

ORIGINAL ARTICLE

Tuber Blight Development in Potato Cultivars in Response to Different Genotypes of *Phytophthora infestans*Jorge Alejandro Rojas¹, William W. Kirk¹, Esther Gachango¹, David S. Douches¹ and Linda E. Hanson^{1,2}¹ Department of Plant, Soil and Microbial Science, Michigan State University, East Lansing, MI 48824, USA² Department of Plant, Soil and Microbial Science, and USDA-ARS, 494 P55B, Michigan State University, East Lansing, MI 48824, USA**Keywords**aggressiveness, genotype, late blight, *Phytophthora infestans***Correspondence**W. W. Kirk, Department of Plant, Soil and Microbial Science, Michigan State University, East Lansing, MI, USA.
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Abstract

Migrations or introduction of new genotypes of *Phytophthora infestans* to a specific region imposes a different perspective for potato production. During 2009–2010, a late blight epidemic affected the Northeastern United States, which quickly spread through several states. The epidemic was characterized by the appearance of a new genotype of *P. infestans* designated US-22, which was isolated from tomato and potato. Potato tubers are an essential component of late blight epidemics where the pathogen cannot overwinter on Solanaceous plants. Six potato cultivars were inoculated with 12 isolates of *P. infestans* (five different genotypes), including isolates of the genotype US-22. Tuber blight development was characterized in terms of tissue darkening expressed as area under the disease progress curve values and lenticel infection. The responses indicated that US-8 was more aggressive than US-22, but US-22 isolates obtained from potato were more aggressive on potato than those acquired from tomato. Tuber periderm responses to infection were limited, yet US-8 isolates infected the periderm more often than US-22 isolates. There were significant differences among the cultivars tested but cv. Jacqueline Lee was the most resistant overall. Although isolates of *P. infestans* genotype US-22 were less aggressive in comparison with US-8 isolates, US-22 isolates still infected potato tubers and were as aggressive as US-8 isolates on some cultivars. Management of late blight caused by isolates of US-22 through host resistance may be feasible but imposes a different set of criteria for consideration from those that US-8 imposed.

Introduction

The oomycete *Phytophthora infestans* (Mont.) de Bary is the causal agent of late blight, which is the most devastating disease on potato worldwide (Fry 2008). Because the disease was first reported in the 1840s (de Bary 1876), outbreaks have occurred intermittently with different degrees of impact. Since the global re-emergence of late blight in the 1980s (Fry and Goodwin 1997b), new and more aggressive genotypes have impacted potato (Hu et al. 2012) and tomato crops (Chowdappa et al. 2013). One genotype designated as US-1 dominated the global *P. infestans* population until the last decade of the 20th century.

Several genotypes then appeared and caused comparatively more severe losses than US-1 (Spielman et al. 1991; Goodwin et al. 1994; Fry and Goodwin 1997a; Fry 2008).

Vleeshouwers et al. (2010) documented the recent impact of late blight during the epidemics in the United States and Europe from 2005 to 2008, showing the capacity of this pathogen to adapt and evolve causing disease. The genotype US-8 (mating type A₂, mefenoxam-insensitive, GPI 100/110/122) has been described as one of the most aggressive genotypes to date, due to the aggressiveness of isolates on foliage (Goodwin et al. 1996; Kirk et al. 2001a). US-8 isolates also proved more aggressive on potato tubers causing

faster appearance of tuber rot symptoms than isolates observed previously (Kirk et al. 2009, 2010). The US-8 genotype quickly became predominant in potato cropping systems following its first detection in 1989 in north and central Mexico (Goodwin et al. 1992, 1998). The appearance of US-8 and the displacement of US-1 were characterized by an increase in the severity of tuber blight (Lambert and Currier 1997).

A similar case was observed recently in Europe: the genotype 13_A2, also known as Blue-13, appeared during 2006–2008 and became the dominant genotype in Great Britain and mainland Europe (Lees et al. 2008; Cooke et al. 2011, 2012) and since then in India (Chowdappa et al. 2013). Genotype 13_A2 characteristically has an increased aggressiveness on potato foliage and tubers in comparison with previous genotypes detected in the region (Cooke et al. 2011). New introduced genotypes can quickly affect epidemic development in a production region, and as the development of resistant host material is historically a lengthy process, disease management becomes very challenging (Lees et al. 2008).

