LATE BLIGHT: POPULATION CHARACTERIZATION AND TUBER DISEASE DEVELOPMENT

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ABSTRACT

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Potato late blight is caused by the oomycete *Phytophthora infestans*, and is considered the most important disease affecting potato worldwide and is also a major pathogen of tomatoes. Isolates of *P. infestans* characterized as the new genotype US-22 collected from potatoes and tomatoes from 2008-10 in Michigan suggested a drastic change on the population. This study analyzed *P. infestans* isolates obtained during 2008 to 2010 in Michigan, and compared them to reference isolates. Characterization included mating type, *Gpi* allozymes, virulence, resistance to mefenoxam, mitochondrial DNA haplotype (mtDNA) and DNA genotyping based on simple sequence repeats (SSRs). Most of the isolates were mating type A2, 100/122 *Gpi* profile and Ia mtDNA haplotype. Response to mefenoxam (EC_{50}) ranged from <0.1 – 91 µg/mL, where most of the isolates were classified as intermediate. Race and tuber virulence were also variable among isolates, but those obtained from tomato were less virulent in tubers than those obtained from potato. To further characterize, SSRs were used and revealed population structure changes over the time that the epidemics occurred. Despite the broad diversity of virulence observed, the genotypic diversity observed was low. Due to the change in the Michigan *P. infestans* populations, the interactions of different cultivars of potato with different genotypes of the potato late blight pathogen, including clonal lineage US-22, on tuber late blight development were evaluated. The success of US-22 may have been due partially to the reduced overall virulence on tubers, for example in comparison to US-8, which may result in increased fitness over time of this genotype. The continuous tracking of changes within *P. infestans* population could produce evidence of genetic shifting due to introduction of new genotypes to the region or due to variability generated by management, environmental conditions and cultivars.

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Chapter I: Literature Review

Phytophthora infestans (Mont.) de Bary, the causal agent of potato late blight is still a devastating pathogen even after 150 years since the Irish potato famine (Fry, 2008). Late blight mainly affects potato foliage, but can also significantly affect the tubers and indeed the tubers play a major role in the disease cycle of this pathogen (Platt and Tai, 1998; Nyankanga *et al.*, 2010). Potato late blight threatens potato production worldwide, which represents around 323 million tones, equivalent to 40 billion dollars of world production (FAO, 2008). The disease appears year after year causing from low effects to important epidemics like late blight epidemic in eastern USA in 2009. In Michigan, growers rate late blight as the most important disease of the potato crop (Kirk and Szendrei, 2011), with a total planted area of 18,000 ha.

Late blight is considered a re-emergent disease (Fry and Goodwin, 1997a) and its causal agent has been broadly studied to understand the infection process at different levels in order to develop effective management of the disease (Kirk *et al.*, 2010; Brurberg *et al.*, 2011b; Cárdenas *et al.*, 2011; Chen and Halterman, 2011). Population analyses have become more important as aids to understanding the epidemiology, disease progression and the biology of the pathogen and have generally indicated that the population is volatile (Guo *et al.*, 2010; Montarry *et al.*, 2010b; Brurberg *et al.*, 2011a). In order to characterize the population, different tests based on phenotypic and genotypic traits, such as allozymes, fungicide resistance, foliar virulence, nuclear DNA fingerprinting, mitochondrial haplotype (Fry, 2008) have contributed to understanding the dynamics of this pathogen.

The complex genetics of *Phytophthora infestans* limited the applicability of these techniques because of their reduced resolution that lead to the underestimation of the variability of the population (Goodwin, 1997), thus the requirement of multiple traits or markers to

overcome this issue (Fry and Goodwin, 1997a). Although, other approaches should be considered to effectively track the population dynamics, single sequence repeats (SSRs) have been successfully used to monitor late blight populations in Europe and China (Knapova and Gisi, 2002; Guo *et al.*, 2009). SSRs are powerful genetic markers, which are used to analyze population structure over time, in this case in the disease context, such as fungicide resistant, new strains, among others. In this study, we propose to use SSRs as genetic markers to monitor *Phytophthora infestans* outbreaks through Michigan in conjunction with classical methods such as race determination, mefenoxam resistance, mitochondrial haplotype, *Gpi* isozyme, mating type, among others (Forbes, 1997; Griffith and Shaw, 1998).

Population changes in production areas are mainly caused after pathogen dispersal from other areas. *P. infestans* is able to survive within the potato tuber and is transmitted when the tubers are transported from one area to another. In recent years, tuber late blight has been studied to address resistance and possible transmission dynamics from the seed to the growing plant in whole tuber infection studies using different genotypes of *P. infestans* and potato cultivars differing in levels of tuber and foliar resistance to *P. infestans* (Kirk *et al.*, 2010), but the system remains not well understood. However, different defense responses have been identified, most of them are chemical defenses observed in tuber slices, such as accumulation of phenolic compounds. The aim of this study is to study the interaction of the tuber and *P. infestans,* using whole tubers to follow the early stages of the infection, such as the formation of sprouts resulting in stem blight and the tuber responses to different *P. infestans* genotypes isolated from recent epidemics. The study of the disease at tuber level will help to understand this important inoculum source, which carries the pathogen to next crop season.

Biology of *Phytophthora infestans*

Phytophthora infestans (Mont.) de Bary is an oomycete, classified in the kingdom Stramenopila, which also comprise diatoms and brown algae. Genera *Phytopthhora* is classified within the phylum *Oomycota*, order *Peronosporales* and family *Peronosporaceae* (Cooke *et al.*, 2000), correcting the misunderstanding that *P. infestans* or other oomycetes are closely related to true fungi. The oomycetes are characterized by cell walls composed of β-1,3-glucan and cellulose, diploidy during most of the life cycle, production of motile spores, coenocytic mycelia, and production of gametangia, among others (Kamoun, 2003).

Phytophthora infestans is a heterothallic, hemibiotrophic pathogen with a complex life cycle that comprises sexual and asexual stages. The asexual stage is characterized by the production of sporangia, which are semi-papillate (lemon-shaped) spores. Sporangia are caducous, dehiscent and can either germinate forming a germ tube or collapse releasing the zoospores, the which formation of which is induced at lower temperatures around 4˚C (Gallegly and Hong, 2008). The zoospores are motile biflagellated cells (tinsel and whiplash flagella) that are able to cause infection, but their motility is limited to a short period and if no host is encountered and infection does not occur they can encyst and extend their survival (Erwin and Ribeiro, 1996; Fry, 2008).

A1 and A2 types occurring in the same population can sexually reproduce, resulting in the most potential variability in the pathogen. Sexual reproduction is induced by the production of hormones (steroids) by the respective mating types, which produce the antheridia and oogonia in the contact zone (Judelson, 1997). The oospore is the result of this fusion, which is often aplerotic, which can germinate to produce new sporangia. Oospores are important in epidemiology because the can overwinter and can resist other environmental extremes promoting

the disease in later seasons (Kirk *et al.*, 2009). However, *P. infestans* can also be self-fertile due to multiple copies of the mating type locus in one isolate or simple expression in aged cultures of either type (Fabritius and Judelson, 1997; Judelson, 1997).

The disease cycle of this pathogen starts with the dispersal of sporangia through air movement and water presence (and movement) to the foliage of the crop, where humid air with temperature around 4 to 14˚C induces the release of zoospores (10 – 12 zoospores per sporangium). Zoospores move in the water films on the leaf surfaces and encyst before infection, penetrating through stomata or directly through epidermal tissue (Agrios, 2005). Sporangia can also germinate and the mycelium can penetrate directly into the leaves, this process occurs at higher temperatures (14 to 21˚C). Once infection is established, low temperatures and rain or heavy dew days promote the infection and the production of sporangia (Andrivon, 1995), although warm weather and low humidity slow the growth without killing the pathogen (Agrios, 2005). Multiple infection events could develop during the crop season due to the production of secondary inoculum, which characterize this pathogen as a polycyclic disease (Agrios, 2005). Sporangia produced in the foliage are washed off either by rain or irrigation water that can lead to inoculum present in the soil and tuber infection (Fig. 1). Spores become water-borne infecting the lower plants of the plant, such as stems and stolons. Tubers close to soil surface are more likely to be infected and be a source of inoculum during storage either for seed or food (Andrivon, 1995; Kirk *et al.*, 1999; Kirk *et al.*, 2001b; Kirk *et al.*, 2010).

Infected tubers are harvested and stored, where the disease could be dispersed and serve as a pathogen-reservoir between seasons. Some of the tubers in storage will be use as seed, which in conjunction with the remaining tubers in the field (volunteer tubers) could be source of inoculum for the next crop season (Kirk *et al.*, 2001b; Kirk *et al.*, 2009). Infected seed pieces

will either rot in the soil or sprout and may develop late blight. Sporangia will be produced again in young plants establishing a new source of inoculum for the current season this renewing the disease cycle (Fig. 1.1).

