Control of Disease Caused by *Pythium ultimum* in Seed-Propagated Geraniums Sprayed or Not Sprayed with Silver Thiosulfate

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ABSTRACT

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Drenches of fenaminosulf, ethazol, and metalaxyl were evaluated for their effectiveness in preventing death of plants, plant stunting, and delay of flowering caused by *Pythium ultimum* in seed-propagated geraniums (*Pelargonium* × *hortorum*) sprayed or not sprayed with silver thiosulfate (STS). Silver thiosulfate, a petal abscission preventative, increases the incidence of plant death caused by *P. ultimum*. Fungicides were applied according to the following drenching schedules to plants grown in *P. ultimum*-infested or noninfested soilless root medium: 1) at seeding, 2) at seeding and transplanting, 3) at seeding, transplanting, and 1 wk after transplanting, 4) at transplanting, 5) 1 wk before STS application, and 6) on the day of STS application. Three metalaxyl drenching schedules (2, 3, and 4) prevented death of plants sprayed or not sprayed with STS and reduced plant stunting. Schedules 2 and 4 also reduced delay of flowering. Plant death was not prevented by any ethazol drenching schedule compared to the control when STS was not applied. Plant death after STS application was reduced, but not eliminated, by all ethazol drenching schedules except schedule 2. Plant stunting was reduced with ethazol drenching schedules 2, 3, and 4 and delay of flowering was decreased with drenching schedules 2 and 3. Fenaminosulf drenching schedules 3 and 4 prevented plant death when STS was not applied. Following STS application, plant death was reduced by all fenaminosulf drenching schedules, with the exception of schedule 1. Fenaminosulf did not reduce delay of plant flowering or consistently reduce plant stunting.

Geranium (*Pelargonium* × *hortorum* L. H. Bailey) petal abscission often occurs during transport of seed-propagated diploid (2N = 18) plants resulting in serious marketing problems (1). Although foliar application of a silver thiosulfate (STS) solution prevents premature petal abscission (5,20), plant death caused by *Pythium ultimum* Trow increased in plants sprayed with STS (8).

Pythium spp. are important and often devastating pathogens of seed-propagated geraniums. Damping-off of geranium seedlings caused by *P. ultimum* is generally not a problem when seeds are germinated under proper temperatures (21). Pythium root rot and lower stem rot of young and mature plants can be a common occurrence causing considerable plant loss. Less severe root rot may result in plant stunting and delay of flowering (8).

Current control practices for Pythium damping-off, root rot, and lower stem rot of seed-propagated geraniums include sanitation and application of fungicides. *Pythium* spp. have been reduced or eliminated by heat or fumigant soil treatments. Reintroduction of the pathogen into greenhouses may occur through water supply sources or irrigation ponds (4,6). Also, *Pythium*-infested soil particles from greenhouse walkways, floors, and beds serve as inoculum that may be unknowingly transported throughout production areas by greenhouse personnel (24,25). A number of fungicides are recommended for control of diseases caused by *Pythium* spp. on seed-propagated geraniums (21,23).

The objectives of this study were to determine 1) the effectiveness of three fungicides when applied in six drenching schedules in preventing plant death caused by *P. ultimum* in seed-propagated geraniums sprayed or not sprayed with STS, and 2) the effectiveness of these fungicides in preventing plant stunting and delay of flowering due to Pythium root rot.

MATERIALS AND METHODS

The fungicides fenaminosulf, 0.43 g a.i./liter (Lesan 35% WP, Mobay Chemical Corp., Kansas City, MO); ethazol, 0.11 g a.i./liter (Truban 30% WP, Mallinkrodt, Inc., St. Louis, MO); and metalaxyl, 0.101 g a.i./liter (Subdue 2E, 25% emulsifiable concentrate, Ciba-Geigy, Agriculture Division, Greensboro, NC) were used in this study.

