Host–Pathogen Interactions Between *Phytophthora infestans* and the Solanaceous Hosts *Calibrachoa* × *hybridus*, *Petunia* × *hybrida,* and *Nicotiana benthamiana*

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ABSTRACT

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Late blight, caused by the pathogen *Phytophthora infestans*, is a devastating disease of potato and tomato, but can also damage other solanaceous hosts. To gain a better understanding of the interaction between *P. infestans* and these other hosts, the susceptibility of species in three solanaceous genera was investigated. Of the 10 *Calibrachoa* × *hybridus* cultivars tested, four were susceptible and six were resistant to the pathogen; susceptible cultivars supported only very limited growth of *P. infestans*. The majority of the *Petunia* × *hybrida* (petunia) cultivars were susceptible, although less so than susceptible potatoes or tomatoes. Two petunia cultivars displayed differential resistance, suggesting the presence of *R* genes against *P. infestans.* The hypersensitive response was present in susceptible, partially resistant, and resistant petunia–*P. infestans* interactions, but was predominant in the resistant interaction. Young petunias (3 weeks) were more susceptible than older petunias (7 weeks). *Nicotiana benthamiana* was susceptible to all four *P. infestans* isolates tested in the lab and became infected during a field epidemic. Several of these isolates were tested for the presence of the *inf1* gene, and were found to have and express the gene in vitro. In addition, culture filtrate from these isolates contained 10-kDa proteins and also elicited the hypersensitive response in *Nicotiana tabacum* and *N. benthamiana*.

Phytophthora infestans Mont. (de Bary), the causal agent of late blight, is often associated with its two most common hosts, potato and tomato (13). However, *P. infestans* has an extensive host range that includes many members of the Solanaceae, a large family that includes many economically important species (21,39). While many hosts are in the genus *Solanum* (1,10,11,13–15,24,40,41), other members of this family, such as petunia (*Petunia* × *hybrida*), Calibrachoa (*Calibrachoa* × *hybridus*), and *Nicotiana benthamiana* have also been implicated as hosts (9,13,24,41,42).

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Calibrachoas resemble miniature petunias and, in fact, are closely related to petunias (57,58). Calibrachoas have become popular ornamental plants in the United States, but their susceptibility to late blight is not known. However, because of their increasing popularity and the fact that greenhouse production practices may put them in close proximity to tomatoes and petunias, their susceptibility to late blight is of practical epidemiological interest.

Petunias, like calibrachoas, could also be in close proximity to tomatoes during production, so their susceptibility to *P. infestans* is likewise of interest. Preliminary observations in our lab confirmed previous reports that petunias are hosts of *P. infestans* (8,13,22,51,52). We also confirmed previous reports that petunias tend to be less susceptible to late blight than susceptible cultivars of potatoes or tomatoes (24,41). Therefore, in addition to the susceptibility of petunias to late blight, several aspects of resistance in the petunia–late blight system were investigated, including differential resistance, the hypersensitive response (HR), and the effect of plant age on resistance.

N. benthamiana has become an important tool for studying host–pathogen interactions involving *P. infestans* (29). Previous work has suggested that naturally occurring isolates of *P. infestans* from Europe, North America, and Mexico cannot induce disease on *N. benthamiana* (29). However, when the *inf1* elicitin gene was silenced in one isolate, that isolate became pathogenic on *N. benthamiana* (29). Elicitins are 10-kDa proteins secreted by *Phytophthora* species that induce the HR, particularly in *Nicotiana* species (3,30). The fact that an *inf1*-silenced isolate could infect and develop sporulating lesions on *N. benthamiana* suggested that resistance of *N. benthamiana* to *P. infestans* was dependent on the recognition of the INF1 protein and that INF1 is an avirulence factor. These results are consistent with findings from the *Phytophthora parasitica–Nicotiana tabacum* pathosystem. Isolates pathogenic on *N. tabacum* produced low levels of the elicitin parasiticein, while nonpathogenic isolates produced high levels of the elicitin parasiticein (3,30,45). However, in preliminary tests, we and others had observed that common North American isolates of *P. infestans* could readily infect and develop sporulating lesions on *N. benthamiana*. It was not known whether these isolates had the *inf1* gene.

The goal of this study was to gain a better understanding of the host–pathogen interactions involving *P. infestans* and species in three non-*Solanum* genera in the Solanaceae. This knowledge will be valuable to the greenhouse industry and may provide incentive to modify production practices to prevent the spread of disease between species. Our specific objectives were: (i) to determine if several calibrachoa cultivars, several petunia cultivars, and *N. benthamiana* could be infected by common North American isolates of *P. infestans*; (ii) to more fully characterize several aspects of resistance in the petunia–late blight interaction; and (iii) to determine whether isolates of *P. infestans* that can cause disease on *N. benthamiana* lack the *inf1* gene.

MATERIALS AND METHODS

***P. infestans* isolates and inoculum preparation.** A diversity of *P. infestans* isolates (Table 1) were obtained from the culture collection at Cornell University. Routine growth of the cultures was on rye agar medium (5). Inoculum consisted of a sporangial suspension prepared from sporulating lesions on detached tomato or potato leaflets. Sporangia were rinsed from the leaflets into 100 ml of distilled water, and the concentration of sporangia was determined using a hemacytometer (aver-

age of three readings) and adjusted as needed. The inoculum concentration for each experiment is described below. Sporangial suspensions were incubated at 4°C for 1 to 2 h to induce zoospore production. Plants were spray inoculated to runoff with a hand held sprayer.

Susceptibility of *Calibrachoa* × *hybridus, Petunia* × *hybrida,* and *N. benthamiana* to late blight. *Growth chamber trials.* The susceptibility of 10 calibrachoa cultivars, 24 petunia cultivars, and *N. benthamiana* to several isolates of *P. infestans* (Table 2) was determined. The plant material in these experiments also included tomatoes, potatoes, and *N. tabacum*. Plants were grown in a soilless mix (Cornell mix) consisting of a 1:1 (vol/vol) peat–vermiculite mix with 0.4 kg each of nitrogen, phosphorus, and potassium per cubic meter of mix. Natural sunlight was supplemented with 400W high-pressure sodium lamps on a 12-h light/dark cycle. Temperatures were maintained between 24 and 29°C. The plants were fertilized once or twice a week with liquid fertilizer containing 300-ppm nitrogen. The calibrachoas were grown from unrooted cuttings (Goldsmith Plants, Inc., Gilroy, CA) in 4-inch (10.16-cm) plastic pots (one plant per pot). The petunias were grown from seeds, plugs, or unrooted cuttings (Ball Seed, Chicago, IL, or Goldsmith Plants) in 5-inch (12.7-cm) plastic pots or 10 × 18 inch (25.4 × 45.72 cm) flats containing eight six-packs. Each pot or six-pack of petunias contained 5 to 10 plants depending on the germination rate. The *N. benthamiana, N. tabacum,* and tomato plants were grown from seed in 6-inch (15.24-cm) plastic pots (containing one, one, and two plants per pot, respectively). The potatoes were grown from tubers in 6-inch (15.24-cm) plastic pots (one plant per pot). Individual pots or individual six-packs were used as experimental units.

