

# The Use of Forced Heated Air to Manage Botrytis Stem Blight of Geranium Stock Plants in a Commercial Greenhouse

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## ABSTRACT

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The impact of forced heated air continuously applied from beneath an open-bottom bench on the incidence of Botrytis stem blight and inoculum production was assessed in a commercial greenhouse planting of geranium stock plants. The forced heated air treatment significantly reduced the incidence of stem blight and sporulation on blighted stems and necrotic leaves of stock plants compared to the control. This reduced incidence of necrotic leaves with sporulating *Botrytis cinerea* with their concomitant decrease in fresh and dry weights at the end of the growing season for those plants exposed to forced heated air clearly shows the efficacy of this treatment. In addition, during days when grower activity was documented, *B. cinerea* conidial concentrations estimated using a Burkard recording spore trap were lower in the modified area than in the control area.

Stem blight, caused by *Botrytis cinerea* Pers.:Fr., is a limiting factor in production of geranium (*Pelargonium × hortorum* L.H. Bailey) stock plants for cuttings. Tetraploid ( $2n = 36$ ) geraniums and selected cultivars of diploid ( $2n = 18$ ) geraniums are propagated asexually by cuttings. Stem blight typically begins in the broken or cut-off stem surface of the stock plant and progresses downward, resulting in dieback of the entire stem. In severe cases, stem blight extends into the base of the plant, eventually killing it (8).

The conventional method of growing geranium stock plants for cutting production is conducive to serious outbreaks of stem blight caused by *B. cinerea*. The terminal buds of stock plants are pinched at regular intervals or treated with the growth regulator ethephon (12) to increase branching and the number of shoot meristems that can be removed as cuttings. This management practice produces short, compact plants with dense canopies that limit light and air penetration and promote senescence of the lower leaves. Close spacing of

stock plants to maximize cutting production greatly enhances these conditions. In a study conducted in a commercial greenhouse, *B. cinerea* readily colonized senescent lower leaves and sporulated (1). Although airborne conidia of *B. cinerea* are present in the stock-plant growing area throughout the growing season, grower activity is the primary cause of peak atmospheric concentrations of conidia (1). The relatively large peak concentrations of conidia associated with harvesting of cuttings pose an especially difficult problem because new infection courts are made available in the form of wounded stems at the same time large concentrations of conidia are airborne.

Traditional methods of controlling stem blight include fungicide application and sanitation. However, fungicide efficacy may be limited by a dense stock-plant canopy shielding senescent, sporulating stems and leaves from adequate fungicide coverage. Observations from a commercial greenhouse indicated that atmospheric conidial concentrations continued to increase following applications of chlorothalonil and iprodione to geranium stock plants (1). Also, the occurrence of fungicide resistance remains a constant threat. Resistance of *B. cinerea* to benomyl and cross-resistance to other benzimidazole fungicides in *Botrytis* populations are now common, while multiple resistance to both benzimidazole and dicarboximide fungicides is not unusual (7).

Sanitation measures typically include the removal and destruction of diseased plant material. Dead leaves at the base of plants and organic matter in and under benches may support *B. cinerea* growth.

For example, from organic matter in and on the sand of cutting benches, moist soils, and elsewhere, Melchers (6) isolated a *Botrytis* sp. (considered to be *B. cinerea*) that potentially may have served as a source of inoculum. For commercial geranium growers, however, maintaining the high standard of hygiene necessary to reduce inoculum sources is time-consuming and costly. The benefit of such sanitation efforts has been questioned by Plaut and Berger (9), who concluded from studies of *B. cinerea* on begonia that sanitation measures may be less effective than previously theorized. Low initial disease incidence apparently was compensated for by an accelerated rate of disease development.

Controlling disease caused by *B. cinerea* through modification of the greenhouse environment is an attractive addition to traditional control methods and typically is achieved by enhanced air circulation and a minimized duration of free moisture on the plants (10). *B. cinerea* depends on a water film for conidial germination and infection; therefore, preventing temperatures from reaching the dew point is an effective mechanism of disease escape (5).

In a research greenhouse, the incidence of sporulating *B. cinerea* on necrotic lower leaves of mature stock plants was significantly lower in comparison to the control for the following treatments: (i) white plastic mulch on top of the pots, (ii) intervals of heated air forced into the plant canopy from 2200 to 0600 h, and (iii) a combination of plastic mulch and forced heated air (4). Although the combination of plastic mulch and intervals of forced heated air limited the incidence of sporulating *B. cinerea* on the necrotic leaves of stock plants significantly more than the individual treatments, forced heated air alone was more effective than plastic mulch alone. Incorporation of forced air would work in management systems in which plants are moved during the growing season, whereas plastic mulch is feasible only in those growing systems in which the stock plants remain stationary throughout the growing season.

