Relationship Between Silver Thiosulfate and Premature Plant Death of Seed-Propagated Geraniums Caused by *Pythium ultimum*

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ABSTRACT

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Topically sprayed silver thiosulfate (STS), a petal abscission preventative, increased premature plant death of seed-propagated geraniums (*Pelargonium* ×*hortorum* 'Ringo Scarlet') grown in a soilless root medium infested with low, medium, and high levels of *Pythium ultimum*. Premature death occurred as quickly as 4 days after STS application. The area under the disease progress curve (AUDPC) was used to express the accumulated premature plant death within 30 days after STS application. According to the AUDPC data, premature plant death resulted when STS was applied to plants grown in the highly infested medium for a minimum of 7 days or in the medium infested with a low or medium level of inoculum for 14 days. A trend toward higher AUDPC values was observed, the longer plants were grown in the highly infested medium (up to 14 days) prior to STS application. Reapplication of STS to surviving plants 30 days after the first application caused a new surge of premature plant death 10–15 days later.

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Diploid ($2N = 18$) geraniums (*Pelargonium* ×*hortorum* L. H. Bailey) are used exclusively for seed-propagated cultivars (8) and are increasing in popularity because of low production costs and the introduction of cultivars with increased diversity, improved horticultural characteristics, and more satisfactory garden performance (3). However, petal shatter has been a problem in transporting and marketing the seed-propagated geranium (1). The degree of shattering varies among cultivars (2). Although double or semi-double flowers lacking nectaries do not shatter easily (21), these "low-shatter" cultivars may not have the most desirable horticultural or aesthetic characteristics.

Foliar application of silver thiosulfate (STS) solution immediately prior to flowering prevents petal abscission and is commercially important in the production of seed-propagated geraniums (6,16). The silver ion is effective in blocking ethylene actions such as senescence and petal abscission (4,5) by inhibiting ethylene effects at a very early stage in the events leading to abscission (17). Precisely how silver inhibits ethylene action is not known, although there are many hypotheses (24).

Heins et al (13) observed symptoms of lower stem rot disease on geraniums treated with STS during STS formulation trials. Commercial growers using STS reported similar findings (Heins, *personal communication*). *Pythium ultimum* Trow was isolated from such plants (10,11). The objective of this study was to determine whether foliar application of STS to geraniums (cv. Ringo Scarlet) grown in a medium infested with *P. ultimum* results in premature plant death caused by lower stem rot.

MATERIALS AND METHODS

Geranium seeds (cv. Ringo Scarlet) obtained from Sluis and Groot B.V., Enkhuizen, The Netherlands, were sown singly in a soilless root medium (Sunshine Media Mix, Blend 1, Fisons-Western Corp., Vancouver, B.C., Canada) containing 2:2:1 (v/v) vermiculite, sphagnum peat, and perlite, in round cells 2 cm in diameter. The seeds were covered with approximately 0.5 cm of fine vermiculite and placed under intermittent mist in a glass greenhouse at 24 ± 2 C. Seedlings were removed from the mist after germination (7 days) and grown under natural light at 24 C during the day and 21 C at night until transplanting.

Inoculum of *P. ultimum* was prepared by a procedure developed for culturing *Rhizoctonia solani* Kühn (14). Finely chopped potatoes (50 g) were mixed with 500 ml of the soilless root medium. The mixture was autoclaved for 1 hr on each of two consecutive days. A pathogenic isolate of *P. ultimum* used in previous studies (19) was grown on 20 ml of water agar in petri plates 10 cm in diameter for 2 days at 24 C. Six mycelial disks (12 mm in diameter) taken from the perimeters of colonies were used to infest 1.5 L of the sterilized potato-medium mixture in 2-L flasks plugged with cotton. After 2 wk the inoculum was air-dried for 1–2 days and sieved through a number 10 (2-mm) screen.

The inoculum was thoroughly mixed with soilless root medium at three infestation levels: 0.75 g/L (low), 1.5 g/L (medium), and 3.0 g/L (high). The base infestation level of 0.75 g/L was determined from cucumber seedling bioassays conducted by Chen et al (7). The medium was then placed into single plastic cells (8 × 8 × 6 cm) of 18-cell flats (25 × 53 cm). Geranium seedlings 35 or 49 days old were transplanted into the infested and the uninfested medium. Entire plugs of soilless root medium containing individual seedlings were transplanted, with one plant per cell. Sixteen single cells were used per treatment, arranged in a completely randomized block design in a walk-in plant growth chamber. These experiments were repeated twice.

