EVALUATION OF SILICON AND BIOFUNGICIDE PRODUCTS FOR MANAGING POWDERY MILDEW CAUSED BY *Podosphaera fusca* IN GERBERA DAISY (*Gerbera jamesonii*)

By

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To my husband Scott

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Gerbera daisy (*Gerbera jamesonii* Bolus ex. Hook f.) is an ornamental plant grown in Florida under greenhouse or shade house conditions as potted plants and for landscape. Gerbera plants are highly susceptible to powdery mildew caused by the fungus *Erysiphe cichoracearum* DC. or *Podosphaera* (Syn. *Sphaerotheca*) *fusca* (Fr.) S. Blumer. This disease affects all plant parts and reductions in yield and in quality are the main components of economic loss. Environmental conditions of high humidity and moderate temperatures, which are highly prevalent in Florida, are the most conducive for powdery mildew development. Consequently, most nurseries use repeated applications of fungicides as the main method for powdery mildew control. However, pathogens may develop resistance to some fungicides after consecutive applications and thus, other alternatives to powdery mildew control are in demand. The objective of this study was to evaluate the efficacy of silicon and other biofungicides for suppressing powdery mildew in gerbera daisy. The effect of silicon as calcium silicate, incorporated into the growing medium, or potassium silicate, applied as a drench, was evaluated in the highly susceptible cultivar 'Snow white.' The effect of silicon in flower quality and the silicon content in gerbera leaves was determined as well. The effect of spray applications of the biofungicide

products Actigard (acibenzolar-S-methyl), K-phite (phosphorous acid), Milstop (potassium bicarbonate), Prevam (boron, orange oil and organic surfactants), Rhapsody (*Bacillus subtilis*) and AgSil (potassium silicate) was evaluated in highly susceptible ('Snow White' and 'Orange') and moderately susceptible ('Hot Pink' and 'Fuchsia') cultivars. Powdery mildew caused by *Podosphaera fusca* developed in the plants from natural inoculum. Disease severity was assessed and ratings were used to calculate the area under disease progress curve (AUDPC). Results suggested that neither calcium silicate nor potassium silicate were effective in suppressing powdery mildew in gerbera daisy. These silicon sources did not have an effect on flower quality nor did they accumulate in gerbera leaves. The biofungicides products Actigard, Agsil, K-phite, Milstop, Prevam, and Rhapsody suppressed powdery mildew of gerbera daisy compared with untreated plants; however, these products were not as effective as the fungicide program of Heritage alternated with Eagle. Among the biofungicides tested, Actigard and Agsil were the least effective treatments. Rhapsody provided moderate disease control and K-phite, Millstop and Prevam were the most effective in reducing disease severity. The level of disease reduction obtained with Prevam in cultivars Fuchsia, Hot pink and Orange compared to that attained with the systemic fungicides. In conclusion, biofungicide products can significantly reduce powdery mildew compared to no treatment and products such as Prevam, K-phite, and Millstop may be used in alternation with systemic fungicides as part of an integrated disease management program and as an alternative to reduce the use of fungicides for suppressing powdery mildew of gerbera daisy.

CHAPTER 1
INTRODUCTION

In 2005, the wholesale value of bedding and garden plants in the US increased four percent giving this industry a total value of about $1.5 billion. Bedding and garden plants are mainly produced in California, Florida, Michigan, New York, Ohio and Texas. The leading producers of potted flowering plants are California and Florida. Florida has the largest floriculture production area with 15, 900 acres and about 10% of this area is in permanent greenhouses; the remainder is either in the open field or under shade or temporary cover. In 2005, floriculture sales in Florida from potted flowering plants reached $95 million (USDA, 2006).

Gerbera daisy (*Gerbera jamesonii* Bolus ex. Hook f.), belonging to the family Asteraceae, is an evergreen perennial grown as an ornamental plant. It produces flowers borne in stems arising from the crown, and it is commercialized as cut flowers, potted plants or for landscape (Tija and Black, 2003). Gerbera daisy originated in South Africa and were introduced to the Americas in the 1920s.In 1932 gerberas were cultivated outdoors in Florida (The gerbera association). Nowadays, gerberas in Florida are produced mainly under greenhouse or shade house as potted and bedding plants.

Gerberas are propagated by seed and vegetatively through tissue culture. Propagative material is produced both by US growers (Florida leads this industry) and in Central America (Costa Rica, Guatemala and Mexico). Immature plants and seedlings are started abroad and are brought into the US to mature further in nurseries prior to final sale (USDA, 2006).

Gerberas are susceptible to several pests and diseases. Powdery mildew is an important fungal disease in gerberas and it can be caused by two species, *Erysiphe cichoracearum* DC and *Podosphaera* (Syn. *Sphaerotheca) fusca* (Fr.) S. Blumer (Daughtrey et al., 1995). This disease

affects all plant parts and reductions in yield and in quality are the most important components of economic loss. Environmental conditions most conductive for powdery mildew development include high humidity (80% to 90%) and moderate temperatures from 20 to 28°C (Daughtrey et al., 1995); these conditions are prevalent in Florida.

Standard practices for powdery mildew control include chemical, cultural and biological methods (Agrios, 2005). Nurseries in Florida mainly control powdery mildew through repeated applications of fungicides (Larson and Nesheim, 2000). However, consumer's perception of the impact of pesticides on the environment and human health has intensified the search for alternative methods of disease control (Gullino et al., 1999).

In recent years, several alternative products including biological control agents, potassium bicarbonate, phosphorous acid, compounds that induce systemic resistance, oils and silicon have been reported to control powdery mildew. These products are often described as biofungicides and they can directly affect fungal organisms or may stimulate the plant's defense mechanisms (McGrath, 2004).

The goal of this study is to evaluate the efficacy of silicon and several biofungicide products for the management of powdery mildew in gerbera daisies grown under greenhouse conditions in Florida.

CHAPTER 2
LITERATURE REVIEW

Powdery Mildew

Powdery mildew describes the organism as well as the disease caused by a group of fungi that are obligate parasites or biotrophs meaning that they can only grow and multiply in a living organism (Agrios, 2005). As many other fungi, the causal agent of powdery mildew has two names and the names are based on its reproductive stage. The teleomorphic or perfect stage describes a fungus when reproducing sexually and anamorphic stage describes the fungus when reproducing asexually. The perfect stage refers to a fungus with white hyphae and courless ascospores (sexual spores) formed within a sac known as ascus (pl. = asci) enclosed in an ascocarp (i.e., cleistothecia) that is present on the surface of plant tissues at the end of the growing season (Heffer et al., 2006). The anamorph is the stage of the fungus that develops as white mycelium (mass of hyphae) on the surface of plant tissue, with one-celled conidia (asexual spore) produced singly or in chains at the tips of conidiophores (specialized hyphae) that arise from the mycelium (Agrios, 2005). Some species of powdery mildew are known to produce only conidia but many others produce conidia and then clestothecia when environmental or nutritional conditions become favorable. Thus two names that differentiate the two reproductive phases describe the same organism. However the sexual name is generally used even though the sexual stage is rarely found (Yarwood, 1978). Powdery mildew of gerbera daisies is caused by two fungal species *Erysiphe cichoracearum* DC.and *Podosphaera* (Syn. *Sphaerotheca) fusca* (Fr.) S. Blumer (Daughtrey et al., 1995).

Taxonomy and Phylogenetic History

Linnaeus, in 1753, gave the first binomial name to the causal agent of powdery mildew as *Mucor erysiphe* based only on the white mycelial stage of the fungus. The name *Mucor* was

dropped since it had been used previously for another fungus similar to what is now called *Rhizopus*; however, the specific epithet e*rysiphe* continued to be used as a genus and later as a family name (Yarwood, 1978). Today's taxonomy is based on the work of Leveille in 1851. He considered powdery mildew fungi as a single and separate family and introduced a classification system with a key for the genera. This system was based on the perfect stage of the fungus, and species were differentiated based on number of asci per ascocarp, number of ascospores per asci and the morphology of appendages; however, the importance of anamorphs was not recognized (Yarwood, 1978). Ten years later, the Tulasne brothers recognized the connection between anamorphic and teleomorphic stages and assigned the species causing powdery mildew to the family Erysiphaceae (Braun et al., 2002). Taxonomic research in powdery mildew is now based not only in morphological features but also on electron microscopy and molecular tools. The current classification is similar to the one proposed by Braun in 1987, but it has been changed based on recent molecular studies (Braun et al., 2002). Saenz and Taylor (1999) studied the phylogenetic relationships among 45 powdery mildews species plus two outgroup species using internal transcribed spacer (ITS) sequences and 17 morphological characters. Results from both morphological and molecular data revealed that the powdery mildew agents formed six evolutionary lineages that include: Clade 1 - *Erysiphe*, *Microsphaera*, and *Uncinula*; Clade 2 - *Erysiphe galeopsidis* and *Erysiphe cumminsiana*; Clade 3 - *Erysiphe* species; Clade 4 - *Leveillula* and *Phyllactinia*, Clade 5 - *Sphaerotheca*, *Podosphaera*, and *Cystotheca*; Clade 6 - *Blumeria graminis*. All clades have an asexual stage called *Oidium* except clade 4 for which the asexual states are known as either *Oidiopsis* or *Ovulariopsis*.

The species causing powdery mildew on gerbera daisies originated from two different lineages (clade 3 and5); both species belonging to the class Ascomycetes; order Erysiphales and

family Erysiphaceae. The species *Erysiphe cichoracearum* is grouped within the tribe Erysipheae, and the species, *Podosphaera* (Syn. *Sphaerotheca) fusca* within the tribe Cystotheceae, subtribe Cystothecinae (Agrios 2005; Braun et al., 2002). The genus *Podosphaera* was introduced in 1823 (Braun et al., 2002). Furthermore, Takamatsu et al. (2000) studied the evolutionary history of the Podosphaera's tribe Cystotheca, and their molecular results suggested that the genus *Sphaerotheca* originated from a *Podosphaera*-like ancestor and that their evolution involved a change in host range from arbor to herb species. Analogous results were reported previously by Saenz and Taylor (1999) who pointed out that since molecular data showed that *Podosphaera* and *Sphaerotheca s*pecies did not form different clades and given that *Podosphaera* is the older name, all *Sphaerotheca* species could be changed to *Podosphaer*,thus, *Podosphaera* (syn. *Sphaerotheca).*

Host Range and Symptoms

Powdery mildew is a worldwide disease and it affects many species of plants including cereals, grapes, ornamentals, trees, and vegetables (Agrios, 2005). Some powdery mildew fungi have a wide host range whereas others are host specific. For instance, *Erysiphe*, *Laveillula* and *Sphaerotheca* are common on annual herbs whereas *Microsphaera*, *Phyllactini, Podosphaera*, and *Uncinula* occur on woody plants, and *Erysiphe graminis* is specific to grasses and cereals (Yarwood, 1978). Flowering potted plants that are especially susceptible to powdery mildew include begonia, dahlia, gerbera, hydrangea and poinsettia (Daughtrey et al., 1995).

Powdery mildew differs from most diseases in that it does not cause symptoms other that a slow decline (or killing) of plant parts and decrease in host productivity (Yarwood, 1978). However, the disease is easy to identify by its conspicuous signs that first appear as spots or patches of white mycelium on upper and lower leaf surfaces that can also spread to shoots, stems, flowers and fruits. The spots gradually enlarge and coalesce as hyphae and conidia are

produced in abundance, forming a white powder-like mat (Agrios, 2005). On artichoke, onion, pepper and tomato, yellow patches and little white powdery growth are produced on the leaves (Davis et al., 2001). Severely infected leaves turned pale yellow, brown and then die. At the end of the growing season, cleistothecia appear singly or in groups on the mycelium. Those structures are spherical, thick-walled, small, and at first white, then orange – red, and finally black (Agrios 2005; Alexopoulos and Mims, 1979).

Disease Cycle

Powdery mildew fungi produces white mycelium from which short and erect specialized hyphae called conidiophores develop (Agrios, 2005). Oval or round-shaped conidia are produced in chains at the tip of each conidiophore (Kendrick, 2000). Conidia are dispersed by air current to other parts of the plant or to other plants of the same species. If conditions of temperature and relative humidity are favorable, the conidia germinate and infect the host plant (Agrios, 2005). The mycelium formed during previous cropping seasons serves as the primary inoculum which infects new emerging leaves. Conidia produced from the primary inoculum bring about secondary infections making powdery mildew a polycyclic disease (Heffer et al., 2006).

Conidia are deposited on the host plant and adhere to its surfaces by sticky exudates that are produced before germination and, according to Carver et al. (1999) these exudates contain several proteins that may play a role in the infection process. The process involved in the recognition of leaf surfaces by the conidia is not well understood; however, it is thought that those interactions initiate the emergence of a germ tube (Green et al., 2002). As conidia germinate development of a germ tube starts which elongates and penetrates the leaf epidermis (Agrios, 2005). Three functions are attributed to the germ tube: the attachment of conidia to plant surface, a means for the conidium to access the host water and establish if the contact surface is suitable for germ tube elongation and development of appressorium (Green et al., 2002). The

germ tube differentiates into an appressorium from which a specialized hyphae, called the penetration peg, crosses the cuticle and penetrates the host epidermal cell (Green et al., 2002). If penetration is successful, the peg differentiates into haustoria, a feeding structure that develops within the plasma membrane and feeds mainly by extracting glucose from its host (Zeyen et al., 2002). The germ tube continues to grow and spreads across the leaf surface producing mycelium and many haustoria (Agrios, 2005). Through haustoria, the fungus establishs a biotrophic relationship with the host. This life cycle takes place with all powdery mildew systems except for *Blumeria graminis*. This species will produce two germ tubes but only one will form appresoria while the other serves as a reserve (Green et al., 2002; Zeyen et al., 2002).