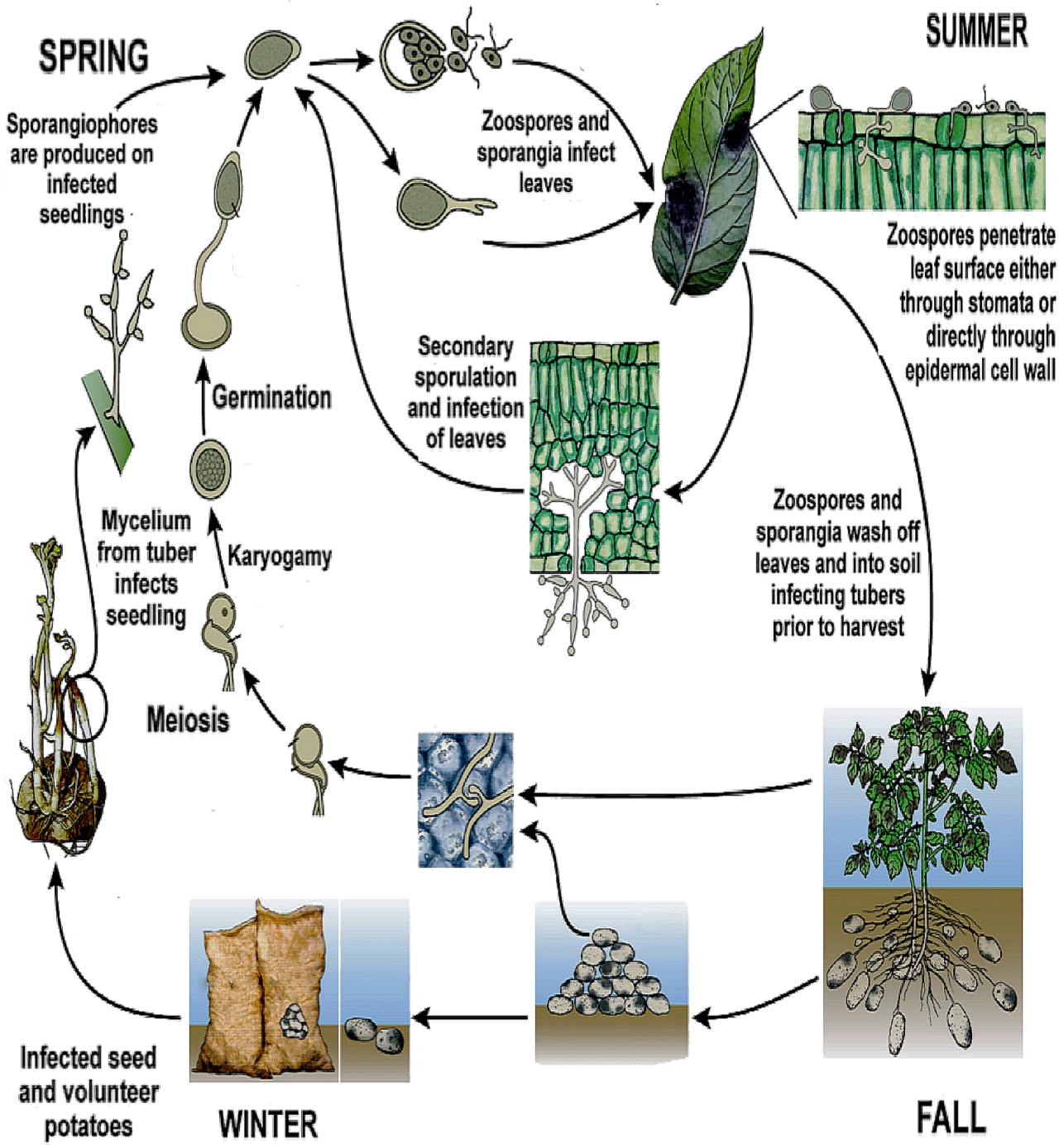

Figure 1.1. Disease cycle of *Phytophthora infestans* (Mont.) de Bary (Kirk et al., 2004). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

Origin of *Phytophthora infestans*

Phytophthora infestans epidemics and the different effects of the disease have been documented since 1845. Since then, late blight has been present in different geographical locations, threatening Solanaceous crops when environmental conditions are disease-conducive. It has been established that cool and humid weather can lead to devastating epidemics, and this pathogen has undergone a diversification process as yet unexplained. Documentation of the various epiphytotic events and studies of the genetics of this pathogen have led to three different theories about the origin of *P. infestans* and its introduction to Europe and North America. Factors underlying these theories include domestication and expansion of potato, timing of outbreaks, and genetics of the pathogen. These factors led scientists to propose three theories: the Andean theory, the Mexico theory, and the Hybrid theory.

The Andean theory was first proposed by scientists in the nineteenth century, suggesting that the region comprising Bolivia, Ecuador and Peru was the source for *P. infestans.* Berkeley (1846) described late blight, postulating the origin in the Andean region, because it is the center of origin of the main host (*Solanum tuberosum*). Most of the descriptions recovered referred to rotten tubers and mildew-like disease on potatoes that occurred after cold and humid weather in this region (Abad and Abad, 1997). From South America, the potato was taken to Europe and widely cultivated in several countries, but few cultivars were available at that time (Gomez-Alpizar *et al.*, 2007). It has been suggested that late blight was present in the potato tubers introduced to Europe, causing the dissemination of the disease. About the same time that the disease was reported in Europe, North America experienced the first epidemics on the east coast. The appearance of late blight in Europe and North America has been related to the trading of tubers as food and seed. Since *P. infestans* clonal lineage US-1 was the dominant lineage in the

Andean region, this theory was not widely accepted because the pathogen had a narrow genetic diversity; hence the theory could not explain the different genotypes of *P. infestans* in the current global populations.

The second theory proposed that Mexico was the center of origin due to the biology of the pathogen in this zone, especially in the Toluca valley (Grünwald and Flier, 2005). Several factors supported this theory and made it widely accepted. One of the most important factors was the high genetic diversity of *P. infestans* in Mexico. For instance, Goodwin *et al.* (1992a) observed *P. infestans* isolates from a single field exhibiting up to 27 different genetic loci, all of them polymorphic. Also, mating type A2 was found in Mexico, and oospores were observed in most of the infected hosts in the Toluca valley. In addition, mating type and allozyme genotypes were in Hardy-Weinberg equilibrium, which is a result of random mating; these facts confirmed that sexual reproduction had occured in this population (Gallegly and Galindo, 1958; Galindo and Gallegly, 1960).

Mexico was also a center of diversity for the Solanaceae, with plants other than potato reported as hosts of *P. infestans*, including wild *Solanum* species. Species reported included *S. acaule, S. bulbocastanum, S. caripense,* and *S. demissum* (Rivera-Peña and Molina-Galan, 1989; Rivera-Peña, 1990). In fact, some of these wild *Solanum* species served as sources of foliar resistance against the pathogen because of non-host resistance or non-race resistance (Colon and Budding, 1988). Additionally, tuber blight was detected neither in potato nor any wild tuber-bearing Solanaceous relative. Since infected leaves and stems have a short life span, the absence of tuber blight constitutes a weakness of this theory, because blighted tubers were the most likely source of inoculum for introduction of *P. infestans* in the nineteenth century. However, it was suggested that *P. infestans* spread from Mexico to the United States, where it

infected tubers that later were transported to Europe and the rest of the world. Another fact not easily explained by the theory is that the clonal lineage US-1 was dominant after its introduction to Europe and North America, yet it is not widely found in Mexico (Grünwald and Flier, 2005).

The third theory is the Hybrid or Three-Step theory, which is based on the two aforementioned theories: Mexico was the center of diversity of the pathogen, and the Andean region was the center of diversity for potato. Taking these premises into account, the theory proposed that the pathogen was introduced from Mexico to South America, from where infected tubers were transported to Europe and North America. The Hybrid theory is supported by population studies that showed relationships between Peruvian, North American and European isolates (Tooley *et al.*, 1989). This theory does not discard the previous presence of *P. infestans* in the Andean region. Indeed, herbarium specimens of potato collected in Europe during the 1840s were analyzed for the presence of *P. infestans*, showing that the lineage was different from US-1 (Ristaino, 2002). None of the theories have been fully accepted, but recent studies using coalescence analysis have shown that *P. infestans* is closely related to *P. andina,* suggesting the Andean region as the center of origin of the pathogen (Gomez-Alpizar *et al.*, 2007).

After the introduction of *P. infestans* worldwide, the clonal lineage US-1 was dominant worldwide until the 1990s, when multiple and more aggressive genotypes were detected (Fry and Goodwin, 1997a). The current populations could be a result of founder effects in Mexico, North America and Europe, leading to the diverse lineages of *P. infestans*. The founder effect supports the hybrid theory. In contrast, the US-1 lineage is still active and widely found in South America, possibly due to environmental conditions and low genetic flow, reducing pathogen variability and resulting in the fixation of this lineage (Andrivon, 1996).

The lack of variability of *P. infestans* in South America could also be explained by the absence of the A2 mating type, which was originally found only in Mexico (Gallegly and Galindo, 1958). This was true until the early 1980s, when the A2 mating type was reported from Switzerland (Hohl and Iselin, 1984), and later from other regions of Europe, North America and South America (Goodwin and Drenth, 1997). The current population structure worldwide gives some insights into the origin of the pathogen, but none of them are enough to fully support any of the theories. In addition, this is even more complicated since a second migration and disease re-emergence occurred as proposed by Fry and Goodwin (1997a). The disease re-emergence and epidemics were characterized by the presence of the clonal lineage US-8, identified in the mid-1990s; US-8 (mating type A2) has been characterized as a more aggressive lineage that is resistant to metalaxyl (Goodwin *et al.*, 1998b).

Population variability in *Phytophthora infestans*

Pathogen variability is one the main factors to consider when monitoring disease, studying epidemics, and developing effective mechanisms to manage a disease. The variability of a pathogen can be evaluated by different means using phenotypic and genotypic approaches. Plant pathogen variability was initially assessed using virulence against different cultivars; for instance, *P. infestans* was classified into physiological races using potato plants bearing different resistance genes derived from *Solanum demissum* (Malcolmson and Black, 1966). This initial assessment not only characterized the diversity of *P. infestans*, but was also used to develop an initial strategy to control the impact of late blight in the field. However, the resistance was not stable and was rapidly overcome by new isolates of the pathogen (Toxopeus, 1956). The virulence test, based on the host genotype is still used to characterize *P. infestans,* by

determining the frequencies of eleven R-genes in the host population. The different combinations of these genes that have been observed have aided documentation of the wide variability of *P. infestans* for further characterizing population structure (Andrivon *et al.*, 2010).

Differences in populations of *Phytophthora infestans* have long been considered a result of asexual variation, but a report of both mating types existing outside of Mexico increased the likeliness of sexual recombination (Brasier, 1992). However, The evidence for sexual recombination is minimal; hence, different methodologies to monitor the diversification are required. Since virulence is a trait subject to intense selection pressure, it is likely to change rapidly and cannot successfully explain the actual genetic flow. For that reason, isozyme analysis was proposed by Tooley *et al.* (1985), who used a neutral loci to evaluate genetic flow. Seventeen of 38 enzymes evaluated were active, but only two enzymes provided enough information to analyze populations, which indicated the limited availability of *P. infestans* genetic markers. Glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*) isoenzymes were selected as makers to monitor variability among *P. infestans,* totaling 10 alleles for both (Goodwin *et al.*, 1995a). These markers were essential for tracking the dominant lineages around the world in relation to mating type, but they lack the resolution needed to characterize populations in depth (Cooke and Lees, 2004).

Molecular markers became relevant to tracking populations worldwide and are widely used. For instance, two methods were used in the 1990s for assessing *P. infestans* diversity: mitochondrial DNA (mtDNA) haplotypes and RG57, which is a probe for restriction fragment analysis (RFLP). The mtDNA haplotypes were established by Griffith and Shaw (1998), who used this PCR-RFLP strategy to define four different regions of the *P. infestans* mitochondrial

genome. The four haplotypes described were Ia, Ib, IIa and IIb; the Ib haplotype, the most common worldwide, is associated with clonal lineage US-1.

On the other hand, the RG57 probe was designed to overcome the low resolution of the other methods and, together with other markers, led to the establishment of clonal lineages in regions with restricted genetic flow (Goodwin *et al.*, 1992b). Digestion of the genomic DNA and subsequent hybridization with RG57 revealed 11 to 16 bands used to characterize *P. infestans* isolates. In summary, a clonal lineage was defined by a set of markers, including mating type, isozyme profile, mtDNA haplotype, and RG57 fingerprint (Fry and Goodwin, 1997a). These markers have been widely used to characterize populations around the world; new clonal lineages have been described that are specific to certain regions (Forbes *et al.*, 1997), or are the result of new epiphytotic events (Gavino *et al.*, 2000).