The impact of tuber late blight on potato production occurs at different levels: on seed production as the potential source of new epidemics, on volunteers that serve as sources of inoculum for tomato and potato crops and on quality and yield of seed, tablestock (or ware) and processing tubers (Bonde and Schultz 1943; Kirk et al. 2009). Latent infections on seed and volunteer tubers are an important mechanism of long-term dispersion and introduction of new genotypes of *P. infestans* (Abad and Abad 1997; Nyankanga et al. 2010). The resistance of tubers against *P. infestans* and development of tuber blight are conditioned by the ability of the pathogen to penetrate the tuber tissue and the localization of the infection within the tuber. The tuber has different components involved in resistance including the periderm, outer cortical cells, medulla, lenticels and eyes (meristematic tissue) and all may respond differently to the pathogen (Pathak and Clarke 1987; Flier et al. 2007; Nyankanga et al. 2008). Different cultivars also vary in these resistance components, and there is variation in the aggressiveness of *P. infestans* genotypes (Kirk et al. 2001a, 2009, 2010). Potato breeding has focused on resistance of foliage with little effort on tuber blight resistance. This trend has changed over time due to the importance of tuber blight that can result in storage rot losses and transmission from season to season through seed (Johnson and Cummings 2009; Kirk et al. 2009, 2010). Therefore, it is important to compare tuber disease development caused by isolates of new genotypes of *P. infestans* with isolates of the existing genotypes to

commonly produced cultivars and those with known tuber resistance to *P. infestans*.

The late blight epidemics of 2009–2010 in the Eastern United States were characterized by the appearance of a new genotype, designated as US-22. The genotype US-22 was initially reported in Florida in 2007 (Ristaino 2010; Hu et al. 2012) and then found in infected potato and tomato along the Eastern US coast (Hu et al. 2012). This new genotype is complex and temporally displaced the US-8 genotype in Michigan (Rojas and Kirk 2011). The change in the genetic structure of the *P. infestans* population in Michigan necessitates the evaluation of currently available cultivars and recently released late blight resistant cultivars from breeding programmes. Therefore, the aim of this study was to compare the ability of the new genotype, US-22, as well as other *P. infestans* genotypes to cause tuber breakdown at 10°C, the storage temperature typically used for chip processing (potato crisp).

Materials and Methods

Germplasm selection

Six cultivars of potato were selected for evaluation. The tubers for this study were obtained from the Michigan State University (MSU) potato breeding and genetics programme and commercial potato fields in Michigan. The MSU potato breeding and genetics programme has identified potato cultivars with different responses against the US-8 genotype of *P. infestans*; the cultivars included in this study with their respective foliar and tuber ratings in parentheses were Jacqueline Lee [resistant (R), moderately resistant (MR); (Douches et al. 2001)] and Missaukee [R, intermediate (I); (Douches et al. 2010)]. Other cultivars used in this study were FL1879 [susceptible (S,S)], Russet Burbank [moderately susceptible (MS),S], Red Norland (S,S) and Monticello (S,S) (Douches et al. 1997; Porter et al. 2004). Potato tubers were stored at 3°C in the dark at 90% relative humidity (RH) until used. Tubers were warmed to 15°C in incremental steps of 2°C for 7 days before inoculation. Tubers for the experiments were within the size grade range 50–150 mm diameter (any plane). Visual examination of a random sample of tubers from each entry for disease symptoms indicated that tubers were free from late blight. The samples were further tested with an ELISA immunodiagnostic for *Phytophthora* sp. (Alert Multiwell kit – *Phytophthora* sp. Neogen Corporation, Lansing, MI, USA) according to the manufacturer's instructions; *P. infestans* was not detected in any of the tubers. Prior to inoculation, all tubers were washed

with water to remove soil. The tubers were surface-disinfested by soaking in a 2% sodium hypochlorite solution for 30 min. Tubers were dried in a controlled environment chamber with continuous airflow at 15°C in dry air (30% RH) for 4 h prior to inoculation. After inoculation, tubers were stored at 10°C and maintained for 30 days in the dark in a controlled environment chamber at 90% RH.

Isolates and inoculum preparation

Characteristics of the twelve different *P. infestans* isolates used in this study are summarized in Table 1. The selected Michigan isolates were from the collection of W.W. Kirk (MSU, USA), US-8 and US-22 reference isolates were provided by Dr. W.E. Fry (Cornell University, USA), Colombian isolates were provided by Dr. Silvia Restrepo (LAMFU, Los Andes University, Colombia), and UK isolates were provided by Dr. David Cooke (The James Hutton Institute, Scotland, UK). For lenticel infection experiment, isolates US-8F and Pi10-012 were selected due to their virulence on tuber tissue. The isolates were re-activated on inoculated leaf tissue and transferred into rye B media for 14 days in the dark at 18°C for sporangia production and transferred to the light for 2 days to encourage sporulation. Sporangia and mycelium were harvested by flooding with cold sterile water (4°C) and gentle scraping of the surface of the culture using a rubber policeman. The mycelium/sporangia suspension was stirred with a magnetic stirrer for 1 h. The suspension was strained through four layers of sterile

cheesecloth, and the sporangia concentration was measured with a hemocytometer and adjusted to approximately 1×10^4 total sporangia/ml (discharged and non-discharged). The sporangial suspensions were stored for 4 h at 4°C to encourage zoospore release from the sporangia.

