A Survey of Tomato and Potato Fields in Florida Reveals Unique Genotypes of Phytophthora infestans between 2005 and 2007

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Abstract. Late blight, caused by Phytophthora infestans, affects tomatoes and potatoes in Florida during the winter–spring crop season. During the 2005 season, severe late blight epidemics were observed in Florida prompting our survey. Isolates from 2005 to 2007 were characterized phenotypically based on growth on three media, mating type, pathogenicity, and sensitivity to metalaxyl and genotypically based on two isozymes, mitochondrial DNA (mtDNA), and genomic profiling using the RG57 probe. Isolates collected in this study were all A2, mtDNA Ia, and either 100/100 (2005), or 100/122 (2006/2007) at the Gpi locus, and homozygous 100 at the Pep locus. Novel genotypes infecting tomato in Florida were recovered during 2005 to 2007. In addition to these novel genotypes recovered from tomato, one isolate was recovered from potato representing the US-8 clonal lineage. The findings of the survey in south Florida and their implications are presented.

Late blight of potato and tomato caused by the stramenopile Phytophthora infestans is regarded as the world’s most destructive crop disease and has been considered a threat to global food security (Birch and Whisson, 2001; Duncan, 1999; Grunwald and Flier, 2005; Judelson and Blanco, 2005; Mizubuti and Fry, 1998; Raman et al., 2000). The genus Phytophthora encompasses more than 80 species with P. infestans representing perhaps one of the more highly evolved species (Blair et al., 2007; Göker et al., 2007; Koon et al., 2004). This is evidenced by its host specialization, formation of haustoria, near-obligate tendencies, and aerial dispersal of asexual spores (Fry, 2008; Fry et al., 1993; Fry and Mizubuti, 1998). Phytophthora infestans is a heterothallic species requiring the presence of both the A1 and A2 mating types for production of sexually recombinant oospores and sexual recombination (Erwin and Ribeiro, 1996; Galindo and Gallegly, 1960; Smoot et al., 1958). Tomato and potato production is chronically plagued by late blight epidemics resulting from conditions during the growing season in south Florida. Late blight impacts Florida’s winter tomato crop, a $464 million industry that accounted for 36% of the national production of fresh tomatoes in 2007 (National Agricultural Statistical Service, USDA).

Early genetic investigations of P. infestans populations relied on the use of isozymes, namely glucose-6-phosphate isomerase (GPI, electrical conductivity (EC) 5.3.1.9] and peptidase (PEP EC, 3.4.3.1) and DNA fingerprinting through Southern hybridization with the RG57 probe (Goodwin et al., 1992a, 1992b, 1994b; Grunwald et al., 1994a). A second marker uses the maternally inherited mitochondrial genome (mtDNA) and has been applied to scenarios varying from population studies to typing herbarium samples from the Irish potato famine (Carter et al., 1990; Gomez-Alpizar et al., 2007; Griffith and Shaw, 1998; Ristaino et al., 2001). These molecular tools when used in combination with the phenotypes for virulence, mating type, and resistance or sensitivity to the phenylamide fungicide metalaxyl have provided a standardized framework for classifying P. infestans genotypes (Forbes et al., 1998; Goodwin et al., 1994a).

Before the 1970s, populations of P. infestans in the United States and many countries of the world were predominated by the A1 mating type and some derivative of the US-1 genotype (Andrivon, 1996; Goodwin et al., 1994a). In Florida, late blight epidemics were infrequent until the early 1990s when conducive conditions likely allowed for the migration of new genotypes from Mexico (Goodwin and Drenth, 1997; Goodwin et al., 1995b). The first novel genotype observed in Florida was US-6 (A1 mating type) in 1991 (Goodwin et al., 1994a; Weingartner and Tombolato, 2004). Both US-6 and US-7 (A2 mating type) had been reported within a single field as early as 1993 (Goodwin et al., 1995b). In Florida, by 1994, the US-8 genotype had appeared and by 1996, the US-17 genotype and possible progeny of US-6 × US-8 was recovered from tomato (Goodwin et al., 1998). We suspect that novel genotypes may be responsible for severe late blight epidemics that have been occurring since 2005. The goal of the current study was to characterize the genotypes of P. infestans recovered from tomato and potato grown in production regions in southern and central Florida over multiple seasons.