The sources of variability in this pathogen are not limited to sexual recombination; parasexuality, self-fertility, and polyploidy are mechanisms that complicate the analysis of genotypes and phenotypes of *P. infestans* (Brasier, 1992; Abu-El Samen *et al.*, 2003). Indeed, the molecular basis of different traits in *Phytophthora* (e.g., segregation of the mating types or resistance against metalaxyl) is not well understood; it is likely that more than one locus is related to both (Judelson *et al.*, 1995; Judelson and Senthil, 2006).

Since the genome of *P. infestans* and some of its sister species has become available, the variability in this genus has become evident. For instance, some remnants that are still present in the genome, like repeats, suggest that an ancestor went through a whole genome duplication event (Martens and Van de Peer, 2010). Evidence includes the presence of multiple repeat-rich regions, adjacent to some gene regions; regions containing genes related to host adaptation that are required for biotrophy (Raffaele *et al.*, 2010). Some methods have successfully

discriminated among isolates, probably due to high repeat content. For instance, Amplified Fragment Length Polymorphism (AFLP) provides sufficient polymorphism information to analyze intra-population diversity (Bosch *et al.*, 2003).

The existence of repeat-rich regions also provided another tool for studying *P. infestans* diversity. Single-sequence repeats (SSRs) or microsatellites are powerful markers used in different fields to analyze the genetics of organisms (Ellegren, 2004). In plant pathology, SSRs have shown promise for tracking populations, epidemiology, ecology and evolution (Schena *et al.*, 2008). For instance, SSRs have been successfully used to monitor late blight populations in Europe and China (Knapova and Gisi, 2002; Guo *et al.*, 2009). In this study, we propose to use SSRs as genetic markers to monitor *Phytophthora infestans* outbreaks through Michigan in conjunction with classical methods such as race determination, mefenoxam resistance, mitochondrial haplotype, *Gpi* isozyme, mating type, among others (Forbes, 1997; Griffith and Shaw, 1998).

Population changes in production areas are mainly caused after pathogen dispersal from other areas where *P. infestans strains* were reshaped to survive. *P. infestans* is able to survive within the potato tuber and is transmitted when the tubers are transported from one area to another. In recent years, tuber late blight has been studied to address the aggressiveness and the transmission dynamics of *P. infestans* from the seed to the growing plant in whole tuber infection studies using different pathogen genotypes and potato cultivars differing in levels of tuber and foliar resistance to *P. infestans* (Kirk *et al.*, 2010). The aim of this study is to study the interaction of the tuber and *P. infestans,* using whole tubers to follow the early stages of the infection, such as the formation of sprouts resulting in stem blight and the tuber responses to different *P. infestans* genotypes isolated from recent epidemics.

References

References

Abad, Z. & Abad, J. (1997) Another look at the origin of late blight of potatoes, tomatoes, and pear melon in the Andes of South America. *Plant Dis.,* **81:**682-688.

Abu-El Samen, F., Secor, G. & Gudmestad, N. (2003) Variability in virulence among asexual progenies of *Phytophthora infestans*. *Phytopathology,* **93:**293-304.

Agrios, G. Plant Pathology*,* San Diego, Elsevier, 2005.

Andrivon, D. (1995) Biology, ecology, and epidemiology of the potato late blight pathogen *Phytophthora infestans* in soil. *Phytopathology,* **85:**1053-1056.

Andrivon, D. (1996) The origin of *Phytophthora infestans* populations present in Europe in the 1840s: a critical review of historical and scientific evidence. *Plant Pathol.,* **45:**1027-1035.

Andrivon, D., Avendaño-Córcoles, J., Cameron, A. M.*, et al.* (2010) Stability and variability of virulence of *Phytophthora infestans* assessed in a ring test across European laboratories. *Plant Pathol.*

Berkeley, J. M. (1846) Observations, botanical and physiological, on the potato murrain. *J. Hort. Soc. Lond.,* **1:**9-34.

Bosch, T., Garay-Serrano, E., Lozoya-Saldaña, H.*, et al.* (2003) The population structure of *Phytophthora infestans* from the Toluca Valley of Central Mexico suggests genetic differentiation between populations from cultivated potato and wild *Solanum spp. Phytopathology,* **93:**382-390.

Brasier, C. (1992) Evolutionary biology of *Phytophthora* part I: Genetic system, sexuality and the generation of variation. *Annu. Rev. Phytopathol.,* **30:**153-170.

Brurberg, M. B., Elameen, A., Le, V. H.*, et al.* (2011a) Genetic analysis of *Phytophthora infestans* populations in the Nordic European countries reveals high genetic variability. *Fungal Biology*.

Brurberg, M. B., Elameen, A., Le, V. H.*, et al.* (2011b) Genetic analysis of *Phytophthora infestans* populations in the Nordic European countries reveals high genetic variability. *Fungal Biology,* **115:**335-342.

Cárdenas, M., Grajales, A., Sierra, R.*, et al.* (2011) Genetic diversity of *Phytophthora infestans* in the Northern Andean region. *BMC Genet.,* **12:**23.

Chen, Y. & Halterman, D. A. (2011) Phenotypic Characterization of Potato Late Blight Resistance Mediated by the Broad-Spectrum Resistance Gene RB. *Phytopathology,* **101:**263-270.

Colon, L. & Budding, D. (1988) Resistance to late blight (*Phytophthora infestans*) in ten wild *Solanum* species. *Euphytica,* **39:**77-86.

Cooke, D., Drenth, A., Duncan, J.*, et al.* (2000) A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genet. Biol.,* **30:**17-32.

Cooke, D. & Lees, A. (2004) Markers, old and new, for examining *Phytophthora infestans* diversity. *Plant Pathol.,* **53:**692-704.

Ellegren, H. (2004) Microsatellites: simple sequences with complex evolution. *Nat. Rev. Genet.,* **5:**435-445.

Erwin, D. C. & Ribeiro, O. K. *Phytophthora* diseases worldwide, APS Press, 1996.

Fabritius, A. L. & Judelson, H. S. (1997) Mating-type loci segregate aberrantly in *Phytophthora infestans* but normally in *Phytophthora parasitica*: implications for models of mating-type determination. *Curr. Genet.,* **32:**60-65.

FAO. Food and Agriculture Organization of the United Nations. FAOSTAT database. In., 2008.

Forbes, G. Manual for laboratory work on *Phytophthora infestans*, 1997.

Forbes, G. A., Escobar, X. C., Ayala, C. C.*, et al.* (1997) Population genetic structure of *Phytophthora infestans* in Ecuador. *Phytopathology,* **87:**375-380.

Fry, W. (2008) *Phytophthora infestans*: the plant (and R gene) destroyer. *Mol. Plant Pathol.,* **9:**385-402.

Fry, W. & Goodwin, S. (1997a) Re-emergence of potato and tomato late blight in the United States. *Plant Dis.,* **81:**1349-1357.

Galindo, J. & Gallegly, M. (1960) The nature of sexuality in *Phytophthora infestans*. *Phytopathology,* **50:**123-128.

Gallegly, M. & Galindo, J. (1958) Mating types and oospores of *Phytophthora infestans* in nature in Mexico. *Phytopathology,* **48:**274-277.

Gallegly, M. & Hong, C. *Phytophthora*: identifying species by morphology and DNA fingerprints, APS Press, 2008.

Gavino, P., Smart, C., Sandrock, R.*, et al.* (2000) Implications of sexual reproduction for *Phytophthora infestans* in the United States: Generation of an aggressive lineage. *Plant Dis.,* **84:**731-735.

Gomez-Alpizar, L., Carbone, I. & Ristaino, J. (2007) An Andean origin of *Phytophthora infestans* inferred from mitochondrial and nuclear gene genealogies. *Proc. Natl. Acad. Sci.,* **104:**3306-3311.

Goodwin, S., Schneider, R. & Fry, W. (1995a) Use of cellulose-acetate electrophoresis for rapid identification of allozyme genotypes of *Phytophthora infestans*. *Plant Dis.,* **79:**1181-1185.

Goodwin, S., Spielman, L., Matuszak, J.*, et al.* (1992a) Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. *Phytopathology,* **82:**955-961.

Goodwin, S. B. (1997) The population genetics of *Phytophthora*. *Phytopathology,* **87:**462-473.

Goodwin, S. B. & Drenth, A. (1997) Origin of the A2 mating type of *Phytophthora infestans* outside Mexico. *Phytopathology,* **87:**992-999.

Goodwin, S. B., Drenth, A. & Fry, W. E. (1992b) Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. *Curr. Genet.,* **22:**107-115.

Goodwin, S. B., Smart, C. D., Sandrock, R. W.*, et al.* (1998b) Genetic Change Within Populations of *Phytophthora infestans* in the United States and Canada During 1994 to 1996: Role of migration and recombination. *Phytopathology,* **88:**939-949.

Griffith, G. & Shaw, D. (1998) Polymorphisms in *Phytophthora infestans*: Four mitochondrial haplotypes are detected after PCR amplification of DNA from pure cultures or from host lesions. *Appl. Environ. Microbiol.,* **64:**4007-4014.

Grünwald, N. & Flier, W. (2005) The biology of *Phytophthora infestans* at its center of origin. *Annu. Rev. Phytopathol.,* **43:**171 - 190.

Guo, J., van der Lee, T., Qu, D.*, et al.* (2009) *Phytophthora infestans* isolates from Northern China show high virulence diversity but low genotypic diversity. *Plant Biol.,* **11:**57-67.

Guo, L., Zhu, X.-Q., Hu, C.-H.*, et al.* (2010) Genetic structure of *Phytophthora infestans* populations in China indicates multiple migration events. *Phytopathology,* **100:**997-1006.

Hohl, H. & Iselin, K. (1984) Strains of *Phytophthora infestans* from Switzerland with A2 mating type behaviour. *Trans. Br. Mycol. Soc.,* **83:**529-530.

Judelson, H. (1997) The genetics and biology of *Phytophthora infestans*: Modern approaches to a historical challenge. *Fungal Genet. Biol.,* **22:**65-76.

Judelson, H. S. & Senthil, G. (2006) Investigating the role of ABC transporters in multifungicide insensitivity in *Phytophthora infestans*. *Mol. Plant Pathol.,* **7:**17-29.

Judelson, H. S., Spielman, L. J. & Shattock, R. C. (1995) Genetic mapping and non-Mendelian segregation of mating type loci in the oomycete, *Phytophthora infestans*. *Genetics,* **141:**503-512.

Kamoun, S. (2003) Molecular genetics of pathogenic oomycetes. *Eukaryot. Cell,* **2:**191-199.

Kirk, W., Abu-El Samen, F., Tumbalam, P.*, et al.* (2009) Impact of different US genotypes of *Phytophthora infestans* on potato seed tuber rot and plant emergence in a range of cultivars and advanced breeding lines. *Pot. Res.,* **52:**121-140.

Kirk, W., Felcher, K., Douches, D.*, et al.* (2001b) Susceptibility of potato (*Solanum tuberosum* L.) foliage and tubers to the US-8 genotype of *Phytophthora infestans*. *Am. J. Pot Res,* **78:**319-322.

