Epidemiology and Management of Petunia and Tomato Late Blight in the Greenhouse

M. C. Becktell, Department of Plant Pathology, Cornell University, Ithaca, NY 14853; M. L. Daughtrey, Department of Plant Pathology, Long Island Horticultural Research and Extension Center, Cornell University, Riverhead, NY 11901; and W. E. Fry, Department of Plant Pathology, Cornell University, Ithaca, NY 14853

ABSTRACT

Becktell, M. C., Daughtrey, M. L., and Fry, W. E. 2005. Epidemiology and management of petunia and tomato late blight in the greenhouse. *Plant Dis.* 89:1000-1008.

Factors affecting the management of petunia and tomato late blight, caused by *Phytophthora infestans*, under greenhouse conditions were investigated. Late blight-infected petunias (*Petunia* × *hybrida*) and tomatoes (*Lycopersicon esculentum*) each produced sporangia that were dispersed throughout the greenhouse via air currents. Infected petunias produced and released fewer sporangia than infected tomatoes, but infected petunias released sporangia two times longer. Surface-directed irrigation reduced disease incidence compared with overhead irrigation that wetted the foliage. The fungicides dimethomorph-mancozeb, fosetyl-Al, azoxystrobin, and dipotassium phosphonate/phosphate suppressed late blight development, as did the plant defense activator acibenzolar-S-methyl. All products were applied twice at 7-day intervals. The other plant defense activator (harpin protein) and the bioantagonists (*Trichoderma harzianum*, *Gliocladium virens*, and *Bacillus subtilis*) were ineffective at the rates tested.

Additional keywords: Burkard spore trap, cultural controls, phytotoxicity

Petunias and tomatoes are two common solanaceous greenhouse crops, both of which are susceptible to *Phytophthora infestans*, the causal agent of late blight. Since the first reports of petunia late blight in 1856 (17), occurrence has been sporadic, leading to the perhaps false assumption that it is not a serious disease in commercial bedding plant production. Tomatoes, in contrast, are generally regarded as highly susceptible to late blight, and the disease is of great concern in the vegetable production industry. Recent disease outbreaks suggest that petunias could have an important role in the epidemiology of late blight in the greenhouse, possibly affecting vegetable crops. During a widespread tomato late blight epidemic in 1998 (13), a commercial production greenhouse in California lost both petunias and tomatoes to late blight. Subsequently, there were cases of late blight on field tomatoes in New York for which late blight-affected petunias might have been the source of inoculum (21). In 2003, *P. infestans* was confirmed on petunias

shipped into the United States from Israel (M. C. Becktell and W. E. Fry, *unpublished results*). Most recently, Deahl and Fravel (11) reported that a commercial grower in Maryland experienced significant losses of a petunia crop when late blight spread quickly throughout the greenhouse from a single inoculum source of infected petunias. These cases indicate that late blight-infected petunias as well as late blight-infected tomatoes could serve as sources of inoculum to other tomatoes and petunias in the greenhouse.

In greenhouse crop production, there are three major categories of irrigation: overhead, surface-directed, and sub-irrigation. Overhead watering results in more wetting of the foliage than either surface-directed or sub-irrigation. Previous results (5) have shown that late blight infection on petunias and tomatoes requires as little as 2 h of leaf wetness, a condition common on overhead-watered plants.

The production of petunias and tomatoes in the enclosed space of a greenhouse makes it possible to adjust cultural practices to avoid conditions that favor disease development and spread. However, cultural control may not always be sufficient; additional strategies such as the use of biological or chemical control agents may be necessary. A few products are labeled for use against tomato late blight, but none has been tested on or labeled for use against petunia late blight. Because petunias, like most ornamental crops, require frequent watering, fertilizing, cleaning, and spacing by greenhouse personnel, there is a need

for products with minimal reentry interval (REI) requirements and low toxicity. Biological controls and alternative, low-risk fungicides have been somewhat successful in controlling potato and tomato late blight in greenhouse and growth chamber experiments (3,7–10,20,22) and have had mixed success in the field (3,10,18; H. Mayton and W. E. Fry, *unpublished data*). The efficacy of these compounds in suppressing petunia late blight has not been investigated.

The objectives of this study were: (i) to compare the roles of infected petunias and infected tomatoes as sources of inoculum for petunias or tomatoes in the greenhouse; (ii) to compare the effects of foliage-wetting overhead irrigation to non-foliage-wetting surface-directed irrigation on late blight incidence and severity; and (iii) to determine the efficacy of several fungicides, biological controls, and plant defense activators for the suppression of petunia and tomato late blight in the greenhouse.

MATERIALS AND METHODS

Plant material. Petunias (*Petunia* × *hybrida*, cv. White Madness) were seeded into flats (25.4 × 50.8 cm) containing eight “six-packs”; each six-pack ultimately contained 12 to 30 plants depending on the germination rate. Tomatoes (*Lycopersicon esculentum* cv. Sunrise) were seeded into flats (25.4 × 50.8 cm) containing 24 individual cells; each cell contained two plants. Seeds were planted in the greenhouse in a soilless mix (Cornell mix) consisting of 1:2:2 (vol/vol) perlite, peat, and vermiculite with 4 kg of dolomitic limestone, 0.33 kg of nitrogen (N) and potassium (K₂O), and 0.16 kg of phosphate (P₂O₅) per cubic meter of mix. Natural sunlight was supplemented with 400W high-pressure sodium lamps on a 12-h day/night schedule. Temperatures were maintained between 25 and 30°C. The plants were fertilized once or twice a week with liquid fertilizer containing 200 to 300 ppm nitrogen.

