Influence of Grower Activity and Disease Incidence on Concentrations of Airborne Conidia of *Botrytis cinerea* Among Geranium Stock Plants

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ABSTRACT

Hausbeck, M. K., and Pennypacker, S. P. 1991. Influence of grower activity and disease incidence on concentrations of airborne conidia of *Botrytis cinerea* among geranium stock plants. Plant Dis. 75:798-803.

The relationship of grower activity, fungicide, and disease incidence to concentrations of airborne conidia of *Botrytis cinerea* among geranium (*Pelargonium* × *hortorum*) stock plants within a commercial greenhouse was studied. Hourly concentrations of conidia of *B. cinerea* were estimated for selected time periods of the 1986 and 1987 growing seasons using a Burkard recording spore trap. The incidence of stem blight caused by *B. cinerea* was estimated during 1987. Also, the incidence of stems and necrotic leaves with sporulating lesions was estimated. During both growing seasons, daily conidial concentrations increased even after applications of fungicide to control Botrytis blight. During 1987, increases in daily conidial concentrations coincided with increases in incidences of stem blight and of blighted stems and necrotic leaves with sporulating lesions. Peak conidial concentrations (>50 conidia per cubic meter per hour) were typically associated with grower activity, including irrigation and fertilization with a plastic tube drip system with emitters that prohibited splashing, spraying of pesticides, and harvesting of cuttings. The occurrence of peak conidial concentrations during and immediately after harvesting of cuttings is an important consideration in disease management because new infection courts are made available in the form of wounded stems at the same time that concentrations of airborne conidia are large. Further, cuttings removed from the stock plants are frequently exposed to large concentrations of airborne conidia that may influence disease occurrence during propagation.

Stem and leaf blights caused by *Botrytis cinerea* Pers.:Fr. are limiting factors in geranium (*Pelargonium* × *hortorum* L.H. Bailey) production. In 1985, a commercial propagator of the florist's geranium calculated that annual dollar losses in the operations ranged between $130,000 and $190,000 and that losses in the North American operations ranged between $300,000 and $500,000. By extrapolating from this data base, losses caused by Botrytis blight of geranium ranged between $5.1 and $7.6 million during 1985. Estimates of the dollar value of indirect losses caused by reduced quality and production are not available.

The conventional method of growing geranium stock plants for cutting production is conducive to serious outbreaks of stem blight caused by *B. cinerea*. Tetraploid ($2n=36$) geraniums 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) (11) to increase plant 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 (9). Close spacing of stock plants to maximize cutting production greatly enhances these conditions. Under specific environmental conditions, *B. cinerea* readily colonizes these senescent leaves and sporulates, providing ample inoculum to infect stems wounded during the harvesting of cuttings.

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. Subsequently, *B. cinerea* sporulates in the diseased tissue. In severe cases, stem blight extends into the base of the plant, resulting in plant death (7). Cuttings harvested from stock plants with sporulating *B. cinerea* may develop a cutting rot or leaf blight disease during propagation. Nichols and Nelson (7) suggested conidia can lodge on the broken stem surface of the cutting at the time of harvest and remain dormant until cuttings are placed in the propagation bench for rooting, where the wet and humid conditions established for optimum propagation promote the germination of conidia and the progression of cutting rot and leaf blight.

Conidia are considered the primary source of inoculum for infecting stems (7) and an important inoculum source for latent infections of cuttings (10). Although incident inoculum of *B. cinerea* is an important consideration in controlling diseases among stock plants and may play a role in incidence of disease during propagation, airborne concentrations of *B. cinerea* conidia in the greenhouse have not been quantified. Therefore, hourly concentrations of airborne conidia of *B. cinerea* among geranium stock plants within a commercial greenhouse were estimated to determine: 1) if conidia are present throughout the growing season and 2) what influence grower activity and disease incidence have on the concentration of airborne conidia. These studies were undertaken to develop effective control measures.