Kirk, W. & Szendrei, Z. (2011) Potato diseases survey report. *Michigan Potato Newsline,* **22:**4-6.

Kirk, W., Wharton, P., Hammerschmidt, R.*, et al.* (2004) Late Blight. *Michigan State University Extension,* **Extension Bulletin E-2945**.

Kirk, W. W., Niemira, B. A., Stein, J. M.*, et al.* (1999) Late blight (*Phytophthora infestans* (Mont) De Bary) development from potato seed-pieces treated with fungicides. *Pestic. Sci.,* **55:**1151-1158.

Kirk, W. W., Rojas, A., Tumbalam, P. G.*, et al.* (2010) Effect of different genotypes of *Phytophthora infestans* (Mont. de Bary) and temperature on tuber disease development. *Am. J. Pot Res,* **87:**509-520.

Knapova, G. & Gisi, U. (2002) Phenotypic and genotypic structure of *Phytophthora infestans* populations on potato and tomato in France and Switzerland. *Plant Pathol.,* **51:**641-653.

Malcolmson, J. & Black, W. (1966) New R genes in *Solanum demissum* lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. *Euphytica,* **15:**199-203.

Martens, C. & Van de Peer, Y. (2010) The hidden duplication past of the plant pathogen *Phytophthora* and its consequences for infection. *BMC Genomics,* **11:**353.

Montarry, J., Andrivon, D., Glais, I.*, et al.* (2010b) Microsatellite markers reveal two admixed genetic groups and an ongoing displacement within the French population of the invasive plant pathogen *Phytophthora infestans*. *Mol. Ecol.,* **19:**1965-1977.

Nyankanga, R. O., Olanya, O. M., Ojiambo, P. S.*, et al.* (2010) Validation of tuber blight (*Phytophthora infestans*) prediction model. *Crop Prot.***:**1-7.

Platt, H. & Tai, G. (1998) Relationship between resistance to late blight in potato foliage and tubers of cultivars and breeding selections with different resistance levels. *Am. J. Pot Res,* **75:**173-178.

Raffaele, S., Farrer, R. A., Cano, L. M.*, et al.* (2010) Genome evolution following host jumps in the irish potato famine pathogen lineage. *Science,* **330:**1540-1543.

Ristaino, J. B. (2002) Tracking historic migrations of the Irish potato famine pathogen, *Phytophthora infestans*. *Microbes Infect.,* **4:**1369-1377.

Rivera-Peña, A. (1990) Wild tuber-bearing species of *Solanum* and incidence of *Phytophthora infestans* (Mont.) de Bary on the Western slopes of the volcano Nevado de Toluca. 3. Physiological races of *Phytophthora infestans*. *Pot. Res.,* **33:**349-355.

Rivera-Peña, A. & Molina-Galan, J. (1989) Wild tuber-bearing species of *Solanum* and incidence of *Phytophthora infestans* (Mont.) de Bary on the western slopes of the volcano Nevado de Toluca. 1. *Solanum* species. *Pot. Res.,* **32:**181-195.

Schena, L., Cardle, L. & Cooke (2008) Use of genome sequence data in the design and testing of SSR markers for *Phytophthora* species. *BMC Genomics,* **9:**620.

Tooley, P., Fry, W. & Gonzalez, M. (1985) Isozyme characterization of sexual and asexual *Phytophthora infestans* populations. *J. Hered.,* **76:**431.

Tooley, P., Therrien, C. & Ritch, D. (1989) Mating type, race composition, nuclear DNA content, and isozyme analysis of Peruvian isolates of *Phytophthora infestans*. *Phytopathology,* **79:**478-481.

Toxopeus, H. (1956) Reflections on the origin of new physiologic races in *Phytophthora infestans* and the breeding for resistance in potatoes. *Euphytica,* **5:**221-237.

Chapter II: Effect of different genotypes of *Phytophthora infestans* (Mont. de Bary) and temperature on tuber disease development

Kirk W.W., Rojas A., Tumbalam P.G., Gachango E., Wharton P.S., El-Samen F.A., Douches D., Coombs J., Thill C., Thompson A. (2010) Effect of Different Genotypes of *Phytophthora infestans* (Mont. de Bary) and Temperature on Tuber Disease Development. Am. J. Pot Res 87:509-520.

Abstract

The interactions of different cultivars/Advance Breeding Lines (ABL) of potato with different genotypes of the potato late blight pathogen (*Phytophthora infestans*) at three storage temperatures on tuber late blight development were evaluated. The contribution of the medullar storage tissues was assessed rather than the periderm and outer cortical cell tissue. Tuber late blight severity measured as tuber darkening [mean Relative Average Reflectance Intensity, RARI (%)] generally increased with temperature. There was little difference in tuber late blight development between 7 and 10^{o}C treatments and in some combinations significantly more tissue darkening developed at 7 than at 10^{o}C but little or no development occurred at 3^{o}C. Resistance in tubers was observed only in Torridon and Stirling and to some extent Jacqueline Lee but the cultivar Missaukee had weak tuber resistance. The US-8 genotype isolates were the most aggressive in tubers in most years causing rapid and significantly more tuber damage than any other genotype of *P. infestans* and similar to the US-6, US-10 and US-14 isolates used in 2006.

Introduction

Potato late blight is the most important and most destructive disease of potato worldwide. The disease caused by the oomycete *Phytophthora infestans* (Mont. de Bary) is the greatest threat to the potato crop, accounting for significant annual losses in North America (Guenthner et al. 2001 (Guenthner *et al.*, 1999; Guenthner *et al.*, 2001) and worldwide (Hijmans, 2001). Tuber late blight results in tuber rotting both in the field and later in storage either in tubers intended for seed or consumption (Melhus, 1915; Murphy and McKay, 1924; Murphy and McKay, 1925; Bonde and Schultz, 1943; Lambert and Currier, 1997; Kirk *et al.*, 1999; Johnson and Cummings, 2009; Olanya *et al.*, 2009). Seed tubers infected with *P. infestans* will either rot in storage, after planting in the field or survive and initiate new epidemics of potato late blight (Doster *et al.*, 1989; Dowley and O'Sullivan, 1991; Stevenson *et al.*, 2007; Kirk *et al.*, 2009). The epidemiology of the foliar phase of the disease is correlated to infection in the tuber phase and vice versa (Bain *et al.*, 1997). Tubers are usually infected by inoculum produced on the plant foliage that is subsequently washed down to the soil by water movement resulting from rainfall and irrigation (Andrivon, 1995; Porter *et al.*, 2005; Stevenson *et al.*, 2007; Fry, 2008). Tubers can become blighted shortly after the disease is established on the foliage. *P. infestans* survives in tubers where it rots tubers intended for commercial use (Niemira *et al.*, 1999) or acts as primary source of inoculum for infection in the following growing season (Bonde and Schultz, 1943).

Three major components contribute to late blight resistance in tubers; 1) a physical barrier consisting of several layers of phellem cells, known as the periderm; 2) the outer cortical cell layers that retard the growth of lesions and can completely block hyphal growth; and 3) medulla storage tissues characterized by reduced hyphal growth and sporulation of *P. infestans*

(Pathak and Clarke, 1987; Flier *et al.*, 1998; Flier *et al.*, 2001). Recent work has indicated that the new immigrant *P. infestans* clones, especially the US-8 genotype, are more aggressive in tubers and sprouts (Lambert and Currier, 1997; Kirk *et al.*, 2001c). Historically, studies of the late blight pathogen on tubers were conducted when *P. infestans* populations were dominated by US-1, a clonal lineage (Goodwin *et al.*, 1994). Today, populations of *P. infestans* have changed and tuber resistance studies need to continue because the US-8 genotype is now predominant and there is a gap in our understanding of these more aggressive genotypes. The dynamics of potato blight development in tubers are largely influenced by temperature (Kirk *et al.*, 2001c) and can result in decay in storage at currently used processing storage temperatures (e.g. 10^{O}C for chip-processing) or non-emergence of plants due to seed and sprout rot (Kirk *et al.*, 2009). The objectives of this study were to evaluate the interactions of different cultivars/Advanced Breeding Lines (ABL) of potato between different genotypes of *P. infestans* and storage temperature on tuber late blight development.

Materials and Methods

Germplasm selection

Potato breeding efforts at Michigan State University and other potato breeding programs in the US have resulted in potato cultivars that are largely resistant to foliar late blight (Kirk *et al.*, 2001a; Kirk *et al.*, 2001b; Douches *et al.*, 2004) but not significantly less susceptible than other cultivars in terms of tuber blight resistance (Kirk *et al.*, 2001b). Potato late blight resistance estimates for the cultivars/Advanced Breeding Lines (ABL) used in this study were breeders' estimates and are given as foliar and tuber ratings below, respectively. US cultivars are exclusively rated against the US-8 genotype of *P. infestans* and were Jacqueline Lee [Resistant (R), Susceptible (S); Douches et al. (2001)]; Kalkaska [R,S; Douches et al. (2009)]; Missaukee [R,I; Douches et al. (2010)]; MSL171-A (R,R); MSL211-3 (R,I); MSL757-1 (R,I); MSL766-1 (R,I); MSM051-3 (R,I); MSM137-2 (I,I); MSM171-A (R,I); MSM182-1 (I,I); MSM183-AY (R,I); MSN105-1 (R,S); MN98642 (S,S); MN15620 (S,S); ND2470-27 (S,S); Dakota Diamond [S,S; Thompson et al. (2008)]; White Pearl [S,S; Groza et al. (2006)] and Megachip [S,S; Groza et al. (2007)]. Both UK cultivars Stirling and Torridon have a NIAB late blight resistance rating of 8 (foliage), 7 (tuber) equivalent to R,R in US scheme. All cultivars were classified as late maturing. Tubers for this study were obtained from the potato breeding programs at Michigan State University, University of Wisconsin, Madison, University of Minnesota and North Dakota State University. Potato tubers from cultivars/ABL harvested during the previous growing seasons were stored at 3^{o}C in the dark at 90% relative humidity until used. Tubers were warmed to 15^{o}C in incremental steps of 2^{o}C for 7 d 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 sample was further tested with the ELISA immuno-diagnostic Alert Multi-well kit (Alert Multiwell Kit - *Phytophthora sp.* Neogen Corporation, Lansing, MI, USA); *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 then surface sterilized by soaking in 2% sodium hypochlorite (Clorox) solution for 30 min. Tubers were dried in a controlled environment with continuous airflow at 15^{o}C in dry air (30% relative humidity) for 4 h prior to inoculation. After inoculation tubers were returned to target temperatures by decreasing temperature by 2^{o}C decrements over a 2, 3 and 4 d for storage treatments of 10, 7 and 3^{o}C, respectively.