Temperature set points in the growth chamber following transplanting were 21 C during the day and 18 C at night. The irradiance was 135 μmol·sec^{-1}·m^{-2} for 12 hr per day, from VHO cool white fluorescent lamps. During the experiment, the pH of the medium varied between 5.5 and 6.5. The plants were fertilized at each watering with N and K, each at 200 mg/L. Foliar applications of chlormequat (2-chloroethyltrimethyl-

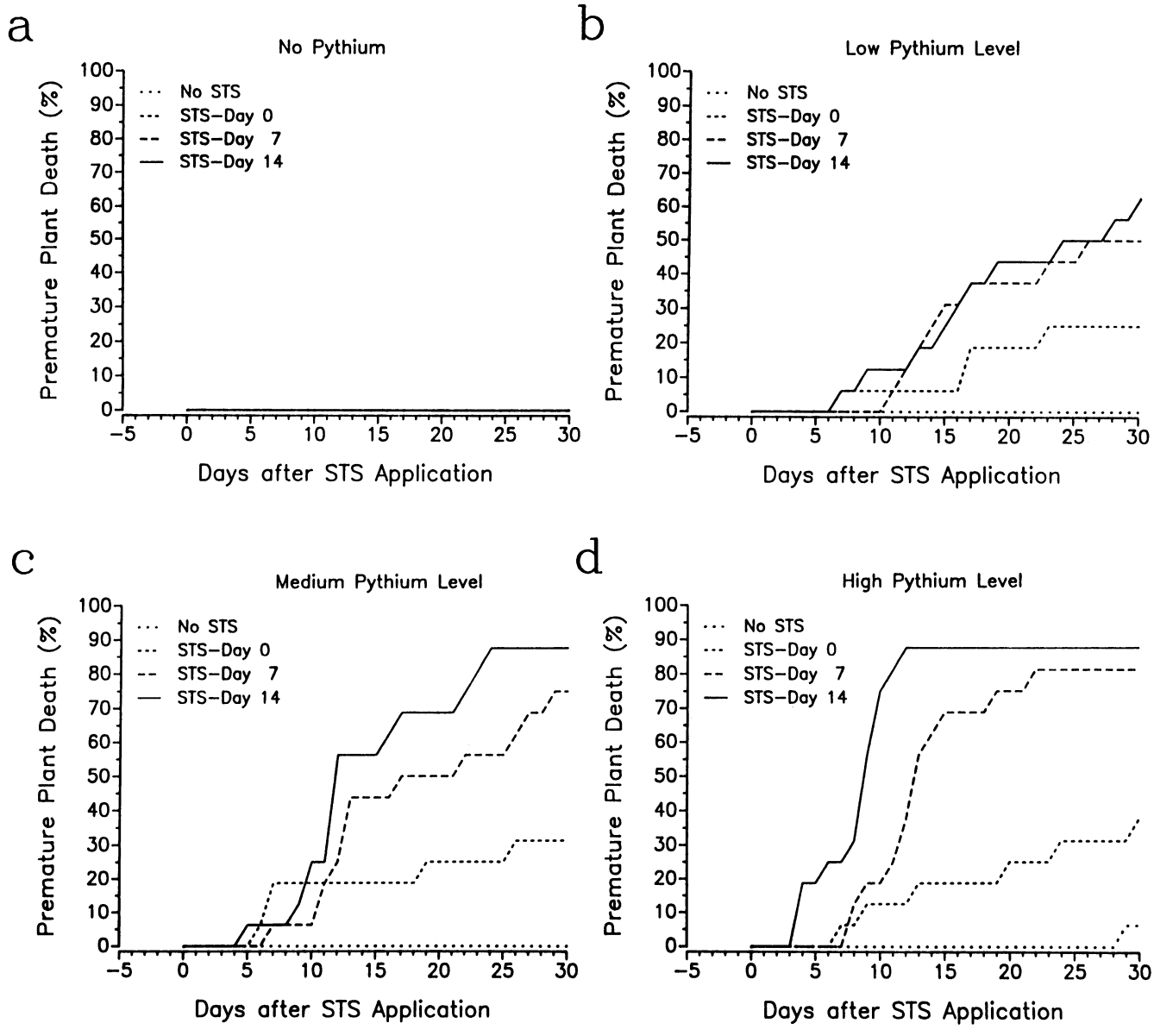

Fig. 1. Cumulative premature plant death over time, expressed as a percentage of geraniums (cv. Ringo Scarlet) grown in an uninfested medium (**A**) and a medium infested with *Pythium ultimum* at low (**B**), medium (**C**), and high (**D**) levels of inoculum (0.75, 1.5, and 3.0 g of inoculum per liter of medium). Plants were grown in the medium for 0, 7, or 14 days prior to an application of silver thiosulphate (STS).

ammonium chloride) at 750 ppm were used in all treatments, including the control, to regulate plant height.

