# SOLUBLE SILICON-BASED DISEASE MANAGEMENT OF FLORICULTURAL CROPS

By

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OF FLORICULTURAL CROPS

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The goal of this study was to evaluate soluble silicon supplementation as a tool for disease management of greenhouse-produced floricultural crops. I hope that the gained information coming out of this study will be helpful and beneficial for greenhouse floricultural production around the world.

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# CHAPTER I

# INTRODUCTION

Silicon (Si) is the second most abundant element in soils and its concentration in plant dry matter ranges from 1% to 10% or higher. However, it is not considered an essential element for most plants, with the exception of some Equisitaceae members (Epstein, 1993). Based on the essentiality criteria given by Arnon and Stout (1939), an element can be considered 'essential' when it has a unique biochemical role which cannot be substituted by other elements, and when its omission causes abnormalities and incompletion of the life cycle. Silicon does not fulfill these criteria in most plants.

However, the prophylactic effects of Si supplementation have been reported against numerous plant diseases. Many of the studies demonstrated Si-mediated protection against the powdery mildews, *Sphaerotheca fuliginea* (Schltdl.) Pollacci, *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff, and *Blumeria graminis* f. sp. *tritici* (DC.) Speer, of cucumber (*Cucumis sativus* L.), pumpkin (*Cucurbita pepo* L.) and wheat (*Triticum aestivum* L.), respectively (Menzies et al., 1991; Heckman et al., 2003; Bélanger et al., 2003). Other pathosystems in which Si supplementation is documented to inhibit disease progression, are pythium root rots of cucumber, and rice blast caused by *Magnaporthe grisea* (Cherif et al., 1992; Cherif et al., 1994; Kim et al., 2002).

Silica accumulation in cell walls is believed to physically impede pathogen infection resulting in plant disease resistance (Ma and Takahashi, 2002). Recent studies report Si may act as a potential signal that activates defense mechanisms and stimulates production of low molecular weight phenolic compounds (Cherif et al., 1994; Datnoff et al., 2007). This additional defense elicitor function does not necessarily exclude mechanical resistance as the primary mechanism of Si-mediated disease suppression. Silicified epidermal cells may mechanically inhibit pathogen penetration sufficiently to permit host cells time to detect and respond to a normally virulent pathogen with induced defenses, thus appearing that Si is acting directly.

In 2006, the estimated wholesale value of US floriculture was estimated at nearly $4 billion (www.ers.usda.gov). Development of pathogen resistance to commercially used fungicides and consumer demands for sustainable crop production have increased interest in alternative solutions to disease management.

The goal of this research was to assess the efficacy of various soluble silicon supplements for the control of three important fungal diseases of greenhouse floricultural crops, both alone and in combination with two rates of standard registered fungicides. The three diseases assessed were as follows: powdery mildew of zinnia and sunflower caused by *Golovinomyces (=Erysiphe) cichoracearum* (DC.) V.P. Heluta, gray mold of sunflower caused by *Botrytis cinerea* Pers., and Phytophthora root rot of gerbera caused by *Phytophthora drechsleri* Tucker. If favorably assessed, inclusion of soluble silicon in the disease management programs of floriculture producers would provide an inexpensive input with marginal environmental impacts.

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# CHAPTER II

# REVIEW OF LITERATURE

## Introduction

One of the biggest challenges during greenhouse floriculture production is the prevention and control of diseases that can cause severe or complete losses of entire crops. Powdery mildew is one of the most economically important diseases affecting greenhouse floricultural crops and a wide variety of protectant and systemic fungicides are often used to manage the causal fungi. *Botrytis* diseases, gray mold and blight, are the most common diseases in commercial greenhouse production, since the causal pathogens are favored by high humidity conditions and can grow in a wide range of temperatures. Phytophthora root rot is also a very threatening disease, especially in greenhouse production, since irrigation systems readily disseminate *Phytophthora* species' water-borne zoospores. Below a concise overview for each of the pathogens causing these economically important diseases is presented, including information about their biology, taxonomy, and available chemical and alternative management practices, with an emphasis on Si-associated disease suppression.

## A. Powdery mildew

### *Biology*

Powdery mildews are some of the most common, widespread diseases that are responsible for major crop losses caused by plant pathogens (Agrios et al., 1997). The principle sign of powdery mildew diseases is a characteristic white to grayish growth (fungal mycelium) mostly on the upper leaf side, but these signs can also appear on the abaxial leaf surface, shoots, buds, flowers, and fruits. The abundant mycelium produces hyaline (colorless) conidia (asexual spores; Coyier, 1985). Severe infections result in general growth stunting, leaf yellowing, flower deformation, and necrosis. Powdery mildew fungi, as obligate parasites (biotrophs), lack the ability to grow on artificial media and require a living host for reproduction. They are ectotrophic parasites, as their mycelium remains outside the plant tissues. In order to obtain nutrients from plant hosts, they form haustoria, specialized feeding structures that penetrate living host cells. They also exhibit xerophytism, causing more severe problems under warm and dry conditions (Takamatsu, 2004).

In older infection courts on senescent host tissues, resting structures called cleistothecia may form. These white-to-orange, spherical structures gradually turn black and are the fungus' ascomata (teleomorph; sexual fruiting bodies), which eventually form asci and ascospores. With free moisture, mature cleistothecia release ascospores that can act as primary inoculum and infect new host plants. Overwintering mycelia in perennial host tissues (e.g. bud meristems) can also activate with spring growth and produce conidia that can also act as primary inoculum. The ectotrophic mycelia infecting host

tissues prolifically produce conidia providing a constant load of secondary inoculum (Agrios, 1997).

### *Taxonomy*

The structure of ascomata has dominated the taxonomy of powdery mildews for many years, until scanning electron microscopy and DNA sequence data caused mycologists to modify the taxonomy of Erysiphales (Braun et al., 2002; Mori et al., 2000). Anamorphic structures, such as the morphology of mycelium, appresoria, haustoria, conidiophores, and conidia, have been used as the basis for the systematics of powdery mildews. Originally, the morphology of the teleomorphic structures, cleistothecia (now called chasmothecia), was used for the discrimination of powdery mildews at the genus level.

Taxonomically, all powdery mildew fungi belong to the phylum Ascomycota, subphylum Pezizomycotina, class Leotiomycetes, order Eryshiphales, and family Erysiphaceae. *Golovinomyces* (=*Erysiphe*) *cichoracearum* (DC.) V.P. Heluta is the causal agent of powdery mildew of numerous floriculturally important composites, including chrysanthemum, zinnia and sunflower.

### *Chemical control and resistance*

Most of the first fungicides used to control powdery mildews were protectants with multiple sites of action, like dithiocarbamates, quinomethionate, and sulfur (Hollomon and Wheeler, 2002). The chemical control strategies were revolutionized

with the introduction of the first systemic, broad-spectrum fungicide, benomyl, which was used successfully to control several powdery mildews. Systemic morpholines, which are sterol biosynthesis inhibitors (SBIs), and triazoles, demethylation inhibitors of sterol precursors (DMIs), and mesosystemic strobilurins are some of the compounds that have also been introduced and used to control powdery mildews.

Continuous chemical control of powdery mildews has often resulted in the development of resistance. Only the older generations of morpholines have retained efficacy (Hollomon and Wheeler, 2002). Development of resistance in powdery mildew strains can be determined using bioassays or molecular techniques like PCR detection of demethylase gene mutations conferring DMI resistance. In order to prevent resistance to fungicides it is advised to alternate fungicides with different modes of action, especially including multi-site-of-action compounds, to which fungicide resistance is rare due to the multigenic mutations required, with single-site-of-action compounds (e.g. DMIs, strobilurins) which are more prone to resistance development.

***Alternative control methods***

Environmentally 'friendly' approaches to the control of powdery mildews, such as some protectant fungicides (like potassium bicarbonate), mycoparasitism (e.g. *Ampelomyces quisqualis*) and antibiosis (e.g. *Tilletiopsis* spp., *Pseudozyma flocculosa*), have been proposed as alternatives and avoid the adverse effects of synthetic chemicals (Bélanger and Labbé, 2002). However, the efficacy, cost, storage and registration of these biologically based controls should be taken under consideration. Another approach, induced resistance against powdery mildews through the application of plant extracts

(e.g. Milsana) or soluble silicon supplements has been investigated in greenhouse horticultural crops (Daayf et al., 1995; Pasini et al., 1997; Bélanger et al., 1995).

***Silicon supplementation against powdery mildew development***

Silicon (Si) supplementation has reduced powdery mildew development in several host plants. Wheat powdery mildew (*Blumeria graminis* DC. f.sp. *tritici* Em. Marchal) was suppressed with both foliar and root Si applications, with the root applications found to be more effective (Guevel et al., 2007). Silicon-amended wheat (*Triticum aestivum* L.), when infected with powdery mildew, displayed such signs of cytological defense mechanisms as, cell wall papillae formation and increased deposition of callose and phenolics (Bélanger et al. 2003). Amending field-grown pumpkin (*Cucurbita pepo* L.) with $CaSiO_3$ reduced the severity of powdery mildew (*Podosphaera xanthii* U. Braun & N. Shishkoff), while combining Si supplements with fungicide treatments improved disease control, compared to Si or fungicide applied individually (Heckman et al., 2003). Powdery mildew of hydroponically grown squash (*Cucurbita pepo* L.) caused by *Podosphaera xanthii* (Castagne) Braun & Shishkoff was suppressed when Si was included in the nutrient solution (Savvas et al., 2009). Silicon supplementation of hydroponically produced cucumber (*Cucumis sativus* L.) suppressed powdery mildew caused by *Podosphaera fuliginea* (Schltdl.) Pollacci (Menzies et al., 1991; Dik et al., 1998). Similarly, Si supplementation of hydroponically grown zinnia (*Zinnia elegans* Jacq.) delayed powdery mildew (*Golovinomyces cichoracearum* (DC.) V.P. Gelyuta) expression and corresponded with deposition of Si in the cuticle of trichomes and adjacent epidermal cells (Locke et al., 2006). A relationship between Si and powdery

mildew (*Podosphaera pannosa* (Wallr.) de Bary) suppression of miniature roses (*Rosa* sp.) has also been reported (Datnoff et al., 2006). However, severity of powdery mildew caused by *Podosphaera* (Syn. Sphaerotheca) *fusca* (Fr.) S. Blumer was not reduced by potassium silicate incorporation in greenhouse production of Gerbera daisy (*Gerbera jamesonii* Bolus ex. Hook f.) (Moyer et al., 2008).

## B. Botrytis blight and gray mold

### *Biology*

*Botrytis* species are necrotrophic fungal pathogens that infect either a narrow range of host of plants (e.g. *B. tulipae* (Lib.) Lind infects only tulips) or a broad range of hosts. One such generalist, capable of infecting over 200 eudicot plant hosts, is *Botrytis cinerea* Pers., the cause of the diseases gray mold and botrytis blight, which are commonly encountered in horticulture. The former name comes from the sporulating gray mycelium produced on infected tissues. As a necrotrophic pathogen, *B. cinerea* infects and kills its hosts' cells through the production of toxins and reactive oxygen species that induce a plant-associated oxidative burst, and the secretion of numerous cell wall degrading enzymes (Choquer et al., 2007; Staats et al., 2005). After killing host plant tissues, *B. cinerea* can subsist on dead tissues as a facultative saprobe (van Kan, 2006). The fungus can survive in absence of nutrients as mycelium, sclerotia (melanized, thick walled hyphal aggregates) and chlamydospores. Some strains of *B. cinerea* can reproduce sexually by producing apothecia, fruiting structures bearing asci and ascospores, from sclerotia. Disease symptoms and signs include stem lesions, soft tissue-

water soaking, grayish-brown coat of conidiophores ('gray mold'), and spongy tubers and roots. *B. cinerea* also has emerged as a potential model for the study of necrotrophic pathogens, since the determination of the genomic sequences of several strains have been determined (van Kan, 2006).

***Taxonomy***

Current classification based on asexual (anamorphic) morphological characters, like macroconidia (mitotically-produced spores), and to a minor extent on physiology and host range. *Sclerotinia* and the teleomorphic genus of *Botrytis*, *Botryotinia* are closely related based on the ribosomal DNA (rDNA) (Holst-Jensen et al., 1998). The small subunit and internal transcribed spacer (ITS) rDNA sequences have been helpful for genus-level discrimination of the Sclerotinaceae, however, the variation, even in the normally polymorphic ITS region, is insufficient for species discrimination.

Taxonomically, *Botrytis* spp. belong to the phylum Ascomycota, subphylum Pezizomycotina, class Leotiomycetes, order Helotiales, family Sclerotiniaceae. *Botrytis cinerea* Pers. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) is the causal agent of botrytis blight and gray mold in many floricultural crops, including zinnia and sunflower.

Several economically important plant pathogen genera occur in the family Sclerotiniaceae with *Botrytis,* including *Monilinia* and *Sclerotinia* (Holst-Jensen et al., 1997). Members of this family possess mostly multinucleate cells that exhibit heterokaryosis, the presence of two or more genetically different nuclei. Several studies

have debated the significance of this biological variation in different strains of *B. cinerea* (Beever and Weeds, 2004).

### *Chemical control and resistance*

According to Leroux (2004) the fungicides effective against *Botrytis* fall into one of the following site of action classes: respiration inhibitors, anti-microtubule toxicants, osmoregulation disruptors, amino acid biosynthesis inhibitors, and sterol biosynthesis inhibitors. Resistance to benzimidazoles, phenylcarbamates and dicarboximides has developed in populations of *B. cinerea*. For that reason, newer compounds like pyrimidines, phenylpyrroles and hydroxyanilides have been developed for inclusion in fungicide rotation schemes designed to prevent further resistance.

### *Alternative control methods*

Resistance of *B. cinerea* to fungicides, such as benzimidazole and dicarboximide (require a single mutation each), environmental concerns regarding excess chemical applications and unsatisfactory levels of control (Elad, 1998), have prompted pursuit of alternative biological and chemical methods. The biocontrol agent, *Trichoderma harzianum* Rifai (a mycoparasitic fungus), provided adequate control of *B. cinerea* in greenhouse grown crops like cucumber and grape (Elad and Zimand, 1991). Phyllophane yeasts can also antagonize *B. cinerea* due to nutrient competition (Filonow, 1998), but with less consistent results compared to fungicides (Sansone, 2005). Several growth

regulators such as gibberellic acid and methyl jasmonate are also reported to suppress the disease (Shaul et al., 1996; Meir et al., 1998).

### *Silicon supplementation against Botrytis blight development*

Few studies have investigated the effect of Si supplements against *Botrytis* diseases. Decreased incidence of Botrytis blight was observed with Si supplementation of greenhouse-produced cucumber (O' Neil, 1991). Silicon supplementation of greenhouse-produced ornamental sunflower resulted in decreased disease incidence and severity of botrytis blight (Kamenidou, 2005).

### *Agrobacterium tumefaciens-mediated transformation (ATMT) of B. cinerea*

*Agrobacterium tumefaciens*-mediated transformation (ATMT) has been a successful tool for obtaining transgenic plants for over thirty years (Zambryski, 1992; Binns, 2002; Gelvin, 2003). *Agrobacterium* genetic transformation is also efficient at producing transgenic non-plant organisms, including fungal species (Lacroix et al., 2006; Michielse et al., 2005). *Botrytis cinerea* transformation has been achieved using various techniques like particle bombardment, protoplast transformation or ATMT (Li et al, 2006; Hamada et al., 1994; Rolland et al., 2003). Transformation of fungi is used to insert reporter genes (e.g. green fluorescent protein, GFP) that can be used to nondestructively follow the cytology of infection events. Or, inserted transgenes are used to functionally characterize endogenous genes, either through disruption or overexpression.