The objective of this study was to test in a commercial geranium-production setting a forced heated air treatment effective under controlled research conditions and to demonstrate to growers the value of environmental modification in managing Botrytis stem blight and inoculum production.

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## MATERIALS AND METHODS

**Geranium culture.** The study was conducted in a 1,672 m<sup>2</sup> commercial greenhouse in Pennsylvania. Rooted tetraploid (2n = 36) cv. Yours Truly geranium cuttings approximately 5 weeks old were planted in 3.78-liter plastic growing pots containing soilless root medium composed of vermiculite, sphagnum peat, and perlite (2:2:1 vol/vol/vol). One plant was transplanted per pot in October 1985 and December 1996 for experiments 1 and 2, respectively. The pots were placed on 1.8 × 18.3 m bench tops constructed of 3.5-cm-wide wooden strips spaced 3 cm apart. Plant densities were approximately 11.8 and 5.1 plants per m<sup>2</sup> for experiments 1 and 2, respectively. One bench per treatment was used.

Greenhouse air temperatures were 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. Venting occurred when temperatures were above 23.9°C. Stock plants were irrigated two to three times weekly, typically for 2 h, using a plastic-tube irrigation system with one tube per plant. Plants were fertilized twice weekly during these irrigations with 300 ppm N and K<sub>2</sub>O applied through the irrigation system two to three times for 1 min at 1-h intervals. During the growing season, pH of the medium varied between 5.4 and 5.8.

A CO<sub>2</sub> generator was used for enriching the greenhouse atmosphere to increase photosynthesis and growth. The CO<sub>2</sub> enrichment began after cuttings were planted and continued until temperatures made venting necessary. CO<sub>2</sub> levels were regulated during experiment 2 by the CO<sub>2</sub> Optimizer (Oglevee Computer Systems) and did not exceed 2,000 ppm. CO<sub>2</sub> levels were not regulated during experiment 1.

The date, time of day, duration of irrigation, fertilization during irrigation, application of pesticides, and harvest of cuttings from stock plants were documented by greenhouse personnel involved in performing the activities. Fungicide sprays were applied to all stock plants at various intervals ranging from 5 to 21 days and included iprodione, 0.598 g a.i./liter (Chipco 26019, Rhone-Poulenc Ag. Co., Research Triangle Park, NC); chlorothalonil, 0.937 g a.i./liter (Daconil 2787 F, ISK Biosciences Corp., Marietta, GA); maneb, 1.92 g a.i./liter (Manzate 80WP, Du Pont de Nemours & Co., Inc., Wilmington, DE); and triadimefon, 0.149 g a.i./liter (Bayleton 50 WP, Bayer Corp., Kansas City, MO). Sixteen or 20 fungicide applications were made to all plants in the study during 22 March to 17 August 1986 (experiment 1) and 5 February to 19 September 1987 (experiment 2), respectively. During monitoring of atmospheric conidial concentrations, one and eight fungicide applications for the control of *B. cinerea* were made for experiments 1 and 2, respectively.

**Environmental modification.** Modification to one bench included continuous forced heated air applied from beneath the bench by an electric heater, fan, and poly tube. The air flowed through a 45.7-cm-diameter poly tube with 5.0-cm-diameter holes spaced 0.83 m apart, which ran the length of the bench with an additional fan placed at the middle to facilitate air flow. Clear plastic extended from the top of the bench down to the floor to contain the heated air under the bench. An adjacent bench without modifications was used as the control treatment. Air temperature within the treatments was periodically monitored approximately 20.3 cm above the soil line. The temperature within the forced heated air treatment did not exceed that of the control area by more than 1.5°C.

**Conidia trapping.** Conidial concentrations were monitored among plants on the forced heated air and control benches from 27 February to 24 March (experiment 1) and 6 March to 11 June (experiment 2). One Burkard 7-day recording spore trap (Burkard Mfg. Co. Ltd., Rickmansworth, Herfordshire, England) was placed in the center of the forced heated air and control bench among geranium stock plants for experiments 1 and 2, respectively. The traps were operated at a flow rate of 10 liter/min, and the orifice was set approximately 7 cm above the plant canopy. Conidia were impacted onto tapes coated with an adhesive mixture of petroleum jelly and paraffin (9:1, wt/wt) dissolved in sufficient toluene to give a thick liquid consistency. Tapes were removed weekly, cut into 48-mm lengths representing 24-h periods, stained with aniline blue in lactic acid (28 mg of aniline blue, 20 ml of distilled water, 10 mg of glycerol, and 10 ml of 85% lactic acid), and mounted on glass slides beneath 22 × 50 mm coverslips. Before mounting, the tape was marked at 2-mm intervals with a razor blade to indicate hourly intervals. Under a compound microscope (×400), conidia were identified as *B. cinerea* based on conidium size, shape, color, and surface texture. The numbers of conidia counted in each 1-h period were recorded. Counts were converted to numbers of conidia per m<sup>3</sup> of air sampled. Because of the time-consuming nature of counting conidia, selected days in which grower activity, including irrigating, spraying fungicide, and harvesting, was documented were analyzed from the monitoring periods.