***P. infestans* isolates and inoculum preparation.** Isolate US970001 of the US-17 clonal lineage (mating type A1) was used for all inoculations; the isolate was originally isolated in 1996 from tomatoes in Florida, and subsequently maintained in the culture collection at Cornell University on rye agar medium (6). Previous work in our lab had demonstrated this isolate’s

Corresponding author: Margot Becktell
E-mail: mbecktel@mesastate.edu

Current address of M. C. Becktell: Department of Biological Sciences, Mesa State College, Wubben 176, 1100 North Ave., Grand Junction, CO 81501.

Accepted for publication 20 May 2005.

DOI: 10.1094/PD-89-1000
© 2005 The American Phytopathological Society

reliability in terms of sporulation in culture and on tomato leaflets; it also was consistently pathogenic on both petunias and tomatoes (4). Sporangia produced on detached 'Sunrise' tomato leaflets were used as inoculum. Sporangia were rinsed from the late blight lesions into 100 ml of distilled water and the concentration of sporangia determined with a hemacytometer (average of three readings) and adjusted as needed. Sporangial suspensions were incubated at 4°C for 1 to 2 h to induce zoospore production. Plants were inoculated to runoff with a hand-held sprayer.

Petunia and tomato late blight in the greenhouse. *Greenhouse environment.* Experiments were conducted between February and April 2003 in a 7.3 × 15.2 × 4.9 m (543 m³), air-inflated, double polyethylene-covered greenhouse with a crushed slate floor. Natural sunlight was supplemented with General Electric low profile 400W high-pressure sodium lights on a 12-h day/night schedule. A moderate level of relative humidity throughout the greenhouse was achieved by maintaining pots of annual rye grass on empty benches, placing damp bales of hay throughout the greenhouse, watering the floor every morning and every evening, and constantly running several Trion model 500 automatic humidifiers (Hummert International, Earth City, MO). The temperature and relative humidity (RH) in the greenhouse—above the plant canopy and within or just above the plant canopy—were monitored with Hobo H8 Pro RH/Temp data loggers (Onset Computer Corporation, Bourne, MA).

Petunias and tomatoes as sources of inoculum. Four trials were conducted, two with infected petunias as a source of inoculum and two with infected tomatoes as a source of inoculum. In each of the four trials, infected plants were placed on two benches (12 flats of petunias or tomatoes per bench) at the end of the greenhouse closest to the intake vents and farthest from the exhaust fan, so that the inoculum coming off the source plants would be dispersed throughout the greenhouse by the ventilation air stream. Tel-Tru T-T smoke sticks (E. Vernon Incorporated, Rohnert Park, CA) were used to check for efficient air circulation.

Inoculum source plants had sporulating lesions when placed in the greenhouse; the disease severity (proportion of plant area infected) on each host was between 10 and 25%; however, because tomato leaves are much larger than petunia leaves, and because lesions on tomatoes are larger, the total lesion area on tomatoes was much larger than the total lesion area on petunias. Healthy plants were spray inoculated to runoff with a sporangium/zoospore suspension (20,000 sporangia per ml) and incubated at 15°C and 100% RH for 4 to 5 days to produce the inoculum source plants. Continuous sporulation of the source plants in the greenhouse was

achieved by maintaining 100% RH each night. Plastic tents were placed over the source plants at night and removed in the morning; household sonicating humidifiers operated periodically throughout the night inside the tents.

Target plants were monitored daily (from 5 to 19 days after introduction of source plants) for the presence of disease. The target plants consisted of four to six flats of disease-free petunias and four to six flats of disease-free tomatoes on each of four tables in the greenhouse. Individual

six-packs (petunias) or individual cells (tomatoes) were used as experimental units. Disease incidence (proportion of units infected) and severity on individual units were recorded.

Sporangia release and dispersal. The number of sporangia in the greenhouse air was continuously monitored using a Burkard 7-day volumetric spore trap (Burkard Manufacturing Company, Rickmansworth, England). The Burkard trap was situated approximately 6 feet from the infected source plants and calibrated to

Table 1. Products and rates tested for phytotoxic effects and suppression of late blight on petunias and tomatoes in the greenhouse

Product name (company)	Active ingredient/organism	Abbrev	REI (h) ^a	Rate (per liter)
Fungicides				
Stature WP (SePro Corp.)	Dimethomorph/mancozeb	DM/MZ	24	2.1 g
Aliette 80 WDG (Bayer Crop Sci.)	Fosetyl-Al	FOS-Al	24	3.17 g
Heritage T/O (Syngenta Crop Prot.)	Azoxystrobin	AZOX	4	0.22 g
Sporan (EcoSMART Technol.)	Rosemary/wintergreen oils	RWG	0	15 ml
ZeroTol (BioSafe Systems)	Hydrogen dioxide	HD	0	3.91 ml
MilStop (BioWorks)	Potassium bicarbonate	PBI	0	5.98 g
Biophos foliar (AgBio)	Dipotassium p/p ^b	DPPf	12	10 ml
Biophos drench (AgBio)	Dipotassium p/p ^b	DPPd	12	5 ml
Bioantagonists				
Plant Shield HC (BioWorks)	<i>Trichoderma harzianum</i>	T22	0	3.7 g
G41 (BioWorks, Inc.)	<i>Gliricladium virens</i>	G41	0	0.15 g
Rhapsody AS (AgraQuest)	<i>Bacillus subtilis</i> QST-173	BSUB	4	9.92 ml
Plant defense activators				
Actigard (Syngenta Crop Prot.)	Acibenzolar-S-methyl	ASM	12	0.075 g
Messenger (Eden Biosci. Corp.)	Harpin protein	HRP	4	0.672 g
Controls				
Triton X-100 ^c	Polyethylene glycol	PG	...	0.5 ml
Water control		WC	...	
Nonsprayed control		UC	...	

