Seed Treatment with Phosphonate (AG3) Suppresses *Pythium* Damping-off of Cucumber Seedlings

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ABSTRACT

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A formulation of phosphonate (AG3) was tested as a seed treatment for the control of *Pythium* damping-off of cucumber (*Cucumis sativus* L.) plants under controlled environment and field conditions. Cucumber seed were treated by soaking for 10 min in phosphonate solution. They were then planted into peat-based mix or sandy-loam soil mixed with *Pythium aphanidermatum* or *P. ultimum* inoculum or into muck soil naturally infested with *P. irregulare*, *P. ultimum*, and other *Pythium* spp. Under growth-room conditions, phosphonate seed treatment provided more than 80% control of damping-off in all infested substrates tested. Effective disease control was obtained even when treated seed were stored for 5 weeks and up to 18 months prior to planting. In microplots containing naturally infested muck soil, phosphonate seed treatment decreased the percentage of diseased cucumber plants and increased total fresh weights compared with untreated seed and phosphonate post-planting drench. In field-plot tests 6 weeks after planting treated seed in *Pythium*-infested muck soil, cucumber stands were 63% compared with 18% in the control, which had no phosphonate exposure, and 53% in the post-planting drench. Tests for potential phytotoxicity in the greenhouse showed that radish and bok choy germination was reduced by phosphonate treatment but corn, cucumber, soybean, sugar beet, tomato, and wheat were not affected. Phosphonate seed treatment is a cost-effective way of protecting cucumber plants from *Pythium* damping-off.

Additional keywords: disease suppression, phosphonate seed soak

Pythium spp. such as *Pythium aphanidermatum* (Edson) Fitzp., *P. ultimum* Trow, and *P. irregulare* Buisman attack young seedlings of several horticultural and ornamental crops in greenhouse and field production systems (23). These pathogens cause seed and seedling diseases in bedding plants and greenhouse-grown transplants (23,37,38). The root of mature plants also may be attacked (23). *Pythium* spp.-induced damping-off diseases occur primarily in cold and wet soils in the field where young seedlings of direct-seeded crops may be killed before or soon after they emerge. *P. aphanidermatum* thrives under warm conditions, whereas *P. ultimum* and *P. irregulare* attack a variety of crops under cool conditions. *Pythium* spp. have been attributed to a sudden wilt of cucumber in Ontario, Canada (23). They have wide host ranges and can cause serious economic losses under conditions favorable for disease development. Although fungicides are available for the control of such diseases,

any technology that involved little risk to the pollution of the environment or to human health would be highly desirable.

Phosphonates, esters of phosphonic acid, generally are known to control plant diseases caused by *Phytophthora* spp. (4,9,17,20,41,45,46) and downy mildew pathogens (32,43,44). Their efficacy against *Pythium* spp. (2,33) and other fungal (11,42) and bacterial (7,30) pathogens also has been described. Various salt-based formulations of phosphorus acid and its esters have been used as agricultural fungicides. Fosetyl-Al or Aliette WP was registered in 1977 (20) and is still used effectively as a fungicide. Phosphonates also have been known to stimulate symbiotic infections by mycorrhizal fungi (22,24) and to interfere with phosphate-starvation responses in plants (40). There is no substantial evidence that phosphonates or phosphites can be utilized readily by plants although these compounds frequently are used as alternative to phosphate fertilizers (20,29,36).

Phosphonates are rapidly absorbed and translocated within the plant in both the xylem and phloem vessels (8). They can persist in plant tissues for extensive periods (6), have protective and curative properties (28), and a complex mode of action ranging from direct toxicity against invading pathogens to indirect effects such as activation of host defence response

(16,18,19,31). These compounds are applied on foliage as sprays (9), in tree trunks as injections (3,10), or in soil as drench (13,14) to control *Phytophthora* diseases. There is no published record on their use as a seed treatment.