Whole-tuber inoculation with *Phytophthora infestans*

Tuber late blight development caused by the different *P. infestans* genotypes on the tuber cultivars was evaluated at 10°C storage temperature using whole-tuber subepidermal inoculation. The washed, surface-disinfested tubers were inoculated by removing a 5-mm-diameter potato plug using a sterile cork borer and placing 2×10^{-5} ml of sporangia suspension (delivering zoospores released from approximately 20 sporangia per inoculation) with a hypodermic syringe and needle at the apical end of the tuber approximately 1 cm from the dominant sprout to a maximum depth of 1 cm. The potato plug was returned to close the wound, and it was sealed with petroleum jelly. A complete randomized block design with three experimental repeats consisted of 10 tubers per cultivar, six different cultivars and 12 different isolates representing four genotypes. A total of three replicates were inoculated for each of the cultivar-isolate combination. Ten control tubers per cultivar were inoculated with cold (4°C) sterile distilled H₂O. After inoculation, tubers were placed in the dark in sterilized, covered plastic crates and returned to controlled environment chambers [Percival Incubator (Model

Table 1 Characteristics of *Phytophthora infestans* isolates used for tuber late blight study including country, state and county of origin, original host, mitochondrial haplotype, glucose-6-phosphate isomerase profile, metalaxyl resistance and genotype

| Origin | Year | Isolate | Host | Location ^a | Mating Type | Mt Hap ^b | Gpi ^c | Met ^d | Genotype |
|-----------------------------|------|----------|-----------------------------|-----------------------|-------------|---------------------|------------------|------------------|----------|
| Michigan, United States | 2009 | Pi09-011 | <i>Solanum tuberosum</i> | Mecosta | A2 | 1a | 100/122 | I | US-22 |
| Michigan, United States | 2009 | Pi09-021 | <i>Solanum lycopersicum</i> | Ingham | A2 | 1a | 100/122 | S | US-22 |
| Michigan, United States | 2010 | Pi10-023 | <i>Solanum lycopersicum</i> | Mecosta | A2 | 1a | 100/122 | S | US-22 |
| Michigan, United States | 2010 | Pi10-012 | <i>Solanum tuberosum</i> | St Joseph | A2 | 1a | 100/122 | I | US-22 |
| North Dakota, United States | 1998 | Pi98-1 | <i>Solanum tuberosum</i> | United States | A2 | 1a | 100/122 | I | US-14 |
| Michigan, United States | 1997 | Pi97-5 | <i>Solanum tuberosum</i> | United States | A2 | 1a | 100/111/122 | R | US-8 |
| Colombia | – | 2568 | <i>Physalis peruviana</i> | COL | A2 | 1a | 100/111/122 | I | US-8 |
| Colombia | – | 1810 | <i>Solanum tuberosum</i> | COL | A1 | 11a | 100/100 | I | CO-2 |
| UK | – | 07-39 | <i>Solanum tuberosum</i> | UK | A2 | 1a | 100/100 | I | Blue-13 |
| UK | – | 3298A | <i>Solanum tuberosum</i> | UK | A2 | 1a | 100/100 | I | Blue-13 |
| New York, United States | – | US-8F | <i>Solanum tuberosum</i> | NY | A2 | 1a | 100/111/122 | R | US-8 |
| New York, United States | – | US-22F | <i>Solanum tuberosum</i> | NY | A2 | 1a | 100/122 | S | US-22 |

^aMichigan location names refer to counties.

^bMt Hap corresponds to mitochondrial haplotype.

^cGpi, glucose-6-phosphate isomerase profile.

^dMefenoxam response: (S) sensitive, (I) intermediate and (R) resistant based on Therrien et al. (1993).

I-36LLVL; Geneva Scientific, LLC, Fontana, WI, USA)]. The chambers were set at 10°C and 95% humidity, and the sample tubers were incubated for 30 days until evaluation.

Eye and lenticel susceptibility to *Phytophthora infestans* genotypes

Tubers of three different cultivars with different responses to *P. infestans* were obtained from MSU potato breeding programme to evaluate periderm susceptibility. The three cultivars with different tuber blight ratings were Atlantic (S), Jacqueline Lee (MR) and Stirling (R) (Kirk et al. 2009). Tubers were prepared as described above. Tubers were submerged in a sporangial suspension containing approximately 1×10^4 total sporangia/ml for 48 h at 18°C. After inoculation, tubers were placed in the dark in sterilized, covered plastic crates with damp towels to maintain high humidity and then placed in controlled environment chambers at 10°C. The experimental design encompassed two different *P. infestans* genotypes (US-8F and Pi10-012), three cultivars and 12 tubers per cultivar. Arbitrary samples of three tubers per genotype–cultivar combination were sampled at 3, 6, 10 and 15 days postinoculation for evaluation. The experiment was conducted four times, and the replicates were analysed together, blocking by repeat. At each time, the number of eyes and the number of lenticels infected were assessed under the dissecting microscope at 20 × magnification (Olympus SZX10; Olympus America Inc., Lake Success, NY, USA) and light microscope at 200 × magnification (Olympus CX22; Olympus America Inc.).