The inoculations involved one or two isolates that are more aggressive on potatoes (US940480, MX980085) and one or two isolates that are more aggressive on tomatoes (US990025, US970001) (35,36, 48,53). Three pots of each species/cultivar were inoculated within a trial; two trials were conducted on the calibrachoa cultivars and three trials were conducted on the petunia cultivars and the *N. benthamiana.* Noninoculated petunias or noninoculated calibrachoas served as negative controls (for the petunia and calibrachoa trials, respectively), *N. tabacum* plants served as negative, nonhost controls, and susceptible potato (cv. Kathadin, Kennebec, or Atlantic) or susceptible tomato (cv. Sunrise) plants were included in all trials as positive controls for the potato and tomato specialized isolates. The experiment was conducted using a split-plot design where the plants were arranged in blocks according to the isolate with which they were inoculated, and the location of the blocks was

Table 1. Characteristics of the *Phytophthora infestans* isolates used in experiments presented in this paper

Isolate	Clonal lineage[a]	Country of origin	Pathotype[b]	GPI locus[c]
US930468	US-1	United States	ND[d]	86/100
US940480	US-8	United States	1,2,3,4,5,6,7,8,10,11	100/111/122
US990025	US-11	United States	ND	100/100/111
US970001	US-17	United States	1,7	100/122
MX990005	ND	Mexico	1,2,3,4,5,6,7,8,9,10,11,12	86/100
MX980085	ND	Mexico	1,2,5	86/100
EC930750	EC-1	Ecuador	1,3,7	90/100
KE980014	ND	Kenya	1,3,5,7	86/100
UG980010	US-1	Uganda	1,2,5,6,7	86/100

[a] Clonal lineage determined using the RG57 genomic fingerprint, allozyme genotype, and mating type (16).
[b] Pathotype determined previously by inoculating detached leaflets from a population of differentially resistant potatoes in which each individual possessed a different *R* gene; race numbers designate potato *R* genes that the isolate is able to overcome (43).
[c] Glucose-6-phosphate isomerase allozyme pattern.
[d] Not determined.

Table 2. Results of several independent growth chamber experiments showing the susceptibility of petunias, calibrachoas, *Nicotiana benthamiana,* and *N. tabacum* to several isolates of *Phytophthora infestans*[a]

	***P. infestans* isolates**			
Host	**US940480**	**US970001**	**US990025**	**MX980085**
Petunia cultivars				
Carpet Plum	+ +	+ +	+ +	--
Super Cascade Blue	+ +	+ +	+ +	--
Super Cascade White	+ +	--	+ +	--
Super Cascade Pink	HR	--	HR	HR
Frost Velvet	NR/+	+ +	+ +	--
Midnight Dream	+ +	+ +	+ +	--
White Dream	+ +	--	+ +	--
Pink Dream	+ +	--	+ +	--
Ultra Red	NR/+	+ +	+/+ +	--
Ultra White	+ +	--	+ +	--
Ultra Pink	+ +	--	+ +	--
Sugar Daddy	NR/+/+ +/ HR	+/HR	NR/+/+ +/HR	NR/+/+ +/HR
Pink Daddy	+ +	--	+ +	--
White Madness	+ +	+ +	+ +	+ +
Sugar Madness	HR	--	HR	HR
Pink Madness	+ +	--	+ +	--
Storm White	+ +	--	+ +	--
Storm Pink	+ +	--	+ +	--
Jamboree Blue Vein	NR/+	NR/+	--	--
Jamboree Burgundy	NR/+/++	+/+ +	--	--
Jamboree Hot Pink	+/+ +	+ +	--	--
Jamboree Light Blue	NR/+/+ +	NR/+/+ +	--	--
Jamboree Violet	+/+ +	+/+ +	--	--
Jamboree White II	+/++	NR/+/+ +	--	--
Calibrachoa cultivars				
Callie Blue	NR	NR/+	--	--
Callie Dark Blue	NR	NR	--	--
Callie Ivory II	NR	NR/+	--	--
Callie Lavender	NR/+	NR/+	--	--
Callie Pink II	NR/+/++	NR/+/+ +	--	--
Callie Rose	NR	NR/+	--	--
Callie Scarlet Red	NR/+/+ +	NR/+	--	--
Callie Sunrise	NR	NR/+	--	--
Callie White	NR/+/+ +	NR/+/+ +	--	--
Callie Yellow	NR	NR/+	--	--
N. benthamiana	+/+ +	+ +	+ +	+ +
Controls				
Potato (PC)	+ +	--	--	+ +
Tomato (PC)	--	+ +	+ +	
Noninoculated (NC)	NR	NR	NR	NR
N. tabacum (NC)	NR	NR	NR	--

[a] NR = no reaction; + = expanding, nonsporulating lesions; HR = macroscopic hypersensitive response (nonsporulating, nonexpanding) lesions; ++ = expanding, sporulating lesions; -- = not inoculated; PC = positive control; NC = negative control.

randomly assigned. The plants were spray-inoculated to runoff (using an inoculum concentration of 6,000 to 10,000 sporangia/ml) in an inoculation chamber within a controlled-environment, walk-in growth chamber (15°C; 16-h light/8-h dark photoperiod). The inoculation chamber consisted of a PVC frame covered with semi-clear plastic sheeting and contained a Trion model 500 automatic humidifier (Hummert International, Earth City, MO) that ran periodically throughout the day and night to maintain 100% relative humidity (RH). The plants were monitored daily for disease development and were visually and qualitatively rated for the presence or absence of disease lesions up to 10 days after inoculation (DAI). Disease lesions were further characterized based on the presence or absence of sporulation and as expanding or nonexpanding. The qualitative ratings were compiled over all trials and are presented in Table 2 (see Results).

Field trial. The susceptibility of 'White Madness' and 'Sugar Daddy' petunias and *N. benthamiana* was determined under field conditions at the Homer C. Thompson Research Farm in Freeville, NY. *N. tabacum* plants were used as nonhost, negative controls, and potatoes (cv. Atlantic) were used as positive controls. Approximately 200 potatoes were planted from tubers as a border around the petunia/*Nicotiana* plots on 30 June 2000. Six- to seven-week-old greenhouse grown *N. benthamiana, N. tabacum,* and petunia plants were transplanted on 7 August 2000 into a 50 × 50 ft. field plot inside the potato border. The petunias were planted in three 12-plant plots, and the *N. benthamiana* and *N. tabacum* were planted in three 2-plant plots using a randomized complete block design. Four days after transplanting, all plants, including the potato border, were spray-inoculated (using an inoculum concentration of 150 sporangia/ml) to runoff with an isolate from the US-11 clonal lineage (US990025). This isolate had been compatible with petunias and *N. benthamiana* in previous inoculations. Inoculation occurred at dusk after the foliage had been wetted via sprinkler irrigation. Conditions favorable for late blight development in the field were maintained by frequent overhead irrigation; the plots were irrigated at an approximate rate of 0.1 inch (0.25 cm)/h for 1 to 4 h every evening, except when it had rained. Visual assessments of disease severity (percentage of plant area showing symptoms) as described previously (25) were taken of each plot every 3 to 5 days after the initial appearance of symptoms for a total of 37 days. Disease severity ratings were also recorded for five potato plants randomly selected from the potato border. Changes in disease severity over time were visualized using disease progress curves and the area under the disease progress curves (AUDPC) (47). Disease progress curves and AUDPCs for each plant type were generated from the disease severity ratings using Statistical Analysis System 8.2 (SAS Institute, Cary, NC).