Fungicide drenches were applied to plants grown in *P. ultimum*-infested or noninfested medium in the following schedules: 1) at seeding, 2) at seeding and transplanting, 3) at seeding, transplanting, and 1 wk after transplanting, 4) at transplanting, 5) 1 wk before STS application, and 6) on the day of STS application. Treatments hereafter will be referred to as drenching schedule 1, 2, etc. Three experiments were conducted using 16 geranium seedlings in each drenching schedule. The dates of geranium seeding, transplanting into infested or noninfested medium, and STS applications are shown in Figure 1.

Geranium seeds (cv. Ringo Scarlet) obtained from Sluis and Groot B.V., Enkhuizen, The Netherlands, were individually sown in round 2.0-cm-diameter cells containing soilless root medium (Sunshine Media Mix, Blend 1,

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Fisons Western Corp., Vancouver, B. C., Canada) containing 2:2:1 (v/v) vermiculite, sphagnum peat, and perlite. The seeds were then covered with approximately 0.5 cm of fine vermiculite and placed under intermittent mist at 24 ± 2 C in a glass greenhouse. Seedlings were removed from the mist after germination (7 days) and grown at 24 C day and 21 C night under natural light until transplanting.

Greenhouse temperature setpoints following transplanting were 22 C day and 20 C night. During the experiment, medium pH varied between 5.5 and 6.5. Plants were fertilized at each watering with 200 mg/L each of N and K. To control plant height, foliar applications of 750 ppm of Chlormequat ([2-chloroethyl]trimethylammonium chloride) were applied to all treatments, including the control.

Pythium ultimum inoculum was prepared using a potato medium procedure developed for culture of *Rhizoctonia solani* Kühn (13). Fifty grams of finely chopped potatoes were added to 500 ml of the soilless root medium. After mixing, the medium was autoclaved for 1 hr on each of 2 consecutive days. A pathogenic isolate of *P. ultimum* used in previous studies (24) was grown on 20 ml of water agar in 10-cm-diameter petri plates for 2 days at 24 C. Six 12-mm-diameter mycelial disks taken from the perimeter of colonies were used to infest 1.5 L of sterilized potato-medium mixture in 2-L flasks plugged with cotton. After 2 wk of growth, the inoculum was air-dried for 1–2 days and sieved through a No. 10 (2 mm) screen.

Preliminary studies indicated that 3 g of inoculum mixed with 1.0 L of the soilless root medium caused high levels of root rot and lower stem rot in geraniums transplanted into this ratio of inoculum and medium. Therefore, the ratio was used throughout this study (8). After thorough mixing, the medium was placed into single cells (8 × 8 × 6 cm) of 18 pack flats (25 × 53 cm) for the first experiment and into plastic pots (10 cm in diameter) for the second and third experiments. Entire soilless root medium plugs containing individual 30-, 21-, or 37-day-old seedlings were transplanted into the infested or noninfested medium for experiments 1, 2, and 3, respectively, with one plant per cell or pot.