^a Reentry interval (hours).

^b Dipotassium phosphonate/phosphate.

^c Used as a surfactant for *Trichoderma* and *Gliricladium* applications.

Table 2. Temperature and relative humidity conditions present in the greenhouse above and within plant canopies during four epidemics of petunia and tomato late blight

Inoculum source and location of sensor	Date	Temperature (°C)		Relative humidity	
		Mean ^a	Range	Mean ^a	Range
Infected petunias trial 1	2 Feb – 14 Feb				
Air above canopy ^b		15 x	11-28	56 x	25-85
Plant canopy/OH ^c		13 y	5-26	85 y	10-100
Plant canopy/S ^d		14 x,y	8-24	82 y	27-100
Infected petunias trial 2	11 Mar – 21 Mar				
Air above canopy		19 x	9-38	62 x	12-100
Plant canopy/OH		16 y	5-32	96 y	23-100
Plant canopy/S		17 y	10-38	95 y	36-100
Infected tomatoes trial 1	21 Feb – 9 Mar				
Air above canopy		17 x	9-35	54 x	14-100
Plant canopy/OH		15 y	3-35	88 y	9-100
Plant canopy/S		16 y	3-37	76 y	16-100
Infected tomatoes trial 2	26 Mar – 6 Apr				
Air above canopy		21 x	4-37	69 x	24-100
Plant canopy/OH		18 x	7-40	98 y	9-100
Plant canopy/S		19 x	11-42	90 z	13-100

^a Mean temperature and relative humidity over the period of the trial calculated from data collected every 2 to 5 min by a Hobo H8 Pro RH/Temp data logger. Values within the same column and trial followed by the same letter are not significantly different at $P = 0.05$, according to Fisher's protected least significant difference test.

^b Conditions of the air in a central location approximately three feet above the level of the plant canopy.

^c Conditions of the air within the plant canopy of the two tables that were overhead (OH) watered.

^d Conditions of the air within the plant canopy of the two tables that were watered via surface-directed (S) irrigation.

sample at a rate of 10 to 11 liters/min (0.4 ft³/min; 0.6 m³/h). Following each collection period (7 days), the spore trapping tape (Melinex tape greased with a 10:1 high vacuum grease:98.5% hexane mixture) was removed from the drum, marked off at 24-h intervals (24, 2-mm increments based on a drum speed of 2 mm/h), and placed onto 7.6 × 2.5 cm microscope slides. The slides were examined at ×10 with an Olympus BH-2 compound microscope (Olympus America, Inc., Melville, NY). The number of sporangia present in the greenhouse at different times over the course of each trial was estimated based on the volume of air contained in the greenhouse (407 m³), the average number of sporangia collected per hour by the Burkard trap (based on spore counts at-

tained from the spore trap tape), and the volume of air collected per hour by the Burkard trap (0.6 m³/h).

Inoculum in the greenhouse was also monitored by observing sporangium deposition onto microscope slides. Glass microscope slides (7.6 × 2.5 cm) coated with high vacuum grease were placed on several tables throughout the entire greenhouse. Fresh slides were set out daily. Each slide was examined at ×10 on a compound microscope, and the number of sporangia were counted and recorded.

Overhead versus surface-directed irrigation. The effect of two different watering techniques on disease development was determined. All plants were hand watered in the morning and evening as needed; plants on two benches were overhead wa-

tered and plants on two other benches were watered via surface-directed watering to provide a contrast with watering that wets the foliage. Common, non-foliage-wetting alternatives to overhead watering include drip-irrigation or sub-irrigation. However, because it was not feasible to set up a drip or sub-irrigation system for this experiment, we employed a surface-directed watering method in which the water was delivered at the surface of the planting mix at the base of the plants.

Products tested for the suppression of petunia and tomato late blight. *Phytotoxicity trials.* Several fungicides, bioantagonists, and plant defense activators were first evaluated for phytotoxic effects on both petunias and tomatoes when applied in the greenhouse. Petunia and tomato trials were conducted simultaneously but were spatially separated. The experiment was conducted twice between October and December 2003. The experiment had a randomized block design consisting of three blocks, with a single replicate of each treatment (one treated flat) in each block. Six fungicides, three bioantagonists, and two plant defense activators were tested on 4- to 5-week-old plants. Each product was applied twice 7 days apart. Plants were sprayed to runoff at label-recommended rates (Table 1). To avoid spray drift between treatments, all of the flats receiving a particular treatment were removed from their blocks, sprayed, and returned to their previous locations. Plastic tents were

Table 3. Effect of inoculum source plant on disease incidence in the greenhouse

	Disease incidence on healthy target plants ^a		Disease severity on healthy target plants ^a	
	Petunia	Tomato	Petunia	Tomato
Inoculum source plant (trial)				
Infected petunias (trial 1) ^b	0	0	0	0
Infected petunias (trial 2)	10 ± 5	42 ± 13	0.1 ± 0	5 ± 0
Infected tomatoes (trial 1)	2 ± 1	31 ± 10	0.1 ± 0	1 ± 0.7
Infected tomatoes (trial 2)	7 ± 2	81 ± 9	2 ± 1	22 ± 12
Overall	7 ± 2	51 ± 8	0.6 ± 0.4	9 ± 5

^a Average disease incidence (percentage of plants infected) or severity (percentage of plant area infected) on infected plants ± standard error of the mean.

^b Disease only occurred on tables where infected plants were immediately adjacent to healthy plants; these tables were not included in this analysis.