When environmental conditions become unfavorable, production of conidia slows down and the fungus may switch to the production of cleistothecia containing one or several asci (Alexopolus and Mims, 1979). The sexual spores or ascospores are produced within the asci. Ascospores are released when the new season or host tissue becomes available and the cycle starts over (Agrios, 2005). Powdery mildew species may survive as mycelium or by production of cleistothecia (Jarvis et al., 2002).

Biology and Epidemiology

During the agricultural growing season, powdery mildew agents reproduce prolifically by asexual conidia. The development of an epidemic depends on environmental conditions as well as on parasite and host factors (Agrios, 2005). A unique set of environmental conditions such as temperature, humidity and light will determine conidial and ascospore formation, release and dispersal (Jarvis et al., 2002). For example, the release of conidia from apple, cucumber, rose and sweet peas is periodic with the highest release occurring at noon and gradually declining in the afternoon and early evening (Pady, 1969).

Conidia once produced remain viable for 7 to 8 days and may be dispersed by air currents (Zitter et al., 1996). The separation of conidia from the mother colony may trigger the germination process since conidia do not germinate when attached to the conidiophores (Green et al., 2002). Requirements of environmental conditions vary among isolates in a specific host (Jarvis et al., 2002). Thus, the range of conditions for powdery mildew development are quite wide and include periods of low to high relative humidity (below 50% to near 100%) and temperatures between 10 to 28°C (Daughtrey et al., 1995; Horst, 1983; Jarvis et al., 2002; Zitter et al., 1996). Powdery mildew is more prevalent in shady areas, which may be related to differences in temperature and humidity (Butt, 1978). Conidia of powdery mildew species have the ability to germinate in the absence of free water. Moreover, water on leaf surfaces is detrimental to the development of powdery mildew (Jarvis et al., 2002). Furthermore, conidial germination varies depending on a particular species. Conidia produced singly germinate faster than conidia borne in chains. For instance, species of *Erysiphe* take about 8 h whereas those of *Podosphaera* take more than 10 h to germinate. (Braun et al., 2002)

The incubation period or time between infection and development of symptoms (i.e. colonies) is generally between 3 to 7 days (Butt, 1978; Zitter et al., 1996; Wheeler, 1978). However, the length of this period depends greatly on environmental conditions. Xu (1999) studied the effect of temperature on the rose- *Sphaerotheca pannosa* dynamics. He found that the shortest incubation period, 3 days, was attained at temperatures between 22 and 24 °C, whereas, the longest, 10 days, was reached at 8 °C. Also, colonies expanded and had greater sporulation when temperatures were kept constant. Consequently, he concluded that temperature influences the development of powdery mildew of rose by the number and size of colonies, rate of colony growth, and sporulation.

Genetics and Physiology

With the exception of *Blumeria graminis*, genetic and molecular analyses of powdery mildew fungi are not well advanced compared to other plant pathogens, and this maybe due to their nature as obligate biotrophic fungi (Fernandez-Ortuño et al., 2007).

Genetic studies using molecular biological techniques have been conducted on powdery mildew fungi not only to study its phylogeny but also to examine the distribution range of the fungus among plants. For instance, Hirata et al. (2000) showed that molecular data were consistent with the groupings of *Sphaerotheca* species based on infectivity. Isolates with a wide host range that co-infected acalypha, cosmos, cucumber, zucchini, sunflower and zinnia, had identical nucleotide sequences, whereas other isolates that infected marigold and gerbera were unable to co-infect and belong to different haplotypes. Moreover, Hirata and Takamatsu (2001) reconfirmed the close relationship between phylogeny and infectivity of powdery mildew fungi. These authors stated that host specialization might be what triggered genetic divergence among the powdery mildews.

In a recent study, Fernandez-Ortuño et al. (2007) amplified the whole genome of *Podosphaera fusca* by using the amplification of phi29 DNA polymerase-mediated multiple displacement amplification (MDA) technique, which allows the production of large amounts of DNA. They demonstrated that the amplification of mitochondrial and nuclear fragments was identical whether using DNA generated from MDA or from the traditional PCR-based technique. Thus, the MDA technique may facilitate molecular genetic studies in powdery mildew fungi, which are very limited at present.

Detection and Diagnosis

Powdery mildew is probably the most easily recognizable plant disease (Agrios, 2005). Its conspicuous signs first appear as whitish colonies growing on living leaves and are particularly

common in shady areas (Kendrick, 2000). The white growth of the fungus consisting of mycelium and conidiophores develops on both leaf surfaces and on shoots, stems, flowers and fruits (Davis et al., 2001). Disease signs usually develop first on older leaves and on some plants powdery mildew may cause distortion of the leaves (Daughtrey et al., 1995; Horst, 1983; Zitter et al., 1996).

Identification of powdery mildew fungi to genera and species used to rely on morphological characteristics of the sexual stage (Yarwood, 1978). Nowadays, new morphological and molecular approaches are used and isolates are differentiated not only by the sexual, but also by the asexual stage (Braun et al., 2002). An important characteristic for identification is whether conidia are produced in chains or singly. However, this distinction can be difficult to observe, and in some genera, particularly in the *Erysipheae*, conidia that are produced singly can "stick together" to form pseudochains. Other characteristics that aid in classification are host specificity and the location of mycelium whether superficial (epiphytically) or within the leaf tissue (endophytically) (Heffer, 2006).

Cook et al. (1997) used light and scanning electron microscopy and host range data to identify and classify sixty powdery mildew fungi in their anamorphic stage. Observations by light microscopy identified isolates based on size and shape of conidia, presence of fibrosin bodies, conidia in chains, patterns of outer wall of wrinkled conidia, characteristics of mycelium and the degree of lobbing of appressoria. Observations by scanning electron microscopy allowed more accurate identification by distinction of patterns on the conidial surfaces such as the end walls separating conidia from conidiophores or from other conidia, and the outer walls of turgid and wrinkled conidia. Despite all characteristics measured, some discrepancies were found among isolates and thus host range data were needed for identification.

In the case of gerbera daisies, the fungi that cause powdery mildew belong to two separate taxa with different morphological characteristics. For instance, *Podosphaera* have conidia with fibrosin bodies and crenate edge lines and, the germ tubes originate from the sidewall whereas *Erysiphe* does not have fibrosin bodies, the conidia have sinuate edge lines and the germ tubes originate from the end wall. These and other differences on the surface of the conidia make the two species relatively easy to recognize by using light and scanning electron microscopy (Braun et al., 2002).

Molecular techniques such as PCR-based analyses are often used for detection and identification of plant pathogens (Agrios, 2005). An accurate identification of plant pathogens is essential in plant quarantine and in locating the origin and source of inoculum (Cook et al., 1997).

Serological tests have also been used for the detection of powdery mildew fungi. Van Roestel et al. (1991) used polyclonal antibodies to detect the location of antigens of the fungus on the surface of germinated conidia of *Erysiphe graminis* on barley. Results suggested that some antigens can be detected on the host before penetration by the fungus and that the levels of antigens increased as disease progressed.

Studies on powdery mildew are difficult because of the obligate, biotrophic nature of the fungus that makes storage and propagation of isolates unpractical and time-consuming. Traditionally, conservation of powdery mildew fungi is done by keeping the fungus on a susceptible host, and frequent transfers of conidia onto fresh plant tissues. However, Perez-Garcia et al. (2006) recently developed a method for long-term preservation of *P. fusca.* After desiccating conidia with silica gel from 6 to 12 h at 22°C and keeping it at -80°C for three years, they obtained a recovery of 100%. After 4 to 5 years, the recovery decreased 20-25%. When

phenotypic and genetic stability essays were conducted, no differences were found among the isolates before and after storage. This preservation technique may be very useful for diagnosis, fungicide resistance, population genetics, or taxonomy studies in powdery mildew fungi.

Powdery Mildew Control

Management of powdery mildew can be achieved by using a variety of strategies such as cultural practices, resistant cultivars, use of chemical products, sprays of defense activating compounds, biocontrol agents, salts and oils (Agrios, 2005; Daughtrey et al., 1995; Malathrakis and Goumas, 1999; Horst, 1983; Zitter et al., 1996).

Cultural methods and resistant varieties

Cultural practices include planting in sunny areas, providing good air ventilation, and avoiding excessive fertilization (Agrios, 2005; Daughtrey et al., 1995; Horst, 1983). Overhead irrigation may reduce powdery mildew as it washes off conidia from the surfaces of leaves, but, it may contribute to other disease problems (Davis et al., 2001).

Control of powdery mildew in cereals and in other annual crops is mainly achieved by the use of resistant cultivars (Agrios, 2005). Hsam and Zeller (2002) stated that, to date, diverse choices of resistance genes, gene combinations and modes of inheritance are available for breeding wheat cultivars resistant to powdery mildew. Resistant cultivars are also used to manage powdery mildew of cucurbits and roses (Horst, 1983; Zitter et al., 1996). Fully resistant cultivars of melon and partially resistant cultivars of cucumber, pumpkin, squash, and watermelon are available (Malathrakis and Goumas, 1999; Jahn et al., 2002). However, disease resistant cultivars are not widely developed for greenhouse crops (Elad et al., 1999) and, moreover, genetic resistance is not common in ornamental crops (Jarvis, 1992). Research on development of gerbera daisy cultivars resistant to powdery mildew is currently underway. In a recent study, Kloos et al. (2005) examined the relationship between the high level of resistance

of *Gerbera hybrida* to powdery mildew and trichome density as they thought there was a relationship between those phenotypes. Although they found that two major genes controlling those traits were unrelated, they provided preliminary information for future powdery mildew resistance research on *G. hybrida.*

Chemical control

Sulfur has been used as treatment to control powdery mildew since 1802. It is inexpensive and relatively effective (Bent, 1978). In the early 1990s, systemic fungicides were developed (Agrios, 2005). Currently, fungicides to control powdery mildew include the group of strobilurins (QoI fungicides) such as trifloxystrobin sold as Flint (Bayer CropScience, North Carolina USA); azoxystrobin as Quadris, Heritage or Abound (Syngenta, Greensboro, NC); and pyraclostrobin sold as Headline(Basf corporation, Florham Park, NJ) or Heritage (Syngenta, Greensboro, NC). Other fungicides used are in the triazole group, such as myclobutanil sold as Nova or Eagle (Dow AgroSciences, Indianapolis, IN) (Agrios, 2005; Crop Data Management Systems, 2007). Inorganic chemicals such as sulfur and copper compounds are often used; however, phytotoxicity has occurred (Nuñez-Palenius, 2006).

In the floriculture industry, growers rely on synthetic chemicals for the control of pests and diseases. However, chemical control is not always completely effective, as some chemicals may no longer work due to the development of resistance by pests and pathogens (Gullino and Wardlow, 1999). The risk of development of resistance increases when a pathogen undergoes many and short diseases cycles per season (Brent, 1995). Systemic fungicides used for the management of powdery mildew have a high risk of developing resistance and powdery mildew has a high potential for development of resistance (McGrath, 2004; Brent, 1995).

Resistance of powdery mildew has already occurred with the fungicides benomyl (Benlate, DuPont, Atlanta GA) and triadimefon (Bayleton, Bayer CropScience, North Carolina USA)

(McGrath, 2001). Studies to determine the sensitivity of *Uncinula necator*, the causal agent of powdery mildew on grapes, to strobilurin fungicides were conducted in California and New York, and both studies agreed that powdery mildew isolates have already developed a higher tolerance to strobilurin fungicides (Miller and Gubler, 2004; Wong and Wilcox, 2002). Also, in Japan, azoxystrobin failed to control *Podosphaera fusca* in cucumber, and a fungicide sensitivity tests and DNA sequence analysis confirmed the development of resistance to strobilurin fungicides (Ishii et al., 2001). Furthermore, the failure of myclobutanil to control powdery mildew in grapevines was confirmed in Canada in 2000 (Northover and Homeyer, 2001).

A successful disease management program must involve strategies for resistance management to delay the build-up of resistant strains (McGrath, 2004). The Fungicide Resistance Action Committee (FRAC) provides fungicide resistance management guidelines to prolong the effectiveness of "at risk" fungicides. The strategies for resistance management include: 1. Use of mixtures of products with one or more fungicides with different modes of action or different chemical groups, or use as one component in a rotation or alternation. 2. Restriction of the number of treatments applied per season, and application only when strictly necessary. 3. Use of products at the manufacturer's recommended rate. 4. Avoidance of systemic fungicides. 5. Use IPM (Integrated Pest Management) (Brent, 1995).