Evaluation of tuber blight

A digital image analysis technique (Niemira et al. 1999; Kirk et al. 2001b) was used to assess tuber tissue infection. The image files were analysed using SigmaScan V3.0 (Jandel Scientific, San Rafael, CA, USA). The area selection cut-off threshold was set to 10 light intensity units, limiting the determination to the non-black parts of the image. The average reflective intensity (ARI) of all the pixels within the image gave a measurement of infection severity of the tuber tissue of each sample. The ARI was measured in sections from the apical, middle and basal regions of the tuber. The amount of late blight infected tissue per tuber was expressed as a single value (mean ARI) calculated as the average ARI of the apical, middle and basal sections evaluated 30 days after inoculation (DAI) (Fig. 1).

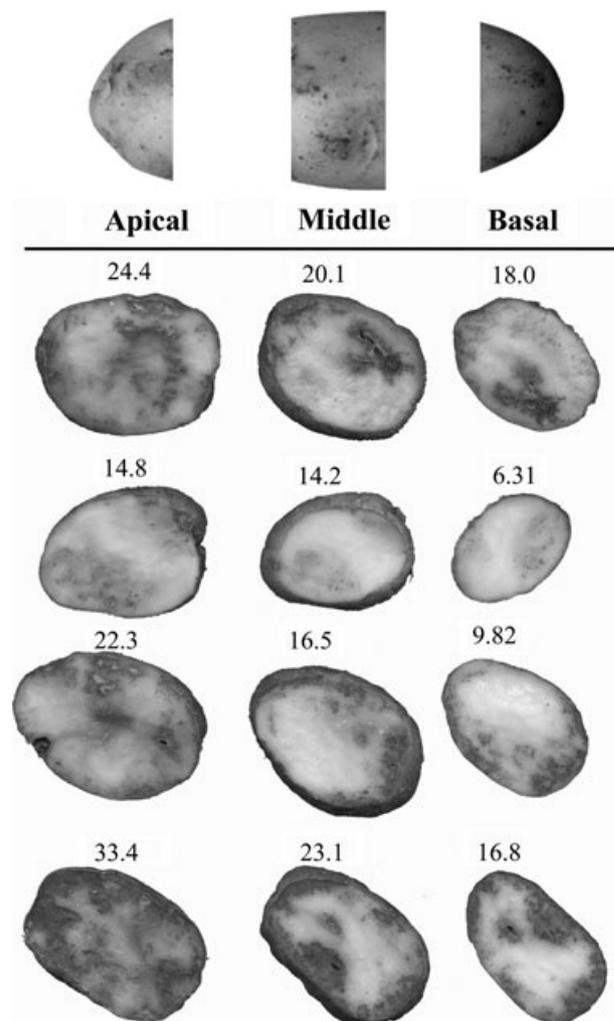


Fig. 1 Digital image of three sections of a tuber inoculated with *Phytophthora infestans* US-8 genotype. Numbers indicate RARI (%) for apical, middle and basal sections.

Late blight infection confirmation

Tissue samples (5-mm-diameter plugs) were taken from infected tubers. A rapid DNA extraction protocol proposed by Wang et al. (1993) and modified by Trout et al. (1997) for potato tissue was used. Samples were homogenized with a plastic micropestle in 100 µl 0.5 N NaOH and centrifuged at $8000 \times g$ for 5 min, and 20 µl of the supernatant was diluted with 80 µl of Tris (pH 8.0). PCR was performed using 2 µl of this extract. The primers PINF and ITS5 were used as reported by Trout et al. (1997). PCR conditions were standardized to initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, and final extension at 72°C for 10 min. PCR products were visualized in

agarose gels (1%) stained with ethidium bromide for the detection of the 600-bp band, as determined for positive amplification of *P. infestans*.

Data analysis

The severity of tuber tissue infection was expressed relative to the ARI (described above) of the control tubers for each cultivar. The relative ARI (RARI) was calculated as follows:

$$\text{RARI (\%)} = \left(1 - \frac{\text{mean ARI treatment}}{\text{mean ARI control}} \right) \times 100$$

RARI (%) has a minimum value of zero (no symptoms) and a maximum value of one hundred (black tuber surface). Data for all experiments were analysed by the analysis of variance (least squares method) using the JMP program version 9.0 (SAS Institute Inc., Cary, NC, USA). Treatment effects were determined by a three-way factorial ANOVA, where the main effects corresponded to cultivar and *P. infestans* genotype and the two-factor interaction. Principal component analysis (PCA; JMP software; SAS Institute) was carried out to describe variability among cultivars and *P. infestans* isolates.

The effect of *P. infestans* genotype on eye and lenticel infection was reported as area under the disease progress curve values (AUDPC) as described by Shaner and Finney (1977). Two-way ANOVA was calculated to determine differences among the genotypes of *P. infestans* and cultivars evaluated using the JMP program.