MATERIALS AND METHODS

The study was conducted within a 1,672-m^2 commercial greenhouse in Pennsylvania. Rooted tetraploid ($2n=36$) geranium cuttings approximately 5 wk old were planted into 3.8-L plastic pots containing soilless root medium composed of 2:2:1 (v/v) vermiculite, sphagnum peat, and perlite. Planting was done during October 1985 and December 1986 for the 1986 and 1987 growing seasons, respectively. The pots were placed on 1.8-m bench tops constructed of 3.5-cm-wide wooden strips spaced 3 cm apart. Plant density was 11.8 and 15.1 plants per square meter during 1986 and 1987, respectively.

Greenhouse temperatures were regulated by means of a greenhouse climate control computer (Oglevee Computer Systems, Connellsville, PA) programmed to provide a minimum of 17 C (60 F) during the day and night. Venting occurred when temperatures exceeded 24 C (75 F). Stock plants were watered two or three times weekly, typically for 2 hr by means of a plastic tube drip irrigation system with emitters that prohibited splashing. Plants were fertilized twice weekly during these irrigations with 300 mg/L of N and K_2O applied through the irrigation system two or three times for 1 min at 1-hr intervals. During the grow-

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Portion of the first author's Ph.D. dissertation submitted to The Pennsylvania State University. Present address of first author: Department of Botany and Plant Pathology, Plant Biology Building, Michigan State University, East Lansing, MI 48824.

Contribution No. 1793, Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station.

This research was supported in part by the Ben Franklin Partnership of the Commonwealth of Pennsylvania and the Pennsylvania Agricultural Experiment Station.

Accepted for publication 26 January 1991 (submitted for electronic processing).

ing season, the pH of the soilless root medium varied between 5.4 and 5.8.

CO_2 enrichment began after the cuttings were planted and continued until temperatures made venting necessary. CO_2 levels were regulated to a maximum of 2,000 μl/L during 1987 by the CO_2 Optimizer (Oglevee). CO_2 levels were not monitored during 1986.

The following fungicides and insecticides were applied to the stock plants throughout the growing season: chlorothalonil (Daconil 2787F), 0.937 g a.i./L; maneb (Manzate 80WP), 1.92 g a.i./L; iprodione (Chipco-26019), 0.598 g a.i./L; acephate (Orthene 75SP), 10.78 g a.i./L; carbaryl (Sevin 5W), 0.958 g a.i./L; demeton-S-methyl sulfoxid/oxydemeton-methyl (Metasystox R2), 0.449 g a.i./L; dicofol (Kelthane), 0.42 g a.i./L; fluvalinate (Mavrik 2E), 0.059 g a.i./L; malathion (Malathion), 0.451 g a.i./L; methomyl (Lannate), 2.2 g a.i./L); methoxychlor + diazinon (Dymet), 1.0 + 0.5 ml a.i./L; and triadimefon (Bayleton 50WP), 0.149 g a.i./L. Chlorothalonil and iprodione primarily target *B. cinerea*.

Information on pesticide application was not available prior to 22 March 1986, the date of the first documented application of fungicide for the control of Botrytis blight after planting of rooted cuttings in 1986; the corresponding date for the 1987 season was 5 February (Table 1). The date, time of day, and duration of irrigating, fertilizing, spraying of pesticides, harvesting of cuttings, and cleaning of benches were documented by greenhouse personnel.

In 1986, conidial concentrations were monitored during 26 February to 24 March, 29 May to 14 June, and 6–19 August. In 1987, conidial concentrations were monitored during 4 March to 5 July. A Burkard 7-day recording spore trap (Burkard Mfg. Co. Ltd., Rickmansworth, England) was placed in the center of a greenhouse bench among stock plants of geranium cultivars Glacier

Table 1. Dates of fungicide applications to geranium stock plants during the 1986 and 1987 growing seasons