ATMT of fungi or plants requires construction of a binary vector system, where the T-DNA (carries transgenes) and virulence (*vir*) regions (required by *Agrobacterium* for gene transfer to the host) are placed on separate plasmids (Michielse et al., 2005). However, there are some differences between ATMT in fungi versus plants. First, transgenes must be controlled by promoters expressed in the host organism (Mullins et al., 2001). In fungi, both negative (e.g. antibiotic resistance) and positive (e.g. gene complementing an auxotrophy) selectable markers are available. In plants, however, negative selection (e.g. herbicide resistance) is most often used. T-DNA integration in plants only occurs randomly and ectopically, while in fungi gene targeting through homologous recombination also can occur if sufficient sequences of the targeted gene are included in the T-DNA (Lacroix et al., 2006). Also, differences in requirements for specific virulence proteins between plants and fungi have been documented (Michielse et al., 2005). Exogenous chemical inducers of *Agrobacterium*'s *vir* genes (acetosyringone) must be supplied exogenously for non-plant hosts, such as fungi (Lacroix et al., 2006). Plants often produce sufficient amounts of such compounds from wounded tissues during transformation to induce *Agrobacterium* virulence.

## C. Phytophthora root rot

### *Biology*

*Phytophthora* spp. have limited saprophytic ability and are generally characterized as hemibiotrophs, though some are necrotrophs. *Phytophthora* spp. belong to the fungus-like Oomycetes ("water molds"), a group of pathogens unique from true fungi (Erwin and Ribeiro, 1996; Erwin et al., 1983). Genetically oomycetes maintain

their nuclear DNA in a diploid state for most of their life cycle, while most fungi are haploid or dikaryotic. Oomycete cell walls contain cellulose and β-glucans, instead of chitin, the major cell wall constituent of fungi. More importantly to the epidemiology of this pathogen, oomycetes produce motile biflagellate zoospores, with whiplash and tinsel flagella, which require free water for dissemination. *Phytophthora* spp. are auxotrophic for sterols and must acquire sterols (β-hydroxysterols) exogenously for sporulation. However, sterols are not required for hyphal growth and thus, oomycetes are insensitive to SBI fungicides and polyene antibiotics, which permeabilize fungal membranes at ergosterol residues. Different *Phytophthora* species cause a variety of diseases on numerous dicot host plants. Possible disease symptoms include seed rots, damping-off of seedlings, foliar blights, and crown and root rots. The term 'life history', rather than life cycle, is used to describe the asexual and sexual phases of Phytophtora (Erwin and Ribeiro, 1996). Sporangia, zoospores, chlamydospores and mycelium are characteristic structures of the asexual phase, whereas, antheridia and oogonia are the sexual structures that form the diploid oospores. Some *Phytophthora* species are homothallic and can be self-fertile, while others are heterothallic and require both mating types (A1 and A2) in order to form oospores. Oospores are resilient survival structures and sources of genetic recombination required to overcome host resistance and fungicides.

***Taxonomy***

Traditionally, morphological characters such as, the mycelium, sporangia, zoospores, chlamydospores, and sex organs (antheridia, oogonia, and oospores), breeding system (homothallic or heterothallic) as well as host range and the ability to grow at high

temperatures, are all used to identify *Phytophthora* species. Under this system almost all *Phytophthora* spp. fall into six groups (I, II, III, IV, V and VI). However, phylogenetic studies of *Phytophthora* species, based on single or multi-loci DNA sequence alignments, contradict much of the morphology-based taxonomy of *Phytophthora*, which does not appear to accurately represent evolutionary relationships inferred by phylogenetics (Förster et al., 2000; Cooke et al, 2000; Villa et al., 2006). The most recent molecular classification of *Phytophthora* species suggests division of the genus into 10 clades (Blair et al., 2008).

Taxonomically, *Phytophthora* belongs to the kingdom Chromista (or Stamenopiles), phylum Oomycota, class Oomycetes, order Peronosporales, and family Peronosporaceae. Two of the most important *Phytophthora* species attacking a broad host range of floricultural crops in greenhouses are *P. cryptogea* Pethybr. & Laff. and *P. drechsleri* Tucker (Erwin and Ribeiro, 1996). These two *Phytophthora* spp. are morphologically and physiologically similar to one another, both belong to group VI, possess nonpapillate sporangia, amphigynous antheridia, grow optimally at high temperatures (28-30°C) and are heterothallic, requiring both A1 and A2 mating types to form oospores. *P. drechsleri* has the ability to grow at slightly higher temperatures than *P. cryptogea* (Tucker, 1931). Recently, DNA sequence data supported *P. drechsleri* is a monophyletic species that differs from the more variable *P. cryptogea* (Mostowfizadeh-Ghalamfarsa et al., 2007).

### *Chemical control and resistance*

Phytophthora diseases can be managed using several protectant fungicides (e.g. copper, dithiocarbamates, chlorothalonil), but they are only effective when applied during zoospore release and germination stages. Systemic fungicides are also used and include the following mode of action classes: carbamates, isoxazoles, phosphonate, and phenylamides. Phenylamides, and especially metalaxyl (racemic mixture) and mefenoxam (purified active R-enantiomer), provide the most effective control. However, development of resistance to the commonly used phenylamides has been reported (Lamour and Hausbeck, 2000). Thus, mixtures of phenylamide and protectants are applied to avoid resistance development.

### *Alternative control methods*

Ideally, *Phytophthora* diseases should be managed with host resistance. In the case of potato late blight caused by *Phytophthora infestans* (Mont.) de Bary, a combination of resistant varieties with horizontal (multigene) resistance and chemical applications is essential for successful disease control (Erwin and Ribeiro, 1996; Agrios, 1997). Also, cultural practices like crop rotation, judicious irrigation (i.e. avoid overwatering), improved drainage and removal of infested plant debris are regularly employed to minimize *Phytophthora*-related disease problems. Alternative disease management strategies against *Phytophthora* spp. have been explored, especially in greenhouse conditions. Most include manipulation of the nutrient irrigation solution in order to disrupt zoospore biology. Adjustment of electrical conductivity, calcium amendments, or addition of surfactants (Stanghellini et al., 1996; von Broembsen and

Deacon, 1997; Thinggaard and Andersen, 1995) have been shown to interfere with zoospore production and behavior experimentally, but are not commonly used as routine practices against *Phytophthora* spp.

***Silicon supplementation against Phytophthora root rot development***

No published studies have investigated the effects of Si supplementation on Phytophthora root rots. However, Si supplementation reduced root rot of cucumber caused by the related oomycete *Pythium* and appeared to promote plant defense reactions against *Pythium* (Cherif et al., 1992; Cherif et al. 1994). Thus, Si supplementation may prove beneficial for the management of *Phytophthora*-caused diseases, as well.

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# CHAPTER III

# SOLUBLE SILICON SUPPLEMENTATION REDUCES POWDERY MILDEW OF GREENHOUSE PRODUCED ZINNIA AND SUNFLOWER


## Abstract

Silicon (Si) supplementation benefits soilless production of floricultural crops by improving horticultural traits and enhancing resistance to biotic stresses, such as diseases. One of the most prevalent and important diseases in greenhouse floriculture is powdery mildew, caused by the fungus *Golovinomyces cichoracearum* (DC.) V.P. Gelyuta. The goal of this study was to assess powdery mildew suppression in greenhouse-produced zinnia (*Zinnia elegans* Jacq.) and sunflower (*Helianthus annuus* L.) grown in peat-based soilless media supplemented with various forms of soluble Si and in combination with half- or full-rate applications of myclobutanil. Supplemental Si was provided through substrate incorporation of potassium silicate at 140 $g \cdot m^{-3}$ Si, substrate incorporation of calcium silicate at 200 $g \cdot m^{-3}$ Si, five weekly substrate drenches of soluble potassium silicate at 50 $mg \cdot L^{-1}$ Si, or five weekly foliar applications of sodium silicate at 50 $mg \cdot L^{-1}$ Si. Powdery mildew incidences and severity ratings were recorded one, two, and three weeks after inoculation of lower and upper leaves. Myclobutanil treatments suppressed powdery mildew in zinnia for one week and in sunflower for three weeks.

In zinnia, Si supplementation reduced powdery mildew severities three- to four-fold after two weeks and two- to three-fold after three weeks. Silicon supplementation of zinnia, when combined with myclobutanil treatments, also appeared to synergistically suppress powdery mildew severity two-fold and prolong fungicide disease protection to three weeks. In sunflower, Si supplementation suppressed powdery mildew severities two- to three-fold after two weeks and around 1.5-fold after three weeks. From previous findings, Si supplementation's greater reduction of powdery mildew in zinnia, versus sunflower, may be related to the higher levels of Si accumulated in zinnia's tissues compared to sunflower. Silicon supplementation with soluble forms of silicon provides an additional practice for integrated disease management programs used by greenhouse floriculture.

**Introduction**

Crop plants grown in silicon-limited substrates, such as histosols and soilless horticultural media (peat-based mixtures and hydroponic solutions), often are predisposed to abiotic stresses and diseases (6). When silicon-limited plants are supplemented with soluble forms of silicon prophylactic effects have been observed against several plant diseases. Most studies have been performed with Si-accumulating plants such as grasses (e.g. rice, wheat, turfgrasses) and cucurbits, and disease suppression has been demonstrated against foliar blights, powdery mildews and *Pythium* root rot (6, 14). Depending on the crop and the pathogen interaction, results range from no effect to total control (3). In particular, silicon supplements have proved beneficial against powdery mildews in several host plants. Si-amended wheat (*Triticum aestivum* L.) infected with

powdery mildew (*Blumeria graminis* DC f.sp. *tritici* Em. Marchal) displayed cytological defense mechanisms such as papillae formation and increased deposition of callose and phenolics (2). Amending field grown pumpkin (*Cucurbita pepo* L.) with $CaSiO_3$ reduced the severity of powdery mildew (*Podosphaera xanthii* U. Braun & N. Shishkoff), while combining Si with fungicide treatments improved disease control, compared to the same treatments applied individually (8). Supplementation of hydroponically produced cucumber (*Cucumis sativus*) with Si suppressed powdery mildew caused by *Podosphaera fuliginea* (Schltdl.) Pollacci (7, 16).

Powdery mildew is one of the most important diseases affecting greenhouse floricultural crops and a wide variety of protectant and systemic fungicides are often used to manage the causal fungi (9). However, powdery mildew strains frequently develop resistance to these fungicides, especially the sterol demethylation inhibitors. In addition to the potentially adverse environmental effects of chemical fungicides, preventative, cultural and biological alternatives are still sought (4). The goal of this study was to assess powdery mildew suppression achieved by supplementation of greenhouse produced zinnia and sunflower with different silicate salts, compared to and in combination with half and full rate of the fungicide myclobutanil.

## Materials and Methods

*Plant material and silicon supplements*:

*Zinnia elegans* Jacq. 'Zowie Yellow Flame' and *Helinathus annuus* L. 'Pacino Cola' were sown into 20.3 cm (1.8 L) pots, one seed per container. The substrate used

was BM1 Mix (Berger Peat Moss, St.Modeste, Quebec) with incorporation of 875 g.m-3 MicroMax (The Scotts Co., Marysville, Ohio). Four Si sources were used in addition to nonsupplemented controls with eight replicate plants per treatment. The sources, method of application, and concentrations of supplemental Si treatments were: potassium silicate ($KSiO_3$, hydrous powder) incorporated into the substrate at 140 $g.m^{-3}$ Si, calcium silicate ($CaSiO_3$) incorporated into the substrate at 200 $g.m^{-3}$ Si, five weekly $KSiO_3$ substrate drenches containing 50 $mg.L^{-1}$ Si, or five weekly $NaSiO_3$ foliar applications of 50 $mg.L^{-1}$ Si solutions sprayed until runoff. All Si sources were supplied from PQ Corporation (Valley Forge, Pa.), except $CaSiO_3$ that was supplied from Sigma-Aldrich Corporation (St.Louis, MO). In addition, dolomitic limestone was added at 3.5 $kg.m^{-3}$ to the substrate in order to equilibrate pH levels among treatments. Plants were grown in a complete randomized block design in ebb-and-flow benches in a polycarbonate covered greenhouse with night/day set temperatures of 18/22°C and fertilized with 150 $mg.L^{-1}$ N from 20N-4.4P-16.6K complete fertilizer (The Scotts Co., Marysville, Ohio).

*Fungicide application and powdery mildew inoculation*

One week prior to inoculation, zero, half and full rates of the systemic fungicide, myclobutanil (Eagle 20EW, 19.7% active ingredient, Dow AgroSciences LLC, Indianapolis, IN), were applied to Si-supplemented and nonsupplemented controls (no Si added) plants. An isolate of the powdery mildew fungus, *Golovinomyces cichoracearum* (DC.) V.P. Heluta, was initially isolated from greenhouse-produced potted sunflowers, and then maintained on zinnia plants. A leaf-to-leaf contact inoculation procedure was used, in which test plants were inoculated by lightly pressing an infected leaf with a

heavily sporulating single-colony against one lower and one upper leaf of each test plant. Immediately after inoculation, inoculated plants and non inoculated controls were incubated at 100% relative humidity for 48 hours to establish infection (in separate humid chambers).

*Disease Assessment and Statistics*

Disease incidence (% of symptomatic leaves) and disease severity (% leaf coverage with mycelium) on upper and lower inoculated leaves were recorded one, two, and three weeks after the inoculation. An index (0-6) previously described by Yan et al. (18) to determine powdery mildew disease severity on rose leaves was used to express the percentage of leaf area covered by mycelial colonies. Ratings were defined as follows: 0 = healthy, 1 = less than 1%, 2 = 1–5%, 3 = 6–20%, 4 = 21–40%, 5 = 41–60% and 6 = greater than 60% leaf area covered by mycelial colonies (Fig. 1). Leaf disease severities were visually scored twice by two people and scores averaged. For disease incidence a chi-square test was used to compare Si supplemented plants to nonsupplemented control plants. The Cochran-Armitage trend test (1, 5) was used to test for linear trends of disease incidence with fungicide rate. Pearson's correlation coefficients of disease incidence for fungicide rate were calculated for each Si source. For disease severity ratings, LSD tests were used to separate differences between Si supplemented and nonsupplemented controls. The data were analyzed using SAS software (SAS Institute, Cary, N.C., 2001).