Culturing of *Phytophthora infestans* and tuber inoculations

Cultures of *P. infestans* isolates corresponding to clonal lineages US−1 (Pi95-3), US−1.7 [Pi88 (2002-06)], US-6 [Pi95-2 (2006-07)], US−8 [Pi02-007 (2002-05), Pi06-02 (2006-07)], US−10 [SR83-84 (2005-06), Banam AK (2006-07)], US−11 (Pi96-1), US−14 [Pi98-1 (2002-05), Pi00-001 (2006-07)] were selected based on the aggressiveness criteria (Young *et al.*, 2009). The selected isolates were from the collection of W. Kirk (Michigan State University). These isolates were acquired from field infections from 1995 to 2006 on foliage and tubers of potatoes of commonly grown in Michigan, USA. Virulence was determined on foliage and tubers in tuber and detached leaf (Young *et al.*, 2009). Since the genotypes US-1, US-1.7 and US-11 are rare in the US only single isolates representative of the range of genotypes were selected for this study. The experiments were carried out in controlled environment chamber studies. The trials were conducted from 2002 to 2007 (total of five experiments).

The isolates were grown in rye B media for 14 days in the dark at 18^{o}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^{o}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 cheesecloth and sporangia concentration was measured with a hemacytometer and adjusted to about 1 x 10^{6} total sporangia ml^{-1} (discharged and non-discharged). The sporangial suspensions were stored for 6 h at 4^{o}C to encourage zoospore release from the sporangia.

Whole tuber inoculation with *P. infestans*

Tuber late blight development caused by the different *P. infestans* genotypes on the tuber cultivars/ABL were evaluated at different commonly used post-harvest potato storage temperatures (3, 7 and 10^{o}C) using whole tuber sub–peridermal inoculation. All tubers were washed in distilled H_2O to remove soil. The tubers were then surface sterilized by soaking in 2% sodium hypochlorite solution for 4 h. Tubers were dried in a controlled environment with forced air ventilation at 5950 l min^{-1} at 15^{o}C in dry air (30% relative humidity) for four hours prior to inoculation.

The washed, surface-sterilized tubers were inoculated by a sub-peridermal injection of a sporangia suspension of 2 x 10^{-5} ml (delivering zoospores released from about 20 sporangia inoculation^{-1}) with a hypodermic syringe and needle at the apical end of the tuber about 1 cm

from the dominant sprout to a maximum depth of 1 cm. Ten tubers of each cultivar/ABL were inoculated with each *P. infestans* genotype per temperature. Ten control tubers per cultivar/ABL were inoculated with cold (4^{o}C) sterile distilled H_2O. After inoculation, tubers were placed in the dark in sterilized covered plastic crates and returned to controlled environment chambers [Percival Incubator (Model I-36LLVL, Geneva Scientific, LLC, PO Box 408, Fontana, WI)]. The chambers were set at 3, 7 or 10^{o}C and 95% humidity and the sample tubers were incubated for 40 days until evaluation. The tuber tissue inoculation experiments were conducted in December 2002 to January 2003 and annually through December to January 2003 to 2008.

Evaluation of tuber blight

A digital image analysis technique was used to assess tuber tissue infection. The method was previously used and standardized (Niemira *et al.*, 1999; Kirk *et al.*, 2001c). The image files were analyzed using SigmaScan V3.0 (Jandel Scientific, San Rafael, CA). The area selection cut-off threshold was set to 10 light intensity units, limiting the determination to the non–dark 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 40 days after inoculation (DAI).

Data Analysis

The presence of *P. infestans* in sample tubers was confirmed by ELISA (described above) and by isolating pure cultures of *P. infestans* from the infected tuber tissue and successful re-

inoculation of potato tubers and leaves. The severity of tuber tissue infection was expressed relative to the ARI (described above) of the control tubers for each cultivar/ABL. The relative ARI (RARI) was calculated as:

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

RARI (%) has minimum value of zero (no symptoms) and maximum value of hundred (completely dark tuber surface).

Data for all experiments were analyzed by analysis of variance (least squares method) using the JMP program version 7.0 (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA). Treatment effects were determined by three-way factorial ANOVA, where the main effects corresponded to: Cultivar/ABL, *P. infestans* genotype and temperature and multiple interactions among the main effects, including the three-factor interaction. Data were not combined across years as different genotypes of *P. infestans* and different cultivars/ABL were used in each year.

Results

Factorial ANOVA analyses resulted in significant differences by year of the three main factors (Cultivar/ABL, *P. infestans* genotype, and temperature) and the multiple interactions among them (Table 2.1). Incubation of inoculated tubers at 10^{o}C resulted in greatest tuber infection and tuber tissue discoloration within 40 days after inoculation (DAI) in 2003 regardless of genotype of *Phytophthora infestans* or cultivar/ABL although differences in some genotypes e.g. US-1.7 were not significant (Table 2.2). In 2004, there were no differences between 7 and 10^{o}C although no measurable disease developed at 3^{o}C. In 2005, tuber late blight only

developed at 10^{o}C (data not shown). In 2006, there were only differences between storage temperature treatments in US-8 and very little disease developed at 3 or 7^{o}C (Table 2.2). In 2007, there was a general increase across all genotypes with increase in temperature. As an example, tubers of White Pearl inoculated with *P. infestans* isolate *Pi*-02-007 (US-8 genotype) and incubated at 10^{o}C for 40 days resulted in significant tuber infection and a range of RARI (%) values.

The effect of genotype of *P. infestans* regardless of temperature in the different cultivars/ABL of potatoes showed a broad range of responses for each year (Tables 2.1 – 2.7). There were significant interactions in tuber late blight development [RARI (%)] among cultivar/ABL, genotypes and storage temperatures (Table 2.1). The US−8 genotype was the most aggressive, regardless of temperature in all years although in 2007, US-6, US-10 and US-14 caused a significant amount of tuber late blight regardless of temperature or cultivar/ABL (Table 2.2).

Table 2.1. Three-way factorial ANOVA of the effect of cultivar/ABL, genotype of *Phytophthora infestans* and storage temperature on tuber tissue late blight as mean Relative Average Reflection Intensity [RARI (%)] by year. Variance ratio (F), degrees of freedom (df), and P–Value.

Variables and interactions	2002-2003			2003-2004			2004-2005			2005-2006			2006-2007		
	F	df	P-Value	F	df	P-Value	F	df	P-Value	F	df	P-Value	F	df	P-Value
Variety	60.9	9	<.0001	75.7	8	<.0001	27.1	8	<.0001	57.5	8	<.0001	678.1	6	0
Genotype	764.7	4	0.0000	1518.9	4	0.0000	509.8	4	<.0001	1036.6	4	0	303.2	50	<.0001
Temperature	680.1	2	<.0001	1360.4	2	0.0000	ND*	0	ND	164.1	2	<.0001	192.2	1	<.0001
Variety X Genotype	27.1	36	<.0001	25.3	32	<.0001	16.1	32	<.0001	16.1	16	<.0001	24.2	13	<.0001
Variety X Temperature	16.4	18	<.0001	24.9	16	<.0001	ND	0	ND	39.7	32	<.0001	42.6	30	<.0001
Isolate X Temperature	76.0	8	<.0001	384.1	8	0.0000	ND	0	ND	123.1	8	<.0001	37.1	5	<.0001
Variety X Genotype X Temperature	11.1	72	<.0001	10.9	64	<.0001	ND	0	ND	6.6	64	<.0001	5.6	65	<.0001

*** ND =** Not determined because tuber late blight developed only at 10^{o}C.

Tuber late blight development 2003

In 2003, 10 cultivars/ABL were tested to measure the tuber response to inoculation with different genotypes of *P. infestans* (Table 2.3). The RARI (%) varied in the different cultivars/ABL among genotypes and the responses were reported relative to each genotype of *P. infestans* (Table 2.3). Among the tuber inoculations, the US−8 genotype was most aggressive, followed by the US−11 and US−14 genotypes. The ABL ND 2470-27 was the most susceptible to *P. infestans* genotypes with the highest RARI (%) value, and Kalkaska was the least susceptible cultivar/ABL in 2003 although still relatively susceptible to US-8 (Tables 2.2 and 2.3). Jacqueline Lee was particularly susceptible to US-1 and US-8 in 2003 but relatively resistant to other genotypes of *P. infestans* (Table 2.3).

Tuber late blight development 2004

The evaluation of *P. infestans* isolates in 2004 at different storage temperatures among different cultivars/ABL differed to that in measured in 2003. No disease was observed in inoculated tubers incubated at 3^{O}C (Tables 2.2 and 2.4). There were no significant differences in tuber late blight development between 7 and 10^{O}C. Overall, US−8 was the most aggressive genotype and was significantly different from the other genotypes. White Pearl was the most susceptible cultivar overall and Megachip was the most resistant (Tables 2.2 and 2.4). Following US−8, genotypes US−14 and US−11 caused moderate tuber late blight and US-1.7 caused moderate disease development at 10^{O}C in MN15620 (Table 2.4).

Table 2.2. Effect of storage temperature on tuber tissue late blight as mean Relative Average Reflection Intensity [RARI (%)] in different cultivars and advanced breeding lines (ABL) of potatoes after inoculation with different genotypes of *Phytophthora infestans* by year.

Year	Variety	Mean RARI (%)[a]		Genotype of *P. infestans*	Mean RARI (%)		Temperature (°C)	Mean RARI (%)	
2002-2003	ND2470-27	10.12	a[b]	US-8	14.59	a	10	10.23	a
	MSJ 461	9.38	ab	US-1	9.80	b	7	9.12	b
	Megachip	9.15	abc	US-14	6.76	c	3	4.00	c
	FL1879	8.50	bcd	US-6	4.64	d			
	Jacqueline-Lee	8.30	cd	US-11	3.13	e			
	MN15620	7.67	de						
	White Pearl	7.57	de						
	ND 5822C-7	6.81	e						
	MN 98642	6.65	e						
	MSJ 371-1	3.69	f						
	LSD 0.05	3.164			2.728			2.344	
2003-2004	White Pearl	7.72	a	US-8	15.93	a	10	7.79	a
	MN15620	7.68	a	US-11	3.73	b	7	7.71	a
	FL1879	6.31	b	US-14	3.32	b	3	0.00	b
	ND2470-27	5.77	bc	US-6	1.49	c			
	MSJ 461	4.92	cd	US-1	1.38	c			
	Jacqueline-Lee	4.38	de						
	MN5822C-7	3.76	def						
	MSJ317-1	3.20	ef						
	Megachip	2.78	f						
	LSD 0.05	3.103			2.729			2.345	
2004-2005	White Pearl	11.55	a	US-8	21.97	a	10	7.06	
	MN 15620	9.34	ab	US-11	5.51	b			
	ND2470-27	8.17	bc	US-14	4.39	b			
	FL1879	8.15	bc	US-6	2.13	c			
	MSJ 461	7.35	bcd	US-1	1.31	c			
	ND 5822C-7	6.00	cde						
	MSJ 371-1	5.56	de						
	Megachip	4.64	ef						
	Jacqueline-Lee	2.79	f						
	LSD 0.05	*3.107*			*2.732*				
2005-2006	MSJ461-1	8.05	a	US-8	15.97	a	10	7.23	a
	MSM137-2	6.87	b	US-11	3.00	b	7	4.53	b

Table 2.2 (cont'd)

	MSM182	6.37	b	US-10	2.50	bc	3	3.63	c
	MSM171-A	5.94	b	US-1	2.11	c			
	MSL766-1	5.82	b	US-14	2.07	c			
	MSL757-1	4.28	c						
	Jacqueline-Lee	3.21	cd						
	Torridon	3.03	d						
	Stirling	2.60	d						
	LSD 0.05	*3.103*			*2.729*			*2.345*	
2006-2007	MSN105-1	16.95	a	US-8	16.14	a	10	13.06	a
	MSM051-3	16.16	a	US-10	13.28	b	7	10.19	b
	MSJ461-1	15.90	a	US-14	12.71	b	3	7.76	c
	MSL 211-3	14.33	b	US-6	10.81	c			
	MSL 183-AY	6.82	c	US-11	7.04	d			
	Torridon	4.48	d	US-1	2.04	e			
	Jacqueline-Lee	4.17	d						
	MSM171-A	3.89	d						
	LSD 0.05	*3.032*			*2.850*			*2.344*	

[a] Normalized tuber tissue darkening score expressed as RARI (%) = [1- Mean ARI $_{treatment}$ / Mean ARI $_{control}$] *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). The numbers are derived from the mean average reflective intensity of three surfaces cut latitudinal at 25, 50 and 75% from the apex of n = 10 tubers per treatment combination.

