The Effect of Plastic Mulch and Forced Heated Air on *Botrytis cinerea* on Geranium Stock Plants in a Research Greenhouse

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ABSTRACT

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Plastic mulch and intervals of forced heated air were incorporated among geranium (*Pelargonium* × *hortorum*) stock plants within a research greenhouse to limit stem blight caused by *Botrytis cinerea* and reduce inoculum produced on necrotic lower leaves of mature stock plants. The area under the leaf blight disease progress curve (AUDPC) revealed that the incidence of sporulating *B. cinerea* on necrotic lower leaves of mature stock plants was significantly decreased compared with that on the control for all treatments including (i) white plastic mulch on top of the pots, (ii) heated air forced into the plant canopy during 2200 to 0600 h, and (iii) a combination of plastic mulch and forced heated air. The AUDPC data indicated that the combination of plastic mulch and forced heated air limited the incidence of sporulating *B. cinerea* on the lower necrotic leaves of stock plants significantly more than did the individual treatments. Forced heated air limited the incidence of sporulating *B. cinerea* on the lower necrotic leaves of stock plants significantly more than did the plastic mulch. Similarly, fresh and dry weights of necrotic leaves with sporulating *B. cinerea* indicated that the combination of plastic mulch and forced heated air was most effective in limiting the incidence of necrotic leaves with sporulating *B. cinerea.* According to these data, forced heated air among stock plants effectively reduced the incidence of sporulating *B. cinerea* on necrotic leaves compared with the plastic mulch and control. Stem blight caused by *B. cinerea* occurred on all inoculated stems and disease progression was not reduced regardless of treatment.

Tetraploid (2N = 36) geraniums (*Pelargonium* × *hortorum* L. H. Bailey) and selected cultivars of diploid (2N = 18) geraniums are propagated asexually by cuttings. The terminal buds of stock plants are pinched at regular intervals or treated with the growth regulator ethephon (Florel) (12) to increase plant branching and the number of shoot meristems that can be removed as cuttings. As stock plants mature, the number of wounds on stems increases with each harvesting and lower leaves become senescent in the reduced light intensity below the dense canopy. Close spacing of stock plants to maximize space usage further enhances the senescence of lower leaves. In a previous greenhouse study, wounded stems and senescent leaves were readily infected by *Botrytis cinerea* Pers.:Fr. that became established in the senescent lower leaves (2). Saprophytic establishment of *B. cinerea* and subsequent sporulation early in the growing season provides inoculum for stems wounded during cutting harvest. Stem blight caused by *B. cinerea* is a limiting factor in growing geranium stock plants for cutting production. Stem blight typically begins in the broken or cut stem surface of the stock plant and progresses downward, causing a dieback of the entire stem. In severe cases, stem blight extends into the base of the plant, resulting in plant death (7).

Conidia are considered the primary source of inoculum for stem infections (7). Atmospheric concentrations of conidia increase during the stock plant growing season and are related to the incidence of blighted stems, and stems and necrotic leaves with sporulating lesions (2). Atmospheric concentrations of conidia peak in the greenhouse during and immediately after harvesting cuttings from geranium stock plants (2) and are an important consideration in disease management because cut stems on stock plants are most susceptible to *B. cinerea* when inoculated within 12 hours of wounding (3).

Traditional methods of controlling stem blight include fungicide application and sanitation. However, in a commercial greenhouse, atmospheric concentrations of conidia of *B. cinerea* continued to increase following applications of chlorothalonil and iprodione to geranium stock plants (2). Fungicide efficacy may be limited by a dense stock plant canopy shielding senescent, sporulating stems and leaves from adequate fungicide coverage. Reliance on fungicides alone to control Botrytis blight increases the potential for development of fungicide resistance and could lead to control failure. Resistance of *B. cinerea* to dicarboximide fungicides, including iprodione and vinclozolin, has been documented since the late 1970s (1,6,8,10).