Alternative measures

The demand by consumers for high plant quality forces growers to intensify their spray programs against diseases. This practice may lead to the faster development of pesticide resistance while increasing overall production costs. However, consumer awareness of potential effects of pesticides on the environment and human health has intensified the search for alternative methods of disease control (Gullino et al, 1999). Biofungicides are certain types of naturally based microbial or biochemical product derived from animals, plants, bacteria, and

certain minerals. These products can affect fungal organisms directly or may stimulate the plant's own defense. They are generally narrow-spectrum, decompose quickly, and thus are considered to have low potential for negative impact on the environment. In addition, biofungicides require less data for registration and are approved in faster than conventional pesticides (McGrath, 2004; EPA, 2007). Biofungicides products such as Actigard, biological control agents, oils, potassium bicarbonate, phosphorous acid, and silicon, have been successfully used against powdery mildew (Belanger et al., 1994; Belanger and Labbe, 2002; Ghanmi et al., 2004; Grove et al., 2005; McGrath and Shishkoff 1999; Menzies et al., 1992; Pasini et al., 1997; Reuveni et al., 1996).

Induced resistance

In nature, most plants defend themselves against pathogens by the effect of constitutive barriers or by activating protective mechanisms, a processes known as induced resistance (Sticher et al., 1997). Defense responses are a set of metabolic alterations that not only accumulate locally at the site of infection but are also induced systemically, such as the development of systemic acquired resistance (SAR) or induced systemic resistance (ISR) that prevents further attacks from fungi, bacteria, and viruses (Vallad and Goodman, 2004; Van Loon and Van Strien, 1999). Defense mechanisms that are elicited include reinforcement of cell wall by papilla formation, callose and lignin deposition, production of antimicrobial phytoalexins or phenolic compounds and synthesis of antifungal proteins such as ß-1,3 glucanases and chitinases (Belanger and Labbe, 2002; Salmeron et al., 2002).

SAR can be triggered by microbes or by natural or synthetic chemicals such as salicylic acid (SA), 2,6-dichloro-isonicotinic acid (INA) or benzo(1,2,3)thiadiazole-7-carbothionic acid S-methyl ester (BTH). The latter has been marketed as Actigard, Bion and Boost. In contrast, ISR is activated by rhizobacteria such as several strains of *Pseudomonas* (Vallad and Goodman,

2004). When SAR is activated, salicylic acid (SA) and pathogenesis related proteins accumulates at the site of infection and moves throughout the plant (Mauch-Mani and Metraux, 1998).

Products such as Actigard, potassium silicate, and microorganims such as *Bacillus subtilis* have been reported to induce resistance against powdery mildew (Belanger and Labbe, 2002). Actigard alone was effective in reducing the incidence of powdery mildew in several lettuce cultivars (Matheron and Porchas, 2000); however, alternating Actigard with commercial fungicides resulted in high levels of powdery mildew suppression in lettuce (Matheron and Porchas, 2004). Moreover, Actigard was effective against powdery mildew in cabbage and in muskmelon (Matheron and Porchas, 2003; Miller and Hernandez, 2001). However, Actigard did not provide a satisfactory level of disease reduction in pumpkin (Babadoost, 2002). Moreover, it was ineffective when used as a protectant against powdery mildew of cucumber suggesting that it failed to enhance the defense responses in this plant (Wurms et al., 1999). Therefore, activation of resistance is independent of the target pathogen and it may be determined by the crop (Oostendorp et al., 2001).

Biological control

The term "biocontrol" that is used in plant pathology entails the use of antagonistic microorganisms to suppress plant diseases. The organisms that suppress pests or plant pathogens are referred to as the biological control agents (BCA) (Agrios, 2005; Pal and McSpadden, 2006). The mechanisms involved in biological control include antibiosis, competition, cross protection and parasitism (Jarvis, 1992; Shoda, 2000). BCA have been used to control powdery mildew since the early 1900s, and their use has increased in the last 20 years especially under greenhouse conditions (Belanger and Labbe, 2002). There are approximately 40 fungal species that have been reported or have been tested as prospective biocontrol agents of powdery mildew (Kiss, 2003). Several BCA have been registered for the control of powdery mildew and it is presumed

that the interaction of these organisms with powdery mildew is not limited to one mode of action. *Ampelomyces quisqualis* (AQ10) colonizes the hyphae and conidiophores of its host. *Tilletiopsis* spp, *Pseudozyma flocculosa* (Sporodex, Plant Products Co., Brampton, Ontario, Canada) and *Bacillus subtilis* QST 713 (Serenade® or Rhapsody®, AgraQuest, Inc. Davis, CA), act by antibiosis meaning that they first kill or weaken their host through the release of antibiotics and then attack the structures of the host fungus (Paulitz and Belanger, 2001). Moreover, *B. subtilis* is capable of inducing disease resistance in some crops (Belanger and Labbe, 2002). The major constraint to BCA is their requirement for high humidity. The efficacy of biological control for powdery mildew on roses varied with the level of relative humidity prevalent in the greenhouse; however, the addition of 1% paraffin oil to the spore suspension reduced the dependency of high humidity and improved the activity of the BCA (Belanger et al., 1994).

Ampelomyces quisqualis was as effective as the commercial fungicide dodemorph in controlling rose powdery mildew. When the BCA was used in rotation with other antifungal compounds, the efficiency increased to a higher level than the control provided by the fungicide alone (Pasini et al., 1997). In a similar study, *P. flocculosa* was used as the BCA for powdery mildew control on roses. The antagonist fungus was as effective as the registered fungicide and only seven applications were sufficient to attain a satisfactory level of control compared to 10 sprays of the fungicide dodemorph acetate (Meltatox, BASF Corporation, Florham Park, NJ) (Belanger et al., 1994). Moreover, the bacteria *Bacillus* spp. provided an 80% reduction in powdery mildew severity of cucumber caused by *P. fusca* as determined by in vitro studies with detached leaves and seedlings (Romero et al., 2004).

The BCA *Bacillus subtilis* has also been shown to reduce disease severity ina a number of host-pathogen systems including *Diaporthe citri* on grapefruit (Agostini et al., 2003); rust caused by *Uromyces appendiculatus* on snap beans (Baker et al., 1985); brown rot caused by *Monilinia fruticola* on stone fruit (Pusey and Wilson, 1984), and stem rot caused by *Fusarium roseum* on carnations (Aldrich and Baker, 1970). Strains of Bacillus are resistant to adverse environmental conditions and are viable for several years under storage conditions, making them advantageous over other BCA's (Shoda, 2000).

Oils

Different types of oils but mostly mineral and plant oils are capable of controlling plant diseases (Calpouzos, 1966). Oils derived from petroleum or from seeds of plants have been used for control of diseases such as leaf spots and powdery mildews. They have not only been effective but also useful in reducing the development of resistance to fungicides (Agrios, 2005). Presumably, oils have four modes of action: prophylactic, curative either before or after visible infection and antisporulative (McGrath and Shishkoff, 2000; Northover and Schneider, 1996). Early studies using mineral oils to control diseases are those reviewed by Calpouzos (1966) where oils were used as fungicides for controlling leaf spot on banana, greasy spot of citrus and other hosts, late blight on celery, rust diseases as well as downy and powdery mildews of cucumber. More recently, reduction of powdery mildew severity has been reported on apple, cherry, cucurbits, grapes and roses. The levels of disease reduction ranged from highly effective (McGrath and Shishkoff, 1999, 2000; Pasini et al., 1997) to slightly satisfactory (Fernandez et al., 2006; Grove et al., 2005). And, in some cases, the efficacy of oils to reduce powdery mildew was comparable to those obtained with standard fungicides (Northover and Schneider, 1996) or even superior (Grove et al., 2000; Wojdyla, 2002).

Prevam, a blend of boron, orange oil and organic surfactants provided effective control of powdery mildew in strawberries (Mertely et al., 2005). Boron is a micronutrient for vascular plants and it is thought to be involved in various metabolic pathways such as lignification, cell wall structure, and phenol metabolism, among others (Marschner, 1995). Our preliminary studies indicated that Prevam provided satisfactory control of powdery mildew in gerbera daisy (unpublished data). The oil ingredient in Prevam breaks down fungal mycelia and spores and exposes them to desiccation and thus prevents further infection.

Inorganic chemicals: Potassium bicarbonate and phosphorous acid

Phosphonate and carbonate compounds such as phosphorous acid, sodium bicarbonate and potassium bicarbonate, are used in plant disease management (Agrios, 2005). Certain inorganic compounds can induce systemic acquired resistance (SAR) in plants (Sticher et al., 1997).

Phosphorous acid (H_3PO_3) is the active ingredient in phosphate or phosphonate and these products are used in agriculture for disease control or as a source of plant phosphorus (P) nutrition; however H_3PO_3 is not a nutritional source of P for plants and it is often confused with phosphoric acid (H_4PO_4)(Brunings et al., 2005; McDonald et al., 2001). Phosphite based products are marketed under the trade names AGRI-FOS, BioPhos or Vital (Agrisel USA, Inc., Brookfield, CT), Aliette or Fosetyl-Al (Bayer CropScience, North Carolina USA), K-phite (Plant Food Systems, Inc. Zellwood, FL), ProPhyt (Luxembourg industries Ltda., Tel Aviv, Israel), and Resist 57 (Actagro LLC., Biola, CA). H_3PO_3 products have been used to control soil-borne diseases caused by oomycota (Brunings et al., 2005). However, limited information is available about their effectiveness against other plant pathogens (Heaton and Dullahide, 1990). Phosphite products have an antifungal activity against *Phytophthora* species (Fenn and Coffey, 1984). Guest and Grant (1991) proposed that phosphites act both directly on the pathogen and

indirectly in stimulating host defense responses. Regardless, phosphite is most effective when applied prior to infection (Jackson et al., 2000; Marks and Smith, 1992).

Phosphite reduced the incidence of Phytophthora root and crown rot on green peppers grown hydroponically (Förster et al., 1998). Furthermore, since ProPhyt was as effective as the synthetic fungicides : Abound, Ridomil Gold (Syngenta, Greensboro, NC) and Cabrio (BASF Corporation, Florham Park, NJ) for the control of strawberry leather rot, it was suggested that alternating fungicides with phosphite should provide effective control of leather rot as well as reduce the risk of development of fungicide resistance (Rebollar-Alviter, 2005). In addition, foliar sprays of Aliette, were effective in reducing disease severity caused by scab, melanose and brown spot on citrus (Agostini et al., 2003). There are few reports of effectiveness of phosphite products to decrease severity of powdery mildew. Schilder et al. (2003) reported that ProPhyt reduced powdery mildew incidence and severity on grapes equivalent to the reduction provided by commercial fungicides. Similarly, Fosphite was effective in reducing powdery mildew on muskmelon when compared with untreated plants (Matheron and Porchas, 2005). Furthermore, foliar sprays of Biophos reduced severity of *E. cichoracearum* on gerbera daisy (Mueller et al., 2003a).

Some potassium bicarbonate-based products are label as fungicides and have been approved by the U.S. Environmental Protection Agency (EPA) (Belanger and Labbe, 2002). Armicarb 100 (Helena Chemical Company, Collierville, TN), Kaligreen(AgBio Inc., Westminster, CO), MilStop (BioWorks, Inc. Fairport, NY), and Remedy (Bonide products, INC., Oriskany, NY) are all trade names for potassium bicarbonate based fungicides and are labeled for powdery mildew control in conventional systems (Crop Data Management Systems, 2007) and organic crops (Kuepper et al., 2001). Kaligreen significantly suppressed powdery mildew

(caused by *Sphaerotheca fusca*) on winter squash, muskmelon, and pumpkin compared with untreated plants (Matheron and Porchas, 2003; McGrath and Shishkoff, 1999). Potassium bicarbonates were the most effective in reducing powdery mildew in cucumber, roses, sweet peppers and tomato (Dik et al., 2003). In addition, foliar sprays of potassium bicarbonate applied preventively in combination with mineral oil were very effective in reducing powdery mildew on cucumber and pumpkin (Ziv and Zitter, 1992). Furthermore, Kaligreen, Armicarb and Milstop have been used successfully to reduce powdery mildew of gerbera daisy (Sconyers and Hausbeck, 2004; Uchida and Kadooka, 2001). Other bicarbonate products such as sodium bicarbonate ($NaHCO_3$) gave satisfactory control of powdery mildew of grapevines, mango, nectarine and roses when applied individually at weekly intervals or alternated with a commercial fungicide (Pasini et al., 1997; Reuveni and Reuveni, 1995). *In vitro* studies showed that bicarbonate products have a fungicidal activity suggesting that this may be the mode of action against plant pathogens (Punja and Grogan, 1982).

Silicon

Silicon (Si) in nature is found combined with oxygen to form SiO_2, which is the simplest source of silica, and is the second most abundant element in the earth's crust (Epstein, 1999; Schottman, 1979). Quartz reacts with water and forms silicic acid (H_4SiO_4) in which Si is found in the soil solution at concentrations between 0.1 to 0.6mM (Epstein, 1999, 1994). Some plants are capable of absorbing Si from the soil solution between a pH of 4 to 9. Following absorption, silicic acid is translocated to the shoots and, as water is lost by transpiration, amorphous silica gel, $SiO_2.H_2O$ is deposited in cell walls, mainly in epidermal and vascular tissues (Ma et al., 2001; Russell, 1988). Si content in plant tissue can range from 0.1 to 10% on a dry weight basis (Epstein, 1994).