[b] Values followed by the same letter are not significantly different at $p = 0.05$ for comparisons of mean RARI values within different *P. infestans* genotypes of cultivar/ABL combinations and temperature treatments (Based on Fishers protected LSD).

Table 2.3. The effect of storage temperature on tuber tissue late blight as mean Relative Average Reflection Intensity [RARI (%)] in different cultivars and advanced breeding lines (ABL) of potatoes after inoculation with different genotypes of *Phytophthora infestans* (2002 – 2003).

Cultivar/ABL	Storage Temperature (°C)	Tuber tissue darkening caused by different genotypes of *P. infestans* [Mean RARI (%)[a]]									
		US-1		US-1.7		US-8		US-11		US-14	
Jacqueline Lee	3	5.71	h-m[b]	1.36	i-k	11.86	f-k	2.78	b-e	2.30	i-k
	7	18.47	ab	3.74	f-k	30.87	a	2.78	b-e	3.67	f-k
	10	10.67	e-h	0.90	jk	23.41	bc	2.77	b-e	3.25	g-k
Kalkaska	3	1.42	m	1.37	i-k	4.74	m	0.70	e	1.53	jk
	7	1.90	m	2.81	g-k	10.05	h-m	1.84	c-e	3.12	h-k
	10	1.78	m	2.01	h-k	14.74	d-h	2.17	c-e	5.17	e-k
Megachip	3	6.35	g-m	5.67	e-g	7.69	j-m	5.21	a-d	4.51	f-k
	7	8.83	f-l	12.65	a	15.28	d-h	3.42	b-e	14.80	b
	10	13.85	b-f	10.54	a-c	14.14	e-i	5.29	a-d	9.14	c-e
Missaukee	3	4.44	k-m	3.11	g-k	5.41	lm	2.70	b-e	2.49	i-k
	7	16.72	a-d	9.37	a-d	19.29	c-e	1.86	c-e	5.85	e-j
	10	18.89	ab	5.86	d-g	32.69	a	3.94	a-e	8.05	c-f
MN15620	3	3.25	l-m	0.65	k	8.42	i-m	2.30	b-e	0.86	k
	7	10.17	e-j	11.07	ab	15.73	d-h	3.57	b-e	6.38	d-i
	10	14.65	b-e	4.64	f-i	19.29	c-e	2.69	b-e	11.37	bc
MN98642	3	4.54	j-m	1.30	i-k	6.43	k-m	2.98	b-e	2.36	i-k
	7	9.46	e-k	5.17	e-h	15.18	d-h	2.26	b-e	7.75	c-g
	10	9.62	e-k	5.33	e-h	10.91	g-l	5.66	a-c	10.77	b-d
ND 2470-27	3	4.11	k-m	2.01	h-k	10.47	h-m	7.72	a	3.05	i-k
	7	11.64	d-g	3.75	f-k	19.83	c-e	1.94	c-e	10.57	b-d
	10	18.35	ab	1.92	h-k	28.58	ab	3.22	b-e	24.58	a
Dakota Diamond	3	5.14	h-m	4.42	f-j	10.20	h-m	1.37	de	1.42	jk
	7	12.21	c-f	7.16	c-f	8.69	i-m	2.31	b-e	3.25	g-k
	10	13.77	b-f	4.67	f-i	17.52	d-f	2.24	b-e	7.71	c-h
White Pearl	3	4.73	i-m	2.12	h-k	6.81	j-m	1.66	de	2.10	i-k
	7	10.23	e-i	10.48	a-c	12.39	f-j	3.90	a-e	9.53	c-e
	10	11.64	d-g	8.64	b-e	16.32	d-g	2.45	b-e	10.60	b-d

[a] Normalized tuber tissue darkening score expressed as RARI (%) = [1- Mean ARI $_{treatment}$ / Mean ARI $_{control}$] *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). The numbers are derived from the mean average reflective intensity of three surfaces cut latitudinal at 25, 50 and 75% from the apex of n = 10 tubers per treatment combination.

[b] Values followed by the same letter are not significantly different at $p = 0.05$ for comparisons of mean RARI values within different *P. infestans* genotypes of cultivar/ABL combinations and temperature treatments (Based on Fishers protected LSD).

Table 2.4. The effect of storage temperature on tuber tissue late blight as mean Relative Average Reflection Intensity [RARI (%)] in different cultivars and advanced breeding lines (ABL) of potatoes after inoculation with different genotypes of *Phytophthora infestans* (2003 – 2004).

		Tuber tissue darkening caused by different genotypes of *P. infestans* [Mean RARI (%)[a]]									
Cultivar/ABL	Temperature (°C)	US-1		US-1.7		US-8		US-11		US-14	
Jacqueline Lee	3	0.00	g[b]	0.00	f	0.00	i	0.00	f	0.00	f
	7	4.39	b	0.92	ef	21.28	ef	5.47	c-e	2.28	ef
	10	1.11	d-g	1.47	c-f	26.49	b-e	1.27	ef	1.06	f
Kalkaska	3	0.00	g	0.00	f	0.00	i	0.00	f	0.00	f
	7	2.57	b-e	1.25	d-f	18.48	fg	1.50	ef	0.37	f
	10	0.89	e-g	1.35	d-f	12.14	h	9.14	b-d	0.24	f
Megachip	3	0.00	g	0.00	f	0.00	i	0.00	f	0.00	f
	7	1.01	e-g	1.22	d-f	14.27	gh	1.56	ef	1.17	ef
	10	0.97	e-g	1.37	d-f	14.51	gh	2.59	ef	2.98	d-f
Missaukee	3	0.00	g	0.00	f	0.00	i	0.00	f	0.00	f
	7	1.19	c-g	1.18	d-f	31.16	ab	1.89	ef	1.59	ef
	10	0.72	fg	0.76	ef	31.26	ab	1.35	ef	2.67	ef
MN15620	3	0.00	g	0.00	f	0.00	i	0.00	f	0.00	f
	7	1.77	c-g	0.83	ef	27.38	b-d	16.33	a	1.50	ef
	10	7.03	a	8.42	a	34.34	a	10.22	bc	7.47	cd
ND2470-27	3	0.00	g	0.00	f	0.00	i	0.00	f	0.00	f
	7	1.76	c-g	0.84	ef	24.28	c-f	13.11	ab	5.64	c-e
	10	1.39	c-g	1.06	ef	23.87	d-f	5.19	de	9.33	c
Dakota Diamond	3	0.00	g	0.00	f	0.00	i	0.00	f	0.00	f
	7	2.02	c-f	1.84	c-f	19.73	fg	1.26	ef	1.58	ef
	10	1.77	c-g	3.13	b-e	19.78	fg	2.13	ef	3.18	d-f
White Pearl	3	0.00	g	0.00	f	0.00	i	0.00	f	0.00	f
	7	2.22	c-f	2.85	b-e	24.13	c-f	8.67	b-d	20.13	a
	10	2.92	b-d	3.53	b-d	27.50	b-d	8.41	b-d	15.36	b

[a] Normalized tuber tissue darkening score expressed as RARI (%) = [1- Mean ARI $_{treatment}$ / Mean ARI $_{control}$] *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). The numbers are derived from the mean average reflective intensity of three surfaces cut latitudinal at 25, 50 and 75% from the apex of n = 10 tubers per treatment combination.

[b] Values followed by the same letter are not significantly different at $p = 0.05$ for comparisons of mean RARI values within different *P. infestans* genotypes of cultivar/ABL combinations and temperature treatments (Based on Fishers protected LSD).

Tuber late blight development 2005

In 2005, no tuber late blight developed at 3 or 7^{o}C and data were collected only at 10^{o}C. The US−8 genotype was the most aggressive across the cultivars/ABL and genotypes US−11 and US−14 caused moderate tuber late blight (Tables 2.2 and 2.5). White Pearl was the most susceptible cultivar and Jacqueline Lee and Megachip were the most resistant (Tables 2.2 and 2.5). Missaukee was particularly susceptible to US-8 and not significantly different from White Pearl or MN15620 (Table 2.5).

Tuber late blight development 2006

Very little late blight developed in inoculated tubers in 2006 at 3, 7 or 10^{o}C in cultivars/ABL inoculated with any genotype of *P. infestans* other than US-8. The US-8 genotype caused tuber late blight of which the severity increased with temperature from 3 to 10^{o}C (Table 2.2). The US−8 genotype was consistently aggressive on different cultivars regardless of temperature (Tables 2.2 and 2.6). The most susceptible cultivar in 2006 was Missaukee and the most resistant were Jacqueline Lee, Torridon and Stirling (Tables 2.2 and 2.6). Missaukee was very susceptible to US-8 but also moderately susceptible to US-11 in 2006 (Table 2.6).

Tuber late blight development 2007

In 2007, new isolates of the US-10 and US-14 genotypes were used and largely tested on cultivars/ABL from the MSU breeding program. The amount of tuber late blight increased with temperature regardless of cultivar/ABL and the US-8, US-10 and US-11 genotypes were the most aggressive (Tables 2.2 and 2.7). The cultivars/ABL MSM171-A, Jacqueline Lee and

Torridon were the most resistant and MSL211-3, Missaukee, MSM051-3 and MSN105-1 were the most susceptible (Tables 2.2 and 2.7).

Table 2.5. The effect of storage temperature on tuber tissue late blight as mean Relative Average Reflection Intensity [RARI (%)] in different cultivars and advanced breeding lines (ABL) of potatoes after inoculation with different genotypes of *Phytophthora infestans* (2004 – 2005).

