Characterization of Isolates of *Phytophthora infestans* from Four Solanaceous Hosts Growing in Association With Late Blight-infected Commercial Potato Crops

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Abstract. The oomycete, *Phytophthora infestans*, is a devastating pathogen of potato worldwide. Several genotypes of *P. infestans* are able to infect other cultivated and weed species of the family Solanaceae and cause symptoms similar to late blight. Changes in *P. infestans* populations have stimulated investigations to determine if potato strains from new immigrant populations infect nonpotato hosts more often than those from the older population. Expansion of the effective host range may be one of the mechanisms involved in pathogenic changes in natural populations of *P. infestans* and to determine its significance, it is necessary to establish if the pathogen strains on nonpotato hosts represent distinct genotypes/populations or are freely exchanging with those on potato. This article reports characterization of *P. infestans* isolates from four solanaceous hosts (black nightshade, hairy nightshade, petunia, and tomato) growing within and around fields of blighted potatoes in four U.S. locations and one U.K. location and their comparison with isolates collected from adjacent infected potatoes. Isolates were characterized for mitochondrial DNA haplotype, mating type, metalaxyl resistance, allozymes of glucose-6-phosphate isomerase and peptidase, and DNA fingerprint with the RG57 probe. Analysis showed close similarity of the petunia, hairy and black nightshade isolates to potato isolates. However, tomatoes from New Jersey and Pennsylvania, respectively, were infected by two distinct and previously unreported pathogen genotypes, which had quite different fingerprints from *P. infestans* isolates recovered from nearby infected potatoes. Potato growers should be aware that both weed and cultivated solanaceous species can be infected with *P. infestans* and may serve as clandestine reservoirs of inoculum. Because some of these plants do not show conspicuous symptoms, they may escape detection and fail to be either removed or treated and so may play a major role in the introduction and spread of pathogens to new locations.

Late blight, incited by the oomycete pathogen *Phytophthora infestans* (Mont.) de Bary, has become an increasingly important problem to agriculture in the United States and many other countries in the past decade. More aggressive fungicide-resistant and host-specialized isolates have appeared attacking potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* Mill.) crops.

In Canada and the United States, two major genotypes of the pathogen, an A2

mating type (US-8) and an A1 mating type (US-11), both resistant to the fungicide metalaxyl, are present (Daayf et al., 2000; Dorrance et al., 1999; Gavino et al., 2000; Goodwin et al., 1998). The US-8 clonal lineage is the most common on potatoes, but US-11 has been particularly troublesome on tomatoes (Gavino et al., 2000). Although these two pathogen genotypes predominate, the infrequent occurrence of other genotypes (Goodwin et al., 1998) indicates that sexually reproducing population pockets or introduced pathogen genotypes pose a continuing threat.

Late blight has suddenly reemerged as a major concern in most tomato-producing areas of the United States. The pathogen has

been reported as being more genetically diverse on tomato than on potato (Wangsomboondee et al., 2002). Epidemics occurred in 2004 when over 50% of the commercial crop was lost in eastern states from New York to Florida; 80% to 90% of early seedbeds in Florida were a complete loss from Nov. 2004 to Apr. 2005. Most growers reported that no available fungicides would control the rapid spread of the epidemic. Heavy losses occurred in transit; symptoms developed on infected but symptomless fruit within 5 d of harvest.

Late blight affects both the leaves and fruit of tomatoes, spreading rapidly. On leaves, greasy-looking, irregularly shaped gray spots appear around which a ring of white mycelium may develop, especially in wet weather. The spots eventually turn dry and papery. Blackened areas may appear on the stems. The fruit also develop large, irregularly shaped, greasy gray spots. *P. infestans* can overwinter in frost-free areas in dead tomato plants and diseased debris (Peterson, 1947). Because it spreads to potatoes, it also overwinters in potato tubers. For transplant tomatoes, the initial source of disease may be infected transplants. Other potential sources of inoculum are potato cull piles and volunteer potatoes and tomatoes.

Solanaceous weed and ornamental species may also be infected with late blight and, where they grow in proximity to potato and tomato crops, may act as reservoirs of inoculum or aid generation of diversity if they permit contact between crop-specialized genotypes. Furthermore, late blight has also been reported on weed species (e.g., nightshade) in the United States and Canada where many potato and tomato production areas are directly linked geographically or by crop marketing. Species reported to be involved include petunia, black nightshade, and hairy nightshade. Although late blight is a highly significant, well-studied disease of potato and tomato, relatively little is known about this disease incited by the same organism on other solanaceous hosts.