Results

Whole-tuber inoculation

Whole-tuber inoculation using different genotypes of *Phytophthora infestans* showed significant differences for the two main factors (genotype and cultivar) and the two-way interaction (Table 2). Among the cultivars evaluated, Dark Red Norland, Russet Burbank and Monticello were the most susceptible, but not significantly different from each other. Jacqueline Lee was the least susceptible of the six cultivars evaluated, but still had tuber blight. Among the different genotypes of *P. infestans* evaluated, several responses were observed. Isolate Pi97-5 (genotype US-8) was the most aggressive, while isolate US-8F, also classified as genotype US-8, had a lower mean RARI (Table 3). The second most aggressive isolate was an isolate designated as genotype US-22, Pi10-012, isolated from

Table 2 Two-way factorial ANOVA for the effect of isolate of *Phytophthora infestans* and cultivars on whole-tuber late blight as revealed by mean relative average reflective intensity [RARI (%)]*. Results represent the combined data set for three repetitions

| Source of variation | Df ^a | Sum of squares | F ratio ^b | P-value ^c |
|---------------------|-----------------|----------------|----------------------|----------------------|
| Cultivar | 5 | 8129.21 | 52.32 | <0.001* |
| Isolates | 11 | 67168.73 | 196.49 | <0.001* |
| Cultivar X Isolates | 55 | 20849.30 | 12.20 | <0.001* |

^aDegrees of freedom.

^bF ratio = the model mean square divided by the error mean square.

^cP = probability value (significance level or $\alpha = 0.05$).

*RARI (%) has a minimum value of zero (no symptoms) and a maximum value of 100 (black tuber surface).

potato in 2010 from St. Joseph county, MI. The European lineages, designated as 13_A2 (also known as Blue-13), were also moderately aggressive on tuber tissue, with mean RARI values between 16.7 and 13.9. Along with Blue-13 genotypes, the isolate Pi09-011 (genotype US-22) obtained during the epidemics on 2009 from potato was moderately aggressive.

The rest of the isolates used in this study caused less tissue darkening on the cultivars tested. The isolates Pi98-1 (US-14 genotype) and US-22F (US-22 genotype) had slightly lower aggressiveness in comparison with the more aggressive isolates. Michigan *P. infestans* isolates Pi10-023 and Pi09-021, characterized as genotype US-22 and isolated from tomato, were significantly different from the aggressive isolate US-8 (Pi97-5) and grouped with isolates from Colombia, as low aggressive isolates on tuber tissue (Table 3).

The two-way interaction visualized as principal components analysis showed that for cultivars axis 1 and axis 2 accounted for 56.9 and 14.6% of the variability, respectively. With respect to the *P. infestans*, isolates axis 1 and axis 2 accounted for 36.4 and 13.1% of the variability, respectively (Fig. 2). Jacqueline Lee was the least variable of the cultivars due to its reduced susceptibility to most of the genotypes of *P. infestans* evaluated. The other cultivars behaved in a similar fashion, where Dark Red Norland, Russet Burbank, FL1879 and Monticello were the most susceptible. Isolates of *P. infestans* were variable, but isolates assigned to genotype US-22 (Table 1) had reduced variability, which indicated that they had a diminished impact on tuber blight among the different cultivars evaluated (Fig. 2). Nonetheless, the isolates Pi09-011 and Pi10-012 identified as genotype US-22 obtained from potato were more aggressive on tuber tissue overall in comparison with other US-22 isolates. Also, the isolates Pi09-021 and Pi10-023

Table 3 Effect of different isolate of *Phytophthora infestans* on extent of tuber tissue affected by late blight as mean relative average reflective intensity [RARI (%)]^a in different potato cultivars; results represent the combined data set for three repetitions of the experiment

| Cultivar | Tuber tissue darkening caused by different genotypes of <i>Phytophthora infestans</i> | | | |
|---------------------|---|---------------------|----------|---------------|
| | Mean RARI (%) ^a | Isolate | Genotype | Mean RARI (%) |
| Dark Red Norland | 13.56a ^b | Pi97-5 | US-8 | 21.93a |
| Russet Burbank | 13.18a | Pi10-012 | US-22 | 20.45a |
| Monticello | 12.82a | 3298A | Blue-13 | 16.79b |
| Missaukee | 10.75b | US-8F | US-8 | 14.16c |
| FL 1879 | 9.67b | 07-39 | Blue-13 | 13.96c |
| Jacqueline Lee | 7.74c | Pi09-011 | US-22 | 13.04c |
| LSD _{0.05} | 2.85 | Pi98-1 | US-14 | 8.62d |
| | | US-22F | US-22 | 5.87e |
| | | 2568 | US-8 | 5.69e |
| | | Pi10-023 | US-22 | 5.29e |
| | | Pi09-021 | US-22 | 5.22e |
| | | 1810 | CO-2 | 5.10e |
| | | LSD _{0.05} | | 3.27 |

^aNormalized tuber tissue darkening score expressed as RARI (%) = $[1 - \text{mean ARI treatment} / \text{mean ARI control}] \times 100$; % RARI has a minimum value of zero (no darkening, but if the value is negative, the tuber tissue was lighter than the control) and maximum value of 100 (cut tuber surface is completely blackened).

