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Evaluation of Acibenzolar-S-Methyl and Silicic Acid for control of Phytophthora Blight Caused by *Phytophthora capsici* in Squash

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ADDITIONAL INDEX WORDS. *Cucurbita pepo*, systemic acquired resistance

Phytophthora blight caused by *Phytophthora capsici* Leonian is a destructive disease of squash (*Cucurbita pepo* L.) in Florida and worldwide. Greenhouse studies were conducted to evaluate the potential of acibenzolar-S-methyl and silicic acid for control of Phytophthora blight in squash. Applied as a soil drench or foliar spray, acibenzolar-S-methyl alone at 20 and 30 mg·L^{-1} significantly ($P < 0.05$) reduced disease severity compared to the nontreated control. Acibenzolar-S-methyl applied as a soil drench at 30 mg·L^{-1} provided the greatest protection with no symptoms developed on treated squash plants. Silicic acid applied as a soil drench at 0.015 and 0.15 mM significantly suppressed disease severity by 47.7% and 53.3%, respectively, and increased Si concentration in squash roots by 36.2% and 44.4%, respectively, when compared with the nontreated control. Soil application of silicic acid in combination with acibenzolar-S-methyl improved the efficacy of silicic acid and acibenzolar-S-methyl each applied separately against Phytophthora blight. In vitro testing indicated that neither silicic acid nor acibenzolar-S-methyl at test rates suppressed *P. capsici* by inhibiting growth of mycelia, production of sporangia or germination of zoospores. Results in this study suggest that acibenzolar-S-methyl and silicic acid were effective against *P. capsici* in squash and may be incorporated into integrated management programs for control of Phytophthora blight in squash.

Phytophthora blight caused by *Phytophthora capsici* Leonian is a devastating disease to cucurbit and solanaceous crop production in the U.S. and other vegetable-growing areas worldwide (Hausbeck and Lamour, 2004). *P. capsici* is a soil-borne pathogen and may survive as oospores for more than 5 years in the soil or as mycelia in plant debris. The pathogen has a wide host range including more than 50 species of major vegetable crops and weeds (Tian and Babadoost, 2004). *P. capsici* can destroy an entire field of squash within days in regions with warm and wet weather such as South Florida. Currently, no single method provides adequate control of *P. capsici*. Highly resistant varieties with ideal horticultural traits are not available for Florida growers (Olson et al., 2007). Crop rotation is an important component in integrated disease management. However, the long-term survival of *P. capsici* oospores limits the effectiveness of crop rotation as an important management strategy (Hausbeck and Lamour, 2004). While a number of fungicides are registered for use on cucurbits, few are highly effective against *P. capsici* under the weather conditions in South Florida. Moreover, resistance to one of the most effective fungicides, mefenoxam, has been documented in *P. capsici* from several vegetable-producing regions of the US including Florida (Hausbeck and Lamour, 2004).

Use of systemically induced plant resistance is a promising strategy for plant disease management. A series of plant defenses are activated upon application of a resistance inducer against pathogens subsequently encountered by the plant through activation of different signaling pathways such as the salicylic acid pathway (Métraux et al., 1990). Acibenzolar-S-methyl, a functional analog of salicylic acid, has been evaluated extensively for control of diseases on various plant types (Oostendorf et al., 2001). It has been reported that acibenzolar-S-methyl enhances the expression of resistance related or pathogenesis-related genes in plants, which cause the accumulation of compounds important for disease resistance including lignin and phenolics, increase activity of peroxidase, polyphenol peroxidase, and phenylalanine ammonia lyase, and trigger a cascade of reactive oxygen species (Benhamou and Nicole, 1999; Jeun et al. 2000; Malolepsza 2006).

Silicon (Si) is one of the most abundant mineral elements in the soil. Many plants can uptake and utilize Si during growth. Beneficial effects of Si on plant disease resistance have been reported from many crops including cucurbit crops (Datnoff et al., 2007; French-Monar et al., 2010; Ma and Takahashi). Control of plant diseases by application of Si has been initially attributed to a mechanical barrier resulting from its polymerization in plants (Kim et al., 2002; Yoshida et al., 1962). Si-mediated disease

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resistance against pathogens was confirmed to be associated with the accumulation of phenolics and phytoalexins as well as activation of PR-genes (Chérif et al., 1994; Dann and Muir, 2002; Fauteux et al., 2006; Fawe et al., 1998), indicating that Si may play an active role in inducing host resistance to plant pathogens by stimulating defense reaction mechanisms. The objectives of present study were to evaluate the effect of acibenzolar-S-methyl and silicic acid applied separately either as a soil drench or as a foliar spray and their combining effects on disease development of Phytophthora blight on squash (*Cucurbita pepo* L.), and to investigate the *in vitro* effect of the acibenzolar-S-methyl and silicic acid application on mycelial growth, sporangial production and zoospore germination of *P. capsici*.

Materials and Methods

***P. capsici* isolate and inoculum preparation.** The isolate of *P. capsici* utilized in the present study was obtained from a squash plant with Phytophthora blight in Homestead, FL in 2008. Inoculum preparation of *P. capsici* for greenhouse assays was performed as described by Ploetz et al. (2002). Counts of zoospores were made using a hemacytometer under a microscope, and the concentration of *P. capsici* inoculum was adjusted to 2 × 10^4 zoospores/mL.