Materials and Methods

Collection of isolates. Samples were collected from solanaceous hosts with lesions similar to late blight in four locations in major potato production areas in the United States and one location in Wales (see subsequently). Blighted potato material was collected from the same locations by cooperators during crop inspections of naturally infected fields. Each sample consisted of infected leaves and stems from one or more plants within a single crop. Information on site, fungicide use, potato cultivar, and blight incidence was collected for each sample. Single lesions were incubated under high humidity for 24 to 48 h to encourage sporulation, then isolates were obtained by collecting sporangia from infected foliage and initially maintained on detached glasshouse-grown potato leaflets or tuber slices of susceptible cultivars free from R-genes.

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Tissue pieces 3 to 4 mm² were cut from the margins of lesions on the detached leaflets, surface sterilized, and transferred to Petri plates containing rye A agar (Caten and Jinks, 1968) amended with antibiotics (100 µg·mL⁻¹ ampicillin, 100 µg·mL⁻¹ nystatin, 50 µg·mL⁻¹ rifampicin). The plates were incubated at 20 °C for 5 to 7 d to allow mycelia to grow into the medium. Small agar blocks containing hyphal tips were cut from the colony margins and transferred to unamended rye A agar for growth and sporulation at 20 °C. Stock cultures were maintained on unamended rye A agar and transferred at 6- to 12-month intervals.

Alternate hosts of Phytophthora infestans. Lesions similar to those incited by *P. infestans* were found on other cultivated and weed species of the family Solanaceae and isolates were obtained from these (Table 1) as detailed subsequently.

Black nightshade. In 1999, brown necrotic leaf lesions with pale green margins were found on black nightshade (*Solanum nigrum* L.) weeds within a potato trial naturally infected with *P. infestans* at Henfaes Research Center, University of Wales, Bangor. Five isolates were obtained from leaf fragments. Isolates were also obtained from infected potato plants in the trial. Similar lesions were observed on black nightshade in the same trial area in 2004 and a further isolate obtained.

Hairy nightshade. An important potato production area of northeastern Maine was surveyed during 2004 for the occurrence of late blight on cultivated and noncultivated host plants. Special attention was directed to solanaceous weed species growing within and around fields of blighted potatoes. Plants of hairy nightshade (*Solanum sarrachoides* Sendtner) with numerous leaf lesions and moderate defoliation were collected from one location within the border of adjoining potato fields. Typical lesions contained extensive, white, superficial mycelia colonizing abaxial and adaxial leaf surfaces. Eight isolates were obtained from sporulating lesions on hairy nightshade and 18 isolates from nearby potatoes.

Petunia. In April 2001, a new disease was detected on petunia (*Petunia × hybrida*)

transplants at a glasshouse in central Maryland. Infected plants had extensive light gray, irregularly shaped, slightly sunken lesions up to 3 cm long on the upper leaves and branches. Stems were distorted or dead above points where lesions had coalesced to cause total or almost complete stem girdling. The disease initially occurred as scattered foci, but rapidly spread through the crop causing significant losses. The disease reappeared in the same and adjacent greenhouses in 2002 and 2003, producing severe damage in several crops. Three isolates were obtained from sporulating lesions on the leaves and stems. Late blight was observed on potato plants in fields in close proximity to the petunia glasshouses and *P. infestans* isolated.

Tomato. Samples of tomato foliage and fruit with late blight lesions were collected from commercial field crops in New Jersey in 2003 and Pennsylvania in 2004. Three isolates were obtained from New Jersey and five isolates from Pennsylvania all from different crops. In each case, *P. infestans* was also isolated from potato crops in the immediate vicinity.

Mating type determination. Unamended rye agar plates were inoculated with a mycelial plug of the test isolate, and a plug of a reference *P. infestans* isolate of either the A1 or A2 mating type placed 20 to 30 mm away (four plates for each test isolate, two with different A1 reference isolates and two with different A2 reference isolates). The dual cultures were incubated at 15 °C in darkness for 7 to 14 d and then examined microscopically for the presence of oospores where the two colonies interacted.

