Influence of Time Intervals Among Wounding, Inoculation, and Incubation on Stem Blight of Geranium Caused by *Botrytis cinerea*

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ABSTRACT

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All stems of geranium (*Pelargonium* × *hortorum*) stock plants inoculated with *Botrytis cinerea* and incubated in a dew chamber under conditions conducive for disease development within 12 hr of excising cuttings (stem wounding) became blighted. Disease incidence maxima were 38 and 29% when plants were inoculated 1 and 3 days, respectively, after stem wounding and placed in an environment of low relative humidity (RH < 60%) before incubation in a dew chamber. The area under the incidence of stem blight disease progress curve (AUDPC) revealed a minimum of 1 day in an environment of low RH between stem wounding and subsequent inoculation and incubation in a dew chamber was necessary to significantly limit stem blight incidence. When averaged, at least 94% of the stems became blighted when plants were exposed to an environment of low RH for 1 day after inoculation, before incubation in a dew chamber. When plants were inoculated and subjected to an environment of low RH for 3, 5, and 7 days before incubation in a dew chamber, an average of 69, 85, and 50% of the stems, respectively, became blighted. According to the AUDPC data, when inoculated plants were placed in an environment of low RH for a minimum of 1 day before incubation in a dew chamber, stem blight incidence was significantly limited. The AUDPC data indicated that the longer inoculated plants were maintained in an environment of low RH before incubation in a dew chamber, the lower the stem blight incidence.

Observations of tetraploid ($2n=36$) and selected cultivars of diploid ($2n=18$) geranium (*Pelargonium* × *hortorum* L. H. Bailey) stock plants within a commercial greenhouse suggest that stem blight caused by *Botrytis cinerea* Pers.:Fr. is a limiting factor in the production of cuttings. Stem blight typically begins in the broken or cut off stem surface of the stock plant and progresses downward, causing a dieback of the entire stem, and in severe cases extends into the base of the plant, resulting in death (10).

Traditional methods of controlling stem blight include fungicide application and sanitation. However, a dense stock plant canopy shields infected stems and leaves with sporulating lesions from adequate fungicide coverage. Observations in a commercial greenhouse in Pennsylvania showed that atmospheric concentrations of conidia of *B. cinerea* continued to increase after applications of chlorothalonil and iprodione (4). Also, occurrence of fungicide resistance remains a constant threat (3,6,7,9,11).

Sanitation measures typically include the removal and destruction of diseased plant material. Dead leaves at bases of plants and organic matter in and under benches may support growth of *B. cinerea*. Melchers (8) isolated a *Botrytis* sp. (considered to be *B. cinerea*) from organic matter in and on the sand of cutting benches, moist soils, and elsewhere that may have served as sources of inoculum. However, maintaining the high standard of hygiene necessary to reduce inoculum sources is time-consuming and costly for commercial geranium growers. The benefit of such sanitation efforts has been questioned by Plaut and Berger (12), who concluded from studies of *B. cinerea* on begonia that sanitation measures may be less effective than previously theorized. Low initial disease was apparently compensated for by the rate of disease development.

Cultural methods of controlling diseases caused by *B. cinerea* have not been thoroughly investigated. Heursel and Kamoen (5) showed that lignified ("hard") cuttings of *Rhododendron* spp. can be better stored than "soft" cuttings without loss caused by *B. cinerea*. Similarly, Cline and Neely (1) showed that when wounded geranium cuttings were allowed to "heal" before inoculation with *Pythium ultimum* Trow, disease incidence and severity decreased.

The objective of this study was to determine if stem blight incidence and severity caused by *B. cinerea* are decreased when wounded geraniums are placed in an environment of low relative humidity (RH < 60%) for increasing periods of time before inoculation and incubation in a dew chamber under conditions favorable for disease development or when inoculated geraniums are placed in an environment of low RH for increasing periods of time before incubation in a dew chamber.