Date	Fungicides[a]
1986	
22 March	Iprodione
12 April	Chlorothalonil
19	Iprodione
3 May	Chlorothalonil
10	Iprodione
17	Chlorothalonil
24	Iprodione
31	Chlorothalonil
13 June	Chlorothalonil
20	Chlorothalonil
4 July	Chlorothalonil
11	Chlorothalonil
18	Iprodione
25	Chlorothalonil
1 August	Chlorothalonil
9	Chlorothalonil
1987	
5 February	Chlorothalonil
27	Chlorothalonil
21	Triadimefon
28	Maneb
4 April	Chlorothalonil
9	Chlorothalonil
22	Triadimefon
30	Maneb
8 May	Chlorothalonil
15	Iprodione
30	Maneb
6 June	Chlorothalonil
12	Iprodione
20	Triadimefon
27	Maneb
10 July	Iprodione
17	Triadimefon
25	Maneb
1 August	Chlorothalonil
7	Iprodione
22	Chlorothalonil

[a] Iprodione and chlorothalonil were specifically targeted for Botrytis blight.

Table 2. Number of hours after onset of activities among geranium stock plants that discrete peak conidial concentrations of *Botrytis cinerea* were observed during 1886 and 1987

	Harvest		Spray		Fertilize, irrigate	
Date	Conidia/m^3/hr	Hr	Conidia/m^3/hr	Hr	Conidia/m^3/hr	Hr
1986						
27 February					63	1
2 March					82	1
5					79	2
10					401	1
14					170	2
22			269	...[a]		
24	746	3			746	3
31 May			95	5	95	5
3 June	1,297	4			1,297	4
4					185	1
6					61	<1
8					97	1
10					146	<1
12	814	3			814	3
13			462	...		
14					54	1
18			526	...		
19					221	1
22					326	5
8 August					1,191	...
9			185	...		
11	504	1			504	1
13					176	5
16					87	<1
18					362	3
1987						
13 March	110	...				
28			144	1	144	1
1 April					125	1
2					414	3
3					1,086	3
8					1,159	7
9			1,659	...		
21	452	...				
24					340	1
30			80	...		
5 May	65	5				
6					53	6
9					67	5
15			55	3	55	3
16					51	3
20					164	6
21	161	...				
10 June					1,647	3
11	1,821	...				
12			178	...		
13					357	4
17					4,353	4
24					2,699	3
26	11,368	...				
27			208	...		
30					201	...
1 July	2,589	...				
3			13,587	...		
4					1,118	...

[a] Onset and duration of activity not documented.

Crimson and Yours Truly for the 1986 and 1987 growing seasons, respectively. The trap was operated at a flow rate of 10 L/min. The orifice was set approximately 7 cm above the plant canopy and fixed in a direction perpindicular to the bench. Conidia were impacted onto tapes coated with an adhesive mixture of petroleum jelly and paraffin (9:1, w/w) dissolved in sufficient toluene to give a thick liquid consistency. Tapes were removed weekly, cut into 48-mm lengths representing 24-hr periods, stained with aniline blue in lactic acid (28 mg aniline blue, 20 ml distilled water, 10 mg glycerol, and 10 ml 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 size, shape, color, surface texture, and characteristic crumpling. The numbers of conidia trapped during each 1-hr period were recorded. When conidial concentrations were exceptionally large (>3,000/m^3/hr), a portion of the 2-mm interval was counted and multiplied by the appropriate factor to provide an estimate of the concentrations for the 1-hr period. Counts were converted to numbers of conidia per cubic meter of air sampled per hour.

Incidence of stem blight was recorded from 19 May to 25 August 1987 for 12 randomly identified stock plants growing on the same bench as the spore trap. Two rows of plants along the perimeter of the bench served as a border to the monitored plants. Infected and noninfected wounded stems were counted for each plant. Presence or absence of sporulation on the blighted stem was noted, and the percentage of necrotic leaves at the base of each monitored plant with sporulating *B. cinerea* was estimated.