Resistance of petunias to late blight. *Differential resistance trials.* The potential for differential resistance against late blight in petunias was investigated by testing whether resistance was effective against some, but not all, isolates of *P. infestans*. The presence of differential resistance would indicate the possible presence of resistance (R) genes against *P. infestans* in petunias. The seven isolates used for this experiment (US930468, US970001, MX990005, MX980085, EC930750, KE980014, UG980010) came from a variety of geographic locations and represented a diversity of pathotypes and genotypes (Table 1) (16,43). The four petunia cultivars (identified based on results from growth chamber trials described above) included a cultivar that had been susceptible to all isolates of *P. infestans* ('White Madness'), a cultivar that had been partially resistant ('Sugar Daddy'), and two cultivars ('Sugar Madness' and 'Supercascade Pink') that had been highly resistant (developed macroscopic HR lesions) to isolates in the growth chamber trials. Susceptible potatoes (cv. Atlantic or Kathadin) and tomatoes (cv. Sunrise) were included as positive controls. All isolates were tested on three plants (pots) of each type within an experiment, and the entire experiment was conducted twice. The experiment was conducted using a split-plot design where the plants were arranged in blocks according to the isolate with which they were inoculated and the location of the blocks was randomly assigned in each trial. Plants (4 to 5 weeks old) were spray-inoculated to runoff (using an inoculum concentration of 10,000 to 20,000 sporangia/ml) with a hand-held sprayer and subsequently incubated in a humid chamber as described above. The plants were monitored daily for 7 to 10 days and qualitatively rated for the presence or absence of disease lesions and/or macroscopic HR lesions. The data were compiled for the two trials and are presented in Table 3 (see Results).

Microscopic characterization of resistance responses in petunia. The presence or absence of the HR in petunias at the microscopic level was investigated. Based on the results of our initial growth chamber inoculations (Table 2), three types of interactions were investigated: compatible ('White Madness'), partially resistant ('Sugar Daddy'), and highly resistant ('Sugar Madness'). One flat of each cultivar was planted, and the petunia seedlings (5 to 7 weeks old) were each spray inoculated (using an inoculum concentration of 20,000 sporangia/ml) with US970001 or US940480 and incubated in a humid chamber as described above. To visualize cells that had undergone the HR, infected leaves were cleared and stained according to the methods of Colon et al. (7). Three leaves were removed from each flat at 6, 12, 24, 48, 72, 96, and 120 hours after inoculation (HAI) and immediately placed into a clearing and fixing solution (100 ml of glacial acetic acid, 600 ml of 95% ethanol, and 300 ml of chloroform per liter) until cleared (several hours to days). Cleared leaves were stained overnight at 60°C with lacto-phenol trypan blue (per 300 ml: 25 ml of phenol, 25 ml of lactic acid, 25 ml of 70% glycerol, 25 ml of distilled water, 0.03 g trypan blue, and 200 ml of 95% ethanol). Stained leaves were then vacuum infiltrated with chloral hydrate (2.5 g/ml) and destained at 42°C for several hours to several days. The destained leaves were mounted on microscope slides (75 × 50 mm) in 70% glycerol and observed with light and fluorescence microscopy. The light microscopy was performed on an Olympus BH-2 compound microscope (Olympus America, Inc., Melville, NY). Fluorescence microscopy was performed using an Olympus BX-60 equipped with a UV lamp, a 425- to 475-nm excitation filter, and a 485-nm barrier filter. The pathogen and affected host cells were located with bright field microscopy. Plant cells that were either dark blue or brown under bright field conditions were subsequently examined under UV light; cells exhibiting the HR fluoresced bright yellow when exposed to UV light.

Plant age and susceptibility. The effect of plant age on late blight susceptibility of petunias was determined. Three-, five-, and seven-week-old susceptible 'White Madness' petunias and susceptible 'Sunrise' tomatoes (for comparison purposes) were used; two flats of petunias (10 × 18 inch

Table 3. Reactions of four petunia cultivars in two trials to inoculations with a diversity of *Phytophthora infestans* isolates[a]

Isolate	White Madness	Sugar Daddy	Sugar Madness	Supercascade Pink
US930468	+	+	HR	HR
US970001	++	+	HR	++
MX990005	++	++	++	++
MX980085	++	++	HR	HR
EC930750	++	++	++	++
KE980014	+	+	HR	HR
UG980010	+	+	HR	+

[a] Results were consistent in both trials. HR = macroscopic hypersensitive response lesions; ++ = sporulating lesions; + = nonsporulating, expanding lesions.

[25.4 × 45.72 cm] containing eight six-packs) and four pots of tomatoes (two plants per pot) were inoculated per age group. The experiment was conducted three times using a split-plot design. The plants were arranged in discrete blocks in the growth chamber based on the isolate with which they were being inoculated; the blocks were randomly located in the chamber in each trial. The plants were spray-inoculated (using an inoculum concentration of 20,000 sporangia/ml) with US970001, an isolate that had been consistently compatible with petunias and tomatoes in previous inoculations. The plants were incubated in a humid inoculation chamber as described above. Visual assessments of disease severity (percentage of plant area showing symptoms) modified from a previously described potato late blight rating method (25) were performed on each flat at 7 DAI.

The plant age data were analyzed with Statistical Analysis System 8.2 (SAS Institute) and SPSS (Chicago, IL). The general linear model (GLM) was used to determine whether age had a significant effect on resistance, and a regression analysis was used to determine the relationship of age to resistance. Because of a large trial effect, one-way analysis of variance (ANOVA) and regression analyses were performed on each petunia trial and on each tomato trial. The assumptions of normality and homogeneity of variance needed for the ANOVA and regression analyses were checked. A transformation (arcsine of the square root transformation) was sufficient to meet the assumption requirements in all trials except the first petunia trial. The nonparametric Mann-Whitney test was used to test the significance of plant age on resistance in the first petunia trial. The results of this test indicated a significant effect; therefore, a regression was ultimately performed on the data from this trial as well.

Tests for the presence of the *inf1* gene. The presence of the *inf1* gene, the expression of the *inf1* gene, the production of 10-kDa proteins in vitro, and the biological activity of the putative INF1 protein were determined for each of four isolates of *P. infestans* (US940480, MX980085, US990025, and US970001) found to be compatible with *N. benthamiana* in the growth chamber trials. In addition, the *inf1* genes from three of the four isolates (US940480, US970001, and MX980085) were sequenced, and the amino acid sequence for the INF1 protein was predicted.

The presence of the *inf1* gene in the four isolates described above was determined using Southern analysis. DNA was extracted from lyophilized mycelia (19) that had been grown in minimal media (per liter, 0.5 g of KH_2PO_4, 0.25 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of asparagine, 1.0 mg of thiamine, 0.5 g of yeast extract, and 25 g of D^+ glucose [31]) for 10 to 14 days. The DNA from each isolate was digested separately with *Bam*H1 and *Sal*1. DNA fragments were separated in an agarose gel and subsequently transferred to a nylon membrane (Hybond N+ membrane; Amersham, Arlington Heights, IL) (46). Southern blots were probed with full-length, fluorescein labeled (Renaissance nonradioactive kit; NEN Life Sciences, Boston, MA) *inf1* cDNA clones (kindly provided by Sophien Kamoun, ORADC, Wooster, OH) as described by the manufacturer.

The expression of the *inf1* gene in the four isolates described above was determined using northern analyses. RNA was extracted from lyophilized mycelia that had been grown in pea broth for 10 to 14 days (19) using a hot phenol protocol modified from Gu et al. (20). RNAs were separated by gel electrophoresis on a 1.2% agarose gel, transferred to a nylon membrane (Hybond N+ membrane, Amersham) (46), and probed with radiolabeled *inf1* cDNA clones.