MATERIALS AND METHODS

***Botrytis* culture.** An isolate of *B. cinerea* was obtained from infected geranium stock plants growing in a commercial greenhouse in Pennsylvania. *B. cinerea* cultures were grown on 20 ml of potato-dextrose agar (PDA) in 10-cm-diameter petri plates for approximately 10–14 days at 20 C. Cultures were placed in a constant temperature walk-in room and exposed to approximately 9 hr of natural daylight and 15 hr of supplemental cool-white light during the night to induce sporulation.

Geranium culture. Rooted tetraploid geranium (cv. Crimson Fire) cuttings approximately 5 wk old were planted into 3.78-L plastic growing pots containing soilless rooting medium (Fafard potting mix, Conrad Fafard, Inc., Anderson, SC) composed of 2:2:1 (v/v) vermiculite, sphagnum peat, and perlite. One plant was transplanted per pot. Planting was done during December 1986 for the 1987 growing season. The pots were placed within a 1,672-m^2 commercial greenhouse of a propagator of geraniums in Pennsylvania. Plant density was approximately 15.1 plants per square meter.

Greenhouse air temperatures were monitored and controlled using a greenhouse climate-control computer (Oglevee Computer Systems, Connellsville, PA) programmed to provide a minimum of 16.7 C during the day and night. Ventilation occurred when temperatures exceeded 23.9 C. Stock plants were irrigated two to three times weekly, typically for a duration of 2 hr, using a plastic tube irrigation system with one tube per pot. Plants were fertilized twice weekly during these irrigations with 300 ppm of N and K_2O applied through the irrigation system two to three times for a duration

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of 1 min at 1-hr intervals. During the growing season, medium pH varied between 5.4 and 5.8.

Fungicides and insecticides were applied to the stock plants throughout the growing season and included methomyl, 2.2 g a.i./L (Lannate, E. I. du Pont de Nemours & Co., Wilmington, DE); iprodione, 0.598 g a.i./L (Chipco 26019, Rhone-Poulenc Agrochimie, Monmouth Junction, NJ); demeton-S-methyl sulfon, oxydemeton-methyl, 0.449 g a.i/L (Metasystox R2, Mobay Corp., Kansas City, MO); chlorothalonil, 0.937 g a.i./L (Daconil 2787 F, Fermenta Plant Protection Company, Mentor, OH); acephate, 10.78 g a.i./L (Orthene 75SP, Chevron Chemical Co., Richmond, CA); maneb, 1.92 g a.i./L (Manzate 200 DF, E. I. du Pont de Nemours & Co.); methoxychlor (1.0 ml a.i./L) + diazinon (0.5 ml a.i./L) (Dymet, Mallinckrodt, Inc., St. Louis, MO); fluvalinate, 0.059 g a.i./L (Mavrik 2E, Sandoz Crop Protection Corp., Des Plaines, IL); carbaryl, 0.958 g a.i./L (Sevin 5W, Rhone-Poulenc Agrochimie); dicofol, 0.42 g a.i./L (Kelthane, Rohm & Haas Co., Philadelphia, PA); malathion, 0.451 g a.i./L (American Cyanamid Co., Wayne, NJ); and triadimefon, 0.149 g a.i./L (Bayleton 50WP, Mobay Corp.).

Inoculation technique. Dry conidia of *B. cinerea* were used as inoculum. Conidia from 10- to 14-day-old sporulating cultures were collected into a pipet under a slight vacuum and were tapped out of the pipet onto weighing paper. Cuttings were excised from stock plants 2 cm above a node with flamed razor blades. Dry conidia were dusted onto the cut surface with a camel's-hair brush.

Growth and dew chamber environment. The environment of the growth chamber (Puffer-Hubbard Refrigeration, Grand Haven, MI) was maintained at 19 C and 50% RH. Irradiance was 1.07×10^3 W/cm^2 at canopy height for 12 hr per day from Sylvania VHO cool-white fluorescent lamps. The environment of the dew chamber (Percival Manufacturing, Boone, IA) was maintained at 20 ± 1 C with continuous dew. The irradiance was 1.175×10^3 W/cm^2 at canopy height for 12 hr per day from General Electric cool-white florescent lamps. Dew formed on the plants within 1 hr of their placement into the dew chamber.