## Results

*Zinnia elegans* 'Zowie Yellow Flame'

Among nontreated fungicide controls one week post inoculation, all Si supplemented plants displayed decreased lower leaf disease incidences (LLDI) of powdery mildew compared to nonsupplemented controls (Table 1). For example, nonsupplemented control plants had 75% LLDI, while plants supplemented with KSiO3 drenches or media incorporation had 0% incidence. Nontreated fungicide control plants supplemented with either $CaSiO_3$ substrate incorporation or $KSiO_3$ weekly substrate drenches continued to display reduced LLDI even after two weeks post inoculation. When half and full rates of fungicides were used there were no significant differences in LLDI between Si supplemented plants and nonsupplemented. The correlation of the applied fungicide rate and LLDI suppression proved significant for nonsupplemented controls for up to two weeks post inoculation. Among Si supplemented plants, only those amended with $NaSiO_3$ showed a negative relationship between fungicide rate and LLDI at two weeks post inoculation. Three weeks post inoculation, LLDI increased for all treatments including fungicide treated plants, and no differences existed between Si supplemented and nonsupplemented controls. The effects of Si supplementation and fungicide rate on the upper leaf disease incidences (ULDI) of powdery mildew displayed similar trends to those observed for the LLDI (Table 2). Among nontreated fungicide controls one week post inoculation, plants supplemented with either $CaSiO_3$ media incorporation or $KSiO_3$ substrate drenches, displayed decreased ULDI compared to nonsupplemented controls (Table 2). Nontreated fungicide control plants supplemented

with $KSiO_3$ substrate incorporation or substrate drenches, or $CaSiO_3$ substrate incorporation displayed reduced ULDI after two weeks post inoculation compared to nonsupplemented controls. Within half rate fungicide treatments, no significant differences were observed in ULDI between Si supplemented plants and nonsupplemented controls, with the exception of reduced ULDI in $CaSiO_3$ supplemented plants at two weeks post inoculation (Table 2). Within full rate fungicide treatments, no significant differences in ULDI between Si supplemented plants and nonsupplemented controls were observed, with the exception of $NaSiO_3$ supplemented plants three weeks post inoculation. The correlation of the applied fungicide rate and ULDI suppression proved significant only for Si nonsupplemented controls one and two weeks post inoculation. Three weeks post inoculation ULDI's increased for all treatments including fungicide treated plants.

Among nontreated fungicide controls, all Si supplemented plants displayed decreased lower leaf disease severities (LLDS), around three-fold less at two weeks post inoculation and two-fold less at three weeks post inoculation compared to nonsupplemented controls . Also among nontreated fungicide controls, all Si supplemented plants displayed decreased upper leaf disease severities (ULDS), around four-fold less at two weeks post inoculation and two-fold less at three weeks post inoculation, compared to nonsupplemented controls (Tables 3 and 4). No differences in LLDS and ULDS were observed between Si supplemented and nonsupplemented plants treated with half and full rates of fungicides up to two weeks after inoculation (Tables 3 and 4). However, after three weeks, Si supplemented plants treated with half and full rates of fungicide displayed reduced ULDS compared to nonsupplemented controls .

*Helianthus annuus* ‘Pacino Cola’

Among nontreated fungicide controls, only plants supplemented with $CaSiO_3$ displayed decreased LLDI at one week post inoculation compared to nonsupplemented controls (Table 5). Specifically, nonsupplemented control plants had 75% LLDI, while $CaSiO_3$ supplemented plants showed 25% LLDI. Also, among nontreated fungicide controls, only plants supplemented with $KSiO_3$ substrate drenches displayed reduced ULDI at two weeks post inoculation compared to nonsupplemented plants (Table 6). Plants treated with half and full rates of fungicides showed no differences in LLDI and ULDI between Si supplemented and nonsupplemented control plants (Table 5 and 6). Fungicide rate correlated with LLDI suppression for both Si supplemented and nonsupplemented control plants, one, two and three weeks post inoculation, with the exception of $CaSiO_3$ supplemented plants, one week post inoculation (Table 5). At one week post inoculation, fungicide rate correlated with ULDI suppression, only in the nonsupplemented Si control plants (Table 6). However, at two and three weeks post inoculation, fungicide rate correlated with ULDI suppression for both Si supplemented and nonsupplemented control plants. Among nontreated fungicide controls, Si supplemented plants (except $NaSiO_3$ supplemented plants) displayed two- to three-fold decreased LLDS one week after inoculation and three- to 1.5-fold decreased ULDS two and three weeks after inoculation, respectively, compared to nonsupplemented controls (Tables 7 and 8). When half and full rates of fungicides were used, no significant differences in LLDS or ULDS between Si supplemented and nonsupplemented control plants were observed during all three weeks after inoculation.

**Discussion**

The extent to which most horticultural crops are able to accumulate Si is not known. Zinnia and sunflower were used in this study because Si uptake and accumulation, ~1% dry weight in leaves, has been previously documented in these species (11, 12). The levels of silicates supplemented to plants in this study were previously shown improve horticultural traits without causing flower deformation, with the exception of $CaSiO_3$ incorporated into media, which has not been investigated as a Si supplement in zinnia or sunflower.

The leaf-to-leaf inoculation method used in this study limited inoculation to specific sites on plants, permitting the identification of inoculation sites for repeated assessment of powdery mildew development over time (17). Although intra-plant LLDS and ULDS were not compared, in control plants, older lower leaves appeared to be more susceptible to powdery mildew than younger upper leaves in zinnias, while younger upper leaves appeared to be slightly more susceptible than older lower leaves in sunflower. Thus, assuming younger upper leaves are generally more competent to resist infections, the particular strain of *G. cichoracearum* used in these studies appears to be more virulent on sunflower, its original host, than on zinnia, the host on which it was maintained. Also, the ability of zinnia to accumulate higher concentrations of Si in its leaves than sunflower (11, 12) may have contributed to the Si supplemented zinnias' higher resistance to powdery mildew compared to Si supplemented sunflower. In the absence of fungicide treatments, several of the tested Si supplements delayed disease development one week after inoculation compared to nonsupplemented plants. Three weeks after inoculation, even though disease incidence percentages were the same across

Si supplemented and nonsupplemented plants, disease severity ratings (semiquantitative measures) were still lower in most of the Si supplemented plants. This indicates that Si supplementation generally delayed disease development. Similarly, Si supplementation of hydroponically grown zinnia delayed powdery mildew expression and corresponded with deposition of Si in the cuticle of trichomes and adjacent epidermal cells (13). Tissue fortification due to Si deposition in plant tissue is thought to provide a protective mechanical barrier against pathogen penetration (14). Thus, the observed delay in disease development in zinnia and sunflower may have resulted from the hindered penetration of epidermal cells, required by the biotrophic ectoparasite, *G. cichoracearum*, for nutrition and growth.

Half and full rate fungicide treatments suppressed powdery mildew disease incidence similarly in Si supplemented and nonsupplemented control plants within host species. However, overall, myclobutanil was less effective against the same strain of *G. cichoracearum* infecting zinnia than sunflower. In zinnia, fungicide rate correlated with reduced disease incidence in only nonsupplemented control plants up to two weeks post inoculation. While in Si supplemented zinnia plants, fungicide rate did not correlate with reduced disease incidence in either lower or upper leaves. However, three weeks post inoculation, half and full rate fungicide treatments suppressed disease severities especially in the upper leaves of Si supplemented zinnia plants, indicating Si supplementation may synergistically prolong myclobutanil's efficacy against powdery mildew of zinnia. On the other hand, fungicide rate correlated with reduced disease incidence over the entire three weeks of the study in both Si supplemented (except $CaSiO_3$ supplemented plants one week after inoculation) and nonsupplemented

sunflowers. Overall, both half and full rates of myclobutanil suppressed powdery mildew almost completely throughout the three weeks of the experiment in sunflower. Thus, no interactions between fungicide rate and Si supplementation could be observed in sunflower. Silicon is absorbed by roots from the soil solution as silicic acid. In dicotyledonous plants, silicic acid passively moves up the transpiration stream until it polymerizes and accumulates as silica gel under the cuticle (15). Thus, foliar plant tissues increase in Si content with age. For several dicotyledonous plants, the older foliar tissues tend to be more susceptible to powdery mildew (10). Similarly, we have observed that in naturally occurring powdery mildew outbreaks in the greenhouse and in inoculated maintenance plants, fungal colonies of *G. cichoracearum* usually appear first on the lower leaves of plants before the upper leaves of plants became infected (Fig. 2). During this study inoculated lower zinnia and sunflower leaves were infected faster than upper leaves in the first week post inoculation. By three weeks post inoculation, mildew colonies occurred uniformly throughout the control plants regardless of tissue age. Si-associated reductions in powdery mildew incidence and severity were observed in both lower and upper leaves with several of the Si supplements tested. Among the Si supplements tested, only weekly foliar sprays of $NaSiO_3$ failed to suppress powdery mildew of sunflower, though it successfully suppressed powdery mildew of zinnia. Overall, Si supplementation delayed powdery mildew development in greenhouse produced zinnia and sunflower. The rates of different silicate salts used to supplement commercial soilless media or to spray foliage must be optimized for each plant species to avoid excess concentrations, which can cause growth abnormalities, such as stunting and deformed flowers (11, 12). The Si supplementation rates used in this study, however, did

not cause any detrimental effects to zinnia or sunflower. Thus, Si supplementation provides greenhouse floriculturists with an additional disease management practice.

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**Table 1.** Effects of Si supplementation and fungicide rate on powdery mildew lower leaf incidence of *Zinnia elegans* 'Zowie Yellow Flame'.

| | Lower Leaf Disease Incidence (% leaves with signs of powdery mildew) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | One week post inoculation Fungicide rate | | | | Two week post inoculation Fungicide rate | | | | Three week post inoculation Fungicide rate | | | |
| Silicon Sources | 0 | Half | Full | r | 0 | Half | Full | r | 0 | Half | Full | r |
| Control Non-supplemented | 75 | 25 | 12 | -0.53** | 100 | 62 | 37 | -0.54*** | 100 | 100 | 87 | $-0.26^{NS}$ |
| Hydrous $KSiO_3$ (140 $gm^{-3}$ Si) in substrate | $0^{\#}$ | 25 | 0 | $-0.00^{NS}$ | 62 | 37 | 37 | $-0.20^{NS}$ | 75 | 75 | 75 | $-0.00^{NS}$ |
| $CaSiO_3$ (200 $gm^{-3}$ Si) in substrate | $12^{\#}$ | 0 | 0 | $-0.26^{NS}$ | $50^{\#}$ | 25 | 25 | $-0.27^{NS}$ | 87 | 100 | 75 | $-0.15^{NS}$ |
| $KSiO_3$ (50 mg $l^{-1}$ Si) weekly substrate drench | $0^{\#}$ | 12 | 0 | $0.00^{NS}$ | $50^{\#}$ | 50 | 50 | $0.00^{NS}$ | 75 | 75 | 75 | $0.26^{NS}$ |
| $NaSiO_3$ (50 mg $l^{-1}$ Si) weekly foliar spray | $25^{\#}$ | 12 | 0 | $-0.31^{NS}$ | 62 | 75 | 0 | -0.51** | 87 | 75 | 62 | $-0.24^{NS}$ |

$^{\#}$Significant from the control in the same column at 5% level by the chi-square test
NS, **, ***. Not significant (NS) or significant at 1% (**) or 0.1% (***)
r: Pearson's correlation coefficient

**Table 2**. Effects of Si supplementation and fungicide rate on powdery mildew upper leaf incidence of *Zinnia elegans* 'Zowie Yellow Flame'.

| | Upper Leaf Disease Incidence (% leaves with signs of powdery mildew) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | One week post inoculation Fungicide rate | | | | Two week post inoculation Fungicide rate | | | | Three week post inoculation Fungicide rate | | | |
| Silicon Sources | 0 | Half | Full | r | 0 | Half | Full | r | 0 | Half | Full | R |
| Control Nonsupplemented | 50 | 0 | 0 | -0.55** | 87 | 62 | 37 | -0.42** | 100 | 100 | 100 | $0.00^{NS}$ |
| Hydrous $KSiO_3$ (140 $gm^{-3}$ Si) in substrate | 12 | 0 | 0 | $-0.18^{NS}$ | $25^{\#}$ | 37 | 0 | $-0.25^{NS}$ | 87 | 75 | 75 | $-0.13^{NS}$ |
| $CaSiO_3$ (200 $gm^{-3}$ Si) in substrate | $0^{\#}$ | 0 | 0 | $0.00^{NS}$ | $25^{\#}$ | $12^{\#}$ | 0 | $-0.31^{NS}$ | 87 | 75 | 62 | $-0.24^{NS}$ |
| $KSiO_3$ (50 mg $l^{-1}$ Si) weekly substrate drench | $0^{\#}$ | 0 | 0 | $0.00^{NS}$ | $12^{\#}$ | 37 | 12 | $0.00^{NS}$ | 87 | 100 | 62 | $-0.27^{NS}$ |
| $NaSiO_3$ (50 mg $l^{-1}$ Si) weekly foliar spray | 12 | 12 | 0 | $-0.18^{NS}$ | 50 | 87 | 12.5 | $-0.30^{NS}$ | 75 | 100 | $50^{\#}$ | $-0.24^{NS}$ |

$^{\#}$ Significant from the control in the same column at 5% level by the chi-square test
NS, **, ***. Not significant (NS) or significant at 1% (**) or 0.1% (***)
r: Pearson's correlation coefficient

**Table 3**. Effects of Si supplementation and fungicide rate on powdery mildew lower leaf severity of *Zinnia elegans* 'Zowie Yellow Flame'.

| | Lower Leaf Disease Severity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | One week post inoculation Fungicide rate | | | Two week post inoculation Fungicide rate | | | Three week post inoculation Fungicide rate | | |
| Silicon Sources | 0 | Half | Full | 0 | Half | Full | 0 | Half | Full |
| Control Nonsupplemented | 0.9 | 0.2 | 0.1 | 3.0 | 1.4 | 1.0 | 4.7 | 3.6 | 2.7 |
| Hydrous $KSiO_3$ (140 $gm^{-3}$ Si) in substrate | 0.0*** | 0.2 $^{NS}$ | 0.0 $^{NS}$ | 1.1*** | 0.6 $^{NS}$ | 0.5 $^{NS}$ | 2.5** | 2.6 $^{NS}$ | 1.9 $^{NS}$ |
| $CaSiO_3$ (200 $gm^{-3}$ Si) in substrate | 0.1*** | 0.0 $^{NS}$ | 0.0 $^{NS}$ | 1.0*** | 0.5 $^{NS}$ | 0.4 $^{NS}$ | 2.6** | 1.9** | 1.6 $^{NS}$ |
| $KSiO_3$ (50 mg $l^{-1}$ Si) weekly substrate drench | 0.0*** | 0.1 $^{NS}$ | 0.0 $^{NS}$ | 1.0*** | 0.8 $^{NS}$ | 1.0 $^{NS}$ | 2.2*** | 1.9** | 2.5 $^{NS}$ |
| $NaSiO_3$ (50 mg $l^{-1}$ Si) weekly foliar spray | 0.2*** | 0.1 $^{NS}$ | 0.0 $^{NS}$ | 1.0*** | 1.1 $^{NS}$ | 0.0 $^{NS}$ | 2.2*** | 2.1* | 1.2* |

NS, *, **, ***. Not significant (NS) or significant at 5% (*), 1% (**) or 0.1% (***) from the control in the same column

**Table 4.** Effects of Si supplementation and fungicide rate on powdery mildew upper leaf severity of *Zinnia elegans* 'Zowie Yellow Flame'.