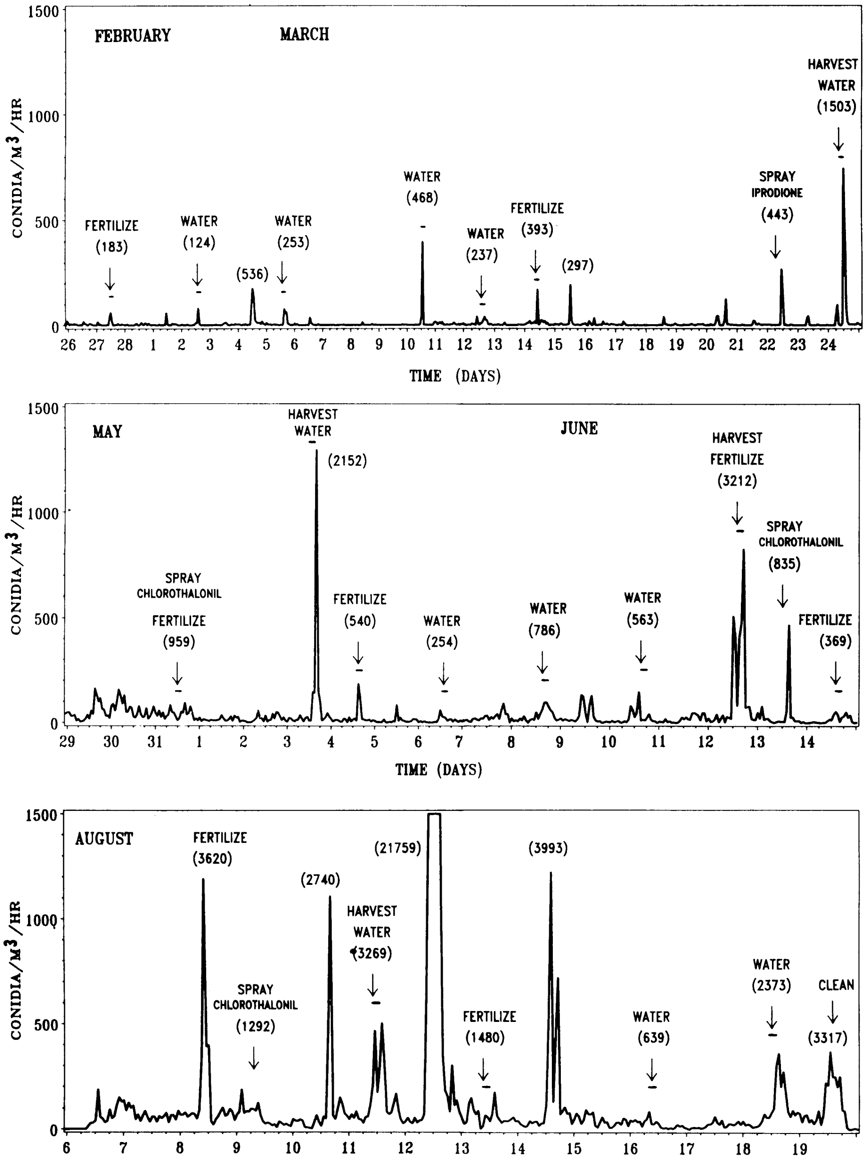

Fig. 1. Number of conidia of *Botrytis cinerea* trapped in hourly periods and corresponding grower activities within a geranium stock-plant growing area during 1986. Lines below arrows indicate recorded time and duration of the activity. Numbers in parentheses indicate number of conidia trapped that day.

RESULTS

Influence of grower activity. *1986 Growing season.* Harvesting was done on 24 March, 3 June, 12 June, and 11 August and was associated with peak conidial concentrations (PCCs) (>50 conidia per cubic meter per hour) ranging from 504 to 1,297/m^3/hr occurring within 1–4 hr after the onset of activity (Table 2, Fig. 1). The largest PCC associated with harvesting occurred on 3 June. All harvesting activities were immediately preceded by irrigation and fertilization.