Effect of a time lag between stem wounding and inoculation on stem blight. On 9 October 1987, geranium stock plants were transferred to a research greenhouse at University Park, PA, and maintained according to previous specifications, although no pesticides were applied. Eight cuttings were excised from each of eight plants leaving wounded stems. Plants were then placed into a growth chamber. After 0 hr, 12 hr, and 1, 3, and 7 days, one plant was removed from the growth chamber, inoculated, and placed in a dew chamber under conditions favorable for disease development. A treatment whereby a plant was inoculated at 0 hr and kept in the growth chamber throughout the experiment was included. In addition, a control (uninoculated) plant was placed into each dew chamber and growth

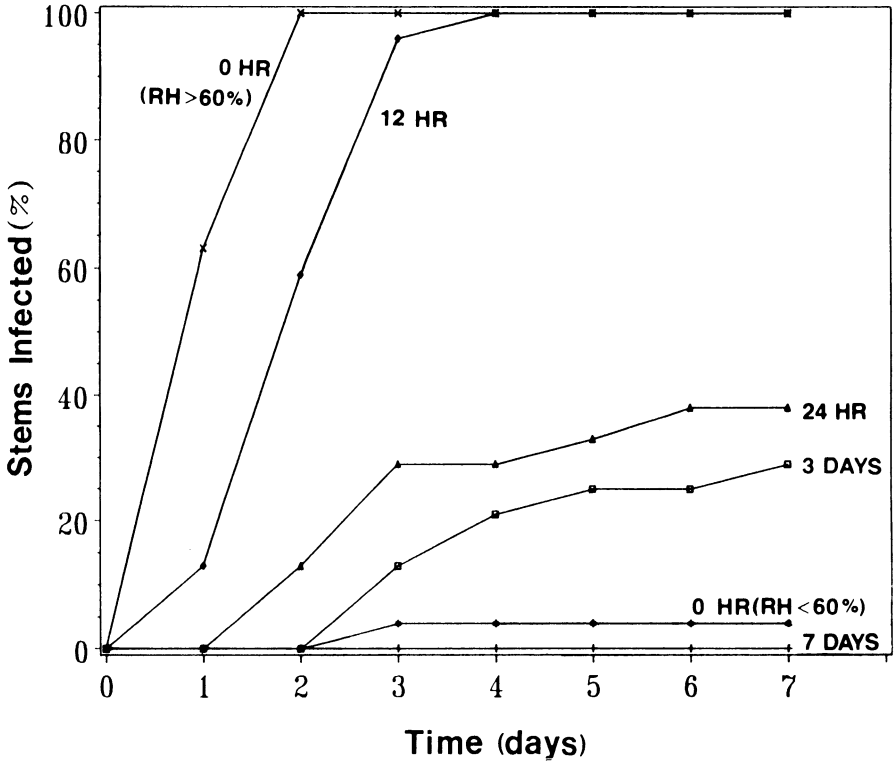

Fig. 1. Disease progress curves for geranium stems inoculated with *Botrytis cinerea* immediately after wounding (0 hr) and incubated in an environment of low relative humidity (RH < 60%), and inoculated 0 hr, 12 hr, and 1, 3, and 7 days after wounding and incubated under conditions favorable for disease development.