Squash plants, inoculation with *P. capsici*, and disease rating. Greenhouse experiments were conducted with the summer squash HMX 5703 (Harris Moran Seed Co., Arcadia, FL). Squash seeds were planted to a depth of approximately 1-2 cm in 10-cm-diameter plastic pots containing soilless pro-mix growing medium (Miracle-Gro Lawn Products, Inc., Marysville, OH) in a greenhouse with a temperature range of 22 to 25 °C. The seedlings were allowed to grow for 2 to 3 weeks after planting (WAP) before being inoculated by soil drench with *P. capsici* to create conditions for root infection. Five milliliters of *P. capsici* inoculum were applied to the soil around the stem of each plant. Inoculated plants were placed on greenhouse benches for 1 to 2 weeks prior to disease rating. Disease severity of Phytophthora blight was assessed based on a rating scale of 0–5 (Zhang et al., 2010), where 0 = no symptoms, 1 = small brownish lesions at the base of stem, 2 = stem lesions extend to cotyledons or the lesion has girdled the stem causing plant collapse, 3 = plant has collapsed with all leaves wilted or turned yellow except for the young leaves, 4 = entire plant has completely collapsed with leaves intact, and 5 = entire plant is dead.

Application of acibenzolar-S-methyl and silicic acid. A commercial product of acibenzolar-S-methyl, Actigard® 50WG (Syngenta Crop Protection, Inc., Greensboro, NC) and silicic acid, a compound of silicon nutrient, purchased from Fisher Scientific Inc. (Fair Lawn, NJ) were evaluated for their effects on Phytophthora blight of squash caused by *P. capsici* in the greenhouse.

Acibenzolar-S-methyl (Actigard® 50WG) was evaluated as soil drenches (Experiment 1) or foliar sprays (Experiment 2) at 0.1, 1, 10, 20, and 30 mg·L^{-1} against *P. capsici*. Silicic acid was investigated as a soil drench (Experiment 3) or foliar sprays (Experiment 4) at concentrations of 0.015, 0.15, and 1.5 mM. As acibenzolar-S-methyl at 30 mg·L^{-1} onsistently showed a significant effect on disease reduction, it was included as a positive check in all experiments in this study. In the soil drench treatment experiments, 10 mL of acibenzolar-S-methyl or silicic acid solutions were added to the soil at the base of the stem for each plant at 1 and 2 WAP, respectively. In the spray treatment experiments, the solutions were applied to foliage by spraying until run-off at 1 or at 2 WAP. Plants treated with fluopicolide (Presidio®, Valent USA Corp. Walnut Creek, CA) at 0.32 mL·L^{-1} (= 4 fl oz/acre) were included as a standard chemical control, and water treatment served as the untreated control. A completely randomized design was used for these experiments with four replications of each treatment and 12 plants per replication. All experiments were performed three or four times.

To evaluate the effect of silicic acid in combination with acibenzolar-S-methyl on disease development, silicic acid was applied into the soil at 0.015, 0.15, and 1.5 mM separately or in combination with acibenzolar-S-methyl at 1 mg·L^{-1} in one experiment (Experiment 5; see Fig. 1 for treatments), and at 10 mg·L^{-1} in the other experiment (Experiment 6; see Fig. 2 for treatments). A total of three applications were made at 7 days intervals by adding 20 mL of solutions into the soil starting at 8 days after seeding. The squash plants were inoculated at 4 d following the last treatment by applying 5 mL of *P. capsici* inoculum to the soil around the stem of each plant. Inoculated plants were placed on greenhouse benches and disease severity of Phytophthora blight was assessed at 7, 9, 12, 16, and 20 d after inoculation (DAI) based on a rating scale of 0–5 described previously. A randomized complete block design was used for these experiments with eight replications of each treatment and one plant per replication. These experiments were performed two times.

Determination of Si concentration in root tissues of squash plants. Two-week old seedlings were treated silicic acid at concentrations of 0.015, 0.15 and 1.5 mM by pippeting 10 mL of the solutions into the soil around the root. All treatments including water treated plants as a nontreated control were arranged in a randomized complete block design with four replications, one plant in a pot for each application. Two days after the last treatment, roots for each replication of all treatments were removed from the pots, shaken to remove the bulk of the soil, washed in deionized water and dried for 72 h at 65 °C. Concentration of Si in roots was determined by a colorimetric analysis at 650 nm on 0.25 g of dried and alkali-digested tissue following the methodology described by Elliott and Snyder (1991). The experiment was conducted twice.

Effect of acibenzolar-S-methyl and silicic acid on mycelial growth of *P. capsici*. The mycelial growth of *P. capsici* colonies was evaluated on potato dextrose agar (PDA) containing acibenzolar-S-methyl or silicic acid as described by Keinath (2007). Briefly, *P. capsici* culture was grown on V8 vegetable juice (Campbell Soup Co., Camden, NJ) agar at 25 °C for 7 d. A 6-mm-diameter plug of *P. capsici* mycelia taken from the edge of a colony was placed at the center of each PDA plate amended with silicic acid at 0, 0.015, 0.05, 0.1, 0.15, 0.5, 1.0, or 1.5 mM, or with acibenzolar-S-methyl at a final concentration of 0, 0.03, 0.3, 3.0, or 30 mg·L^{-1}. Three plates were used for each concentration and the plates with inoculated *P. capsici* were incubated at 25 °C in the dark. The diameter of each colony at each concentration was measured at 7 d after incubation twice with the second diameter perpendicular to the first and the mean value of the two measurements was used as an estimate of the colony diameter. The experiment was repeated one time.