| | Upper Leaf Disease Severity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | One week post inoculation Fungicide rate | | | Two week post inoculation Fungicide rate | | | Three week post inoculation Fungicide rate | | |
| Silicon Sources | 0 | Half | Full | 0 | Half | Full | 0 | Half | Full |
| Control Nonsupplemented | 0.5 | 0.0 | 0.0 | 2.1 | 1.0 | 0.1 | 2.9 | 2.4 | 2.1 |
| Hydrous $KSiO_3$ (140 $gm^{-3}$ Si) in substrate | 0.1** | $0.0^{NS}$ | $0.0^{NS}$ | 0.4*** | $0.6^{NS}$ | $0.0^{NS}$ | 1.4*** | 1.2** | 0.7*** |
| $CaSiO_3$ (200 $gm^{-3}$ Si) in substrate | 0.0*** | $0.0^{NS}$ | $0.0^{NS}$ | 0.4*** | $0.6^{NS}$ | $0.0^{NS}$ | 1.2*** | 0.9*** | 0.7*** |
| $KSiO_3$ (50 mg $l^{-1}$ Si) weekly substrate drench | 0.0*** | $0.0^{NS}$ | $0.0^{NS}$ | 0.4*** | $0.6^{NS}$ | $0.1^{NS}$ | 1.4*** | 1.6* | 1.1** |
| $NaSiO_3$ (50 mg $l^{-1}$ Si) weekly foliar spray | 0.1** | $0.1^{NS}$ | $0.0^{NS}$ | 0.6*** | $1.2^{NS}$ | $0.4^{NS}$ | 1.2*** | $2.4^{NS}$ | 0.5*** |

NS, *, **, ***. Not significant (NS) or significant at 5% (*), 1% (**) or 0.1% (***) from the control in the same column

**Table 5.** Effects of Si supplementation and fungicide rate on powdery mildew lower leaf incidence of *Helianthus annuus* 'Pacino Cola'.

| | Lower Leaf Disease Incidence (% leaves with signs of powdery mildew) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | One week post inoculation Fungicide rate | | | | Two week post inoculation Fungicide rate | | | | Three week post inoculation Fungicide rate | | | |
| Silicon Sources | 0 | Half | Full | r | 0 | Half | Full | r | 0 | Half | Full | r |
| Control Nonsupplemented | 75 | 12 | 0 | -0.67*** | 100 | 12 | 0 | -0.84*** | 100 | 25 | 0 | -0.83*** |
| Hydrous $KSiO_3$ (140 $gm^{-3}$ Si) in substrate | 50 | 12 | 0 | -0.50** | 75 | 12 | 0 | -0.67*** | 87 | 12 | 0 | -0.76*** |
| $CaSiO_3$ (200 $gm^{-3}$ Si) in substrate | 25[#] | 0 | 12 | -0.16[NS] | 75 | 0 | 12 | -0.55** | 100 | 0 | 12 | -0.74*** |
| $KSiO_3$ (50 mg $l^{-1}$ Si) weekly substrate drench | 50 | 0 | 0 | -0.55** | 87 | 0 | 0 | -0.79*** | 87 | 0 | 12 | -0.65*** |
| $NaSiO_3$ (50 mg $l^{-1}$ Si) weekly foliar spray | 75 | 37 | 0 | -0.63*** | 100 | 37 | 0 | -0.82*** | 100 | 25 | 0 | -0.83*** |

[#]Significant from the control in the same column at 5% level by the chi-square test
NS, **, ***. Not significant (NS) or significant at 1% (**) or 0.1% (***)
r: Pearson's correlation coefficient

**Table 6**. Effects of Si supplementation and fungicide rate on powdery mildew upper leaf incidence of *Helianthus annuus* 'Pacino Cola'.

| | Upper Leaf Disease Incidence (% leaves with signs of powdery mildew) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | One week post inoculation Fungicide rate | | | | Two week post inoculation Fungicide rate | | | | Three week post inoculation Fungicide rate | | | |
| Silicon Sources | 0 | Half | Full | r | 0 | Half | Full | r | 0 | Half | Full | R |
| Control Nonsupplemented | 37 | 0 | 0 | -0.46* | 100 | 0 | 0 | -0.87*** | 100 | 0 | 0 | -0.87*** |
| Hydrous $KSiO_3$ (140 $gm^{-3}$ Si) in substrate | 0 | 0 | 0 | 0.00 $^{NS}$ | 62 | 0 | 0 | -0.63*** | 75 | 0 | 0 | -0.71*** |
| $CaSiO_3$ (200 $gm^{-3}$ Si) in substrate | 0 | 0 | 0 | 0.00 $^{NS}$ | 62 | 0 | 0 | -0.63*** | 100 | 12 | 0 | -0.84*** |
| $KSiO_3$ (50 mg $l^{-1}$ Si) weekly substrate drench | 0 | 0 | 0 | 0.00 $^{NS}$ | 37$^{\#}$ | 0 | 0 | -0.46* | 100 | 0 | 0 | -0.87*** |
| $NaSiO_3$ (50 mg $l^{-1}$ Si) weekly foliar spray | 25 | 0 | 0 | -0.37 $^{NS}$ | 87 | 0 | 0 | -0.79*** | 100 | 0 | 0 | -0.87*** |

$^{\#}$ Significant from the control in the same column at 5% level by the chi-square test
NS, *, ***. Not significant (NS) or significant at 5% (*) or 0.1% (***)
r: Pearson's correlation coefficient

**Table 7**. Effects of Si supplementation and fungicide rate on powdery mildew lower leaf severity of *Helianthus annuus* 'Pacino Cola'.

| | Lower Leaf Disease Severity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | One week post inoculation Fungicide rate | | | Two week post inoculation Fungicide rate | | | Three week post inoculation Fungicide rate | | |
| Silicon Sources | 0 | Half | Full | 0 | Half | Full | 0 | Half | Full |
| Control Nonsupplemented | 1.2 | 0.1 | 0.0 | 3.1 | 0.2 | 0.0 | 3.2 | 0.2 | 0.0 |
| Hydrous $KSiO_3$ (140 $gm^{-3}$ Si) in substrate | 0.5** | 0.1 $^{NS}$ | 0.0 $^{NS}$ | 2.6 $^{NS}$ | 0.1 $^{NS}$ | 0.0 $^{NS}$ | 3.8 $^{NS}$ | 0.1 $^{NS}$ | 0.0 $^{NS}$ |
| $CaSiO_3$ (200 $gm^{-3}$ Si) in substrate | 0.2*** | 0.0 $^{NS}$ | 0.1 $^{NS}$ | 2.9 $^{NS}$ | 0.0 $^{NS}$ | 0.1 $^{NS}$ | 3.2 $^{NS}$ | 0.0 $^{NS}$ | 0.1 $^{NS}$ |
| $KSiO_3$ (50 mg $l^{-1}$ Si) weekly substrate drench | 0.6** | 0.0 $^{NS}$ | 0.0 $^{NS}$ | 2.4 $^{NS}$ | 0.0 $^{NS}$ | 0.0 $^{NS}$ | 3.0 $^{NS}$ | 0.0 $^{NS}$ | 0.1 $^{NS}$ |
| $NaSiO_3$ (50 mg $l^{-1}$ Si) weekly foliar spray | 1.6 $^{NS}$ | 0.7 $^{NS}$ | 0.0 $^{NS}$ | 3.8 $^{NS}$ | 0.9 $^{NS}$ | 0.0 $^{NS}$ | 4.0 $^{NS}$ | 0.9 $^{NS}$ | 0.0 $^{NS}$ |

NS, *, **, ***. Not significant (NS) or significant at 5% (*), 1% (**) or 0.1% (***) from the control in the same column

**Table 8.** Effects of Si supplementation and fungicide rate on powdery mildew upper leaf severity of *Helianthus annuus* 'Pacino Cola'.

| | Upper Leaf Disease Severity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | One week post inoculation Fungicide rate | | | Two week post inoculation Fungicide rate | | | Three week post inoculation Fungicide rate | | |
| Silicon Sources | 0 | Half | Full | 0 | Half | Full | 0 | Half | Full |
| Control Nonsupplemented | 0.4 | 0.0 | 0.0 | 3.2 | 0.0 | $0.0^{NS}$ | 4.9 | 0.0 | 0.0 |
| Hydrous $KSiO_3$ (140 $gm^{-3}$ Si) in substrate | 0.0** | $0.0^{NS}$ | $0.0^{NS}$ | 1.6*** | $0.0^{NS}$ | $0.0^{NS}$ | 3.7** | $0.0^{NS}$ | $0.0^{NS}$ |
| $CaSiO_3$ (200 $gm^{-3}$ Si) in substrate | 0.0*** | $0.0^{NS}$ | $0.0^{NS}$ | 0.9*** | $0.0^{NS}$ | $0.0^{NS}$ | 3.6** | $0.2^{NS}$ | $0.0^{NS}$ |
| $KSiO_3$ (50 mg $l^{-1}$ Si) weekly substrate drench | 0.0*** | $0.0^{NS}$ | $0.0^{NS}$ | 1.0*** | $0.0^{NS}$ | $0.0^{NS}$ | 3.0** | $0.0^{NS}$ | $0.0^{NS}$ |
| $NaSiO_3$ (50 mg $l^{-1}$ Si) weekly foliar spray | $0.3^{NS}$ | $0.0^{NS}$ | $0.0^{NS}$ | $2.9^{NS}$ | $0.0^{NS}$ | $0.0^{NS}$ | $4.4^{NS}$ | $0.0^{NS}$ | $0.0^{NS}$ |

NS, *, **, ***. Not significant (NS) or significant at 5% (*), 1% (**) or 0.1% (***) from the control in the same column

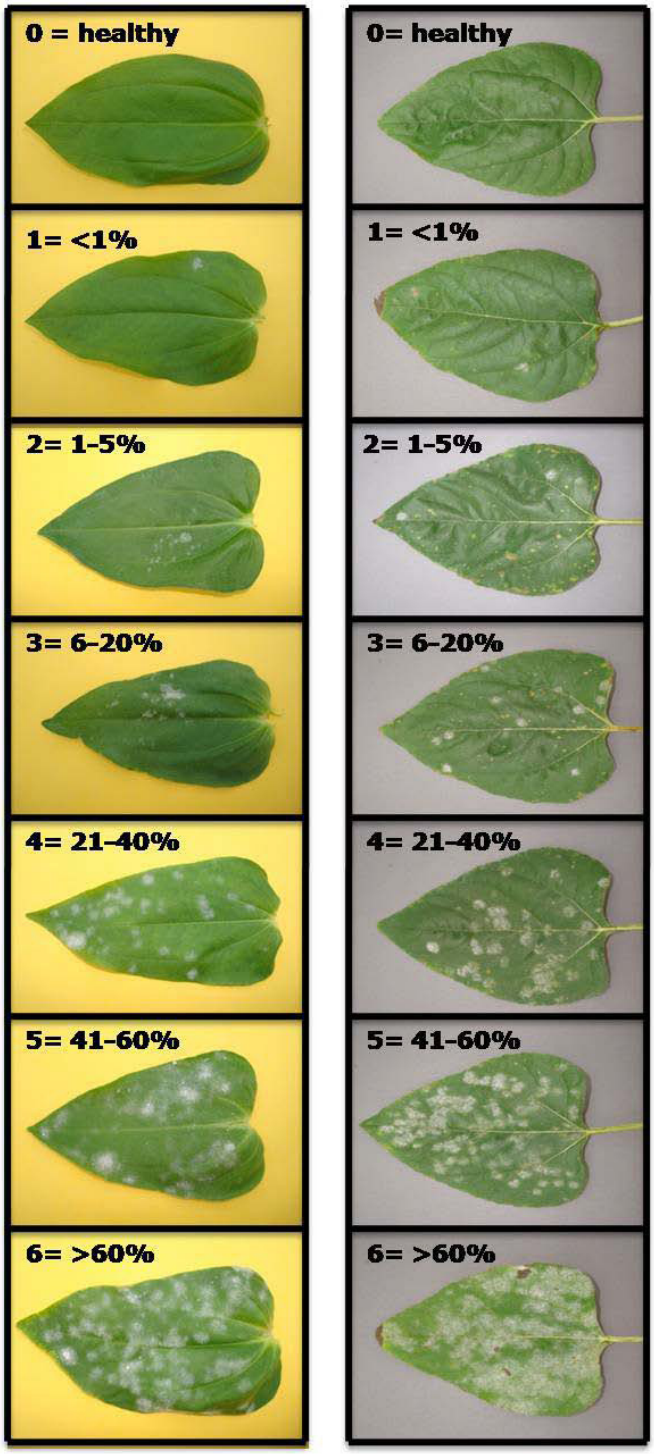


***Figure 1.*** Powdery mildew index used to score disease severities based on the leaf area covered by mycelial colonies. Representative leaves of zinnia (yellow background) and sunflower (gray background) are pictured.

***Figure 2.*** Maintenance plant of *Zinnia elegans* 'Zowie Yellow Flame' infected with the *Golovinomyces cichoracearum* strain used in this study. Powdery mildew disease progression from older lower leaves to younger upper leaves is illustrated.

# CHAPTER IV

# SILICON SUPPLEMENTATION DELAYS PHYTOPHTHORA ROOT ROT ONSET OF GREENHOUSE GROWN GERBERA


## Abstract

Silicon (Si) supplementation can improve plant resistance to certain biotic stresses, such as some diseases. Root rot caused by *Phytophthora drechsleri* Tucker can cause losses of greenhouse produced gerbera *Gerbera jamesonii* Bolus ex Hooker, even at warm temperatures. The goal of this study was to assess Phytophthora root rot in greenhouse-produced gerbera grown in peat-based soilless media supplemented with various forms of soluble Si and in combination with half- or full-rate soil drenches of mefenoxam. Supplemental Si was provided through substrate incorporation of potassium silicate at 140 $g \cdot m^{-3}$ Si, substrate incorporation of calcium silicate at 200 $g \cdot m^{-3}$ Si, or five weekly substrate drenches of soluble potassium silicate at 50 $mg \cdot L^{-1}$ Si. Disease incidences and severity ratings were recorded six, ten, and fourteen days post inoculation. The *P. drechsleri* isolate used in this study was sensitive to mefenoxam in vitro, and both half and full rate of mefenoxam treatments suppressed disease in the greenhouse study. Among nontreated fungicide controls, $KSiO_3$ weekly substrate drenches was the only Si source that reduced disease incidence and severity up to ten days post inoculation compared to nonsupplemented controls. However, two weeks post inoculation, Si

supplementation with soluble forms of silicon failed to reduce Phytopthora root rot of greenhouse produced gerbera, and media incorporated $KSiO_3$ slightly increased disease incidence and severity.

## Introduction

The non-essential element Si is abundant in most soil types, thus it has been undervalued as a mineral supplement for many years (Ma and Takahashi, 2002). However, Si amendments have proved beneficial to the production of both agronomic and horticultural crops, particularly those accumulating Si to high concentrations. For crops grown in Si-depleted soils or in soilless substrates routinely used in greenhouses, Si supplementation has been shown to enhance plant resistance to numerous abiotic and biotic stresses, such as water and chemical stresses, nutrient imbalances, metal toxicities, diseases and pest problems (Ma and Takahashi, 2002; Hodson and Sangster, 1999; Seebold et al., 2001; McAvoy and Bible, 1996). Silicon-associated prophylaxis has been reported against *Pythium*, an oomycete genus causing root rots (Cherif et al.,1992; Cherif et al., 1994).

*Phytophthora* spp. are also oomycete pathogens that can cause foliar blights and crown and root rots in a wide range of hosts, including many greenhouse floricultural crops (Erwin and Ribeiro, 1996). Humid and warm greenhouse conditions and irrigation practices that disseminate *Phytophthora* species' water-borne zoospores, provide a highly conducive environment for polycyclic disease development (van der Gaag et al., 2001; Hwang and Benson, 2005). Fungicides are commonly used to control Phytophthora root rot on greenhouse produced flowers such as gerbera; however, development of fungicide

resistance to the commonly used phenylamides (e.g. metalaxyl, mefenoxam) has been reported (Dole and Wilkins, 1999; Lamour and Hausbeck, 2000). According to United States Department of Agriculture Economic Research Service, in 2006 gerbera had the third highest wholesale value among cut flowers and cut greens in the U.S., thus losses to Phytophthora root rot are costly.