**Disease assessment.** The incidence of stem blight was recorded every 2 weeks from 19 May to 25 August in experiment 2 for 12 randomly selected plants growing near the spore trap within the modified and control growing areas. Two rows of stock plants along the perimeter of each bench served as a border to the monitored plants. Infected and healthy wounded stems were counted for each stock plant. Presence or absence of sporulation on the blighted stem

was noted. In addition, the percentage of necrotic leaves at the base of each monitored stock plant with sporulating *B. cinerea* was estimated.

The area under the disease progress curve (AUDPC) was calculated to express the cumulative incidence of wounded stems infected, infected stems with sporulating *B. cinerea*, and necrotic leaves with sporulating *B. cinerea* from the 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 at the  $i$ th observation;  $t_i$  is the time (in days after the first observation) at the  $i$ th observation; and  $n$  is the total number of times disease incidence was assessed (13). Data were analyzed by using a  $t$  test of the Statistical Analysis System (11).

At the termination of experiment 1 (7 August), seven plants each from the control and treated growing areas were chosen at random. All visibly senescent leaves were removed from these plants and were separated into those showing *B. cinerea* sporulation and those that did not. These data were also collected at the end of experiment 2 (9 September) for the 12 plants monitored in each growing area. Fresh and dried samples were weighed and analyzed by using a  $t$  test of the Statistical Analysis System (11).

**Isolation of *B. cinerea*.** At the end of all experiments, blighted stems were sampled randomly to detect colonization by *B. cinerea*. One 2-cm segment of the stem at the interface of the diseased and healthy tissue was surface-disinfected in 9.5% sodium hypochlorite for approximately 30 s, rinsed in sterile distilled water, and plated on 20 ml of potato-dextrose agar (250 g of potatoes, 15 g of dextrose, and 15 g of agar per liter of medium) in 10-cm-diameter petri plates for approximately 10 to 14 days at 20°C. Cultures were placed in a constant-temperature walk-in type room and exposed to approximately 9 h of natural daylight and 15 h of supplemental cool-white fluorescent light during the evening and night hours to induce sporulation. *B. cinerea* isolated from the plant tissue was identified based on conidia and conidiphore color, structure, and size (14).

## RESULTS

**Incidence of necrotic leaves with sporulating *B. cinerea*.** In experiment 2, the average incidence of necrotic lower leaves with sporulating *B. cinerea* was lower for plants within the forced heated air treatment than for those within the control treatment (Fig. 1A). A minimum average of 1 and 5% of the necrotic leaves on plants within the forced heated air treatment and control treatment, respectively, showed sporulating *B. cinerea* on 19 May. The incidence of necrotic leaves with sporulating *B. cinerea* on plants within the