Metalaxyl sensitivity in vitro. Metalaxyl (as 'Ridomil 2E', Syngenta, Greensboro, N.C., 25.1% w/w metalaxyl) was added to rye seed agar to yield a final concentration of 10 mg·L⁻¹. Each isolate was inoculated onto three Petri plates of metalaxyl-amended agar and three unamended control plates using agar plugs (5-mm diameter) cut from the outer zones of active growth from cultures aged 10 to 20 d. Standard metalaxyl-resistant and -sensitive *P. infestans* isolates were included with each set of tests. Plates were incubated at 21 °C in darkness and colony

diameters measured once average growth diameters reached 15 mm on untreated plates (typically after 5 to 6 d for faster-growing isolates) and again 2 to 3 d later. The percentage reduction in growth on 10 mg·L⁻¹ metalaxyl compared with growth on the unamended control plates was calculated. Each isolate was tested at least twice. Isolates were designated as metalaxyl-resistant if growth was >60% of the control, -intermediate if growth was 10% to 60% of the control, or -sensitive if growth was <10% of the control using the criteria of Shattock (1988).

Allozyme assays. Genotypes at two polymorphic allozyme loci, *Gpi-1* (glucose-6-phosphate isomerase, GPI, E.C. 5.3.1.9.) and *Pep-1* (peptidase, PEP, E.C. 3.4.3.1.), were determined using the protocols of Goodwin et al. (1995a). Chilled supernatants containing protein released from mycelial fragments in sterile distilled water were loaded onto cellulose acetate plates equilibrated in the appropriate buffer: Tris-glycine (TG) buffer (25 mM Tris-HCl, 192 mM glycine, pH 8.5) was used for both GPI and PEP. Enzymatic activities were revealed after electrophoresis and staining with the appropriate agar overlays (Goodwin et al., 1995a). The genotypes of unknown isolates were determined by comparing their banding patterns with those of reference isolates kindly provided by SB Goodwin, USDA, Purdue University; isolate (P-83-PI), *Gpi* 90/100 *Pep* 83/100; R Young, West Virginia University; US-1 isolate WV-63, *Gpi* 86/100 *Pep* 92/100; WE Fry, Cornell University, US-6 isolate CAL 7-5 *Gpi* 100/100 *Pep* 92/100; US-7 isolate KKK-W4B, *Gpi* 100/111 *Pep* 100/100; and US-8 isolate NY-01, *Gpi* 100/111/122 *Pep* 100/100 and US-11 isolate 110B, *Gpi* 100/100/111 *Pep* 100/100; D. Inglis, Washington State University.

Identification of mitochondrial DNA haplotypes. Mitochondrial DNA (mtDNA) haplotypes of isolates were determined by polymerase chain reaction–restriction fragment length polymorphism using a modification of the method of Griffith and Shaw (1998). Two-week-old mycelium, grown in pea broth supplemented with 2 g·L⁻¹ calcium carbonate and 0.05 g·L⁻¹ β-sitosterol, was lyophilized following the methods of Goodwin et al. (1995a). DNA extraction was performed using the Qiagen DNAeasy Plant Mini Kit (Qiagen, Valencia, Calif.). DNA was amplified using two pairs of oligonucleotide primers F2/R2 and F4/R4 synthesized by GibcoBRL Life Technologies (Gaithersburg, Md.) according to the sequences given by Griffith and Shaw (1998). The sequences for the primer pairs were as follows:

F2 (forward) 5'-TTCCCTTTGTCCTCTACCGAT-3'

R2 (reverse) 5'-TTACGGCGGTTTAGCACATACA-3'

F4 (forward) 5'-TGGTCATCCA GAGGTTTATGTT-3'

R4 (reverse) 5'-CCGATACCGATAC CAGCACCA-3'

Polymerase chain reactions (PCRs) were carried out in a Perkin Elmer Gene Amp 9600

Table 1. Host origin, location, and collection date of isolates of *Phytophthora infestans* evaluated in this study.

