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

Kenneth L. Deahl1, Richard W. Jones, and Frances M. Perez

Vegetable Laboratory, USDA, ARS, Beltsville, MD 20705-2350

David S. Shaw
School of Biological Sciences, University of Wales, Bangor, U.K., and Sárvari Research Trust, Siambra Gwynion, Llandygai, Bangor, LL57 4BG, U.K.

Louise R. Cooke
Department of Applied Plant Science, School of Agriculture & Food Science, Queen's University, Newforge Lane, Belfast, BT9 5PX, U.K.

Additional index words: tomato, petunia, nightshade, Solanum tuberosum, Lycopersicon esculentum, disease susceptibility

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 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.
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.) within a potato field 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 controls (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 (peptidease, 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 overlay (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), Gpi 90/100 Pep 83/100; R Young, West Virginia University; 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 100/100 Pep 100/100; and US-8 strain NY-01, Gpi 100/100 Pep 100/100 and US-11 isolate 110B, Gpi 100/100 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'-TCCCTTTTGCTCTTCTACCGAT-3'
- R2 (reverse) 5'-TTACGGCGGTAGACACATA-3'
- F4 (forward) 5'-TGTTCTACCCAAGGTTATGTT-3'
- R4 (reverse) 5'-CAGCATACCAGCAGACACAA-3'

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

<table>
<thead>
<tr>
<th>Hosts</th>
<th>Location</th>
<th>Years when sampled</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black nightshade</td>
<td>Wales</td>
<td>1999, 2004</td>
<td>6</td>
</tr>
<tr>
<td>Potato⁶</td>
<td></td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>Petunia</td>
<td>Maryland</td>
<td>2002, 2003</td>
<td>3</td>
</tr>
<tr>
<td>Potato⁶</td>
<td></td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>Hairy nightshade</td>
<td>Maine</td>
<td>2004</td>
<td>8</td>
</tr>
<tr>
<td>Potato⁶</td>
<td></td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>Tomato</td>
<td>New Jersey</td>
<td>2003</td>
<td>3</td>
</tr>
<tr>
<td>Potato⁶</td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Tomato</td>
<td>Pennsylvania</td>
<td>2004</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>19</td>
</tr>
</tbody>
</table>

¹Late-blighted potato hosts that were located in bordering, neighboring, or close-by plantings.
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 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/00/100, 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).

Single-lesion isolates from late blighted tomato fields in Pennsylvania were also all A2 mating type, metalaxyl-sensitive, mtDNA haplotype Ia and were Gpi/00/122, Pep 100/100, characteristics atypical of isolates of P. infestans from late blighted tomato fields in New Jersey. 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 attribute 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).

Table 2. Evaluation of the mitochondrial DNA (mtDNA) haplotype, mating type, metalaxyl resistance, alzymes 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.

<table>
<thead>
<tr>
<th>Host</th>
<th>MtDNA haplotype</th>
<th>Alzyme genotype</th>
<th>Metalaxyl sensitivity</th>
<th>Mating Type</th>
<th>Genotype</th>
<th>RG57 fingerprint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black nightshade</td>
<td>Ia/100</td>
<td>100/100</td>
<td>S</td>
<td>A1</td>
<td>N/A</td>
<td>1.5,9,10,13,14,16,20,21,24,25</td>
</tr>
<tr>
<td>Potato*</td>
<td>Ia/100</td>
<td>100/100</td>
<td>S</td>
<td>A1</td>
<td>N/A</td>
<td>1.5,9,10,13,14,16,20,21,24,25</td>
</tr>
<tr>
<td>Petunia</td>
<td>Ia/100</td>
<td>100/100</td>
<td>R</td>
<td>A2</td>
<td>US-8</td>
<td>1.5,10,13,14,16,20,21,24,25</td>
</tr>
<tr>
<td>Potato*</td>
<td>Ia/100</td>
<td>100/100</td>
<td>R</td>
<td>A2</td>
<td>US-8</td>
<td>1.5,10,13,14,15,20,21,24,25</td>
</tr>
<tr>
<td>Hairy nightshade</td>
<td>Ia/100</td>
<td>100/100</td>
<td>R</td>
<td>A2</td>
<td>US-8</td>
<td>1.5,10,13,14,15,20,21,24,25</td>
</tr>
<tr>
<td>Tomato (New Jersey)</td>
<td>Ia/100</td>
<td>100/100</td>
<td>R</td>
<td>A2</td>
<td>N/D</td>
<td>1.5,13,7,10,13,14,18,20,21,24,25</td>
</tr>
<tr>
<td>Potato*</td>
<td>Ia/100</td>
<td>100/100</td>
<td>R</td>
<td>A2</td>
<td>US-8</td>
<td>1.5,10,13,14,16,20,21,24,25</td>
</tr>
</tbody>
</table>

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

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

*Late-blighted potato hosts that were located in bordering, neighboring, or close-by plantings.
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 Al and readily infected tomato. Isolates collected from hairy nightshade in Washington State in 1994 (Deahl and Inglis, 1995) were metalaxyl-resistant and Al 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-
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 incepted *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.

Literature Cited