Infested and noninfested treatments

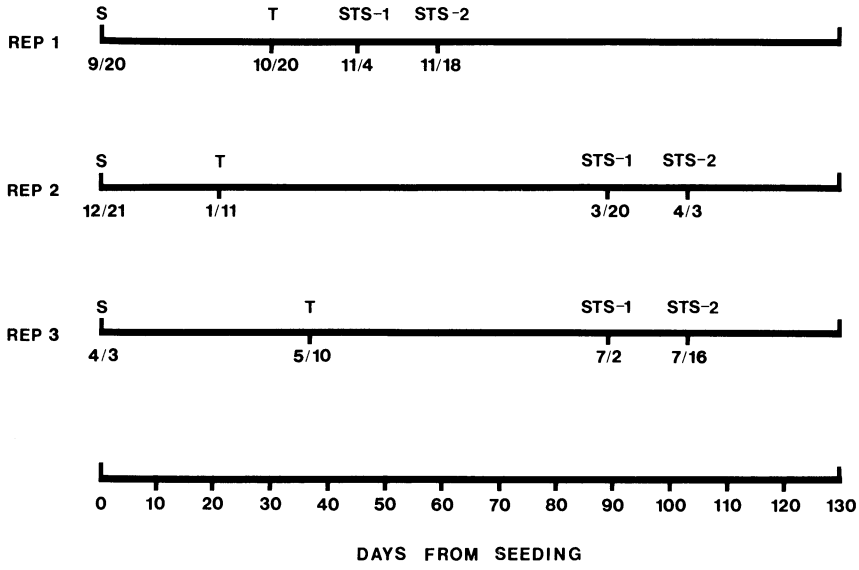

Fig. 1. Relationship between days from seeding and the actual date geranium plants were seeded (S), transplanted (T), and sprayed with STS for the first time (STS-1) and second time (STS-2) for experiments 1, 2, and 3.

Table 1. Percent of plant death of fungicide-treated geraniums (cv. Ringo Scarlet) when grown in *Pythium ultimum*-infested soilless root medium, and sprayed or not sprayed with silver thiosulfate (STS)

	Percent plant death[b]											
	Control			Fenaminosulf			Metalaxyl			Ethazol		
Fungicide drenching schedule[a]	1[c]	2	3	1	2	3	1	2	3	1	2	3
					−STS							
None	0	12	38	...	...	...	...	...	...	...	...	...
1. At seeding	...	...	...	0	0	12 NS[d]	0	0	12 NS	0	12	88 NS
2. At seeding, transplanting	...	...	...	0	12	0 NS	0	0	0 *	0	0	62 NS
3. At seeding, transplanting, and 1 wk before STS application	...	...	...	0	0	0 *	0	0	0 *	0	0	25 NS
4. At transplanting	...	...	...	0	0	0 *	0	0	0 *	0	12	12 NS
5. 1 Wk before STS application	...	...	...	0	0	12 NS	12	0	38 NS	12	0	12 NS
6. Day of STS application	...	...	...	0	0	12 NS	12	0	0 NS	12	12	0 NS
					+STS							
None	88	62	100	...	...	...	...	...	...	...	...	...
1. At seeding	...	...	...	50	25	100 NS	25	38	100 (25) NS[e]	25	12	100 *
2. At seeding, transplanting	...	...	...	0	38	100 *	0	0	0 **	0	62	100 (12) NS
3. At seeding, transplanting, and 1 wk before STS application	...	...	...	0	0	100 **	0	0	0 **	0	0	75 **
4. At transplanting	...	...	...	12	25	88 **	0	0	0 **	0	38	100 (50) *
5. 1 Wk before STS application	...	...	...	12	0	88 **	0	0	17 (25) **	0	12	80 (38) **
6. Day of STS application	...	...	...	0	0	88 **	0	0	12 **	0	0	86 (86) **

[a] Fungicide drenches at seeding, transplanting, 1 wk before STS treatment, and day of STS treatment correspond to 0, 30, 38, and 45 days after seeding, respectively, for experiment 1; 0, 21, 82, and 89 days after seeding for experiment 2; and 0, 37, 82, and 89 days after seeding for experiment 3.

[b] Data represent percent of plant death that occurred over a 32-day period after STS application.

[c] Experiments 1, 2, and 3 were seeded on 20 September, 21 December, and 3 April, respectively.

[d] NS = not significant, or significant at the 5% (*) or 1% (**) level, based on a chi-square comparison between each fungicide treatment and the control. Data from the three experiments were combined for the test. The −STS treatments were compared with the −STS control, and the +STS treatments were compared with the +STS control.

[e] Percent of plant death caused by *P. ultimum* that occurred before STS application.

were randomized on greenhouse benches constructed of 14.0-cm-wide wooden planks spaced 4.0 cm apart. Contamination of noninfested containers was minimized by placing infested and noninfested treatments on alternating wooden planks.

