Evaluation of Biofungicides for Control of Powdery Mildew of Gerbera Daisy

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ADDITIONAL INDEX WORDS. Gerbera jamesonii, Podosphaera fusca, greenhouse daisies, ornamental diseases

Powdery mildew caused by the fungus *Erysiphe cichoracearum* DC. or *Podosphaera fusca* (Fr.) S. Blumer is a common disease on gerbera daisies (*Gerbera jamesonii* Bolus ex. Hook f.) grown in Florida. This disease affects all parts of the plant and reduced plant quality is the main component of economic loss. The effect of calcium silicate, potassium silicate, and the biofungicide products Actigard (acibenzolar-S-methyl), K-phite (phosphorous acid), Milstop (potassium bicarbonate), Tricon (boron, orange oil and organic surfactants), Cease (*Bacillus subtilis*) and AgSil (potassium silicate) were evaluated in highly susceptible ('Snow White' and 'Orange') and moderately susceptible ('Hot Pink' and 'Fuchsia') gerbera cultivars. Results suggested that neither calcium silicate nor potassium silicate were effective in suppressing powdery mildew in gerbera daisy. The biofungicides products Actigard, Agsil, Cease, K-phite, Milstop and Tricon, 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 biofungicide products tested K-phite, Millstop and Tricon were the most effective in reducing disease severity. Thus, biofungicide products may be used as an alternative to reduce the use of fungicides for suppressing powdery mildew of 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 major fungal disease in gerbera (Figs. 1, 2) and can be caused by two species, *Erysiphe cichoracearum* DC. and *Podosphaera* (Syn. Sphaerotheca) *fusca* (Fr.) S. Blumer (Daughtrey et al., 1995). 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 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 harm to the environment and human health through the use of pesticides 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, Tricon and oils, are labeled for control of powdery mildew in ornamentals in Florida (Crop Data Management Systems, 2007). However, information on the effectiveness of these products in managing powdery mildew in ornamentals, and more specifically on gerbera daisy, is limited. 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.



Fig.1. Powdery mildew symptoms on gerbera leaves.

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Fig.2. Powdery mildew symptom on gerbera flower.

Materials and Methods

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

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 Apr. 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, Tricon, Milstop, K-Phite, AgSil (plus Tween20), or Cease (plus Biotune) (Table 1). Heritage alternated with Eagle was used as the standard control and untreated controls were

sprayed with water only. 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 Apr. (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 eight (8) treatments and four (4) cultivars. Disease severity ratings were analyzed by week by analysis of variance (ANOVA) with mean separation by Fisher's Protected LSD ($P \le$ 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 = $\sum_{i}^{n-1}[(y_i + y_{i+1})/2](t_{i+1} - t_i)$ 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 ($P \le 0.05$) (Statistix 8.1, Tallahassee, FL) to separate means.

Results

Powdery mildew developed from natural inoculum. Disease severity was assessed once a week for 6 weeks. There were significant differences ($P \le 0.05$) between treatments and cultivars. The interaction between treatment and cultivar was significant ($P \le 0.05$). 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 2 and 3).

Until 40 DAT, the average relative humidity was below 80% and temperature fluctuated from 72 to 76 °F (22 to 24 °C). At about 44 (DAT), the relative humidity increased to above 80% (Fig. 3). On 44 DAT, powdery mildew increased on all cultivars and then it gradually progressed every week thereafter (Fig. 4).

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

Table 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 | 0.3 g | azoxystrobin 50% | Syngenta, Greensboro, NC |
| Eagle | 0.4 g | myclobutanil 40% | Dow AgroSciences, Indianapolis, IN |
| Tricon | 4 mL | sodium tetraborohydrate decahydrate 0.99% | Oro Agri, Inc., Trophy Club, TX |
| AgSil 21 + Tween 20 | $3 \text{ mL} + 100 \mu \text{L}$ | potassium silicate (12.65%K ₂ O, 26.5%SiO ₂ /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 |
| Cease + Biotune | 10 mL + 1.3 mL | Bacillus subtilis (QST 713) 1.34% + Adjuvant | AgraQuest, Inc., Davis, CA |

Table 2. Effect of biofungicides and conventional fungicides on powdery mildew severity in gerbera daisy.

| | | | Days after t | ansplant ^{z, y} | | | |
|---------------------|--------|--------|--------------|--------------------------|--------|--------|-----------------------|
| Treatment | 28 | 37 | 44 | 51 | 58 | 65 | AUDPC ^{y, x} |
| 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 |
| Tricon | 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 |
| Cease + Biotune | 0.0 c | 0.0 c | 0.1 bcd | 0.5 cd | 0.7 c | 1.0 c | 3.50 c |

²Disease 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.

^yMean separations in columns followed by the same letter are not significant according to Fisher's protected LSD ($P \le 0.05$).

