *et al*., 1998) and severe infection can reduce the

yield by more than 50% (Sundheim, 1982). Therefore, the ability of our isolates to suppress PM level by 40-90% shows their potential to be used against PM under greenhouse conditions, giving the growers an alternative option to managing PM effectively.

Most of our isolates controlled PM better when they were applied to plants treated with Si. Drenching the pots of zucchini weekly with 250 mℓ of a Si solution at a concentration of 100 mg ℓ$^{-1}$ reduced PM by an average of 12-14% over all treatments. Such additive effects were likely due to a combination of mechanisms that are involved by the mixed treatment in controlling PM as opposed to the modes of action provided by Si or individual antagonist, applied alone. Although the mechanism by which Si exerts its protective effects against plant diseases is the subject of debate and controversy (Ghanmi *et al*., 2004), prevention of penetration of PM fungi as a result of physical barrier (Bowen *et al*., 1992; Samuels *et al*, 1991a & b), priming the resistance of the host (Cherif *et al*, 1994; Fawe *et al*., 1998; Menzies, *et al*., 1991b) and ameliorating the biotic and abiotic stresses of the plant (Epstein, 1994, 1999, 2001) are currently considered to be the main modes of action of the element. In case of biological control, the known mechanisms of action employed by different antagonists in suppressing PM include: antibiosis (Dik *et al*., 1998; Hajlaoui *et al*., 1992 & 1994; Urquart *et al*., 1994), competition (Nofal and Haggag, 2006), mycoparasitism (Askary *et al*., 1997; Falk *et al*., 1995a & b; Kiss 1998; Romero *et al*., 2003; Sundheim, 1982; Sztenjberg *et al*., 1989; Verhaar *et al*., 1997) and inducing host resistance (Elad *et al*., 1998; Silva *et al*., 2004; Vogt and Buchenauer, 1997). Therefore, when some of these mechanisms of action are used in controlling PM, better disease control could be obtained. Sometimes, co-application of BCAs together or with Si might not give an improved efficacy against the target disease, PM. However, it might still benefit the plant because one of the treatments in the combination could provide protection against non-target pathogens. For instance, co-application of AQ10 and Trichodex™ did not improve efficacy of the application in controlling PM of cucumber but gave better control of grey mold (Elad *et al*., 1998).

The level of control obtained by Si alone, or in combination with the BCAs, was promising. However, there is still a possibility that such level of control can be improved by manipulating the environment of the greenhouse further. For instance, setting the operating temperature at 20-25°C can give better results (Schuerger and Hammer, 2003).

According to these authors, suppression of PM of cucumber by Si was independent of light intensity, but was significantly inhibited when the temperature was higher than $24^{\circ}C$.

Co-application of BCAs with other control options has also given an improved success in controlling PM of different crops in similar environment (Dik *et al*., 1998; Elad *et al*., 1998; Sundheim, 1982). This is because when some BCAs are used alone, they cannot offer protection for the entire season so that they can be viable alternatives to chemicals (Pertot *et al*., 2008). This is common especially when mycoparasites are used as BCAs, which need a certain level of disease since they can only attack established infections (e.g., *A. quisqualis*) (Fokkema, 1993). In contrast, epidemics of PM develop very fast and can overtake the mycoparasite. For this reason, repeated applications of BCAs may increase the likelihood of their controlling PM. However, if the mode of action of the BCA is antibiosis, the antagonist does not have to be in direct contact with the pathogenic fungi because the molecules will diffuse over the leaf surface (Dik *et al*., 1998). In this study the use of Break-Thru® has improved efficacy of BCAs by enhancing their deposition on the leaf and the pathogen and directly affecting the PM fungi.

In most reports where Si produced promising results, the research has been conducted under controlled environments by growing plants in hydroponics (Kanto *et al*., 2004; Schuerger and Hammer, 2003) or recycling-nutrient solutions (Adatia and Besford, 1986). In both systems, there is continuous supply of Si to the plant, giving an improved efficacy compared to the technique used in this study. In our case, Si was supplied to the rhizosphere once a week, from which some of the solution was drained out of the pots due to gravity, or leached as a result of irrigation, leaving little opportunity for the plant to utilize the supply. This means that the amount of Si that could be absorbed by the plant was lower than when the element is supplied continuously to the roots. When Si is used to provide control of diseases, the availability of Si over time is more important than its amount. This is because if the supply is interrupted, even for one day, the plant may be infected by the pathogen (Samuels *et al*., 1991b). Once infection occurs, protective measures have little impact on PM. Samuels *et al*. (1991b) have described the type protection offered by Si against diseases as "non-systemic resistance". Therefore, we believe that improved efficacy can be obtained by increasing the frequency of supply of Si to plant roots. Alternatively, reducing the loss of Si by reducing the drainage can play a significant role in improving the availability of Si to the plant.

The overall disease levels were high in Trial 2, which made most BCAs perform better. However, Si performed worse under the high disease pressure of Trial 2. In Trial 2, the disease severity level increased by 31%, reflecting a 28% reduction in the efficacy of Si. This was assumed to be a direct response to the increase in disease pressure compared to the first trial. High levels of infection at the start of Trial 2 might have played a role in reducing the efficacy of the Si treatment by increasing the development of PM, which ultimately resulted in a high final disease level. Kanto *et al*. (2007) demonstrated that the impact of Si applications is reduced when the plants are already infected with PM. Therefore, for best control of PM, Si must be applied prior to infection, by relying on the previous history of the growing environment or disease forecasts. If Si fails to provide protection against infection by the pathogen or if the pressure of PM is too high, the use of other control options, including fungicides, may be needed as emergency measures so that Si can function at a manageable level of PM. In spite of the reduced efficacy of Si in Trial 2, the fact that both control options provided promising results gives hope that they can provide economic control under natural levels of infection, where PM levels are usually much lower than the levels tested here.

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CHAPTER SIX

USE OF SELECTED BIOCONTROL AGENTS AND SILICON FOR THE MANAGEMENT OF POWDERY MILDEW OF ZUCCHINI UNDER FIELD CONDITIONS

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Abstract

Two field trials were conducted at the Ukulinga Research Farm to evaluate the efficacy of five selected biocontrol agents and soluble silicon (Si) in controlling powdery mildew of zucchini caused by *Podosphaera xanthii*. Biocontrol agents were applied as foliar sprays at a concentration of 10^8 propagules mℓ^{-1}. One litre of Si at 100 mg ℓ^{-1} was drenched weekly into the rhizosphere of treated plants. Although statistically not significant, disease reductions of 32-70% by Si alone, 30-53% by Isolate B15, and 33-65% by Isolate B15 + Si were achieved. Other BCAs applied alone or together with Si also reduced disease levels by 9-68%. Plants treated with most of the BCAs showed significantly lower AUDPC values. For most antagonists, better efficacy was obtained when Si was drenched weekly into the rhizosphere of the plants. Efficacy of some of the BCAs and Si were affected by the environmental conditions of the field. A low temperature with a high humidity enhanced the performances of Isolate B15 plus Si whereas these conditions suppressed the fungal BCAs. However, at a high temperature and a low relative humidity, the efficacy of the fungal antagonists was superior, and the rest of the treatments provided reduced disease control. Although promising results were achieved by all treatments, repeated trials and better understanding of the use of Si and the BCAs in their dosage and application frequency, as well as interactions with the host plant and the environment, are needed before they can be implemented on a commercial scale for sustainable control of the powdery mildew.

6.1 INTRODUCTION

The development of fungicide resistant fungal mutants combined with increased concerns over health and environmental hazards caused by fungicides have intensified the interest in identifying biocompatible products that can supplement or replace conventional fungicides, especially to control diseases such as powdery mildew (PM) (Shishkoff and McGrath, 2002).

Effective control of PM with several biocontrol agents has been achieved under greenhouse conditions (Bélanger *et al*., 1997; Elad *et al*., 1996 & 1998; Falk *et al*., 1995; Jarvis and Slingsby, 1977; Verhaar *et al*., 1996). This has resulted in the development of some promising biocontrol products on the market. However, efficacy of many of these products has been inconsistent when tested under field conditions, partly due to the variability of the many factors that govern the establishment of BCAs (Bélanger *et al*., 1994; Schuerger and Hammer, 2003). Unlike greenhouse conditions, where humidity, temperature and other growth factors are relatively stable, field conditions are variable and unpredictable. Humidity, temperature, light intensity and rainfall are some of the main factors that determine the success of a BCA. These factors have a direct impact on disease development, and the survival and antagonistic activities of BCAs on the phylloplane.

Adding silicon (Si) into nutrient solutions has reduced PM of cucurbits grown in hydroponics (Bélanger *et al*., 1995; Menzies *et al*., 1991a & b; Samuels *et al*., 1991a; Schuerger and Hammer, 2003). As a foliar spray, Si has also been reported to effectively control PM on cucumber, muskmelon and zucchini (Menzies *et al*., 1992), and on grape (Bowen *et al*., 1992).

However, under both greenhouse and field conditions, the level of control achieved with BCAs and Si is often incomplete. This is as a result of the slow establishment of BCAs as opposed to the fast development of the disease. One of the limitations of Si has been the failure to provide curative protection once the plant is infected by the pathogen (Kanto *et al*., 2007). Co-application of BCAs and Si may complement each other to give a better disease control.

To date, we have found no publications in which Si was directly supplied to the roots of zucchini plants growing under field conditions. Objectives of this study were to evaluate the efficacy of five selected isolates and Si against PM of zucchini caused by *Podosphaera xanthii* (Castagne) under field conditions, and to study the possibility of using these two control options in the development of an integrated field management strategy against this disease.

6.2 MATERIALS AND METHODS

6.2.1 Trial site, land preparation and transplanting

The field trials were conducted at Ukulinga Research Farm located at E 29^o40E and 30^o24' S, 715 m above sea level, in the Southern Tall Grassveld of South Africa (Morris, 2002) on heavy, deep soil of Bonheimer clays (Dr R. Melis[1], 2002, pers. comm.).

The field was thoroughly plowed twice with a tractor and all the existing weeds were removed by hand. To moisten the field and enhance survival of the seedlings, irrigation was commenced one day before transplantation. Seedlings of zucchini, raised as described in previous sections, were transplanted into the field once they had a fully developed second leaf. Planting distance was 1.25m between plants and 1.5m between rows. During the experimental periods, moisture level of the field was monitored daily. The field was irrigated with overhead irrigation and kept free of weeds with herbicides, as needed.

6.2.2 Preparation of *Podosphaera xanthii* inoculum

Collection, storage and preparation of *P. xanthii* were the same as described in the previous Chapters except that inoculations were performed by spraying the conidia onto the zucchini crop with a CP3 knapsack sprayer instead of a hand sprayer.

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6.2.3 Application of biocontrol agents and soluble silicon

Microbial suspensions of three *Serratia marcescens* (Bizio) isolates (i.e., Isolates B15, Y15 and Y41) and two fungi (*Clonostachys rosea* (Link) Schroers, Samuels, Seifert & Gams (syn. *Gliocladium roseum*) (Isolate EH) and *Trichothecium roseum* (Pers.) Link (syn. *Cephalothecium roseum*) (Isolate H20)) were prepared as described in the previous sections and were applied onto plant leaves at a concentration of 10^8 propagules mℓ^{-1}, together with a wetter (Break-Thru®, 0.25 mℓ ℓ^{-1}) 3 d before inoculation and continued weekly 4 d after inoculation. All BCAs were applied using a CP3 Knapsack sprayer until runoff.

One litre of Si at 100 mg ℓ^{-1}, in the form of KSi, was drenched onto the roots of each plant 3 d before inoculation and weekly thereafter. Where Si was not used as a treatment, the same volume of clean water was drenched onto the rhizosphere. All treatments were made during the late afternoon or early evening to provide favourable conditions for the establishment of BCAs and to increase the availability of Si to the plant roots by reducing evaporation.

6.2.4 Disease assessment

The first disease rating was performed 10 d after inoculation with conidia of *P. xanthii* and continued weekly for 4 wk. Percentage of the area of leaves covered by PM was recorded weekly as the disease severity and area under disease progress curve (AUDPC) was calculated from the disease percentage during the course of the trial using an AUDPC Program (Shaner and Finney, 1977).

6.2.5 Statistical analysis

Two trials were conducted, with each treatment having three replications, arranged in a randomized complete blocks design. Each replication was represented by a plot of 12 plants. To reduce inter-plot interference, data was collected from the central row of each plot. Data was analyzed using Genstat(R) Statistical Analysis Software (Genstat, 2006). Comparison between treatments with Si and without Si was performed using contrast analysis. Where the CV (%) was > 20%, data was transformed using a square root

transformation. Means of treatments were compared using Fisher's Protected Least Significant Difference (FLSD) and efficacy of each treatment on percentage reductions of FDL and AUDPC values were calculated by comparing the values of each treatment with that of the Untreated Control.

6.3 RESULTS

Symptoms of PM started to appear one week after inoculation and developed slowly. In all plots, the disease was mainly concentrated on the older leaves, which senesced with increasing PM severity. Late-emerging leaves were susceptible to infection, but severity of the disease was relatively low on them. To assess levels of infection, disease progress within the same period was analysed for each plot.

The impact of the selected BCAs and Si on the severity levels of PM after 5 wk of inoculation are presented in Table 6.1. Some of the treatments significantly reduced the severity of the disease in the first trial ($P < 0.05$), but not in the second trial (Table 6.1). In contrast, the effects of treatments on disease progress, represented by AUDPC values, were not significant for the first trial. However, they were significant for the second trial conducted during January - March, 2007. In both experiments, the effects of Si on individual treatments were not significant, as shown by contrast analysis. However, most BCAs provided improved control when applied to plants treated with Si (Figures 6.1 & 6.2).

In the first trial (March - May, 2006), Si alone and Isolate B15 with/without Si controlled the disease significantly. Disease reduction of 73, 53 and 65% was obtained by treating the plant with Si, Isolate B15 and Isolate B15 + Si, respectively. The levels of disease after treatment by the rest of the individual treatments were not statistically lower than that of the control. However, they reduced the disease severity by 9 - 51%. In this trial, the bacterial isolates showed better antagonistic activity than the two fungal isolates (Figure 6.1). Similarly, significant reductions in AUDPC values of 67 and 60% was recorded when plants were treated with Si and B15 + Si, respectively. In contrast, application of Isolate EH + Si resulted in an AUDPC value similar to that of the Untreated Control. In most cases, treatments that showed high level of disease had high AUDPC values (Table 6.1).

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However, if the disease levels recorded throughout the trial remained almost constant, it resulted in low or high AUDPC values depending on the initial disease level.

In the second trial, which was conducted between January - March, 2007, the effects of treatments on the severity of PM were not significant at $P < 0.05$. However, Isolates H20 and EH, without Si, reduced disease severity by 68 and 61%, respectively. Although not significant, application of the rest of BCAs, with or without Si, reduced PM by 22-41% and Si alone reduced disease severity by 32% (Figure 6.1). The effect of treatments on the development of disease, as represented by AUDPC, was significant at $P < 0.05$ when the original data was used, but none were significant when transformed data was analysed. In spite of these statistical differences, all BCAs, with or without Si, reduced the AUDPC values by 44-77%, in parallel with reductions in disease severity.

In both trials, the effect of Si on individual treatments was not significant when contrasts were made between treatments that contained Si against those without Si. In spite of difference in disease levels between treatments, there were no obvious differences in terms of time to flowering and fruit setting among plots.

Table 6.1 Effects of biocontrol agents and silicon on final disease level (FDL) and AUDPC values of powdery mildew of zucchini under field conditions.