^bValues followed by the same letter are not significantly different at $\alpha = 0.05$ for comparisons of mean RARI values within different *P. infestans* genotypes of cultivar combination (based on Fishers protected LSD).

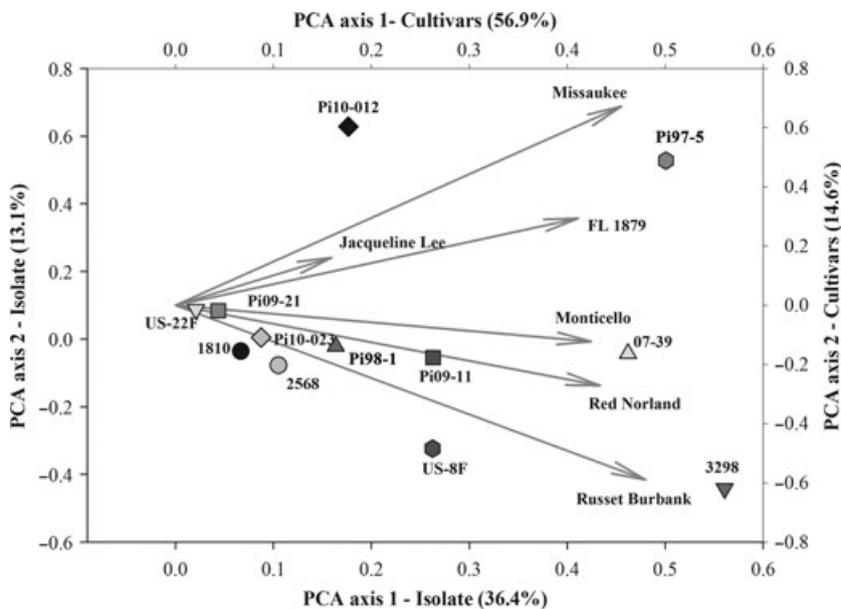


Fig. 2 Principal component analysis (PCA) for potato cultivar and genotype of *Phytophthora infestans* isolates in evaluations of tuber late blight of mean relative area under the disease progress curve RARI (%). Cultivars are represented by lines and isolates by symbols.

(US-22 genotype) from tomato were less aggressive than isolates US-8F and Pi97-5 (US-8 genotype) and 07-39 and 3298 (Blue-13). In general, Pi97-5 (US-8), 07-39 and 3298 (Blue-13) contributed most to variability among isolates and cultivars.

The interactions between cultivars and isolates of the different genotypes of *P. infestans* are shown in

Table 4. The isolate Pi97-5 (US-8) was highly aggressive in the different cultivars with mean RARI values ranging from 10 to 27%, and this isolate was chosen as an aggressive control in these studies. With respect to the US-22 isolates obtained in Michigan, Pi10-012 was moderately aggressive on most of the cultivars evaluated, with values 14.6 – 29.0%, but the isolates

Table 4 Effects of different isolates of *Phytophthora infestans* on extent of tuber tissue affected by late blight revealed by mean relative average reflective intensity [RARI (%)] in different potato cultivars

| <i>Phytophthora infestans</i> isolates | Mean RARI (%) ^a | | | | | |
|--|----------------------------|----------------|-----------|------------|------------------|----------------|
| | FL 1879 | Jacqueline Lee | Missaukee | Monticello | Dark Red Norland | Russet Burbank |
| 07-39 | 8.5 l–v ^b | 5.5p–v | 13.4i–o | 17.8e–i | 21.6c–f | 16.9f–j |
| 1810 | 4.1t–v | 4.9q–v | 2.7v | 6.6p–v | 6.5p–v | 5.8p–v |
| 2568 | 3.2uv | 4.1t–v | 4.6s–v | 5.7q–v | 7.4n–v | 9.1 l–u |
| 3298A | 12.5i–p | 7.4n–v | 11.4j–q | 18.6e–i | 21.5c–f | 29.4a |
| Pi09-021 | 5.5q–v | 4.7r–v | 5.9p–v | 5.9p–v | 5.9p–v | 3.5uv |
| Pi10-023 | 4.0s–v | 4.9r–v | 5.0q–v | 5.4q–v | 6.6p–v | 5.9p–v |
| Pi10-012 | 29.0ab | 14.1h–m | 21.1c–g | 23.5a–e | 20.3d–h | 14.6 h–l |
| Pi98-1 | 6.3p–v | 7.0o–v | 6.9o–v | 10.1k–t | 11.0j–r | 10.5k–s |
| US-22F | 3.3uv | 8.2 m–v | 8.7 l–v | 3.7u–v | 6.9p–v | 4.4t–v |
| US-8F | 5.8p–v | 14.4h–m | 13.6i–n | 20.1d–h | 15.1 g–k | 15.9f–k |
| Pi97-5 | 22.4b–f | 10.8j–s | 26.0a–d | 20.8d–g | 24.5a–d | 27.0a–c |
| Pi09-011 | 15.2g–k | 7.2n–v | 10.1k–t | 14.8g–l | 15.2g–k | 15.7f–k |

^aNormalized tuber tissue darkening score expressed as RARI (%) = $[1 - \text{mean ARI}_{\text{treatment}} / \text{mean ARI}_{\text{control}}] \times 100$; % RARI has a minimum value of zero (no darkening, but if the value is negative, the tuber tissue was lighter than the control) and maximum value of 100 (cut tuber surface is completely blackened).