A 0.25 mM STS solution (13) was sprayed to runoff on one-half of the plants in each treatment 0, 7, or 14 days following transplanting into the infested or the uninfested medium. A second STS application was made 30 days after each original STS treatment. Disease symptoms were noted and the percentage of dead plants determined daily following transplanting. Percentage premature plant death was then plotted against time from the STS application. As the results from the two experiments were similar, only the results from the experiment with seedlings transplanted at 35 days are reported.

The area under the disease progress curve (AUDPC) was calculated to express the cumulative incidence of plant death occurring during the 30 days after the first STS application, from the following formula:

$$\text{AUDPC} = \sum_{i=1}^{n-1} [(X_{i+1} + X_i)/2] * (t_{i+1} - t_i)$$

where X_i is cumulative disease incidence, expressed as a proportion of the ith observation; t_i is the time (in days after the STS application) at the ith observation; and n is the total number of times plant death was assessed (20). Variances between the two experiments were homogeneous, and therefore the data were pooled. The AUDPC data were analyzed by analysis of variance of the combined experiments, with a protocol of the Statistical Analysis System (18). There was a significant interaction between STS and *P. ultimum*, and so mean comparisons were made among STS treatments within each *P. ultimum* level with the Waller-Duncan Bayesian k-ratio t-test using a k ratio of 100, corresponding to $\alpha = 0.05$.

Geraniums that died during the study were sampled to detect colonization by *P. ultimum*. At the end of the experiment, surviving geraniums growing in the infested and the uninfested medium were sampled randomly (a minimum of three plants per treatment). Three 2-cm segments of root, stem, and petiole tissues were surface-disinfested in 0.5% sodium hypochlorite for approximately 20 sec, rinsed in sterile distilled water, and then plated on water agar. *Pythium* spp. isolated from the plant tissue were identified according to Middleton's key (15).

RESULTS

No premature plant death or visibly adverse effects occurred among geraniums grown in the uninfested medium following STS application (Fig. 1A). In contrast, STS application to plants growing in the infested medium, regardless of the inoculum level, resulted in premature plant death caused by Pythium lower stem rot (Fig. 1B–D). The percentage of dead plants 30 days after the first STS application increased from 0% among plants grown in the uninfested medium to a maximum of 90% among plants grown in the highly infested medium. Premature plant death occurred as quickly as 4 days after STS application among plants grown in the highly infested medium (Fig. 1D), with a surge in plant death occurring between 7 and 14 days after STS application (Fig. 1B–D).

The analysis of variance of the AUDPC data from the combined experiments showed a highly significant interaction between the infestation level of the soilless root medium and the length of time geraniums were grown in the infested medium prior to an STS application (Table 1). According to the AUDPC data, premature plant death resulted when STS was applied to plants grown in the highly infested medium for a minimum of 7 days or in the medium infested with the low or medium level of inoculum for 14 days. A trend toward higher AUDPC values was observed, the longer plants were grown in the highly infested medium (up to 14 days) prior to STS application (Table 2).

Reapplication of STS to surviving plants 30 days after the first STS application resulted in a new surge of premature plant death among plants growing in the infested medium (Fig. 2). This new surge occurred 10–15 days after the reapplication.

Regardless of STS treatment, *P. ultimum* was consistently isolated from surface-disinfested root and stem tissue and occasionally from petiole tissue of dead and surviving plants grown in the infested medium. Plants grown in the uninfested medium were free of any detectable *P. ultimum*.

DISCUSSION

STS application to geraniums grown in the medium infested with *P. ultimum* resulted in premature plant death. This relationship between STS application and premature plant death caused by *P. ultimum* has not been previously reported.

The increase in premature plant death observed when plants were grown in the infested medium for a longer time (up to 14 days) prior to STS application probably resulted from an increase in the pathogen population during this interval. Chen et al (7), using an initial inoculum level of 0.75 g/L, showed that the population density of *P. ultimum* in a Canadian peat soilless root medium increased from 640 to 990 colony-forming units per gram during the first 10 days following infestation of the medium. The population doubling time for *P. ultimum* in the Canadian soilless root medium planted with cucumber was 6 days (7).

Results of this study suggest that foliar sprays of STS increase the decline of plants already infected, because *P. ultimum* was reisolated from surviving geraniums grown in the infested medium but not treated with STS. Further, it is our opinion that the commercial crop losses caused by Pythium lower stem rot, which triggered this research, resulted from STS application to plants already infected with *P. ultimum* but not showing typical symptoms of Pythium lower stem rot. Previous studies have shown that

Table 1. Combined analysis of variance of the area under the disease progress curve expressing premature plant death of geraniums grown in an uninfested medium and in a medium infested with *Pythium ultimum* at low, medium, and high levels of inoculum (0.75, 1.5, and 3.0 g/L) for 0, 7, or 14 days prior to the application of silver thiosulfate (STS)