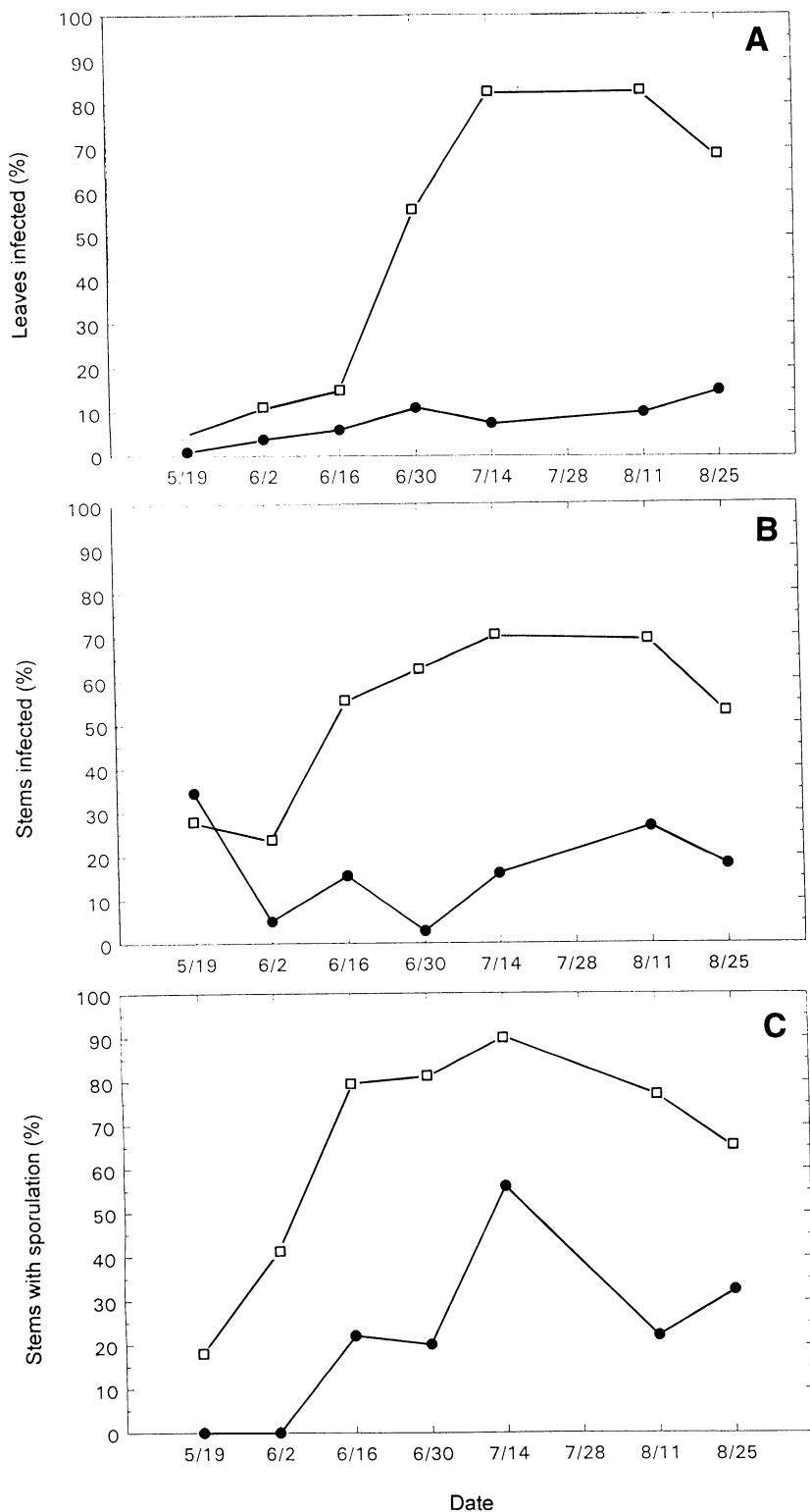


Fig 1. Incidence of (A) necrotic leaves with sporulating *Botrytis cinerea*, (B) wounded stems infected with *B. cinerea*, and (C) infected stems with sporulating *B. cinerea* on plants within the control (□) or forced heated air (●) treatments in experiment 2.

forced heated air treatment had reached an average maximum of only 16% by the last sampling period on 25 August. In contrast, an average maximum of 88% of the necrotic leaves on plants within the control treatment showed sporulating *B. cinerea* on 14 July. Cumulative incidence of necrotic lower leaves with sporulating *B. cinerea*

was significantly lower for plants within the forced heated air treatment in comparison to plants within the control treatment (Table 1).

Fresh and dry weights of necrotic leaves with sporulating *B. cinerea* collected from plants at the end of both experiments indicated that forced heated air treatment ef-

fectively reduced the incidence of necrotic leaves with sporulating *B. cinerea* in comparison to the control treatment (Table 2).

**Incidence and severity of stem blight on wounded stems.** In experiment 2, the average incidence of wounded stems infected with stem blight was lower for plants within the forced heated air treatment than for plants within the control treatment (Fig. 1B). An average of 7 to 34% of the wounded stems on plants within the forced heated air treatment was infected with *B. cinerea* during the sampling periods. In contrast, plants within the control treatment had an average of 24 to 71% of the wounded stems infected with *B. cinerea* during the sampling periods.

According to the AUDPC data, the incidence of wounded stems infected with *B. cinerea* was significantly lower for plants within the forced heated air treatment in comparison to plants within the control treatment (Table 1).