Effects of acibenzolar-S-methyl and silicic acid on sporangial production of *P. capsici*. *P. capsici* culture was grown on V8 vegetable juice (Campbell Soup Co., Camden, NJ) agar plates wrapped with parafilm for 5 d at 25 °C in the dark. Then, 6-mm-diameter plugs were cut from the edge of *P. capsici* colonies. Three 5-mm plugs were placed with the mycelium side up in each petri dish containing sterilized solutions (3-4 mm deep) of

acibenzolar-S-methyl or silicic acid at concentrations listed above. The petri dishes were incubated at room temperature under lab lighting and not wrapped with parafilm to induce the production of sporangia (Keinath, 2007). Agar plugs with *P. capsici* were placed on glass slides, stained and fixed with 0.01% (w/v) acid fuchsin in 87.5% lactic acid after 48 h incubation. The number of sporangia was counted under a microscope at 100× magnification from two randomly selected fields for each agar plug. Nine agar plugs were examined for sporulation in each concentration, and the experiment was conducted twice under similar conditions.

Effect of acibenzolar-S-methyl and silicic acid on zoospore germination of *P. capsici*. Zoospores of *P. capsici* were produced on V8 vegetable juice (Campbell Soup Co., Camden, NJ) agar as described by Keinath (2007) with minor modifications. A 6-mm-diameter plug cut from the edge of a *P. capsici* colony was placed on a V8 vegetable juice agar plate, and the plates were incubated at room temperature for 5 d under lab lighting. The plates were flooded with 10–15 mL sterile water, chilled at 4 °C for 45 min, and then placed at room temperature for 15–20 min to allow the release of zoospores. Four drops of suspensions of zoospores were streaked out on each PDA plate amended either with acibenzolar-S-methyl or with silicic acid at concentrations mentioned previously. The plates were incubated at 25 °C for 2 h when germinated zoospores were counted for 100 zoospores on each plate under a microscope at 100× magnification, and three counts were made randomly for each plate. The germination rate of zoospores was expressed as the percentage of germinated spores per 100 zoospores for each concentration, calculated from the number of germinated zoospores out of a total number of 100 zoospores counted on the PDA plates amended with either silicic acid or acibenzolar-S-methyl. The experiment was conducted twice with three plates for each concentration.

Statistical analysis. Data from Phytophthora blight greenhouse experiments and plate studies with *P. capsici* on PDA amended with acibenzolar-S-methyl or silicic acid were analyzed separately for each repeated experiment, and were submitted to analysis of variance (ANOVA) using the Statistical Analysis System software (JMP version 5.1, SAS Institute Inc., Cary, NC). The significance of treatment effects was determined by the magnitude of the F value ($P < 0.05$). When a significant F test for treatments was obtained, separation of the means was accomplished using Fisher's protected least significant difference (LSD) at $P = 0.05$.

Results

Effect of acibenzolar-S-methyl on Phytophthora blight of squash. *Soil drench.* When applied as a soil drench at 1 and 2 WAP, acibenzolar-S-methyl at 10, 20, and 30 mg·L^{-1} significantly ($P < 0.05$) reduced the disease severity of Phytophthora blight in all four repeated trials compared to the untreated control (Table 1). No disease developed on squash plants treated with acibenzolar-S-methyl at 30 mg·L^{-1}. The effect of acibenzolar-S-methyl at lower concentrations i.e., 0.1 and 1 mg·L^{-1} on the disease severity was not significant from the nontreated control.

Foliar spray. In the foliar spray experiments, disease severity of Phytophthora blight on squash was significantly ($P < 0.05$) reduced in the treatments of acibenzolar-S-methyl at 20 and 30 mg·L^{-1} in all three repeated trails (Table 2). However, acibenzolar-S-methyl at the concentrations lower than 20 mg·L^{-1} generally did not significantly decreased disease severity of Phytophthora blight on squash compared to the untreated control.

Suppression of Phytophthora blight of squash by silicic acid. *Soil drench.* In Experiment 3, silicic acid at 0.015 and 0.15 mM significantly ($P < 0.05$) reduced Phytophthora blight compared to the nontreated control when it was applied as soil drenches (Table 3), whereas silicic acid at 1.5 mM significantly suppressed the disease severity only in one of three repeated trials. Acibenzolar-S-methyl at 30 mg·L^{-1}, included as a positive control, was as effective in suppression of the disease as fluopicolide was.

Foliar spray. No significant ($P < 0.05$) effect of silicic acid was observed on reduction of Phytophthora blight in comparison with the untreated control except at 0.15 mM in one of the three trials (Table 4).

Si concentration in roots of squash treated with silicic acid. When applied as a soil drench, treatments with silicic acid at 0.015 and 0.15 mM significantly ($P < 0.05$) increased the concentration of Si in the roots of squash compared to the

Table 1. Effects of acibenzolar-S-methyl applied as soil drenches on Phytophthora blight of squash (Experiment 1).

	Disease severity[z]			
Treatment[y]	Trial 1	Trial 2	Trial 3	Trial 4
ASM 0.1 mg·L^{-1}	1.9 b[x]	3.2 a	3.0 a	3.3 a
ASM 1 mg·L^{-1}	1.0 bc	2.6 a	2.2 ab	2.7 a
ASM 10 mg·L^{-1}	1.5 b	0.2 b	1.1 bc	1.2 b
ASM 20 mg·L^{-1}	0.2 c	0 b	0.9 bc	0 b
ASM 30 mg·L^{-1}	0 c	0 b	0 c	0 b
Fluopicolide 0.32 mL·L^{-1}	0.6 c	0.2 b	0.1 c	0.4 b
Untreated CK	3.1 a	2.5 a	2.7 a	3.6 a

[z]Squash plants were inoculated with *P. capsici* at 4 d following the last treatment by adding 5 mL of inoculum (2×10^4 zoospores/mL) into Pro-mix soilless potting medium around the stem base of each plant. The disease was rated on a scale of 0–5 at 14 d after inoculation.