The objectives of this study were to investigate the efficacy of a new phosphonate formulation (AG3) as a seed treatment to control *Pythium* damping-off of cucumber under growth room, microplot, and field conditions. Toxicity of phosphonate seed treatment to various plant species also was investigated.

MATERIALS AND METHODS

Plant material. All seed used in this study were fungicide free. Chinese leaf cabbage (*Brassica rapa* var. *chinensis* L. 'Bok Choy') seed was obtained from a grower located near Holland Marsh, ON, Canada. Corn (*Zea mays* L. '39T68' hybrid) seed was provided by Pioneer Hi-Bred, Chatham, ON. Cucumber (*Cucumis sativus* L. 'Straight Eight' or 'Marketmore 76') and radish (*Raphanus sativus* L. 'Cherry Belle') seed were purchased from William Dam Seeds Ltd., Flamborough, ON. Soybean (*Glycine max* L. Merr. 'Harosoy') seed was obtained from Dr. Mark Gijzen, Agriculture and Agri-Food Canada, London, ON. Sugar beet (*Beta vulgaris* L. 'Bronco') seed was kindly provided by Dr. Suha Jabaji-Hare, McGill University, Montreal, QC, Canada. Tomato (*Lycopersicon esculentum* Mill. 'Bonny Best') seed was purchased from OSC Seeds, Waterloo, ON. Wheat (*Triticum aestivum* L. 'Annett') seed was obtained from Morley McLean, Agriculture and Agri-Food Canada, Harrow, ON.

Phosphonate formulation and seed treatment. A new phosphonate formulation, AG3 (Calirus 150, liquid formulation; 10.45% a.i. phosphorus acid and copper sulphate with citrate as a chelating agent) was supplied by Bromine Compounds Ltd., Beer Sheva, Israel. Cucumber seed were treated by immersion in an AG3 solution at formulated strength (10.45% a.i.) for 10 or 20 min. They were then air dried in a laminar flow hood and planted immediately. Control seed were treated with water for 15 min. These preliminary tests indicated that the seed-soaking time above 10 min did not improve disease suppression; therefore, in all subsequent experiments, seed were treated for only 10 min, including controls. To determine the effect

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of storage time on germination and disease control, phosphonate-treated seed were stored at 24°C in a laboratory and planted 1 to 5 weeks and 18 months after treatment. To evaluate the possibility of phytotoxicity of phosphonates to seed, seed of bok choy, corn, cucumber, radish, soybean, sugar beet, tomato, and wheat were treated with AG3 solutions at formulated strength (10.45% a.i.) and at half strength (5.225% a.i. after 1:1 dilution in water) by soaking for 10 min. Treated seed were air dried and used immediately.

Growth of fungal cultures, preparation of inoculum, and dilution plating.

An isolate of *P. aphanidermatum* pathogenic to cucumber was obtained from Dr. James Traquair, Agriculture and Agri-Food Canada, London, ON, whereas *P. ultimum* was isolated from infected cucumber plants grown in muck soil naturally infested with *Pythium* spp. *Pythium* cultures were maintained on potato dextrose agar (PDA) medium. Inocula of *P. aphanidermatum* and *P. ultimum* were prepared in a mixture of soil and finely chopped potato as described previously (27), with some modifications (1). In brief, the mixture of finely chopped potato and clay loam soil (particle size 1 to 2 mm) was added to a liter flask. The soil:potato medium (10:1) was moistened by adding 50 ml of water and autoclaved for 1 h for 2 consecutive days. The flask was inoculated with 15 to 20 plugs obtained from the edge of the freshly grown *Pythium* spp. colony on PDA medium and kept in an incubator at 24°C. After 10 to 15 days, when the *Pythium* spp. had completely colonized the soil, the soil inoculum was placed on paper and air dried to 10 to 15% moisture level in a laminar flow hood with sterile air. The dried soil inoculum had to be used within a week of preparation. Various quantities of the *Pythium* spp. inocula were added to a peat-based mix in preliminary experiments in order to determine the amounts required to give more than 80% disease incidence within 2 weeks after planting. The propagule density of *P. aphanidermatum* in the inoculum was determined by dilution plating on PARP (corn meal agar amended with 5 mg pimarin, 250 mg ampicillin, 10 mg rifampicin, and 100 mg PCNB) medium, selective for isolating pythiaceous organisms (25). The propagule density of *Pythium* spp. in naturally infested muck soils also was determined by dilution plating on PARP medium. Individual colonies of *Pythium* spp. from PARP plates were transferred to PDA medium and species identification was made using grass blade culture technique (35) and taxonomic keys based on morphological structures (12,39). The identity of *P. ultimum* and *P. irregulare* was confirmed by polymerase chain reaction (PCR) analyses of individual colonies from PARP plates using species-specific primers K1 and K3 for *P. ultimum* (26) and internal