Fig. 1. Sporangia released from infected source plants and deposition of sporangia throughout the greenhouse when late blight infected petunias (**A and C**) and late blight infected tomatoes (**B and D**) were used as sources of inoculum. **A and B,** Each data point represents an estimation of the total number of sporangia in the entire greenhouse area (543 m³) over the course of one epidemic and assumes a homogeneous mixture of air and sporangia throughout the greenhouse. **C and D,** Each data point represents the total number of sporangia collected on 7.6 × 2.5 cm greased slides over the course of one epidemic located at various distances from source plants.

placed over plants at night to maintain high humidity conditions; humidifiers (Hummert International) operated periodically throughout the night inside the tents. Relative humidity was monitored with a Hobo H8 Pro RH/Temp data logger (Onset Computer Corporation).

All products were mixed with distilled water and delivered with a hand-held, CO₂-pressurized boom sprayer with a single XR11003VS flat fan nozzle (R & D Sprayers, Inc., Opelousas, LA). Phytotoxicity ratings were performed 7 days after each treatment. The phytotoxicity rating scale was adapted from Georgis (14): 0 = 0%, perfect plant; 1 = <2% of plant area affected, plants still marketable; 2 = 2 to 4% of plant area affected, plants have reduced marketability; 3 = 4 to 6% of plant area affected, plants not marketable; 4 = >6% of plant area affected, plants not marketable.

Efficacy trials. All products were tested for efficacy in suppressing late blight on petunias and tomatoes. The experiment was conducted twice. The experimental design, fungicide application procedure, spray schedule, and nightly humidity conditions were as described for the phytotoxicity trial. Because the rosemary/wintergreen oil treatment (Sporan; EcoSMART Technologies, Longwood, FL) resulted in some phytotoxicity on both petunias and tomatoes at the full rate (see Results), half the recommended rate (which did not produce phytotoxic effects) was used. Two to three days after the second spray application, plants were inoculated to runoff with a zoospore suspension of *P. infestans* (4,000 sporangia per ml) using a hand-held sprayer. Each flat was rated for disease severity 7 days after inoculation (DAI).

In anticipation that the fungus *Trichoderma harzianum* (Plant Shield HC T22, BioWorks, Inc., Fairport, NY), the fungus *Gliocladium virens* (G41, BioWorks, Inc., Fairport, NY), or the bacterium *Bacillus subtilis* (Rhapsody AS, AgraQuest Inc., Davis, CA) might not control late blight on petunias or tomatoes, steps were taken to verify the presence of bacteria on plants treated with *B. subtilis* and the presence of fungi on plants treated with either *T. harzianum* or *G. virens*. Leaf samples were taken 2 days after each application of biological materials and 2 days after inoculation with *P. infestans*. The leaf samples were rinsed in 30 ml of distilled water, and four concentrations (nondiluted, 1:10, 1:100, and 1:1,000) of each sample were plated onto three replicate plates of medium. To detect fungi (*Trichoderma* or *Gliocladium*), samples were plated on selective acidified, potato dextrose/igepal (API) medium consisting of potato dextrose agar (39 g/liter), igepal (1 ml/liter; Sigma-Aldrich, St. Louis, MO), and 10% tartaric acid (added after autoclaving; 17 ml/liter; VWR International, Westchester, PA). Inoculated plates were incubated in a

28°C incubator for 48 to 72 h before colonies were counted. The *Trichoderma* colonies were characterized by green concentric circles of growth, while the *Gliocladium* colonies were orange. To detect bacteria (*Bacillus*), samples were plated onto tryptic soy agar medium (TSA) consisting of 40 g of TSA/liter and incubated at room temperature (23 to 25°C) for 24 h before colonies were counted. The *Bacillus* colonies were differentiated from other bacteria by their colony morphology (undulated, dry edges and sacs of fluid).

Statistical analyses. All statistical analyses were performed using Statistical Analysis System 8.2 (SAS Institute, Cary, NC) or SPSS statistical software (SPSS Inc., Chicago, IL). The data from the petunia and tomato epidemics (source host, target host, watering regime, and spore release) were analyzed using a one-way analysis of variance (ANOVA) or the Mann-Whitney test. When the assumption of homogeneity of variance was met (with original data or transformed data), an ANOVA was performed. When the assumption of homogeneity of variance

could not be met, even after transformation, the data were analyzed using the non-parametric Mann-Whitney test.

The mean temperature and relative humidity during the petunia/tomato late blight epidemics were analyzed using an ANOVA. The ANOVA indicated a significant effect ($P < 0.001$) of location (above or within the plant canopy) on temperature and relative humidity within each epidemic; therefore, Fisher's protected LSD was used to compare the mean temperature and relative humidity and determine which treatments were significantly different from each other.

The general linear model (GLM) was used to determine whether the various control products had an effect on late blight disease severity on petunias and tomatoes. The petunia and tomato data were analyzed separately, but the two trials within the petunia experiment and the two trials within the tomato experiment were combined for analysis (there were no treatment-trial interactions). A significant ($P < 0.001$) treatment effect was indicated by the ANOVA; therefore, Fisher's pro-

Fig. 2. Daily sporangia release trends on sunny days (days 6 and 1) and cloudy days (days 9 and 2) during epidemics with late blight infected petunias (A) and late blight infected tomatoes (B) as the sources of inoculum. Bars represent total spores collected over a 1-h period (i.e., 830A to 930A) by the Burkard trap. In each case, the infected plants were enclosed in a humidity tent overnight; the tent cover was removed around 830A and replaced between 430P and 530P every evening.

tected LSD was used to compare the mean disease severity ratings and determine which treatments were significantly different from the controls.