Spraying of pesticide on 22 March and 13 June was associated with PCCs of 269 and 462/m^3/hr, respectively (Table 2, Fig. 1). Although daily conidial concentrations (DCCs) were relatively large in association with spraying of pesticides on 31 May (959/m^3) and 9 August (1,292/m^3), discrete PCCs differing from background concentrations of conidia were lacking. Fertilization also was done on 31 May.

During February and March, PCCs occurred within 1–3 hr after the onset of irrigation and fertilization and ranged from 63 to 746/m^3/hr (Table 2, Fig. 1). During June, PCCs typically occurred within 1 hr after the onset of irrigation and fertilization and ranged from 54 to 1,297/m^3/hr. The largest PCC associated with irrigation and fertilization occurred on 3 June. During August, PCCs occurred within 1–5 hr after the onset of irrigation and fertilization and ranged from 87 to 1,191/m^3/hr. A discrete PCC differing from background concentrations of conidia was lacking in association with irrigation and fertilization on 31 May, although a relatively large DCC of 959/m^3 occurred. Pesticides were also sprayed on 31 May. A DCC of 237/m^3 was associated with irrigation and fertilization on 12 March, although a PCC was absent.

The growing season effectively ended on 19 August and growers began to remove stock plants from the greenhouse for disposal. A PCC of 370/m^3/hr was associated with this activity (Fig. 1).

1987 Growing season. Harvesting of cuttings from geranium stock plants was associated with several PCCs (Table 2, Fig. 2). Prior to June, PCCs associated with harvesting ranged from 65 to 452/m^3/hr. During June, PCCs associated with harvesting of cuttings increased and ranged from 1,821 to 11,368/m^3/hr. The largest PCC associated with harvesting of cuttings occurred on 26 June. Although DCCs of 81 and 180/m^3 were associated with harvesting on 6 March and 14 May, respectively, PCCs were not observed.

PCCs were also associated with spraying of pesticides (Table 2, Fig. 2). Prior to 3 July, PCCs associated with spraying of cuttings were less than 208/m^3/hr, with the exception of a relatively large PCC (1,659/m^3/hr) that occurred on 9 April. On 28 March, a PCC occurred within 1 hr after spraying of pesticides and fertilizing. The largest PCC (13,587/m^3/hr) observed during 1987 was associated with spraying of pesticides on 3 July. Sprays on 12 and 27 June followed days during which large numbers of conidia had been trapped and were associated with PCCs smaller (<210/m^3/hr) than those associated with other activities during that sampling period. Although DCCs (23–290/m^3) were associated with spraying of pesticides on 21 March, 4 and 22 April, and 8 May, PCCs were not observed.

A PCC in association with irrigation and fertilization was first observed on 28 March (Table 2, Fig. 2). Pesticides were also sprayed that day. During April, PCCs associated with irrigation and fertilization typically occurred within 1–4 hr. During May and June, PCCs associated with irrigation and fertilization typically occurred within 3–6 hr. The largest PCC (4,353/m^3/hr) associated with irrigation and fertilization during 1987 occurred on 17 June. Fertilization of plants on 18 April and 2 and 12 May was not associated with discrete PCCs. DCCs (127–194/m^3) were observed on 11 and 29 April and 23 May in association with fertilization, although discrete PCCs were not observed.

Influence of fungicide applications. *1986 Growing season.* Prior to an application of iprodione on 22 March, the maximum DCC was 536/m^3 (Fig. 1). The DCC increased to 1,503/m^3 by 24 March. Between 29 May and 14 June, the maximum DCC increased further to 3,212/m^3 despite an application of chlorothalonil and iprodione for control of *B. cinerea* in April and weekly applications of chlorothalonil or iprodione in May (Table 1). Following two applications of chlorothalonil in August, DCCs continued to be large, with a maximum DCC of 21,759/m^3 on 12 August.