transcribed spacer region-derived primers for *P. irregulare* (R. Reeleder, unpublished data; primer sequences are available on request from Dr. R. Reeleder, Agriculture and Agri-Food Canada, London, ON). The pathogenic potential of these field isolates was tested on PDA medium using cucumber seed. Three surface-sterilized seed were placed in each PDA plate and plates were inoculated with a field isolate by placing a single plug from a freshly grown colony in the middle. An isolate was scored pathogenic if it caused seed decay or damping-off of the germinating seed. The pathogenicity of individual isolates was not tested in peat-based mix or soil. However, cucumber 'Straight Eight' seed were planted into the naturally infested muck soils and *Pythium* spp. were isolated from the diseased plants on PARP medium.

Growth room or greenhouse tests. The potential of phosphonate seed treatment to control *Pythium* damping-off of cucumber was determined in a number of substrates, including peat-based mix (Pro-Mix 'BX'; Premier Horticulture Inc., Rivière-du-Loup, QC), a sandy-loam soil (organic matter 1%, pH 6, water-holding capacity 18%) from a commercial potato field near Palmer Rapids, ON, and muck soils (organic matter 50%, pH 6.8) from a commercial field near Holland Marsh, ON. Peat-based mix and sandy-loam soil were artificially infested with freshly prepared inoculum of *P. aphanidermatum* or *P. ultimum* (0.8 to 1% wt/wt substrate), whereas muck soils from Holland Marsh were naturally infested with *P. ultimum*, *P. irregulare*, and other *Pythium* spp.

Tests for disease impacts of *Pythium* spp. on cucumber were performed as described previously (1). Circular plastic pots, 10 cm in diameter, were filled with the infested muck soil or substrate (0.4 liters/pot, five pots per treatment) and planted with phosphonate-treated and untreated cucumber seed (eight seed per pot). In some experiments, rectangular plastic pots (32 by 12.5 by 10.5 cm) were filled with 1,700 g of muck soil per pot and 16 seed were planted in each of three pots per treatment. AG3 drench (0.07% a.i. vol/wt substrate) was applied with a watering can after planting. Each circular pot received 20 ml and the rectangular pot received 200 ml of mixture. Pots were kept in a growth room (15 h of fluorescent light at 22 to 24°C and 9 h of dark at 19°C) and arranged in a completely randomized design. They were watered as required to maintain appropriate moisture level for growth. Plants were rated 2 weeks after sowing for damping-off using a 1-to-4 scale where 1 = healthy, 2 = yellowing of stem or root, 3 = plant dead, and 4 = seedling not emerged. Data were expressed as percent diseased seedlings. All experiments were repeated once.