Treatment	Silicon	Trial 1		Trial 2	
		FDL	AUDPC	FDL	AUDPC
Water	-	25.7 (4.91)^b	233.3 (14.87)^b	29.0(5.34)^b	532.3(22.99)^b
Water	+	7.0 (2.59)^a	77 (8.59)^a	19.7(4.16)^{ab}	306.0(16.26)^b
B15	-	12.0 (3.45)^{ab}	126.0 (11.17)^{ab}	20.3(4.27)^{ab}	266.3(16.13)^{ab}
B15	+	9.0 (2.98)^a	92.2 (9.5)^a	19.3(4.29)^{ab}	229.0(14.86)^{ab}
EH	-	17.0 (4.1)^b	129.5 (11.37)^{ab}	11.3(3.35)^a	168.0(12.71)^{ab}
EH	+	23.3 (4.79)^b	190.2 (13.68)^b	20.0(4.47)^{ab}	266.7(16.31)^{ab}
H20	-	20.7 (4.51^b	165.7 (12.75)^{ab}	9.3(2.90)^a	127.7(11.16)^a
H20	+	16.0 (3.97)^{ab}	141.2 (11.67)^{ab}	17.0(4.08)^{ab}	201.0(14.02)^{ab}
Y15	-	16.0 (3.9)^{ab}	136.5 (11.37)^{ab}	22.0(4.67)^{ab}	217.3(14.73)^{ab}
Y15	+	16.3 (4.03)^b	151.7 (12.12)^{ab}	22.7(4.72)^{ab}	304.0(17.35)^b
Y41	-	14.3 (3.75)^{ab}	143.5 (11.89)^{ab}	20.0(4.28)^{ab}	220.7(14.57)^{ab}
Y41	+	12.7 (3.55)^{ab}	124.8 (11.14)^{ab}	20.0(4.4)^{ab}	283.7(16.64)^{ab}
Effects		**P-value**	**P-value**	**P-value**	**P-value**
Treatment		0.030	0.200	0.587	0.068
-Si vs. +Si		0.084	0.168	0.576	0.655
F.LS.D.		1.266	3.992	1.98	5.891
CV (%)		19.4	20.3	27.6	22.2

- = no Si, += Si present

Means within column followed by a common letter were not significantly different according to Fisher's protected least significant difference (P < 0.05).

Values in brackets are means of data transformed using square root transformations
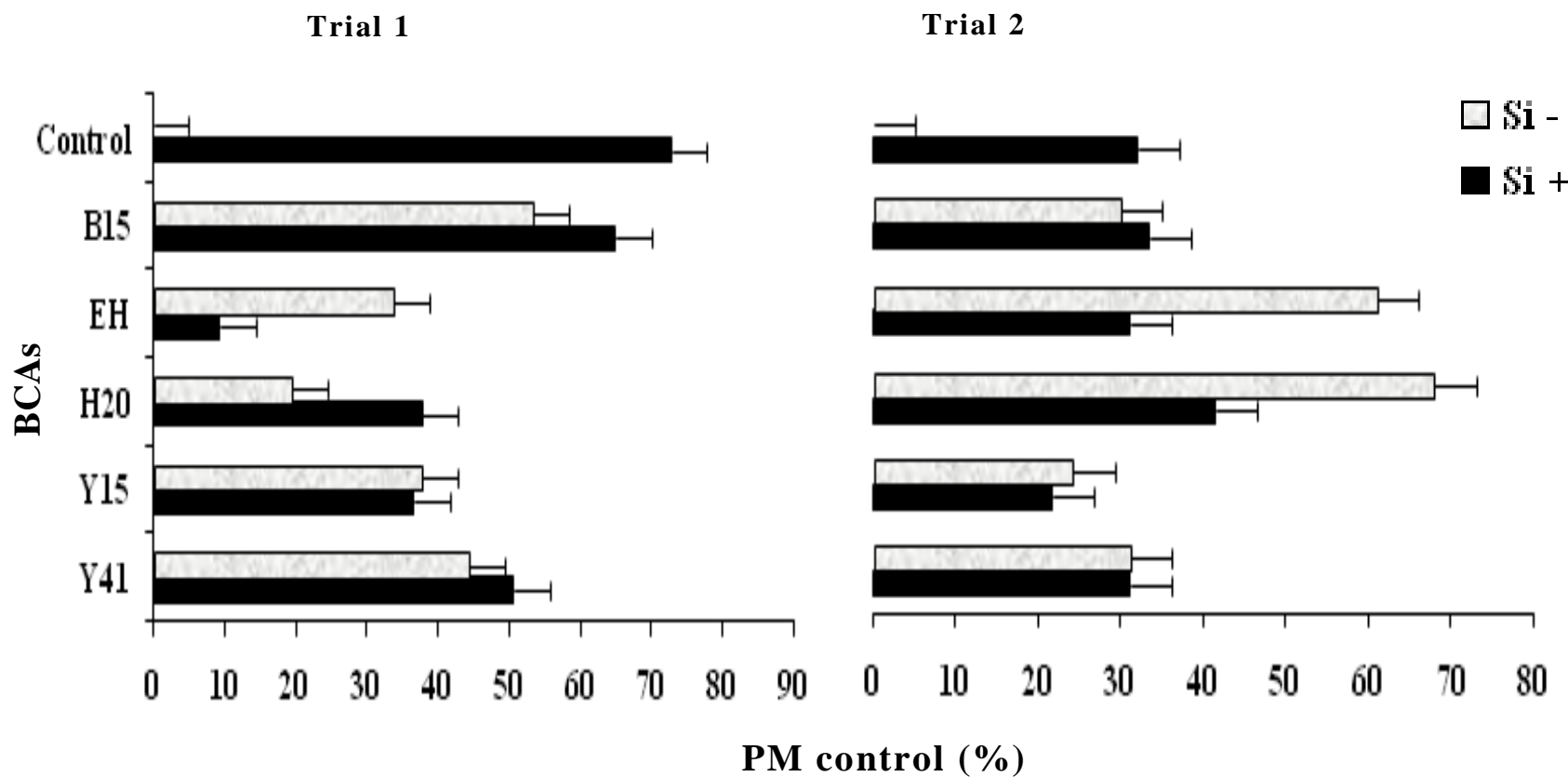
Figure 6.1 Efficacy of five biocontrol agents and silicon in reducing the severity of powdery mildew of zucchini under field conditions five weeks after inoculation with *Podosphaera xanthii* in two trials.
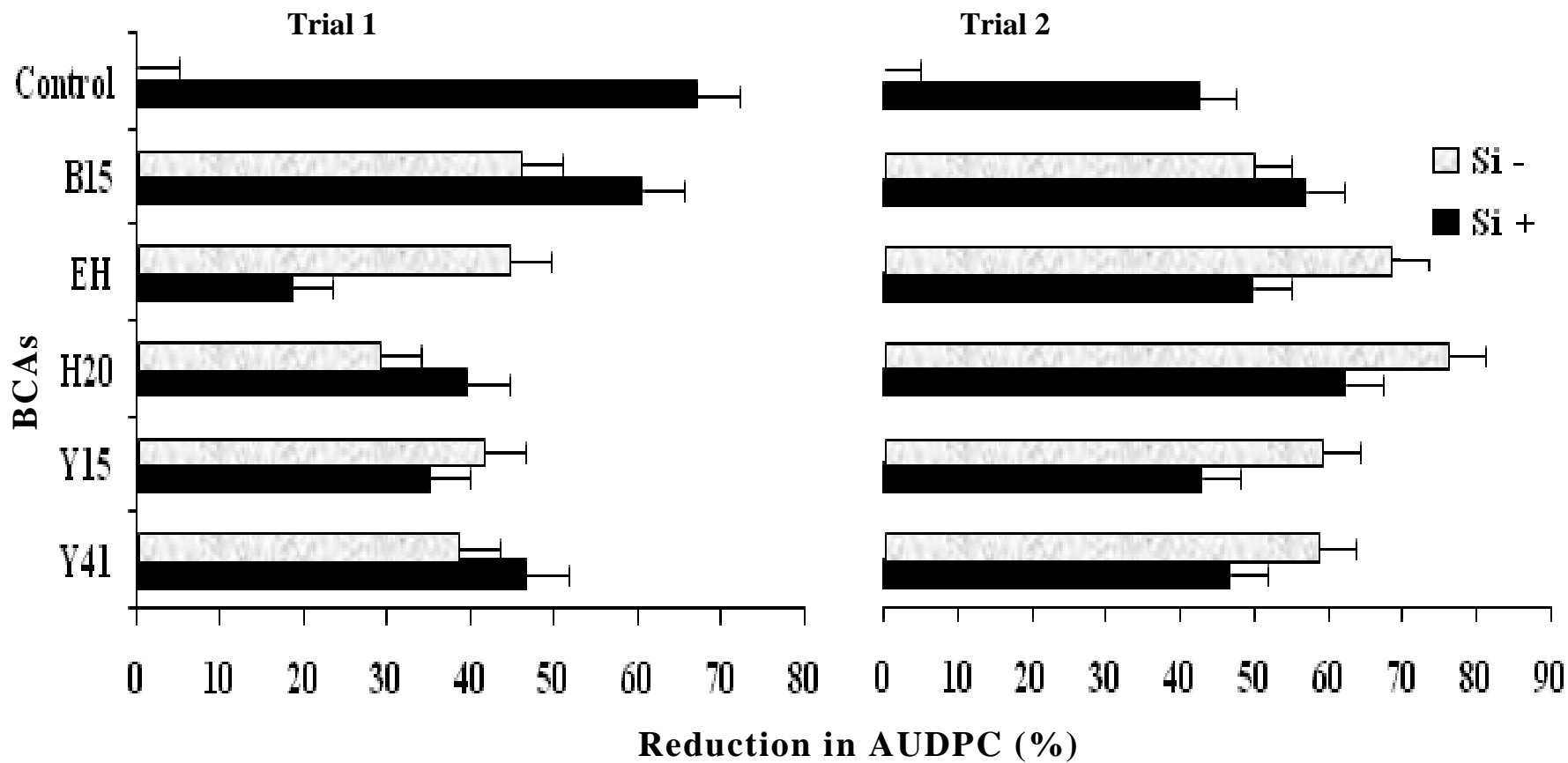
Figure 6.2 Efficacy of five biocontrol agents and silicon in reducing the AUDPC values of powdery mildew of zucchini after five weeks of inoculation with *Podosphaera xanthii* under field conditions.

6.4 DISCUSSION

The BCAs tested and a Si drench demonstrated the potential to reduce the final disease levels of PM of zucchini effectively. Isolate B15 alone, or together with Si, provided significant disease control. Compared to the rest of the isolates tested, Isolate B15 showed superior biocontrol activity, although its efficacy was lower in the second trial. However, the efficacies of most treatments were reduced in the second trial, with the exceptions of Isolates EH and H20 without Si. Five weeks after inoculation with *P. xanthii*, the epidemic of the disease, as represented by AUDPC values, was significantly reduced by all treatments.

These two trials showed that, in spite of their potential to control the disease, inconsistency was observed with some of the isolates tested. For example, Isolate B15 was the best in the first trial, but performed poorly in the second trial. In contrast, Isolates EH and H20 were inferior at the first trial and were the best in the second trial. Interestingly, the performance of Isolates Y15 and Y41 were relatively stable in both trials. For most treatments, the disease level of the second trial was slightly lower than the first one. Cook and Baker (1983) suggested that effective biocontrol activity can only be obtained within a specific level of disease. According to these authors, if the disease level caused by a target pathogen is minimal compared to the Uninfected Control, then the efficiencies of various treatments in controlling the disease appear to be negligible, masking the efficacy of some genuinely effective isolates. In contrast, if the disease level is higher than certain level, especially for fast developing diseases such as PM, then the contribution of each control measure becomes minimal, and comparison between treatments usually results in non-significant differences.

Significant control of diseases and subsequent increases in yield has been obtained in a number of field studies of biological control. The major problem, however, is the failure to repeat these results consistently in different soils or in different years in naturally infected fields and to make biological control of plant diseases competitive with chemical control (Schippers, 1988). Unlike greenhouse trials, where most environmental conditions are controlled, field trials results are affected by several environmental factors operating independently or in combination with each other. Reports of previous studies on biocontrol of several diseases indicated that fluctuations in environmental conditions in the field have been the main reason for the failure or inconsistent performance of biocontrol agents

(Paulitz and Bélanger, 2001). Because of their requirements for specific environmental conditions, BCAs are usually effective within a limited range of temperature and RH (Blakeman and Fokkema, 1982). For instance, most antagonists of PM operate more efficiently when RH is maintained above 80% (Bélanger *et al*., 1997; Jarvis and Slingsby, 1977), which can be achieved by manipulating the greenhouse environment, but this is not possible under field conditions. The requirement of many BCAs for a high RH has been confirmed by Jarvis *et al*. (1989) and Bélanger *et al*. (1994), who showed that the efficacy of *Stephanoascus flocculosus* Traquair , L.A. Shaw and Jarvis, *Sporothrix flocculosa* Traquair, Shaw & Jarvis and *S. rugulosus* Traquair, Shaw & Jarvis against PM was reduced when RH levels dropped below 60%. Hijwegen (1992) also showed that survival and antagonistic activities of *Tilletiopsis minor* Nyland against *P. xanthii* was reduced significantly when RH levels were reduced below 70-75%. Relative humidity is often the primary limiting factor under field conditions, when it drops below a minimum requirement of the BCAs.

Climatic data secured for the two periods of the trials showed that during the first trial, the mean temperature was $< 15^{o}C$ and the mean RH was $> 70\%$. Although the prevalent RH was ideal for germination of PM spores and infection of the plant by the pathogen, development of the disease was retarded because of the low temperature, which was less than optimum for the fungus (Cheah and Falloon, 2001). Most BCAs prefer warmer environmental conditions for their establishment and best biocontrol activities. In spite of that, the performances of the bacterial isolates were favoured by high RH of the field, which was within the range of their requirement for their survival and biocontrol activities. In the second trial, where day time temperatures in the field were high (i.e. $27\text{-}29^{o}C$) and RH was lower than $< 54\%$, the efficacy of antagonistic fungi was better than that of bacterial isolates. This might have been related to the sensitivity/tolerance of these BCAs towards RH. Based on the results of these two trials, it was concluded that the fungal BCAs were relatively tolerant to low RH but were sensitive to low temperature; specifically, Isolate B15 was more sensitive to a low RH; and Isolates Y15 and Y41 were less sensitive to fluctuations in temperature and RH.

Although the contrast analysis between treatments containing Si against treatments without Si showed no significant effect, application of Si reduced the disease severity consistently and improved the efficacy of most of the BCAs, especially in the first trial. Reduced

performances of Si in the second trial might have been related to the high temperatures in the field. Schuerger and Hammer (2003) reported similar results, where the efficacy of Si in controlling PM of glasshouse-grown cucumber crop was reduced at higher temperatures (24-32°C). According to these researchers, the optimum temperature for best control of the disease using Si was in the range of 20°C, which supports the results of our first trial.

Despite its potential, this study showed that the potential role of Si in controlling PM of zucchini under field condition may be limited, especially when compared to previous reports that were conducted under greenhouse conditions (Bélanger *et al*., 1995; Guevel *et al*., 2007; Menzies *et al*., 1991a & b; Schuerger and Hammer, 2003). Some of the following reasons might have contributed for this shortcoming.

For effective control or disease prevention, Si must be supplied to the plant continuously. Interruption of the supply, even for one day, can result in infection of the plant by the pathogen. Samuels *et al*. (1991b) reported that enhanced resistance of cucumber against PM lasted only 24 h after Si was removed from the nutrient solution. Firstly, in this field study, one litre of Si was applied to the rhizosphere at a frequency of once per week. Most of the water content of the solution can be lost soon to evaporation, leaving dry Si in the rhizosphere. In this form, Si cannot be absorbed by plant roots because the element is not in the form of a solution. Secondly, some of the applied Si may have become permanently unavailable to the plant roots if it bounds to Al^{3+} or Mn^{2+} ions in the soil (M.D. Laing, 2008, pers. comm.). Therefore, if the applied Si is not being taken up by the plant due to unavailability, the plant could be infected easily by the pathogen after 1-2 d of treatment. Once the symptoms appear on the susceptible plant, applying Si as curative measure has little impact because the disease can cover the whole plant within few days. Kanto *et al*. (2006) and Liang *et al*. (2005) noted that Si is more effective when used preventively than curatively. Therefore, increasing the application frequency or switching to using slow release formulations of Si could be used to improve the availability of Si to plants over an extended period of time.

In greenhouse trials, Kanto *et al*. (2006) showed that the efficacy of Si in controlling PM of strawberry was affected by the susceptibility of the plants to the disease. These researchers showed that the less susceptible cultivar responded better to Si treatment than more susceptible cultivar.

In both trials, there was little variation in terms of time of flowering and production of fruit among the treatments. From the lack of correlation between the treatments, and time of flowering and fruit setting, it would appear that the linkage between PM infection and the development of zucchini under field conditions is weak. Severe infection of PM causes indirect effects on the quality and yield of fruit by reducing the photosynthetic area of the plant through premature senescence of leaves (Cheah and Falloon, 2001; Keinath et $al.$, 2000). Fruit set is indirectly affected as a result of the reduced photosynthetic activities of the plant. Therefore, one explanation for our observation would be that the cultivar used in these studies may be highly susceptible, but has a relatively high level of tolerance to the disease, continuing to yield well despite high disease levels.