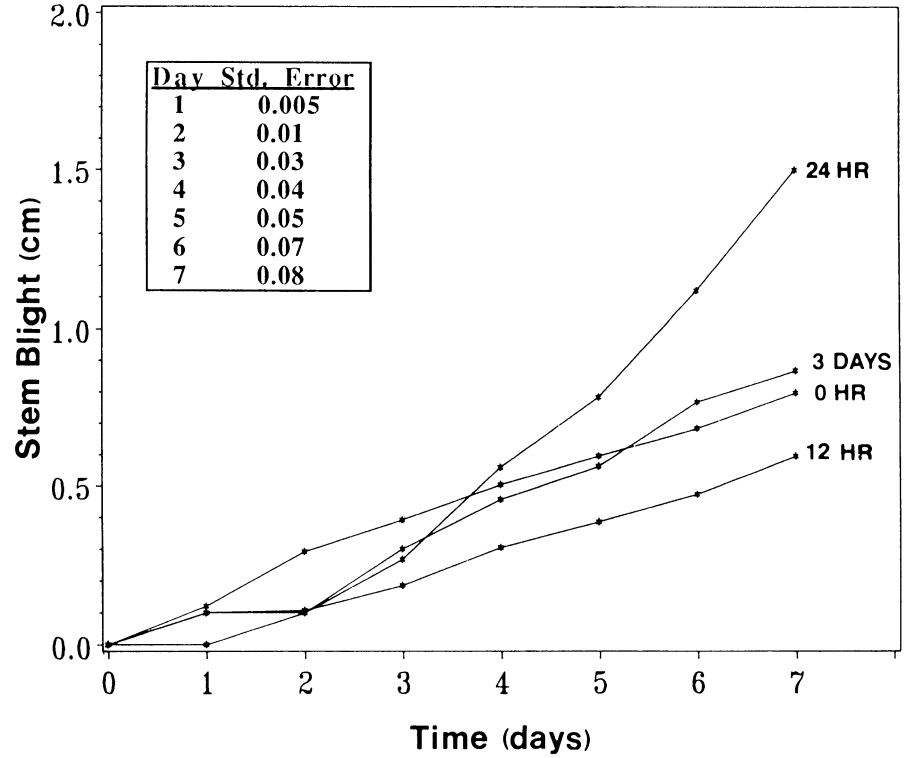

Fig. 2. Blight progression on geranium stems inoculated with *Botrytis cinerea* immediately after wounding and incubated in an environment of low relative humidity (RH < 60%), and inoculated 0 hr, 12 hr, 1 day, and 3 days after wounding and incubated under conditions favorable for disease development.

chamber and monitored throughout the experiment.

Disease symptoms were noted and the percentage of stems infected for each treatment was determined daily for 7 days after inoculation. The percentage of stems infected was then plotted against time from inoculation. Because of space constraints, one plant was used per treatment. The study was conducted three times and initiated on 5 December 1986 and 10 and 25 March 1987. All plants were disposed of at the conclusion of each repetition. The experiment was analyzed as a completely randomized block design with time as blocks.