RESULTS

Petunia and tomato late blight in the greenhouse. Greenhouse environment. The daily average temperatures in the greenhouse over the course of each trial were

quite stable (Table 2) and were within the range of temperatures previously determined to be optimal for petunia and tomato late blight development (5). However, the temperatures in the greenhouse ranged from 3 to 40°C.

The daily average relative humidities were influenced by the plant canopy (Table 2). Above the canopy, the average relative humidity ranged from 54 to 69%, whereas

within the canopy it ranged from 76 to 98%. In all four trials, the average relative humidity was significantly ($P < 0.02$) lower above the plant canopy than within the plant canopy.

Petunias and tomatoes as sources of inoculum. The type of host used as a source of inoculum did not have a significant effect on disease incidence or severity on healthy target petunias or tomatoes. The type of target plant had a significant effect on disease incidence ($P < 0.001$) and severity ($P = 0.006$); disease incidence and severity were higher on tomatoes than on petunias. The average disease incidence on tomatoes was $51 \pm 8\%$ compared with $7 \pm 2\%$ on petunias (Table 3). The average disease severity on infected tomatoes was $9 \pm 5\%$ compared with $0.6 \pm 0.4\%$ on petunias (Table 3).

Sporangia release and dispersal. Infected source tomatoes released more sporangia into the air than did infected source petunias ($P < 0.001$), but infected source petunias survived long enough to allow secondary infections that presumably contributed to an increased sporulation period (12 to 14 days) compared with tomatoes (7 days) (Fig. 1A and B). During peak sporangium release days, there were 80,000 sporangia in the greenhouse (greenhouse volume = 543 m^3) when petunias were the source of inoculum (day 6, Fig. 1A) compared with nearly 2,500,000 sporangia when tomatoes were the source of inoculum (day 1, Fig. 1B).

The smoke test indicated efficient air movement throughout the greenhouse. This was confirmed during the epidemics, as sporangia from both sources were dispersed well throughout the greenhouse (Fig. 1C and D).

Release of sporangia coincided with a large decrease in relative humidity. Large changes in humidity usually occurred in the morning, so more sporangia were released during the morning than the afternoon (Fig. 2). Additionally, more sporangia were released on sunny days when the temperature increased and the relative humidity decreased (day 6 and day 1; Figs. 2 and 3) than on overcast days when the overall changes in temperature and relative humidity were not large (day 9 and day 2; Figs. 2 and 3).

Overhead versus surface-directed irrigation. Disease incidence was consistently higher on plants that were overhead watered compared with plants that were watered via surface-directed watering. Petunias that were overhead watered had an average disease incidence of $6 \pm 3\%$ compared with $3 \pm 1\%$ disease incidence on petunias that were watered at the soil surface ($P = 0.03$) (Table 4). Tomatoes that were overhead watered had an average disease incidence of $68 \pm 12\%$ compared with $35 \pm 8\%$ on tomatoes that were watered at the soil surface ($P = 0.06$) (Table 4). In four of six comparisons, the disease

Fig. 3. Relative humidity (RH) and temperature trends just above the plant canopy of inoculum source plants on sunny days (days 6 and 1) and cloudy days (days 9 and 2) during epidemics with late blight infected petunias (A and B) and late blight infected tomatoes (C and D) as a source of inoculum. Note that the period of 100% RH observed in (C) may not reflect actual conditions, but rather saturation of the sensor and the time required for it to dry out.

incidence for overhead watered plants was at least twice as large as that for plants given surface-directed irrigation (Table 4). Watering technique did not have a significant effect on disease severity in three of the four trials; however, in one of the four trials (tomato trial 2) there was a trend ($P = 0.1$) toward increased disease severity on petunias and tomatoes that were overhead watered compared with plants that were watered via surface-directed irrigation (Table 5).

The relative humidity within the canopies of plants that were overhead watered tended to be higher than the relative humidity within the canopies of plants that were watered at the planting mix surface. The difference in relative humidity was significant in the tomato trials ($P < 0.05$), but was not significant in the petunia trials (Table 2).

Products tested for the suppression of petunia and tomato late blight. *Phytotoxicity trials.* Most products tested were not phytotoxic to either petunias or tomatoes. However, the rosemary/wintergreen oil product was phytotoxic at the recommended rate of 15 ml/liter. At this rate, petunias and tomatoes had reduced marketability and average phytotoxicity ratings of 2.16 and 2.77, respectively. Subsequently, treatments with one-half or one-quarter the recommended dosage were included, and there were no phytotoxic effects observed with these reduced dosages.

Efficacy trials. Overall, late blight was more severe on tomatoes than on petunias. The nonsprayed tomato controls had an average disease severity rating of 84% at 7 DAI; the nonsprayed petunia controls had an average disease severity rating of 23% at 7 DAI (Fig. 4). Four fungicides (dimethomorph/mancozeb, Stature WP, SePro Corporation, Carmel, IN; fosetyl-Al, Aliette 80 WDG, Bayer Crop Science, Kansas City, MO; azoxystrobin, Heritage T/O, Syngenta Crop Protection, Inc., Greensboro, NC; and dipotassium phosphonate/phosphate, Biophos, AgBio, Westminster, CO) significantly suppressed late blight on both petunias and tomatoes (Fig. 4). Dimethomorph/mancozeb and azoxystrobin suppressed disease to less than 1% on petunias; dimethomorph/mancozeb completely prevented late blight development on tomatoes; and azoxystrobin very nearly prevented disease on tomatoes (Fig. 4). Fosetyl-Al suppressed disease to less than 4% on petunias and to less than 50% on tomatoes (Fig. 4). The dipotassium phosphonate/phosphate drench suppressed petunia late blight to 10%, and the dipotassium phosphonate/phosphate foliar application suppressed tomato late blight to 50% (Fig. 4).