Sanitation measures to control *B. cinerea* 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 growth of *B. cinerea.* Maintaining the high standard of hygiene necessary to reduce inoculum sources is time consuming and costly for commercial geranium growers. Regularly removing senescent leaves with sporulating *B. cinerea* may result in peak conidial concentrations in the atmosphere. Large peak conidial concentrations have been associated with roguing plant tissue with sporulating *B. cinerea* prior to shipping (4). The mechanical action associated with this activity releases and disperses the conidia. Plaut and Berger (9) concluded from studies of *B. cinerea* on begonia that sanitation measures may be less effective than previously theorized. Low initial disease incidence was apparently compensated for by an accelerated rate of disease development.

Reducing inoculum concentration of *B. cinerea* produced on lower necrotic leaves through modification of the geranium microenvironment is an attractive addition to traditional control methods. Environmental modification to control disease caused by *B. cinerea* is typically aimed at enhancing air circulation and minimizing the duration of free moisture on the plants (11). *Botrytis cinerea* depends on a water film for conidial germination and infection; therefore, preventing temperatures from reaching the dew point and reducing the relative humidity are effective mechanisms for disease escape (5). The objectives of this study were to determine the effects of reducing the relative humidity by limiting the exposure of the canopy to the wet soil surface using plastic mulch and/or enhancing air circulation, using forced heated air incorporated among stock plants within a research greenhouse, on stem blight progression and sporulation of *B. cinerea* on the necrotic lower leaves.

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MATERIALS AND METHODS

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

Geranium culture. Rooted tetraploid geranium cuttings (cv. Crimson Fire) approximately 5 weeks old were planted into 3.78-liter plastic pots containing soilless root medium (Fafard Potting Mix, Conrad Fafard, Inc., Anderson, SC) composed of 2:2:1 (vol/vol/vol) vermiculite, sphagnum peat, and perlite. One plant was transplanted per pot on 15 November 1986 and 23 February 1988 for experiments 1 and 2, respectively. The pots were placed on 0.96-m by 3.8-m bench tops within a research greenhouse at The Pennsylvania State University (University Park, PA) and arranged in two groups (four rows of seven plants) on each of four benches. Plant density was approximately 15.1 plants/m^2.

Greenhouse air temperatures were monitored and controlled using a greenhouse climate control computer (Oglevee Computer Systems, Connellsville, PA) programmed to provide a minimum of 15.5°C during day/night. Air temperature within the treatments incorporating forced heated air was monitored 20.3 cm above the soil line during the treatment period of 2200 to 0600 h. Temperatures within these treatments typically averaged 2°C above that for the plastic mulch treatment and the untreated control. Ventilation occurred when temperatures exceeded 22°C. Stock plants were irrigated two to three times weekly, typically for 30 min, using a plastic tube irrigation system with one tube per pot. Plants were fertilized twice weekly during these irrigations with 300 ppm N and K_2O applied through the irrigation system. During the growing season, the growing medium pH varied between 5.3 and 5.9. Fungicides and insecticides were not applied to the plants.

Environmental modification. The following treatments were investigated: (i) no treatment; (ii) white plastic mulch on top of groups of pots with the rooting systems of the cuttings inserted through holes in the plastic and placed into the soilless growing medium as in conventional planting; (iii) forced heated air, applied through a perforated, 7.5-mm PVC pipe placed on top of the pots between rows of plants by means of an electric heater and fan, throughout the pipe and plant canopy from 2200 to 0600 h; and (iv) a combination of plastic mulch and forced heated air. Treatments were initiated immediately after transplanting.

Inoculation technique and disease assessment. Plants were inoculated on 20 March 1987 and 18 May 1988 for experiments 1 and 2, respectively. Three cuttings were excised with a razor blade from each of the 10 innermost plants in each group. Five of these plants were randomly identified and inoculated by placing a 12-mm-diameter mycelial disk, taken from the perimeter of *B. cinerea* colonies, onto each cut stem surface.