[y]Five milliliters of aqueous solutions of treatment were applied into the Pro-mix soil at the stem base of each plant at 1 and 2 weeks after planting. ASM = acibenzolar-S-methyl.

[x]Values represent means of disease rating from four replications per treatment and 12 plants per replication. Means in columns followed by different letters are significantly different at $P = 0.05$ according to the LSD test.

Table 2. Effects of acibenzolar-S-methyl applied as foliar sprays on Phytophthora blight of squash (Experiment 2).

	Disease severity[z]		
Treatment[y]	Trial 1	Trial 2	Trial 3
ASM 0.1 mg·L^{-1}	4.4 ab[x]	3.4 a	2.9 ab
ASM 1 mg·L^{-1}	4.2 ab	2.8 abc	2.2 abc
ASM 10 mg·L^{-1}	2.9 abc	2.8 ab	1.3 bcd
ASM 20 mg·L^{-1}	2.1 bc	1.0 bc	0.5 cd
ASM 30 mg·L^{-1}	1.1 c	0.9 c	0 d
Fluopicolide 0.32 mL·L^{-1}	0.7 c	0.5 c	0.4 cd
Untreated CK	4.5 a	4.0 a	4.2

[z]Squash plants were inoculated with *P. capsici* at 4 d following the last treatment by adding 5 mL of inoculum (2×10^4 zoospores/mL) into Pro-mix soilless potting medium around the stem base of each plant. The disease was rated on a scale of 0–5 at 14 d after inoculation.

[y]Aqueous solutions of treatment were sprayed until runoff onto squash plants grown in Pro-mix soilless potting medium 1 and 2 weeks after planting. ASM = acibenzolar-S-methyl.

[x]Values represent means of disease rating from four replications per treatment and 12 plants per replication. Means in columns followed by different letters are significantly different at $P = 0.05$ according to the LSD test.

Table 3. Effects of silicic acid applied as soil drenches on Phytophthora blight of squash (Experiment 3).

Treatment[y]	Disease severity[z]		
	Trial 1	Trial 2	Trial 3
Silicic acid 0.015 mM	2.3 b[x]	0.6 bc	1.9 b
Silicic acid 0.15 mM	1.4 c	1.2 b	1.8 b
Silicic acid 1.5 mM	2.7 b	2.1 a	2.5 a
ASM 30 mg·L^{-1}	0.1 d	0 c	0.1 c
fluopicolide 0.32 mL·L^{-1}	0.4 d	0 c	0 c
Untreated CK	3.4 a	2.9 a	2.7 a

[z]Squash plants were inoculated with *P. capsici* at 4 d following the last treatment by adding 5 mL of inoculum (2×10^4 zoospores/mL) into Pro-mix soilless potting medium around the stem base of each plant. The disease was rated on a scale of 0–5 at 14 d after inoculation.
[y]Five milliliters of aqueous solution of silicic acid, Actigard® 50WG, fluopicolide (Presidio®) and water each were applied into the Pro-mix soilless potting medium at the stem base of each plant at 1 and 2 d after inoculation. ASM = acibenzolar-S-methyl.
[x]Values represent means of disease rating from four replications per treatment and 12 plants per replication. Means in columns followed by different letters are significantly different at $P = 0.05$ according to the LSD test.

Table 4. Effects of silicic acid applied as foliar sprays on Phytophthora blight of squash (Experiment 4).

Treatment[y]	Disease severity[z]		
	Trial 1	Trial 2	Trial 3
Silicic acid 0.015 mM	3.5 ab[x]	3.1 ab	4.7 a
Silicic acid 0.15 mM	2.3 b	1.5 c	3.4 b
Silicic acid 1.5 mM	3.3 ab	3.7 a	4.8 a
ASM 30 mg·L^{-1}	0.4 c	1.8 bc	0.5 c
fluopicolide 0.32 mL·L^{-1}	0.6 c	0.7 c	0.9 c
Untreated CK	3.8 a	2.8 abc	4.4 ab

[z]Squash plants were inoculated with *P. capsici* at 4 d following the last treatment by adding 5 mL of inoculum (2×10^4 zoospores/mL) into Pro-mix soilless potting medium around the stem base of each plant. The disease was rated on a scale of 0–5 at 14 d after inoculation.
[y]Aqueous solutions of treatment were sprayed until runoff onto squash plants grown in Pro-mix soilless potting medium 1 and 2 weeks after planting. ASM = acibenzolar-S-methyl.
[x]Values represent means of disease rating from four replications per treatment and 12 plants per replication. Means in columns followed by different letters are significantly different at $P = 0.05$ according to the LSD test.

nontreated control (Fig. 1). The concentration of Si in roots of squash treated with silicic acid at 1.5 mM was similar to that was in the nontreated control roots.