The efficacy of the tested antagonistic fungi was generally superior in the second trial compared to the first one. In the first trial conducted from March to May 2006, the relative humidity was in the range of 70-75%, with a temperature of 4-12°C. In the second trial, the mean temperature and RH during the growing period of January to February 2007 was 27-29°C and 54-59%, respectively. From this information, it was concluded that Isolates B15, Y15 and Y41 have the potential to perform well at lower temperatures, provided that there are high RH levels.

Some of the tested isolates provide some degree of control, especially when they were used together with Si. This is an encouraging result because it provides the growers with an option to include this combination as an alternative in PM control. Farmers who do not want to use conventional fungicides can use this option. However, since there were inconsistent performances by some of the BCAs tested, there is a need for repeated investigation under various environmental conditions. Repeated application of Si may give a better control as compared to the frequency used in this study. Hence, further research on the dosage/frequency of Si applications is needed before its use is adopted widely as a control strategy against this disease. In addition, the cultivar used in this study was very susceptible to the disease and the potential of the treatments might have been hindered due to the extremely fast development of the disease. It is possible that the efficacy of BCAs and Si treatments would have been improved if the cultivar used was less susceptible to the disease. In studies conducted by Bélanger et $al.$ (1994) and Dik et $al.$ (1998), it was observed that the degree of control provided by BCAs was better when resistant cultivars were used compared to susceptible ones. Similarly, the efficacy of sprays of inorganic salts

such as potassium bicarbonate was affected by disease pressure (McGrath and Shishkoff, 1999; Muza and Travis, 1995). Other reports also showed that efficacies of *Ampelomyces quisqualis* Ces ex Schlect, *Trichoderma harzianum* Rifai and *Bacillus subtilis* (Ehrenberg) Cohn were dependant on environmental conditions and disease pressure (Elad *et al*., 1998; Pertot *et al*., 2008).

Moreover, co-application of some of the antagonists tested in this study might have provided enhanced efficacy in controlling the disease. In a number of studies, synergistic effects have been observed by combining antagonists. For instance, the use of *Pseudomonas fluorescens* Migula and a mixture of *Trichoderma* spp. provided effective control of PM and downy mildew (Abd-El-Moity *et al*., 2003). They also reported that when *B. subtilis* was added to that combination and applied at an early age (i.e. 4wk old), the crop gave its highest yields. The additive effects were probably due to a combination of multiple mechanisms that affect the pathogens, as opposed to the fewer control mechanisms provided by a single antagonist.

There is also a need to understand more about the epidemiology of the disease, the resistance level of the plant and to identify the optimal environmental requirements of the BCAs in order to improve their efficacy by manipulating some of these variables. Moreover, since disease suppression by an introduced beneficial organism depends on the amount of inoculum applied onto the phylloplane, it may be necessary to alter the application protocol using higher rates, shorter spray intervals or applying chemical fungicides when the disease pressure is too high.

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CHAPTER SEVEN

UPTAKE AND DISTRIBUTION OF SILICON IN ZUCCHINI AND ZINNIA, AND ITS INTERACTION WITH THE UPTAKE OF OTHER ELEMENTS

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Abstract

Elemental analysis was conducted to determine the impact of differing application levels of silicon (Si) in nutrient solutions on: (1) the uptake and distribution of Si into different organs (tissues) of zucchini and zinnia; (2) its impact on the uptake and accumulation of other elements; (3) the effect of powdery mildew on the levels of selected elements on these two plant species; and (4) the effects of Si uptake on the growth of zucchini and zinnia plants. Plants were grown in re-circulating nutrient solutions, supplied with Si at different concentrations. Samples were taken from different organs of each plant and analysed using energy dispersive X-ray fluorescence scanning electron microscopy (EDX) and inductively coupled plasma-optical emission spectrometers (ICP-OES). Increased levels of Si in the solution increased accumulation of Si in leaves and roots of both plants without affecting its distribution in other parts. In zucchini, roots accumulated higher levels of Si g dw^{-1} than leaves. With zinnia, accumulation of Si g dw-^{1} was highest in leaves. Accumulation of 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 Ca in both plants.

Adding Si into the nutrient solution at a lower level (i.e., 50 mg ℓ^{-1}) increased the growth of zucchini plants and resulted in a maximal uptake of P, Ca, and Mg in both plants. However, application of higher levels of Si did not provide further growth enhancement. Levels of Si in the nutrient solution had no effects on elemental composition and characteristics of the fruits of zucchini. However, when Si was applied at > 50 mg ℓ^{-1}, then

the P level of the fruit was reduced by 50%. In both plants, infected leaves accumulated 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 of both plants. For optimum disease control and maximum accumulation of different elements in these two plants, application of Si at 50-150 mg ℓ^{-1} is recommended.

7.1 INTRODUCTION

Silicon (Si) is the second most abundant element in the earth's crust. It has been considered as not essential to the growth of plants (Epstein, 1994). This is because of a perception that many plants can grow normally in its absence (Epstein, 1999) and symptoms for its deficiency and toxicity are not apparent (Ma and Yamaji, 2006). Even today, some scientists do not consider Si as an essential element for plant growth, although its beneficial roles on plant growth and resistance to biotic and abiotic stresses have received considerable attention (Datnoff et al., 1997; Epstein, 1994, 1999 & 2001; Ma, 2004; Ma and Yamaji, 2006; Meyer and Keeping, 2005). However, these researchers have shown that when crops with a high Si demand are repeatedly grown in soils with low levels of plant-available Si, then symptoms of Si deficiency are being manifested by low productivity, and susceptibility of the crops to biotic and abiotic stresses.

The mechanisms of uptake, translocation and accumulation of Si on different plants have been investigated by several researchers (Liang et al., 2005; Ma and Yamaji, 2006; Rains et al., 2006; Raven, 2001; Tamai and Ma, 2003). In some plants, the uptake of Si can be equal or even greater than Ca, Mg, S and P (Epstein, 1994 & 1999; Ma and Yamaji, 2006), ranging 0.1-10 % (Tamai and Ma, 2003), with the cell walls of epidermal layers being the main site of Si deposition (Adatia and Besford, 1986). The level and mechanisms of uptake varies among plants species (Adatia and Besford, 1986; Ma and Yamaji, 2006). Beneficial effects of the element on growth and yield of several crops have been linearly related to the level of Si supplied to the plants (Bélanger et al., 1997; Ma, 2004; Ma and Takahashi, 2002). However, an excessive supply of Si to cucumber (Bélanger et al., 1997; Samuels et al., 1993) and strawberry plants (Lieten et al., 2002) may result in poor fruit quality.

Therefore, there is a need to determine the optimum level of Si supply in order to obtain maximum benefits without compromising the quantity and quality of yield, or the cost of applying Si to plants. With the increased application of Si to soil-less media in order to enhance plant growth and disease protection, careful investigation of the uptake and distribution of this element in different part of the plants is needed.

Increased accumulation of Si in trichomes, around pathogen sites of infection and at different parts of the cell has been reported on powdery mildew (PM) infected plants (Cherif *et al*., 1992a; Menzies *et al*., 1991; Samuels *et al*., 1991a, 1991b & 1993). The presence of Si may result in different levels of activation, or speed of activation, of defence reactions in infected and uninfected plants (Cherif *et al*., 1992b). Where a plant is infected by *P. xanthii*, its uptake of Si increases, and then resistance to the disease is enhanced (Cherif *et al*., 1994; Rodrigues *et al*., 2005). To date, there is little information available that maps out the distribution of Si to different parts of different plant species. Understanding the level of uptake and accumulation of Si into different organs of plants, and its effects on the total elemental uptake of plants can lead to the better use of this element in crop production. The objectives of this research were (1) to assess uptake and distribution of Si to different parts of zucchini and zinnia, in relation to Si supply and its impact on the uptake and accumulation of other elements by these plants; (2) to determine the relationship between infection of these plants by *P. xanthii* and accumulation of Si and selected elements by each plant; and (3) to study the effects of Si supply on characteristics of fruits of zucchini and flowers of zinnia and accumulation of other elements in these organs.

7.2 MATERIALS AND METHODS

7.2.1 Preparation of plants

Seeds of zucchini (*Cucurbita pepo*, F1-Hybrid Partenon) and zinnia (*Zinnia elegans* cv. Jakobrekop Sunbow), obtained from Starke Ayres[1] and McDonald Seeds[2], respectively, were used for this study. Seeds of both plants were planted in Speedling® trays containing

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[1] Starke Ayres, P. O. Box 304, Eppindust 7475, South Africa.
[2] McDonald Seeds (Pty) Ltd, P. O. Box 238, Pietermaritzburg, 3200, South Africa.

composted pine bark. These were transferred into a tunnel operating at 26-28°C and 75-85%. Trays were irrigated with a balanced fertilizer [3:1:3 (38) from Ocean Agriculture[3], at 0.5 g ℓ^{-1}] + [Ca(NO$_3$)$_2$ at 0.5 g ℓ^{-1}] until seedlings were transplanted into pots filled with composted pine bark. Transplanting was commenced after seedlings of zucchini developed their first leaf, and when zinnias reached a height of 50-70 mm. Finally, pots were transferred into a nutrient re-circulating system that is in a greenhouse (24-30°C and RH of 60-70%) and supplied with a complete hydroponics nutrient solution. Soluble Si was added in the form of KSi$_,$ at 11 different concentrations (0, 50, 100, 150, 200, 250, 300, 400, 500, 750 and 1000 mg ℓ^{-1}). To see the effect of infection with *P. xanthii* on elemental uptake by these two plant species, both plants were inoculated with the pathogen and grown on separate benches while being supplied with Si (150 mg ℓ^{-1}). Inoculations were made by dusting conidia of the pathogen from infected leaves onto healthy zinnia leaves, or spraying a conidial suspension onto zucchini leaves using a hand sprayer. The levels of Si used were based on previous results for both plants. Plants of zucchini and zinnia were kept in the system for 6 wk, by which time all the plants had produced well-developed fruits and flowers.

7.2.2 Energy dispersive X-ray fluorescence (EDX) analysis

Leaf samples were taken from different positions of each plant and washed thoroughly with distilled water to remove superficial dust, cut into pieces of approximately 10 mm diameter, and fixed overnight in 3% glutaraldehyde in cacodylate buffer (0.1 M; pH 7). Samples were then dehydrated in a graded alcohol series. Specimens were critical point dried in a Hitachi HCP-2 using carbon dioxide as the transfusion fluid. Dried specimens were mounted onto copper stubs using double-sided carbon tape. All stubs were then coated with gold-palladium in a Polaron E500 Sputter Coater and the accumulation of Si and other elements in the samples was assayed using an environmental scanning electron microscope with an energy dispersive X-ray analysis system (ESEM-EDX). Analysis was performed on leaves of uninfected plants and areas of infected leaves that were covered or uncovered with colonies of the pathogen.

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[3] Ocean Agriculture (Pty) Ltd., P. O. Box 742, Muldersdrift, 1747, South Africa.

Quantitative calculations were made using the fundamental parameter method of the EDX program as used by Marguí *et al*. (2005). Elements that were considered for analysis and quantification included: carbon (C), nitrogen (N), calcium (Ca), magnesium (Mg), potassium (K), oxygen (O), phosphorus (P) and silicon (Si).

To further study the effects of infection and presence of the pathogen on Si accumulation in leaves, elemental mapping was performed on (1) entirely disease-free leaves, (2) disease-free areas of infected plants, and (3) infected areas of infected plants covered by PM colony. Elemental mapping was also performed to examine the distribution of Si to different parts of the leaf.

7.2.3 Inductively coupled plasma-optical emission spectrometers (ICP-OES) analysis of plant tissues for elemental composisions

(a) Preparation of samples

Plants of zinnia and zucchini were removed from their growing medium and washed thoroughly using tap water and finally rinsed in distilled water, to remove superficial dust. The plant materials were cut into four parts: leaves, roots, flower/fruits, and stem + petioles. Samples were then oven-dried at $70^{\circ}C$ for 72 h, ground into particles less than 1.0 mm in diameter, using a blender and then thoroughly homogenized. A sample of 0.5 g of each plant material was put into a crucible and kept overnight in a furnace set at $650^{\circ}C$. Ashes of the samples were processed using microwave digestion.

(b) Microwave digestion

All sample digestions were carried with the CEM Microwave Digester (CEM MARS5™ Microwave) using tarred 100 mℓ Teflon® PFA digestion vessels. The vessels were thoroughly washed using acid and rinsed with distilled water to free them from any particulate matter and then dried. Ash was transferred into the vessels and 5 mℓ of 65% HNO_3 and 0.1 mℓ HF were added to each sample and the vessels were thereafter sealed with their lids. Thirteen vessels, including a reagent blank vessel, were arranged in a scrubber and digestion was performed using the following procedures. The temperature was ramped to $165^{\circ}C$ within 10 min with the application of 1200W power, followed by a

dwell time of 20 min at 165°C. The temperature and pressure limits were set to 175°C and 15.2 bar (220 psi), respectively. Following completion of the digestion program, the vessels were allowed to remain in the microwave cavity until the internal temperature cooled to < 65°C. Then the scrubber was removed from the microwave, placed in a fume exhaust system and then the vessels were vented slowly to release the residual pressure. Roots and leaves of zinnia and zucchini produced a small quantity of precipitate at the end of the cycle, so the procedure was repeated after adding 0.9 mℓ HF to the digest. Once the samples were fully digested, they were diluted to 100mℓ with de-ionized distilled water (DDW) to make a dilution factor of 100 (v/v) and transferred into high-density polyethylene (HDPE) bottles. After every cycle, the vessels were washed with tap water and rinsed with DDW.

(c) Preparation of standards

A 100 mℓ reagent blank was prepared by mixing DDW with 5 mℓ of HNO$_3$ and 0.1 or 1 mℓ of HF, depending on the volume of HF used for the digest. Two sets of five standard solutions (i.e., 2, 15, 50, 100 and 200 mg ℓ$^{-1}$) were prepared to avoid formation of Si precipitates, with the first set containing only Si and the second set including Ca, K, Mg and P.

(d) ICP-OES system

All measurements were made using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Varian Model 720-ES). To avoid instrument damage and contamination from the free HF of the solution, a V-groove nebulizer, a Sturman-Masters Spray Chamber, and a Radial Torch were used instead of the glassy materials of the machine. The system components used were recommended by the supplier as being suitable for solutions containing up to 30% HF. Samples were taken automatically at a rate of 1 mℓ min-1 using an SPS3 AutoSampler. The plasma forward power was 1000W with plasma and auxiliary gas flow rates of 15 and 1.5 ℓ min-1, and a pump rate of 15 rpm was used to aspire the sample solutions. The ICP system was calibrated using ICP Expert II software, with each calibration curve being constructed linearly through zero after subtraction of the reagent blank, as used by Feng et al. (1999).

7.2.4 Statistical analysis

Data collected from the ICP-OES was analysed using polynomial regression to study the relationships of Si in the nutrient solution with elemental compositions of different tissues of zucchini and zinnia plants.