Materials and Methods

Stramenopile cultures. Isolates were obtained from leaf, stem, and fruit of tomato (85%) and potato (15%) plants in central and south Florida during the fall/spring (August/May) growing seasons spanning 3 years (2004–2005 = 2005; 2005–2006 = 2006; and 2006–2007 = 2007). Samples were obtained from southwest Florida, including Hendry, Collier, and Lee counties and from the west–central Florida county of Manatee and the east coast, Palm County. Infected leaf tissue was placed in a petri plate on a moistened sterile filter paper, wrapped with parafilm, and incubated at 20 °C in darkness. Fruit and stem samples were treated similarly, with the exception that plastic clam food boxes (14 × 14 × 7 cm; Dart Constrained Corp., Mason, MI) were used in place of petri plates. Tissue was monitored daily for sporulation, and sporangia were picked with the tip of a sterile scalpel and transferred onto...
agar plates of either Rye seed A agar (RSA) or barley potato dextrose agar (BPDA) amended with 150 μg·mL⁻¹ ampicillin (Caten and Jinks, 1967; Erwin and Ribeiro, 1996). Single-spore isolates were performed by picking sporangia from a sporulating lesion and placing in a 1.5-mL microcentrifuge tube containing 1 mL of 0.8% water agar amended with 300 μg·mL⁻¹ ampicillin warmed to 35 °C. The suspension was vortexed for 30 s and poured on BPDA amended with ampicillin. Plates were incubated at 20 °C for 24 h and when a single, germinating, sporangium was detected through a stereomicroscope, lifted with a bit of agar using the tip of a sterile scalpel, and placed either into pea broth (Wangsomboondee et al., 2002) or on BPDA.

We evaluated BPDA, RSA, and V8-juice agar (V8A) media to determine if there was a nutritional preference for two isolates representing US-20 (Pi052 and Pi058) and two representing US-21 (Pi0626 and Pi06339). The same media, also tested in the same experiments, were amended with 5% activated charcoal (AC) to test for growth enhancement because AC is reported to improve the growth of many plant pathogens without interfering with sporulation (Caten and Jinks, 1967; Erwin and Ribeiro, 1996). Three plates per treatment were inoculated with a 5-mm plug of mycelia taken from an actively growing colony of P. infestans and incubated at 20 °C in the dark. After 7 d, the colony diameter was measured in two orthogonal directions; these experiments were repeated four times. The data were analyzed using SAS (Version 9.1; Cary NC) using PROC GLM and the means were separated by Fisher’s protected least significant difference.

**Phenotypic characterization.** Mating types were determined as has been described previously (Grunwald et al., 2001). Plugs of isolates representing 42 cultures were paired on RSA agar and V8A plates either with US-20 isolates or with US-21 isolates (Pi0626 and Pi06339) were tested in pathogenicity assays. Inoculum was prepared by growing cultures on RSA for 2 weeks at 18 °C in darkness, then flooding plates with 10 mL of 4 °C H₂O₂, and rubbing to dislodge sporangia. The suspension was then filtered through four layers of cheesecloth. Sporangia were counted with a hemacytometer, and distilled water was used to adjust the suspension to 1 × 10⁴ sporangia/mL. In detached leaf assays, two leaflets were placed abaxial side up in a 100 × 15-mm plate with moistened filter paper. A 10-μL aliquot of the sporangial suspension was spotted onto each leaf. Inoculated leaflets were maintained in an incubator at 18 °C and a 14/10-h light/dark cycle. Lesion size was estimated as the percentage of the leaf area exhibiting symptoms. Detached leaf assays were repeated three times.

For intact plant assays, tomatoes were grown in 10-cm pots containing Metro-Mix 3000 with 6 g Scotts Miracle Gro Osmocote 14N–4.2P–11.6K in the greenhouse. At 4 weeks of age, plants were moved 3 d before inoculation to a growth chamber set at 20/15 °C with a 14/10-h light/dark cycle. Five plants per isolate were sprayed until runoff using a handheld aerosol mister with the sporangial suspension prepared as described previously. Untreated plants were sprayed with distilled water. After inoculation, plants were placed in clear plastic bags that had been sprayed with distilled water and returned to the growth chamber. Symptoms were evaluated 5 d after inoculation, estimating the disease severity as the percentage of total tissue that was symptomatic. Intact plant assays were repeated three times. The mean difference in disease severity was analyzed using an unpaired Student’s t test.

**Genotypic characterization.** Genotypes at two polymorphic allozyme loci, Gpi-1 (EC 5.3.1.9.) and Pdp-1 (PEP, EC 3.4.3.1.), were determined on 2005 growth chamber isolates grown on RSA-AC and V8-AC, whereas growth was significantly reduced (P < 0.0001) on other media.