Effects of silicic acid applied separately or in combination with acibenzolar-S-methyl on phytophthora blight of squash. In the experiment in which acibenzolar-S-methyl was evaluated at 1.0 mg·L^{-1}, silicic acid alone at 0.15 mM significantly ($P < 0.05$) reduced the disease severity of Phytophthora blight assessed at 16 and 20 DAI compared to the untreated water control (Fig. 2). In general, silicic acid applied in combination with acibenzolar-S-methyl significantly reduced the disease compared to each individual treatment alone. Compared to the untreated control, a significant reduction in disease severity was achieved by silicic acid at either 0.015 or 1.5 mM for ratings since 9 DAI, and at 0.15 mM for ratings at 12 and 16 DAI. However, levels of the disease reduction were lower than fluopicolide, the fungicide control, for most of the ratings.

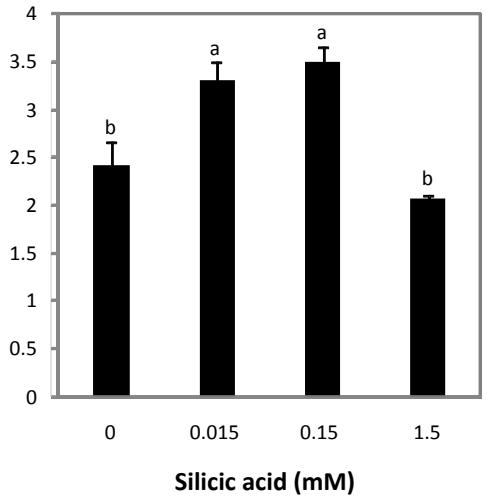

Fig. 1. Si concentration in roots of squash treated with silicic acid. Vertical bars represent standard error of the mean. Soil treatments: nontreated control (0 mM), silicic acid at 0.015, 0.15 and 1.5 mM at 1 and 2 weeks after planting. Root samples were collected at 2 d after the last treatment, and Si concentration were determined by a colorimetric analysis at 650 nm on 0.25 g of dried and alkali-digested tissue.

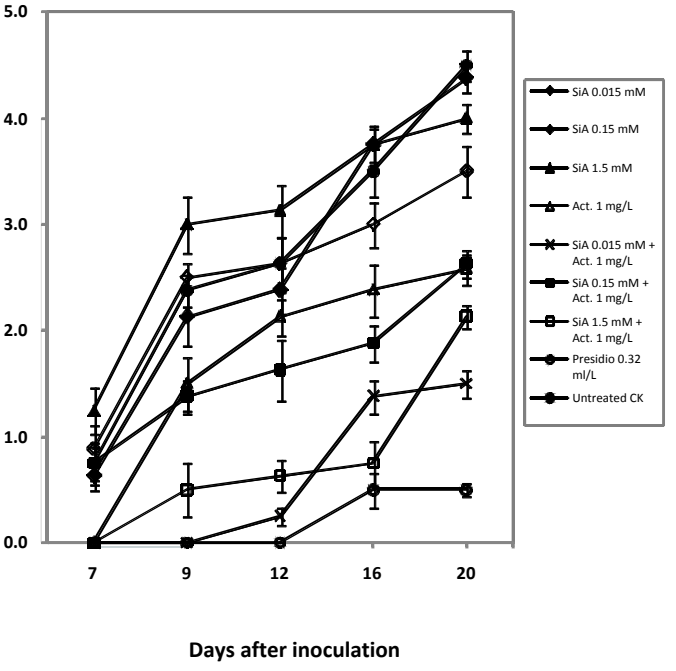

Fig. 2. Evaluation of silicic acid applied as soil drenches separately or in combination with acibenzolar-S-methyl (Actigard® at 1 mg·L^{-1}) for suppression of Phytophthora blight of squash (Experiment 5). Twenty milliters of aqueous solutions of silicic acid, acibenzolar-S-methyl, fluopicolide (Presidio®) and water each were applied into the Pro-mix soilless potting medium at the stem base of each plant at 8, 15 and 22 d after planting (DAP); and each plant was inoculated at 26 DAP by adding 5 mL of *P. capsici* inoculum (2×10^4 zoospores/mL) around the stem base. Disease severity was assessed at 7, 9, 12, 16 and 20 d after inoculation according to a rating scale of 0–5. RCB design was used with eight replications. Data are means of two repeated experiments. The error bar on each data point represents the standard error of the mean.