The goal of this study was to assess the effects of different chemical forms of soluble silicon supplements compared to and in combination with half- and full-rates of mefenoxam on the levels of Phytophthora root rot affecting greenhouse produced gerbera.

## Materials and Methods

*Plant material, silicon supplements and fungicide applications*:

*Gerbera jamesonii* cv. Emperor plugs (Fred C. Gloeckner & Co. Inc., Harrison, NY) were planted into 20.3 cm in diameter (1.8 L) pots. The substrate used was BM1 Mix (Berger Peat Moss, St.Modeste, Quebec) with incorporation of 875 $g \cdot m^{-3}$ MicroMax (The Scotts Co., Marysville, Ohio). Nonsupplemented controls and three Si supplements were evaluated with eight replicate plants per treatment. The sources, methods of application and concentrations of the Si supplements used were as follows: substrate incorporation of potassium silicate ($KSiO_3$) at 140 $g \cdot m^{-3}$ Si, substrate incorporation of calcium silicate ($CaSiO_3$) at 200 $g \cdot m^{-3}$ Si, or five weekly substrate drenches of soluble $KSiO_3$ at 50 $mg \cdot L^{-1}$ Si. All Si sources were supplied from PQ Corporation (Valley Forge,

Pa.), except $CaSiO_3$ that was supplied from Sigma-Aldrich Corporation (St.Louis, MO). Dolomitic limestone was added (3.5 $kg \cdot m^{-3}$) to the substrate to equilibrate pH levels among treatments. Plants were grown in a randomized complete block design in ebb-and-flow benches located in a polycarbonate covered greenhouse and fertilized with 150 $mg \cdot L^{-1}$ N from 20N-4.4P-16.6K complete fertilizer (The Scotts Co., Marysville, Ohio). Plants were overhead irrigated during production without recirculation of the nutrient solution. Ebb-and-flow benches were only flooded prior to and during the inoculation with *Phytophthora* (see below). One week prior to inoculation, zero, half and full rates of the systemic fungicide Subdue MAXX (22.0% mefenoxam), were applied to all Si-supplemented and nonsupplemented control (no Si added) plants as 300 ml soil drenches per pot, resulting in 0, 2.75 and 5.5 µg mefenoxam per pot. Noninoculated control plants were maintained and treated identically in separate ebb-and-flow benches.

*Pathogen cultures, inoculum preparation and inoculation*

A culture of *Phytophthora drechsleri* 22A, originally isolated from snap bean (*Phaseolus vulgaris* L.) grown in eastern Oklahoma (provided by Jennifer Olson, Oklahoma State University), was maintained on V8 agar at 28°C. Zoospore inoculum was prepared by inducing V8 cultures with soil extract. Briefly, soil extract was prepared by stirring 15 g sandy loam in 1 liter of water overnight. Soil particles were allowed to settle for five days at room temperature and then the upper 500 ml of nonsterile extract was transferred to a sterile container. Fifteen plugs (~1 $cm^2$ in diameter) from a 7 day old V8 agar culture of *P. drechsleri* 22A were placed in a sterile petri plate, flooded with 20 ml of the soil extract and incubated 24h at room temperature. Zoospore release was

monitored with a microscope. Zoospores were harvested from ten such petri plates by pouring soil extract from plates through two layers of cheesecloth. Zoospore concentration was quantified using a haemocytometer and the suspension adjusted to 10,000 zoospores/ml with sterile water for inoculation. Prior to inoculation, ebb-and-flow benches containing plants were flooded overnight with water to saturate the substrate in pots. Into each pot, 2 ml of zoospore suspension (i.e. 20,000 zoospores/pot) were applied to the substrate surface near each plant's crown, as two 1 ml aliquots at antipodal sites. Noninoculated controls were supplied with 2 ml of soil extract under identical conditions. Both inoculated and noninoculated control plants were maintained in flooded ebb-and-flow benches for 48 hours.

*Fungicide sensitivity*

To test the sensitivity of *Phytophthora drechsleri* 22A to mefenoxam, agar plugs (~1 $cm^2$ in diameter) from 3 day old corn meal agar (CMA) cultures, were transferred to the center of five replicate CMA agar plates containing 0, 0.22, 2.2, or 22 μg $ml^{-1}$ mefenoxam and incubated at 20°C. Plates were observed daily for 10 days.

*Disease Assessment and Statistics*

Phytophthora root rot disease incidences and severities were scored at six, ten and fourteen days post inoculation (dpi). Disease incidence was recorded as percentage of symptomatic plants. Disease severities were scored using an index (1-3) based on foliar symptoms, defined as follows: 1, healthy with no visible symptoms, 2, foliage partially wilted and 3, foliage completely wilted and crown rot present (Fig.4). To confirm

symptomatic gerbera plants were infected by *P. drechsleri*, root segments were collected from several plants, surface-sterilized with 70% ethanol for 30 seconds, placed on oomycete selective media (PBRCV; CMA containing 10 μg $ml^{-1}$ pimaricin, 5 μg $ml^{-1}$ benomyl, 25 μg $ml^{-1}$ rifampicin, 50 μg $ml^{-1}$ cephalexin, and 25 μg $ml^{-1}$ vancomycin), incubated at 20°C, and emerging mycelium observed under a microscope.

The Cochran-Armitage trend test (Armitage 1955; Cochran, 1954) was used to test the linear trend of disease incidence with fungicide rate. Pearson's correlation coefficients of disease incidence for fungicide rate were calculated for each Si source. For disease severity ratings LSD tests were used to separate differences between Si supplemented and nonsupplemented controls. The data were analyzed using SAS software (SAS Institute, Cary, N.C., 2001).

**Results**

Among inoculated, nontreated fungicide controls, the first plants began wilting six days post inoculation and plants supplemented with weekly substrate drenches of $KSiO_3$ displayed decreased disease incidences (DI) compared to nonsupplemented Si controls (Table 9), with a DI of 37% for nonsupplemented control plants and a DI of 0% for potassium silicate drenched plants. At ten dpi, nontreated fungicide control plants supplemented with weekly substrate drenches of $KSiO_3$ continued to display less DI compared to nonsupplemented controls, with 25% and 62% DI, respectively. However, at 14 dpi, the DI of nonsupplemented control plants and plants supplemented with weekly substrate drenches of $KSiO_3$ were similar.

In fungicide sensitivity assays, the *P. drechsleri* isolate used in this study (22A) was sensitive in vitro to the lowest tested rate of mefenoxam, 0.22 μg $ml^{-1}$. Even after 10 days, while *P. drechsleri* 22A mycelia had covered the area of control CMA plates, no hyphal growth was observed on any of the mefenoxam containing plates.

Applications of the fungicide mefenoxam decreased Phytophthora root rot. Disease incidence between Si supplemented and nonsupplemented control plants treated with fungicide was similar (Table 9). At 6 dpi, fungicide application rates was not correlated with DI suppression for either Si supplemented or nonsupplemented control plants. At 10 dpi, fungicide rate was negatively related to DI suppression in plants supplemented with substrate incorporated $KSiO_3$ and nonsupplemented controls. Fungicide rate was negatively correlated with DI suppression in all Si supplemented and nonsupplemented control plants 14 dpi. Among nontreated fungicide controls 14 dpi , DI increased for all Si supplemented and nonsupplemented plants. However, plants supplemented with substrate incorporated $KSiO_3$ showed an increased DI compared to nonsupplemented controls.

Trends in disease severities (DS) were similar to those observed with DI (Table 10). Decreased DS was only observed among nontreated fungicide controls, at 6 and 10 dpi, in plants supplemented with weekly substrate drenches of $KSiO_3$ compared to nonsupplemented control plants (Fig. 3). At 14 dpi, the DS of plants supplemented with substrate incorporated $KSiO_3$ were higher than nonsupplemented control plants.

**Discussion**

*Phytophthora drechsleri* and *P. cryptogea* Pethybr. & Laff. are morphologically similar, phylogenetically related, grow at high temperatures and are important root rot pathogens with a broad host ranges (Tucker 1931; Erwin and Ribeiro, 1996; Mostowfizadeh-Ghalamfarsa et al., 2007). *Gerbera jamesonii* is a host of both *Phytophthora* species (Farr et al., 2008). The strain 22A of *P. drechsleri* used in this study could grow at the high temperatures present in greenhouses (optimal temperatures, 28-30°C; J. Olson, personal communication), prolifically produced zoospores and was found to be pathogenic on gerbera in preliminary studies (data not shown).

In this study, mefenoxam, the phenylamide fungicide active against most Oomycetes (Schwinn and Staub, 1987), inhibited the in vitro growth of *P. drechsleri* 22A and suppressed Phytophthora root rot of gerbera inoculated with *P. drechsleri* 22A. Insensitivity to mefenoxam has been reported for several *Phytophthora* spp. (Lamour and Hausbeck, 2000; Hwang and Benson, 2005). Thus, alternative disease management strategies against *Phytophthora* spp. have been explored (von Broemsen and Deacon, 1997; Stanghellini et al., 1996; Thingaard and Andersen, 1995). Equivocally, Si supplementation has been reported to either suppress diseases caused by the oomycete *Pythium* spp. (Sun et al., 1994; Chérif et al., 1994) or have no effect on disease development (Menzies et al., 2001).

In this study, Si supplementation failed to control Phytophthora root rot. Though, among nontreated fungicide controls, gerbera plants supplemented with $KSiO_3$ as weekly substrate drenches displayed had less DI and DS, up to ten days post inoculation. However, four days later, 14 dpi, no differences in the DI or DS between $KSiO_3$-

drenched to nonsupplemented control plants were observed. $CaSiO_3$ substrate incorporation had no effect on either DI or DS compared to nonsupplemented controls, even though calcium amendments have been shown to negatively affect the zoospore biology of *Phytophthora parasitica* Dastur (von Broembsen and Deacon, 1997). Finally, at 14 dpi, substrate incorporation of $KSiO_3$ resulted in increased DI and DS compared to nonsupplemented controls. How this Si supplement apparently accelerated Phytophthora root rot is unclear.

Si supplemented gerbera plants accumulated less Si than zinnia and sunflower plants supplemented with the same Si treatments (Kamenidou, 2005). Crops with limited ability to increase their Si concentration, like lettuce and tomato (Voogt and Sonneveld, 2001), have also shown no benefits due to Si supplementation against Pythium root rot and Fusarium root rot respectively (Menzies et al., 2001).

In summary, mefenoxam controlled Phytophthora root rot of greenhouse produced gerbera. Potassium silicate weekly substrate drenches delayed disease development, but overall Si supplements failed to provide protection against this disease.

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**Table 9.** Effects of Si supplementation and fungicide rate on Phytophthora root rot disease incidence (%) of *Gerbera jamesonii* 'Emperor'.

| | Disease Incidence (% plants with symptoms of Phytophthora root rot) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Six days post inoculation Fungicide rate | | | | Ten days post inoculation Fungicide rate | | | | Fourteen days post inoculation Fungicide rate | | | |
| Silicon Sources | 0 | Half | Full | r | 0 | Half | Full | r | 0 | Half | Full | r |
| Control Nonsupplemented | 37 | 12 | 12 | -0.25 $^{NS}$ | 62 | 12 | 12 | -0.45* | 62 | 12 | 12 | -0.45* |
| $KSiO_3$ (50 mg $l^{-1}$ Si) weekly substrate drench | 0$^{\#}$ | 0 | 0 | 0.00 $^{NS}$ | 25$^{\#}$ | 0 | 0 | -0.37 $^{NS}$ | 87 | 0 | 0 | -0.79*** |
| $CaSiO_3$ (200 $gm^{-3}$ Si) in substrate | 12 | 0 | 0 | -0.26 $^{NS}$ | 50 | 0 | 12 | -0.38 $^{NS}$ | 75 | 12 | 12 | -0.54** |
| Hydrous $KSiO_3$ (140 $gm^{-3}$ Si) in substrate | 37 | 25 | 12 | -0.24 $^{NS}$ | 87 | 25 | 12 | -0.62*** | 100$^{\#}$ | 25 | 12 | -0.72*** |

$^{\#}$ Significant from the control in the same column at 5% level by the chi-square test
NS, **, ***. Not significant (NS) or significant at 1% (**) or 0.1% (***)
r: Pearson's correlation coefficient

**Table 10.** Effects of Si supplementation and fungicide rate on Phytophthora root rot disease severity of *Gerbera jamesonii* 'Emperor'.

| | Disease Severity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Six days post inoculation Fungicide rate | | | Ten days post inoculation Fungicide rate | | | Fourteen days post inoculation Fungicide rate | | |
| Silicon Sources | 0 | Half | Full | 0 | Half | Full | 0 | Half | Full |
| Control Nonsupplemented | 1.37 | 1.12 | 1.12 | 1.87 | 1.12 | 1.12 | 2.25 | 1.12 | 1.12 |
| $KSiO_3$ (50 mg $l^{-1}$ Si) weekly substrate drench | 1.00* | 1.00 $^{NS}$ | 1.00 $^{NS}$ | 1.25* | 1.00 $^{NS}$ | 1.00 $^{NS}$ | 2.50 $^{NS}$ | 1.00 $^{NS}$ | 1.00 $^{NS}$ |
| $CaSiO_3$ (200 $gm^{-3}$ Si) in substrate | 1.12 $^{NS}$ | 1.00 $^{NS}$ | 1.00 $^{NS}$ | 1.50 $^{NS}$ | 1.00 $^{NS}$ | 1.12 $^{NS}$ | 2.50 $^{NS}$ | 1.12 $^{NS}$ | 1.12 $^{NS}$ |
| Hydrous $KSiO_3$ (140 $gm^{-3}$ Si) in substrate | 1.37 $^{NS}$ | 1.25 $^{NS}$ | 1.12 $^{NS}$ | 2.12 $^{NS}$ | 1.12 $^{NS}$ | 1.12 $^{NS}$ | 2.87* | 1.25 $^{NS}$ | 1.12 $^{NS}$ |

Not significant (NS) or significant at 5% (*) from the control in the same column