7.3 RESULTS

7.3.1 Observations from EDX-ESEM analysis and elemental mapping

Results of the EDX analysis and elemental mapping showed that leaves of both plants accumulated high levels of Si. Even in the control, where Si was not added into the nutrient solution, leaves of both plants accumulated some levels of Si (Figures 7.1 & 7.2). As the concentration of Si in the nutrient solution was increased, the level of Si in the leaves of both plants increased. In some cases, the level of Si was even higher than that of C and O. In both plants, infected leaves accumulated more Si than the uninfected leaves, with Si mainly concentrated around the infected areas. Further investigations of these areas showed that Si was accumulated in the plant and not in the hyphae of the PM fungus (Figure 7.3B). Regardless of Si concentration in the nutrient solution, Si was always highly concentrated at the base of trichomes (Figure 7.3A).
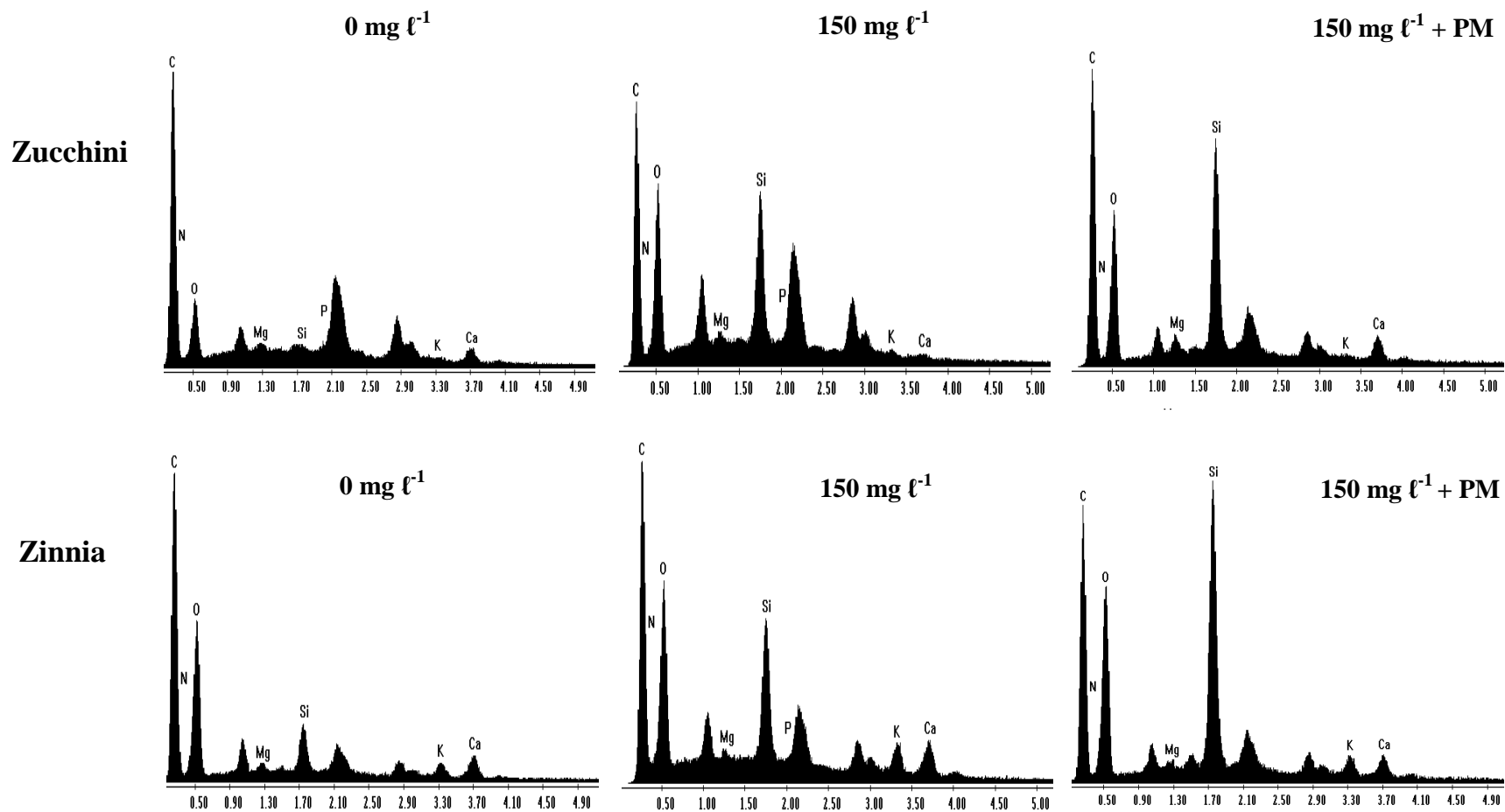
Figure 7.1 Energy dispersive X-ray (EDX) spectrums of silicon and other elements on leaves of zucchini and zinnia plants that received Si treatments of 0 (control), 100 mg ℓ^{-1} and 100 mg ℓ^{-1} plus *Podosphaera xanthii* and *Glovinomyces cichoracearum*, respectively.

	0 mg ℓ$^{-1}$	150 mg ℓ$^{-1}$	150 mg ℓ$^{-1}$ + PM
Zucchini			
Zinnia			
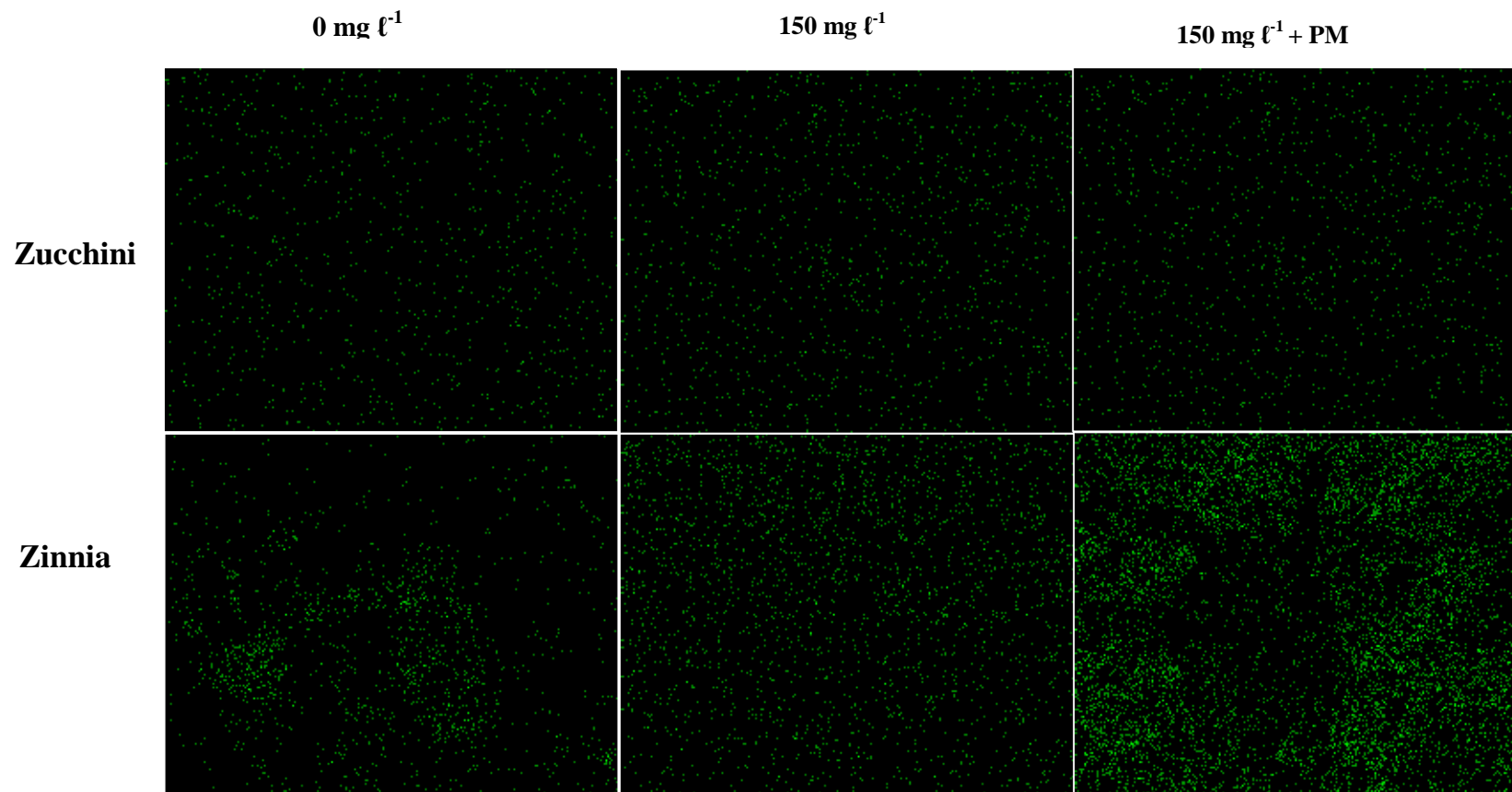
Figure 7.2 EDX mapping of silicon deposition on leaves of zucchini and zinnia plants that received Si treatments of 0 (control), 100 mg ℓ$^{-1}$ and 100 mg ℓ$^{-1}$ plus *Podosphaera xanthii* and *Glovinomyces cichoracearum,* respectively.
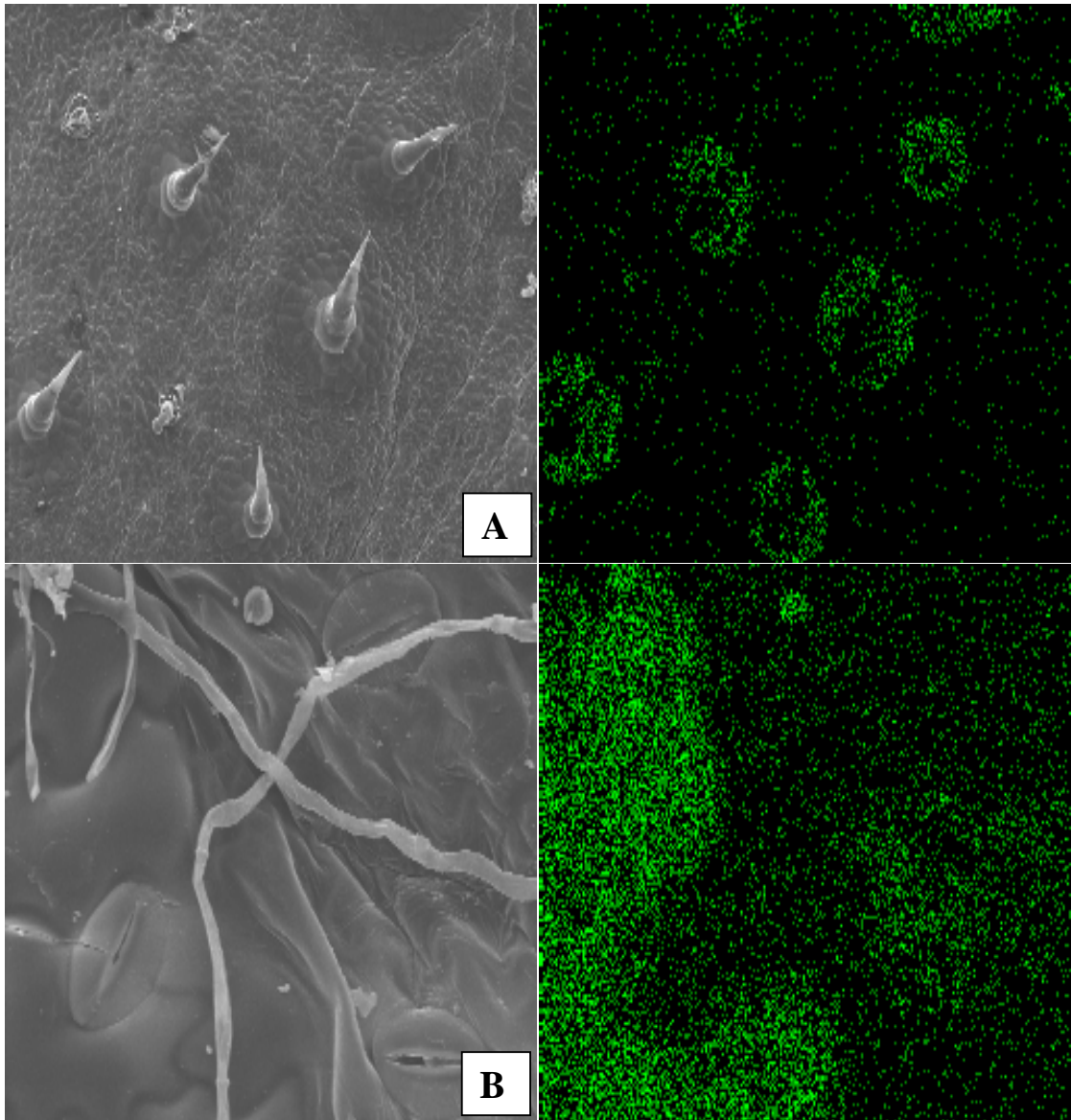
Figure 7.3 Scaning electron microscope and EDX mapping showing silicon deposition of Si at the base of trichomes of non-infected leaf of zucchini (A) and in the leaf containing structures of the pathogen (B).

7.3.2 Results of ICP-OES analysis

When samples were completely digested through a microwave treatment, the digest became colourless. An amount of 5 mℓ HNO3 and 0.1 mℓ HF were sufficient to yield full digests of samples of flowers of zinnia and fruits of zucchini and the stem and petioles of these two plants. However, leaf and root samples required an extra 0.9 mℓ of HF for a complete digestion.

As with the EDX analysis, both plants accumulated considerable amounts of Si in their tissue, even if the element was not added to the nutrient solution. The source of this element in the control was probably from the composted pine bark that was used as the growing medium, plus the Si in the irrigation water. Accumulation of Si by both plants was directly related to the level of the element in the nutrient solution. With zucchini, adding as little Si as 50 mg ℓ-1 increased growth of the plant. However, extra applications of Si did not result in further increases in plant growth. Adding Si into the nutrient solution at 50 mg ℓ-1 doubled the total amount of Si accumulated in different tissues of the plant, with leaves accumulating more than six times as much. As the levels of Si in the nutrient solution was increased up to 400 mg ℓ-1, the level of Si accumulated by leaves was increased by 8-9 times, reaching its maximum level of 68.8 mg g-1 dry weight (dw). However, the extra accumulation of Si in the leaves obtained by supplying Si at 150-400 mg ℓ-1 was negligible, and extra applications had little impact. Similarly, accumulation of Si in roots was almost doubled when the concentration of Si in the solution was kept between 50-200 mg ℓ-1. However, application of Si at higher rates (i.e., > 200 mg ℓ-1) had little impact on the level of Si accumulated by roots. In addition, the level of Si accumulated in stems and petioles of zucchini remained constant when Si was supplied at 0-200 mg ℓ-1. However, at 250-500 mg ℓ-1, that amount increased by 35-65%. The amount of Si in the fruit of zucchini was not affected by nutrient concentrations of Si (Figure 7.4).

The effect of Si levels in the nutrient solutions on the accumulation of Si by zinnia was similar. As the level of Si in the nutrient solution increased, leaves of zinnia accumulated as high as 92.6 mg g-1 dw, which was more than double the value obtained for the control treatment. Optimum concentration of Si in the nutrient solution was considered to be

250 mg ℓ-1 because increasing the Si level further had a negligible impact on accumulation of the element. Roots of this plant also accumulated high amount of Si (24.2 mg g-1 dw) as the level of Si in the solution was set at 150 mg ℓ-1. Flowers of zinnia doubled their Si content when the plant was supplied with 50 mg ℓ-1. However, subsequent increases in Si supply did not result in increased Si levels in zinnia flowers. Changing Si level in the nutrient solution did not affect the Si content of the stems and petioles of zinnia (Figure 7.4).

Both plant species responded positively to the levels of KSi in the solution by increasing the total amount of K obtained in their tissues. With zucchini, adding Si at 100 mg ℓ-1 to the solution increased the amount of K found in the leaves by 44%. However, applying more Si did not increase accumulation of K further. In contrast, leaves of zinnia accumulated higher levels of K when the plant was supplied with higher levels of Si. In addition, the K content of the stems and petioles of both plants was increased as the concentration of the Si supply was increased. When KSi was added to the nutrient solution at the maximum concentration (i.e., 1000 mg ℓ-1), the amount of K accumulated by stem and petioles of both plants almost doubled. In contrast, the levels of K in the roots and fruits of zucchini, and flowers of zinnia were not affected by Si levels of nutrient solutions (Figures 7.5 & 7.6).

The levels of Mg in leaves of both plants were increased in both plants as a result of increasing Si level in the nutrient solution. At a Si level of 50 mg ℓ^{-1}, the level of Ca in different parts of both plants was increased. However, extra levels of Si in the solution did not increase their Ca contents. Instead, reduced accumulation of Ca was observed, especially in roots, flowers and fruits. Level of P in fruits of zucchini and flowers of zinnia were increased slightly with increased levels of Si in the nutrient solution. In addition, the Mg content in leaves of both plants increased as the level of Si of the nutrient solutions increased. Measurement of levels of Ca, Mg and P in both plants showed that the highest levels of these elements were obtained when Si was added to the nutrient solution at 50 mg ℓ^{-1} (Figures 7.5 & 7.6).