Of the two plant defense activators, only acibenzolar-S-methyl, also known as benzoethiadiazole (Actigard, Syngenta Crop Protection), significantly suppressed late

blight development on petunias and tomatoes. Acibenzolar-S-methyl completely prevented late blight development on petunias, and suppressed late blight development on tomatoes to less than 60% (Fig. 4). The second plant defense activator, harpin protein (Messenger, Eden Bioscience Corporation, Bothell, WA), had no suppressive effect on tomato late blight, but a slightly suppressive effect on petunia late blight (Fig. 4).

Analyses of bioantagonistic bacteria and fungi on the leaves indicated that foliage of plants treated with *Bacillus subtilis* had higher populations of *Bacillus* than did the nonsprayed control plants, and foliage of plants treated with *Trichoderma harzianum* and *Gliocladium virens* had higher populations of *Trichoderma* and *Gliocladium* than did the nonsprayed control plants (Fig. 5). Despite the presence of the bioantagonists, these treatments failed to suppress late blight on tomatoes; they were, however, slightly suppressive on petunias (Fig. 4).

DISCUSSION

Our results show that late blight infected petunias can be a source of inoculum to other petunias and tomatoes in the greenhouse. Late blight infected petunias released fewer sporangia than did late blight infected tomatoes: these results are consistent with the observations that lesion area on petunias is only 20% of that on tomatoes and petunia late blight lesions produce fewer sporangia per lesion than tomato late blight lesions (5). The infected source petunias did not succumb to infection to the extent that the tomatoes did and re-

leased sporangia for six additional days compared with the tomatoes. In addition, the sporangium release pattern from infected source petunias suggested a polycyclic disease cycle with secondary infections resulting in bursts of sporangium release, whereas the source tomatoes did not survive long enough to develop such a cycle. Despite the high numbers of sporangia released from the infected tomatoes and the extended sporangium release period from the infected petunias, there was not a significant difference in disease incidence or severity when infected petunias were the source of inoculum compared with when infected tomatoes were the source. In contrast, the type of healthy target plant did have a significant effect on disease incidence and severity. Disease incidence and severity were higher on tomatoes than on petunias. These results support previous conclusions that petunias are generally less susceptible than tomatoes (4,19).

Irrigation method strongly affected the epidemic. Disease incidence was higher on plants that were overhead watered, and there was also a tendency toward higher disease severity on plants that were overhead watered. In general, overhead irrigation created conditions of free moisture and higher relative humidity that were more conducive to disease development than did surface-directed watering. The difference in relative humidity created by the two watering techniques was greater on tomatoes than on petunias. However, given the difference in canopy structure (tomatoes have an open canopy compared to petunias), this difference is not surprising.

Table 4. Effect of watering technique on disease incidence in the greenhouse

	Disease incidence ^a on petunias		Disease incidence ^a on tomatoes	
	Overhead	Surface-directed	Overhead	Surface-directed
Inoculum source plant (trial)				
Infected petunias (trial 1) ^b	0	0	0	0
Infected petunias (trial 2)	19 ± 0.5	2 ± 0.7	63 ± 7	20 ± 9
Infected tomatoes (trial 1)	4 ± 0.3	1 ± 0.5	45 ± 16	18 ± 1
Infected tomatoes (trial 2)	9 ± 3	6 ± 3	96 ± 2	66 ± 0
Overall	6 ± 3	3 ± 1	68 ± 12	35 ± 8

^a Percentage of plants infected on two tables ± standard error of the mean.

^b Disease only occurred on tables where infected plants were immediately adjacent to healthy plants; these tables were not included in this analysis.

Table 5. Effect of watering technique on disease severity in the greenhouse

	Disease severity ^a on petunias		Disease severity ^a on tomatoes	
	Overhead	Surface-directed	Overhead	Surface-directed
Inoculum source plant (trial)				
Infected petunias (trial 1) ^b	0	0	0	0
Infected petunias (trial 2)	0.1 ± 0	0.1 ± 0	5 ± 0	5 ± 0
Infected tomatoes (trial 1)	0.1 ± 0	0.1 ± 0	1.5 ± 1.5	0.5 ± 0.4
Infected tomatoes (trial 2)	3.2 ± 2	0.1 ± 0	42 ± 11	2 ± 2
Overall	1.5 ± 0.8	0.1 ± 0	16 ± 9	2 ± 1

^a Average disease severity (percentage of plant area infected) on two tables during each epidemic ± standard error of the mean.

^b Disease only occurred on tables where infected plants were immediately adjacent to healthy plants; these tables were not included in this analysis.

Overhead irrigation that wets the foliage is commonly employed for petunia and tomato bedding plants. However, our results suggest that modification of this method of watering could be an important tool in the management of late blight in greenhouses, particularly for smaller growers who use hand watering.

This is the first report of sporangial release patterns from late blight infected petunias or tomatoes in the greenhouse. In this study, sporangial release increased as relative humidity decreased, with the peak release period occurring by noon and decreasing thereafter. This pattern of release

was most apparent on sunny days, when there was a greater change in humidity than on cloudy days. The results of this study agree with work that has been done with potato late blight in the field, where the concentration of sporangia released decreased after noon and with decreasing relative humidity (2,15,16).

Because cultural control strategies alone may be insufficiently effective in preventing disease, several disease control products were evaluated for effectiveness against late blight. Of the 12 products tested in these trials, five (azoxystrobin, dimethomorph/mancozeb, fosetyl-Al, di-

potassium phosphonate/phosphate and acibenzolar-S-methyl) provided statistically significant disease reduction (compared to the nonsprayed controls) on both petunias and tomatoes. The remaining seven products showed little to no efficacy against petunia and tomato late blight. The lack of control provided by the bioantagonists was not because they failed to survive on leaf surfaces, as they appeared to be present in increased numbers on the treated versus control plants.