The four isolates compatible with *N. benthamiana* were also tested for the production of 10-kDa proteins in vitro. Isolates were grown for 10 to 14 days in minimal media as described above. The liquid culture medium from each of the isolates was separated from mycelium by

Fig. 1. Symptoms and signs of disease caused by *Phytophthora infestans* isolate US940480. **A,** lesions on 'Callie Pink II' calibrachoa. **B,** Lesions with light sporulation on 'Callie Scarlet Red' calibrachoa 7 days after inoculation (DAI). **C and D,** lesions and sporulation on 'White Daddy' petunias 5 to 7 DAI. **E and F,** lesions and sporulation on *Nicotiana benthamiana* 10 DAI.

sequential filtrations through Whatman no. 1 filter paper (Whatman, Maidstone, England) and 0.22-µm cellulose acetate filter (Corning Incorporated, Corning, NY). Proteins in the culture filtrates were separated using Novex, 10 to 20% Tris-Tricine precast gradient gels (Invitrogen, Carlsbad, CA). The gels were loaded with 20-µl samples consisting of 13 µl of culture filtrate, 2 µl of NuPage 10× sample reducing agent, and 5 µl of Tricine SDS 2× sample buffer (Invitrogen) and run for 60 to 70 min at 128 volts. Two molecular weight markers were included: the Mark 12, unstained, molecular weight standard (20 µl) (Invitrogen) and the Precision Protein standard, unstained, broad-range marker (10 µl) (Bio-Rad Laboratories, Hercules, CA). Following electrophoresis, the proteins present in the gels were silver stained according to the directions using a Bio-Rad Silver Stain Plus kit (Bio-Rad Laboratories).

To determine if the sequence of *inf1* in isolates pathogenic to *N. benthamiana* was the same as the previously published sequence (28), the gene was cloned from three isolates (US940480, US970001, and MX980085). The sequence of *inf1* from isolate US990025 was not obtained. Polymerase chain reaction (PCR) amplification was performed using oligonucleotide primers designed based on the *inf1* sequence (28). The forward and reverse primer sequences were 5′ GATGAACTTTCGTGCTCTGT 3′ and 5′ TACCGCAGCTGCTACACTATTACA 3′, respectively. Each 30-µl PCR reaction included 100 ng of genomic DNA, 1× PCR reaction buffer (Invitrogen), 2.5 mM $MgCl_2$, 2 µM dNTP, and 3 U AmpliTaq DNA polymerase (Invitrogen). The amplification conditions included one denaturing cycle at 95°C for 5 min followed by 35 cycles consisting of 72°C for 1 min, 55°C for 1 min, and 95°C for 30 s. The final extension cycle ran at 72°C for 5 min. The PCR products were cleaned using a Wizard PCR clean-up kit (Promega, Madison, WI) and cloned into a TOPO PCR-II vector (Invitrogen) according to the manufacturers' instructions. Cleaned PCR products were sequenced at the Cornell University BioResource center, and the sequence analyses were performed using Lasergene v5.0 (DNASTAR, Inc., Madison, WI).

The biological activity of the culture filtrates of the four isolates described above was evaluated via infiltration assays. Culture filtrates from each isolate were infiltrated into leaves of *N. tabacum* and *N. benthamiana* as described previously (31,32). Culture filtrate containing INF1 produced by *Escherichia coli* with a pFB53 expression vector (provided by Sophien Kamoun, ORADC, Wooster) was included as a positive control. The expression of *inf1* by *E. coli* was achieved according to previously described methods (28). The total protein concentration of each sample was determined using a Bradford assay (Bio-Rad Laboratories), and all samples were diluted to 1 µg/ml. The culture filtrate was infiltrated into two different leaves on each of two plants within a trial, and three trials were conducted. Distilled water, LB/ampicillin medium (pFB53 growth medium), and minimal medium were used as negative controls. The presence (necrotic lesion at the site of infiltration) or absence of the hypersensitive response was assessed at 24 and 48 h after infiltration.

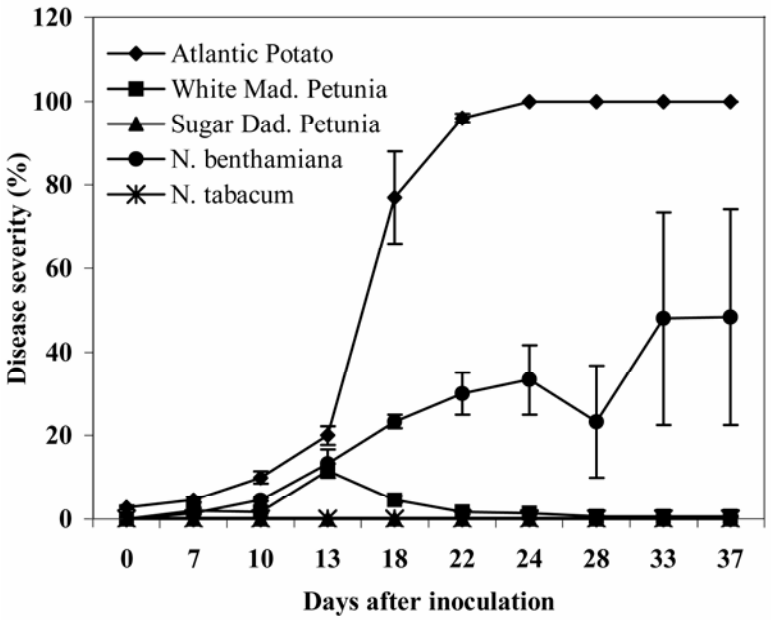

Fig. 2. Change in severity of late blight in the field on a susceptible petunia cultivar ('White Madness'), a partially resistant petunia cultivar ('Sugar Daddy'), a susceptible potato cultivar ('Atlantic'), *Nicotiana benthamiana,* and *N. tabacum*. Error bars represent the standard error of the mean disease severity of three replicate plots of each petunia cultivar and *Nicotiana* species or five replicate potato plants at each date.

RESULTS

Susceptibility of *Calibrachoa* × *hybridus, Petunia* × *hybrida,* and *N. benthamiana* to late blight. *Growth chamber trials.* Calibrachoas were less susceptible to *P. infestans* than the petunia, potato, and tomato cultivars tested here. Three of the 10 calibrachoa cultivars tested were susceptible to both isolates used (US940480 and US970001), six cultivars were either completely or partially resistant to both isolates, and one cultivar was partially resistant to one isolate and completely resistant to the other (Table 2). Lesions were typically visible within 5 to 7 DAI and were similar to those seen on petunias; however, sporulation on calibrachoa late blight lesions was sparse compared with sporulation on petunia late blight lesions (Fig. 1A and B).

Overall, petunias were susceptible to *P. infestans*, but less so than commonly grown, susceptible cultivars of potatoes or tomatoes; 22 of the 24 cultivars were infected by at least one of the four isolates of *P. infestans* (Table 2). Symptoms of infection were typically evident within 5 DAI. A compatible interaction was characterized by necrotic, irregularly shaped, sporulating lesions with the sporulation largely limited to the necrotic lesion area (Fig. 1C and D). Sporulation was typically evident on both the upper and lower surfaces of the infected leaves. The partially resistant cultivar, 'Sugar Daddy', displayed a mix of slowly expanding, lightly sporulating and nonsporulating lesions and nonexpanding, nonsporulating lesions. Two cultivars, 'Sugar Madness' and 'Supercascade Pink', developed only small, necrotic "flecks" that did not expand.

N. benthamiana plants were susceptible to all four of the *P. infestans* isolates tested in the growth chamber (Table 2). Lesions typically started as small, well-defined circles that quickly coalesced into larger lesions; sporulation tended to be more apparent on the abaxial side of the leaf surface (Fig. 1E and F). Symptom development on *N. benthamiana* usually took between 5 and 10 days.