Stem blight, progression, and the approximate percentage of necrotic leaves with sporulating *B. cinerea* were recorded from 26 May to 15 July 1987 for experiment 1. Data for experiment 2 were collected from 1 June to 2 August 1988. The area under the disease progress curve (AUDPC) was calculated to express the cumulative incidence of sporulating *B. cinerea* on necrotic leaves at the base of the stock plants from inoculation to 116 and 75 days after inoculation of the stems for experiments 1 and 2, respectively, using the formula AUDPC = $\sum_{i=1}^{n-1} [(X_{i+1} + X_i)/2] \times (t_{i+1} - t_i)$, 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 disease incidence was assessed (13).

At the termination of each experiment, all visibly senescent leaves were removed from the plants. Leaves were separated into those showing sporulation of *B. cinerea* and those without sporulation. Fresh

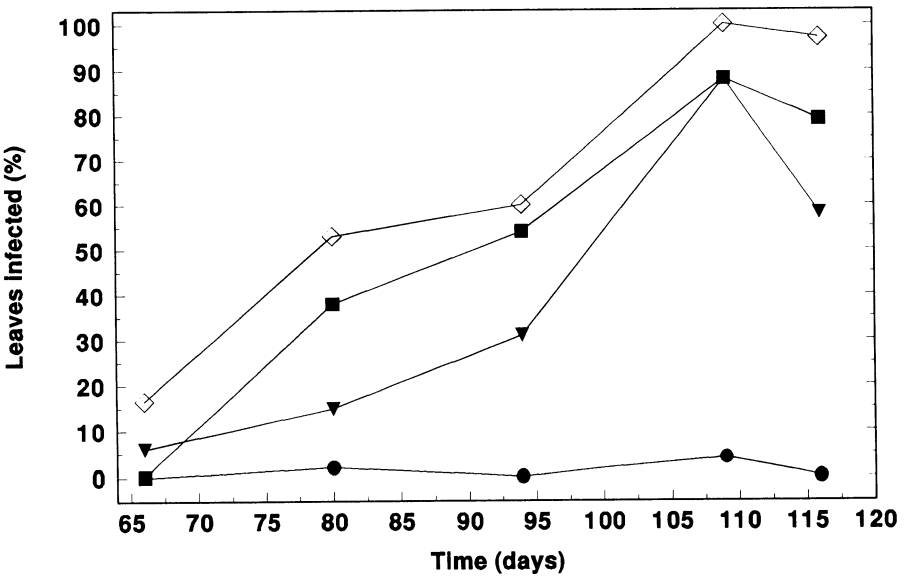

Fig 1. Necrotic leaves with sporulating *Botrytis cinerea* on inoculated plants in experiment 1 with no treatment (◇), plastic mulch (■), forced heated air (▼), and plastic mulch and forced heated air (●). Time (x-axis) represents days after inoculation.

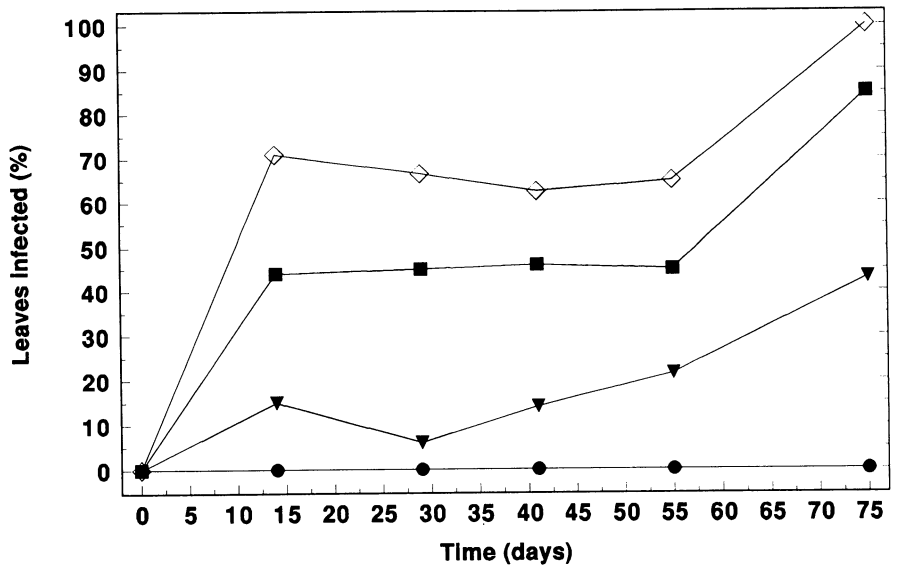

Fig 2. Necrotic leaves with sporulating *Botrytis cinerea* on inoculated plants in experiment 2 with no treatment (◇), plastic mulch (■), forced heated air (▼), and plastic mulch and forced heated air (●). Time (x-axis) represents days after inoculation.

and dried samples were weighed.