A 0.25-mM STS solution (9) was sprayed on plants to runoff on half of all treatments at 15, 69, or 54 days following transplanting into the infested or noninfested medium for experiments 1, 2, and 3, respectively. Previous studies showed that a time interval of 14 days between transplanting into infested medium and STS treatment was the minimum time necessary for significant plant death due to the STS/root rot and lower stem rot interaction (8). This minimum period of 14 days was used in experiment 1. However, experiments 2 and 3 followed the commercial practice of STS application at bud color. A second STS spray was applied 14 days after the first STS spray had been applied.

After transplanting into infested or noninfested medium, plants were observed daily for symptoms of Pythium root and lower stem rot and dead plants were counted. Percent of plant death presented in Table 1 is based on that occurring 32 days after the first STS application. Plant height and width were recorded 68, 114, and 68 days after transplanting for experiments 1, 2, and 3, respectively. Plant volume (size) was calculated from plant height and canopy width measurements by assuming volume occupied by the plant was a cylinder. Only data from experiments 1 and 3 are presented because results from experiment 3 were similar to those of experiment 2. Number of days from seeding to first floret opening also was recorded for experiments 1 and 3. Only data from experiment 1 are shown, as similar trends in time to flowering were noted in experiment 3. Plant size and flowering data are shown for plants not sprayed with STS to prevent confounding due to possible growth regulatory effects of STS. All treatments were set up in a completely randomized experimental design. Chi-square tests (22) were run to establish significance between treatments on the plant death data and an HSD mean separation (22) was used to establish significance on the plant size and flowering data.

Geraniums that died during the study were sampled randomly (minimum of one plant per treatment) to detect colonization by *P. ultimum*. At the termination of the experiment, surviving geraniums in infested and noninfested media also were sampled randomly. Three 2-cm segments of root, stem, and petiole tissues were surface-disinfested in 10% sodium hypochlorite for approximately 20 sec and then plated on water agar. Hyphal growth from plant tissue was identified according to Middleton's key (17).

RESULTS

Disease severity in controls. Up to 38% of plants grown in infested media without fungicide treatment or STS application died. In contrast, 62–100% of the plants grown in infested media without fungicide treatment died after STS was applied (Table 1). Irrespective of STS application, no plants grown in noninfested media died (data not shown).

Plants grown in infested media and not treated with a fungicide were stunted and flowering was delayed. Sixty-eight days after transplanting, surviving plants in these treatments were 53% (experiment 1) and 82% (experiment 3) smaller than plants grown in noninfested media (Table 2). Surviving plants grown in infested media also flowered 17.5 days later than plants grown in noninfested media (Table 3).

Disease severity in fenaminosulf-treated plants. Fenaminosulf drenching schedules 3 and 4 completely prevented death among plants grown in infested media and not sprayed with STS (Table 1). The high percentage of plant death following STS application observed among control plants was reduced by all fenaminosulf drenching schedules, except schedule 1.

Plant stunting symptoms associated with root rot disease were not reduced consistently by the drenching schedules tested. In all experiments, fenaminosulf drenching schedules 1, 3, and 6 failed to reduce plant stunting (Table 2). Delay of flowering was not prevented by any fenaminosulf drenching schedule and was significantly increased by schedules 3 and 5 (Table 3).

Disease severity in metalaxyl-treated plants. Metalaxyl drenching schedules 2, 3, and 4 completely prevented death of plants grown in infested media and sprayed or not sprayed with STS (Table 1). Metalaxyl drenching schedules 5 and 6 significantly reduced the increase in plant death observed following STS application to control plants. Irrespective of STS application, metalaxyl drenching schedule 1 was ineffective in preventing or reducing plant death.