1987 Growing season. Prior to an application of chlorothalonil on 4 April, the maximum DCC was 5,616/m^3 (Fig. 2). By 9 April, the maximum DCC was 6,395/m^3. Between 9 April and 25 May, two applications of chlorothalonil and one application of iprodione were made and DCCs were relatively low (<2,157/m^3). During the sampling periods of 10–18 June and 24 June to 5 July, however, DCCs increased, reaching a max-

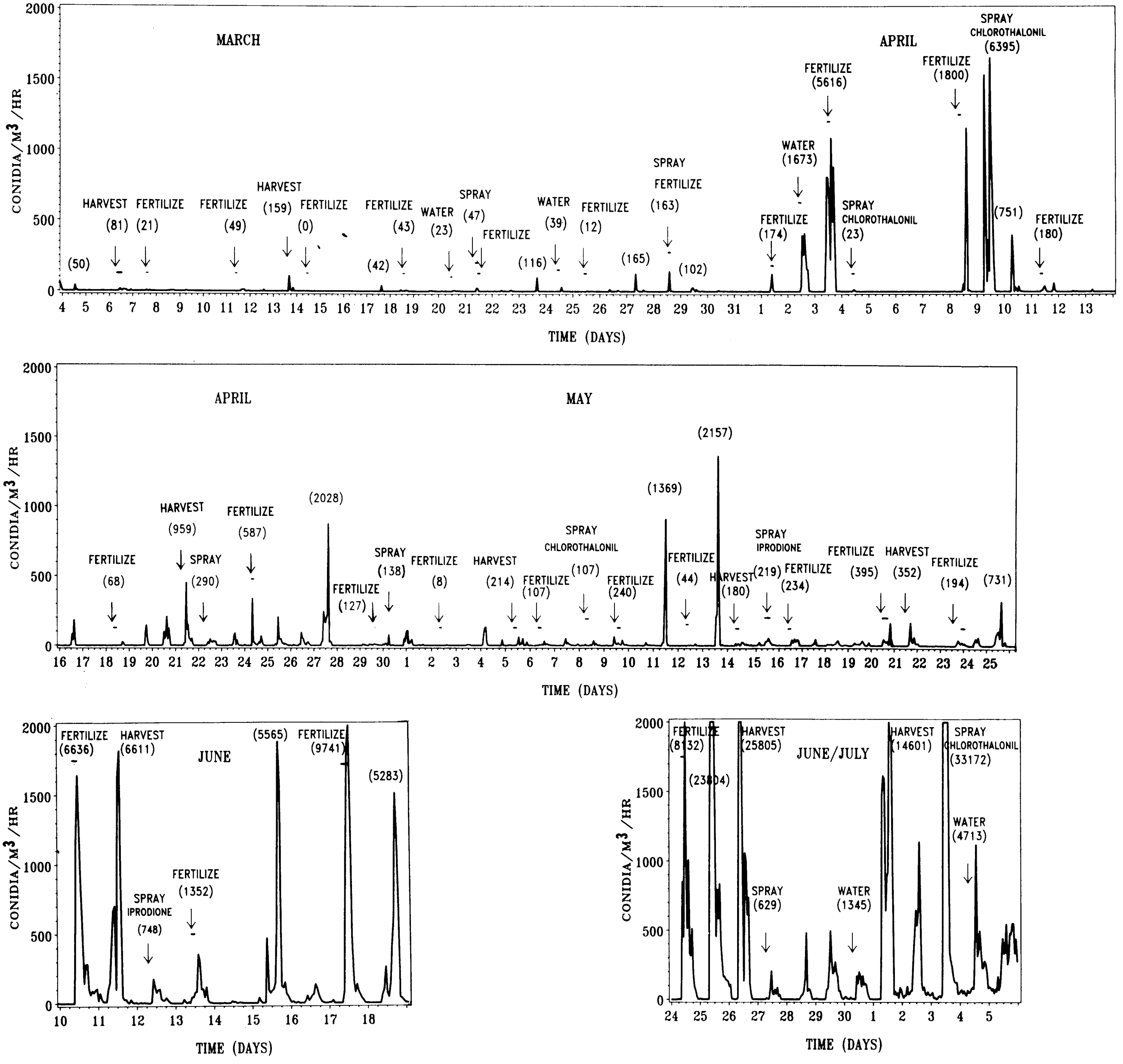

Fig. 2. Number of conidia of *Botrytis cinerea* trapped in hourly periods and corresponding grower activities within a geranium stock-plant growing area during 1987. Lines below arrows indicate recorded time and duration of the activity. Numbers in parentheses indicate number of conidia trapped that day.