Field trial. 'White Madness' petunias and *N. benthamiana* were susceptible to late blight in the field. Symptom development on petunias and on *N. benthamiana* occurred within 10 DAI, which was later than symptom development on the susceptible potato controls (disease evident at 5 to 7 DAI) (Fig. 2). Lesions on petunias and *N. benthamiana* sporulated in the field (Fig. 3A and B). However, the 'White Madness' petunias did not support an epidemic in the field; new growth foliage that developed after the initial inoculation did not become infected, and disease severity declined over time (Fig. 2). In contrast,

new growth of *N. benthamiana* plants developed secondary infections caused by inoculum produced during the epidemic (Fig. 2). At the conclusion of the experiment, the average area under the disease progress curve (AUDPC) (47) for each plant type was 2,359 proportion days (PD) for the potatoes, 848 PD for *N. benthamiana*, 99 PD for the 'White Madness' petunias, 0.01 PD for the 'Sugar Daddy' petunias, and 0.0 PD for *N. tabacum*. 'Sugar Daddy' petunias remained essentially symptomless (a few necrotic lesions), and *N. tabacum* was completely resistant (no macroscopic symptoms).

Resistance of petunias to late blight. *Differential resistance trials.* 'Sugar Madness' and 'Supercascade Pink' petunias were highly resistant to the two isolates tested in the initial growth chamber trials (Table 2), but displayed differential resistance to the seven additional isolates tested (Table 3). 'Sugar Madness' petunias were highly resistant (developed macroscopic, HR lesions) to five of the seven isolates (US930468, US970001, MX980085, KE980014, and UG980010), but were susceptible (sporulating lesions) to two of the isolates (MX990005 and EC930750) of *P. infestans* (Table 3). 'Supercascade Pink' petunias were highly resistant to three of the seven isolates (US930468, MX980085, and KE980014), but were susceptible to three isolates (US970001, MX990005, and EC930750) and partially resistant (expanding, nonsporulating lesions) to one isolate (UG980010) of *P. infestans* (Table 3). 'White Madness' and 'Sugar Daddy' petunias displayed a mix of susceptible and partially resistant phenotypes to the seven isolates. The results were consistent in both trials.

Microscopic characterization of resistance responses in petunia. The HR was observed microscopically in all types of interactions between petunias and *P. infestans*: susceptible, partially resistant, or highly resistant. In all cases, the HR was not evident until 48 HAI. In the susceptible interaction, the HR could be seen in association with some, but not all, hyphae, and the pathogen was able to grow extensively throughout the leaf tissue (Fig. 4A and B). In the partially resistant interaction, each infection site was associated with some level of the HR; however, hyphae could be seen growing beyond the HR in several cases (Fig. 4C and D). In the highly resistant interaction, no hyphae were seen growing beyond the HR (Fig. 4E and F).

Plant age and susceptibility. In general, as petunias increased in age, their susceptibility to late blight decreased ($P < 0.05$) (Fig. 5). In all three petunia trials, the linear regression analyses indicated that the slopes were significantly ($P < 0.001$) negative. In contrast, plant age had no significant effect on susceptibility of tomatoes. The regression equations ($y = a + bx$, where y = disease severity, a = intercept, b = slope, and x = age), R^2 values, and significance for each petunia trial were as follows: trial 1, $y = 11.020 + -1.254x$, $R^2 = 0.537$, and the significance of the slope was $P < 0.001$; trial 2, $y = 30.701 + -2.515x$, $R^2 = 0.433$, and the significance of the slope was $P < 0.001$; and trial 3, $y = 42.270 + -4.604x$, $R^2 = 0.803$, and the significance of the slope was $P < 0.001$.

Tests for the presence of INF1. The presence and expression of the *inf1* gene

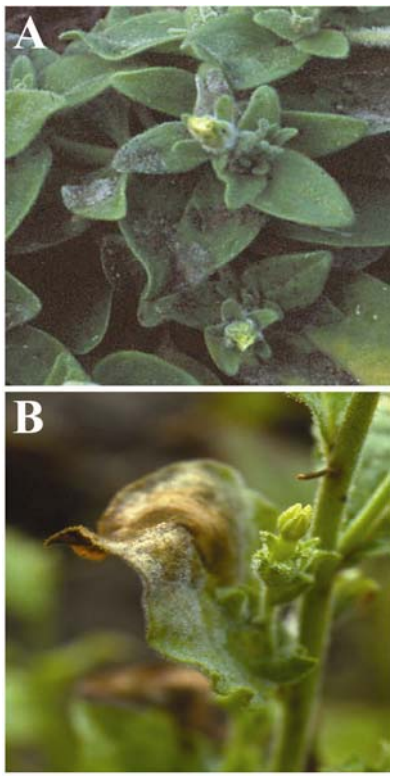

Fig. 3. Sporulating late blight lesions in the field on **A,** 'White Madness' petunias and **B,** *Nicotiana benthamiana* 16 days after inoculation.

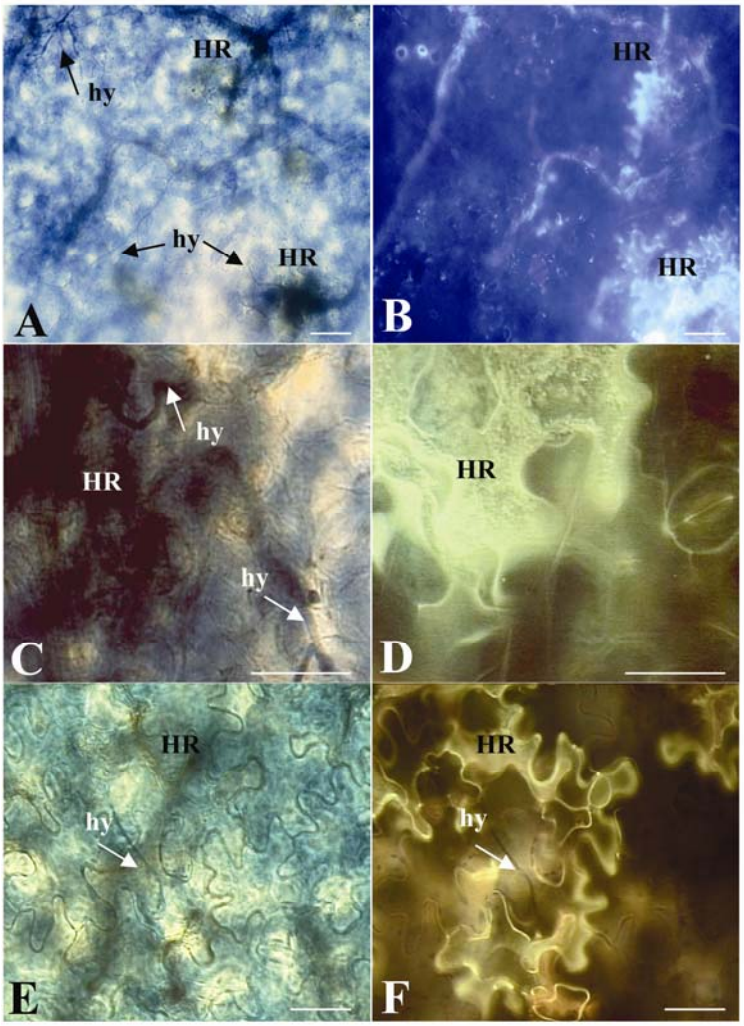

Fig. 4. Hypersensitive response (HR) in three cultivars of petunia 48 hours after inoculation (HAI) with *Phytophthora infestans* isolate US940480 **(A to D)** or US970001 **(E and F).** In the bright field views **(A, C, E),** the HR is evident by darker blue or brown regions; the same views are shown with UV light **(B, D, F)** where the HR cells fluoresce a bright yellow. **A and B,** Susceptible 'White Madness' petunias with mild HR or no HR of epidermal cells in association with growing hyphae. **C and D,** Example of a hypha growing beyond a group of HR cells in the partially resistant cultivar 'Sugar Daddy'. **E and F,** A single hyphal thread contained by the HR in surrounding epidermal cells in the highly resistant cultivar 'Sugar Madness'. hy = hypha. Bar = 50 µm.