A

Si in plant tissue (mg g^{-1} dw)

Zucchini

- Leaves: $y = -0.898x^2 + 6.866x + 6.275$
  $R^2 = 0.58$
- Roots: $y = -0.192x2 + 2.596x + 12.70$
  $R^2 = 0.215$
- Fruits: $y = -0.014x2 + 0.138x + 4.958$
  $R^2 = 0.225$
- Stem+ petioles: $y = -0.056x2 + 0.794x + 2.334$
  $R^2 = 0.437$

Si level in sol. (mg ℓ$^{-1}$)

B

Si in plant tissue (mg g^{-1} dw)

Zinnia

- Leaves: $y = -0.532x^2 + 10.476x + 28.94$
  $R^2 = 0.728$
- Roots: $y = -0.354x^2 + 4.726x + 24.52$
  $R^2 = 0.290$
- Flowers: $y = -0.036x^2 + 0.678x + 9.780$
  $R^2 = 0.384$
- Stem+ petioles: $y = 0.046x^2 - 0.936x + 11.662$
  $R^2 = 0.676$

Si level in sol. (mg ℓ$^{-1}$)
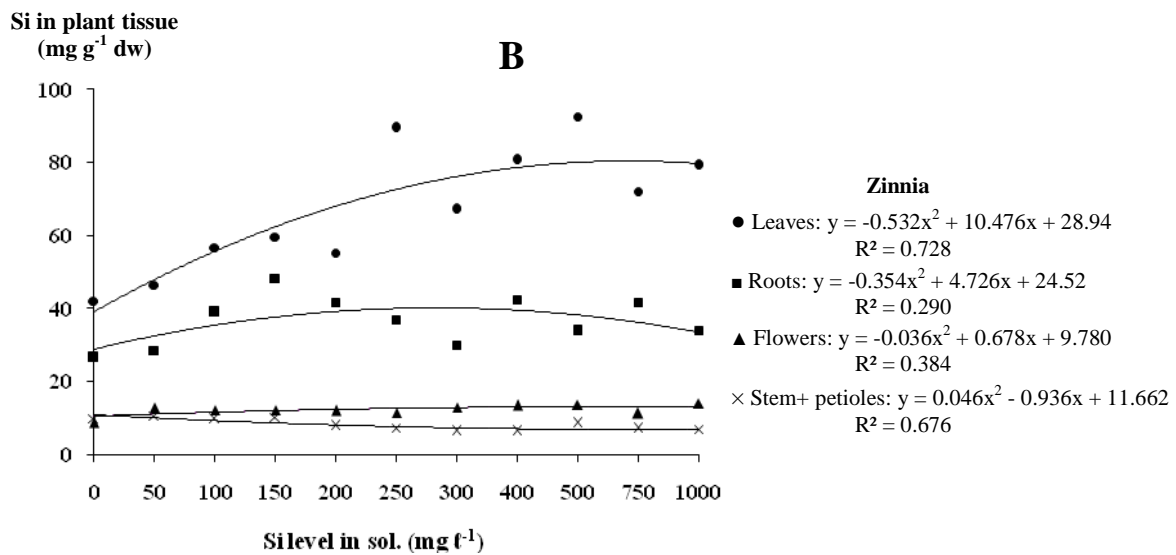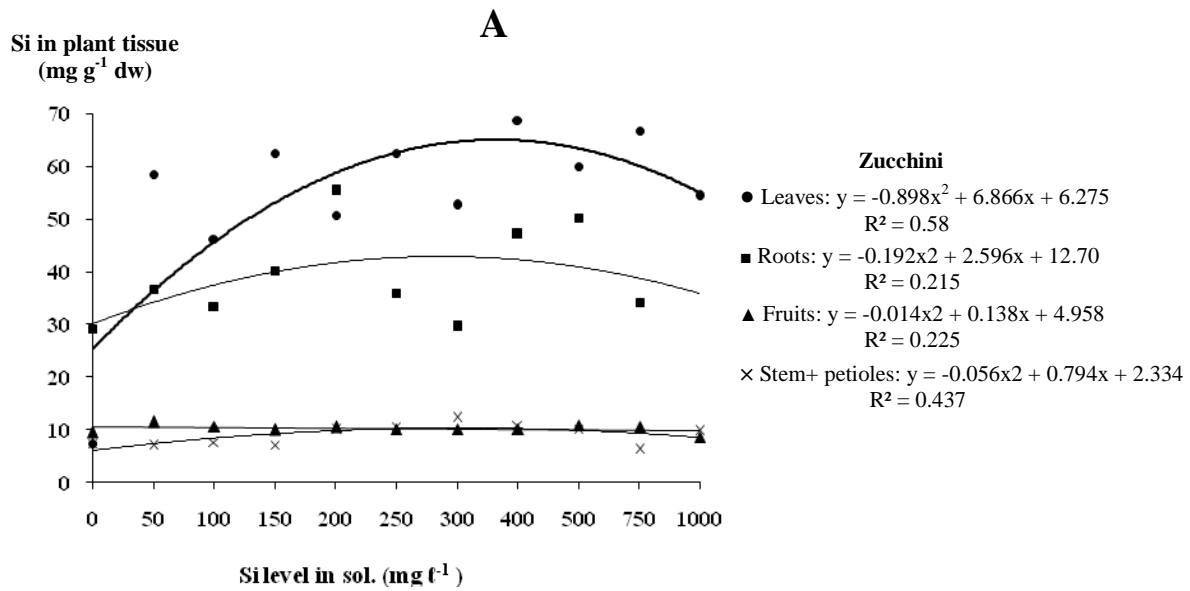
Figure 7.4 Effects of Si level in nutrient solution on the accumulation of Si in different parts of zucchini (A) and zinnia (B) plants after six weeks of growth in a nutrient recirculating system.

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K in plant tissue (mg g⁻¹ dw)

Zucchini

× Stem + petioles: $y = 0.044x^2 - 0.082x + 4.496$
$R^2 = 0.713$

● Leaves: $y = -0.016x^2 + 0.308x + 3.96$
$R^2 = 0.168$

■ Roots: $y = 0.024x^2 - 0.214x + 2.456$
$R^2 = 0.169$

▲ Fruits: $y = 0.014x^2 - 0.224x + 3.438$
$R^2 = 0.075$

Si level in sol. (mg l⁻¹)

Mg in plant tissue (mg g⁻¹ dw)

Zucchini

● Leaves: $y = 0.002x^2 + 0.154x + 5.95$
$R^2 = 0.204$

■ Roots: $y = 0.002x^2 - 0.10x + 2.288$
$R^2 = 0.358$

▲ Fruits: $y = -0.012x^2 + 0.106x + 2.486$
$R^2 = 0.033$

× Stem + petioles: $y = -0.022x^2 + 0.062x + 4.402$
$R^2 = 0.643$

Si level in sol. (mg l⁻¹)

Ca in plant tissue (mg g⁻¹ dw)

Zucchini

● Leaves: $y = -0.200x^2 + 2.844x + 20.90$
$R^2 = 0.188$

■ Roots: $y = -0.002x^2 - 0.182x + 7.934$
$R^2 = 0.268$

▲ Fruits: $y = -0.030x^2 + 0.318x + 1.378$
$R^2 = 0.080$

× Stem + petioles: $y = -0.078x^2 + 0.88x + 5.012$
$R^2 = 0.505$

Si level in sol. (mg l⁻¹)

P in plant tissue (mg g⁻¹ dw)

Zucchini

● Leaves: $y = -0.128x^2 + 1.78x + 3.778$
$R^2 = 0.108$

■ Roots: $y = 0.070x^2 - 0.766x + 9.362$
$R^2 = 0.14$

▲ Fruits: $y = 0.018x^2 - 0.328x + 7.392$
$R^2 = 0.144$

× Stem + petioles: $y = 0.002x^2 + 0.174x + 5.868$
$R^2 = 0.161$

Si level in sol. (mg l⁻¹)

Figure 7.5 Effects of concentrations of Si applied in nutrient solution on accumulations of K, P, Ca, and Mg in different parts of zucchini after six weeks of growth in a nutrient re-circulating system.

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K in plant tissue
(mg g^{-1} dw)
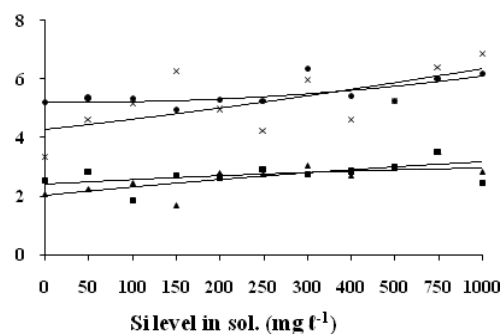
Zinnia

● Leaves: $y = 0.01x^2 - 0.038x + 5.264$
  $R^2 = 0.435$
■ Roots: $y = -0.002x^2 + 0.088x + 2.316$
  $R^2 = 0.209$
▲ Flowers: $y = -0.002x^2 + 0.158x + 1.852$
  $R^2 = 0.585$
✕ Stem + petioles: $y = 0.004x^2 + 0.156x + 4.108$
  $R^2 = 0.426$

Si level in sol. (mg ℓ^{-1})

P in plant tissue
(mg g^{-1} dw)
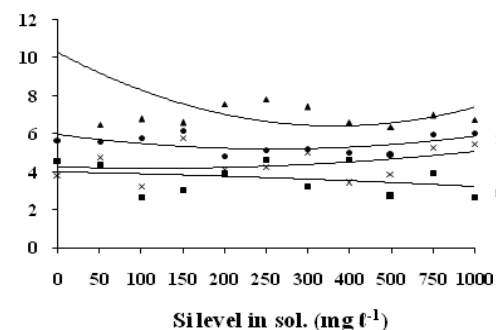
Zinnia

▲ Flowers: $y = 0.088x^2 - 1.352x + 11.554$
  $R^2 = 0.41$
● Leaves: $y = 0.028x^2 - 0.352x + 6.276$
  $R^2 = 0.302$
✕ Stem + petioles: $y = 0.014x^2 - 0.092x + 4.344$
  $R^2 = 0.112$
■ Roots: $y = -0.002x^2 - 0.034x + 4.016$
  $R^2 = 0.093$

Si level in sol. (mg ℓ^{-1})

Ca in plant tissue
(mg g^{-1} dw)
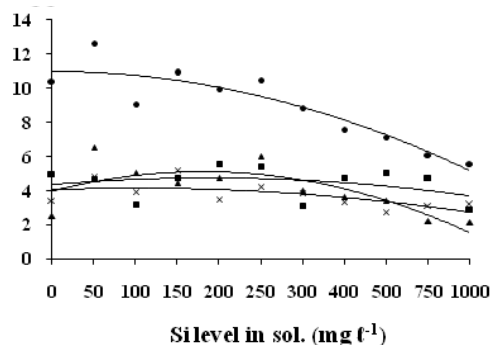
Zinnia

● Leaves: $y = -0.056x^2 + 0.112x + 10.926$
  $R^2 = 0.840$
■ Roots: $y = -0.026x^2 + 0.254x + 4.144$
  $R^2 = 0.121$
✕ Stem + petioles: $y = -0.022x^2 + 0.142x + 3.952$
  $R^2 = 0.444$
▲ Flowers: $y = -0.086x^2 + 0.794x + 3.34$
  $R^2 = 0.585$

Si level in sol. (mg ℓ^{-1})

Mg in plant tissue
(mg g^{-1} dw)
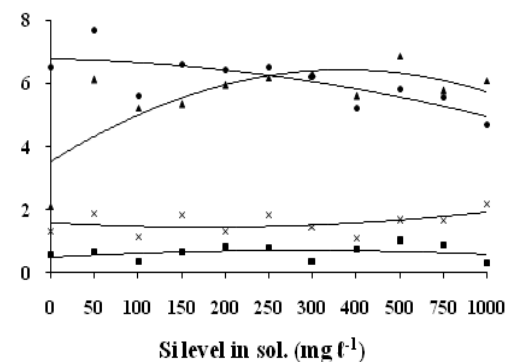
Zinnia

▲ Flowers: $y = -0.064x^2 + 0.994x + 2.574$
  $R^2 = 0.577$
● Leaves: $y = -0.014x^2 + 0.002x + 6.764$
  $R^2 = 0.578$
✕ Stem + petioles: $y = 0.01x^2 - 0.104x + 1.668$
  $R^2 = 0.201$
■ Roots: $y = -0.006x^2 + 0.092x + 0.402$
  $R^2 = 0.101$

Si level in sol. (mg ℓ^{-1})

Figure 7.6 Effects of concentration of Si applied in nutrient solution on accumulations of K, P, Ca and Mg in different parts of zinnia after six weeks of growth in a nutrient re-circulating system.
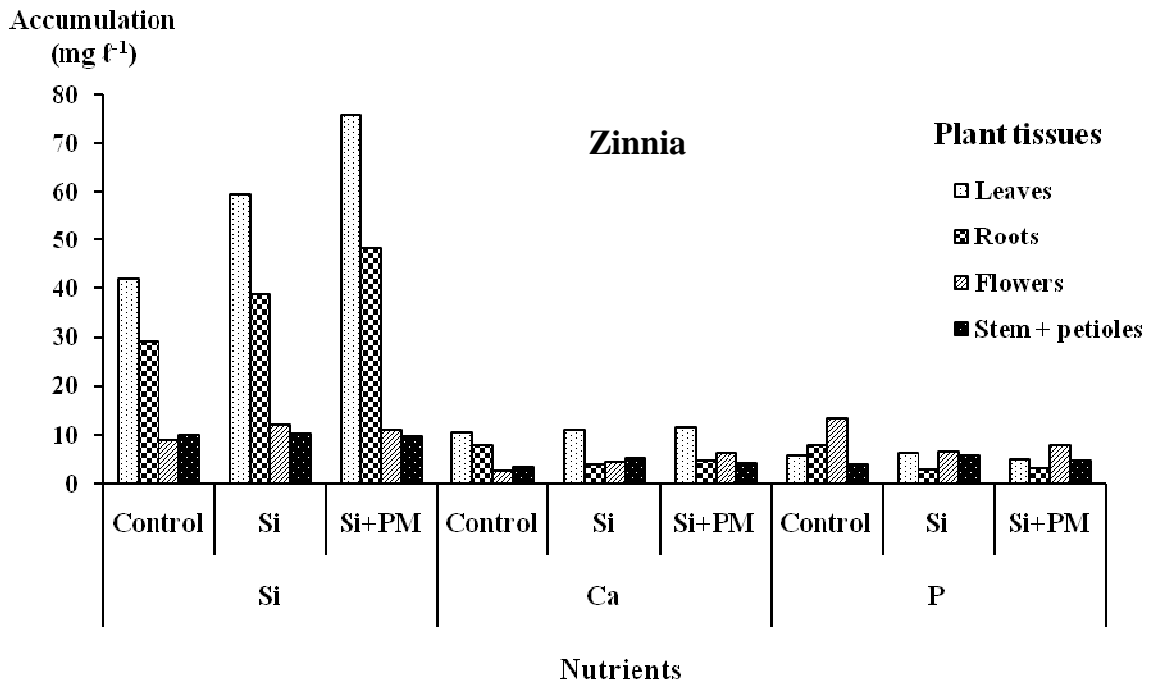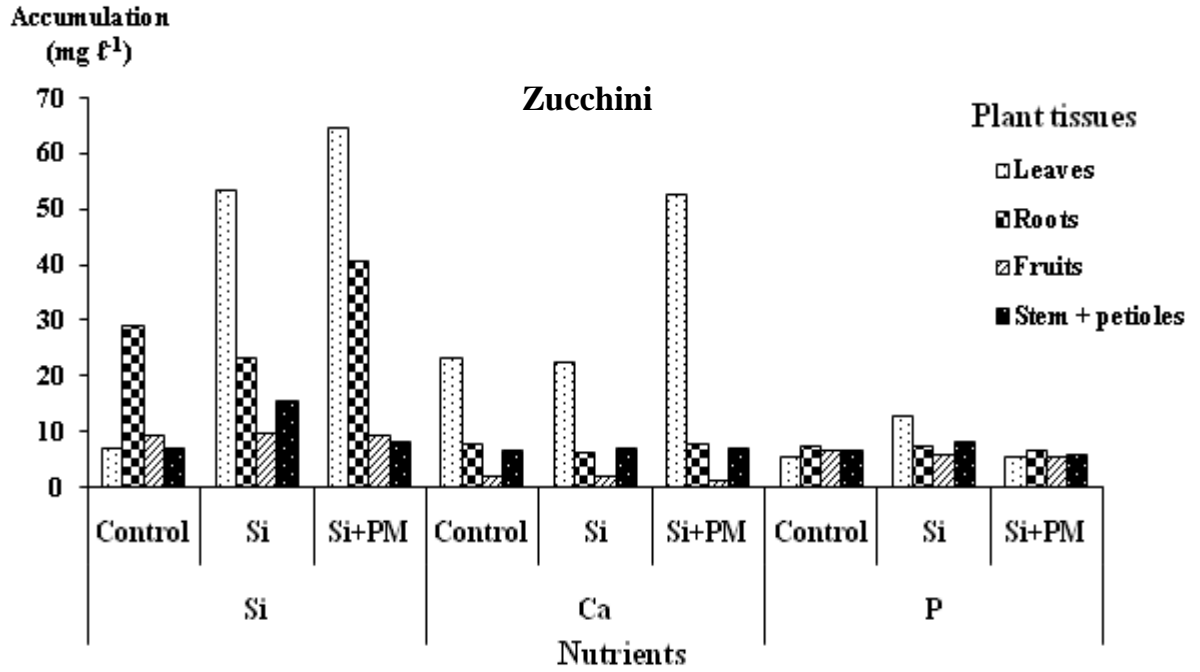
Figure 7.7 Effects of infection with powdery mildew fungi on the accumulation of Si, Ca and P in different parts of zucchini and zinnia plants grown in a nutrient re-circulating system for six weeks and supplied with Si at 150 mg ℓ^{-1}.