imum of 33,172/m^3 on 3 July despite applications of chlorothalonil and iprodione on 6 and 12 June, respectively (Table 1, Fig. 2).

Influence of disease incidence. *1987 Growing season.* An increase in DCCs coincided with increases in stem blight incidence and percentages of stems and necrotic leaves with sporulating lesions. On 19 May and 2 June, the incidences of blighted stems (24–27%) and of stems (7–16%) and necrotic leaves (5–11%) with sporulating lesions were at their minimum (Fig. 3). Corresponding DCCs did not exceed 800/m^3. The incidences of stem blight (54%) and of stems (44%) and necrotic leaves (15%) with sporulating lesions were greater on 16 June, and corresponding DCCs frequently exceeded 5,000/m^3 despite applications of fungicides on 6 and 12 June (Table 1). The incidences of blighted stems (71%) and of stems (64%) and necrotic leaves (88%) with sporulating lesions were greatest on 14 July. Although observations of concentrations of airborne conidia did not extend beyond 5 July, DCCs during 24 June to 5 July were generally larger (629–33,172/m^3) than those during previous sampling periods (748–9,741/m^3).

DISCUSSION

Airborne conidia of *B. cinerea* were present in the stock-plant growing area throughout the monitored periods of 1986 and 1987. A primary factor influencing the occurrence of PCCs in this study was grower activity, including irrigation and fertilization by a plastic tube drip system with emitters that prohibited splashing, spraying of pesticides, and harvesting of cuttings.

The association of PCCs with harvesting of cuttings from geranium stock plants in this study was similar to conidial fluctuations observed among raspberries (3) and strawberries (2) during harvesting. PCCs observed during the harvesting of geranium cuttings may have resulted from mechanical action releasing and dispersing conidia from sporulating colonies of *B. cinerea* on blighted stems and necrotic leaves. These PCCs may have also resulted from a decrease in relative humidity (RH) within the microclimate because of canopy disturbance. Jarvis (1) observed that release of *B. cinerea* conidia is maximum when the RH is rising or falling rapidly between the limits of 85 and 65%. Only a 5% change in RH within this 20% range is necessary for vigorous hygroscopic movement of the conidiophores resulting in conidial release. Jarvis (5) thought that the RH beneath the leaf canopy and around the sporulating sites of strawberries was constantly high until harvesters disturbed the foliage, causing a sudden drop in RH and thereby releasing and dispersing the conidia. Unfortunately, our attempts to investigate the influence of grower activity on the microclimate were unsuccessful because we could not maintain the necessary sensitive equipment in a commercial setting.

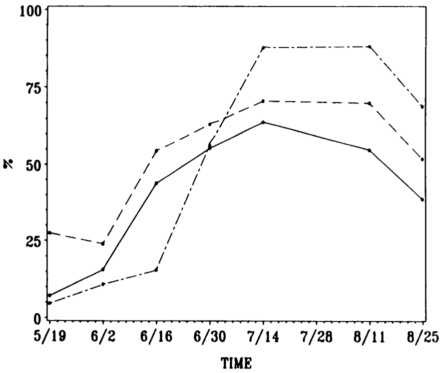

Fig. 3. Stems infected by *Botrytis cinerea* (dashed line) and stems (solid line) and necrotic leaves (dotted line) with sporulating lesions from 19 May to 25 August 1987.