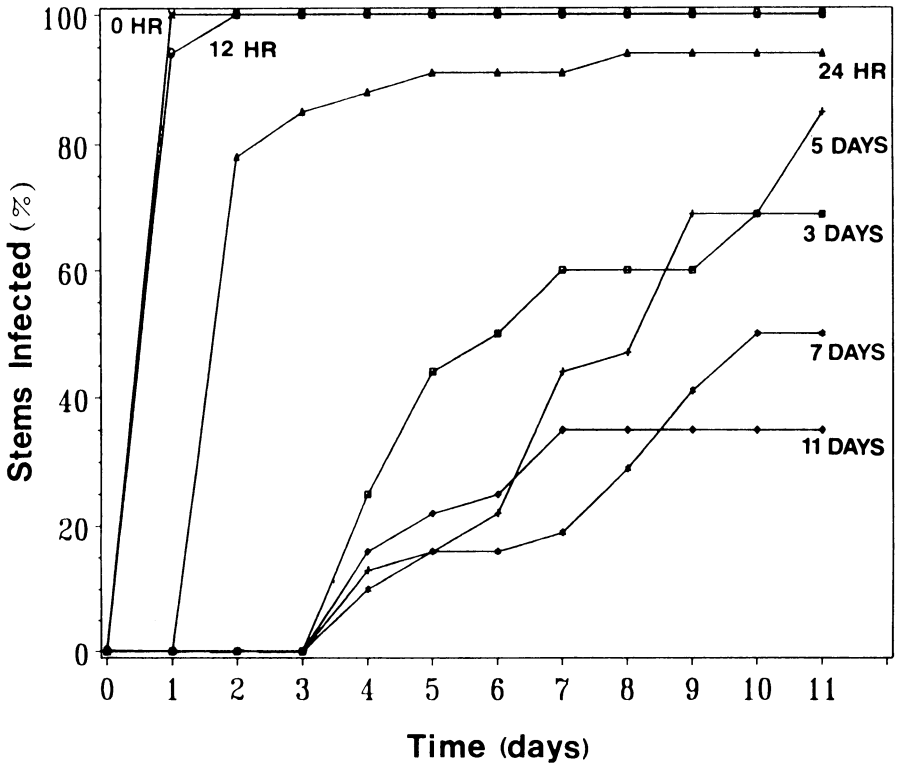

Fig. 3. Disease progress curves for geranium stems inoculated with *Botrytis cinerea* when placed in an environment of low relative humidity (RH < 60%) for 0 hr, 12 hr, and 1, 3, 5, 7, and 11 days before incubation under conditions favorable for disease development.

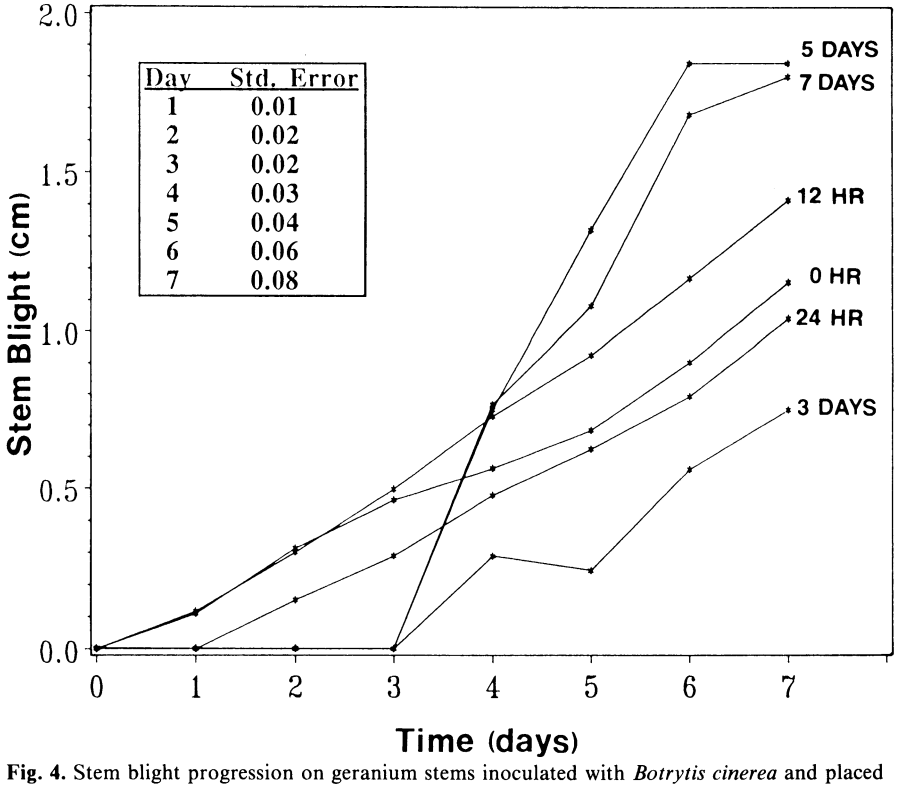

Fig. 4. Stem blight progression on geranium stems inoculated with *Botrytis cinerea* and placed in an environment of low relative humidity (RH < 60%) for 0 hr, 12 hr, and 1, 3, 5, and 7 days before incubation under conditions favorable for disease development.

Effect of a time lag between inoculation and incubation on stem blight. On 21 September 1987, geranium stock plants were transferred to a research greenhouse at University Park, PA, and maintained according to previous specifications, although no pesticides were applied. Eight cuttings were excised from each of nine plants and the wounded stems inoculated as described previously. Plants were then placed into a growth chamber. After 0 hr, 12 hr, and 1, 3, 5, 7, and 11 days, one inoculated plant was removed from the growth chamber and placed into a dew chamber. Also, a control (uninoculated) plant was placed into each dew and growth chamber and monitored throughout the experiment.