The AUDPC and the fresh and dry weight data were analyzed by analysis of variance with a protocol of the Statistical Analysis System (SAS Institute, Cary, NC). 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.

Isolation of *B. cinerea*. At the end of both experiments, geranium stems showing stem blight were randomly sampled 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 0.5% sodium hypochlorite for approximately 30 s, rinsed in sterile distilled water, and then plated on PDA. *Botrytis cinerea* isolated from the plant tissue was identified based on conidia and conidiophore color, structure, and size.

RESULTS

Incidence of necrotic leaves with sporulating *B. cinerea*. The incidence of sporulating *B. cinerea* on necrotic lower leaves of mature stock plants decreased compared with 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.

A combination of plastic mulch and forced heated air incorporated among stock plants effectively limited *Botrytis* sporulation to a maximum average of 4% of the necrotic leaves showing sporulating *B. cinerea* at the termination of the experiments (Figs. 1 and 2). The forced heated air treatment resulted in an average of 58 and 43% of the necrotic leaves showing sporulating *B. cinerea* in experiments 1 and 2, respectively. The incorporation of plastic mulch resulted in an average of 79 and 85% of the necrotic leaves showing sporulating *B. cinerea* for experiments 1 and 2, respectively. In comparison, for both experiments an average of at least 97% of the necrotic leaves on plants within the control area showed sporulating *B. cinerea*.

According to the AUDPC data for experiments 1 and 2, the incidence of sporulating *B. cinerea* on necrotic lower leaves of stock plants was significantly less for all treatments compared with the control (Table 1). The AUDPC data indicated that the combination of plastic mulch and forced heated air limited the incidence of sporulating *B. cinerea* on the lower necrotic leaves of stock plants significantly more than had the individual treatments. Forced heated air among stock plants was significantly more effective in reducing the incidence of sporulating *B. cinerea* on the lower necrotic leaves of stock plants than was the plastic mulch.

From both experiments, fresh and dry weights of necrotic leaves with sporulating *B. cinerea* indicated that the combination of plastic mulch and forced heated air most effectively limited the incidence of necrotic leaves with sporulating *B. cinerea* (Table 2). According to those data, the intervals of forced heated air among stock plants effectively reduced the incidence of sporulating *B. cinerea* on necrotic leaves compared with the plastic mulch treatment or no treatment. Data from experiment 1 indicated that the fresh and dry weights between the plastic mulch treatment and no treatment (control) were not significantly different. However, in experiment 2, the fresh and dry weights of necrotic leaves with sporulating *B. cinerea* collected from plants with the plastic mulch treatment were significantly greater than those from plants with no treatment.

Stem blight occurred on all inoculated stems regardless of treatment. In experiment 1, stem blight progression ranged from 13.18 to 14.48 cm 116 days following inoculation and was not significantly different among treatments (Table 3). In experiment 2, stem blight progression ranged from 8.58 to 15.30 cm 75 days following inoculation and was significantly greater for the treatment intervals of forced heated air compared with the other treatments and may be a result of the stress imposed on the plants by the warm air pulses under late summer greenhouse conditions. Since naturally occurring inoculum was not present at the onset of the experiments, stems that were wounded but not inoculated did not become infected regardless of treatment. Once the Botrytis epidemic was established, the wounded control stems appeared to be "healed" and resistant to infection.

Isolation of *B. cinerea*. *B. cinerea* was consistently isolated from surface-disinfected stem tissue showing stem blight symptoms.