Source of variation	df	Mean square	PR > F
Experiment	1	234,706	0.1485
Block	3	81,997	0.5251
Inoculum level	3	1,760,016	0.0001
STS application	3	3,258,379	0.0001
Inoculum level × STS	9	472,624	0.0004
Error	44	108,526	

Table 2. Premature plant death expressed as the combined average area under the disease progress curve with mean separation among applications of silver thiosulfate (STS) within each level of infestation

Days in medium prior to STS application	Levels of infestation[z]			
	Control	Low	Medium	High
No STS	0.0 a	170.3 a	53.1 a	243.7 a
0	0.0 a	329.7 a	384.3 a	232.8 a
7	0.0 a	378.1 ab	509.4 a	728.1 b
14	0.0 a	1,038.8 b	1,629.7 b	1,815.6 c

[z]Means within a column followed by the same letter do not differ significantly according to the Waller-Duncan Bayesian k-ratio t-test using a k ratio of 100, corresponding to $\alpha = 0.05$.

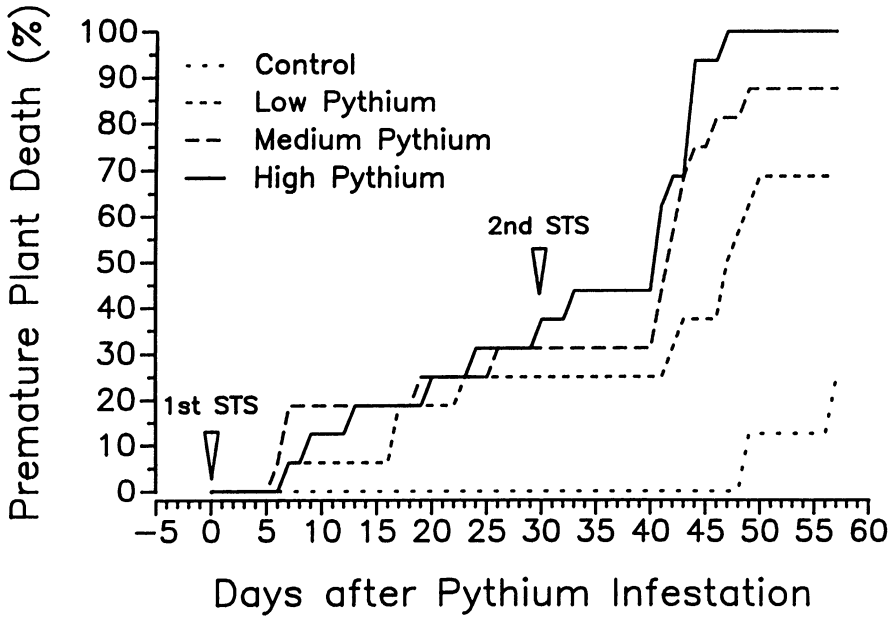

Fig. 2. Cumulative premature plant death over time, expressed as a percentage of geraniums (cv. Ringo Scarlet) grown in an uninfested medium (control) and a medium infested with *Pythium ultimum* at low, medium, and high levels inoculum (0.75, 1.5, and 3.0 g of inoculum per liter of medium) 0 and 30 days prior to applications of silver thiosulphate (STS).

geranium plants may be infected with *P. ultimum* without showing the black stem lesions commonly associated with Pythium lower stem rot (9). Instead, stunting and delay of flowering may be the only visual symptoms of disease in otherwise healthy-appearing geraniums (9). Mild root rot, causing only slight plant stunting, may go unnoticed unless uninfected plants are available for comparison or STS is applied. In this study, STS application to symptomless seed-propagated geraniums infected with even low levels of *P. ultimum* increased premature plant death caused by Pythium lower stem rot.

Fungicide drenches applied to seed-propagated geraniums that prevent or reduce the incidence of plant death, plant stunting, and delay of flowering caused by *P. ultimum* also prevent or reduce premature plant death caused by *P. ultimum* following an STS application (12). A fungicide drench applied the day of STS application to plants grown in the highly infested medium for approximately 50 days significantly reduced the premature plant death observed among plants not treated with a fungicidal drench (12).

The mechanism involved in premature plant death following STS application to plants infected with *P. ultimum* was not determined in this study. However, preliminary (unpublished) studies suggested that this mechanism is an STS-specific response rather than simply a toxicity response that could be duplicated with other heavy metal solutions. Preliminary (unpublished) studies also showed that STS applied to *P. ultimum* cultures does not affect mycelial growth, and pathogen virulence was not enhanced by STS (22). Whalen and Wulster (22) proposed that the effects of STS in premature plant death caused by *P. ultimum* result from an altered host metabolism mediated by silver. They further proposed that phenolic constituents play a role in the defense of geranium to attack by *Pythium* (23). Differences resulting from STS treatments were mainly found in the accumulation of phenolic esters and, to a lesser degree, of phenolic glycosides (23).

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