Ma and Takahashi (2002) differentiated Si-accumulating and non-accumulating plants based on Si content and Si/Ca ratio. Plants with a Si content higher than 1% and a Si/Ca ratio higher than 1 are defined as Si accumulators. Intermediate accumulators are those plants that contain 0.5%-1% Si and the Si/Ca ratio is less than 1. Non-accumulators or Si excluders are plants that have less than 0.5% Si and a Si/Ca ratio lower than 0.5. In a recent study, Hodson et al. (2005) examined the phylogenetic variation of Si content on leaf or shoot tissues among 735 plant species and concluded that Si concentration was low in angiosperms, gymnosperms and ferns compared to non-vascular plant species (mosses and algae) and horsetails.

Among plant species cropped for human consumption, cucumber, grape, maize, rice, strawberry and wheat have the capability of Si uptake (Belanger et al., 2003; Bowen et al., 1992; Kanto et al., 2007; Liang et al., 2006; Mitani and Ma, 2005). Currently, the gene that controls Si accumulation has only been characterized for rice. Ma et al. (2006) identified the low Si rice 1 (Lsil1) gene that was expressed mainly in rice roots. Lsil1 is the Si transporter gene in rice and was localized at the distal side of plasma membrane of both exodermal and endodermal cells. Solutes are unable to cross the plasma membrane and transporters are needed for translocation of solutes from roots to shoots (Marschner, 1995).

Si has been reported as a beneficial element in plants (Havlin et al., 2005; Marschner, 1995; Mengel and Kirkby, 1987) as it affords protection against biotic and abiotic stresses (Ma et al., 2001). A recent study demonstrated that Si could enhance the growth and appearance of flowering ornamentals. Savvas et al. (2002) reported that gerbera plants supplied with Si in the nutrient solution had significantly thicker flower stems and a higher proportion of flowers graded Class I. Moreover, Richter (2001) revealed that vase life of different gerbera cultivars can be increased by supplying plants with Si and thus reducing the number of flowers with bent neck.

Si has been shown to reduce plant diseases in a number of plants including arabidopsis, avocado, barley, Bermuda grass, coffee, cucumber, grape, muskmelon, rice, strawberry, sugarcane, St. Agustine grass, wheat, zucchini and flowering ornamentals such as poinsettia and rose (Bekker et al., 2005; Belanger et al., 2003; Botelho, et al., 2005; Bowen et al., 1992; Brecht et al., 2004; Cherif et al., 1992a,b; Datnoff et al., 2005; Ghanmi et al., 2004; Gillman et al., 2003; Kanto et al., 2006; McAvoy and Bible, 1996; Menzies et al., 1992; Remus-Borel, 2005; Seebold et al., 2001; Wiese et al., 2005). However, most Si research has been conducted on rice and cucumber.

Datnoff et al. (1997) reported that calcium silicate applied to Si-deficient organic soils in south Florida resulted in significant reductions of two foliar fungal diseases of rice, blast and brown spot. Moreover, Si provided disease control as effective as that of synthetic fungicides in rice and turf (Brecht et al. 2004; Seebold et al., 2004) suggesting that fungicide rates could be reduced when used in combination with Si (Seebold et al., 2004). Furthermore, when Si was applied to susceptible and partially resistant rice cultivars, the level of resistance was increased to that of the resistant cultivar (Rodrigues et al., 2001; Seebold et al., 2001, 2000).

Si affects the parasitic fitness of different fungi. Seebold et al. (2001) examined the effect of different rates of Si on the components of resistance to rice blast. When the rate of calcium silicate was increased, the incubation period was longer and the number of sporulating lesions decreased for the susceptible and partially resistant cultivars. Similarly, Si affected several components of resistance of cucumbers to powdery mildew caused by S. *fuliginea*. Colony number, colony area and percentage of conidial germination were reduced by increasing Si amendments from 0.5 to 2.3 mM (Menzies et al., 1991a). In addition, foliar or root applications

of potassium silicate reduced the number of colonies of powdery mildew fungi on cucumber, muskmelon and zucchini squash (Menzies et al., 1992) and on grape leaves (Bowen et al., 1992).

Fungal development on *Arabidopsis thaliana* was limited and rarely observed when plants were watered with a nutrient solution containing soluble Si (Ghanmi et al., 2004). In Japan, liquid potassium silicate was effective in suppressing powdery mildew on a susceptible strawberry cultivar 'Toyonoka' in soil as well as in hydroponic cultivation (Kanto et al., 2004, 2006). Moreover, Belanger et al. (2003) found that Si amendments protected wheat plants from powdery mildew caused by *Blumeria graminis* f.sp. *tritici*.

Si has also been reported to control other diseases. Black spot infection of *Rosa hybrida* 'Meipelta' was suppressed by the addition of 150 mg/L Si as potassium silicate to irrigation water (Gillman et al., 2003). *In vitro* effect of soluble Si in several plant pathogenic fungi revealed that Si inhibited growth of *Alternaria, Curvularia, Fusarium, Phytophthora, Pythium, Stemphylium and Verticillium* species at concentrations between 5 to 20 ml of Si per liter of agar suggesting that Si has selective fungicidal properties (Kaiser et al., 2005). Nevertheless, this contradicts the results found by Bowen et al. (1992), Menzies et al. (1992) and Kanto et al. (2007) as their in vitro assays demonstrated that conidial germination and germ tube elongation of powdery mildew fungi was unaffected by potassium silicate.

Currently, there are two running hypothesis on how Si can enhance resistance of plants to diseases. Initially, the protective role of Si was attributed to the accumulation of Si in the leaves creating a physical barrier to pathogens (Adatia and Besford, 1986; Epstein, 1994; Samuels et al., 1991a,b; Raven, 1983). However, recent studies indicate that Si has a more active role in protecting plants against diseases by inducing the plant's own defense mechanisms (Fauteux et al., 2006; Fawe et al., 1998; Remus-Borel, 2005; Rodrigues et al., 2004). So, the role of Si is not

solely limited to a physical barrier since Si also induces metabolic changes in Si- fertilized plants (Epstein, 1999; Fauteux et al., 2006).

Because of Si deposition in plant cell walls, it is expected that Si strengthens plant tissues and thus creates a physical barrier that impedes or slows down penetration of plant pathogens (Ma et al., 2001). Adatia and Besford (1986) reported anatomical and morphological effects of Si on cucumber leaves as they had a rougher texture, were more erect, with a darker color and their senescence was delayed. Bowen et al. (1992) examined the effect of root and leaf applications of soluble Si on powdery mildew severity on grapes. Reduction of disease severity was attributed in part to a physical barrier created by thick potassium silicate deposits on the leaf that limited hyphal penetration. However, they also considered the role of Si in inducing a resistance response because of the movement of Si and its deposition at fungal penetration sites. Kim at al. (2002) investigated the location of Si in rice leaves by electron microscopy and X-ray microanalysis. Their findings confirmed that Si was deposited in epidermal cell walls, middle lamella and intercellular spaces of rice leaves providing a physical barrier for the penetration and invasion of the fungus *Magnaporthe grisea*.

Cherif et al. (1994) reported that supplying cucumber plants with Si produced an intense activation of peroxidases (POD), polyphenoloxidases (PPO) and chitinases after infection with *Pythium* spp. Similar results were found by Fawe et al. (1998) who demonstrated an enhanced resistance to *Sphaerotheca fuliginea* in cucumber by increased antifungal activity from production of phytoalexins. Similarly, Liang et al. (2005) found that root-applied Si enhanced the activity of pathogenesis-related (PR) proteins and thus increased resistance to pathogen attack on cucumber plants.

The mechanism of induced resistance has been extensively studied in other pathosystems. Infection of wheat plants by *Blumeria graminis* was reduced by Si amendments and this response was associated with specific defense reactions (callose and papilla formation, deposition of chitin, B-1,3 glucans and phenolic compounds) found around fungal cell walls, haustoria , and epidermal cell walls (Belanger et al., 2003). Similarly, reduction of powdery mildew of wheat was attributed to the presence of phenolic compounds around degraded haustoria (Remus-Borel, 2005). Rodrigues et al. (2004) reported that Si stimulated the accumulation of momolactone phytoalexins in rice plants inoculated with *M. grisea*. Moreover, Ghanmi et al. (2004) detected accumulation of phenolic compounds around collapsed haustoria of powdery mildew infected epidermal cells of *Arabidopsis thaliana.* Fauteux et al. (2006) reported that infection of *Arabidopsis* plants with powdery mildew fungi enhanced the expression of several defense-related and metabolic genes and the addition of Si produced a more efficient defense response by the plant. However, they stated that Si alone had no effect on the metabolism of unstressed plants.

In conclusion, alternative products have been effective in powdery mildew control of various crops. They have been as effective as fungicides when applied alone or in combination with other products. Therefore, it is worthwhile to evaluate alternative methods for control of powdery mildew of gerberas in that products other than synthetic fungicides may help growers reduce their dependency on chemicals and consecutively, reduce the development of resistance by the pathogens. Also, the use of non-synthetic fungicides may give growers an economic advantage by providing a potential market for environmentally friendly gerbera production.

CHAPTER 3
EVALUATION OF SILICON FOR SUPPRESSING POWDERY MILDEW DEVELOPMENT IN GERBERA DAISIES

Introduction

Gerbera daisy (*Gerbera jamesonii* Bolus ex. Hook f.) is an ornamental plant produced as a cut flower, potted plant and for landscape use (Tija and Black, 2003). In Florida, gerberas are mainly produced under greenhouse or shade house conditions as potted and bedding plants. Gerberas are susceptible to a variety of pests and diseases. Powdery mildew is an important fungal disease in gerberas and it can be caused by two species, *Erysiphe cichoracearum* DC.or *Podosphaera* (Syn. *Sphaerotheca) fusca* (Fr.) S. Blumer (Daughtrey et al., 1995). This disease affects all plant parts and reductions in yield and in quality are the most important components of economic loss. Environmental conditions that are most conductive for powdery mildew development include high humidity (80% to 90%) and moderate temperatures 20 to 28°C (Daughtrey et al., 1995). These conditions are prevalent in Florida. Methods for powdery mildew management are chemical, cultural, and biological control. The majority of the nurseries in Florida use fungicides for powdery mildew control such as chlorothalonil, copper, mancozeb and metalaxyl (Larson and Nesheim, 2000). The demand for high plant quality forces growers to intensify their spray programs against diseases which may lead to faster development of fungicide resistance while increasing overall production costs. However, consumer's awareness of the implications of pesticides (i.e. fungicides) in environment and human health has intensified the search for alternative methods of disease control (Gullino et al, 1999). Several alternative products have been reported to be effective for the control of powdery mildew such as electrolyzed oxidizing water in gerbera daisy (Mueller et al., 2003b); clay in English cucumber and wine grapes (Ehret et al., 2001); phosphate and potassium salts in apple, cucumber, grapevines, mango, nectarine and wheat (Reuveni et al., 1996; Mitchell and Walters, 2004;

Reuveni et al., 1998; and Reuveni and Reuveni, 1995); sodium bicarbonate in roses (Horst et al., 1992); salicylic acid, soy lecithin and sulfur, in chicory (Trdan et al., 2004); oils in cherry, cucurbits, grapevines and roses (Grove et al., 2005; Pasini et al., 1997; McGrath and Shishkoff, 2000; and Grove et al., 2000), cow's milk in zucchini squash (Bettiol, 1999) and biocontrol agents in rose and cucurbits (Pasini et al., 1997; Romero et al., 2004). Another potential alternative for the control of powdery mildew of gerberas is the use of silicon, which has given satisfactory results in the management of powdery mildew of different crops such as *Arabidopsis thaliana* (Ghanmi et al., 2004); cucumber (Liang et al., 2005; Adatia and Besford 1986); cucumber, muskmelon, and zucchini squash (Menzies et al., 1992); grapes (Bowen et al., 1992); strawberry (Kanto et al., 2006) and wheat (Belanger et al., 2003). Addition of silicon to the soil or the nutrient solution has shown beneficial effects on plants under biotic and abiotic stress (Ma et al., 2001). Among dicotyledonous plants, extensive research has been conducted in greenhouse cucumbers where applications of silicon have been shown to reduce disease levels of powdery mildew caused by *Sphaerotheca fuliginea* (Schlecht Fr. Poll) also known as *Sphaerotheca fusca* (Fr.) S. Blumer (Epstein, 1999). Foliar and root applications of potassium silicate reduced the number of colonies of powdery mildew fungi in cucumber, muskmelon and zucchini squash (Menzies et al., 1992). Colony number of *Uncinula necator* in grape leaves was reduced to 11% of the control leaves when foliar silicon sprays were used (Bowen et al., 1992). Moreover, the effect of silicon has also been tested in other components of parasitic fitness, and was found that increasing silicon amendments from 0.5 to 2.3 mM decreased colony number, colony area and percentage of conidial germination (Menzies et al., 1991a). Powdery mildew development in *Arabidopsis thaliana* was limited and rarely observed when plants were watered with a nutrient solution containing soluble potassium silicate (Ghanmi et al., 2004). In Japan,

liquid potassium silicate was effective in suppressing powdery mildew in the highly susceptible 'Toyonoka' strawberry cultivar, when used in soil as well as in hydroponic cultivation (Kanto et al., 2004; Kanto et al., 2006). Belanger et al. (2003) found that silicon amendments to the soil mix or added to the nutrient solution protected wheat plant from powdery mildew caused by the fungus *Blumeria graminis* f.sp. *tritic.*

The use of silicon in gerbera daisies was reported previously by Savvas et al. (2002) and Ritcher (2001). However, the objective of their investigation was to evaluate the effect of silicon on yield and flower quality and on vase life, respectively. To our knowledge, the effect of silicon for the control of powdery mildew in gerbera daisy has not been investigated. Based on the effectiveness of silicon in controlling powdery mildew in other crops, a hypothesis was formulated that silicon amendment to gerbera plants could decrease disease severity caused by *Podosphaera fusca.* Consequently, the objective of this study was to evaluate the efficacy of silicon for the management of powdery mildew in gerbera daisies grown under greenhouse conditions in Florida.