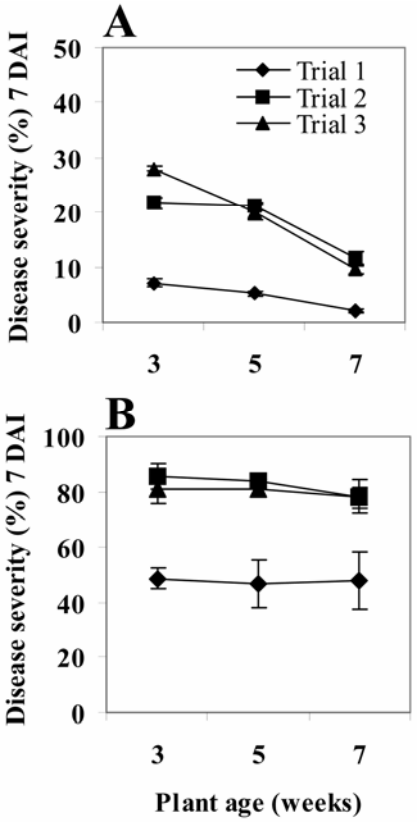

Fig. 5. Effect of plant age on susceptibility of **A,** petunias and **B,** tomatoes to *Phytophthora infestans*. Slopes for petunia trials one, two, and three were –1.2, –2.5, and –4.6 disease severity/weeks, respectively. The R^2 values for petunia trials one, two, and three were 0.537, 0.433, and 0.803, respectively. Significance of the slopes in all three trials was $P < 0.001$. Error bars represent the standard error of the mean disease severity 7 days after inoculation of 16 petunias and three trials ($n = 48$) or four tomatoes and three trials ($n = 12$).

and the biological activity of the culture filtrate was investigated. The *inf1* gene was detected via Southern analysis (data not shown), and its expression was detected via northern analysis in all four *P. infestans* isolates tested (Fig. 6A and B). The genomic banding patterns observed in the Southern analysis (one major *Bam*H1 fragment of 2.3 kb and three *Sal*1 fragments of 3.4, 4.2, and ~7.0 kb) were similar to those previously described for the *inf1* gene (28). In addition, the amino acid sequences predicted from the *inf1* cDNA sequences from the two North American isolates and the one Mexican isolate cloned in the current study were identical to the sequences determined previously for a European isolate (28). The culture filtrate of each isolate contained 10-kDa proteins (US940480 data not shown) (Fig. 6B) and the culture filtrate produced the HR when infiltrated into *N. tabacum* and *N. benthamiana* (Table 4). Thus, all indications of the current study are that the *inf1* elicitin gene, transcript, and protein are present in isolates that infect *N. benthamiana*.

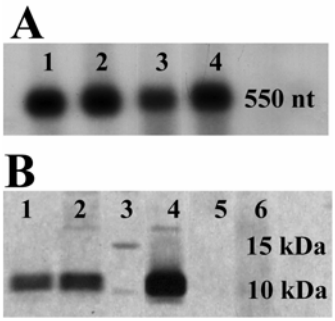

Fig. 6. Presence and expression of the *inf1* gene in isolates of *Phytophthora infestans* that infect *Nicotiana benthamiana.* **A,** Northern analysis showing expression of the *inf1* gene in four isolates of *P. infestans*: lane 1, US940480; lane 2, US970001; lane 3, US990025; lane 4, MX980085. **B,** Silver-stained 10–20% Tris-Tricine gradient gel (Invitrogen) containing culture filtrates obtained from isolates grown in liquid medium: lane 1, US970001; lane 2, US990025; lane 3, Precision Protein standard, unstained, broad range marker (Bio-Rad Laboratories); lane 4, MX980085; lane 5, Mark 12, unstained, molecular weight standard (Invitrogen); lane 6, minimal media control. (The band for US940480 was present, but very faint and difficult to reproduce in a figure).

DISCUSSION

The results presented here demonstrate that calibrachoas, petunias, and *N. benthamiana* can be infected by *P. infestans*. To our knowledge, this is the first published report of calibrachoas as hosts and the first growth chamber/field demonstrations that show *N. benthamiana* and petunias are consistent hosts of *P. infestans*. In addition to serving as hosts in the controlled environment of a growth chamber, 'White Madness' petunias and *N. benthamiana* were also infected under field conditions. However, only the initial inoculation seemed to be successful on petunias, while secondary infections occurred on the *N. benthamiana* plants during the epidemic. The resistance of the petunias to secondary infection may be the result of an increase in resistance as the plants aged or the development of systemic acquired resistance following the initial inoculation.

Differential resistance to *P. infestans* in petunias was found in two cultivars ('Sugar Madness' and 'Supercascade Pink'). Each of these cultivars was highly resistant and developed HR lesions when inoculated with some isolates, but were susceptible (developed sporulating lesions) when inoculated with other isolates. The group of isolates compatible with 'Sugar Madness' petunias was somewhat distinct from the group of isolates compatible with 'Supercascade Pink' petunias. *R* gene resistance is typified by being differential: effective against some isolates and not others. Therefore, results presented here are consistent with a set of distinct *R* genes in the hosts interacting with pathogen isolates containing different *Avr*-genes.

The HR has been associated with resistance in other late blight systems (26,48,55). In petunias, the HR was present in all three interaction types (compatible, partially resistant, and highly resistant), but was more prominent in the highly resistant interaction. The hyphae observed growing beyond the HR cells in the partially resistant interaction in 'Sugar Daddy' petunias is consistent with the mix of nonsporulating and sporulating lesions seen on this cultivar. Our results are consistent with those found in the potato and tomato late blight systems in which the HR is associated with resistance to *P. infestans* (26,48,55).

Reports on age-related late blight resistance in potatoes and tomatoes are contradictory but suggest that age might have an effect on resistance (4,12,18,38,49,50, 54,56). Recent occurrences of petunia late blight in commercial floriculture production have involved very young plants (personal observations). In the current study, plant age had a significant effect on resistance in petunias, with resistance increasing with plant age. This type of resistance has been termed age-related resistance (ARR) (33). In some systems, ARR has been correlated to increased production of defense related compounds with increasing plant age (17,23,34,37,59) and flowering (17,37). In addition, work on *Arabidopsis* suggests that ARR depends on the accumulation of salicylic acid (SA) (33). Interestingly, treatment of the SA analog, acibenzolar-*S*-methyl (ASM), results in complete resistance to late blight in petunias (2). Perhaps late blight resistance in petunias is related to the presence of SA. Further work is needed to determine the role of SA in petunia resistance to late blight.