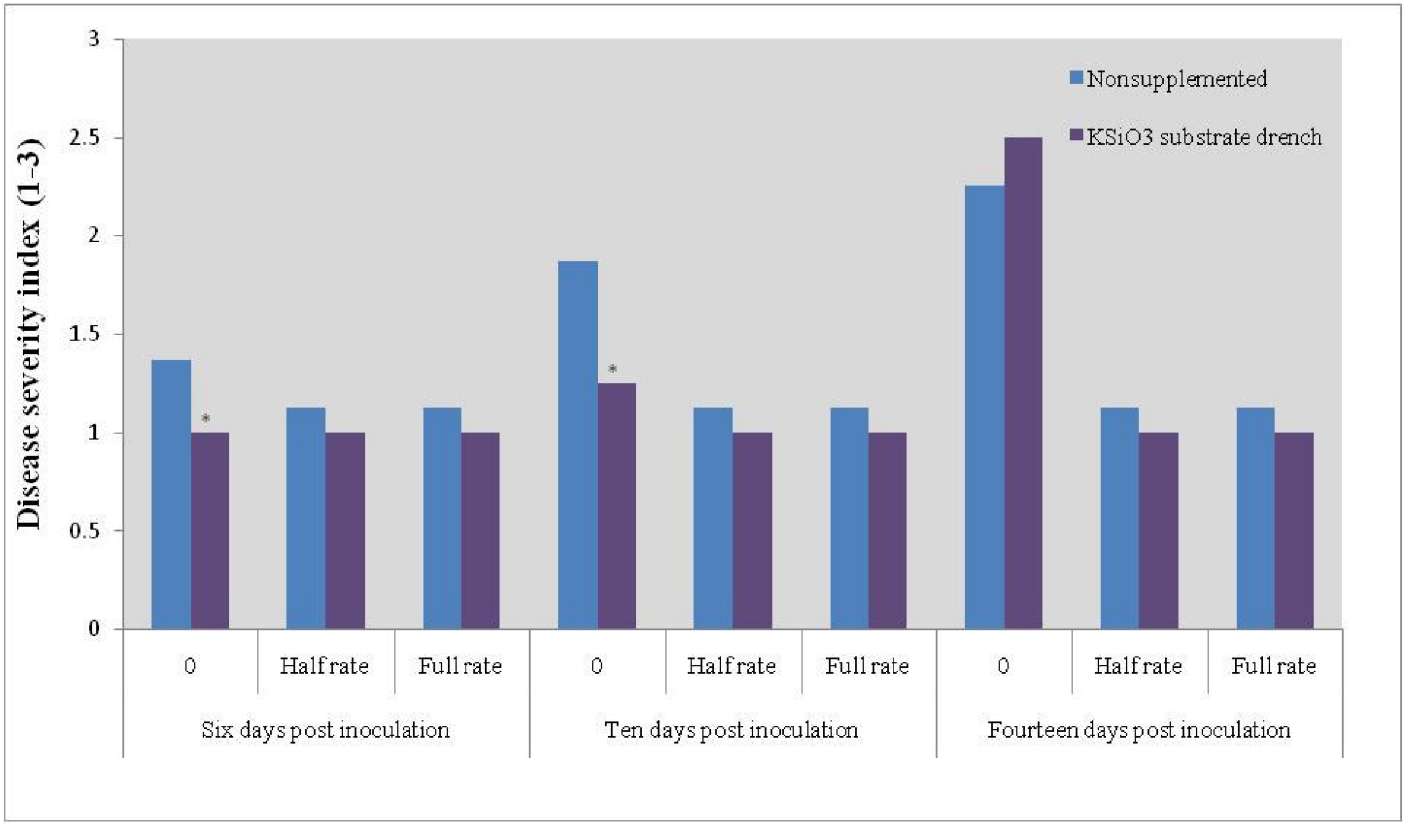


**Figure 3**. Effects of five weekly substrate drenches of $KSiO_3$ and half- and full-rate mefenoxam applications on Phytophthora root rot disease severity of gerbera.
Significant differences (*) between Si supplemented and nonsupplemented plants determined by LSD test (p = 0.05)

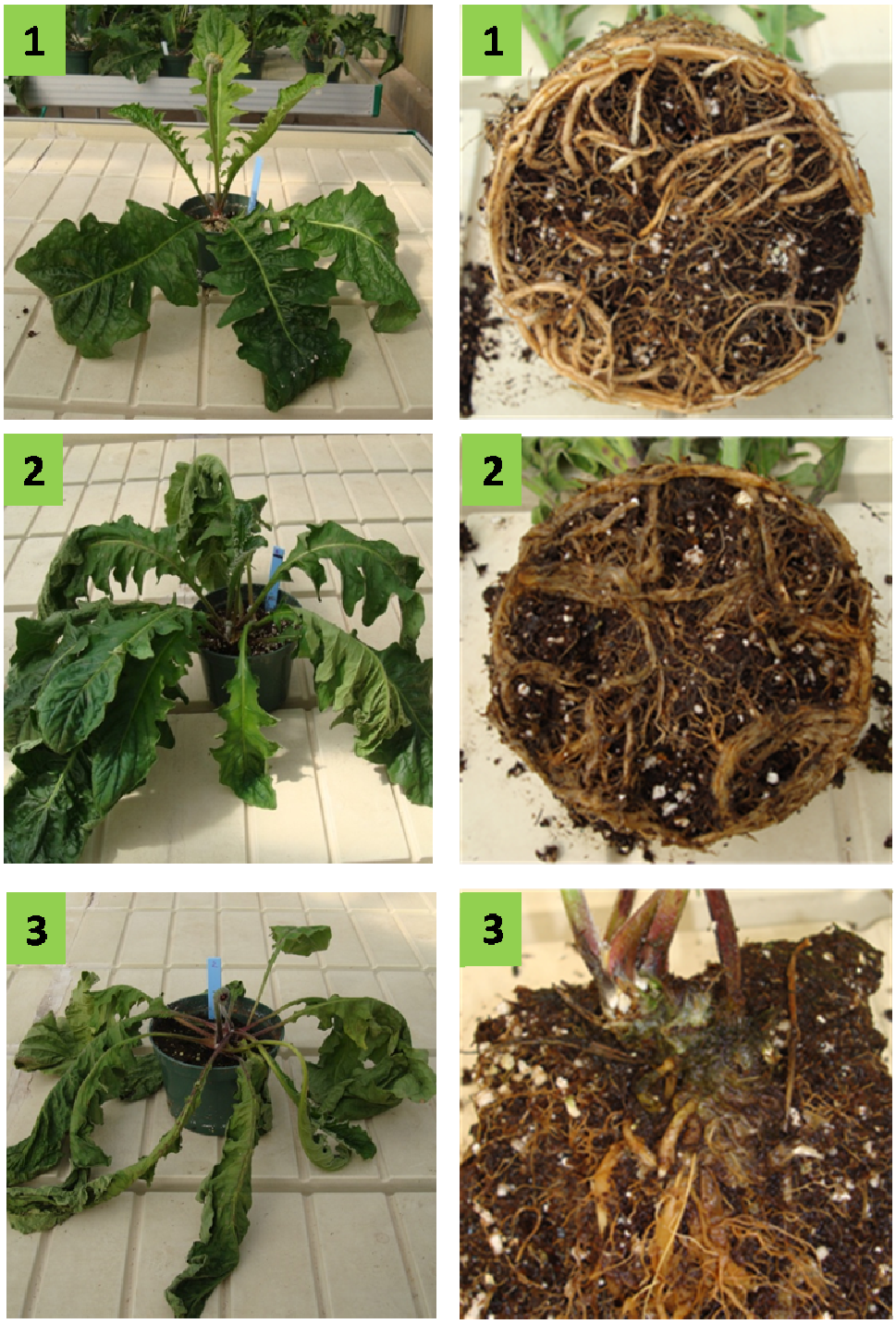


***Figure 4.*** Phytophthora root rot severities were scored using an index (1-3) based on foliar symptoms, defined as follows: 1, healthy with no visible foliar or root symptoms, 2, foliage partially wilted and root rot present, 3, foliage completely wilted and root and crown rot present.

# CHAPTER V

# SILICON SUPPLEMENTATION DELAYS GRAY MOLD DEVELOPMENT IN ORNAMENTAL SUNFLOWERS


## Abstract

Silicon (Si) is a nonessential mineral element reported to reduce disease severity in numerous plant species, especially those plants that accumulate Si. Greenhouse produced floricultural crops grown in soilless media, such as peat moss-based mixes, provide limited amounts of available Si. *Botrytis cinerea* Pers., the causal pathogen of gray mold, is one of the most economically important diseases in greenhouse production. The effects of different silicon supplements alone and in combination with two rates of chlorothalonil on gray mold of ornamental sunflower (*Helianthus annuus* L.) were previously assessed and found to reduce disease but not gray mold-related postharvest mortality. In this study, *Agrobacterium*-mediated transformants of *B. cinerea* expressing a green fluorescent protein (GFP) were generated to study the cytology of Si-associated gray mold suppression. A brightly fluorescent transformant was found to be as virulent as the wild type and both were used to inoculate whole plants or detached leaves of sunflowers, that were supplemented with Si and treated or not treated with chlorothalonil. Disease progression was delayed in Si supplemented plants by delaying conidial

germination. However, gently wounding detached leaves of Si supplemented plants returned disease progression and conidial germination to control levels. Therefore, Si deposition may fortify sunflower trichomes, an observed location of conidial accumulation, and leaf and stem surfaces, creating a mechanical barrier against *B. cinerea*.

## Introduction

One benefit associated with silicon (Si) supplementation of plants is less severity of several diseases (Datnoff et al., 2007). Plant diseases caused by *Botrytis* spp. are some of the most common diseases in production greenhouses (Agrios, 1997). *Botrytis cinerea,* which causes gray mold, is a generalist necrotroph, infecting over 200 eudicot species, that proliferates on senescing or wounded tissues (Elad et al., 2004). It is a major problem in cut flower production, since it reduces the ornamental value and limits the postharvest life of flowers (Meir et al., 1998). Silicon supplementation has been previously reported to decrease gray mold intensity in cucumber greenhouse production (Voogt and Sonneveld, 2001).

The mechanism associated with Si-mediated resistance against plant pathogens still remains illusive. Several studies suggest that Si deposition in plant tissue creates a mechanical barrier against pathogen penetration (Ma and Takahashi, 2002). Other studies provide evidence of systemic acquired resistance, without necessarily rejecting the theory of mechanical resistance (Datnoff et al., 2007). Understanding how plant pathogens, such as *B. cinerea*, interact with Si supplemented host tissues may provide further insight into

Si-mediated resistance. Genetically transforming *B. cinerea* to express fluorescent reporter genes would permit live cell observations of such interactions.

*Agrobacterium tumefaciens*-mediated transformation (ATMT) has been a successful tool to obtain transgenic plants for over thirty years (Zambryski, 1992; Binns, 2002; Gelvin, 2003). ATMT has also been used to efficiently produce transgenic non-plant organisms, including numerous fungal species (Lacroix et al., 2006; Michielse et al., 2005). *Botrytis cinerea* transformation has been achieved using several different techniques like particle bombardment, protoplast transformation and ATMT (Li et al, 2006; Hamada et al., 1994; Rolland et al., 2003).

In this study ATMT was used to produce transgenic *B. cinerea* expressing bright green fluorescent protein (GFP) and these transformants used to study the cytology of Si-associated gray mold resistance in sunflower.

## Materials and Methods

### *Plant material preparation*

*Helianthus annuus* 'Solita' seeds were sown into 20.3 cm (1.8 L) pots using BM1 Mix (Berger Peat Moss, St. Modeste, Quebec). This dwarf and pollen-free sunflower cultivar was selected for growth chamber experiments due to space limits and to avoid interference from autofluorescent pollen in the cytology studies. Plants were grown in growth chambers (Conviron, Winnipeg, Manitoba, Canada) with a 12 h photoperiod at 20$^{\circ}$C and 80% relative humidity and fertilized with 150 $\mu g \cdot L^{-1}$ N from 21N-2.5P-16K (The Scotts Co., Marysville, OH). The sources, rates and methods of application of Si supplements were as follows: substrate incorporation of hydrous $KSiO_3$ at 140 $g \cdot m^{-3}$ Si,

substrate incorporation of $CaSiO_3$ at 200 $g \cdot m^{-3}$ Si, and five weekly drenches of a $KSiO_3$ solution containing 50 $mg \cdot L^{-1}$ Si. All Si sources were supplied from PQ Corporation (Valley Forge, Pa.), except $CaSiO_3$ that was supplied from Sigma-Aldrich Corporation (St.Louis, MO).

*Wild-type B. cinerea cultures and inoculations*

Single-spore cultures of wild-type *Botrytis cinerea* G1, originally isolated from commercial potted *Gerbera jamesonii* plants, were maintained by serial culture on V8 agar (160 ml V8 tomato juice, 1.6 g $CaCO_3$, 16 g agar, 840 ml $H_2O$). To promote sporulation, V8 agar cultures of *Botrytis cinerea* were grown 1 week under fluorescent lighting at 20°C. *B. cinerea* conidia were suspended in water from culture plates with a glass rod and filtered through sterile cheese cloth to avoid mycelia. Spore concentrations were adjusted to $10^5$ $ml^{-1}$ using a hemacytometer. Spore suspensions were sprayed on whole flowering plants. After which, control and *Botrytis*-inoculated plants were incubated 48 hours at 95% relative humidity to establish infection inside the growth chambers described above.

*Fungal cultures for transformation*

*Botrytis cinerea* G1 cultures were maintained on MYPA medium (0.1% yeast extract, 0.1% tryptone and 0.1% sucrose, 1.5% malt extract and 1.8% agar) and incubated at 18°C under 20 W fluorescent lights with a 12 h light cycle. *B. cinerea* conidia were suspended in water with a glass rod and filtered through sterile cheese cloth. For

transformations, spore concentrations were adjusted to $10^5$ $ml^{-1}$ water, after counting with a hemacytometer.

*Fungicide application*

Full (1.68g $L^{-1}$) and half (0.84g $L^{-1}$) rates of foliar chlorothalonil (Daconil Ultrex®, Syngenta Crop Protection, Inc., Greensboro, NC) were included alone for comparison to standard industry practice and in combination with silicon treatments to discern any novel synergisms. Fungicide treatments were applied one week before inoculation. Si-supplemented and non-supplemented control plants were inoculated with conidial suspensions of either wild type or transformed *B. cinerea*, the transformation of which is described below.

*Transformation*

All *Agrobacterium tumefaciens* AGL-1 strains (Table 11) were cultured at 28°C for three days on minimal medium (MM; contains per 1 liter: 2.05g $K_2HPO_4$, 1.45g $KH_2PO_4$, 0.5 g $NH_4NO_3$, 1.5g NaCl, 0.01g $CaCl_2$, 0.25g $MgSO_4$, 2.5mg $FeSO_4$, 2g glucose, 20 µL Vogel trace elements stock solution and 18g agar) supplemented with 50 µg $ml^{-1}$ carbenicillin. Strains carrying either of the binary plasmids, pBHt2-sGFP, pOHT-sGFP or pOHT-DsRed, were cultured on media also supplemented with 200 µg $ml^{-1}$ kanamycin. Single colonies of each AGL-1 strain were used to inoculate 5 ml MM supplemented with the appropriate antibiotics, and incubated overnight at 28°C on an orbital shaker (250 rpm). Optical densities (OD) of the AGL-1 cultures were measured using a spectrophotometer (UV-265, UV Visible spectrophotometer, Shimadzu, Kyoto,

Japan) and were diluted to $OD_{600}$ = 0.2 with induction medium (IM; contains per 1 liter: 2.05 g $K_2HPO_4$, 1.45g $KH_2PO_4$, 0.5g $NH_4NO_3$, 0.15g NaCl, 0.0025g $FeSO_4$, 0.01g $CaCl_2$, 0.25g $MgSO_4$, 0.9g glucose, 5.33g MES, 5 ml glycerol, 20 µl Vogel trace elements solution, and 200µM acetosyringone), incubated overnight at 28°C on an orbital shaker (250 rpm), and the resulting cultures diluted again to $OD_{600}$ = 0.2. Equal volumes (500 µl) *Agrobacterium* IM cultures and *B. cinerea* conidial suspensions ($10^5$ conidia $ml^{-1}$) were mixed and 200 µl aliquots of this mixture were spread on 60 mm IM agar plates containing the appropriate antibiotics and overlaid with 47 mm nitrocellulose membranes (Fisher Scientific, Pittsburgh, PA).