Given consumer demand for blemish-free plants and the potential devastation that could occur if late blight spread from petunias or tomatoes in the greenhouse to tomatoes or potatoes in nearby fields, there is a very low threshold for disease in the greenhouse. In this study, using conditions very favorable to the pathogen, only three products (azoxystrobin, dimethomorph/mancozeb, and acibenzolar-S-methyl) provided a practical and desirable level of late blight control that might be sufficient when combined with scouting and roguing out visibly diseased plants.

The complete suppression of late blight on petunias, but not tomatoes, treated with the plant defense activator acibenzolar-S-methyl is an intriguing result. While the mechanisms of action of acibenzolar-S-methyl (ASM) on petunia are not known at this time, there is information about its mode of action on other solanaceous plants. ASM induces the expression of pathogenesis related (PR) genes in tomatoes and in tobacco; in tobacco, the PR gene expression results in the development of systemic acquired resistance (SAR) against oomycetes (1,12). Therefore, we can speculate that ASM might activate PR genes and SAR in petunias as it does in tobacco and that SAR may play a role in protecting petunias against late blight. A profile of the genes expressed in response to ASM in petunias would help answer this question. Furthermore, elucidating the pathway and genes induced by ASM in petunias could have important implications for the control of late blight on tomatoes and potatoes through plant breeding or genetic engineering.

The greenhouse experiments presented here indicate that even under variable greenhouse conditions, late blight on petunias and tomatoes can occur. The timing (February to April) and the environmental conditions of our greenhouse experiments coincide with the time frame and range of environmental conditions used in commercial production in the northeast, where it is not uncommon for tomatoes and petunias to be produced in the same greenhouse (M. C. Bechtell and W. E. Fry, *unpublished survey*). Therefore, growers should be aware of the possibility of petunia and tomato late blight outbreaks in the greenhouse and how to prevent and/or suppress the disease.

Based on the work presented here, we have identified several cultural control

Fig. 4. Effect of a variety of fungicides, bioantagonists, and plant defense activators on disease severity of late blight on petunias (A) and tomatoes (B). Error bars represent the standard error of three replicate flats and two trials (n = 6). * = significantly different from the controls at $P = 0.05$. DM/MZ = dimethomorph/mancozeb, FOS-Al = fosetyl-Al, AZOX = azoxystrobin, RWG = rosemary/wintergreen oil, HD = hydrogen dioxide, PBI = potassium bicarbonate, T22 = *Trichoderma harzianum*, G41 = *Glucoladium virens*, BSUB = *Bacillus subtilis*, ASM = acibenzolar-S-methyl, DPPf = dipotassium phosphonate/phosphate (foliar), DPPd = dipotassium phosphonate/phosphate (drench), HRP = harpin protein, PG = polyethylene glycol control, WC = water control, UC = nonsprayed control.

measures that can be used to help prevent and suppress petunia and tomato late blight in the greenhouse. First, to render the environmental conditions less conducive to infection and development of late blight, adequate airflow should be maintained within and around the plant canopy. This will keep the relative humidity levels low and minimize long periods of leaf wetness (this is especially important if overhead watering is the primary form of irrigation). Second, overhead watering resulted in more disease compared with surface-directed watering; therefore, we recommend that overhead watering be avoided. Drip irrigation may be an economically feasible method for watering petunia hanging baskets, and sub-irrigation may be feasible for petunia or tomato pots or flats. However, due to the risk of *Ralstonia solanacearum* spread in shared irrigation systems (David Norman, University of Florida, Apopka, *personal communication*), separate subirrigation systems should be used for tomatoes and petunias.

Third, to decrease the spread of late blight from one host to another, petunias and tomatoes should not be grown in close proximity if possible (i.e., avoid hanging petunia baskets over tomatoes, and grow bench or floor-level crops in separate greenhouses). Fourth, scouting for disease during cool, wet periods will help with early detection. Detection, removal, and disposal of infected plants will remove potential sources of inoculum and minimize the spread of disease throughout the greenhouse. However, the disposal of diseased plant material near greenhouse air intakes or near potato and tomato field production areas should be avoided.

In addition to cultural controls, we have identified five products with potential for assisting in the management of petunia and/or tomato late blight in the greenhouse. These include four fungicides that contain azoxystrobin, dimethomorph/mancozeb, fosetyl-Al, or dipotassium phosphonate/phosphate as their active ingredients and one plant defense activator with

acibenzolar-S-methyl as the active ingredient. Current U.S. Environmental Protection Agency (EPA) registrations for products containing these active ingredients, with the exception of those containing dimethomorph/mancozeb, have short reentry intervals (4 to 12 h); azoxystrobin products have also been categorized as "reduced risk" by the EPA. For control of petunia late blight, products containing all of the active ingredients except acibenzolar-S-methyl are registered for use at the present time. For managing tomato late blight, only acibenzolar-S-methyl is labeled in such a way that treatment of greenhouse transplants would be permissible.

ACKNOWLEDGMENTS

This work was supported by the Department of Plant Pathology at Cornell University and in part by the Fred C. Gloeckner Foundation. We thank Linda Bork at Ball Seed (Chicago, IL) for kindly providing petunia seeds. Thanks to all the people and companies who donated products for the fungicide-biocontrol trials. We also thank Paul Becktell, Kevin Myers, and Kristin Ondik for their technical assistance and John Sanderson and K. C. Bennett for the use of their greenhouse space.