The occurrence of PCCs during and immediately after harvesting of cuttings is an important consideration in disease management because cut stems on stock plants are susceptible to infection by *B. cinerea*. Also, conidia impacted on leaves of cuttings during harvest may germinate and infect the leaf tissue under suitable environmental conditions during propagation. The wet and humid conditions established for optimum propagation promote germination of conidia and favor expansion and coalescence of lesions, resulting in dieback and collapse of entire leaves and enhancing progression of cutting rot. Results from this study suggest that such disease management strategies as applying a fungicide or modifying the environment should be used immediately after harvest to inhibit and reduce conidial germination on stock plants and the cuttings harvested from stock plants.

PCCs associated with spraying stock plants with pesticide may have resulted from spray droplets dispersing dry conidia on air shock waves and turbulent currents in a manner similar to the mechanism observed with splash droplets (4). Also, composite projectiles of conidia and spray droplets might have formed in which dry conidia coated the spray droplets (4). In addition to mechanical shock, spray droplets may have released conidia by causing a rapid increase and subsequent decrease in the RH within the plant microclimate.

The association of PCCs with irrigation and fertilization was unexpected because the plastic tube drip irrigation system had emitters that prohibited splashing. Perhaps irrigation or fertilization caused a rapid increase and subsequent decrease in the RH within the stock plant microclimate, thereby resulting in conidial release as discussed previously. Also, the movement of the grower within the greenhouse during irrigation and fertilization may have resulted in mechanical action releasing and dispersing the conidia.

The magnitude of DCCs and PCCs increased during the growing season and appeared to be related to the incidences of blighted stems and of stems and necrotic leaves with sporulating lesions. Similarly, Jarvis (2) concluded that the number of airborne conidia of *B. cinerea* during picking of strawberries was related to the number of affected berries beneath the canopy. In general, DCCs and PCCs among geranium stock plants remained relatively low until the plants had been established for 6–7 mo. As the stock plants matured, the number of wounds on stems was increased by each harvesting and the lower leaves became senescent in the reduced light intensity below the dense canopy. Historically, wounded and senescent plant tissues have been considered favorable infection and sporulation sites for *B. cinerea.* In the greenhouse where this study was done, wounded stems and senescent leaves were readily infected by *B. cinerea*, which became established in the senescent lower leaves. Saprophytic establishment and subsequent sporulation early in the growing season apparently provided inoculum for stems wounded during harvest.

Increased spacing between the plants would result in a less dense plant canopy and allow better light penetration, thereby reducing senescence of the lower leaves and removing potential infection sites. Also, a less dense plant canopy would allow better air circulation and result in a less suitable environment for germination, infection, and sporulation of conidia of *B. cinerea* (12). Finally, a less dense plant canopy would allow better fungicide coverage of wounded stems and of sporulating stems and senescent lower leaves.

The effect of a commercial fungicide spray program for control of *B. cinerea* conidial concentrations could not be determined because a control treatment was not available for comparison. However, the increase in DCCs and disease incidence as the growing season progressed, despite frequent applications of fungicides, warrants a critical examination of the current role of fungicides in control programs. 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 dicarboximides, including iprodione and vinclozolin, has been documented since the late 1970s (8). *B. cinerea* isolated from geraniums grown in the commercial greenhouses of the propagator where this study was done was found to be resistant to vinclozolin and the benzimidazole fungicide benomyl (6).

Floricultural crops are intensively managed, typically requiring the frequent involvement of growers. This study identified grower activity as a primary factor in the occurrence of PCCs among geranium stock plants. Although factors such as RH, temperature, vapor pressure deficit, and irradiance may ultimately determine the magnitude of PCCs, the role of grower activity in the occurrence of PCCs is an important consideration in disease control. The relatively large PCCs 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. Further, cuttings removed from the stock plants are frequently exposed to large conidial concentrations that may influence disease occurrence in the propagation greenhouse. Information from this study should be helpful in timing fungicide applications to maximize their effectiveness, potentially reducing the number of applications necessary and reducing the threat of fungicide resistance.

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