**Incidence of blighted stems with sporulating *B. cinerea*.** In experiment 2, the average incidence of blighted geranium stems with sporulating *B. cinerea* was lower for plants within the forced heated air treatment than for plants within the control treatment (Fig. 1C). The average incidence of blighted stems with sporulating *B. cinerea* within the forced heated air treatment ranged from 9 to 56% during the sampling periods. In contrast, an average of 18 to 90% of the blighted stems of plants within the control treatment had sporulating *B. cinerea*. Cumulative incidence of blighted stems with sporulating *B. cinerea* was significantly lower for plants within the forced heated air treatment in comparison to plants within the control treatment (Table 1). *B. cinerea* consistently was isolated from surface-disinfected stem tissue showing stem blight symptoms.

***B. cinerea* conidial concentrations.** During selected days when grower activity was documented, *B. cinerea* atmospheric conidial concentrations estimated for the forced heated air treatment were lower than those estimated for the control treatment in both experiments (Table 3).

## DISCUSSION

Results of this study indicate that forced heated air can reduce the sporulation incidence of *B. cinerea* on necrotic leaves and atmospheric conidial concentrations among geranium stock plants within a commercial greenhouse. It was also noted in experiment 2 that the incidence of stem blight and the sporulation incidence of *B. cinerea* on blighted stems was reduced by forced heated air. We propose that the reduction of stem blight incidence among plants within the forced heated air treatment-modified growing area is a result of reduced inoculum and unfavorable environmental conditions for conidial germination and infection. The sporulation of *B. cinerea* on the necrotic leaves at the base of the stock

**Table 1.** Cumulative incidence of geranium tissue infected with *Botrytis cinerea* and supporting sporulation in experiment 2

Treatment	AUDPC <sup>z</sup> (% * days)		
	Necrotic leaves with sporulating <i>B. cinerea</i>	Wounded stems infected	Infected stems with sporulating <i>B. cinerea</i>
Forced heated air	616.2 a	1,867.6 a	2,424.5 a
Control	4,911.9 b	5,213.0 b	6,570.4 b

<sup>z</sup> Means followed by the same letter within a column do not differ significantly according to Student's *t* test *P* < 0.05.

**Table 2.** Fresh and dry weights of necrotic leaves with sporulating *Botrytis cinerea*

Treatment	Experiment 1		Experiment 2	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Forced heated air	4.4 a <sup>z</sup>	3.4 a	8.9 a	7.4 a
Control	18.1 b	14.2 b	24.0 b	19.3 b

<sup>z</sup> Means followed by the same letter within a column do not differ significantly according to a Student's *t* test *P* < 0.05.

**Table 3.** Number of conidia trapped within a control and forced heated air stock-plant growing area during days when grower activity occurred

Date	Activity	Forced heated air /m <sup>3</sup> /h	Control /m <sup>3</sup> /h
Experiment 1			
27 February	Irrigate	62	183
2 March	Irrigate	13	124
5 March	Irrigate	29	253
10 March	Irrigate	5	468
12 March	Irrigate	29	237
14 March	Irrigate	13	393
22 March	Spray fungicide	139	443
24 March	Irrigate/harvest	523	1,503
Experiment 2			
6 March	Harvest	0	81
13 March	Harvest	73	159
5 May	Harvest	5	214
14 May	Harvest	38	180
21 May	Harvest	22	352
11 June	Harvest	95	6,611

plants appears to be a major source of inoculum for infecting geranium stems wounded during harvest of cuttings. If a wounded stem was inoculated, the forced heated air would provide an unfavorable environment for infection, thereby reducing stem blight incidence. In a previous study (2), when inoculated geranium stock plants were placed within a low relative humidity (RH) (60%) environment before incubation in a dew chamber, stem blight incidence was reduced. Also, when wounded stems were placed in a low RH environment for a minimum of 24 h following wounding of stems, stem blight incidence was decreased following inoculation with dry conidia and subsequent incubation in a dew chamber (2).

Further investigations are warranted to determine the cost of implementing forced heated air within a commercial stock plant greenhouse as part of a strategy to control *B. cinerea*. In determining the cost efficiency of this control measure, several factors must be considered. Reducing the sporulation on the necrotic leaf tissue and blighted stems of geranium stock plants could have a significant impact on the entire production chain. A reduced conidial concentration within the stock greenhouse could result in a lower stem blight incidence, thereby increasing the number of growing points that can be removed later as cuttings. The large peak conidial concentrations observed within a commercial stock-plant greenhouse during harvest of

cuttings (1) could be reduced greatly or eliminated, thereby reducing the number of conidia that could become impacted on the leaf surface of the cutting while in the stock house. Fewer *B. cinerea* conidia on the phylloplane of the cutting could result in decreased disease incidence during propagation. A reduction in disease caused by *B. cinerea* on cuttings could decrease the large peak conidial concentrations associated with shipping cuttings from a commercial propagation greenhouse (3) and thereby decrease postharvest diseases caused by *B. cinerea*.

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