Hosts	Location	Years when sampled	Number of isolates
Black nightshade	Wales	1999, 2004	6
Potato ^z			23
Petunia	Maryland	2002, 2003	3
Potato ^z			19
Hairy nightshade	Maine	2004	8
Potato ^z			18
Tomato	New Jersey	2003	3
Potato ^z			11
Tomato	Pennsylvania	2004	5
Potato ^z			19

^zLate-blighted potato hosts that were located in bordering, neighboring, or close-by plantings.

thermal cycler (Perkin Elmer, Wellesley, Mass.) in 21 µL final reaction volumes. Each reaction consisted of 1 µL (≈55 ng DNA) of template DNA, 2 µL 10× PCR buffer (Takara, Madison, Wis. 10× Ex *Taq* Buffer containing 20 mM MgCl₂), 1.6 µL dNTPs (2.5 mM each), 0.2 µL each of the oligonucleotide primers, 3.2 µL BSA (160 µg·mL⁻¹), 0.2 µL *Taq* DNA polymerase (Takara Ex *Taq* polymerase; 5U µL⁻¹), and 12.6 µL sterile water. Amplification of DNA was optimized by the following PCR conditions: a denaturation cycle at 94 °C for 90 s followed by 35 cycles of 94 °C for 40 s, annealing temperatures of 60 °C (primers F2/R2) or 55 °C (primers F4/R4) for 60 s, and 72 °C for 90 s. The reactions were held for 10 min at 72 °C as a final extension step before cooling down to room temperature. A 10-µL aliquot of each amplified DNA was thoroughly mixed with 20 µL of master mix containing 1 µL (50 U µL⁻¹) of the restriction enzyme *Msp*I (for primer pair F2/R2 products) or *Eco*RI (for primer pair F4/R4 products), 3 µL 10× restriction buffer (10× React 1 for *Msp*I enzyme or 10× SH buffer for *Eco*RI enzyme), and 16 µL sterile water to volume. Restriction enzyme digestions were incubated overnight at 37 °C. Five microliters of gel-loading buffer (20% Ficoll, 0.02% bromophenol blue, 0.002% xylene cyanol) were added to each digested DNA sample and 15 µL of each mixture was loaded into each well of a 2% agarose gel in 1× Tris-acetic acid-EDTA (TAE) buffer stained with ethidium bromide (0.1 µg·mL⁻¹). A 100-bp ladder (Promega, Madison, Wis.) was used as a size marker. Digested DNA patterns were resolved by electrophoresis run at ≈5 V cm for 2 to 2.5 h and visualized using an ultraviolet transilluminator at 254 nm. Images were captured using the Bio-Rad Fluor-S System (Bio-Rad, Hercules, Calif.) with Quantity One software.

RG57 Fingerprinting. DNA fingerprinting using the moderately repetitive probe RG57 was carried out as described by Goodwin et al. (1992) except as follows. The RG57

insert was PCR-amplified with the oligonucleotide primers M13 (forward and reverse). The product was purified with the Promega Wizard DNA Purification Kit and labeled with the NEN Random Primer Fluorescein Labeling Kit (Antifluorescein-AP, NEN Life Science Products, Boston, Mass.). *P. infestans* DNA was extracted using Sigma's GeneElute Plant Genomic DNA Extraction Kit (Sigma, St. Louis, Mo.). The DNA was transferred to Hybond N+ nylon membrane (Amersham Pharmacia, Piscataway, N.J.). The manufacturers' instructions for these kits were followed for membrane transfer, Southern hybridization, and nucleic acid detection.

Results

Black nightshade. Six isolates obtained from leaf fragments were subsequently confirmed as *P. infestans* by morphological, biochemical, and molecular characteristics and Koch's postulates were completed (Deahl et al., 2004). The isolates were A1 mating type, metalaxyl-sensitive, mtDNA haplotype IIa (Table 2) with an RG57 fingerprint identical to that reported by Day et al. (2004) as RF006. Isolates collected from potatoes in the vicinity of the infected black nightshade plants had identical characteristics to those from black nightshade. RF006 was the third most common fingerprint (9%) among over 1400 isolates collected from potato crops in England, Scotland, and Wales during 1995 through 1998; all isolates with this fingerprint were A1 mating type and 94% were mtDNA IIa.