^bValues followed by the same letter are not significantly different at $\alpha = 0.05$ for comparisons of mean RARI of cultivars with the different *P. infestans* genotypes (based on Fishers protected LSD).

Pi09-021 and Pi10-023 isolated from tomato had consistently lower aggressiveness among cultivars (3.5–5.9 and 4–6.6%, respectively), which agreed with the PCA analysis. Overall, the US-22 genotype isolates from potato were more aggressive than those from tomato. The local isolate Pi97-5 (US-8) was the most aggressive isolate in most of the of potato cultivars.

Tuber eyes and lenticel infection

The infection of periderm was evaluated in terms of the number of eyes and lenticels infected using isolates representing genotypes US-8 and US-22 of *P. infestans* to determine whether the new introduced genotype in Michigan was likely to infect tubers through the periderm without wounding. The isolates Pi97-5 (US-8) and Pi10-012 (US-22) (Table 1) previously identified as aggressive strains on tuber tissue were selected to infect three potato cultivars with different levels of resistance. In general, moderate lenticel infection was observed, but infected tubers had mycelial growth on the surface 10 days after inoculation [DAI (Fig. 3)]. The ANOVA of the main effects resulted in a significant difference between the genotypes US-8 and US-22 but not for cultivars. Also the interaction of genotype and cultivar for lenticel infection rated as AUDPC was not significant (Table 5). Mean values for AUDPC for genotype US-22 were lower in all cultivars, but the lowest were from cvs. Atlantic and Stirling. In contrast, the US-8 isolates

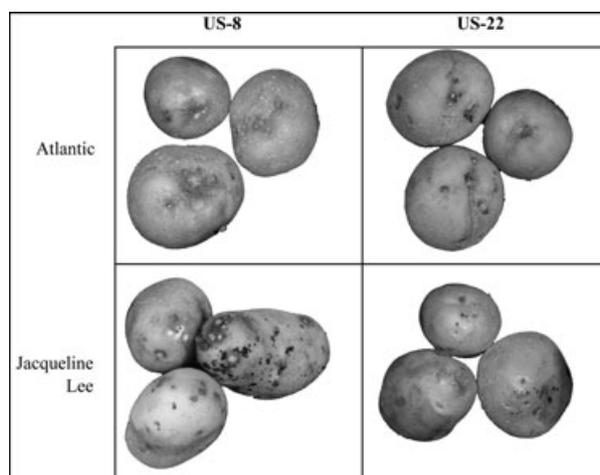


Fig. 3 Periderm infections on lenticels and eyes of two different potato cultivars infected by *Phytophthora infestans* genotypes US-8 and US-22.

were more aggressive on Atlantic and less aggressive on Stirling (Fig. 4).

Discussion

The impact of tuber blight on epidemics caused by *Phytophthora infestans* emphasizes the importance of characterizing the interaction of different genotypes against different cultivars and the effect that new genotypes could have on the existing host-plant material. Tuber blight importance has been identified

Table 5 Two-way factorial ANOVA of three different cultivars evaluated for tuber eye and lenticel infection against two genotypes of *Phytophthora infestans* (US-8 and US-22) expressed as area under disease progress curve values (AUDPC); results represent the combined data set for three repetitions

| Source of variation | Df ^a | Sum of squares | F ratio ^b | P-value ^c |
|---------------------|-----------------|----------------|----------------------|----------------------|
| Isolate | 1 | 1669.136 | 7.944 | 0.016* |
| Cultivar | 2 | 1117.355 | 2.659 | 0.111 |
| Isolate X Cultivar | 2 | 573.373 | 1.364 | 0.292 |

^aDegrees of freedom (Df).

^bF ratio = the model mean square divided by the error mean square.

^cP = probability value (significance level or $\alpha = 0.05$).

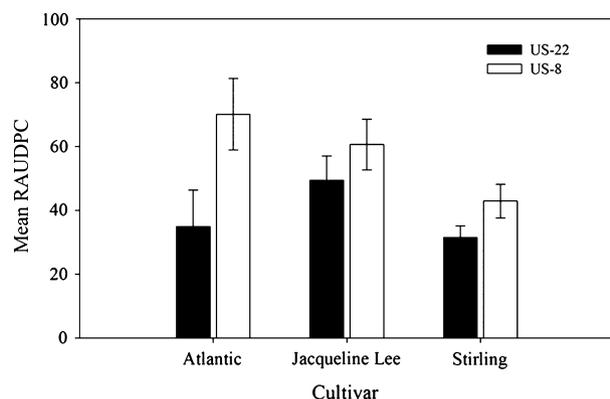


Fig. 4 Mean area under the disease progress curve values for progression of eyes and lenticel infection caused by *Phytophthora infestans* genotypes US-8 and US-22 on three different cultivars of potato. Non-overlapping error bars indicate significant differences among genotypes within the three cultivars ($P < 0.05$) as calculated using two-way ANOVA.

previously as a critical factor in storage and season-to-season transmission (Johnson and Cummings 2009; Kirk et al. 2009, 2010; Nyankanga et al. 2010).