**Table 11.** *Agrobacterium tumefaciens* strains used

| **Strain** | **Binary plasmid** | **Bacterial Genotype[a]** | **T-DNA (fungal transformant phenotypes)[b]** |
|---|---|---|---|
| AGL-1 | None (control) | $carb^R$ | None (control) |
| AGL-1 | pBHt2-sGFP | $carb^R$, $kan^R$ | $hyg^R$, SGFP |
| AGL-1 | pOHT-sGFP | $carb^R$, $kan^R$ | $hyg^R$, SGFP |
| AGL-1 | pOHT-DsRed | $carb^R$, $kan^R$ | $hyg^R$, DsRed |

[a] selective antibiotic resistances, carbenicillin resistance ($carb^R$) carried on the pTiBo542DT and kanamycin resistance ($kan^R$) carried on the binary plasmid

[b] Transgenes: hygromycin resistance ($hyg^R$) is the hygromycin phosphotransferase gene *hph* driven by the *trpC* (pBHt2) or the *oliC* (pOHT) promoter from *Aspergillus nidulans*; green fluorescent protein (SGFP) is driven by the *ToxA* promoter from *Pyrenophora tritici-repentis*; red fluorescent protein (DsRed) is driven by the *gpdA* promoter from *A. nidulans*.

After a co-incubation period of 3 days at 20°C in the dark, nitrocellulose membranes with fungal and bacterial cells were transferred to 60 mm plates containing selection medium: YPS (0.1% yeast extract, 0.1% tryptone and 0.1% dextrose, and 1.8% agar) supplemented with 50 μg $ml^{-1}$ hygromycin, as a selection agent for fungal transformants, and 200 μg $ml^{-1}$ zosyn and 50 μg $ml^{-1}$ cefotaxime, to eliminate *Agrobacterium*. After 12 days, hygromycin-resistant colonies of *B. cinerea* were transferred to 24-well plates containing the selection medium. Transformants were purified by subculturing single spores twice by on streaking 60mm petri plates containing selection medium. Transformants were subcultured three times on nonselective YPS, and then transferred to YPS supplemented with 100 μg $ml^{-1}$ hygromycin to test for stable integration. The relative green (sGFP) fluorescence of stable transformants was assessed by microscopy and by scanning culture plates using a FluorImager (Molecular Dynamics, Sunnyvale, CA) with a DF300 filter (ex. 488nm, em. 514nm). DNA was isolated from the wild type G1 strain and each transformant using a microbial DNA isolation kit according to manufacturer's instructions (Mo Bio Laboratories, Inc., Carlsbad, CA) and T-DNA integration confirmed with PCR using the primers sGFP-TYG-R (5'-AAGTCGTGCTGCTTCATGTG-3') and hph-R (5'-GCCGATGCAAAGTGCCGATAAACA-3') primers to amplify the *hph-sGFP* region.

*Transformed pathogen inoculations*

For cytological studies, detached leaves were inoculated. Fully expanded sunflower leaves were excised from plants close to the stem using a razor blade. Leaves from all treatments were trimmed to fit in an 11cm diameter petri plate and placed inside

with a water-saturated filter paper (Whatman 1) on the bottom for high humidity. Leaf surfaces were either left unwounded or lightly wounded at the center of leaf using a pipette tip prior to inoculation. Agar plugs, 5mm in diameter, from a 5-day old V8 agar culture of wild type or transformed *B. cinerea* were used for the inoculation. One plug was placed, mycelium-side down, directly over the center of each leaf, including wound site of wounded leaves. Leaves were incubated overnight inside a plastic storage container at room temperature. Plugs were removed after overnight incubation. Plates containing non-inoculated control leaves were incubated overnight in a separate plastic storage container. All treatments were replicated 3 times in a completely randomized design.

*Microscopy*

To examine the cytology of inoculated leaves, inoculated whole leaves were directly observed under low magnification (40x or 100x ), or razor-excised leaf infection courts were mounted in water on a microscope slide and observed under higher power (200x, 400x, and 600x). All specimens were examined using a Nikon Eclipse E800 epifluorescent microscope with an Endow GFP longpass filter set (ex. 450-490nm/dichroic 495nm/em.>500nm) and a Retiga-2000R CCD camera (QImaging, Burnaby, Canada).

**Results**

ATMT with the pBHt2-sGFP binary vector was successful and resulted in twenty one transformants. ATMT with the pOHT-sGFP and pOHT-DsRed binary vectors was also

successful generating twenty and eighteen transformants respectively (data not presented). All transformants expressed stable resistance to hygromycin that was not lost after three nonselective subcultures, and T-DNA integration was confirmed by PCR (Fig. 5). The GFP gene was successfully expressed in the hyphae and conidia of transformed *B. cinerea* both in vitro and during plant infection conditions (Fig. 6).

The goal of this study was to use GFP expression as a visual marker in order to study differences in the interaction between Si-supplemented and non-supplemented plants and the pathogen. For that purpose the brightly fluorescent transformant T17, which maintained the morphology and pathogenicity of the wild-type, was selected for cytological studies. *Botrytis cinerea* T17 was found to be comparable to the wild-type G1 isolate in culture and during infection of sunflower plants under growth chamber conditions and in detached leaf bioassays (Fig. 7).

When sunflower leaves of nontreated fungicide controls were inoculated with *B. cinerea* T17 plugs, decreased leaf lesion diameters were observed on plants supplemented with Si using potassium silicate drenches and calcium silicate medium incorporation (Table 12). Similar results also were observed when the wild-type isolate was used for the inoculation (data not shown). When half and full rates of fungicides were used, only the potassium silicate drenched plants, treated with half rate fungicide, showed slightly reduced lesion diameters compared to Si non-supplemented controls one week post inoculation. However, when leaf surfaces were wounded prior to inoculation there was no delay in disease development and all wounded detached leaves displayed lesions one day after inoculation regardless of Si supplements or fungicide applications (data not shown). However, one day post inoculation, unwounded leaves from potassium silicate drenched

plants showed no disease (Table 13). Also, unwounded control leaves from plants treated with full rate fungicide showed no disease, while leaves from potassium silicate or calcium silicate substrate treated with full rate fungicide showed increased disease incidence.

Sunflower leaves were observed to be covered with abundant trichomes, especially on the adaxial surface, and possessed two discrete types, smaller glandular hairs and larger conical spines (Fig. 8). In cytological examinations, *B. cinerea* conidia often were observed adhered to and aggregated on leaf trichome surfaces especially along leaf veins where trichomes appeared more aggregated. On detached leaves from plants supplemented with either potassium silicate drenches or calcium silicate medium incorporation, conidia of *B. cinerea* T17 germinated slower compared to leaves from nonsupplemented controls. For example, one day post inoculation, most conidia adhering to the trichomes of Si supplemented leaves remained ungerminated, while conidia adhering to the trichomes of nonsupplemented control leaves already had formed extensive germ tubes. These initial germ tubes appeared to rapidly grow from tichome-to-trichome on control leaves, without contacting the leaf's epidermal cells.

**Discussion**

Previous studies showed that even though Si supplementation failed to protect the highly susceptible flowers of greenhouse-produced sunflowers, Botrytis blight incidence and severity on stem and leaf surfaces were reduced (Kamenidou et al. 2005). Sunflower accumulates Si at similar concentrations to cucumber (Ma and Takahashi, 2002; Kamenidou et al., 2008), and Si supplementation has been reported to decrease gray mold

severity of greenhouse produced cucumber (Voogt and Sonneveld, 2001). Both sunflower and cucumber have leaf surfaces with abundant trichomes. Trichomes have been associated with *B. cinerea* infection in other plants. In greenhouse grown *Antirrhinum*, *B. cinerea* was found to colonize and penetrate through trichomes (McWhorter, 1939). Mutant *Arabidopsis* lacking trichomes was found to be more tolerant of *Botrtyis* infection than wild type plants (Calo et al., 2006). Trichomes appeared to facilitate adhesion of conidia and hyphae to *Arabidopsis* leaves . A trichome-overproducing mutant of *Arabidopsis* was more susceptible to *B. cinerea* than wild type plants.

In sunflower and other horticultural species, Si is deposited in leaf trichomes and polymerized into silica in higher concentrations than in the surrounding epidermal surfaces (Lanning and Eleuterius, 1989; Frantz et al., 2008). Consequently, Si deposition may fortify the trichomes in the leaf and stem surfaces creating a mechanical barrier against gray mold development, a hypothesis supported by the observation that wounding overcomes gray mold suppression due to Si supplementation or contact fungicide (chlorothalonil) treatments. Si-fortified trichomes may resist the cell wall degrading enzymes secreted by adhered *B. cinerea* germlings (Doss, 1999), thereby inhibiting further germ tube development, possibly due to the lack of inducing monomers released from the cell wall, as with cutinase (Kolattukudy, 1985).

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**Table 12.** Effect of silicon supplementation and chlorothalonil on leaf lesion diameter caused by *B. cinerea* (plug-inoculated) on unwounded detached sunflower leaves.

| | Leaf Lesion Diameter (cm) One Week Post Inoculation | | |
|---|---|---|---|
| | Fungicide rate | | |
| Silicon Sources | 0 | Half | Full |
| Control Si-Untreated | 4.0 | 3.6 | 3.5 |
| $KSiO_3$ (50 ml $l^{-1}$ Si) weekly drench | 3.1* | 3.0* | 3.1 $^{NS}$ |
| Hydrous $KSiO_3$ (140 $gm^{-3}$ Si) in substrate | 3.7 $^{NS}$ | 3.2 $^{NS}$ | 3.3 $^{NS}$ |
| $CaSiO_3$ (200$gm^{-3}$ Si) in substrate | 3.3* | 3.2 $^{NS}$ | 3.4 $^{NS}$ |

$^{NS, *}$ Not significant (NS) or significant at 5% (*) from the control in the same column by the LSD test

**Table 13.** Effect of silicon supplementation and chlorothalonil on incidence of leaf lesions caused by *B. cinerea* on inoculated unwounded detached sunflower leaves.

| | % Leaves with Lesions One Day Post Inoculation | | |
|---|---|---|---|
| | Fungicide rate | | |
| Silicon Sources | 0 | Half | Full |
| Control Si-Untreated | 100 | 100 | 0 |
| $KSiO_3$ (50 ml $l^{-1}$ Si) weekly drench | 0* | 0* | 0 $^{NS}$ |
| Hydrous $KSiO_3$ (140 $gm^{-3}$ Si) in substrate | 100 $^{NS}$ | 100 $^{NS}$ | 100* |
| $CaSiO_3$ (200$gm^{-3}$ Si) in substrate | 100 $^{NS}$ | 100 $^{NS}$ | 33* |

$^{NS, *}$ Not significant (NS) or significant (*) from the control in the same column at 5% level by the chi-square test

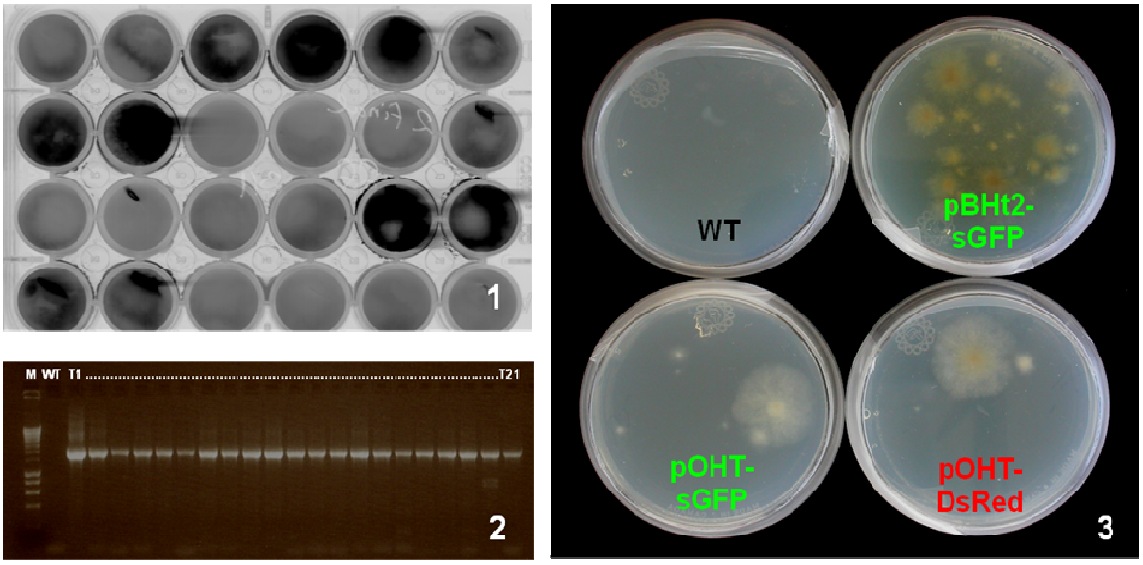



***Figure 5.*** Characterization of ATMT transformants of *B. cinerea*. 1. Fluorimager 488nm laser scan of a 24-well plate containing *B. cinerea* GFP-expressing transformants. Bright fluorescence appears darker. 2. PCR screening for T-DNA integration of pBHT2-sGFP T-DNA using primers hphR and sGFP-TYG: Markers (M), *B. cinerea* wild type (WT) and transformants (T1…T21). 3. Initial hygromycin selection plates showing transformed colonies of *B. cinerea* with pBHt2-sGFP producing the most primary transformants.

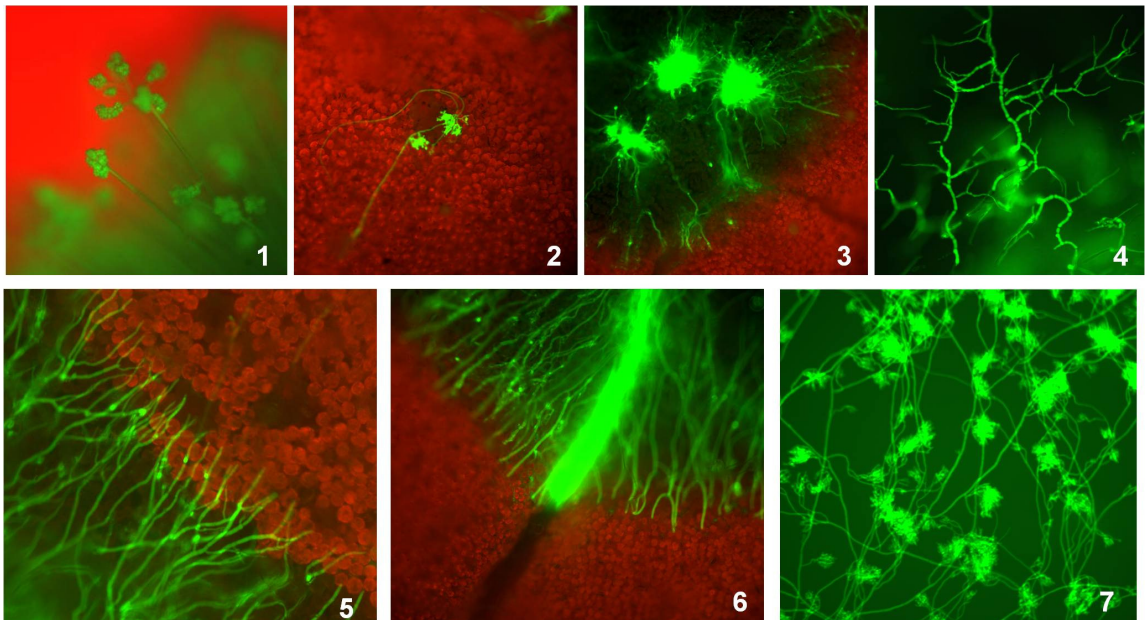



***Figure 6***. Cytology of *B. cinerea* transformants expression GFP. 1: Conidiophores (400×), 2: Initial infection cushions on leaf's surface (100×), 3: infection foci radiating hyphae inside lesion (100×), 4: hyphae in culture (200×), 5: invasive hyphae at lesion margin (400×), 6: hyphae proliferating along leaf vein (200×) and 7: infection cushions induced on the culture plate lid (200×). Leaf chlorophyll produces red autofluorescence (1,2,3,5,6).