Hairy nightshade. When samples of hairy nightshade plants were placed in a moist chamber to induce sporulation, lemon-shaped sporangia typical of *P. infestans* developed. The identity of the pathogen was confirmed after reisolation onto rye-lima bean medium. Koch's postulates were completed on *S. sarrachoides*, potato, and tomato with a random selection of the isolates obtained from *S. sarrachoides* (Deahl et al., 2005). Isolates obtained from the hairy night-

shade were shown to be metalaxyl-resistant (Table 2). Single sporangial isolates derived from each isolate had the allozyme genotype *Gpi* 100/111/122 and *Pep* 100/100, were A2 mating type and mtDNA Ia. DNA fingerprint analysis with probe RG57 further established that all the hairy nightshade isolates had the fingerprint characteristic of the US-8 clonal lineage, the clonal lineage most widely occurring on potato in the United States, and were identical to local *P. infestans* isolates from potato. Hairy nightshade infected with *P. infestans* was only found when blight was already widespread in potato fields.

Petunia. The pathogen sporulating on leaves and stems of the petunia plants was shown to be *P. infestans* (Deahl and Fravel, 2003). Three isolates obtained from symptomatic petunia stem and leaf tissue were all of the A2 mating type. The petunia isolates were shown to be *Gpi* 100/111/122, *Pep* 100/100, metalaxyl-resistant, and mtDNA haplotype Ia, characteristics associated with US-8; this attribution was confirmed by RG57 analysis of three single spore isolates (Table 2). The petunia isolates were indistinguishable from potato isolates collected in the same area in terms of RG57 fingerprints, morphology, and all other assessed characteristics (Table 2).

Tomato. *P. infestans* isolates obtained from late blighted tomato fields in New Jersey in 2003 were all A2 mating type, metalaxyl-resistant, and mtDNA haplotype Ia. However, these isolates were homozygous at the loci coding for both glucose-6-phosphate isomerase and peptidase, having *Gpi* 122/122, *Pep* 100/100. RG57 analysis showed that all the tomato isolates from New Jersey had a unique and previously unreported fingerprint (Fig. 1). Although severe late blight symptoms were present in nearby potato crops, the isolates were typical of the US-8 genotype (Table 2).

Single-lesion isolates from late blighted tomato fields in Pennsylvania were also all A2 mating type, metalaxyl-sensitive, mtDNA haplotype Ia and were *Gpi* 100/122, *Pep* 100/100, characteristics atypical of isolates of

Table 2. Evaluation of the mitochondrial DNA (mtDNA) haplotype, mating type, metalaxyl resistance, allozymes of glucose-6-phosphate isomerase and peptidase, and DNA fingerprint with the RG57 probe of isolates of *Phytophthora infestans* collected from potatoes, tomatoes, and weed hosts in four U.S. locations and one U.K. location.

Host	MtDNA haplotype	Allozyme genotype		Metalaxyl sensitivity ^z	Mating Type	Genotype ^y	RG57 fingerprint
		<i>Gpi</i>	<i>Pep</i>				
Black nightshade	IIa	100/100	100/100	S	A1	N/A	1,5,9,10,13,14,16,20,21,24,25
Potato ^x	IIa	100/100	100/100	S	A1	N/A	1,5,9,10,13,14,16,20,21,24,25
Petunia	Ia	100/111/122	100/100	R	A2	US-8	1,5,10,13,14,16,20,21,23,24,25
Potato ^x	Ia	100/111/122	100/100	R	A2	US-8	1,5,10,13,14,15,20,21,23,24,25
Hairy nightshade	Ia	100/111/122	100/100	R	A2	US-8	1,5,10,13,14,15,20,21,23,24,25
Potato ^x	Ia	100/111/122	100/100	I/R	A2	US-8	1,5,10,13,14,15,20,21,23,24,25
Tomato (New Jersey)	Ia	122/122	100/100	R	A2	N/D	1,3,5,7,10,13,14,18,20,21,24,25
Potato ^x	Ia	100/111/122	100/100	R	A2	US-8	1,5,10,13,14,16,20,21,23,24,25
Tomato (Pennsylvania)	Ia	100/122	100/100	S	A2	N/D	1,5,13,14,18,20,21,24,25
Potato ^x	Ia	100/111/122	100/100	R	A2	US-8	1,5,10,14,16,20,21,23,24,25

^zSensitivity to metalaxyl of isolates of *P. infestans*: R = resistant; S = sensitive; I = intermediate (as defined by Shattock, 1988).

^yN/A = not applicable, genotype designations not assigned for UK; N/D = not determined, does not conform to any published U.S. genotype.

^xLate-blighted potato hosts that were located in bordering, neighboring, or close-by plantings.