The effects of AG3 phosphonate seed treatment on germination and growth of

various plant species were determined in an uninoculated peat-based mix in 72-cell transplant plug trays. Plug trays were filled with peat-based mix and planted with phosphonate-treated and untreated seed (12 seed per replicate tray). Treatments within each plant species were separated by a single row of blank cells in a tray. There were four replicates per treatment per plant species. Phosphonate-treated and untreated corn and wheat seed also were planted into a sandy-loam soil in circular pots (eight seed per each of five replicate pots). Trays or pots were watered and kept in a greenhouse at 22 to 24°C under a combination of daylight and supplemental lighting (light intensity 225 $\mu\text{E m}^{-2} \text{s}^{-1}$ for 12 h per day) in a completely randomized design. Percent germination was determined 17 days after planting. All plants from each replicate were weighed together to determine the average fresh weights per treatment. The average shoot lengths of corn, soybean, and wheat plants also were determined. The experiments were repeated once.

Microplot tests. Plastic drainage tiles (25 cm in diameter by 25 cm long) buried into a sandy-loam soil constituted microplots. The bottom 12 cm of each tile was filled with the same sandy-loam soil. Naturally infested muck soils from field sites (Barn and Creek) near Holland Marsh, ON were added to the top 12 cm of each tile (4 kg per tile). Treatments included a control, phosphonate post-planting drench (0.07% a.i. vol/wt soil), and phosphonate seed treatment and were arranged in a completely randomized design with five replicates per treatment. Sixteen cucumber seed treated and not treated with phosphonate were planted by hand in each tile to a depth of 2.5 cm. Phosphonate drench was mixed with water and applied with watering cans after planting. Each tile received a 400-ml aliquot. Plants were rated 16 days after planting for damping-off and the percentage of diseased seedlings was determined, as described above. After rating, cucumber plants also were weighed to determine total fresh weight per tile. The microplot tests were repeated with muck soils from both field locations.

Field tests. The efficacy of AG3 phosphonate seed treatment to improve cucumber seedling stand counts was determined in muck soil naturally infested with damping-off pathogens at two field locations (Barn and Creek) near Holland Marsh, ON. Plots were established in 2003 and treatments were arranged in a randomized block design with four replicates per treatment. The size of each plot was 3 by 1.5 m at the Barn site and 7.5 by 1.5 m at the Creek site. Treatments at both sites included a control, phosphonate post-planting drench (0.07% a.i.), and phosphonate seed treatment. Phosphonate-treated and untreated cucumber seed were planted

5 cm apart on twin rows with a planter. Phosphonate drench solutions were mixed with water (total volume per plot was 10 and 25 liters at the Barn and Creek sites, respectively) and applied immediately after planting to each plot with watering cans. At 2 and 6 weeks after planting, seedling stand counts from each plot were determined on the middle 1.5-m sections at the Barn site and 4.5-m sections at the Creek site. The number of emerged seedlings were expressed as a percentage of the total number of seed estimated to have been planted. The accuracy of the planter, however, was not determined. Infested soil samples from both locations also were brought to London for testing under controlled environmental conditions and in the microplots, as described previously.

Data analyses. The data were analyzed using MINITAB statistical software (version 13.0; Minitab Inc., State College, PA). Two-way analysis of variance (ANOVA) was performed on data from repeated growth-room and greenhouse experiments and, if *P* values indicated a significant difference ($P \leq 0.05$), means were separated by Fisher's protected least significant difference (LSD) test. Microplot (two soils from two field sites) and field (two sites) data were subjected to ANOVA using general linear model procedure of MINITAB and means were separated according to Tukey's test.