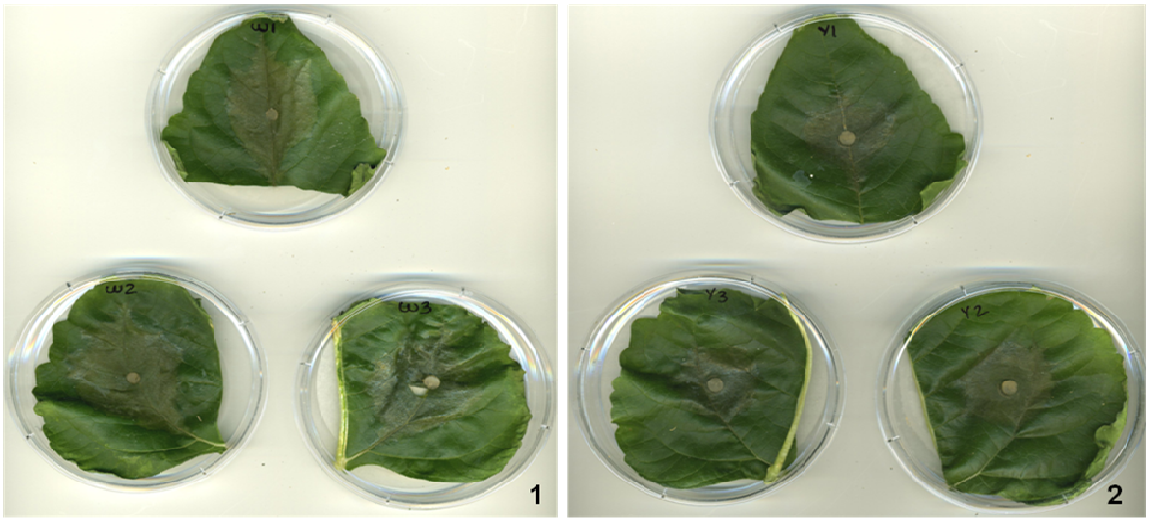



***Figure 7***. Detached leaf bioassays showing leaf sections inoculated with plugs of *B. cinerea* T17. Leaf sections of nonsupplemented control plants (1) show larger lesion areas compared to leaf sections of plant supplemented with weekly drenches of potassium silicate (2).

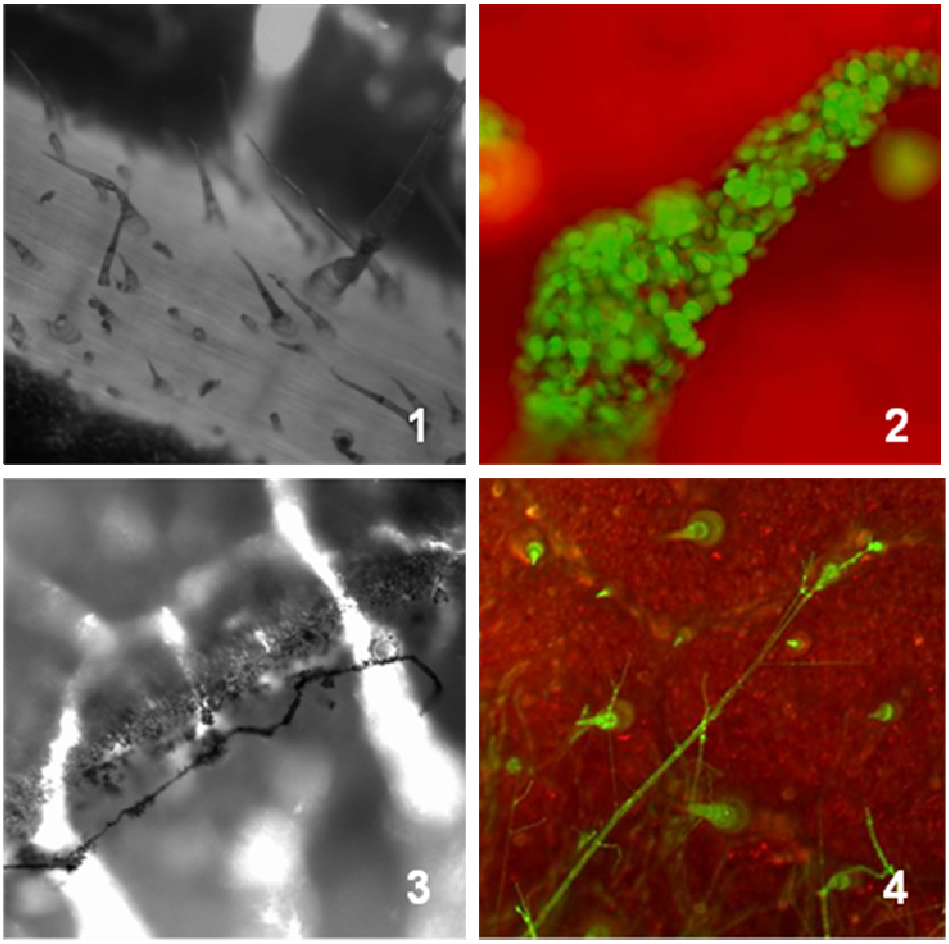
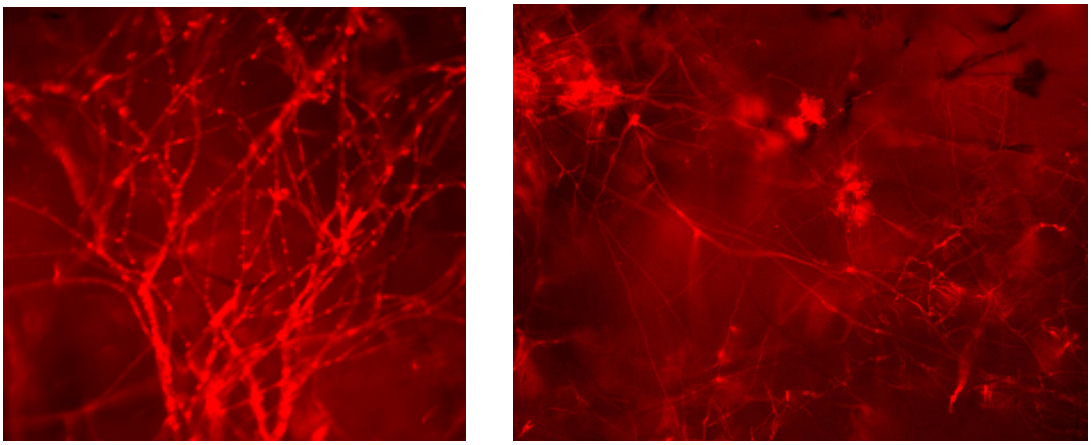
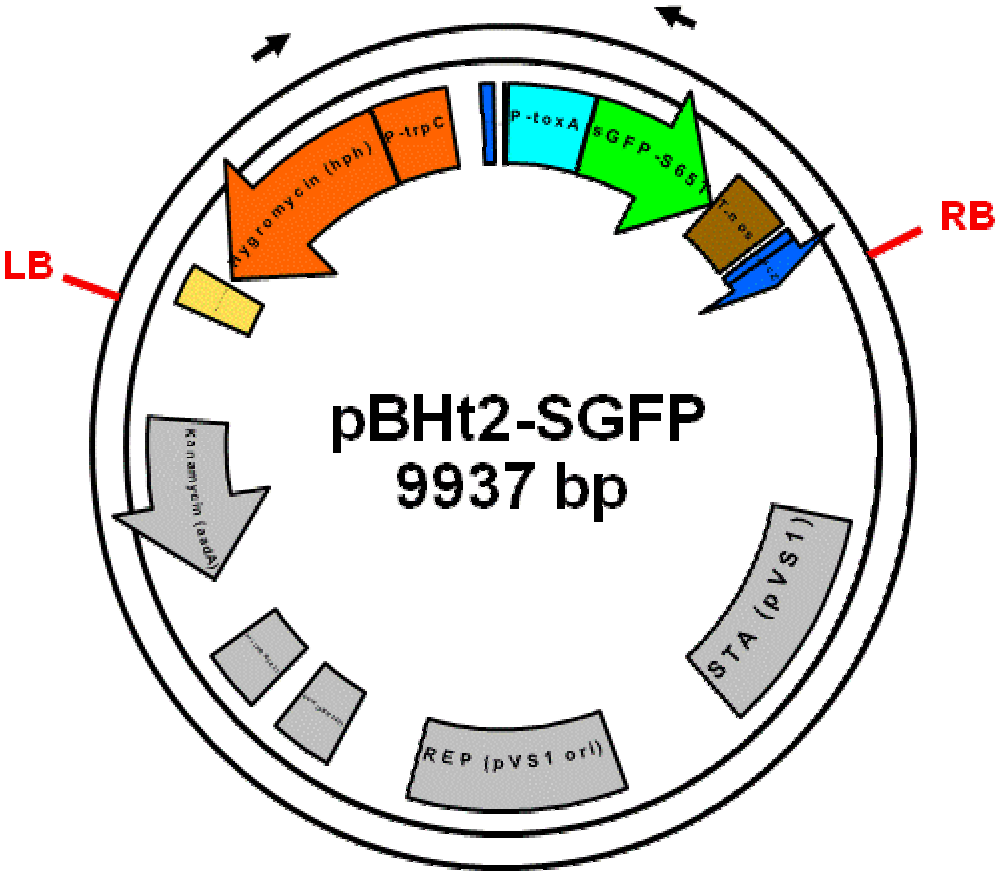



***Figure 8***. Epiphytic interactions of *B. cinerea* conidia with sunflower leaves. 1: Leaf surface (midrib) covered with trichomes (100×), 2: Leaf trichome covered with conidia of *B. cinerea* expressing GFP (400×), 3: Conidia of the wild-type *B. cinerea* adhere and germinate on the leaf trichomes (100×) 4: Conidia and hyphae of *B. cinerea* T17 adhering to and germinating on the leaf trichomes (100×).

# APPENDICES



Mycelia (bright red) of *B.cinerea* expressing DsRed protein (100X)




Binary vector: pBHt2-sGFP

Effect of Si supplementation and fungicide application rate on leaf severity of *Zinnia elegans* 'Benary's Giant Lime'

| | Leaf severity (0-6 scale) | | | | | |
|---|---|---|---|---|---|---|
| | Lower Leaf fungicide rate | | | Upper Leaf fungicide rate | | |
| Silicon Sources | 0 | Half | Full | 0 | Half | Full |
| Two weeks after inoculation | | | | | | |
| Control | 3.4 | 0.0 | 0.0 | 2.2 | 0.0 | 0.0 |
| $NaSiO_3$ folliar application | 3.4 | 0.0 | 0.0 | 2.0 | 0.0 | 0.0 |
| $KSiO_3$ drench | 2.4 | 0.0 | 0.0 | 2.2 | 0.0 | 0.0 |
| $KSiO_3$ substrate | 2.8 | 0.0 | 0.0 | 2.8 | 0.0 | 0.0 |
| $CaSiO_3$ substrate | 2.4 | 0.0 | 0.0 | 1.8 | 0.0 | 0.0 |
| Three weeks after inoculation | | | | | | |
| Control | 4.7 | 0.6 | 0.0 | 4.4 | 0.6 | 0.0 |
| $NaSiO_3$ folliar application | 4.6 | 0.0 | 0.0 | 3.0 | 0.0 | 0.0 |
| $KSiO_3$ drench | 4.0 | 0.0 | 0.0 | 4.3 | 0.0 | 0.0 |
| $KSiO_3$ substrate | 2.8* | 0.4 | 0.0 | 3.8 | 0.0 | 0.0 |
| $CaSiO_3$ substrate | 3.6 | 0.0 | 0.4 | 3.2 | 0.0 | 0.0 |

*: Significant from the control in the same column at 1% level

Fungicide rate impact on powdery mildew incidence of *Zinnia elegans* 'Benary's Giant Lime'

| | Disease Incidence[1] (% diseased plants) | | | |
|---|---|---|---|---|
| | Fungicide Rate | | | |
| Silicon Sources | 0 | Half | Full | r |
| Control Si-untreated plants | 100 | 0 | 0 | -0.87*** |
| $NaSiO_3$ folliar application | 100 | 0 | 0 | -0.87*** |
| $KSiO_3$ substrate drench | 80 | 0 | 0 | -0.74*** |
| $KSiO_3$substrate incorporation | 100 | 0 | 0 | -0.87*** |
| $CaSiO_3$ substrate incorporation | 80 | 0 | 0 | -0.74*** |

*** , Significant at 0.1%

r: Pearson's correlation coefficient

[1]: two weeks after innoculation

VITA

SOPHIA KAMENIDOU

Candidate for the Degree of

Doctor of Philosophy

Dissertation: SOLUBLE SILICON-BASED DISEASE MANAGEMENT OF FLORICULTURAL CROPS

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Institution: Oklahoma State University Location: Stillwater, Oklahoma

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Major Field: Plant Science

Scope and Method of Study: The goal of this research was to assess the efficacy of various forms and applications of soluble silicon (Si), both alone and in combination with two rates of registered fungicides, to control three economically important fungal diseases that affect greenhouse production. The three diseases assessed were as follows: powdery mildew of zinnia and sunflower caused by *Golovinomyces* (=*Erysiphe*) *cichoracearum*, Phytophthora root rot of gerbera caused by *Phytophthora drechsleri,* and gray mold of sunflower caused by *Botrytis cinerea*.

Findings and Conclusions:

Powdery mildew study: In zinnia, Si supplementation reduced powdery mildew severities three- to four-fold after two weeks and two- to three-fold after three weeks. Silicon supplementation of zinnia, when combined with myclobutanil treatments, also appeared to synergistically suppress powdery mildew severity two-fold and prolong fungicide-mediated disease suppression to three weeks. In sunflower, Si supplementation suppressed powdery mildew severities two- to three-fold after two weeks and around 1.5-fold after three weeks. Myclobutanil suppressed powdery mildew of sunflower all three weeks.

Phytophthora root rot study: Mefenoxam suppressed Phytophthora root rot of greenhouse produced gerbera. Five weekly potassium silicate substrate drenches delayed disease development, but overall Si supplements did not provide protection against this disease.

Gray mold study: Disease progression and conidial germination and mycelial growth of wild type and transgenic *B. cinerea* expressing GFP were delayed in Si supplemented sunflower plants. However, gently wounding detached leaves of Si supplemented plants overcame disease suppression and returned conidial germination and disease progression to control levels. Therefore, Si deposition may fortify sunflower trichomes, an observed location of conidial accumulation, and leaf and stem surfaces, creating a mechanical barrier against *B. cinerea*.

ADVISER'S APPROVAL: Dr. Mike Smith