Materials and Methods

Experiments were conducted under greenhouse conditions from May 2006 to January 2007 at the University of Florida, Gulf Coast Research and Education Center, Wimauma, Florida.

Effect of Calcium Silicate in Powdery Mildew Development in Gerbera Plants

Calcium silicate ($CaSiO_3$) Slag 43.41% SiO_2 (Calcium Silicates Corporation, Lake Harbor, FL) at rates of 0, 0.9, 1.8,3.6, 5.53 and 7.3 g/pot was added to the growing media CMA mix: peat moss 65%, perlite coarse 20%, A3 Coarse 15%, and rock 3% (Verlite Company, Tampa, FL). Afterwards, Osmocote Plus (15-9-12) controlled release fertilizer (The Scott's Company, Marysville,OH) at 11g/pot was added and all mixed with a concrete mixer (Gilson mixer 59015C, CF Gilco, Inc. Grafton, WI.). Gerbera seedlings Snow White Sunburst series (Twyford

International, Apopka, FL) highly susceptible to powdery mildew were transplanted into 15 cm diameter plastic pots previously filled with the growing medium 8 May 2006. Pots were placed on greenhouse benches and watered by drip irrigation. Powdery mildew caused by *Podosphaera* (*Sphaerotheca) fusca*, developed on plants from natural inoculum and symptoms were first seen two weeks after transplanting. Disease evaluations were made at 7-day intervals beginning on 24 May and ending on 19 July 2006. The disease severity was rated using a 0 to 5 scale, where 0= no powdery mildew symptoms present, 1= 1 to 20%, 2= 21 to 40%, 3= 41 to 60%, 4= 61 to 80 % and 5= 81 to 100% of upper leaf surface covered with powdery mildew. This experiment was conducted as a randomized complete block design with six treatments and four replications. Disease ratings were used to calculate the area under disease progress curve (AUDPC) for each treatment by the midpoint rule method (Campbell and Madden, 1990) as follows: AUDPC = $\Sigma_{i=1}^{n-1} [(y_i + y_{i+1})/2] (t_{i+1} - t_i)$ where **n** is number of disease assessment times, **y** is the disease severity and **t** is the time duration of the epidemic. The AUDPC values obtained were analyzed by linear regression (SAS institute, Cary, NC).

Effects of Silicon in Horticultural Traits of Gerbera Flowers

Gerbera plants started blooming four weeks after being transplanted. Flowers were harvested once they were fully developed, i.e. when the second circle of disks in the flower showed pollen development (Rogers and Tjia, 1990). Flowers were counted and the flower diameter, stem diameter, and flower height were recorded. Data were analyzed by linear regression (SAS institute, Cary, NC).

Evaluation of Silicon Accumulation over Time

This experiment was designed to determine a timeline for silicon uptake into gerbera leaves. In August 2006, gerbera seedlings Snow White Sunburst series (Twyford International, Apopka, Florida) were transplanted into 15-cm diam plastic pots filled with the growing media

as previously described. Silicon was applied as Calcium silicate ($CaSiO_3$) slag 43.41% SiO_2 (Calcium Silicates Corporation Lake Harbor, FL) at 5.4 g/pot or as potassium silicate (K_2SiO_4) 26.5% SiO_2 supplied as Kasil®6 (PQ Corporation, Valley Forge, PA) at 0.27 mL/L (1.22 mM SiO_2). Calcium silicate was incorporated into the growing medium before transplanting and potassium silicate was applied three times a week as a drench. Control plants received no silicon. All plants were watered by drip irrigation. Each treatment had 45 plants that were evaluated for silicon content on 2, 5, 9, 16, and 23 days after transplanting (DAT). On each sampling date, four to five new leaves were collected from 9 plants of each treatment to determine silicon uptake. This experiment was repeated in January 2007. Data were subjected to analysis of covariance using SAS Version 9.0 (SAS Institute, Cary, NC). Disease evaluations were made on 9, 16 and 23 DAT. The disease severity was rated using a 0 to 5 scale, as described previously. This experiment was conducted as a randomized complete block design with three treatments and three replications. Disease ratings were used to calculate the area under disease progress curve (AUDPC) for each treatment as described previously. The AUDPC values obtained were analyzed by analysis of variance (ANOVA) and means separated by the Waller–Duncan k ratio t test ($P \leq 0.05$).

Silicon Extraction from Gerbera Leaves

Four to five new leaves from gerbera plants were oven dried at 70ºC for 3 days. Dried tissue was ground finely with a sample mill (Cyclotec 1093, Foss analytical, Denmark) to pass through a 0.425 mm. Silicon content was determined by a modification of the autoclave-induced digestion procedure of Elliot and Snyder (1991). Briefly, 100 mg of dried, ground leaf tissue were placed in 100-mL polyethylene tubes and added 2 mL of 50% hydrogen peroxide (H_2O_2) and 3 mL of 50% sodium hydroxide (NaOH). Each tube was vortex gently and covered with loose fitting plastic caps. The tubes were placed in an autoclave at 103 kPa (15 psi) for 30 min. If

tissue was not completely digested, 2 mL of hydrogen peroxide were added to each tube, vortexed and placed in the autoclave again. Otherwise, tubes were removed and the contents brought to 50 mL with distilled water. Silicon was determined colorimetrically as follows: a 1-mL aliquot was taken from the digested plant tissue and mixed in 10 mL of distilled water. Then, 0.25 mL hydrochloric acid (1:1), 0.5 mL of ammonium molybdate solution (100g/L, pH 7.0), 0.5 mL tartaric acid (200g/L) and 0.7 mL of a reducing solution were added. The reducing solution was prepared by dissolving 4 g sodium sulfite (Na_2SO_3), 0.8 g 1-amino-2-naphthol-4-sulphonic acid, and 50 g sodium bisulfite ($NaHSO_3$) in 500 mL water. Five minutes elapsed between the addition of the ammonium molybdate and the tartaric acid. A series of standard silicon (silicon reference solution, Fisher Scientific) contents were developed and used to generate a regression equation for determining final silicon content in leaves (ppm). After 10 min, the absorbance was measured at 650 nm with a spectrophotometer (PC 910, Brinkmann Instruments, Inc.).

Results

Effect of Silicon on Powdery Mildew Development in Gerbera Plants

Powdery mildew developed from natural inoculum and the first symptoms were observed 12 DAT. 26 DAT, disease severity was about 50% in all treatments. Disease increased until the end of the experiment when powdery mildew symptoms covered most of the leaves. Disease severity was not related to the amount of silicon applied at any of the evaluation dates (Table 3-1). At the end of this experiment, leaves were collected to determine the silicon concentration and linear regression analysis showed no relationship of the silicon content in the leaves of the different silicon treatments to the amount applied (Table 3-2).

Effects of Silicon in Horticultural Traits of Gerbera Flowers

Gerbera flowers were evaluated for the effect of silicon on flower number, height, flower diameter and stem diameter. There was an average of 3 flowers per plant with an average height

of 21 cm, flower diameter of 7 cm and stem diameter of 0.5cm. There were no significant relationship of the amount of silicon applied and the average number of flowers, flower height, flower diameter and stem diameter (Table 3-3).

Evaluation of Silicon Accumulation 2, 5, 9, 16 and 23 Days after Transplant

Plants were treated with two sources of silicon, calcium silicate and potassium silicate, to evaluate silicon uptake over time (2, 5, 9, 16 and 23 days after transplant-DAT). This experiment was conducted twice and since the interaction between treatment and experiment was significant ($P \leq 0.05$), results are presented separately. For the first experiment, ANOVA showed no significant difference among the treatments applied and nor was there a significant interaction between treatment and DAT. However, the DAT was significant showing that silicon accumulation decreased over time as determined by the regression equation: Si (%) = 0.055-0.00104(DAT). For the second experiment, silicon treatment (potassium silicate) had a significant effect, as did the interaction of potassium silicate and DAT (Table 3-4). The accumulation of potassium silicate in gerbera leaves showed a slightly increase over time as described by the regression equation: Si (%) = 0.037+0.00036(DAT). According to this equation, the expected Si concentration of gerbera leaves at 2 DAT would be 0.038 % of dry weight and it would increase to 0.045% at 20 DAT. Consequently, although the accumulation of potassium silicon in gerbera leaves is significant, the amount of silicon did not vary greatly over time

Powdery mildew severity was evaluated for the last three weeks of this experiment to see if silicon uptake was related to disease control. Although plants treated with potassium silicate showed a slightly higher silicon uptake when compared to untreated control (Table 3-5), at the end of the experiment, the potassium silicate treatment did not significantly reduce disease (Tables 3-6, 3-7).

Discussion

Silicon mixed with the soil or applied as a drench to gerbera plants did not reduce the severity of powdery mildew nor did it improve the quality of the flowers. Disease symptoms in plants were observed 12 to 18 DAT and the symptoms were uniform among all treatments.

The lack of control of powdery mildew with silicon can possibly be explained by three hypotheses. First, the concentration of inoculum may be too high at the beginning of the experiment and there was not enough time for silicon to act before the fungus infected the plants. This is assuming that silicon moved systemically from the roots throughout the plant to either induce the plant's defense mechanisms or accumulated in plant tissues to create a physical barrier to the pathogen. In the wheat-powdery mildew pathosystem, calcium silicate was responsible for the accumulation of phenolic compounds that produced abnormalities in haustoria and thus reduced infection (Belanger et al., 2003). Liang et al. (2005) reported that Si applied to the roots of cucumber plants enhanced the production of pathogenesis-related proteins (PRs) in response to infection by *Podosphaera xantii*. Silicon also induced the production of an electron-dense fungitoxic substance that appeared to be toxic to powdery mildew fungi infecting *Arabidopsis thaliana* (Ghanmi et al., 2004). Silicon has the potential to trigger the plant's defense mechanisms (Cherif et al., 1994; Ghanmi et al., 2004). Nevertheless, the exact time between uptake of silicon, movement throughout the plant and the production of defense response is not clear. In some cases, plants are supplied with a silicon treatment a week or two before inoculation. Ma, et al. (2004) characterized the silicon uptake in rice in a time-course experiment where roots and xylem sap were analyzed for silicon. Silicon in the roots increased over time with an increase in the silicon concentration in the external solution, reaching saturation of about 5.5 mM after 25 h. The concentration of silicon in the xylem sap was measured after 8 h of silicon exposure and again, the silicon concentration in the xylem increased

with increasing of silicon in the external solution, but it was saturated at a higher concentration of about 35mM. This is in agreement with a similar study in cucumber where silicon uptake increased with time depending upon the silicon supplied in the external solution (Liang et al., 2005). Hence, in rice and in cucumber, the uptake of silicon can be a rapid active process and can provide good disease control. This apparently is not the case for gerberas especially since silicon did not seem to have any effect in suppressing the development of powdery mildew.

Silicon has been proposed to act as a physical barrier in leaf surfaces for providing protection against biotic stress (Ma et al., 2001). Adatia and Besford (1986) found major effects of silicon in cucumber leaves as they had a rougher texture were more erect with a darker color and their senescence was delayed. Bowen et al. (1992) studied the effect of root and leaf applications of soluble silicon in powdery mildew severity of grapes. Reduction of disease severity was attributed in part to a physical barrier created by thick potassium silicate deposit in the leaf that limited hyphal penetration compared with areas of leaf surface that were not coated and fungal development was more extensive. However, they also considered the role of silicon in inducing a resistance response because of the movement of silicon and its deposition at fungal penetration sites. Kim at al. (2002) investigated the location of silicon in rice leaves using electron microscopy and X-ray microanalysis and found that silicon was deposited in epidermal cell walls, middle lamella and intercellular spaces of rice leaves providing a physical barrier for the penetration and invasion of the pathogen *Magnaporthe grisea,* the cause of rice blast. Because of the deposition of silicon in plant cell walls, it is reasonable to expect that silicon may strengthen plant tissues and thus creates a physical barrier that impedes direct penetration by fungal pathogens. Savvas et al. (2002) stated that silicon improved gerbera flower quality by providing mechanical strength to the stems since their diameter increased with increasing silicon

concentration in the nutrient solution. However, in our study, stem diameter was not affected by silicon.