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Effects of powdery mildew on accumulation of selected elements

Infected plants of zucchini accumulated higher levels of Si in their leaves and roots than disease-free plants. However, the amount of Si in other parts of infected plants was almost unaffected. The total amount of K found in different parts of infected zucchini plants was not significantly affected as a result of infection with the pathogen. The total amount of P accumulated by all parts of infected zucchini plants declined by 30%, with leaves of infected zucchini plants accumulating 56% less than uninfected zucchini plants. In addition, the P level in stems and petioles of infected zucchini plants was reduced by 29%. However, the level of Ca accumulated in different parts of infected zucchini plants was increased by 81%. Infection doubled the amount of Ca in leaves and increased that in roots by 26%. However, fruit of infected zucchini plants accumulated less Ca. Infection resulted in increased levels of Mg in leaves (+45%), while reducing that of roots and fruits (-62% and -34%, respectively) (Figure 7.7).

Plants of zinnia infected with *G. cichoracearum* accumulated higher levels of Si in their roots and leaves than similar tissues of disease-free plants. However, other parts of infected and non-infected plants accumulated similar levels of Si. Similarly, infected plants showed increased levels of Ca and Mg in their different parts than the uninfected plants. Infection with zinnia with *G. cichoracearum* did not have a major impact on the total amount of K and P accumulated by this plant, although the level of P in leaves was reduced slightly. The effect of infection on accumulation of different elements by zucchini and zinnia plants are presented on Figures 7.7.

7.4 DISCUSSION

To our knowledge, this is the first investigation to determine the uptake and distribution of Si, K, Ca, P and Mg to different parts of zinnia and zucchini plants. The analytical techniques employed in this study provided unbiased and reliable information on the concentration of different elements in different tissues of both plants. EDX analysis was faster and required lesser chemicals in comparison to the ICP-OES. However, ICP-OES has a higher precision and lower detection limit than EDX (Einhäuser, 1997). This is because the sample size taken by EDX is often small and superficial. For instance, if the reading is taken from the surface of

a leaf, the value may vary depending on the presence of the pathogen on the leaf. In some cases, where the leaf was covered by the pathogen, the Si values were much higher than that of a disease-free leaf. In addition, the data obtained from EDX was relative (i.e. the graph represents a percentage of each element from the total in consideration). Moreover, depending on the age of the plant, distribution of Si on both sides of the leaf can differ (Hodson and Sangster, 1988), with older tissues accumulating more Si than the young tissues (Ma and Yamaji, 2006). According to these authors, the increased accumulation of Si in specific tissues is related to immobility of Si in older tissues. Therefore, it is not possible to make firm conclusions on the exact amount of a specific element based on EDX assessment alone. Despite these limitations, the EDX can give useful information about a sample's composition and its elemental distribution (Einhäuser, 1997). Furthermore, it provides an opportunity to observe the effect of Si treatments on the morphology of the pathogen and the plant, as well identifying the distribution of the element in specific plant tissues. The main advantage of ICP over EDX was that it provided accurate results by taking representative samples, while minimizing the level of sampling error. Hence, only the results of the ICP analyses were considered for interpretation.

Although the mechanism of uptake and distribution of Si in different plants remains poorly understood, Epstein (1999) suggests that Si is absorbed by plant roots in the form of monosilicic acid $[Si(OH)_4]$ and translocated to different parts of the plant through the xylem (Hodson & Sangster, 1989), making for uneven distribution within shoots (Ma and Takahashi, 2002; Ma and Yamaji, 2006). In monocots, once silicic acid is translocated from roots to shoots, it becomes more concentrated as a result of loss of water through transpiration and polymerizes to form silica gel $(SiO_2.nH_2O)$. In most monocots, polymerization starts once the concentration of $Si(OH)_4$ in the xylem exceeds 2 mM, although higher concentrations are needed in some plants such as rice and wheat (Ma and Yamaji, 2006). If Si is deposited in a polymerized form, it is not available for redistribution or for physiological activity (Ma and Yamaji, 2006). However, these studies were conducted in monocots, which routinely deposit Si as phytoliths in their leaves. In contrast, most dicots do not deposit phytoliths (some cucurbits are the exceptions), and it is therefore unlikely that the same process applies to dicots.

Leaves have been the main organ selected for analysis when determining uptake and accumulation of elements by plants (Adatia and Besford, 1986; Ranganathan *et al*., 2006; Samuels *et al*., 1991b). However, such observations may lead to biased and inconclusive results because elements are distributed to different organs of plants at different levels. Even when samples are being taken only from leaves, their orientation can affect the outcome. Adatia and Besford (1986) discovered that leaves of cucumber positioned at the bottom of shoots had a higher Si content than the top leaves. Such differences might be related to differences in the age of the leaves, or to their proximity to the source of Si (Ma and Yamaji, 2006). In young leaves of wheat, Si is mainly concentrated in the abaxial (lower) epidermal cells, whereas in old leaves both abaxial and adaxial (upper) epidermal cells have the same levels of Si (Hodson, and Sangster, 1988). Moreover, Epstein (1994) noted that distribution of this Si in different parts of the roots and reproductive parts is often variable. In this study such variables were avoided by taking representative samples of each organ from the entire plant and blending it thoroughly after drying.

Accumulation of Si by some plant species may reach levels, or higher, than recognized plant macronutrients such as P, K, Mg, Ca and S (Epstein, 1994 & 1999). Our investigation showed that there was accumulation of Si in both plants. Even in the control, where no Si was added into the nutrient solution, the levels of Si in the roots and leaves of zinnia and zucchini plants were higher than that of Ca, P, K and Mg. We believe that these plants obtained the element from the composted pine bark and water, which were used in the growing system. This is in agreement with the observation that Si is ubiquitous in nature (Epstein, 1994 & 1999).

Regardless of the level of Si in the nutrient solution, the daily uptake of the element by both plants was related to their water consumption. However, the fact that both plants reached saturation at a certain level of Si in the solution demonstrates that they absorb the element actively rather than passively. The difference in accumulation of Si by different species of plants has been attributed to differences in the ability of the roots to take up this element (Ma and Takahashi, 2002). The uptake, translocation and polymerization of Si in rice is affected by the transpiration rate and other metabolic activities of the plant (Ma and Yamaji, 2006; Rains *et al*., 2006). However, Ma and Yamaji (2006) have identified a Si transporter gene in rice that is believed to be responsible for increased accumulation of Si in this plant.

In zucchini and zinnia plants, Si was mainly accumulated in roots and leaves. In contrast, the remaining parts of these two plant species accumulated relatively little Si. High levels of Si in the root were assumed to be as a result of a slow rate of translocation of the element from the roots to shoots, or that the amount of Si required by other organs of the plant had reached its saturation stage. A high level of Si in leaves might be because of high transpiration rate of this organ (Raven, 2001), coupled with other metabolic activities that take place in this part of the plant (Liang *et al*., 2005; Rains *et al*., 2006).

Although both plants accumulated more Si as the levels of Si in the nutrient solution were increased, zucchini attained its optimal level of Si with a lower level of Si in the nutrient solution than zinnia. Supplying Si at levels as low as 50 mg ℓ^{-1} increased the growth of zucchini because the uptake of most of the elements studied was maximal at that level of Si. Adding Si at > 50 mg ℓ^{-1} did not result in increased growth because the levels of most of the other macronutrient elements in the plant remained almost constant. With cucumber, application of Si increased the weight of leaves of the plant without affecting their size (Adatia and Besford, 1986). According to these authors, leaves of Si- fed plants were stronger than the control because they had higher chlorophyll contents. Surprisingly, the effect of Si on the growth of zinnia was not noticeable. It is possible that the difference could not be detected easily because the plant does not produce a big crown. Accumulation of Si and K in the petiole and stems of Si supplemented plants was not different compared to the control. In addition, there was no apparent difference in the morphology of the zinnia flowers as a response to the increased supply of Si in the nutrient solutions. Plant size also appeared similar. However, this may have been because the beneficial effects of Si treatment of plants are usually expressed when the plants are subjected to various stress conditions (Epstein, 1994). However, in this study, plants were not stressed because they were raised under optimum growth conditions and kept free of diseases, except PM.

In addition to protection against biotic and abiotic stresses, Si has also been shown to strengthen the plant by thickening its stem and stiffening the leaves (Takahashi, 1995). This role can be of great importance especially for zinnia and other ornamental plants, and vegetables, where their ornamental value or their fruit quality are affected by the ability of the

plant to stand erect and support its organs properly. Application of Si can also improve the yield of zucchini, as well as increasing the quantity, as has been mentioned for other crops (Datnoff *et al*., 1997; Ma, 2004; Ma and Takahashi, 2002; Samuels *et al*., 1993).

Although the mode of action by which Si exerts its protective effects against diseases and pests are complex and remain controversial (Fawe *et al*., 1998; Epstein, 1994; Ghanmi *et al*., 2004), the relationship between the accumulation of Si in specific areas of the plant and consequent disease protection remains of great importance. Therefore, accumulation of a high level of Si in leaves of both plants may protect these plants against PM and other foliar diseases.

Adding Si into the nutrient solution did not affect the elemental composition of fruits of zucchini. With the exception of P, accumulation of all other elements (i.e. Ca, Mg, K and Si) was slightly increased by supplying the plant with low levels of Si (i.e. 50 mg ℓ^{-1}). As the concentration of Si in the nutrient solution was raised beyond 50 mg ℓ^{-1}, the levels of Ca and P in zucchini fruit were slightly lower than that of the control, while the level of Mg remained unaffected and levels of both K and Si increased slightly. Even increased in K and Si levels in response to extra Si application were negligible.

Time of flowering and fruit settings and the size of the fruit were not affected by Si supply in the solution. In addition, visual estimations were that the colour and texture of Si-treated and untreated zucchini fruit were the same, suggesting that treatment with Si did not have major impact on the characteristics of the zucchini fruit. Contrary to our findings, a study by Bélanger *et al*. (1997) reported that application of Si at > 100 mg ℓ^{-1} increased the cucumber yield, but at the same time, it hardened the fruit of cucumber, resulting in poor fruit quality which limited farmers to using Si at low levels. Samuels *et al*. (1993) found that cucumber plants produced unusual, dull appearing fruits, when they were grown in hydroponics supplied with Si. Therefore, the response of cucurbits to Si treatment may vary among species or even the cultivar level (Ago *et al.,* 2008). Hence, no generalization can be made on each plant species and cultivar without proper testing.

Elemental analysis of the whole plant showed that application of 100-150 mg ℓ^{-1} Si was optimal for zinnia. Application of Si at 150 mg ℓ^{-1} provided effective control of PM and resulted in accumulation of high levels of Si in all parts of the plant. Moreover, concentrations of Ca, P, K, and Mg in all parts of the plant were little affected at that level. For zucchini, supplying Si at 200 mg ℓ^{-1} resulted in the highest level of Si accumulation in roots, although leaves could accumulate more with an extra supply of Si. However, the differences in accumulated Si as a result of applications of 50-400 mg ℓ^{-1} were small, indicating that translocation of Si from roots to leaves was not increased significantly as a result of increasing the supply of Si. Since our main objective was to control PM, the impact of lower levels Si on growth and composition of zucchini and zinnia were not investigated. We believe that when the risk of infection by PM is minimal, the use of Si at lower levels (i.e., < 50 mg ℓ^{-1}) can increase the growth of both plants because accumulations of most of the elements studied seemed to be optimal. However, when the risk of PM is high, Si should be used at higher rates (100-150 mg ℓ^{-1}). This level could be altered, based on the need to balance the uptake and distribution of other elements, which ultimately determines the health of the plant. For example, K was part of the solution (i.e. KSi) and its level in the plant was increased by increasing the concentration of potassium silicate applied. However, this can result in reduced uptake of Ca, Mn, Fe and other elements by the plant (Ma and Takahashi, 1993). For instance, uptake of Ca by rice plants was reduced when Si was added into the nutrient solution (Ma and Takahashi, 1993). With cucumber, reduced uptake of Ca as a response to Si supply resulted in increased growth of the plant (Marschner *et al*. (1990). However, adding Ca to the solution did not affect uptake and distribution of Si in rice (Ma and Takahashi, 1990).

Previous research on the effects of Si on uptake and accumulation of P in various plants provided contradictory results (Ma and Takashi, 1990; Islam and Saha, 1969). This was because Si is possibly involved in the metabolic or physiological changes in the plants by promoting or suppressing uptake and transportation of selected elements, depending on the stress conditions (Liang, 1999). Islam and Saha (1969) discovered that the application of Si resulted in increased levels of P, Ca and Mg in rice. Interestingly, Ma and Takashi (1990) found that the concentration of Si in the shoots of cucumber was slightly reduced with increased level of P in the same organ, although uptake of Si was not significantly affected by the presence of P in the nutrient solution. They concluded that Si could increase availability of

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P when Si is deficient or reduce uptake of P when levels of Si are high, reflecting that Si plays a major role in balancing P uptake. Within a small range, adding low levels of Si to the nutrient solution improved accumulation of P in most parts of both plants studied here.

Infections of zinnia and zucchini with PM resulted in increased accumulation of Si and Ca in their leaves. Similar observations have been reported for various plant species (Samuels *et al*., 1991b; Koga, 1994: Cherif *et al*., 1992a). Even in the same leaf, the concentration of Si around infected areas was higher than disease-free areas. This agrees with previous investigation by Menzies *et al*. (1991) and Samuels *et al*. (1991b) who demonstrated that infection of the plant with the pathogen results in increased accumulation of Si in the leaves. These authors observed high levels of Si at the infection sites and around the hyphae of the pathogen. Our observations using EDX mapping gave extra information, showing conclusively that Si was accumulated in the leaf and not in the pathogen. The conclusion was based on the observation that when the concentration of Si in the pathogen and the leaf tissue (infected site) were compared using contrast mapping, Si levels were low in the pathogen, but were at high levels in leaf tissue adjacent to PM hyphae. Even though the total uptake of K and Mg by both plants was not affected by infection, their accumulation on leaves was increased by infection. This indicates that they may also have some roles in disease control. Regardless of infection, the highest concentration of Si was observed at the base of trichomes, confirming observations of other researchers (Iwasaki and Matsumura, 1999; Samuels *et al*., 1991a, 1991b & 1993).

Leaves of zucchini and zinnia infected their respective pathogens accumulated more Ca and less P than leaves of uninfected plants. Similar observations were reported for other crops infected with different pathogens (Goodenough and Maw, 2008; Kalamera and Heath, 1998). Reduced uptake of P by infected plants may have been affected by the increased uptake of Ca by the infected plant. Zhang *et al*. (2006) showed that the uptake of P was reduced when plants were supplied with calcium silicate (CaSiO). It has been noted that under stress conditions, Si enhances absorption of Ca by the plant at the expenses of Fe and Mn (Islam and Saha, 1969; Liang, 1999). However, whether the increase in Ca content of infected leaves is related to the expression of the resistance to PM or not is still not clear.

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CHAPTER EIGHT

EFFECTS OF SELECTED BIOCONTROL AGENTS AND SILICON ON POWDERY MILDEW OF ZINNIA PLANTS GROWN HYDROPONICALLY

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Abstract

Five selected biocontrol agents, and soluble silicon (Si) at various concentrations, were tested for their ability to control powdery mildew of hydroponically grown zinnia plants. Biocontrol treatments were applied before infection and continued weekly, while Si was supplied to plants continuously. Both BCAs and Si reduced the severity and AUDPC values of PM significantly. Application of BCAs resulted in reductions in severity 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 soluble Si (50-200 mg ℓ^{-1}). It is proposed that the provision of a continuous supply of Si and the ability of zinnia to accumulate high levels of Si in its leaves were the major reasons for the good response of zinnia plants to Si treatments against PM. Silicon played a protective role before infection and suppressed development of PM after infection. The combination of the tested BCAs and Si can be used as effective control options against PM of zinnia grown hydroponically.