In addition to completely preventing plant death, metalaxyl drenching schedules 2, 3, and 4 reduced plant stunting symptoms (Table 2). Drenching schedules 2 and 4 also reduced the delay in flowering observed in plants grown in infested media and not treated with fungicides (Table 3). In all experiments, metalaxyl drenching schedule 1 was ineffective in reducing plant stunting or decreasing delay in flowering. Drenching schedules 5 and 6 were not consistently effective in reducing plant stunting among experiments (Table 2) and did not significantly reduce delay in flowering

Table 2. Relative plant size of fungicide-treated geraniums (cv. Ringo Scarlet) 68 days following transplanting into *Pythium ultimum*-infested soilless root medium

Fungicide drenching schedules[a]	Plant volume relative to *P. ultimum*-infested control (%)[b]					
	Experiment 1			Experiment 3		
	Fenaminosulf	Metalaxyl	Ethazol	Fenaminosulf	Metalaxyl	Ethazol
1. At seeding	162 NS[c]	185 NS	178 NS	197 NS	103 NS	61 NS
2. At seeding and transplanting	174 NS	257 *	395 *	244 *	298 *	209 *
3. At seeding, transplanting, 1 wk before STS application	119 NS	228 *	339 NS	181 NS	489 *	266 *
4. At transplanting	161 NS	287 *	324 *	217 *	414 *	245 *
5. 1 Wk before STS application	131 NS	140 NS	141 NS	245 *	210 *	259 *
6. Day of STS application	96 NS	340 *	216 *	116 NS	···	199 NS
	Control (infested medium) 100[d]			Control (infested medium) 100[d]		
	HSD (5%) = 104[e]			HSD (5%) = 106[e]		

[a] Fungicide drenches at seeding, transplanting, 1 wk before STS treatment, and the day of STS treatment correspond to 0, 30, 37, or 44 days after seeding, respectively, for experiment 1, and 0, 37, 86, or 93 days after seeding for experiment 3.

[b] Data is shown for plants not treated with STS to prevent confounding due to possible growth regulatory effects of STS.

[c] NS = not significant, or significant at the 5% (*) level based on an HSD comparison between each fungicide treatment and the control.

[d] Control plants grown in *P. ultimum*-infested medium and not treated with fungicides had plant volumes of 285 cm^3 (100%) or 487 cm^3 (100%) for experiments 1 and 3, respectively. Control plants grown in noninfested medium and not treated with fungicides had plant volumes of 625 cm^3 (221%) or 2,902 cm^3 (593%) for experiments 1 and 3, respectively.

[e] Minimum value for significant difference ($P = 0.05$) between plants treated or not treated with fungicide.

(Table 3).

Disease severity in ethazol-treated plants. Ethazol drenching schedules did not significantly reduce death of plants grown in infested medium without STS application, compared with the control. Following STS application, all ethazol drenching schedules, with the exception of schedule 2, reduced plant death compared with the control (Table 1). Plant stunting was consistently reduced by ethazol drenching schedules 2, 3, and 4 (Table 2). Ethazol drenching schedule 1 failed in all experiments to reduce plant stunting in comparison with the control. Drenching schedules 5 and 6 were not consistently effective in reducing plant stunting among experiments. Delay of flowering was prevented only by drenching schedules 2 and 3 (Table 3).

Reisolation. *Pythium ultimum* was reisolated from plants grown in *P. ultimum*-infested medium in all cases. *Pythium ultimum* was not isolated from plants grown in the noninfested medium.

DISCUSSION

The fungicides evaluated in this study effectively control diseases caused by *Pythium* spp. in a variety of greenhouse crops (3,18,19,26,27). Only those fungicide drenching schedules that totally prevent death of seed-propagated geraniums sprayed or not sprayed with STS, reduce plant stunting, and decrease delay in flowering caused by *Pythium ultimum* realistically can be incorporated into a disease management program. Only the metalaxyl drenching schedules with treatments at both seeding and transplanting, and a treatment at transplanting, met this criteria. However, exclusion from grower recommendations of a metalaxyl drenching schedule with treatments at seeding, transplanting, and 1 wk after transplanting solely because time to flowering was not significantly reduced from that of plants grown in infested medium without fungicide treatment may not be warranted. Time to flowering for this treatment was not significantly different from plants grown in noninfested medium without fungicide treatment.