Disease symptoms were noted and the percentage of stems infected for each treatment was determined daily for 11 days after inoculation. The percentage of stems infected was then plotted against time from inoculation. Because of space constraints, one plant was used per treatment. The study was conducted four times and initiated on 24 September and 6 and 30 October 1987. All plants were disposed of at the conclusion of each repetition. The experiment was analyzed as a completely randomized block design with time as blocks.

Isolation of *Botrytis*. At the end of each experiment, blighted geranium stems were randomly sampled to detect colonization by *B. cinerea*. A 2-cm segment of the stem at the interface of the diseased and healthy tissue was surface-disinfested in 0.5% NaOCl for approximately 30 s, rinsed in sterile distilled water, and plated on PDA. *B. cinerea* isolated from the plant tissue was identified based on conidia and conidiophore color, structure, and size.

Calculation of the area under the incidence of stem blight disease progress curve (AUDPC). AUDPC was calculated to express the cumulative incidence of stem infection occurring over a 7- and 11-day period after inoculation for studies on the effect of a time lag between stem wounding and inoculation, and inoculation and incubation on stem blight, respectively, from the formula: AUDPC $= \Sigma_{i=1}^{n-1} [(X_{i+1} + X_i)/2] * (t_{i+1} - t_i)$ where X_i is cumulative disease incidence, expressed as a proportion at the ith observation; t_i is the time (in days after inoculation) at the ith observation; and n is the total number of times stem infection was assessed (16). The AUDPC data were analyzed by analysis of variance with a protocol of the Statistical Analysis System (14). Mean comparisons were made among treatments with the Waller-Duncan Bayesian k-ratio t test

using a k-ratio of 100, corresponding to $\alpha = 0.05$.

RESULTS

Effect of a time lag between stem wounding and inoculation on stem blight. All stems of geranium stock plants inoculated with *B. cinerea* and incubated in a dew chamber under conditions favorable for disease development within 12 hr of excising cuttings (stem wounding) displayed stem blight symptoms within 4 days (Fig. 1). Disease incidence maxima were 38 and 29% when plants were inoculated 1 and 3 days, respectively, after stem wounding and placement in an environment of low RH before incubation in a dew chamber. None of the stems became infected when they were inoculated 7 days after wounding. Only 4% of the stems became infected when plants were inoculated immediately after wounding and maintained in an environment of low RH. Seven days after inoculation, average stem blight progression ranged from 0.60 to 1.50 cm among inoculated stems (Fig. 2). Uninoculated plants maintained in either an environment of low RH or the dew chamber did not show symptoms of stem blight.

AUDPC values were significantly greater (560 and 510%) after a minimum lag time of 0 and 12 hr, respectively, in an environment of low RH between stem wounding and subsequent inoculation and incubation in a dew chamber than when the interval was 1, 3, 7, or 14 days, which had AUDPC values of 160, 98, 0, and 19, respectively.

Effect of a time lag between inoculation and incubation on stem blight. When averaged, at least 94% of the stems became blighted when plants were placed in an environment of low RH for 1 day after inoculation, before incubation in a dew chamber (Fig. 3). When plants were inoculated and subjected to an environment of low RH for 3, 5, and 7 days before incubation in a dew chamber, an average of 69, 85, and 50% of the stems, respectively, showed stem blight symptoms within 11 days. An average of 35% of the inoculated stems became infected when plants were maintained in an environment of low RH for the duration of the experiment. The average progression of stem blight 7 days after inoculation ranged from 0.75 to 1.85 cm for the treatments (Fig. 4). Uninoculated plants maintained in an environment of low RH or the dew chamber for the duration of the experiment did not show symptoms of stem blight.

According to the stem blight incidence AUDPC data, a minimum of 1 day in an environment of low RH after inoculation was necessary to significantly reduce stem blight incidence (Table 1). The AUDPC data indicated that the longer inoculated plants were maintained in an environment of low RH, the lower the stem blight incidence. *B. cinerea* was consistently isolated from surface-disinfested stem tissue showing stem blight symptoms.