RESULTS

Inoculum density, identity, and pathogenicity of *Pythium* spp. The average propagule density of *P. aphanidermatum* in artificially prepared soil inoculum enumerated on PARP medium was 1×10^4 CFU/g. In naturally infested muck soils, the average propagule density of all *Pythium* spp. combined was 1.4×10^3 CFU/g soil. Based on morphological structures such as oogonia and sporangia produced in grass blades on PDA medium, 39% of the colonies from PARP plates were identified as *P. ultimum* and 41% as *P. irregulare*. In all, 20% of the colonies from PARP plates could not be identified and were described as unidentified *Pythium* spp. The identity of *P. ultimum* and *P. irregulare* isolates was further confirmed by PCR analyses using species-specific primers. Although pathogenicity of individual isolates was not tested in peat-based mix or soil, all of *P. ultimum* and *P. irregulare* and 8 of 11 unknown field isolates of other *Pythium* spp. caused decay of the germinating cucumber seed in the PDA plate inoculated with field isolates. The *Pythium* isolate recovered from the diseased cucumber plants produced in the naturally infested muck soils from untreated seed was identified as *P. ultimum* by PCR analysis. Muck soils also were naturally infested with *Plasmodiophora brassicae* and *Rhizoctonia solani*, which were pathogenic on bok choy but did not effect cucumber. The

presence of other pathogens in muck soils could not be ruled out.

Effect of phosphonate seed treatment on *Pythium* damping-off of cucumber in the growth room. Treatment of cucumber seed with AG3 phosphonate solution significantly enhanced their survival compared with untreated controls when planted into a *Pythium aphanidermatum*-infested peat-based mix or a muck soil naturally infested with *Pythium* spp. (Fig. 1). Less than 20% of the plants were diseased with the phosphonate seed treatment compared with more than 85% in the control (Fig. 1). Increasing the seed-soaking time did not improve the disease suppression above that obtained with a 10-min exposure (Fig. 1) and, as a result, in subsequent experiments seed were treated for only 10 min. AG3 drench application and seed treatment also effectively protected cucumber plants from damping-off when grown in a *P. ultimum*-infested peat-based mix. Less than 20% of plants were diseased with the AG3 treatments compared with more than 60% in the control. Plants grown from phosphonate-treated seed were identical in appearance to those from untreated seed.

Experiments on the effect of storage time after phosphonate seed treatment on germination and disease control were evaluated by planting seed stored at 22 to 24°C in a laboratory for 1 to 5 weeks and up to 18 months. When such seed were

Fig. 1. Efficacy of phosphonate (AG3) seed treatment for controlling damping-off of cucumber seedlings in A, a peat-based mix artificially infested with *Pythium aphanidermatum* or B, muck soil naturally infested with *Pythium* spp. Cucumber 'Straight Eight' seed were treated with AG3 solution by immersion for 10 or 20 min and planted into infested peat-based mix or muck soil (eight seed per pot and five pots per treatment). Control seed were treated with water for 15 min. Pots were incubated in a growth room and plants were rated for damping-off 2 week after planting. Error bars are standard error of mean from two experiments ($n = 10$). Values followed by the same letter do not differ significantly according to Fisher's protected least significant difference test at $P \leq 0.05$.

planted into *P. aphanidermatum*-infested peat-based mix, they consistently provided effective protection against the damping-off disease even after 5 weeks of storage. On average, less than 20% of the plants from treated seed were diseased (Fig. 2). Seed planted 18 months after seed treatment with phosphonate into infested muck soil had 39% diseased plants compared with 82% of plants diseased in the untreated control. The storage time after the phosphonate treatment of cucumber seed did not decrease efficacy of the phosphonate treatment.

The efficacy of the phosphonate seed soak was compared with phosphonate drench treatment in an artificially infested sandy-loam soil and a naturally infested muck soil. In sandy-loam soil artificially infested with *P. aphanidermatum*, damping-off incidence was high and 95% of cucumber seedlings were diseased in the control (Fig. 3A). Both phosphonate post-planting drench and phosphonate seed-soak treatments reduced the incidence of *P. aphanidermatum* damping-off, with 41 and 29% diseased plants, respectively (Fig. 3A). In muck soils naturally infested with *Pythium* spp., more than 70% of cucumber plants from untreated seed were diseased, whereas those treated by phosphonate drench and by seed soak were 33 and 20% diseased, respectively (Fig. 3B). Though not statistically significant, the phosphonate seed soak treatment provided better control than the post-planting drench treatment in both soils in this study.