A second theory was that environmental conditions such as temperature and light might have inhibited silicon uptake. Some experiments were conducted during the summer and plants were placed in a shady area of the greenhouse in an effort to lower temperatures and provide better conditions for the development of powdery mildew. Schuerger and Hammer (2003) conducted an experiment to understand the discrepancy of powdery mildew control by silicon between studies conducted in Florida and Canada. Consequently, three horticultural parameters (cultivar, nutrient solution and rooting medium) and two environmental factors (light intensity and temperature) were tested to determine their influence in silicon effectiveness to reduce powdery mildew severity in cucumber. Among all factors tested, temperature had the most significant effect in reducing powdery mildew severity. Temperatures of 20 °C and silicon at 100 mg/L was demonstrated to be the most effective combination in decreasing the number of powdery mildew colonies per leaf compared to temperatures of 25 or 30°C. These results could be expected based on the interaction between temperature, transpiration and silicon uptake. Transport of water and solutes in the plants is regulated by active and passive processes (Ting, 1982) and transpiration plays a role in those transport processes (Taiz and Zeiger, 1991). The process of uptake and movement of silicon in the plant is in the form of the uncharged molecule, H_4SiO_4, and its translocation within the plant is affected more by the transpiration stream than with any other element (Epstein, 1999). Therefore, silicon uptake can be influenced by the plant's transpiration rate (Ma and Takahashi, 2002). Okuda and Takahashi (1962) demonstrated that silicon uptake in rice and tomato was influenced by transpiration. When transpiration rate was reduced by high humidity, the silicon uptake in rice did not change, but it was reduced in

tomatoes. Transpiration is also highly influenced by humidity, temperature, light and wind (Ting, 1982). Transpiration and photosynthesis in potato plants decreased when temperature increased above 25°C (Ku et al., 1977). Light stimulates transpiration by opening the stomata hence increased transpiration might enhance Si uptake (Ma and Takahashi, 2002).

The last theory is that gerbera plants do not uptake silicon. Savvas et al. (2002) reported that the inclusion of silicon in the nutrient solution improved flower quality by increasing the proportion of class I flowers and the thickness of flower stems. The authors stated that silicon also enhanced the uptake of calcium by the plants. Silicon content in plant tissue varies greatly with the plant species and external silicon concentration (Ma et al., 2001, Liang et al., 2006). Even genotypes within a species may have a different ability to uptake and accumulate silicon in their tissues (Epstein, 1994; Ma and Takahashi, 2002; Hodson et al., 2005). Comis (2007) reported that ornamentals such as New Guinea impatiens, marigold and zinnia accumulate silicon as X-ray analysis showed significant concentrations on the leaves. Moreover, silicon reduced powdery mildew severity in Zinnia while no effect was found in begonia and geranium as they did not accumulate silicon. Because of this specificity in silicon uptake and accumulation, testing different species and cultivars to determine their capacity to accumulate silicon is worthwhile. The amount of silicon in leaf tissues found in gerbera daisies, Snow-White Sunburst series, supplied with different rates of calcium silicate averaged 0.05% and did not increase with higher silicon rates. This result is different than those reported for rose, sunflower or rice. With rose and sunflowers, an increase in the amount of silicon applied to the soil or the nutrient solution resulted in increased silicon uptake from 0.02 to 0.09% and 0.5 to 3% respectively (Gillman et al., 2003; Liang et al., 2006). Similarly in rice, Seebold et al. (2000) demonstrated that an increase in silicon supply produced an increase in silicon content of the

leaves. Calcium silicate at 500 kg/ha resulted in a silicon content of 3.1% and at double the rate, the concentration in the leaves increased to 3.5%. In our study, the same applied rates resulted in a silicon concentration in the leaves of 0.05% of dry weight and were not significantly different than the untreated control. So, while we have documented that gerbera daisy do not accumulate Si, the mechanisms of exclusion need to be established.

Experiments on silicon uptake over time were conducted to determine a time course for silicon uptake in gerbera leaves. Silicon content in leaf or shoot tissues for 735 plant species was recently reported (Hodson et al., 2005). However, the time required for those plants to uptake silicon was not reported. Tamai and Ma (2003), in their characterization of silicon uptake by rice roots, determined that the uptake of silicon increased linearly with time and it took only hours for silicon to be absorbed. This is in agreement with Liang et al.(2006) who showed that for maize, rice, sunflower and wax gourd, it only took a few hours (2 to 10) for silicon to accumulate and the amount absorbed increased with an increase in silicon supply.

Since silicon alone does not have fungicidal activity (Bowen et al., 1992), it must be absorbed before the pathogens attack to provide protection against diseases and reduce disease severity. Usually, silicon treatments start at the time when seedlings are transplanted and then plants are inoculated hours (Menzies et al., 1992) or weeks (Gillman et al., 2003; Rodrigues et al., 2001) later. Disease symptoms are then observed within hours (Datnoff and Rutherford, 2004) or days (Gillman et al., 2003; Rodrigues et al., 2001; Menzies et al., 1992) suggesting that, in most cases, silicon uptake and silicon response occurs in a matter of hours or days. However, this was not the case in our study with gerbera daisies. Plants treated with potassium silicate showed some evidence of silicon uptake 14 DAT and, at that time, symptoms of powdery mildew became apparent. However, no disease reduction was observed thereafter suggesting that

even though the plants had some silicon accumulation, it was not enough to reduce disease. Alternatively, the timing of observation may have been too short to make such conclusions and perhaps a longer time course experiment would have shown greater silicon uptake, higher levels of disease and therefore differences in disease control. However, the first experiment to determine the effect of silicon on powdery mildew development showed that there were no significant differences in silicon concentration in the leaves of treated plants compared with the untreated control after two months. In our study on silicon content in gerbera leaves receiving calcium silicate and potassium silicate, the silicon content ranged from 0.01% to 0.06% in a dry matter basis. This concentration is very low compared with silicon concentrations from 0.5% to 6% found in cucumber, strawberry, sunflower or rice tissues (Kanto et al., 2006; Liang et al., 2006; Seebold et al., 2000; Menzies et al., 1991b). In those experiments, silicon was successful in reducing disease severity. In the study with gerberas by Savvas et al. (2002), the authors never quantified silicon accumulation nor clearly stated that the positive results in flower quality could be due to an indirect effect of silicon and not to its own properties. Therefore, the low silicon content in gerbera leaves may explain the lack of powdery mildew control. In conclusion, gerbera plants apparently do not accumulate silicon as fast and at high amounts as rice, cucumber or sunflower and, therefore, does not have an application for powdery mildew management under greenhouse conditions in Florida.

Table 3-1. Effect of amending potting soil with calcium silicate ($CaSiO_3$) on powdery mildew of gerbera daisy.

$CaSiO_3$ g/pot	Disease severity (0-5) [z]									AUDPC[y]
	Days after transplanting									
	12	19	26	33	40	47	54	61	68	
0	0.8	1.7	2.2	2.9	3.2	3.9	3.9	4.2	3.9	130.73
0.9	1.0	2.2	2.4	2.8	3.3	3.8	3.8	3.9	3.9	118.95
1.8	0.7	1.8	2.1	2.5	3.0	3.7	3.8	3.9	3.9	109.75
3.6	1.1	2.3	2.6	3.0	3.1	3.8	3.9	3.7	3.8	122.38
5.5	0.8	1.9	2.3	2.8	3.0	3.9	4.0	3.9	4.0	115.58
7.3	0.9	1.9	2.3	2.8	3.3	3.9	3.9	3.9	3.9	117.5
	ns[x]	ns	ns	ns	ns	ns	ns	ns	ns	ns

[z]Disease severity rated on a 0 to 5 scale, where 0= no powdery mildew symptoms and 5 = 81 to 100% of upper leaf surface covered with powdery mildew symptoms.

[y]AUDPC = area under the disease progress curve

[x]ns: not significant

Table 3- 2. Silicon (Si) content in new leaves of gerbera daisy.

Si[z] (g/pot)	% Si in leaves
0	0.049
0.9	0.046
1.8	0.079
3.6	0.041
5.5	0.048
7.3	0.037
	ns[y]

[z]Calcium silicate
[y] ns: not significant

Table 3-3. Effect of amending potting soil with calcium silicate ($CaSiO_3$) in horticultural traits of gerbera flowers.

Si (g/pot)	Flower number No /plant	Flower Height (cm)	Flower diameter (cm)	Stem diameter (cm)
0	3.27	19.81	7.13	0.54
0.9	3.21	19.89	7.08	0.53
1.8	3.63	21.46	7.27	0.54
3.6	3.33	22.53	7.81	0.56
5.5	3.23	21.54	7.45	0.53
7.3	3.67	22.16	7.14	0.55
	ns[z]	ns	ns	ns

[z]ns: not significant

Table 3-4. Analysis of covariance for silicon content in gerbera leaves at 2, 5, 9, 16, and 23 days after transplant (DAT).

Source	Exp 1				Exp 2			
	DF	Mean Square	F value	Pr < F	DF	Mean Square	F value	Pr < F
Silicon treatment	2	0.00017337	1.05	0.352	2	0.00037337	4.49	0.013[z]
DAT	1	0.00992459	60.25	<0.0001[z]	1	0.00003427	0.41	0.5221
Treat*DAT	2	0.00004737	0.29	0.7505	2	0.00034887	4.19	0.0172[z]
Error		0.00016472				0.00008317		

[z] Significant different.

Table 3-5. Silicon content in gerbera leaves at 2, 5, 9, 16, and 23 days after transplant (DAT)

DAT	Silicon treatment					
	0		Calcium silicate		Potassium silicate	
	exp 1	exp 2	exp 1	exp 2	exp 1	exp 2
2	0.035	0.030	0.040	0.021	0.042	0.031
5	0.052	0.025	0.046	0.028	0.051	0.048
9	0.048	0.039	0.048	0.037	0.060	0.036
16	0.027	0.022	0.027	0.027	0.037	0.044
23	0.018	0.024	0.024	0.029	0.027	0.043
R^2 exp. 1	0.36					
R^2 exp. 2	0.33					

Table 3-6. Effect of silicon on powdery mildew severity in gerbera leaves. Experiment I

Si treatment	Days after transplanting			AUDPC
	18	25	32	
0	0.6 b[z]	1.0 a	1.8 a	15.37 a
Calcium silicate	0.7 a	1.0 a	2.0 a	16.73 a
Potassium silicate	0.6 ab	0.9 a	1.6 a	14.03 a

[z]Means in columns followed by the same letter are not significantly different according to the Waller-Duncan k ratio t test (P≤0.05).

Table 3-7. Effect of silicon on powdery mildew severity in gerbera leaves. Experiment II

Si treatment	Days after transplanting			AUDPC
	18	25	32	
0	0.2 a[z]	0.3 ab	0.3 b	2.95 a
Calcium silicate	0.1 ab	0.3 a	0.5 a	3.30 a
Potassium silicate	0.1 b	0.2 b	0.5 a	3.00 a

[z]Means in columns followed by the same letter are not significantly different according to the Waller-Duncan k ratio t test (P≤0.05).

CHAPTER 4
EVALUATION OF BIOFUNGICIDE PRODUCTS FOR MANAGING POWDERY MILDEW IN GERBERA DAISY

Gerbera daisy (*Gerbera jamesonii* Bolus ex. Hook f.) is an ornamental plant grown as cut flowers, potted plants and for landscape use (Tija and Black, 2003). In Florida, gerbera daisies are mainly produced under greenhouse or shade house conditions as potted and bedding plants. Gerbera is susceptible to a variety of pests and diseases. Powdery mildew is an important fungal disease in gerbera and can be caused by two species, *Erysiphe cichoracearum* DC.and *Podosphaera* (Syn. Sphaerotheca*) fusca* (Fr.) S. Blumer (Daughtrey et al., 1995). This disease may affect all plant parts and reductions in yield and in quality are the most important components of economic loss. Environmental conditions most conducive for powdery mildew development include high relative humidity (80% to 90%) and moderate temperature (20 to 28°C) (Daughtrey et al., 1995); these conditions that are common in Florida. The two main methods for powdery mildew control are repeated applications of fungicides and the use of resistant or tolerant cultivars. Fungicides used in Florida for powdery mildew control include chlorothalonil (Bravo), Mancozeb+ Thiophanate Methyl (Duosan) and Propiconazole (Banner) (Larson and Nesheim, 2000). However, chemical control is not always completely effective since pathogens may develop resistance to some fungicides (Gullino and Wardlow, 1999). In addition, consumer awareness of the implications of pesticides in the environment and human health has intensified the search for alternative methods of disease control (Gullino et al, 1999).

Biofungicides are naturally based microbial or biochemical products derived from animals, bacteria, plants, or minerals. These products can affect fungal organisms directly or may stimulate the defense response of the plant. They are generally narrow-spectrum, have low toxicity to non target organisms, decompose quickly, and thus are considered to have low

potential for negative impacts on the environment (McGrath, 2004; EPA, 2007). Biofungicides such as biological control agents (i.e. *Bacillus subtilis*), potassium bicarbonate, phosphorous acid, Prevam and oils, are labeled for control of powdery mildew in ornamentals in Florida (Crop Data Management Systems, 2007). However, limited information is available on the effectiveness of these products in managing powdery mildew in ornamentals and more specifically on gerbera daisies. Consequently, the objective of this study was to evaluate the efficacy of biofungicides for the management of powdery mildew in gerbera daisy grown under greenhouse conditions in Florida.