Fig. 1. DNA fingerprint patterns (probe RG57) for *Phytophthora infestans* isolates: standard isolates US-1 (lane 1), US-8 (lane 2), US-11 (lane 8), tomato isolates collected in New Jersey (lanes 3 and 5) and Pennsylvania (lane 4), petunia isolate (lane 6), and hairy nightshade isolate (lane 7).

P. infestans from potato in the same area (Table 2), which were all of the US-8 genotype. The tomato isolates had the allozyme banding pattern and mating type associated with the US-14 genotype. As with the late blighted tomatoes in New Jersey, RG57 analysis (Fig. 1) showed that the tomato isolates had a unique fingerprint (different from that found in the New Jersey isolates and not that of US-14), which does not appear to have been reported previously.

Discussion

Weeds and ornamental plants belonging to the family Solanaceae occur very widely. Solanaceous weeds often grow in the close vicinity of potato crops, yet reports of natural infection of late blight of these plants are infrequent. Although *P. infestans* has been found to be capable of infecting a wide range of solanaceous hosts (Erwin and Ribeiro, 1996), particularly when artificially inoculated (Dandurand et al., 2006), natural infection of noncrop Solanaceae has seldom been observed in the field and integrated control programs for potato late blight rarely consider a possible role for alternative hosts (e.g., Johnson, 2006). However, with the introduction of the new *P. infestans* populations in the last 20 to 30 years, the situation may be changing.

Black nightshade (*S. nigrum*) is a common annual weed of arable fields in the United Kingdom and the United States and is resistant to residual herbicides commonly used on potatoes. Natural infection of black nightshade by *P. infestans* was observed by Hirst and Stedman (1960) in England but has not been reported there since. There are

apparently no published reports of *P. infestans* naturally infecting *S. nigrum* in the United States, although Peterson (1947) reported that this species allowed sporulation when it was artificially inoculated. Indeed, some authors have considered *S. nigrum* as a nonhost of *P. infestans* (Platt, 1999; Vartanian and Endo, 1985; Vleeshouwers et al., 2000). In the present study, the infected black nightshade plants found in Henfaes, North Wales, were low-growing with large, succulent leaves 4 to 5 cm long instead of having a more erect habit and smaller leaves but were confirmed as *S. nigrum*; their atypical appearance may relate to the known phenotypic plasticity of this species (Rogers and Ogg, 1981). Although late blight has occurred in potato plots at Henfaes every year since 1999 and the black nightshade weeds have been regularly monitored, only in 2004 were a few *P. infestans*-like lesions again observed; *P. infestans* was isolated from one of these. During a nationwide late blight survey in The Netherlands in 1999 and 2000, infection of black nightshade by *P. infestans* was observed on several occasions but was considered to be a relatively rare event and not to contribute to the overall disease pressure (Flier et al., 2003). The results of the present study would support that view, because late blight was only found when the disease was already widespread in potato and the *P. infestans* genotypes isolated were typical of those infecting local potatoes.

Hairy nightshade (*S. sarrachoides*) is another common solanaceous weed of potato crops in the United States, but, as with *S. nigrum*, infection by *P. infestans* appears relatively infrequent, particularly in the eastern United States. Platt (1999) reported sporulation on this host when excised leaf and stem tissue was artificially inoculated with *P. infestans* isolates of the US-1 and US-8 clonal lineages. Similarly, Dandurand et al. (2006) also showed infection of detached leaves and wounded berries of *S. sarrachoides* inoculated with US-1 and US-8 *P. infestans* isolates. In the western United States, *S. sarrachoides* naturally infected by *P. infestans* was found in cultivated tomato fields in southern California in 1981 and 1982 (Vartanian and Endo, 1985); all isolates were A1 and readily infected tomato. Isolates collected from hairy nightshade in Washington State in 1994 (Deahl and Inglis, 1995) were metalaxyl-resistant and A1 mating type. Subsequently, Derie and Inglis (2001) investigated virulence of *P. infestans* isolates collected from potato, tomato, and other solanaceous hosts in 1998 and 1999 in the same area. Three isolates were obtained from *S. sarrachoides*, two US-8 and one US-11; virulence testing of one US-8 and one US-11 isolate showed that they were able to overcome eight and six R-genes, respectively, which was similar in the number of R-genes overcome by US-8 from potato and US-11 from tomato. Punja et al. (1998) obtained a few (<10) isolates from hairy nightshade in their study of *P. infestans* in British Colum-