Effect of phosphonate seed treatment on germination and plant growth in the greenhouse. The phosphonate seed soak treatments were not phytotoxic to seed of corn, cucumber, soybean, sugar beet, to-

Fig. 2. Effect of delaying the sowing of phosphonate (AG3)-treated seed on incidence of damping-off of cucumber seedlings in a peat-based mix artificially infested with soil inoculum of *Pythium aphanidermatum*. Cucumber 'Straight Eight' seed were treated with AG3 solution by immersion for 10 min and kept at 24°C for 1 to 5 weeks before planting into infested peat-based mix (eight seed per pot and five pots per treatment). Pots were incubated in a growth room and plants were rated 2 weeks later for damping-off. Error bars are standard error of mean from two experiments ($n = 10$). Values followed by the same letter do not differ significantly according to Fisher's protected least significant difference test at $P \leq 0.05$.

mato, and wheat and their germination and fresh weights were not different from the control treatments (Table 1). However, both phosphonate treatments reduced the germination and fresh mass of radish and bok choy seed. Phosphonate toxicity to radish and bok choy seed also was observed in microplot and field tests (*data not shown*). Corn, soybean, and wheat plants also were measured for height and there were no differences between control plants and plants produced from phosphonate-soaked seed (*data not shown*). Phosphonate-treated and untreated corn and

wheat seed also were planted into sandy-loam soil and their germination and plant fresh weights and heights were similar (*data not shown*).

Effect of phosphonate seed treatment on *Pythium* damping-off of cucumber seedlings in muck soil from commercial fields in the microplots. The data from both Barn and Creek sites were identical and, thus, were combined for analysis. On average, phosphonate post-planting drench and seed treatment resulted in a decrease of diseased plants by 84 and 60% compared with the control plots (Fig. 4). The decrease in diseased plants was accompanied by an increase in the fresh weights of cucumber plants. Plants from phosphonate post-planting drench and phosphonate seed treatments were 13 and 32 g heavier, respectively, than the plants harvested from the control plots (Fig. 4). Seed-soak treatment was significantly better than the post-planting drench treatment in reducing the percentage of diseased plants and increasing the fresh weights (Fig. 4).

Effect of phosphonate seed treatment on *Pythium* damping-off of cucumber seedlings in the field. Stand counts from both 2 and 6 weeks after planting were similar and only data collected after 6 weeks was presented. Stand counts at both locations were similar and phosphonate post-planting drench and seed treatment significantly improved the average stand counts compared with the control. On average at both locations, the drench application of phosphonate and seed treatment produced 55 and 63% plants, respectively, whereas the control produced 18% plants (Fig. 5).

DISCUSSION

Phosphonates, used primarily against Phytophthora diseases, have been found to be most effective as prophylactic agents before infection (28), although their curative effects postinfection also have been reported (4,44). We recently demonstrated that the AG3 phosphonate formulation effectively controls *Pythium* damping-off of cucumber when applied as either a pre-planting soil application or post-planting

drench (2). However, in a naturally infested muck soil, phosphonate pre-planting soil application was superior to the post-planting drench treatment. The efficacy of AG3 phosphonate as a seed treatment against *Pythium* damping-off of cucumber was investigated in this study. Seed of cucumber, when soaked in AG3 phosphonate for 10 min, were protected from *P. aphanidermatum* damping-off when planted into artificially infested peat-based mix or sandy-loam soil, and from *P. irregularare*, *P. ultimum*, and other *Pythium* spp. when planted into naturally infested muck soil and incubated under controlled environmental conditions. Treated seed stored for 5 weeks or 18 months prior to planting were equally protected compared with those planted immediately after treatment. AG3 seed treatment also was effective in protecting cucumber seedlings in muck soils naturally infested with *Pythium* spp. under field conditions. The efficacy of phosphonate seed-soak was comparable to or better than that of phosphonate post-planting drench. Cucumber plants produced from phosphonate-treated seed looked normal and healthy. The increase in fresh weights of cucumber plants was due to disease control.