Materials and Methods

Experiments were conducted under greenhouse conditions from April to June 2007 at the University of Florida, Gulf Coast Research and Education Center, Wimauma, Florida.

Effect of Biofungicides in Powdery Mildew Development in Gerbera Plants: The growing medium (CMA mix: peat moss 65%, perlite coarse 20%, A3 Coarse 15%, and rock 3%. Verlite Company, Tampa, FL) plus OP (Osmocote Plus (15-9-12) controlled release fertilizer (The Scott's Company, Marysville, OH) at 11g/pot, were combined using a concrete mixer (Gilson mixer 59015C, CF Gilco, Inc. Grafton, WI). Two highly susceptible ('Snow White' and 'Orange') and two moderately susceptible ('Hot Pink' and 'Fuchsia') Sunburst series (Twyford International, Apopka, FL) gerbera seedlings, were transplanted into 15-cm diam. plastic pots previously filled with the growing medium on 11 April, 2007. Sulfur (52%) at the 6.2 ml/l rate (Micro Flo Company, Memphis, TN) was sprayed until runoff, on the leaves of all plants to eliminate any powdery mildew inoculum already present. Seven days after transplant (DAT), gerbera plants were sprayed with Actigard, Prevam, Milstop, K-phite, AgSil (plus Tween20), Rhapsody (plus Biotune), Heritage alternated with Eagle as standard control or water (Table 4-1). Pots were placed on greenhouse benches and hand watered three times per week. Treatments

and cultivars were randomized and divided into two benches (96 plants per bench). Treatments were applied weekly on the upper surface of the leaves to runoff with a hand sprayer. Powdery mildew, caused by *Podosphaera* (*Sphaerotheca*) *fusca*, developed on the plants from natural inoculum 28 DAT. Disease evaluations were made at seven day intervals beginning 9 April (28 DAT)and ending 15 June(65 DAT) 2007. Disease severity was rated on a 0 to 6 scale, where 0= no powdery mildew symptoms, 1= 1 to 20%, 2= 21 to 40%, 3= 41 to 60%, 4= 61 to 80 %, 5= 81 to 99% and 6= 100% of upper leaf surface covered with powdery mildew symptoms. This experiment was conducted as a completely randomized design with 8 treatments and 4 cultivars. Disease severity ratings were analyzed by week by analysis of variance (ANOVA) with mean separation by Fisher's Protected LSD ($P \leq 0.05$) (Statistix 8.1, Tallahassee, FL). Disease ratings were used to calculate the area under disease progress curve (AUDPC) for each treatment by the midpoint rule method (Campbell and Madden, 1990) as follows:

$AUDPC=\Sigma_i^{n-1}[(y_i + y_{i+1})/2] (t_{i+1} - t_i)$ where **n** = the number of disease assessment times, **y** = disease severity and **t** = time duration of the epidemic. AUDPC values were transformed to square roots to normalize variance and then subjected to analysis of variance followed by Fisher's Protected LSD ($P \leq 0.05$) (Statistix 8.1, Tallahassee, FL) to separate means.

Results

Powdery mildew developed from natural inoculum. Disease severity was assessed once a week for six weeks. There were significant differences ($P \leq 0.05$) between treatments and cultivars. The interaction between treatment and cultivar was significant ($P \leq 0.05$). However, the F value for the interaction was low ($F=3.11$) thus disease severity ratings of treatments and cultivars were pooled for comparisons among treatments and cultivars (Tables 4-2, 4-3).

Until 40 DAT, the average relative humidity was below 80% and temperature fluctuated from 72 to 76 °F (22-24 °C). At about 44 (DAT), the relative humidity increased to above 80% (Figure 4-1).On 44 DAT, powdery mildew increased on all cultivars and then it gradually progressed every week thereafter (Figure 4-2).

The first symptoms were observed 28 DAT, but incidence was very low and during the first two weeks of evaluations (28 to 37 DAT), disease was observed only on untreated plants and those treated with Agsil and Actigard. Most of the plants at 44 DAT were infected except for plants that received the Heritage-Eagle treatment. A week later, disease increased for all treatments including the Heritage-Eagle-treated plants (Table 4-2). During the last three weeks of the experiment (51 to 65 DAT), disease severity increased gradually regardless of treatment or cultivar (Tables 4-2, 4-3). By the end of the experiment, non-treated plants had a disease severity of 4.2. Plants treated with Actigard and AgSil had a disease severity of 2.1 and 1.7, respectively. Rhapsody-treated plants had a disease severity of 1.0 whereas less than 0.6 was observed on plants treated with Heritage-Eagle, K-phite, Milstop, and Prevam (Table 4-2). As indicated by the AUDPC values, all treatments were significantly different from untreated control. However, none of the biofungicide treatments were as effective as the commercial fungicides. Nevertheless, K-phite, Milstop, Prevam, and Rhapsody reduced disease severity from 0.3 to 1.0, approximately between 76 to 93%, compared with untreated plants (Table 4-2).

Regardless of treatment, cultivar Snow White and Orange were the most susceptible to powdery mildew throughout the experiment. Cultivars Hot Pink and Fuchsia were not significantly different from each other when evaluated on a weekly basis. However, the AUDPC values showed that Fuchsia was the least susceptible among all cultivars tested (Table 4-3).

Heritage alternated with Eagle significantly reduced disease severity on all cultivars when compared with untreated control (Figure 4-3). For the cultivar Fuchsia, Milstop and Prevam significantly reduced powdery mildew severity to a level equivalent to that of commercial fungicides. Moreover, Prevam was as effective as the standard fungicides in reducing disease severity in more susceptible cultivars such as Hot Pink and Orange (Figure 4-3). K-phite and Milstop were the only biofungicide products capable of reducing powdery mildew on the most susceptible cultivar, Snow White. Moreover, K-phite was as effective as the rotation of fungicide Heritage and Eagle.

Discussion

Disease symptoms appeared almost a month after transplant and the powdery mildew epidemic developed slowly thereafter. During the first six weeks of the experiment, the relative humidity was below 80% and since powdery mildew develops best at a high humidity (80 to 90%) (Daughtrey et al., 1995), the low relative humidity probably was a constrain to a faster epidemic development. However, adverse microclimatic conditions were probably useful for the plants cells that were already attacked by powdery mildew fungi in that they reduced the speed of infection process giving the plant more time to transport material to the infection site and stop penetration by formation of papillae (Aust and Hoyningen-Huene, 1986).

The biofungicides products Actigard, Agsil, K-phite, Milstop, Prevam, and Rhapsody suppressed powdery mildew of gerbera daisies compared with untreated plants under greenhouse conditions in Florida. However, these products were not as effective as the fungicide program of Heritage alternated with Eagle.

Among all biofungicides, Actigard and Agsil were the least effective treatments. Rhapsody provided moderate disease control and K-phite, Millstop and Prevam were the most effective in reducing powdery mildew severity. Moreover, for the cultivar Fuchsia, Milstop and Prevam were

as effective as the fungicide program. For the most susceptible cultivar, Snow White, K-phite and Milstop were the only biofungicide products capable of reducing powdery mildew severity.

Actigard did not provide satisfactory control of powdery mildew in gerbera daisies when compared to the other biofungicide treatments. Similarly, Babadoost (2002) reported that Actigard did not provide a satisfactory level of powdery mildew control in pumpkin. In addition; this material was ineffective when used against powdery mildew of cucumber, suggesting that it failed to enhance plant defense responses (Wurms et al., 1999). However, when Actigard was used in muskmelon, it provided moderate powdery mildew control when compared to other treatments (Matheron and Porchas, 2003). When used in cabbage and lettuce, Actigard was effective in controlling powdery mildew (Matheron and Porchas, 2004; Miller and Hernandez, 2001). Thus, these results are in agreement with those of Oostendorp et al. (2001) and Görlach et al. (1996) who suggested that the crop may determine the activation of resistance. Consequently, Actigard may not elicit a strong defense response in gerbera plants.

Powdery mildew severity on gerbera daisies treated with AgSil was significantly different that untreated plants, although the level of disease reduction obtained was low when compared with other biofungicide treatments. However, potassium silicate has been demonstrated to be effective in suppressing powdery mildew in other crops (Belanger et al., 2003; Bowen et al., 1992; Ghanmi et al., 2004; Kanto et al., 2006; Menzies et al., 1992). Furthermore, potassium silicate was effective in suppressing powdery mildew on the highly susceptible strawberry cultivar, Toyonoka, in soil as well as in hydroponic cultivation (Kanto et al., 2006, 2004).

The lack of satisfactory powdery mildew suppression by potassium silicate on gerbera daisies was demonstrated previously in chapter 3. In the previous experiment, drench treatment with potassium silicate was ineffective but plants were exposed for only 32 days. In the present

experiment, plants were exposed for 56-days and foliar treatment with potassium silicate still did not provide the disease reduction reported for other crops (Belanger et al., 2003; Bowen et al., 1992; Ghanmi et al., 2004; Kanto et al., 2006; Menzies et al., 1992).

Given that Bowen et al. (1992), Kanto et al. (2007) and Menzies et al. (1992) in their *in vitro* assays demonstrated that conidial germination and germ tube elongation of powdery mildew fungi was unaffected by potassium silicate, suppression of powdery mildew by potassium silicate may be due to its ability to induce systemic acquired resistance. Subsequently, the lack of effective response of AgSil might be in agreement with the lack of response of Actigard and ultimately, the plant species may determine the activation of resistance (Görlach et al., 1996; Oostendorp et al., 2001).

In a previous study, Rhapsody failed to control powdery mildew of gerbera and a high level of disease severity was observed when compared to other treatments (Sconyers and Hausbeck, 2004). However, *Bacillus* spp., provided 80% reduction in powdery mildew severity of cucumber, caused by *P. fusca,* as determined by *in vitro* studies on detached leaves and seedlings (Romero et al., 2004). In this study, gerbera plants treated with Rhapsody had significantly lower disease severity than untreated plants; however, the effect was moderate compare to other biofungicide treatments. This is in agreement with Utkhede and Koch (2006) who showed that *Bacillus subtilis* (Quadra 137), significantly reduced powdery mildew severity in cucumber when compared with untreated plants although other non chemical products tested provided better results than *B. subtilis*. The efficacy of biological control varies with the level of relative humidity prevalent in the greenhouse (Belanger et al., 1994). However, *Bacillus* strains are resistant to adverse environmental conditions (Shoda, 2000). Regardless, in our study, Biotune was added to *Bacillus subtilis* to enhance its efficacy and reduce the dependency of high

humidity. Therefore, the moderate response of Rhapsody for control of powdery mildew of gerberas may be due to causes other than low humidity in the greenhouse.

In this study, K-phite was effective in reducing powdery mildew of gerbera compared with untreated plants and other biofungicide products. However, it did not provide the same level of control as the commercial fungicides. Similar results were reported previously by Mueller et al. (2003a) who showed that phosphorous acid applied as Biophos was effective for control of powdery mildew of gerbera daisies as was Fosphite on powdery mildew on muskmelon (Matheron and Porchas, 2005). Schilder et al. (2003) reported that ProPhyt reduced powdery mildew incidence and severity on grapes as much as the fungicide program (Dithane/Abound/Ziram). Our results did not correspond with those of Schilder et al. (2003) in that K-phite was not as effective as the commercial fungicide products for powdery mildew control. Consequently, the fungicide program used in their study was not as effective as the one used in this study or phosphite products acted differently for each pathosystem.

Our study demonstrated that potassium bicarbonate formulated as Milstop reduced powdery mildew levels in gerbera daisies and, for cultivar Fuchsia, the level of disease reduction was comparable to that of the systemic fungicides. Sconyers and Hausbeck (2004) showed that gerbera plants ‘Jaguar Mix’ treated with Milstop had low levels of infection similar to those found in plants treated with Heritage and Eagle. Furthermore, Uchida and Kadooka (2001) showed that Kaligreen reduced levels of powdery mildew to less than 5% in gerbera plants grown in Hawaii. Potassium bicarbonate products have also been successful in reducing powdery mildew on other crops such as cucumber, muskmelon, pumpkin, roses, sweet peppers, tomato and winter squash (Matheron and Porchas, 2003; McGrath and Shishko, 1999; Dik et al., 2003).

Among the biofungicide products evaluated for gerbera powdery mildew, Prevam was the most effective. Moreover, the level of disease reduction for cultivars Fuchsia, Hot pink and Orange compared to that achieved with the systemic fungicides. Prevam was previously reported to provide effective control of powdery mildew of strawberries (Mertely et al., 2005). Based on its components (boron; 0.99%, orange oil and organic surfactants; 99.01%), it is possible the oil ingredient in Prevam breaks down fungal mycelia and spores and exposes them to desiccation and thus prevents further infection. Oil has been used to control diseases for many years (Calpouzos, 1966) and it has been effective in reducing powdery mildew of apple, cherry, cucurbits, grapes and roses. The level of disease reduction ranged from highly effective (McGrath and Shishkoff, 1999, 2000; Pasini et al., 1997) to slightly satisfactory (Fernandez et al., 2006; Grove et al., 2005). In some cases, the efficacy of oils to reduce powdery mildew, compared to the levels obtained with standard fungicides (Northover and Schneider, 1996), were even superior (Grove et al., 2000; Wojdyla, 2002).