Fenaminosulf and ethazol were less effective in controlling the symptoms of disease caused by *P. ultimum*. However, fenaminosulf and ethazol are not to be wholly discounted because a rotation of fungicides is recommended to avoid selection and build-up of fungicide-resistant pathogen populations (12).

There were differences in plant death among the three experiments. Typical methods of handling and seasons of geranium production were encompassed by this study. Use of plug systems and improved growing techniques currently allow production of several geranium crops staggered within a single year (11,14). Crops grown in different seasons will experience varying environmental conditions, as did the three experiments of the study. It is our opinion that environmental conditions may have been a factor in the incidence of Pythium root rot and lower stem rot, and subsequently influenced fungicide efficacy.

Environmental information collected with a computerized greenhouse environmental control system showed that during experiment 1 natural irradiance levels decreased rapidly and Michigan experienced extended periods of cloudy weather. Greenhouse temperatures were easily controlled during the day and typically fluctuated less than 3 C above the 22 C set point within and among days. In contrast, plants in experiments 2 and 3 were exposed to rapidly rising natural irradiance levels and rising outside temperatures. Plants were exposed to more widely fluctuating temperatures (up to 15 C) within and among days. Greater water stress fluctuations probably accompanied the higher temperatures experienced during experiments 2 and 3.

Historically, *P. ultimum* has been considered a cool season pathogen (2,10,15), although studies with red clover suggest that high temperatures can also be favorable (7). It is our opinion, however, that the change in environmental conditions created plant stress and increased host susceptibility enough to favor the pathogen. There are many examples of root rot disease resulting in plant death after some change in cultural practice (16).

Another factor that may have influenced the incidence and severity of Pythium root rot and lower stem rot in these three experiments was the timing of STS sprays. Silver thiosulfate was applied later in experiments 2 and 3 than in experiment 1, possibly allowing more time for *P. ultimum* to infect and colonize the plants. Further, fenaminosulf and ethazol applied at transplanting may have leached from the medium and metalaxyl may have diluted in plant tissue allowing adequate time for pathogen reestablishment in the 52–68 days before STS application. Realistically, a plant may become infected with *P. ultimum* at virtually any production stage. Therefore, those fungicides and drenching schedules shown to completely control damping-off, root rot, and root or lower stem rot constitute, in many situations, reliable grower recommendations.

Table 3. Difference in time to flower of fungicide-treated geraniums (cv. Ringo Scarlet) grown in *Pythium ultimum*-infested soilless root medium compared with plants not treated with fungicide (experiment 1)

Fungicide drenching schedules[a]	Difference in days to flower[b]		
	Fenaminosulf	Metalaxyl	Ethazol
1. At seeding	+11.0[c]	0.0	−2.1
2. At seeding and transplanting	+3.5	−20.8	−17.1
3. At seeding, transplanting, and 1 wk before STS application	+13.1	−7.0	−13.8
4. At transplanting	−1.1	−15.3	−10.9
5. 1 Wk before STS application	+13.7	−1.0	+9.0
6. Day of STS application	−2.7	−10.0	−2.0
Control (noninfested medium) HSD (5%) = 11.5[d]	−17.5	...	...

[a] Fungicide drenches at seeding, transplanting, 1 wk before STS treatment, and day of STS application correspond to 0, 30, 37, or 44 days after seeding, respectively.

[b] Comparison plants were grown in *P. ultimum*-infested medium without fungicides. Plants flowered an average of 140.3 days from seeding, whereas plants grown in noninfested medium flowered in an average of 122.8 days. Data is shown for plants not treated with STS to prevent confounding due to possible growth regulatory effects of STS.

[c] Days to flower of fungicide-treated plants after (+) or before (−) plants grown in infested medium without fungicide treatment.

[d] Minimum value for significant difference ($P = 0.05$) between plants treated or not treated with fungicide.

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