^xArea under disease progress curve (AUDPC) values.

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

| | | | Days after | transplant ^{z, y} | | | |
|------------|-------|--------|------------|----------------------------|-------|-------|-----------------------|
| Cultivar | 28 | 37 | 44 | 51 | 58 | 65 | AUDPC ^{y, x} |
| 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 | 0.0 b | 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 |

²Disease 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. ^yMean separations in columns follow by the same letter are not significantly according to Fisher's protected LSD ($P \le 0.05$).

*Area under disease progress curve (AUDPC) values.

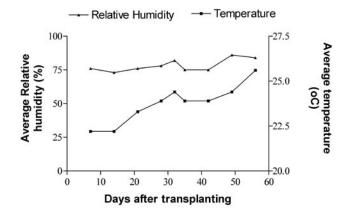


Fig.3. Daily average temperature and relative humidity in greenhouse from Apr. to June 2007.

were infected except the plants that received the Heritage-Eagle treatment. A week later, disease increased for all treatments including the Heritage-Eagle-treated plants (Table 2). During the last three weeks of the experiment (51 to 65 DAT), disease severity increased gradually regardless of treatment or cultivar (Tables 2 and 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. Cease-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 Tricon (Table 2). As indicated by the AUDPC values, all treatments were

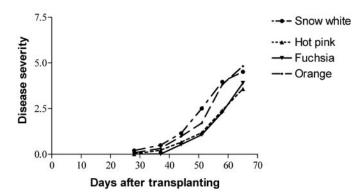


Fig. 4. Disease progression of powdery mildew for the gerbera cultivars used in the biofungicide experiment.

significantly different from untreated control. However, none of the biofungicide treatments were as effective as the commercial fungicides. Nevertheless, K-phite, Milstop, Tricon, and Cease reduced disease severity from 0.3 to 1.0, approximately between 76 to 93%, compared with untreated plants (Table 2).

Regardless of treatment, cultivars 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 3).

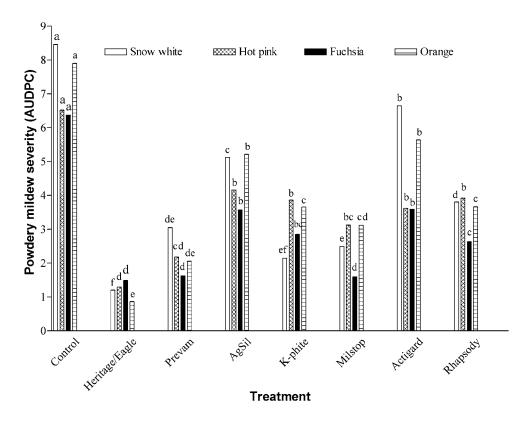


Fig. 5. Area under disease progress curve (AUDPC) values for severity of powdery mildew in gerbera daisy treated with biofungicides and conventional fungicides. Bars with the same letter in each cultivar do not differ significantly according to Fisher's protected LSD ($P \le 0.05$).

Heritage alternated with Eagle significantly reduced disease severity on all cultivars when compared with untreated control (Fig. 5). For the cultivar Fuchsia, Milstop and Tricon significantly reduced powdery mildew severity to a level equivalent to that of commercial fungicides. Moreover, Tricon was as effective as the standard fungicides in reducing disease severity in more susceptible cultivars such as Hot Pink and Orange (Fig. 5). Kphite 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 was probably a constraint to a faster epidemic development. This adverse microclimatic condition (low humidity) was probably useful for the plant cells that were already infected by the 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, Tricon, and Cease suppressed powdery mildew of gerbera daisy 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. Cease provided moderate disease control and K-phite, Millstop and Tricon were the most effective in reducing powdery mildew severity. Moreover, for the cultivar Fuchsia, Milstop and Tricon 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 daisy 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 daisy treated with AgSil was significantly different than 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., 2004, 2006).

In a previous experiment (Moyer et al., 2008) drench treatment with potassium silicate was ineffective but plants were exposed for only 32 d. In the present experiment, plants were exposed for 56 d 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 (Sconyers and Hausbeck, 2004), Cease failed to control powdery mildew of gerbera and a high level of disease severity was observed when compared to other treatments. 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 Cease had significantly lower disease severity than untreated plants; however, the effect was moderate compared 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, such as low humidity (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 Cease 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 daisy as was Fosphite for 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 daisy 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 (Dik et al., 2003; Matheron and Porchas, 2003; McGrath and Shishko, 1999).

Among the biofungicide products evaluated for gerbera powdery mildew, Tricon was the most effective. Moreover, the level of disease reduction for cultivars Fuchsia, Hot Pink, and Orange was comparative to that achieved with the systemic fungicides. Tricon 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 Tricon 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 Tricon significantly reduced powdery mildew severity in gerbera daisy. Alternative products such as Cease, 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, 2003b; 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.

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