bia, Canada, in 1993 to 1997; several genotypes were identified, including US-11. There are few reports of infection of *S. sarrachoides* by *P. infestans* in the major potato-producing areas of the northeastern United States. Goodwin et al. (1995b) obtained three US-1 isolates from hairy nightshade in a field in Wisconsin in 1993, whereas Goodwin et al. (1998) characterized six isolates from New York State collected in 1994, which all proved to be US-8. Although in the 2004 survey of potato crops in northeastern Maine, particular attention was directed to solanaceous weed species, plants of late blight-infected hairy nightshade were collected from only one location and the isolates of *P. infestans* recovered were again typical of the genotypes on the surrounding potato crops. This suggests that there may be a host-related factor rather than pathogenic specialization limiting infection of *S. sarrachoides* in the northeastern United States.

Thus, although solanaceous weeds, including *S. nigrum*, *S. sarrachoides*, and also *S. dulcamara* (Cooke et al., 2002; Flier et al., 2003), can act as hosts of *P. infestans*, they are generally only infected when late blight is widespread and by pathogen genotypes already occurring in potato crops in the same area. The basis of the resistance of these species to *P. infestans* is not well understood, although Flier et al. (2003) suggested that *S. nigrum* may contain R-genes. It appears unlikely that these solanaceous weeds contribute significant inoculum to late blight epidemics, because the results of this study found limited diversity, i.e., close similarity of the petunia, hairy and black nightshade isolates with potato isolates. Nonetheless, even infrequent infection of solanaceous weeds by *P. infestans* warrants investigation, particularly because some of these species have been used as sources of supposed nonhost resistance (e.g., Zimnoch-Guzowska et al., 2003).

Petunia (grown to provide flowers and bedding plants for local markets) and tomato are two common solanaceous glasshouse crops in the United States, both of which are susceptible to *P. infestans* and could serve as alternate hosts to carry inoculum over seasons to subsequent crops, including potatoes. Correspondingly, inoculum from blighted potato fields can initiate infection in greenhouse-grown petunias and tomatoes. Since the first reports of petunia late blight in 1856 (Hirst and Moore, 1957), occurrence has been sporadic in the United States; however, recent disease outbreaks (Becktell et al., 2005a, 2005b; Deahl and Fravel, 2003) suggest that petunias are very susceptible to current U.S. *P. infestans* genotypes and could have an important role in the epidemiology of potato and tomato late blight. During an extensive tomato late blight outbreak in 1998 (Gavino et al., 2000), a commercial production greenhouse in California lost both petunias and tomatoes to late blight. Subsequently, there were occurrences of late blight on field-grown tomatoes in New York for which late

blighted petunias were thought to have been the main source of inoculum (Smart and Fry, 2001). Most recently, *P. infestans* has been reported to cause leaf blight on petunia in the northeastern United States (Deahl and Fravel, 2003).

In this study, late blight on petunia initially occurred as scattered foci but rapidly spread through the crop causing significant losses. The disease reappeared in the same and adjacent greenhouses the next year, producing severe damage in several crops. In both years, late blight was observed on potato plants in fields in close proximity to the petunia glasshouses and the pathogen sporulated on leaves and stems of the petunia plants. The present study showed the close similarity of the petunia and potato isolates. Greenhouse growers who cultivate more than one solanaceous species should be aware that petunia transplants may have incipient *P. infestans* infections, which can serve as reservoirs of inoculum, provide a means of long-distance movement of the pathogen, and support genotypes that are resistant to metalaxyl-based fungicides.

The investigation of *P. infestans* genotypes from tomato and potato hosts in our study demonstrated evidence of host specialization, as previously reported by various groups, including Legard et al. (1995), Oyarzun et al. (1998) and Lebreton et al. (1999) and investigated by Lee et al. (2002). The *P. infestans* genotypes isolated from adjacent tomato and potato crops were quite distinct and the presence of additional RG57 bands in the fingerprints of isolates from tomato compared with the US-8 isolates from potato suggests that the tomato genotypes were not derived from the potato ones. The origins of these new tomato genotypes are unclear and worthy of further investigation, because they are highly aggressive and caused severe disease epidemics, which proved extremely difficult to control and resulted in significant/major economic losses.

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