8.1 INTRODUCTION

Zinnia (*Zinnia elegans* L) is a popular ornamental flower grown in many parts of the world. Production of attractive flowers, fast growth, a short production cycle and minimal labor requirements makes zinnia a commercially important crop as a cut flower (Pinto *et al*., 2005), as well as a popular bedding plant. However, its susceptibility to several diseases often reduces its value as an ornamental plant (Linderman and Ewart, 1990). Powdery mildew (PM), caused by *Golovinomyces cichoracearum* (DC.) VP Heluta is one of the common diseases of zinnia, resulting in plant losses and decreased ornamental values by weakening plant growth, resulting in poor flowering (Boyle and Wick, 1996; Kamp, 1985).

The use of fungicides to control PM of annual bedding plants is limited by their toxicity to the flower crop (Kamp, 1985) and other non-target organisms. Furthermore, development of fungicide resistance by several species of PM fungi as a response to intensive use of fungicides has also been a major challenge (McGrath, 1996; McGrath, 2001; McGrath and Shishkoff, 2001; O'Hara *et al*., 2000; Wong and Wilcox, 2002). As an alternative approach to the control of PM of zinnia, Kamp (1958) successfully used a polymer-based anti-transpirant. Similarly, applications of biocontrol agents (BCAs) and soluble silicon (Si) to PM susceptible crops have produced promising results in suppressing the disease caused by different PM species, under controlled environmental conditions (Dik *et al*., 1998; Guével *et al*., 2007; Liang *et al*., 2005; Menzies and Bélanger, 1996; Verhaar *et al*., 1996, 1997). However, in spite of the challenges that PM poses to growers of zinnia, no alternative control measures have been developed to date to supplement or replace fungicides.

Based on prior research, the objectives of this study were to test the efficacy of five selected biocontrol agents, and Si applied at various concentrations, for control of PM and to assess whether a continuous supply of Si could improve its efficacy. Zinnia was selected as an ornamental test plant for this study under hydroponics condition because of its relatively small size and its susceptibility to PM.

8.2 MATERIALS AND METHODS

8.2.1 Preparation of plants

Seeds of Zinnia (*Zinnia elegans* L*.,* cv. Jakobrekop Sunbow), obtained from McDonald Seeds[1], were planted in Speedling[®] trays containing composted pine bark and transferred into a greenhouse tunnel operating at 26-28°C and an RH of 75-85%. Trays were irrigated with a balanced fertilizer [3:1:3 (38) from Ocean Agriculture[2] at 0.5 g ℓ^{-1}] + [Ca(NO$_3$)$_2$ at 0.5 g ℓ^{-1}] for 3 wk. Seedlings were transplanted into pots (150 mm diameter) once they had produced 2-3 well developed leaves and attained a height of approximately 50-70 mm. Thereafter, pots were transferred into a greenhouse (mean temperature, 28°C and RH of 60-70%). All pots were placed into horizontal mini troughs with the nutrient solution being supplied in a re-circulating system (Figure 8.1).

8.2.2 Design of the hydroponic system

Horizontal mini troughs, constructed from 1mm thick black plastic, were placed on a table. The troughs were arranged in alternating directions to provide enough space for the reservoirs (8ℓ) to fit next to each other. Each reservoir contained a Project Powerhead submersible pump (100w) fitted to supply lines which carried the nutrient solution to the top end of the trough from where it flowed through the trough and back to the reservoir (Neumann, 2003). The pumps ran continuously and the level of nutrient solution in each reservoir was maintained by adding the nutrient solution as needed. Where Si was used as a treatment, its concentration in the nutrient solution was set at 50, 100, 150 and 200 mg ℓ^{-1} by adding KSi. Finally, the seedlings of zinnia, in pots, were placed into the troughs with each row containing four or five pots for the first and second trials, respectively.

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[1] McDonald Seeds (Pty) Ltd, P. O. Box 238, Pietermaritzburg, 3200, South Africa

[2] Ocean Agriculture (Pty) Ltd., P. O. Box 742, Muldersdrift, 1747, South Africa.

Figure 8.1. Horizontal mini troughs and their accessories used to supply nutrient solution to the plants in a re-circulating system.

8.2.3   Preparation of biocontrol agents

Microbial suspensions of three *Serratia marcescens* (Bizio) isolates (i.e., Isolates B15, Y15 and Y41) and two fungi (*Clonostachys rosea* (Link) Schroers, Samuels, Seifert & Gams (syn. *Gliocladium roseum*) (Isolate EH) and *Trichothecium roseum* (Pers.) Link (syn. *Cephalothecium roseum*) (Isolate H20)) were prepared as described in Chapter 3. Biocontrol agents were applied to plants once a week at a concentrations of 10^8 and 10^6 spores mℓ^{-1} for bacterial and fungal BCAs, respectively. Treatments with BCAs were applied 2 d before plants were inoculated with PM, and repeated 4 d after the first symptoms of PM developed and continued weekly. To enhance survival of BCAs on the phylloplane, spraying was commenced during the late afternoon using hand sprayers.

8.2.4   Preparation of powdery mildew inoculum and inoculation technique

Seedling of zinnia were prepared as described in the above and put into ice cream containers (2ℓ) and kept in an open environment to allow natural infection by placing them in batches, placed at different positions in the Controlled Environmental Facility (CEF) of the university. Plants were watered manually by drenching them with a complete nutrient solution. After the first symptoms of PM, infected plants were kept in a separate glasshouse to enhance development of the disease and to avoid contamination between experiments. The inoculum

was maintained by infecting fresh plants through direct contact of infected and uninfected leaves.

Three inoculation techniques were tested: (1) conidia of PM were sprayed onto both sides of zinnia leaves until run-off, using a hydraulic hand sprayer; (2) dusting of dry conidia collected from infected leaves onto disease-free plants; and (3) physical contact between infected and disease free leaves. However, the spray technique of inoculation did not result in infection of zinnia. Therefore, the last two methods were combined as the standard inoculation technique for zinnia. Infected leaves of the same age were collected from the source plants, and one infected leaf was used to infect one plant by dusting the conidia using a brush and by gently rubbing the infected leaf against 3 leaves of the test plant. Inoculation was performed during the late afternoon to enhance establishment of the disease.

8.2.5 Disease assessment

Percentage of leaf area covered by PM was assessed weekly before spraying of BCAs for 5 wk, and AUDPC was calculated using an AUDPC Program (Shaner and Finney, 1977). The final disease level recorded at the end of the experiment was recorded as the final disease level.

8.2.6 Statistical analysis

The experiment was conducted twice, with each treatment having four replications in the first trial, and five replications in the second trial. The arrangement was determined by access to space in the hydroponics system. Because of their homoginuity, the results of these two trials were mixed together and ANOVA analysis was performed using GenStat[(R)] Statistical Analysis Software (GenStat, 2006). To reduce the coefficient of variation, data was transformed a using square root transformation. Means of treatments were compared using Fisher's Protected LSD at $P < 0.05$. Percentage reduction in disease severity and AUDPC values were calculated by comparing each treatment against the control.

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8.3 RESULTS

Symptoms of PM started to appear on the leaves one week after inoculation and developed slowly. After 5 wk, disease level of the control plants reached > 45% in the first trial and 31% in the second trial.

In both trials, the BCAs and Si had significant effects on the severity of PM and its AUDPC values. In the first trial, all BCAs reduced the severity of PM significantly, with reductions of 35-84%. Isolates EH and H20 reduced the severity of PM by 83% and 60%, respectively. Similarly, treatments with the Isolates B15, Y15 and Y41 caused significant reductions in disease severity of 35-76%. Among the three bacterial isolates tested against the disease, Isolate Y41 performed best. In the second trial, only Isolates EH, H20 and Y41 reduced the severity of PM significantly. Treatments with these three isolates provided disease reductions of 41-47%. Although statistically not significant, Isolate B15 and Isolate Y41 also reduced the final disease level by 30% and 34%, respectively. In both trials, application of BCAs reduced the AUDPC values significantly ($P < 0.001$). Among the BCAs tested, Isolate EH gave the best results in suppressing the development of PM (Figure 8.2).

Adding Si at concentrations of 50-200 mg ℓ^{-1} into the nutrient solution reduced the severity and AUDPC values of PM significantly. After one month of infection, the final disease level recorded for plants treated with Si was reduced by 86-100% and 75-90%, in the first and second experiments, respectively. AUDPC values were also significantly reduced when plants were treated with Si (Figure 8.2). The performance of Si was better in the first trial, providing disease control as much as 100% when Si was added into the nutrient solution at 150 mg ℓ^{-1}.

Table 8.8 Effects of selected biocontrol agents and silicon on the final disease level (FDL) and area under disease progress curve (AUDPC) of powdery mildew of zinnia, five weeks after inoculation with *Golovinomyces cichoracearum*

Treatments	FDL	AUDPC
Control	36.9 (5.92) [e]	358.2 (18.02) [e]
BCAs		
B15	22.8 (4.45) [d]	220.5 (13.86) [cde]
EH	12.1 (3.00) [bc]	126.4 (9.74) [bc]
H20	17.8 (4.07) [cd]	182.0 (13.06) [cd]
Y15	22.6 (4.53) [de]	250.8 (15.18) [de]
Y41	15.7 (3.47) [cd]	145.8 (11.00) [cd]
Silicon		
50 mg ℓ^{-1}	4.3 (1.92) [ab]	43.2 (6.04) [ab]
100 mg ℓ^{-1}	2.3 (1.29) [a]	21.4 (3.87) [a]
150 mg ℓ^{-1}	4.1 (1.39) [a]	31.9 (3.93) [a]
200 mg ℓ^{-1}	4.7 (1.82) [ab]	47.4 (5.69) [ab]
Effects	**P-values**	**P-values**
F	< 0.001	< 0.001
CV%	46.8	46.6
MSE	2.222	21.885
FLSD	1.398	4.389

Means within column followed by a common letter were not significantly different according to Fisher's protected least significant difference ($P < 0.05$).

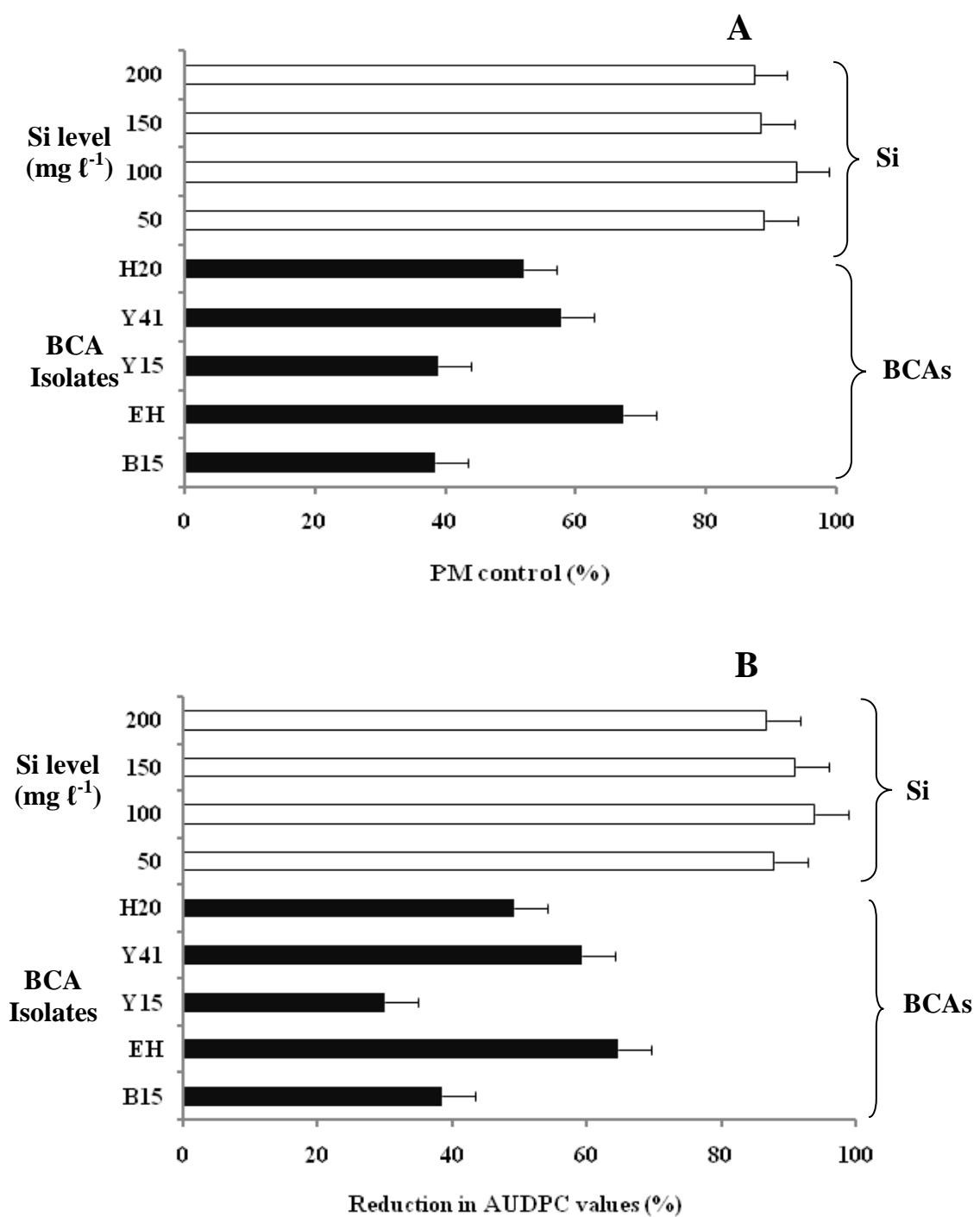Values in brackets are means of data transformed using a square root transformation.

Figure 8.2 Percentage reductions in the disease severity (A) and Area under disease progress curve (AUDPC) (B) of powdery mildew of zinnia after five weeks of treatment with five biocontrol agents soluble silicon, compared to a control inoculated with *Golovinomyces cichoracearum*.

8.4 DISCUSSION

Unlike other crops, where inoculation PM fungi can be performed by spraying on a conidial suspension of the pathogen in water, inoculation of zinnia with conidial spray onto leaves was not effective. It has been reported that high leaf wetness has detrimental effects on the germination of conidia of PM fungi (Bushnell and Rowell, 1967; Quinn and Powell, 1982; Sakurai and Hirata, 1959; Sivapalan, 1993). In this study, even though the time between harvest of conidia and spraying was minimized to less than 30 min and a high concentration of conidia was used, the inoculation method did not produce consistent infection, indicating that this pathogen was sensitive to water. Therefore, dusting conidia by shaking the infected leaf over the test plant, and physical contact between infected and disease-free leaves were used as standard technique of inoculation. Although these techniques lacked information on the exact amount of inoculum used to cause a certain level of infection, both techniques consistently produced a level of infection that developed to $> 45\%$ within one month after inoculation.

From the hydroponic trials, it was demonstrated that all the tested BCAs and Si reduced the level of PM significantly. In both trials, the level of control obtained from Si treatments was better than that of BCAs. If zinnia plants absorb Si before infection, then they become highly resistant to infection by *G. cichoracearum*. Richmond and Sussman (2003) noted that plants treated with Si before infection displayed an accelerated activation of SAR resistance of the plant, thereby inducing fungal cell death. In contrast, although some BCAs which can antagonize the pathogen within one week after application (Sundheim and Krekling, 1982), most antagonists require an average of 10-14 d to establish themselves on the phylloplane and to start their antagonistic activities (Verhaar *et al*., 1997). Therefore, by the time the BCAs started their activities, the pathogen could have already covered a large part of the phylloplane, making biocontrol less effective. In previous chapters, fast development of the disease versus slow establishment of the BCAs has been shown to be one of the challenges in controlling PM with BCAs. To overcome a similar problem, Verhaar *et al*. (1997) suggested that BCAs should be used as preventatively (i.e. applied approximately 1 wk before mildew inoculation) or as an early curative treatment (within 2 d after PM inoculation).

Both BCAs and Si gave some control of PM. Most isolates tested in this study showed promising performance by reducing the severity of PM and suppressing its progress. Among the BCA isolates tested, Isolate EH performed best, providing disease control that was comparable to that provided by Si. In spite of fluctuations in disease levels between the two experiments, the relative efficacies of all isolates were almost constant and were less than Si treatments.