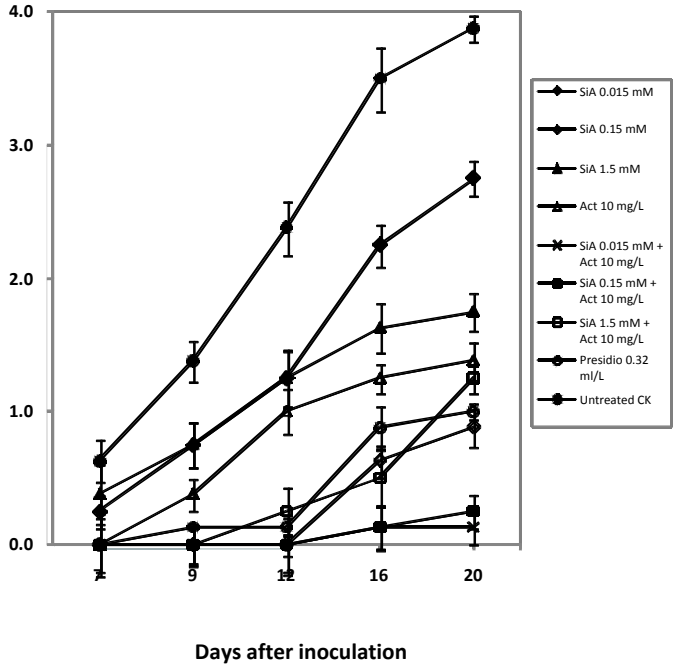

Fig. 3. Evaluation of silicic acid applied as soil drenches separately or in combination with acibenzolar-S-methyl (Actigard® at 10 mg·L^{-1}) for suppression of Phytophthora blight of squash (Experiment 6). Twenty milliters of aqueous solutions of silicic acid, acibenzolar-S-methyl, fluopicolide (Presidio®) and water each were applied into the Pro-mix soilless potting medium at the stem base of each plant at 8, 15 and 22 d after planting (DAP); and each plant was inoculated at 26 DAP by adding 5 mL of *P. capsici* inoculum (2×10^4 zoospores/mL) around the stem base. Disease severity was assessed at 7, 9, 12, 16, and 20 d after inoculation according to a rating scale of 0–5. RCB design was used with eight replications. Data are means of two repeated experiments. The error bar on each data point represents the standard error of the mean.

In another experiment in which acibenzolar-S-methyl was evaluated at 10 mg·L^{-1}, all treatments significantly ($P < 0.05$) suppressed Phytophthora blight of squash compared to the untreated water control (Fig. 3). Similarly, silicic acid applied in combination with acibenzolar-S-methyl significantly reduced the disease compared to either treatment alone. Silicic acid at 0.015 mM in combination with acibenzolar-S-methyl significantly reduced the disease severity for ratings since 9 DAI. When applied at 0.15 mM, a significant reduction was achieved for ratings of 16 and 20 DAI, and at 1.5 mM for ratings at 9, 12, and 16 DAI. Levels of disease reduction by silicic acid at 0.015 and 0.15 mM applied in combination with acibenzolar-S-methyl were generally greater than fluopicolide.

In vitro effect of silicic acid and acibenzolar-S-methyl on mycelial growth, sporangial production and zoospore germination. Mycelial growth of *P. capsici* was not affected by acibenzolar-S-methyl or silicic acid at the concentrations tested except for the highest concentration (30 mg·L^{-1}) of acibenzolar-S-methyl, which appeared to increase the growth of mycelia on PDA plates (Figs. 4 and 5). Neither silicic acid nor acibenzolar-S-methyl inhibited sporangial production or zoospore germination at test concentrations.

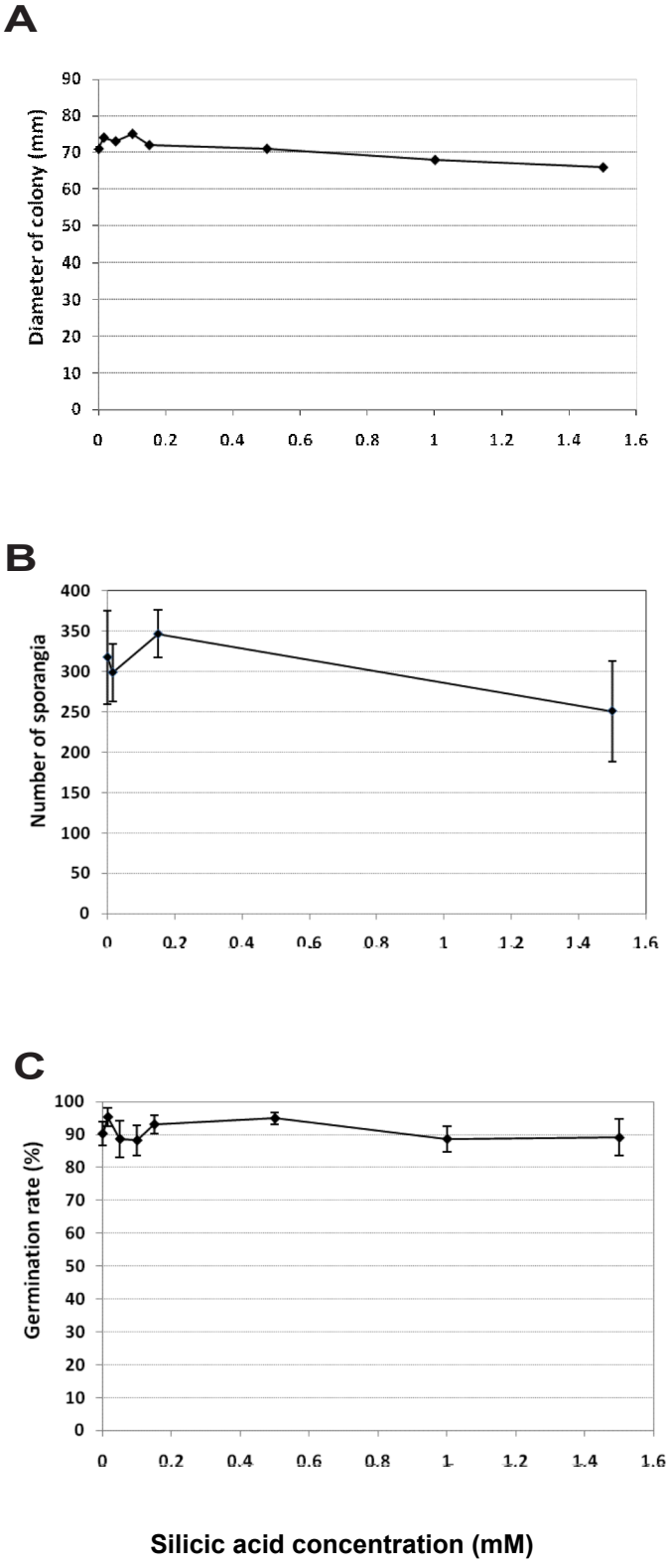

Fig. 4. Effect of silicic acid on mycelial growth, sporangial production and zoospore germination of *P. capsici*. The bar on each data point represents the standard error of the mean. (**A**) Diameter (mm) of *P. capsici* colonies on PDA amended with silicic acid at 7 d after incubation (DAI). (**B**) Production of sporangia (average number per vision field under microscope) of *P. capsici* on PDA with silicic acid at different concentrations 2 DAI. (**C**) Germination rate (%) of *P. capsici* on PDA with silicic acid at different concentrations 2 h after incubation