DISCUSSION

A lag time of 1 day or more between excision of the cutting (stem wounding) from the geranium stock plant and subsequent inoculation with conidia of *B. cinerea* limited stem blight incidence. This timing corresponds to observations on geranium cuttings showing that within 1 day of wounding, suberin occurs in the cells near the wounded surface, in the intercellular spaces of the cortex and pith, and in the lumen of xylem vessels (2). Two days after wounding, the mean length of the intercellular suberin deposits in the pith and cortex increases, and after 3 days, all intercellular spaces around the cortex and pith cells are completely filled with suberin. Cline and Neely (2) concluded that the formation of suberized deposits on cell walls and in intercellular spaces during the first few days after wounding geranium cuttings decreases susceptibility to blackleg disease caused by *P. ultimum*.

This study also showed a lower level of stem blight incidence when inoculated plants were placed in an environment of low RH (<60%) for a minimum of 1 day before incubation in a dew chamber under conditions favorable for disease development. However, this method of control did not appear to be as effective in limiting disease incidence as exposing wounded plants to an environment of low RH for a minimum of 1 day before inoculation and subsequent incubation in a dew chamber.

Stem blight progression did not appear to be hindered by increasing beyond 1 day the time between stem wounding and inoculation. Similar trends were observed when the time between inoculation and incubation in a dew chamber were increased. Treatments most effective in reducing stem blight incidence sometimes showed a relatively rapid disease progression. If the plant was actively transpiring, conidia may have been pulled into the cut ends of the xylem vessels to a depth of several millimeters by the transpiration stream. Wilson (17) observed such conidia germinating in the vessels of stems of tomato plants.

The results of this study suggest that if atmospheric concentrations of conidia occurring within 1 day of harvesting cuttings could be minimized, fewer stems would become infected. However, in a commercial greenhouse, minimizing atmospheric concentrations of conidia immediately after harvest may be difficult. The conventional method of growing geranium stock plants for the production of cuttings is conducive to production of *B. cinerea* inoculum. Stock plants are pinched at regular time intervals or treated with the growth regulator ethephon (Florel) (15) to increase plant branching, and the number of growing points that can be removed as cuttings. This management practice produces low, compact plants with dense canopies that limit light and air penetration and promote senescence of the lower leaves (13). Close spacing of stock plants to maximize cutting production greatly enhances this problem. Under specific environmental conditions, *B. cinerea* readily infects these senescent leaves and sporulates, providing ample inoculum to infect stems wounded during the harvest of cuttings.

A recent study showed that grower activity, including harvesting cuttings, irrigating, fertilizing, and applying pesticides, is a primary factor influencing the occurrence of peak atmospheric concentrations of conidia of *B. cinerea* among geranium stock plants within a commercial greenhouse (4). Atmospheric concentrations of conidia increase throughout the growing season and in the absence of grower activity display a bimodal periodicity with peak concentrations at approximately midmorning and midafternoon.

A minimum lag time of 1 day in an environment of low RH between stem wounding and subsequent inoculation with conidia of *B. cinerea* decreased stem blight incidence. Atmospheric concentrations of conidia may be reduced by preventing sporulation on infected leaves and minimizing grower activity within the greenhouse immediately before and after stem wounding (4). Results also suggest that an environment of low RH immediately after stem wounding may limit stem blight incidence. A lower level of stem blight incidence occurred when inoculated plants were placed in an environment of low RH for a minimum of 1 day before incubation in a dew chamber. Stem blight progression did not appear to be hindered by the treatments in this study.

Table 1. Stem blight incidence[x] of geranium stock plants inoculated with *Botrytis cinerea* and placed in an environment of low relative humidity (<60%) for increasing time intervals before incubation in a dew chamber under conditions favorable for disease development

Time[y]	AUDPC (% days)
0 hr	1,000.0 a[z]
12 hr	985.9 ab
24 hr	850.0 b
3 days	382.8 c
5 days	284.4 cd
7 days	195.3 de
11 days	195.3 de

[x] Expressed as area under the disease progress curve.
[y] Between inoculation and incubation.
[z] Means followed by the same letter do not differ significantly according to the Waller-Duncan Bayesian k-ratio of 100, corresponding to $\alpha = 0.05$.

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