DISCUSSION

According to the AUDPC data, the incidence of sporulating *B. cinerea* on necrotic lower leaves of mature stock plants within a research greenhouse was significantly lower than that on the control for the following treatments: (i) white plastic mulch on top of the pots; (ii) intervals of forced heated air into the plant canopy from 2200 to 0600 h; and (iii) a combination of plastic mulch and forced heated air. The AUDPC data indicated that 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 did the individual treatments. Forcing heated air among stock plants limited the incidence of sporulating *B. cinerea* on the necrotic leaves significantly more than did the plastic mulch.

Although plastic mulch was effective in reducing the incidence of *B. cinerea* sporulation on necrotic leaves compared with the control treatment, it may not be cost-effective because the incidence of sporulation was still relatively high. However, forcing heated air among mature stock plants may be cost-effective because the incidence of sporulation on necrotic leaves dramatically decreased. Further, the incorporation of forced air would work in management systems in which plants are moved during the growing season, where-

Table 1. Incidence of necrotic leaves with sporulating *Botrytis cinerea* on inoculated geranium stock plants expressed as the area under the disease progress curve (AUDPC)

	AUDPC (% days)[z]	
	Experiment	
Treatment	1	2
Control	2,599.3 a	4,026.5 a
Plastic mulch	2,014.5 b	2,748.5 b
Air	1,396.8 c	924.3 c
Plastic mulch + air	44.5 d	0.0 d

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

Table 2. Fresh and dry weights of necrotic leaves (g) with sporulating *Botrytis cinerea* 116 and 75 days following inoculation for experiments 1 and 2, respectively

	Leaf weight (g)[z]			
	Experiment 1		Experiment 2	
Treatment	Fresh	Dry	Fresh	Dry
Control	10.82 a	9.03 a	13.40 b	11.52 b
Plastic mulch	11.43 a	10.14 a	18.47 a	15.89 a
Air	6.06 b	4.71 b	5.22 c	4.94 c
Plastic mulch + air	0.37 c	0.36 c	0.03 d	0.02 d

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

Table 3. Stem blight progression (cm) 116 and 75 days following inoculation for experiments 1 and 2, respectively

	Experiment[z]	
Treatment	1	2
Control	13.43 a	9.85 b
Plastic mulch	14.48 a	8.58 b
Air	14.02 a	15.30 a
Plastic mulch + air	13.18 a	10.42 b

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

as plastic mulch is feasible only in those growing systems in which the stock plants remain stationary throughout the growing season.

Sporulating *B. cinerea* on necrotic leaves at the base of stock plants provides a major source of inoculum for infecting geranium stems wounded during harvest of cuttings. If a wounded stem was inoculated, the forced heated air treatment investigated in this study would provide an unfavorable environment for infection, thereby reducing stem blight incidence. In a previous study (3), when geranium stock plants inoculated with dry conidia were placed within a low relative humidity (<60%) environment prior to incubation in a dew chamber, incidence of stem blight was reduced. Also, when wounded stems were placed within a low relative humidity 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 (3). The inability of treatments included in this study to reduce the incidence of stem blight is probably a result of the agar plug inoculation method used.

Although stem blight progression was not reduced by the treatments tested, reducing sporulation and disease incidence was possible. Therefore, control efforts should be aimed toward reducing the incidence of infection and sporulation of *B. cinerea*. 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-effectiveness of this control measure, several factors must be considered. Reducing sporulation on the necrotic leaf tissue and blighted stems of geranium stock plants could have a large 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 could later be removed as cuttings. The high peak conidial concentrations observed within a commercial stock plant greenhouse during harvest of cuttings (2) could be greatly reduced or eliminated, thereby reducing the number of conidia that could become lodged on the leaf surface of the cuttings while in the stock house. Fewer *B. cinerea* conidia on the phylloplane of the cutting could result in decreased disease incidence during propagation. Within a commercial propagation greenhouse, a reduction in the incidence of disease caused by *B. cinerea* on cuttings could decrease the high peak conidial concentrations associated with shipping cuttings (4) and thereby decrease the incidence of postharvest diseases caused by *B. cinerea*.

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