All four isolates tested in the present study induced disease on *N. benthamiana*. Furthermore, these isolates possessed and expressed the *inf1* gene, and the amino acid sequence predicted from the cDNA of the gene from three isolates matched a previously published sequence (28). All isolates tested produced 10-kDa proteins in culture and the culture filtrate caused the HR in *N. benthamiana* and *N. tabacum*: criteria previously used to ascertain the presence of elicitins (27,29,30,42). The banding patterns observed in the Southern analyses are consistent with those obtained by Kamoun et al. (28), who suggested that the three bands associated with the *Sal*1 reflect the presence of at least two copies of the *inf1* gene in the genome. Thus, we have identified several isolates that produce INF1, yet still have the ability to infect *N. benthamiana*. Previous work on the potato–*P. infestans* and the *N. tabacum*–*P. parasitica* systems has shown that the *inf1* and the *parA1* (gene encoding the elicitin parasiticein) genes are downregulated during infection (6,28). Therefore, we can hypothesize that the *inf1* gene is being downregulated during infection of

Table 4. Response of *Nicotiana benthamiana* and *N. tabacum* to infiltration with culture filtrates of various *Phytophthora infestans* isolates[a]

	Hypersensitive response (HR) 48 h after inoculation	
Infiltration solution	***N. tabacum***	***N. benthamiana***
US940480 culture filtrate	HR	HR
US970001 culture filtrate	HR	HR
US990025 culture filtrate	HR	HR
MX980085 culture filtrate	HR	HR
pFB53 supernatant[b]	HR	HR[c]
LB/ampicillin broth	--	--
Minimal media	--	--
Distilled water	--	--

[a] HR = hypersensitive response; -- = no HR.
[b] *Escherichia coli* expression vector producing INF1.
[c] No HR at 1 µg/ml, only at 15 and 25 µg/ml.

N. benthamiana by the isolates tested in the current study, an interesting hypothesis to test in future studies.

The results of our research indicate that the host range of *P. infestans* should be expanded to include *N. benthamiana* and calibrachoas. The expansion of the host range of *P. infestans* to include *N. benthamiana* is beneficial to experimental host–pathogen studies involving *P. infestans* because Virus Induced Gene Silencing (VIGS) is more readily accomplished with *N. benthamiana* than with tomatoes (for example see 44). The continued inclusion of petunias and the addition of calibrachoas as host plants has important practical implications when these crops are produced in proximity to tomatoes.

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LITERATURE CITED

1. Adler, N. E., Chacon, G., Flier, W. G., and Forbes, G. A. 2002. The Andean fruit crop, pear melon (*Solanum muricatum*) is a common host for A1 and A2 strains of *Phytophthora infestans* in Ecuador. Plant Pathol. 51:802.
2. 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.
3. Bonnet, P., Bourdon, E., Ponchet, M., Blein, J. P., and Ricci, P. 1996. Acquired resistance triggered by elicitins in tobacco and other plants. Eur. J. Plant Pathol. 102:181-192.
4. Carnegie, S. F., and Colhoun, J. 1982. Susceptibility of potato leaves to *Phytophthora infestans* in relation to plant age and position. J. Phytopathol. 104:157-167.
5. 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.
6. Colas, V., Conrod, S., Venard, P., Keller, H., Ricci, P., and Panabieres, F. 2001. Elicitin genes expressed in vitro by certain tobacco isolates of *Phytophthora parasitica* are down regulated during compatible interactions. Mol. Plant-Microbe Interact. 14:326-335.
7. Colon, L. T., Eijlander, R., Budding, D. J., Vanijzendoorn, M. T., Pieters, M. M. J., and Hoogendoorn, J. 1993. Resistance to potato late blight (*Phytophthora infestans* (Mont) deBary) in *Solanum nigrum*, *S. villosum* and their sexual hybrids with *Solanum tuberosum* and *S. demissum*. Euphytica 66:55-64.
8. Conners, I. L. 1932. Annual report of the Canadian plant disease survey, 12th. Dominion of Canada Department of Agriculture Experimental Farms Branch.
9. Deahl, K. L., and Fravel, D. R. 2003. Occurrence of leaf blight on petunia caused by *Phytophthora infestans* in Maryland. Plant Dis. 87:1004.
10. Deahl, K. L., Shaw, D. S., and Cooke, L. R. 2004. Natural occurrence of *Phytophthora infestans* on black nightshade (*Solanum nigrum*) in Wales. Plant Dis. 88:771.
11. Derie, M. L., and Inglis, D. A. 2001. Persistence of complex virulences in populations of *Phytophthora infestans* in western Washington. Phytopathology 91:606-612.
12. Dowley, L. J., Routley, D. G., and Peirce, L. C. 1975. Ontogenetic predisposition of tomato foliage to race-o of *Phytophthora infestans*. Phytopathology 65:1422-1424.
13. Erwin, C. D., and Ribeiro, O. K. 1996. *Phytophthora* Diseases Worldwide. American Phytopathological Society, St. Paul, MN.
14. Flier, W. G., van den Bosch, G. B. M., and Turkensteen, L. J. 2003. Epidemiological importance of *Solanum sisymbriifolium*, *S. nigrum* and *S. dulcamara* as alternative hosts for *Phytophthora infestans*. Plant Pathol. 52:595-603.
15. Fontem, D. A., Olanya, O. M., and Njualem, B. F. 2004. Reaction of certain solanaceous and asteraceous plant species to inoculation with *Phytophthora infestans* in Cameroon. J. Phytopathol. 152:331-336.
16. Forbes, G. A., Goodwin, S. B., Drenth, A., Oyarzun, P., Ordonez, M. E., and Fry, W. E. 1998. A global marker database for *Phytophthora infestans*. Plant Dis. 82:811-818.
17. Fraser, R. S. S. 1981. Evidence for the occurrence of the pathogenesis-related proteins in leaves of healthy tobacco plants during flowering. Physiol. Plant Pathol. 19:69-76.
18. Fry, W. E., and Apple, A. E. 1986. Disease management implications of age-related changes in susceptibility of potato foliage to *Phytophthora infestans*. Am. Potato J. 63:47-56.
19. Goodwin, S. B., Drenth, A., and Fry, W. E. 1992. Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. Curr. Genet. 22:107-115.
20. Gu, Y. Q., Yang, C., Thara, V. K., Zhou, J., and Martin, G. B. 2000. Pti4 is induced by ethylene and salicylic acid, and its product is phosphorylated by the Pto kinase. Plant Cell 12:771-785.
21. Hawkes, J. G. 1999. The economic importance of the family Solanaceae. In: Solanaceae IV: Advances in Biology and Utilization. M. Nee, D. E. Symon, R. N. Lester, and J. P. Jessop, eds. The Royal Botanic Gardens, Kew, England.
22. Hirst, J. M., and Moore, W. C. 1957. 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.
23. Hwang, B. K. 1995. Effects of age-related resistance and metalaxyl on capsidiol production in pepper plants infected with *Phytophthora capsici*. Pages 503-523 in: Handbook of Phytoalexin Metabolism and Action. R. P. Purkayastha and M. Daniel, eds. Marcel Dekker, Inc., New York.
24. Inglis, D., Derie, M., Gundersen, B., Vestey, E., Ludy, R., and Powelson, M. 2001. Solanaceous hosts as sources of inoculum for late blight on potato in the Pacific Northwest. Phytopathology 91:S184-S185.
25. James, W. C. 1971. An illustrated series of assessment keys for plant diseases, their preparation and usage. Can. Plant Dis. Surv. 51:39-65.
26. Kamoun, S., Huitema, E., and Vleeshouwers, V. G. A. A. 1999. Resistance to oomycetes: A general role for the hypersensitive response? Trends Plant Sci. 4:5.
27. Kamoun, S., van der Lee, T., van den Berg-Velthuis, G., de Groot, K. E., and Govers, F. 1998. Loss of production of the elicitor protein INF1 in the clonal lineage US-1 of *Phytophthora infestans*. Phytopathology 88:1315-1323.
28. Kamoun, S., van West, P., de Jong, A. J., de Groot, K. E., Vleeshouwers, V. G. A. A., and Govers, F. 1997. A gene encoding a protein elicitor of *Phytophthora infestans* is down-regulated during infection of potato. Mol. Plant-Microbe Interact. 10:13-20.
29. Kamoun, S., van West, P., Vleeshouwers, V. G. A. A., de Groot, K. E., and Grovers, F. 1998. Resistance of *Nicotiana benthamiana* to *Phytophthora infestans* is mediated by the recognition of the elicitor protein INF1. Plant Cell 10:1413-1425.
30. Kamoun, S., Young, M., Forster, H., Coffey, M. D., and Tyler, B. M. 1994. Potential role of elicitins in the interaction between *Phytophthora* species and tobacco. Appl. Environ. Microbiol. 60:1593-1598.
31. Kamoun, S., Young, M., Glascock, C. B., and Tyler, B. M. 1993. Extracellular protein elicitors from *Phytophthora*: Host-specificity and induction of resistance to bacterial and fungal phytopathogens. Mol. Plant-Microbe Interact. 6:15-25.
32. Klement, Z., Farkas, G. L., and Lovrekov, L. 1964. Hypersensitive reaction induced by phytopathogenic bacteria in tobacco leaf. Phytopathology 54:474-477.
33. Kus, J. V., Zaton, K., Sarkar, R., and Cameron, R. K. 2002. Age-related resistance in *Arabidopsis* is a developmentally regulated defense response to *Pseudomonas syringae*. Plant Cell 14:479-490.
34. Lazarovits, G., Stossel, R., and Ward, E. W. B. 1981. Age-related changes in specificity and glyceollin production in the hypocotyl reaction of soybeans to *Phytophthora megasperma* var. *sojae*. Phytopathology 71:94-97.
35. Lee, T. Y., Simko, I., and Fry, W. E. 20002. Genetic control of aggressiveness to tomato in *Phytophthora infestans*. Can. J. Plant Pathol. 24:471-480.
36. Legard, D. E., Lee, T. Y., and Fry, W. E. 1995. Pathogenic specialization in *Phytophthora in-*