**Discussion**

Our analysis of P. infestans isolates recovered from tomato and potato in Florida...
through growing seasons 2005 to 2007 identified unique genotypes not previously described within the United States. In 2005, isolates were similar to the US-13 genotype based on mating type, mtDNA, and isozyme profiles but could not be identified conclusively because a RG57 profile for US-13 genotype could not be obtained from the literature. Lacking a complete reference profile for US-13, we propose to recognize the 2005 isolates from tomato in Florida as US-20 following the suggestions put forth previously (Forbes et al., 1998; Goodwin et al., 1994a). In 2006 to 2007, the US-20 clonal lineage was essentially displaced by a new genotype, and we propose the name US-21 for the clonal lineage collected in 2006 and 2007 in Florida. In addition to the US-21 genotype, we also observed the historic US-8 genotype, which has been recovered from potato in Florida for over 12 years (Goodwin et al., 1998). It would appear that the US-8 clonal lineage is endemic in Florida’s potato production.

Isolates of *P. infestans* have been observed to be resistant to the phenylamide metalaxyl since the 1990s. Phenylamide resistance is related to a single dominant gene and levels of sensitivity may involve minor genes (Fabritius et al., 1997; Lee et al., 1999). Isolates of the same genotype in Florida varied in sensitivity to phenylamide, similar to reports elsewhere (Fraser et al., 1999; Goodwin et al., 1998). Metalaxyl-resistant isolates have higher fitness compared with sensitive isolates, and this might contribute to the increased difficulty in managing the novel isolates (Goodwin et al., 1996; Kadish and Cohen, 1988, 1992).

Mitochondrial haplotypes Ia and Ila are associated with the migration of *P. infestans* populations into Europe around 1976, whereas haplotype Iib was limited to North America (Day and Shattock, 1997; Griffith and Shaw, 1998). All our isolates are of the mtDNA Ia haplotype, which agrees with the findings of others that the "old" genotype isolates representing those present before the identification of A2 mating types outside Mexico have been or are being displaced by "new" genotypes (Day and Shattock, 1997; Fry et al., 1993; Goodwin et al., 1995c; Spielman et al., 1991).

Late blight has been documented infecting potatoes in Florida as early as 1937 (Eddins, 1945). Likewise, *P. infestans* has been a persistent problem for tomato and potato growers Florida since 1993. This work was initiated as a result of an outbreak of late blight that occurred in the 2004–2005 (2005) growing season. This outbreak was unusual because the disease was particularly destructive to tomato, extremely difficult to control despite intensive spray programs, and it persisted through the end of the season. South Florida growers typically encounter late blight only during the coldest months of the season (December to March) and can generally achieve good control with fungicides. The growth chamber inoculations demonstrated that the US-20 isolates from 2005 caused statistically significant more disease severity on tomato under the same conditions when compared with US-21 isolates from 2006 (Fig. 2). All of the isolates produced symptoms on tomato, although the potato isolate Pi0639 caused the least disease severity in this test.

The observation of the potato isolate resembling US-8 and its production of oospores in single culture prompts inquiry as to whether these sexual structures are occurring in natural settings and playing a role in the next year’s epidemics. Oospores in a single culture of *P. infestans* have been reported in the literature throughout the years (Gallegly and Galindo, 1958; Groves, 2002; Smart et al., 1998; Vartanian and Endo, 1985). Oospore formation is driven by pheromones produced and perceived by the opposite mating type; however, factors such as...
genotype, growth medium, presence of fungicides, and age of the culture influence the health and abundance of oospores observed (Smart et al., 1998). The presence of a self-fertile isolate or of both mating types does not guarantee the production of viable oospores because abortion, sexual incompatibility, and lack of mating partners have been reported in P. infestans (Erwin and Ribeiro, 1996; Flier et al., 2001). Additionally, oospore production and fecundity depend on the particular combination of parental genotypes (Flier et al., 2001). The detection of a single isolate that may be self-fertile may have implications for field survival in the field in south Florida. Factors important to late blight epidemics such as the origin of inoculum, rate and pattern of spread, and mechanisms of survival between seasons by “oversummering” are still unknown in Florida. Although our observations are based on a survey, intensive sampling throughout the season would likely provide insight on the source(s) of inoculum and survival mechanisms in Florida and perhaps other production regions in the United States. Future studies may allow for the elucidation of migration pathways responsible for the introduction of novel P. infestans genotypes into Florida and possibly the United States.

Literature Cited