The resistance of six different cultivars against twelve isolates representing five different genotypes of *P. infestans* was assessed in this study. The newly identified genotype US-22 was compared with other genotypes already identified and collected from the field. We focused on the resistance responses in medullar tissue and periderm, to determine the risk of the new genotype US-22 to potato growers. Large differences in susceptibility measured as medullar tissue darkening were observed among the different isolates of *P. infestans* on the potato cultivars.

The evaluation of tuber blight on medullar tissue revealed that isolates of genotype US-8 were the most aggressive in medullar tuber tissue in comparison with the other genotypes tested. Colombian isolates of *P. infestans* were less aggressive, probably due to a lack of pathogenic fitness to infect tubers, a phenomenon

that has been observed previously in other lineages found in South America (Oyarzún et al. 2005). The UK isolates designated as genotype Blue-13 were highly aggressive and similar in aggressiveness in medullar tuber tissue to US-8 genotypes of *P. infestans*. These isolates impacted potato crops in Europe during 2007–2008 (Gisi et al. 2010; Cooke et al. 2011). The variability observed among the genotype US-22 isolates used could be due to different factors, mainly due to recent introduction of this genotype to the region. For instance, isolates of genotype US-22 obtained from potato were more aggressive on potatoes than those obtained from tomato. This could be explained by host specificity, similar to previous observations in some isolates of *P. infestans* (Cooke et al. 2006). The degree of aggressiveness of the isolates on the tuber could be an important factor for transmission as aggressive isolates may not be spread from season to season due to reduced number of shoots killed by virulent *P. infestans* strains (Montarry et al. 2007). Therefore, isolates of genotype US-22 might survive better than more aggressive strains by overwintering in seed or volunteer tubers, as it has been shown that transmission after overwintering tends to be low and varies according to the season (Kirk 2003; Montarry et al. 2007).

Periderm responses (evaluated as incidence of eyes and lenticels infected) were similar among cultivars for the two *P. infestans* genotypes evaluated, but genotype US-8 was more aggressive and effective in terms of infection. One isolate of each genotype (US-8 and US-22) was used to inoculate tubers; however, these isolates were identified as the most aggressive strains on tuber tissue during this research. The establishment of infection during the season is an important step in an epidemic. Infection of potato tubers during the growing season by *P. infestans* may occur when inoculum (sporangia, zoospores or mycelia) is washed from the foliage into the soil (Andrivon 1995). Unwounded tubers would only be infected through natural openings like lenticels and eyes (Lacey 1967). Tuber resistance to *P. infestans* in cultivars is related to tuber maturity and therefore also to resistance of the periderm (Walmsley-Woodward and Lewis 1975). Genotype US-8 was more likely to infect through the periderm than genotype US-22. The AUDPC for lenticel infection incidence was low overall, which could have been related to the maturity of the tubers that were harvested approximately 3 weeks after desiccation [an adequate duration to promote periderm maturation (Johnson and Powelson 2008)]. The inoculation method used assures that the tuber eyes and lenticels were ‘open’, which would promote infection;

this has been carried out previously using high humidity to promote infection (Montarry et al. 2007). Tubers with intact skin are less susceptible to infection by *P. infestans* than those with open eyes and lenticels, but those with fresh wounds are most likely to be infected (Darsow 2004). Using intact tubers in this experiment gives a good indication of likely tuber response to infection with *P. infestans* in the field.

Generally, the aggressiveness of isolates of US-22 genotype measured across different cultivars was lower in comparison with the previously predominant US-8 genotype. The reduced aggressiveness disagrees with population changes observed during recent years in Europe and the United States (Lambert and Currier 1997; Cooke et al. 2011). The consistent aggressiveness of isolates of the US-8 genotype agrees with previous studies, and such aggressive isolates can be considered as references for breeding programmes to determine tuber resistance. To our knowledge, this was the first study to compare aggressiveness of US-22 across tubers of different potato cultivars. However, the aggressiveness of the US-22 genotype and potential overwintering properties of isolates should not be underestimated because there is little information on the epidemiology of this genotype, and its impact could become a greater issue for potato growers in the future.

Tuber blight caused by newly introduced genotypes of *P. infestans* may impose a change in emphasis of breeding efforts to generate more tolerant cultivars. The variability of susceptibility observed among the cultivars to the different isolates of US-22 could have implications for breeding programmes especially given the limited number of cultivars screened in these tests and the capacity for mutation in *P. infestans* (Catal et al. 2010).

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