festans: Aggressiveness on tomato. Phytopathology 85:1356-1361.
37. Lotan, T., Ori, N., and Fluhr, R. 1989. Pathogenesis-related proteins are developmentally regulated in tobacco flowers. Plant Cell 1:881-887.
38. Mooi, J. C. 1965. Experiments on testing field resistance to *Phytophthora infestans* by inoculating cut leaves of potato varieties. Eur. Potato J. 8:182-183.
39. Olmstead, R. G., Sweere, J. A., Spangler, R. E., Bohs, L., and Palmer, J. D. 1999. Phylogeny and provisional classification of the Solanaceae based on chloroplast DNA. Pages 111-137 in: Solanaceae IV: Advances in Biology and Utilization. M. Nee, D. E. Symon, R. N. Lester, and J. P. Jessop, eds. The Royal Botanic Gardens, Kew, England.
40. Ordonez, M. E., Hohl, H. R., Velasco, J. A., Ramon, M. P., Oyarzun, P. J., Smart, C. D., Fry, W. E., Forbes, G. A., and Erselius, L. J. 2000. A novel population of *Phytophthora*, similar to *P. infestans*, attacks wild *Solanum* species in Ecuador. Phytopathology 90:197-202.
41. 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.
42. Rathbone, M. C., Smart, C. D., and Fry, W. E. 2002. Isolates of *Phytophthora infestans* that infect *Petunia* × *hybrida* and *Nicotiana benthamiana* also produce INF1. (Abstr.) Phytopathology 92:S145.
43. Rauscher, G. M. 2004. Qualitative and quantitative resistance to *Phytophthora infestans* in reciprocal backcross progenies of *Solanum tuberosum* and *S. berthaultii*. Ph.D. thesis. Cornell University, Ithaca, NY.
44. Restrepo, S., Myers, K. L., del Pozo, O., Martin, G. B., Hart, A. L., Buell, C. R., Fry, W. E., and Smart, C. D. Gene profiling of a compatible interaction between *Phytophthora infestans* and *Solanum tuberosum* suggests a role for carbonic anhydrase. Mol. Plant-Microbe Interact. In press.
45. Ricci, P., Trentin, F., Bonnet, P., Venard, P., Moutonperronnet, F., and Bruneteau, M. 1992. Differential production of parasiticein, an elicitor of necrosis and resistance in tobacco, by isolates of *Phytophthora parasitica*. Plant Pathol. 41:298-307.
46. Sambrock, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Springs Harbor Laboratory, Cold Spring Harbor, NY.
47. Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051-1056.
48. Smart, C. D., Myers, K. L., Restrepo, S., Martin, G. B., and Fry, W. E. 2003. Partial resistance of tomato to *Phytophthora infestans* is not dependent upon ethylene, jasmonic acid, or salicylic acid signaling pathways. Mol. Plant-Microbe Interact. 16:141-148.
49. Stewart, H. E. 1990. Effect of plant age and inoculum concentration on expression of major gene resistance to *Phytophthora infestans* in detached potato leaflets. Mycol. Res. 94:823-826.
50. Stewart, H. E., Taylor, K., and Wastie, R. L. 1983. Resistance to late blight in foliage (*Phytophthora infestans*) of potatoes assessed as true seedlings and as adult plants in the glasshouse. Potato Res. 26:363-366.
51. Sztejnberg, A., and Wahl, I. 1966. Studies on the host range of the potato late blight fungus and its oversummering on wild and ornamental Solanaceae plants. Pages 156-157 in: Proceedings of the Mediterranean Phytopathological Union. Bari, Naples.
52. Vartanian, V. G., and Endo, R. M. 1985. Overwintering hosts, compatibility types, and races of *Phytophthora infestans* on tomato in southern California. Plant Dis. 69:516-519.
53. Vega-Sanchez, M. E., Erselius, L. J., Rodriguez, A. M., Bastidas, O., Hohl, H. R., Ojiambo, P. S., Mukalazi, J., Vermeulen, T., Fry, W. E., and Forbes, G. A. 2000. Host adaptation to potato and tomato within the US-1 clonal lineage of *Phytophthora infestans* in Uganda and Kenya. Plant Pathol. 49:531-539.
54. Visker, M. H. P. W., Keizer, L. C. P., Budding, D. J., Van Loon, L. C., Colon, L. T., and Struik, P. C. 2003. Leaf position prevails over plant age and leaf age in reflecting resistance to late blight in potato. Phytopathology 93:666-674.
55. Vleeshouwers, V. G. A. A., van Dooijeweert, W., Govers, F., Kamoun, S., and Colon, L. T. 2000. The hypersensitive response is associated with host and nonhost resistance to *Phytophthora infestans.* Planta 210:853-864.
56. Walter, J. M., and Conover, R. A. 1952. Hereditary resistance to late blight of tomato. Phytopathology 42:197-199.
57. Wijsman, H. J. W. 1990. On the interrelationships of certain species of *Petunia* 6. New names for the species of *Calibrachoa* formerly included into *Petunia* (Solanaceae). Acta Bot. Neerlandica 39:101-102.
58. Wijsman, H. J. W., and Dejong, J. H. 1985. On the interrelationships of certain species of *Petunia* 4. Hybridization between *Petunia linearis* and *Petunia calycina* and nomenclatorial consequences in the *Petunia* Group. Acta Bot. Neerlandica 34:337-349.
59. Wyatt, S. E., Pan, S. Q., and Kuc, J. 1991. Beta-1,3-glucanase, chitinase, and peroxidase-activities in tobacco tissues resistant and susceptible to blue mold as related to flowering, age and sucker development. Physiol. Mol. Plant Pathol. 39:433-440.