LITERATURE CITED

- Alexander, D., Goodman, R. M., Gutrella, M., Glascock, C., Weymann, K., Friedrich, L., Maddox, D., Ahlgo, P., Luntz, T., Ward, E., and Ryals, J. 1993. Increased tolerance to two oomycete pathogens in transgenic tobacco expressing pathogenesis-related protein-1a. *Proc. Natl. Acad. Sci. U.S.A.* 90:7327-7331.
- Aylor, D. E., Fry, W. E., Mayton, H., and Andrade-Piedra, J. L. 2001. Quantifying the rate of release and escape of *Phytophthora infestans* sporangia from a potato canopy. *Phytopathology* 91:1189-1196.
- Baider, A., and Cohen, Y. 2003. Synergistic interaction between BABA and mancozeb in controlling *Phytophthora infestans* in potato and tomato and *Pseudoperonospora cubensis* in cucumber. *Phytoparasitica* 31:399-409.
- Becktell, M. C. 2005. The host pathogen interactions and epidemiological implications of the *Petunia × hybrida*, *Calibrachoa hybridus* and *Nicotiana benthamiana* - late blight systems. Ph.D. thesis. Cornell University, Ithaca, NY.
- Becktell, M. C., Daughtrey, M. L., and Fry, W. E. Temperature and leaf wetness requirements for pathogen establishment, incubation period, and sporulation of *Phytophthora infestans* on *Petunia × hybrida*. *Plant Dis.* In press.
- Caten, C. E., and Jinks, J. L. 1968. Spontaneous variability of single isolates of *Phytophthora infestans* I. Cultural variation. *Can. J. Bot.* 46:329-348.
- Cohen, Y. 1994. Local and systemic control of *Phytophthora infestans* in tomato plants by DL-3-amino-n-butanoic acids. *Phytopathology* 84:55-59.
- Cohen, Y., Gisi, U., and Mosinger, E. 1991. Systemic resistance of potato plants against *Phytophthora infestans* induced by unsaturated fatty-acids. *Physiol. Mol. Plant Pathol.* 38:255-263.
- Cohen, Y., Gisi, U., and Niderman, T. 1993. Local and systemic protection against *Phytophthora infestans* induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. *Phytopathology* 83:1054-1062.
- Cohen, Y. R. 2002. β -aminobutyric acid-induced resistance against plant pathogens. *Plant Dis.* 86:448-457.
- Deahl, K. L., and Fravel, D. R. 2003. Occur-

Fig. 5. Test for enhanced numbers of *Trichoderma*, *Gliocladium*, or *Bacillus* on petunias and tomatoes treated with T22, G41, or *Bacillus subtilis*, respectively. Three samples were taken in each trial (**A** and **B**): 1) 2 days following the first application of biological material, 2) 2 days following the second application of biological material, and 3) 2 days following inoculation with *Phytophthora infestans*. Error bars represent the standard error of three replicate dilution plates and two trials. * = *Bacillus* colonies formed lawns.

- rence of leaf blight on petunia caused by *Phytophthora infestans* in Maryland. Plant Dis. 87:1004.
12. Friedrich, L., Lawton, K., Ruess, W., Masner, P., Specker, N., Rella, M. G., Meier, B., Dincher, S., Staub, T., Uknes, S., Mettraux, J. P., Kessmann, H., and Ryals, J. 1996. A benzothiadiazole derivative induces systemic acquired resistance in tobacco. Plant J. 10:61-70.
 13. Gavino, P. D., Smart, C. D., Sandroock, R. W., Miller, J. S., Hamm, P. B., Lee, T. Y., Davis, R. M., and Fry, W. E. 2000. Implications of sexual reproduction for *Phytophthora infestans* in the United States: Generation of an aggressive lineage. Plant Dis. 84:731-735.
 14. Georgis, R. 2002. Phytotoxicity of eleven EcoSMART experimental formulations on twelve plant varieties under greenhouse conditions. AgroSci. Research & Development, Elmhurst, IL.
 15. Harrison, J. G. 1992. Effects of the aerial environment on late blight of potato foliage - a review. Plant Pathol. 41:384-416.
 16. Hirst, J. M. 1953. Changes in atmospheric spore content: Diurnal periodicity and the effects of weather. Trans. Br. Mycol. Soc. 36:375-392.
 17. Hirst, J. M., and Moore, W. C. 1957. *Phytophthora infestans* on petunia and datura. Page 76 in: Plant Pathology - A record of current work on plant diseases and pests. Vol. 6. Ministry of Agriculture, Fisheries and Food Plant Pathology Laboratory, Harpenden, England.
 18. Johnson, D. A., Inglis, D. A., and Miller, J. S. 2004. Control of potato tuber rots caused by oomycetes with foliar applications of phosphorous acid. Plant Dis. 88:1153-1159.
 19. Platt, H. W. 1999. Response of solanaceous cultivated plants and weed species to inoculation with A1 or A2 mating type strains of *Phytophthora infestans*. Can. J. Plant Pathol. 21:301-307.
 20. Si-Ammour, A., Mauch-Mani, B., and Mauch, F. 2003. Quantification of induced resistance against *Phytophthora* species expressing GFP as a vital marker: Beta-aminobutyric acid but not BTH protects potato and Arabidopsis from infection. Mol. Plant Pathol. 4:237-248.
 21. Smart, C. D., and Fry, W. E. 2001. Invasions by the late blight pathogen: Renewed sex and enhanced fitness. Biol. Invasions 3:235-243.
 22. Yan, Z., Reddy, M. S., Ryu, C.-M., McInroy, J. A., Wilson, M., and Kloepper, J. W. 2002. Induced systemic protection against tomato late blight elicited by plant growth-promoting rhizobacteria. Phytopathology 92:1329-1333.