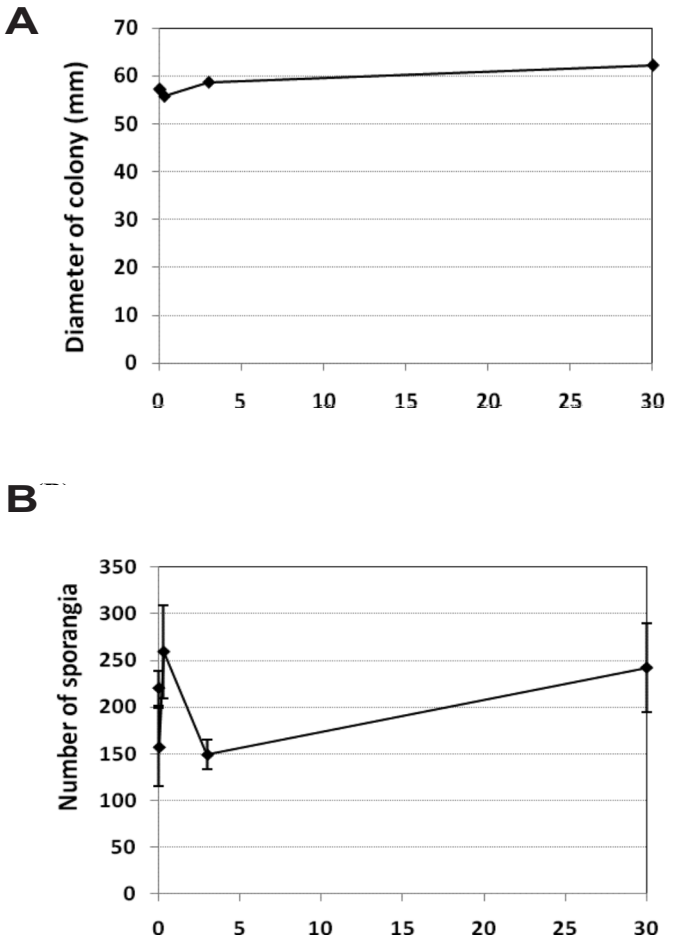

Fig. 5. Effect of acibenzolar-S-methyl on mycelial growth, sporangial production and zoospore germination of *P. capsici*. The bar on each data point represents the standard error of the mean. (**A**) Diameter (mm) of *P. capsici* colonies on PDA amended with acibenzolar-S-methyl at 7 d after incubation. (**B**) Production of sporangia (average number per vision field under microscope) of *P. capsici* on PDA with acibenzolar-S-methyl at different concentrations 2 d after incubation. (**C**) Germination rate (%) of *P. capsici* on PDA with acibenzolar-S-methyl at different concentrations 2 h after incubation

Discussion

Results from this study indicate that silicic acid applied as a soil drench and acibenzolar-S-methyl as a soil drench or foliar spray each significantly reduced disease severity of Phytophthora blight in summer squash under greenhouse conditions. The combined application of silicic acid with acibenzolar-S-methyl demonstrated an improved effect on suppression of Phytophthora blight. Data from this study suggest that silicic acid and acibenzolar-S-methyl could be used separately or jointly as important components in developing integrated programs for managing Phytophthora blight of squash.

Application of acibenzolar-S-methyl leads to broad spectrum resistance in many plant species against fungal, bacterial and viral pathogens (Oostendorf et al., 2001). Acibenzolar-S-methyl induced defense-related enzymes such as phenylalanine ammonia lyase, pathogenesis- related proteins and phenol accumulation, and this may contribute to enhanced resistance against *P. capsici*. However, little is known about the potential and the mechanisms of acibenzolar-S-methyl for control of Phytophthora blight on squash. Data from our studies indicate that Phytophthora blight of squash can be significantly suppressed by acibenzolar-S-methyl either as a soil drench or as a foliar spray. However, the efficacy of acibenzolar-S-methyl on Phytophthora blight reduction is greater when acibenzolar-S-methyl was applied as a soil drench (Table 1) than a foliar spray (Table 2). When applied as a soil drench, acibenzolar-S-methyl at 10, 20, and 30 mg·L^{-1} consistently suppressed Phytophthora blight disease, and no disease symptoms were developed on squash plants treated by acibenzolar-S-methyl at 30 mg·L^{-1}. Acibenzolar-S-methyl applied as a foliar spray significantly reduced the disease only at the higher rates of 20 and 30 mg·L^{-1} with a disease rating of 1.1 on plants treated with acibenzolar-S-methyl at 30 mg·L^{-1}. Since Phytophthora blight was mainly root and crown rot incited by soil inoculation with *P. capsici* in our studies, it is not a surprise to see the difference in the effect of acibenzolar-S-methyl between the application methods, i.e., the soil drench and foliar spray. This result warrants more efforts to extend this research to field trials for confirming the performance of acibenzolar-S-methyl on control of Phytophthora blight in squash.