Cultivar/ABL	Temperature (°C)	Tuber tissue darkening caused by different genotypes of *P. infestans* [Mean RARI (%)[a]]									
		US-1		US-1.7		US-8		US-11		US-14	
Jacqueline Lee	10[c]	1.11	k[b]	1.47	k	9.02	f-i	1.27	k	1.06	k
Kalkaska	10	1.52	k	1.87	k	13.51	d-f	10.47	e-g	0.43	k
Megachip	10	0.97	k	1.37	k	14.51	d-f	2.59	jk	3.76	h-k
Missaukee	10	0.72	k	0.76	k	31.26	a	1.35	k	2.67	jk
MN15620	10	0.92	k	1.46	k	28.35	ab	15.50	de	0.49	k
Dakota Diamond	10	1.77	k	3.13	h-k	19.78	cd	2.13	jk	3.18	h-k
ND2470-27	10	1.39	k	1.06	k	23.87	bc	5.19	g-k	9.33	e-h
White Pearl	10	2.92	i-k	3.53	h-k	27.50	ab	8.41	f-j	15.36	de

[a] Normalized tuber tissue darkening score expressed as RARI (%) = [1- Mean ARI $_{treatment}$ / Mean ARI $_{control}$] *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). The numbers are derived from the mean average reflective intensity of three surfaces cut latitudinal at 25, 50 and 75% from the apex of n = 10 tubers per treatment combination.

[b] Values followed by the same letter are not significantly different at $p = 0.05$ for comparisons of mean RARI values among all *P. infestans* genotypes of cultivar/ABL combinations (Based on Fishers protected LSD).

[c] No tuber tissue infection occurred at 3 or 7°C in 2004-05 tests.

Table 2.6. The effect of storage temperature on tuber tissue late blight as mean Relative Average Reflection Intensity [RARI (%)] in different cultivars and advanced breeding lines (ABL) of potatoes after inoculation with different genotypes of *Phytophthora infestans* (2005 – 2006).

		Tuber tissue darkening caused by different genotypes of *P. infestans* [Mean RARI (%)[a]]									
Cultivar/ABL	Temperature (ºC)	US-1		US-8		US-10		US-11		US-14	
Jacqueline Lee	3	3.45	a-e[b]	5.20	h-k	3.37	b-d	1.89	cd	2.66	
	7	2.22	a-f	7.19	h-k	2.47	b-d	3.18	b-d	2.14	
	10	0.89	ef	11.30	f-i	1.02	cd	0.36	cd	0.75	
Missaukee	3	0.85	ef	19.32	d-f	1.97	b-d	2.74	cd	1.71	c-f
	7	0.08	f	26.91	a-d	1.99	b-d	3.77	bc	3.37	a-e
	10	1.04	d-f	35.27	ab	1.29	cd	18.14	a	2.22	c-f
MSL757-1	3	4.10	a-d	0.88	k	3.37	b-d	2.55	cd	1.28	d-f
	7	1.02	d-f	7.24	h-k	0.82	d	1.38	cd	0.41	f
	10	2.08	b-f	23.56	cd	7.48	a	2.64	cd	5.45	ab
MSL766-1	3	1.71	c-f	9.63	g-k	1.19	cd	1.01	cd	1.34	d-f
	7	2.88	a-f	26.21	b-d	2.76	b-d	3.70	bc	1.91	c-f
	10	0.30	ef	27.03	a-d	5.73	ab	1.35	cd	0.56	ef
MSM137-2	3	0.85	ef	22.03	de	1.05	cd	2.49	cd	0.64	ef
	7	0.44	ef	23.81	cd	1.59	cd	2.67	cd	1.02	ef
	10	4.33	a-c	31.46	a-c	3.42	b-d	2.66	cd	4.58	a-c
MSM171-A	3	5.39	a	9.00	h-k	1.81	b-d	2.26	cd	3.14	a-f
	7	3.12	a-f	21.67	de	5.04	a-c	2.29	cd	2.29	c-f
	10	1.94	b-f	26.26	b-d	2.87	b-d	1.08	cd	1.00	ef
MSM182-1	3	3.35	a-e	5.59	h-k	2.48	b-d	6.38	b	2.07	c-f
	7	1.86	b-f	10.76	f-j	1.75	b-d	2.76	cd	1.74	c-f
	10	4.99	ab	35.95	a	3.88	a-d	6.26	b	5.72	a
Stirling	3	3.10	a-f	1.63	jk	3.08	b-d	3.73	bc	1.90	c-f
	7	0.47	ef	4.36	i-k	0.61	d	0.71	cd	0.41	f
	10	1.67	c-f	14.27	e-h	0.91	d	1.47	cd	0.71	ef
Torridon	3	3.04	a-f	2.62	i-k	2.82	b-d	1.14	cd	1.54	d-f
	7	1.16	c-f	3.44	i-k	2.06	b-d	2.23	cd	4.11	a-d
	10	0.66	ef	18.47	d-g	0.72	d	0.22	d	1.22	d-f

[a] Normalized tuber tissue darkening score expressed as RARI (%) = [1- Mean ARI $_{treatment}$ / Mean ARI $_{control}$] *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). The numbers are derived from the mean average reflective intensity of three surfaces cut latitudinal at 25, 50 and 75% from the apex of n = 10 tubers per treatment combination.

[b] Values followed by the same letter are not significantly different at $p = 0.05$ for comparisons of mean RARI values within different *P. infestans* genotypes of cultivar/ABL combinations and temperature treatments (Based on Fishers protected LSD).

Table 2.7. The effect of storage temperature on tuber tissue late blight as mean Relative Average Reflection Intensity [RARI (%)] in different cultivars and advanced breeding lines (ABL) of potatoes after inoculation with different genotypes of *Phytophthora infestans* (2006 – 2007).

Cultivar/ABL	Temperature (°C)	Tuber tissue darkening caused by different genotypes of *P. infestans* [Mean RARI (%)[a]] US-1	US-6	US-8	US-10	US-11	US-14
Jacqueline Lee	3	1.48 c-f[b]	4.10 fg	4.44 ij	3.17 j	1.90 fg	3.35 kl
	7	1.27 c-f	4.68 fg	5.56 i	5.09 ij	2.67 fg	2.97 kl
	10	2.32 b-f	4.84 fg	15.00 e-g	3.10 j	1.38 g	7.69 h-k
Missaukee	3	1.36 c-f	13.09 de	17.44 d-f	18.40 c-e	3.41 f-g	12.14 e-h
	7	1.87 b-f	13.85 cd	20.74 cd	20.60 b-e	10.40 de	17.84 c-e
	10	5.60 a	27.15 a	33.11 a	32.47 a	12.55 b-d	24.15 ab
MSL183-AY	3	2.36 b-f	4.46 fg	11.42 gh	3.30 j	3.13 fg	6.63 h-l
	7	3.13 b-d	5.84 fg	14.73 fg	9.51 g-i	4.19 fg	7.60 h-k
	10	1.04 d-f	4.58 fg	24.42 b	7.91 h-j	2.51 fg	6.00 i-l
MSL211-3	3[c]	-	-	-	-	-	-
	7	2.46 b-f	21.16 b	14.24 fg	19.91 b-e	5.00 fg	17.13 cf
	10	2.25 b-f	18.43 bc	32.09 a	22.04 bc	9.67 de	23.03 a-c
MSM051-3	3	0.94 d-f	8.63 ef	18.23 de	14.94 e-g	11.25 cd	14.44 d-g
	7	2.15 b-f	13.10 de	23.41 bc	20.85 b-d	15.86 ab	26.69 a
	10	2.93 b-e	18.14 bc	31.64 a	22.28 bc	17.37 a	28.00 a
MSM171-A	3	1.28 c-f	3.95 fg	7.79 hi	3.66 j	2.69 fg	2.33 kl
	7	0.94 d-f	3.00 g	7.15 i	3.07 j	3.60 fg	4.59 j-l
	10	0.54 f	1.82 g	6.29 i	2.35 j	3.14 fg	11.80 f-i
MSN105-1	3	3.39 a-c	21.90 b	18.47 de	15.31 d-f	9.84 de	15.48 d-g
	7	0.75 ef	22.71 ab	20.93 b-d	25.29 b	11.37 b-d	18.33 b-d
	10	3.92 ab	18.45 bc	30.67 a	25.59 b	15.57 a-c	27.10 a
Torridon	3	0.41 f	3.81 fg	1.45 j	2.94 j	2.12 fg	1.23 l
	7	1.74 b-f	5.39 fg	5.85 i	11.70 f-h	6.18 ef	1.99 kl
	10	1.39 c-f	4.10 fg	4.76 ij	10.45 f-i	4.76 fg	10.46 g-j

[a] Normalized tuber tissue darkening score expressed as RARI (%) = [1- Mean ARI $_{treatment}$ / Mean ARI $_{control}$] *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). The numbers are derived from the mean average reflective intensity of three surfaces cut latitudinal at 25, 50 and 75% from the apex of n = 10 tubers per treatment combination.

[b] Values followed by the same letter are not significantly different at $p = 0.05$ for comparisons of mean RARI values within different *P. infestans* genotypes of cultivar/ABL combinations and temperature treatments (Based on Fishers protected LSD).

[c] Insufficient tubers of MSL 211-3 to inoculate and store at 3°C.

Discussion

The significance of tuber late blight in initiating storage problems has been reported in many studies (Kirk *et al.*, 1999; Kirk *et al.*, 2001c) and recently reviewed (Olanya *et al.*, 2009). Infection of potato tubers by *P. infestans* may be initiated by zoospores, sporangia or oospores washed in precipitation or irrigation water from plant foliage and deposited in soil (Fry, 2008). Although three major components contribute to late blight resistance in tubers; the phellem cells (periderm), the outer cortical cell layers and the medulla storage tissues characterized by reduced hyphal growth and sporulation of *P. infestans* (Pathak and Clarke 1987) in this study, only the contribution of the medullar storage tissues was assessed.

Temperature has a profound influence on the physiology of potato tubers (Kaur *et al.*, 2009; Knowles *et al.*, 2009; Kumar, 2009) and also on the pathology of tubers as pertaining to late blight (Lambert and Currier, 1997; Kirk *et al.*, 2001c). The inclusion of the three temperature conditions was intended to simulate late blight development in tubers stored for seed, table-stock and processing, 3, 7 and 10^{o}C, respectively. In this study, tuber late blight severity measured as tuber darkening [RARI (%)] generally increased with temperature as previously reported (Kirk *et al.*, 2001c). However, in some years no late blight developed at 3 or 7^{o}C even in susceptible cultivars/ABL. Temperature in the controlled environments was measured through the season with data loggers and while the temperature in the 7^{o}C environment was consistently between 6 and 7^{o}C in 2004-05 late blight developed only at 10^{o}C, although in most other years disease developed at the 7^{o}C storage treatment. The reason for the failure of late blight development in tubers is therefore unclear. However, acclimation of tubers may have varied

depending on the size of the tuber and impacted initial and subsequent disease development after inoculation.