Although application of phosphonates through seed treatment is easy and cost effective, phytotoxicity to seed of individual plant species can occur. We found that bok choy and radish but not tomato and sugar beet were more sensitive to phosphonate seed-soak. Reduced germination from phosphonate-treated radish and bok choy seed also was observed in our microplot and field tests (*data not shown*). This may be due to seed size or, possibly, it may be that crucifers are sensitive to phosphonates. None of the large-sized seed, such as corn, cucumber, soybean, and wheat, showed any germination or growth reductions. Phosphonates generally are considered to have low phytotoxicity to mature plants (20); however, phosphorus deficiency has been documented under phosphate-limiting conditions (6,15), which could be corrected by phosphate supplementation. Also, foliar phytotoxicity

Fig. 3. Effect of phosphonate (AG3) seed treatment on damping-off of cucumber seedlings in **A**, a sandy-loam soil artificially infested with soil inoculum of *Pythium aphanidermatum* and **B**, muck soil naturally infested with *Pythium* spp. AG3-treated and untreated cucumber 'Marketmore 76' seed were planted in infested soils (for sandy-loam soil, 8 seed per pot and five circular pots per treatment; for muck soil, 16 seed per rectangular pot and three pots per treatment). AG3 (0.07% a.i. vol/wt soil) drench was applied immediately after planting. Pots were incubated in a growth room and plants were rated 2 weeks later for damping-off. Error bars are standard error of mean from two experiments ($n = 10$) for sandy-loam soil and three experiments ($n = 9$) for muck soil. Values followed by the same letter do not differ significantly according to Fisher's protected least significant difference test at $P \leq 0.05$.

Table 1. Effect of phosphonate (AG3) seed soak on the percent germination and fresh weights of various plant species^w

AG3 ^x	Bok choy	Corn	Cucumber	Radish	Soybean	Sugar beet	Tomato	Wheat
Germination (%) ^y								
0.0% a.i.	91 ± 2 a	98 ± 1 a	83 ± 6 a	100 a	97 ± 1 a	93 ± 2 a	94 ± 2 a	89 ± 3 a
5.225% a.i.	66 ± 6 b	99 ± 1 a	88 ± 5 a	54 ± 7 b	96 ± 3 a	92 ± 2 a	97 ± 2 a	93 ± 3 a
10.45% a.i.	66 ± 5 b	97 ± 2 a	90 ± 4 a	32 ± 5 c	95 ± 2 a	90 ± 2 a	96 ± 2 a	88 ± 4 a
Fresh weights (g) ^z								
0.0% a.i.	3 ± 0.2 a	29 ± 1 a	7 ± 0.8 a	6 ± 0.5 a	24 ± 1 a	2 ± 0.2 a	2 ± 0.1 a	5 ± 0.5 a
5.225% a.i.	2 ± 0.2 b	27 ± 2 a	7 ± 0.7 a	2 ± 0.3 b	24 ± 2 a	2 ± 0.3 a	2 ± 0.2 a	5 ± 0.8 a
10.45% a.i.	2 ± 0.2 b	28 ± 2 a	6 ± 0.6 a	1 ± 0.2 b	24 ± 1 a	2 ± 0.2 a	2 ± 0.1 a	5 ± 0.7 a

^w Means ± standard error are the average of two experiments ($n = 8$). Values, if followed by the same letter within each column, for germination and fresh weights do not differ significantly according to Fisher's protected least significant difference test at $P \leq 0.05$.

^x Seed were immersed in AG3 solutions for 10 min. Treated seed were air dried and planted immediately in a peat-based mix in 72-cell plug trays (12 seed per replicate).