It has been reported that application of Si can reduce disease intensity of important crops such as rice, cucurbits, corn, wheat, sorghum, turfgrass, and grape (Datnoff et al., 2007). In cucurbits, the ability of Si to enhance host resistance has been addressed in several studies against root rot caused by *Pythium aphanidermatum* and *P. ultimum* (Bélanger et al., 1995; Chérif et al., 1994). Lee et al. (2004) evaluated potassium silicate as a means to control *P. capsici* infections on pepper plants, and found that supplying the solutions with 100 or 200 ppm of potassium silicate significantly reduced mortality, root decay, and yield losses of pepper infected by *P. capsici*. However, little is known about Si-mediated disease suppression against *P. capsici* on squash. We have demonstrated that, for the first time, application of silicic acid to squash significantly reduced disease severity of root rot caused by *P. capsici*. Silicic acid applied as a soil drench at 0.015 and 0.15 mM significantly suppressed disease of Phytophthora blight when compared with the nontreated control (Table 4), whereas no significant disease reduction was observed for silicic acid at higher concentrations tested, i.e., 0.5, 1.0, or 1.5 mM (Tables 3, 4). The reason for the difference in reducing the disease at these widely divergent concentrations is not clear. However, the fact that silicic acid at 0.015 and 0.15 mM (not 1.5 mM) increased the Si concentration in the roots from our study revealed the possibility that the excess of silicic acid at the high concentration (1.5 mM) was polymerized (Lewin and Reimann 1969) to form Si gel or biogenic opal, an amorphous SiO_2 that is hydrated with various numbers of water molecules. Polymerized Si is not available for plants since Si is only absorbed by plants as monosilicic acid or its anion (Yoshida, 1975).

The mechanisms underlying elevated disease resistance induced by Si in plants are poorly understood. Most of the relevant research has focused on rice. Reduction of diseases in rice by Si appears to derive solely from a mechanical barrier formed by polymerization of Si in planta (Ma and Takahashi, 2002; Yoshida et al., 1962). However, many studies have shown that Si-mediated resistance against plant pathogens is associated with the accumulation of phenolics and phytoalexins, and with the activation of pathogenesis-related genes as well, indicating that Si may play an active role in increasing host resistance to plant diseases by stimulating defense reaction mechanisms (Bélanger et al., 1995; Chérif et al., 1994; Dann and Muir, 2002; Fawe et al., 1998).

A few studies examined the effects of Si on fungal pathogens such as spore germination, but this information is very limited. Bowen et al. (1992) reported in vitro effects of Si treatment on stimulation of conidial germination of *Uncimala necata*, whereas Rachniyom and Jaenaksorn (2008) indicated that soluble Si significantly reduced mycelial growth and sporangial production of *P. aphanidermatum*. Likewise, Kaiser et al. (2005) found that potassium silicate (20.7% silicon dioxide) inhibited the mycelial growth of Pythiaceae fungi including *P. capsici*. However, results from other studies indicate no significant influence of Si on the target pathogens. Heine et al. (2007) observed no direct effect of silicic acid at 1.4 mM on zoospore germination of *P. aphanidermatum*, the causal pathogen of root rot in cucumber. Although foliar supplication of Si significantly reduced disease severity of powdery mildew on cucurbits including squash, no influence was found on the germination of the pathogen *Sphaerotheca fuligena* (Menzies et al., 1992). Data from our studies indicate that Si did not exhibit in vitro inhibition to mycelia growth of *P. capsici*, which is different from the findings of Kaiser et al. (2005). This may be due to the differences in Si compounds used for the research. In our experiments, silicic acid was used and its solubility in water was very low with test concentrations not higher than 1.5 mM, whereas concentrations of silicates in water can be much higher. Si at high concentrations may exhibit inhibition to fungal pathogens. Further research on accumulation of phytoalexins and activation of PR genes in systemically induced defense needs to be carried out to elucidate the increased resistance in squash by Si against *P. capsici*.

In this study, we demonstrated that an elevated effect on disease reduction was observed between silicic acid and acibenzolar-S-methyl (Figs. 2 and 3). Based on the results from this study, levels of disease suppression for Phytophthora blight by silicic acid were inferior to the fungicide fluopicolide. Acibenzolar-S-methyl consistently reduced the disease severity at the concentrations above 10 $mg \cdot L^{-1}$. However, the use of acibenzolar-S-methyl especially at high concentrations may result in adverse consequences such as growth retardation and yield reduction (Gent et al., 2005; Nair et al., 2007; Romero et al., 2001). This could be costs for crops as a result of stimulation of resistance in crops, i.e., the additional resource allocation for the activation of resistance by acibenzolar-S-methyl (Heil et al., 2000). Combining application of Si and acibenzolar-S-methyl at the lower concentrations would be a way to improve the efficacy of disease suppression by Si and to avoid the retardation effect of acibenzolar-S-methyl on plant growth. Suppression of Phytophthora blight by silicic acid at various doses was significantly improved by combining with acibenzolar-S-methyl either at 1 or 10 mM. Some combinations such as silicic acid at 0.015 and 0.15 mM with acibenzolar-S-methyl at 10 mM resulted in greater effect on disease reduction than fluopicolide (Fig. 3). This study provides a solid case that certain combinations of Si and acibenzolar-S-methyl are superior to each individual one in squash against *P. capsici*, and could be incorporated into integrated management strategies for better control of Phytophthora blight of squash.

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