Several researchers have reported that treatments with Si produced promising results in controlling PM of different crops (Bélanger *et al*., 1995; Guével *et al*., 2007; Menzies *et al*., 1991). However, most of these researchers indicated that the level of control was usually incomplete. In contrast to these reports, our results demonstrated the possibility of obtaining complete control of PM when zinnia plants were grown in hydroponics supplied with Si. Unlike hydroponics, where a plant is supplied with a nutrient solution continuously, in most glasshouses and field trials, Si is usually supplied to plants as a spray or drench, at limited frequencies per week. Samuels *et al*. (1991) observed a rapid decline in Si-induced resistance against PM when cucumber plants growing with a Si containing solution were transferred into a Si-free solution. However, the same plant species supplied with Si continuously at a concentration of 100 mg ℓ^{-1} or higher, showed a reduction in PM severity by as much as 98% (Menzies *et al*., 1991). Similarly, as much as 100% control was obtained when zinnia plants were continuously supplied with Si (50-150 mg ℓ^{-1}), leading to a conclusion that for maximum disease protection, a continuous supply of Si is needed. This, according to Heine *et al*. (2006), is because Si-enhanced resistance of plants against diseases is linked to the maintenance of a high Si status in the plant. Another reason for the inconsistency in efficacy of Si is the effect of the soil. Soil pH and nutritional balance can determine the availability of Si to the plant. For instance, soils that have large amounts of Fe, Al, and Mn can bind Si and make it unavailable to the plant (M.D. Laing, 2008, pers. comm.).

In this study, complete control of PM was obtained where plants did not show any symptom of the disease one week after artificial inoculation with the pathogen. Once symptoms of PM were observed on the leaves, it was not possible to cure the plant completely regardless of the concentration of Si added into the nutrient solution, indicating that Si was more effective as

protective treatment. Although Si did not provide a complete curative treatment, it kept PM levels low, probably by inducing resistance of the plants against the disease (Bélanger *et al*., 2003; Fawe *et al*., 1998; Liang *et al*., 2005).

The response of zinnia to Si treatment for the management of PM was promising. To date, there is no information on the uptake and accumulation of Si by zinnia and subsequent disease suppression. In Chapter 7, however, we showed that leaves of zinnia could accumulate Si to levels of 10% of dry matter. Although the mode of action by which Si provides its protective role against plant pathogens remains a controversial issue (Fauteux *et al*., 2005; Ghanmi *et al*., 2004; Rodrigues *et al*., 2004 and 2005), reports by Ma and Yamaji (2006) indicated that there is a positive relationship between the amount of Si accumulated in the plant and the level of disease resistance. Therefore, the high level of PM control observed on zinnia by Si treatment could be the result of high uptake and accumulation of the element by this plant species.

The experiments were conducted as a preliminary testing of both control options and they were not used together as part of an integrated approach in managing the disease because there was a lack of space in the glasshouse. However, given the additive effects of BCAs and Si shown in our previous research, the promising results obtained in this study, suggest that co-application of these two components should provide better control of PM, leading to increased growth and quality of zinnia plants.

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CHAPTER NINE

GENERAL OVERVIEW

The impact of powdery mildew (PM) on crop production and the control strategies that have been used against this disease has been widely covered in the literature (Bélanger *et al*., 1997; McGrath and Thomas, 1996; Yarwood, 1957 & 1978). Increasingly, reports in development of fungicide resistance by a number of PM species, coupled with public concerns for environmental and health hazards, have made the use of fungicides less popular. To meet the need for safe and environmentally friendly control measures, biocontrol agents and soluble silicon have emerged as viable alternatives that can be used individually, or combined with other control strategies for integrated disease management (Bélanger *et al*., 1997; McGrath, 2001; McGrath *et al*., 1996).

The use of biological control against PM of several species has been well documented (Bélanger *et al*., 1997; Jarvis and Slingsby, 1977; Sztejnberg *et al*., 1989; Verhaar *et al*., 1996 & 1998). This has resulted in development of some promising biocontrol products on the market. However, their use has been limited due to their inconsistency under variable environmental conditions. To improve the efficacy of biocontrol agents (BCAs), which has been associated with their specific requirements for specific environmental conditions, various techniques have been used. To a certain extent, the lack of appropriate screening protocols, combined with insufficient environmental experiments during evaluation, and a lack of knowledge of the modes of action of the specific BCA, have all contributed to this problem. Similarly, promising progress has been made in the use of soluble silicon (Si) against PM and other diseases (Bélanger *et al*., 1997; Epstein, 1994 & 1999; Ma and Takahashi, 2002). In some plants, increased growth and yields have been reported as a result of enhanced resistance against biotic and abiotic stresses, especially when Si was applied to soils that were deficient in Si (Datnoff *et al*., 2001). The ability of Si to benefit plants without affecting the environment negatively has made it an "agronomically essential element" (Ma and Takahashi, 2002) or quasi-essential (Epstein, 1999). In spite of this, due to the incomplete and

inconsistent control it provides, the use of Si against PM and other pathogens has been limited. The main reasons for the low efficacy of Si are a lack of knowledge on the optimum application conditions (i.e., concentration, frequency and methods of application), mode of action in controlling the disease and the relationship between Si and the plant, and other biotic and abiotic factors that are involved in the production system.

The research presented in this thesis focused on the isolation and *in vitro* screening of antagonists, using a series of *in vitro* and glasshouses experiments, for the evaluation and improvement of the efficacy of BCAs and Si against PM under different environmental conditions. It was established that:

- At the preliminary stages, 29 BCA isolates reduced severity of PM by 30-77%. Under glasshouses and field conditions, five isolates (i.e., *Gliocladium roseum* (Isolate EH). *Trichothecium roseum* (Isolate H20) and 3 isolates of *Serratia marcescens* (Isolates B15, Y15 and Y41)) reduced the severity of PM by 50-90%.

- Break-Thru$^®$ (BK) at lower concentrations (i.e., 0.25 mℓ ℓ^{-1}) improved the efficacy of spray applications of BCAs and Si by enhancing their deposition on the leaves and the pathogen, and by directly affecting the pathogen. Break-Thru$^®$ was compatible with the zucchini and BCA isolates when used at < 0.40 mℓ ℓ^{-1}. However, at higher rates, it was toxic to zucchini and to the BCAs, especially Isolates EH and H20.

- When Si was applied as a foliar treatment, it inhibited PM through direct contact with the pathogen. Efficacy of Si spray was improved by increasing the spray frequency. Although no Si was absorbed directly through the leaf, part of the foliar treatment was absorbed by the plant roots as runoff and drift, and played a role in enhancing host resistance of the plant against PM.

- The efficacy of Si was better when added into the nutrient solution than when it was drenched onto roots of plants or sprayed on as a foliar treatment. In all these application methods, increased concentrations provided better disease control. Drenching 250 mℓ (under greenhouse conditions) or 1 ℓ (under field conditions) of Si at 100 mg ℓ^{-1} per week provided significant control of PM of zucchini. Spraying 750 mg ℓ^{-1} of Si also gave significant reduction in the severity of PM. When added into

the nutrient solution, 50-150 mg ℓ^{-1} of Si reduced the severity of PM of zinnia by 85-100%.

- A combination of BCAs + Si + BK provided better control of PM under both field and glasshouse conditions, and can be used for integrated management of PM.

- Adding KSi into the nutrient solution increased the levels of Si and K in zucchini and zinnia. It also enhanced the growth of zucchini without affecting morphological characteristics of the fruits. However, it did not cause obvious changes to the growth of zinnia plants and their flowers.

- Infections of zucchini and zinnia plants with PM resulted in increased uptake of Si and Ca by these plants, while reducing their P uptake. However, it did not affect the uptake of K and Mg in these plants.

The development of a proper isolation and *in vitro* screening protocol that provides rapid, repeatable and reliable results is an important initial step in screening effective antagonists for biocontrol of PM. Since the performance of biocontrol isolates on agar has less predictive value than *in vivo* tests (Verhaar *et al*., 1998), the screening protocol adopted in this thesis involved three components: the biocontrol agent (BCA), the pathogen and the plant itself. If one of these three components were to be missing, then the screening procedure would have been incomplete. Isolates that showed consistent results against PM of zucchini (*P. xanthii*) under different growth conditions also controlled PM of zinnia caused by *G. cichoracearum*, confirming a previous hypothesis that if a BCA isolate can antagonize PM of one species of plant, then it can often be used against PM species of different plants (Sztejnburg *et al*., 1989; Szentivanyi and Kiss, 2003). The process of screening and rating systems followed in this study can serve in developing a more comprehensive protocol, especially where isolates are to be screened against obligate pathogens, and their antifungal activity is not noticeable during *in vitro* assays.

Clonostachys rosea has been reported as mycoparasite of many fungal species (Kwasna *et al*., 1999) and could be used as BCA against PM (Sutton and Peng, 1993) and other foliar (Cota *et al*., 2008; Morandi *et al*., 2008; Yu and Sutton, 1997) and root diseases (Tarantino *et al*., 2007). *Serratia marcescens* has been identified as a growth promoting rhizobacterium that

produces several antibiotics and can induce resistance of plants against several diseases (Battaglino *et al*., 1991; Jeun, 2004; Kobayashi *et al*., 1995; Maji *et al*., 2003; Ordentlich *et al*., 1988; Roberts *et al*., 2005; Strobel *et al*., 1999; Sujay *et al*., 2003; Wei *et al*., 1996). Similarly, a report by Iida *et al* (1996) identified *Trichothecium roseum* as producing antifungal antibiotics and inducing host resistances in plants. In other research, it has provided promising results when tested against PM and other diseases (Hijwegen and Buchenauer, 1984; Huang *et al*., 2000; Vanneste *et al*., 2002).

All the adjuvants tested (i.e., Break-Thru®, Partner® and Tween-80®) improved spray efficiency of BCAs and Si by enhancing coverage of the spray and reducing the total volume. Both Break-Thru® and Partner® suppressed PM directly. However, Break-Thru® was chosen for further studies because it was more compatible with our BCAs and the plant. Electron microscopy investigations showed that Break-Thru® increased the deposition of BCAs on the pathogen and on the leaves. It also inhibited germination of conidia, and caused the collapse and disintegration of conidia and mycelia of the pathogen. The fact that it was biocompatible at lower dosages provides the opportunity to be used in the spray mix in controlling PM with spray applications.

Si was more effective against PM when supplied to plants as a nutrient solution in a hydroponics system than when applied as drench or foliar spray. For best PM control, plants need an uninterrupted supply of Si, which was manageable under hydroponics. Regular drenching of Si onto the roots of plants was also effective in reducing the severity of PM of zucchini under both glasshouse and field conditions. When Si is added to the soil as a fertilizer, it usually improves the resistance of plants against PM, and other biotic and abiotic stresses of the plant, resulting in increased growth and yield of the plants. These benefits are noticeable when the plants are grown under sub-optimal conditions (Datnoff *et al*., 2001). However, if the aim is to control PM, then Si should be applied before infection because once the plant is infected, curative treatment with Si is less effective (Liang *et al*., 2005). In addition, continuity of supply of Si to plants is more important than the total volume or level of Si in the solution. Therefore, to improve the continuity of plant-available Si in the fields, the development of slow release formulations of Si, or increasing the application frequencies

are recommended. As a foliar treatment, increasing the concentration and frequency of Si applications provided the best control of PM. This was partly due to increasing availability of Si to plant roots via drift and runoff. Spraying Si three times per week at lower levels of Si (i.e., 250mg ℓ^{-1}) provided the best results while reducing the total amount of Si that gave equivalent PM control when applied once per week. When KSi is used as a foliar treatment against PM, Si has been identified as the active ingredient of the solution (Menzies *et al*., 1992). Direct contact between Si and the pathogen, or the changes in osmotic characteristics of the leaf created by Si deposition, are one of the mechanisms of disease control (Liang *et al*., 2005). In addition, increased deposition of Si on the leaf surface can prevent penetration of fungal hyphae into the host (Bowen *et al*., 1992). However, there is no evidence that shows penetration of the leaf surface by Si directly. Therefore, any priming of resistance of the plant by foliar applications of Si can only be the result of Si that is absorbed by plant roots after the element is intercepted by the plant roots as a result of runoff or drift.

Although the use of BCAs and Si against PM has been studied intensively, there are not many studies that incorporated both control options as part of an integrated control package. Combinations of BCAs and Si were more effective, and should result in a better PM control than when these two control measures are used separately. As expected, better control was obtained under both glasshouse and field conditions, when BCAs were applied with Si. The additive effects were probably due to the combination of different modes of action that affect the pathogen, as opposed to the fewer control mechanisms provided by the BCA or Si. Unlike most fungicides, Si has a broad spectrum of activities that affect economically important diseases (Datnoff *et al*., 2001). Therefore, the use of BCAs and Si for disease control should be developed and practiced in an integrated disease management programmes.

The main challenge in the use of BCAs and Si against PM has been the speed of PM development. In this study, the use of Break-Thru® at lower concentrations improved the performances of BCAs and Si spray by reducing the rate of PM development and contributed substantially towards an integrated programme under which BCAs and Si can actually control this disease. It might be possible to use combination of BCAs + Si + Break-Thru® against other foliar diseases of economic crops. Treatment with Si resulted in less infection of the

plant and the presence of Break-Thru[®] weakened the pathogen, increasing the vulnerability of the pathogen to attacks by the antagonists. If the combination fails, the addition of 1-2 sprays of effective, systemic fungicides may provide better control, leading to an integrated disease management programme that would minimize the frequency of fungicide application, reduce the risk of fungicide resistance, and reduce environmental pollution, while providing a high level of PM control.

In using Si, a lack of knowledge on optimum concentrations for optimal disease control and plant growth, without compromising quality has been the main challenge. Epstein (1994) and Feng (2004) have noted that disease resistance is linked with the amount of Si accumulated by the plant, which is species and cultivar dependant, and is linked to the level of physiologically available Si. Although high levels of Si provide effective PM control, at high concentrations, Si can reduce the quality of fruits of some crops (Bélanger *et al*., 1995 & 1997; Lieten *et al*., 2002). In this study, the use of Si at 100-150 mg ℓ^{-1} as a soil drench, or in nutrient solutions, provided effective control of PM on zucchini and zinnia. Increasing the level of Si in the nutrient solution resulted in an increased accumulation of Si in roots and leaves of both plant species, without affecting its distribution to other parts of these plants. The fact that growth of zucchini and the uptake of most other elements in both plants were optimal when Si was added into the nutrient solution at 50 mg ℓ^{-1} showed that, where the likelihood of infection by the PM fungi is low, then application of Si at lower levels (50 mg ℓ^{-1}) can improve growth of the plant, while minimizing the cost of Si applications. However, since the experiment was conducted under optimal condition, these results need to be confirmed on different species of plants growing under different growth conditions.

Electron microscopic observations on increased accumulation of Si in the base of trichomes and around the leaf areas infected by PM confirmed previous reports by Menzies *et al*. (1991) and Samuels *et al*. (1991a & b). The role of Si in infected plant was to priming defense mechanisms of the plant (Cherif *et al*., 1992). Leaves of zucchini and zinnia infected by their respective pathogens accumulated more calcium and less phosphorus than leaves of uninfected plants. Similar observations were reported on other crops infected with different pathogens (Goodenough and Maw, 2008; Kalamera and Heath, 1998). Cell wall strengthening and thickness are some of the functions of Ca (White and Broadley, 2003). However, whether the

increase in Ca content of infected leaves is related to the expression of the resistance to infection or not is still not clear.

The way forward

- This study presents some promising isolates that have the potential to be used against PM and other foliar diseases. Some of them may also be used as growth promoters and in priming the resistance status of the plant (Jeun *et al*., 2004; Kobayashi *et al*., 1995; Iida *et al*., 1996). However, before these BCAs are developed further for commercialization, a series of studies is needed to assess their efficacy against other pathogens under various conditions. More investigations on the formulation, shelf life and cost-benefit analysis of these isolates are needed before they are released as bio-products. Toxicological studies will also be essential.

- Most of the trials on Si were conducted under controlled growth conditions, where the plants were almost free of stresses except for the infection with PM. We believe that effects of Si would have been more visible if the plants were grown under more stressful conditions.

- Most available information on uptake, transportation and accumulation of Si in plants are based on studies conducted in monocots. It is not known whether the same principle applies to dicots or not. Since the form of Si inside the plant is more important than total, more investigations is recommend on dicots in order to determine the mechanisms of uptake and levels of different forms of Si in different plants species.

- In our study, the level of Si application did not cause obvious effects on the quality of the fruits of zucchini. However, since this effect may differ from species to species, it is important to assess this effect on other crops and determine the impacts of the fluctuation of selected nutrients in the fruit quality as influenced by Si application.

- It is only recently that Si has been studied as an option for disease management. It is concluded that much research is still needed to fully understand the impact of this element on plant metabolic activities. Even in areas where much research has been conducted, e.g., disease control mechanisms, there is still much to be learned. However, since the effects of

Si on plants seemed more complex than it was initially imagined, inter-disciplinary research is needed in order to exploit the full potential of Si for control of abiotic and biotic stress, and to prpmote plant growth.

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