^y Seedling stand counts were determined 17 days after planting.

^z Plants from each replicate tray were removed and weighed together.

has been reported in horticultural, ornamental, and native plant species in Australia (21,34).

Seed application of phosphonates has numerous benefits as a disease control strategy. It is inexpensive because the amounts of chemical required are much

Fig. 4. A, Disease incidence of *Pythium* damping-off and B, fresh weight of cucumber plants from phosphonate (AG3)-treated seed, from soil drenched with phosphonate, and from untreated control seed and soil in a naturally infested muck soil collected from two field sites near Holland Marsh, ON and placed into microplots located at London Research Farm. AG3-treated and untreated cucumber 'Straight Eight' seed were planted in drainage tiles filled with infested muck soil from two field sites (15 seed per tile). AG3 (0.07% a.i. vol/wt soil) drench was applied immediately after planting. Plants were rated 3 weeks after planting. Error bars are standard error of mean from two sites ($n = 10$). Values followed by the same letter do not differ significantly according to Tukey's test at $P \leq 0.05$.

Fig. 5. Stands of cucumber plants derived from phosphonate (AG3)-treated seed from soil drenched with AG3 or left untreated in naturally infested muck soil at two field sites near Holland Marsh, ON. AG3-treated and untreated cucumber 'Straight Eight' seed were planted on twin rows of each of four replicate plots at both sites with a planter. AG3 (0.07% a.i.) drench solutions were applied immediately after planting. Stand counts were determined from middle 1.5-m (Barn site) and 4.5-m (Creek site) sections 6 weeks after planting. Error bars are standard error of mean from four replicates at both sites ($n = 4$). Values followed by the same letter do not differ significantly according to Tukey's test at $P \leq 0.05$.

smaller than that needed for application via drench applications. Because the protectant is localized near the germinating seed, it can provide a protective shield around the plant when it is most susceptible to damping-off. The degree of protection, however, needs to be evaluated further because a considerable variation in sensitivity to phosphonates has been observed with different isolates of *Phytophthora* spp. (8,18). Seed treatment also reduces the risk of developing resistance in the pathogen against phosphonates because the amounts of chemical used are too small to exert selective pressure on the pathogen or only a small fragment of the pathogen population is exposed to the active ingredient. Large applications of pesticide to soil and foliage had resulted in loss of efficacy to Fosetyl-Al within some isolates of the lettuce downy mildew pathogen, *Bremia lactucae* Regel (5). Seed treatment also will preclude enhanced degradation of phosphonates by soil microorganisms because there will be less exposure to the chemical.

How seed application of phosphonates protects cucumber plants against *Pythium* damping-off is not understood. Preliminary studies that tested treated seed pre-germinated in vermiculite and challenged with *P. aphanidermatum* on agar plates indicated that the seed released toxic compounds that inhibited the pathogen. The germinating phosphonate-treated seed were not affected by *P. aphanidermatum* on these plates (G. Lazarovits and P. Abbasi, unpublished results). However, induction of plant defence responses have not been ruled out (16,19,20). When phosphonate-treated cucumber seed were stored for 5 weeks or 18 months at 22 to 24°C before planting into infested substrate or soil, they were still effective in providing protection against *Pythium* damping-off. Whether the effect of phosphonate against the pathogen is direct or indirect, disease control against *Pythium* damping-off was evident.

Despite the many years during which phosphonates have been used, we were unable to locate other studies that have used these chemicals as a seed treatment to control *Pythium* damping-off diseases. The treated cucumber plants in this study were not affected negatively by the chemical but some plant seed, such as radish, did show phytotoxicity effects. Thus, tests are required for such negative effects before large volumes of seed are treated. Further studies are underway to investigate the combined effects of various treatments, such as phosphonate seed soak and biological control agents or phosphonate seed soak and organic amendments, to maximize protection against *Pythium* damping-off as well as against other diseases.

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