All cultivars performed as expected; Snow white and Orange were the most susceptible and Fuchsia and Hot pink were less susceptible.

Our study is the first evaluation of several biofungicide products for the control of powdery mildew of gerberas under greenhouse conditions in Florida. In addition, this is the first study demonstrating that Prevam significantly reduced powdery mildew severity in gerbera daisies. Alternative products such as Rhapsody, Milstop, Kaligreen, Biophos and electrolyzed oxidizing water were previously reported for control of powdery mildew of gerberas in other states including Georgia, Hawaii and Michigan (Mueller et al., 2003a,b; Sconyers and Hausbeck, 2004; Uchida and Kadooka, 2001).

In conclusion, the biofungicide products tested when applied prior to disease infection may reduce powdery mildew significantly compared to no treatment. As a consequence these products can be used as part of an integrated disease management program as an alternative to reduce the use of standard fungicides for the control of powdery mildew in gerbera daisy.

Table 4-1. Source, rate, active ingredient, and manufacturer of biofungicides used to suppress powdery mildew in gerbera daisy.

Product name	Rate /liter	Active ingredient	Manufacturer
Heritage alt.	0.3 g	azoxystrobin 50%	Syngenta, Greensboro, NC
Eagle	0.4 g	myclobutanil 40%	Dow AgroSciences. Indianapolis, IN
Prevam	4 ml	sodium tetraborohydrate decahydrate 0.99%	Oro Agri, Inc. Trophy Club, TX
AgSil 21 + Tween 20	3 ml + 100 µl	potassium silicate (12.65%K_2O, 26.5%SiO_2 / polyoxyethylene polyoxyethylene (20) sorbitan monolaurate	PQ Corporation. Valley Forge, PA./ Fisher Scientific Inc.
K-phite	5 ml	mono- and dipotassium salts of phosphorous acid 53%	Plant Food Systems, Inc. Zellwood, FL
Milstop	3 g	potassium bicarbonate 85%	BioWorks, Inc. Fairport, NY
Actigard	0.1 g	acibenzolar-S-methyl 50%	Syngenta, Greensboro, NC
Rhapsody + Biotune	10 ml + 1.3 ml	*Bacillus subtilis* (QST 713) 1.34% + Adjuvant	AgraQuest, Inc. Davis, CA. / AgraQuest, Inc. Davis, CA

Table 4-2. Effect of biofungicides and conventional fungicides on powdery mildew severity in gerbera daisies.

Treatment	Days after transplant[zy]						AUDPC[yx]
	28	37	44	51	58	65	
Control	0.1 ab	0.3 a	0.8 a	1.6 a	3.1 a	4.2 a	7.31 a
Heritage alt.							
Eagle	0.0 c	0.0 c	0.0 d	0.1 e	0.1 e	0.2 e	1.21 f
Prevam	0.0 c	0.0 c	0.1 d	0.2 de	0.4 de	0.3 de	2.23 e
Agsil + Tween 20	0.0 bc	0.0 bc	0.3b	0.7 bc	1.1 b	1.7 b	4.52 b
K-phite	0.0 c	0.0 bc	0.1 cd	0.4 dc	0.6 cd	0.6 d	3.13 cd
Milstop	0.0 c	0.0 c	0.0 d	0.3 de	0.3cde	0.6 cd	2.58 de
Actigard	0.1 a	0.1 ab	0.3 bc	0.8 b	1.4 b	2.1 b	4.87 b
Rhapsody + Biotune	0.0 c	0.0 c	0.1 bcd	0.5 cd	0.7 c	1.0 c	3.50 c

[z]Disease severity rated on a 0 to 6 scale, where 0= no powdery mildew symptoms, 1= 1 to 20%, 2= 21 to 40%, 3= 41 to 60%, 4= 61 to 80 %, 5= 81 to 100% and 6= 100% of upper leaf surface covered with powdery mildew symptoms.

[y]Mean separations in columns follow by the same letter are not significantly according to Fisher's protected LSD (P≤ 0.05).

[x]Area under disease progress curve (AUDPC) values.

Table 4-3. Effect of treatments on powdery mildew severity in gerbera cultivars treated with biofungicides and conventional fungicides.

Cultivar	Days after transplanting[zy]						AUDPC[yx]
	28	37	44	51	58	65	
Snow white	0.1 a	0.1 a	0.3 a	0.8 a	1.2 a	1.5 a	4.12 a
Hot pink	0.0b	0.1 ab	0.3 a	0.5 bc	0.8 b	1.1 b	3.58 b
Fuchsia	0.0 b	0.0 b	0.1 b	0.4 c	0.6 b	1.0 b	2.96 c
Orange	0.0 b	0.1 ab	0.2 a	0.6 ab	1.2 a	1.7 a	4.01 a

[z]Disease severity rated on a 0 to 6 scale, where 0= no powdery mildew symptoms present, 1= 1 to 20%, 2= 21 to 40%, 3= 41 to 60%, 4= 61 to 80 %, 5= 81 to 100% and 6= 100% of upper leaf surface covered with powdery mildew symptoms.

[y]Mean separations in columns follow by the same letter are not significantly according to Fisher's protected LSD ($P \leq 0.05$).

[x]Area under disease progress curve (AUDPC) values.

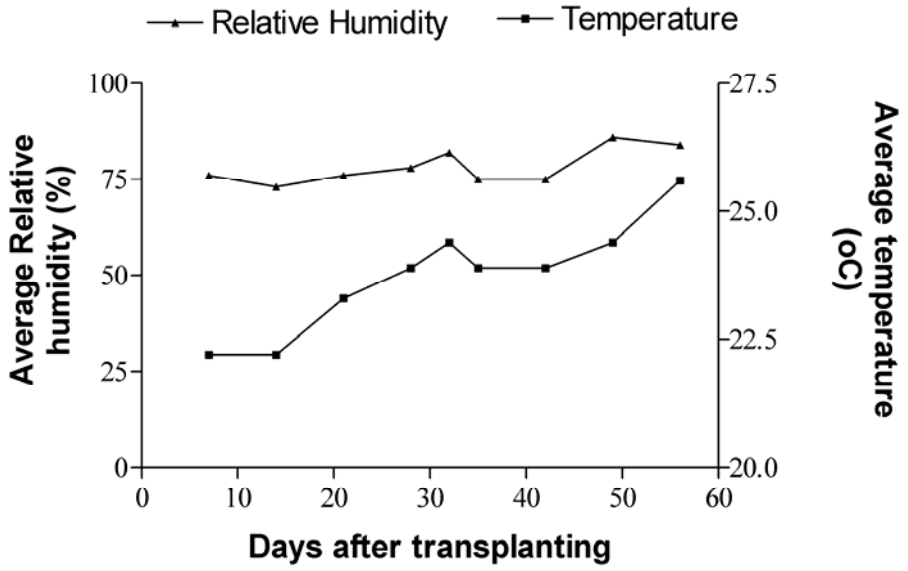

Figure 4-1. Daily average temperature and relative humidity in greenhouse from April to June, 2007.

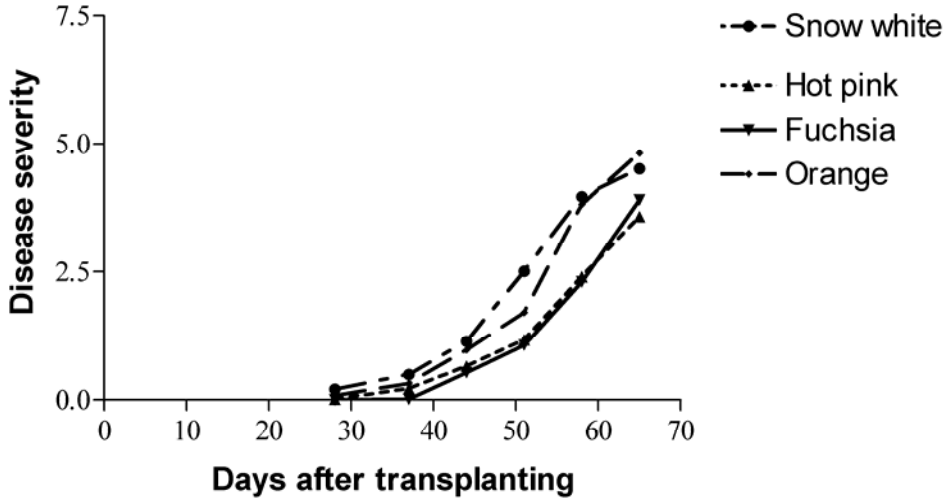

Figure 4-2. Disease progression of powdery mildew for the gerbera cultivars used in the biofungicide experiment.

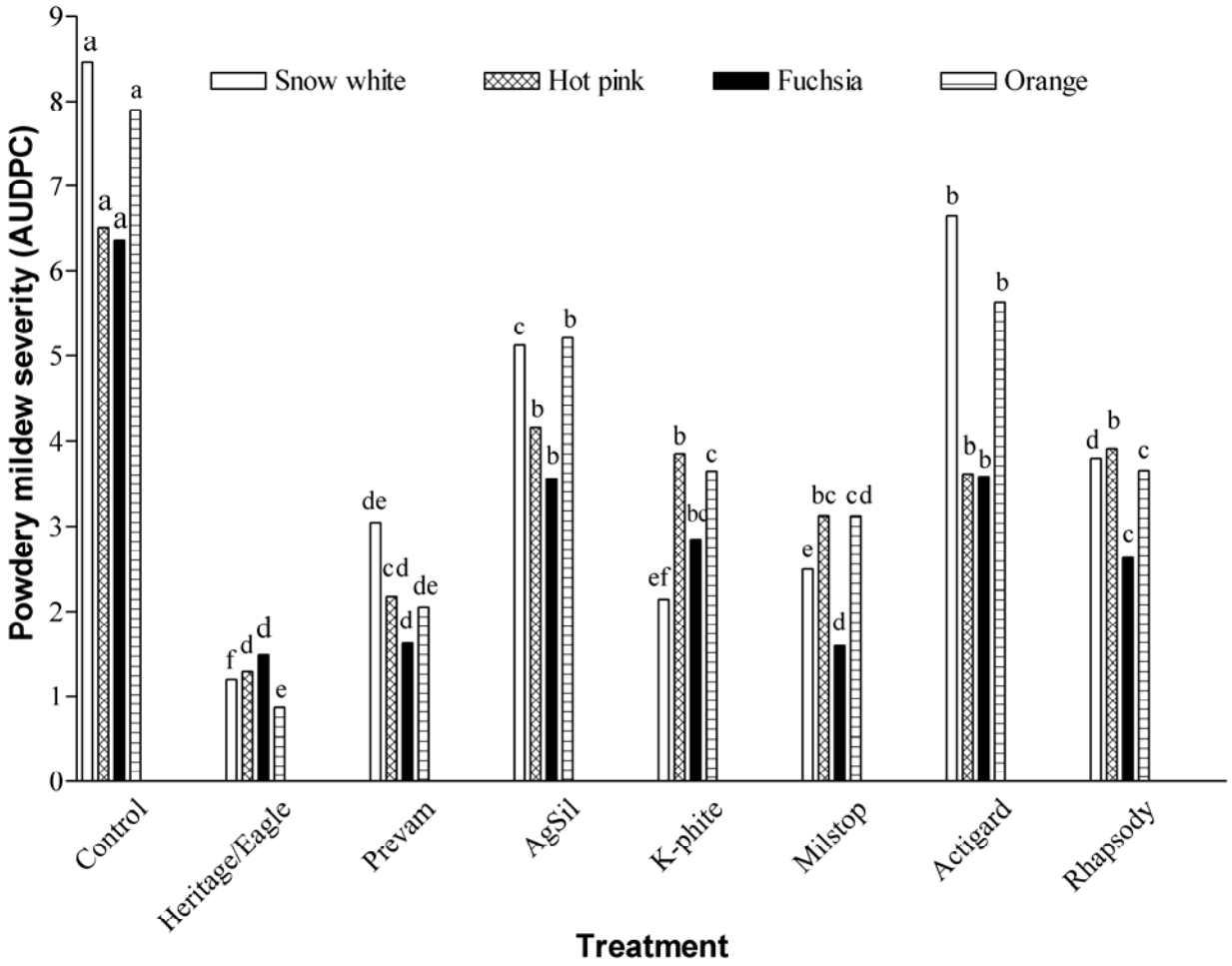

Figure 4-3. Area under disease progress curve (AUDPC) values for severity of powdery mildew in gerbera daisies treated with biofungicides and conventional fungicides. Bars with the same letter in each cultivar do not differ significantly according to Fisher's protected LSD ($P \leq 0.05$).

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BIOGRAPHICAL SKETCH

Catalina was borne in Medellin, Colombia. She earned a B.S. degree in agronomy from Zamorano University–Honduras, in 1999. Catalina came to the U.S. in 2001 to work as an intern at the hydrology department with the Forest Service and the Bureau of Land Management in Roseburg, Oregon. There, she met her husband, Scott Moyer, who she married in 2003. A year later, they moved to Florida. Catalina worked at Dr. Natalia Peres' strawberry pathology lab for ten months before she became a graduate student. In 2007, Catalina finished her graduate program and received a M.S degree in plant pathology. Catalina will continue working with Dr. Peres at GCREC in Wimauma, FL.

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