Generally, there was little difference in tuber late blight development between 7 and 10^{o}C treatments and in some combinations significantly more tissue darkening developed at 7 than at 10^{o}C e.g. Jacqueline Lee by US-1 and US-11 (Table 1.4). In future experiments, it may be useful to incubate tubers from cooler temperatures at e.g. 10^{o}C as seed used for planting would be warmed prior to planting and as it is known that *P. infestans* can survive temperature exposure down to -3^{o}C for 5 days (Kirk, 2003), it is very likely that mycelium would spread through the tubers and infect sprouts. In addition, seed-borne inoculum of the late blight pathogen has been linked to the initiation of late blight disease in the field (Boyd, 1974; Boyd, 1980; Doster *et al.*, 1989; Dowley and O'Sullivan, 1991; Platt *et al.*, 1999; Johnson, 2009; Johnson and Cummings, 2009; Kirk *et al.*, 2009; Keil *et al.*, 2010).

Unlike foliage resistance, the genetics of tuber blight resistance have not been extensively studied (Olanya *et al.*, 2009). Generally, cultivars with foliage blight resistance show some tuber blight resistance (Collins *et al.*, 1999), but this depends on plant genotype (Swiezynski and Zimnoch-Guzowska, 2001) and in some instances the relationship does not hold (Platt and Tai, 1998; Kirk *et al.*, 2001b). In this study, the inoculation technique utilized aimed to examine the resistance of medulla storage tissues which is characterized by mainly by reduced hyphal growth of *P. infestans* and therefore tuber symptoms e.g. tissue necrosis (Pathak and Clarke, 1987; Niemira *et al.*, 1999; Flier *et al.*, 2001; Kirk *et al.*, 2001c). Such resistance may be linked to major gene resistance as recently reviewed by Olanya *et al.* (2009). Park et al. (2005) analyzed

tuber resistance in three mapping populations carrying *R* genes or a major QTL for foliar resistance to late blight. In one mapping population, tuber blight resistance was inherited independently of foliar blight and the other two populations tuber and foliage resistance were linked. In these two populations, the *R1* (or *R1-like*) gene acted on both foliage and tuber resistance. Resistance in both foliage and tubers is a very desirable trait in potatoes, but in this study only Torridon and Stirling appeared to have this quality and to some extent Jacqueline Lee (Douches *et al.*, 2001). Jacqueline Lee and Missaukee (Douches *et al.*, 2001; Douches *et al.*, 2010) have strong foliar resistance to the US-8 genotype of *P. infestans* but in this study Jacqueline Lee had only moderate or in the case of Missaukee weak resistance. This suggests that the genes responsible for foliage resistance are not present or at least active in the tubers.

Inoculation with the US-8 genotype of *P. infestans,* the dominant genotype in North America (Young *et al.*, 2009), resulted in significant tuber late blight development for most cultivars and ABL tested. These findings are in agreement with Lambert and Currier (1997) and Lambert *et al.* (1998) who found that the US-8 genotype isolates were the most aggressive in tubers causing rapid and significantly more tuber damage than any other genotype of *P. infestans*. In this study, the isolates of the US-10 and US-14 genotypes of *P. infestans* used in from 2005 to 2007 were as aggressive as the US-8 isolates used throughout. Results of recent tuber rot severity experiments demonstrated similar trends in cultivar susceptibility and genotype aggressiveness on plant emergence (Kirk *et al.*, 2009). Data from the two experiments conducted were strongly negatively correlated, where cultivars/ABL that demonstrated the highest level of plant emergence had the least tuber rotting and vice-versa. Results from this study and that of (Kirk *et al.*, 2009) circumstantially suggest that highly aggressive genotypes of *P. infestans*, such as the US-8 genotype, may produce limited primary inoculum due to severe tuber rotting and

deterioration of tubers before emergence. However, this scenario will depend mostly on the amount of inoculum of *P. infestans* found in or on potato tubers. In both studies, tuber seed pieces and stored tubers were exposed to an excessive amount of inoculum and results suggest that this amount of inoculum was sufficient to cause severe tuber rotting in some cultivars/ABL. The significant extent of tuber rotting and deterioration appears to be the primary symptom after inoculation with *P. infestans.* The variability of susceptibility of tubers to different genotypes of *P. infestans* has implications for plant breeding efforts in that the major emphasis in the past has been to breed for foliar resistance with limited emphasis on the reaction of the tuber. It is clear that at least as much emphasis should be apportioned to the tuber resistance phenotype.

References

References

Andrivon, D. (1995) Biology, ecology, and epidemiology of the potato late blight pathogen *Phytophthora infestans* in soil. *Phytopathology,* **85:**1053-1056.

Bain, R., Fairclough, R., Holmes, S.*, et al.* The role of *Phytophthora infestans* inoculum from stem lesions in the infection of tubers. In, Proceedings of the Workshop on the European Network for Development of an Integrated Control Strategy of Potato Late Blight. 1997, pp. 98-105.

Bonde, R. & Schultz, E. (1943) Potato cull piles as a source of late-blight infection. *Am. J. Potato Res.,* **20:**112-118.

Boyd, A. E. W. (1974) Sources of potato blight (*Phytophthora infestans*) in the East of Scotland. *Plant Pathol.,* **23:**30-36.

Boyd, A. E. W. (1980) Development of potato blight *Phytophthora infestans* after planting infected seed tubers. *Ann. Appl. Biol.,* **95:**301-309.

Collins, A., Milbourne, D., Ramsay, L.*, et al.* (1999) QTL for field resistance to late blight in potato are strongly correlated with maturity and vigour. *Mol. Breed.,* **5:**387-398.

Doster, M., Sweigaird, J. & Fry, W. (1989) The influence of host resistance and climate on the initial appearance of foliar late blight of potato from infected seed tubers. *Am. J. Potato Res.,* **66:**227-233.

Douches, D., Coombs, J., Felcher, K.*, et al.* (2004) Foliar reaction to *Phytophthora infestans* in inoculated potato field trials in Michigan. *Am. J. Pot Res,* **81:**443-448.

Douches, D., Coombs, J., Felcher, K.*, et al.* (2010) Missaukee: A round white potato variety combining chip-processing with resistance to late blight, *Verticillium* wilt and Golden cyst nematode. *Am. J. Potato Res.,* **87:**10-18.

Douches, D., Jastrzebski, K., Coombs, J.*, et al.* (2001) Jacqueline Lee: A late-blight-resistant tablestock variety. *Am. J. Potato Res.,* **78:**413-419.

Dowley, L. & O'Sullivan, E. (1991) Sporulation of *Phytophthora infestans* (Mont.) de Bary on the surface of diseased potatoes and tuber to tuber spread of infection during handling. *Pot. Res.,* **34:**295-296.

Flier, W., Turkensteen, L. & Mulder, A. (1998) Variation in tuber pathogenicity of *Phytophthora infestans* in the Netherlands. *Pot. Res.,* **41:**345-354.

Flier, W., Turkensteen, L., Van Den Bosch, G*., et al.* (2001) Differential interaction of *Phytophthora infestans* on tubers of potato cultivars with different levels of blight resistance. *Plant Pathol.,* **50:**292-301.

Fry, W. (2008) *Phytophthora infestans*: the plant (and R gene) destroyer. *Mol. Plant Pathol.,* **9:**385-402.

Goodwin, S., Cohen, B. & Fry, W. (1994) Panglobal Distribution of a Single Clonal Lineage of the Irish Potato Famine Fungus. *Proc. Natl. Acad. Sci.,* **91:**11591-11595.

Guenthner, J., Michael, K. & Nolte, P. (2001) The economic impact of potato late blight on US growers. *Pot. Res.,* **44:**121-125.

Guenthner, J., Wiese, M., Pavlista, A*., et al.* (1999) Assessment of pesticide use in the U.S. potato industry. *Am. J. Potato Res.,* **76:**25-29.

Hijmans, R. (2001) Global distribution of the potato crop. *Am. J. Potato Res.,* **78:**403-412.

Johnson, D. A. (2009) Transmission of *Phytophthora infestans* from Infected Potato Seed Tubers to Emerged Shoots. *Plant Dis.,* **94:**18-23.

Johnson, D. A. & Cummings, T. F. (2009) Latent Infection of Potato Seed Tubers by *Phytophthora infestans* During Long-Term Cold Storage. *Plant Dis.,* **93:**940-946.

Kaur, A., Singh, N., Ezekiel, R*., et al.* (2009) Properties of starches separated from potatoes stored under different conditions. *Food Chem.,* **114:**1396-1404.

Keil, S., Benker, M. & Zellner, M. (2010) Double setting of potato seed tubers as a new approach to research primary stem blight *Phytophthora infestans* (Mont.) De Bary. *Am. J. Potato Res.,* **87:**27-31.

Kirk, W., Abu-El Samen, F., Tumbalam, P*., et al.* (2009) Impact of different US genotypes of *Phytophthora infestans* on potato seed tuber rot and plant emergence in a range of cultivars and advanced breeding lines. *Pot. Res.,* **52:**121-140.

Kirk, W., Felcher, K., Douches, D.*, et al.* (2001a) Effect of host plant resistance and reduced rates and frequencies of fungicide application to control potato late blight. *Plant Dis.,* **85:**1113-1118.

Kirk, W., Felcher, K., Douches, D.*, et al.* (2001b) Susceptibility of potato (*Solanum tuberosum* L.) foliage and tubers to the US-8 genotype of *Phytophthora infestans*. *Am. J. Pot Res,* **78:**319-322.

Kirk, W., Niemira, B. & Stein, J. (2001c) Influence of storage temperature on rate of potato tuber tissue infection caused by *Phytophthora infestans* (Mont.) de Bary estimated by digital image analysis. *Pot. Res.,* **44:**87-96.

Kirk, W. W. (2003) Tolerance of mycelium of different genotypes of *Phytophthora infestans* to freezing temperatures for extended periods. *Phytopathology,* **93:**1400-1406.

Kirk, W. W., Niemira, B. A., Stein, J. M.*, et al.* (1999) Late blight (*Phytophthora infestans* (Mont) De Bary) development from potato seed-pieces treated with fungicides. *Pestic. Sci.,* **55:**1151-1158.

Knowles, N. R., Driskill Jr, E. P. & Knowles, L. O. (2009) Sweetening responses of potato tubers of different maturity to conventional and non-conventional storage temperature regimes. *Postharvest Biol. Technol.,* **52:**49-61.

Kumar, D. (2009) Changes in antioxidant system of potato tuber during low temperature storage in relation to sweetening development. *J. Food Sci. Technol. (Mysore),* **46:**392-394.

Lambert, D. & Currier, A. (1997) Differences in tuber rot development for North American clones of *Phytophthora infestans*. *Am. J. Potato Res.,* **74:**39-43.

Lambert, D., Currier, A. & Olanya, M. (1998) Transmission of *Phytophthora infestans* in cut potato seed. *Am. J. Potato Res.,* **75:**257-263.

Melhus, I. E. Germination and infection with the fungus of the late blight of potato (*Phytophthora infestans*), Agricultural Experiment Station of the University of Wisconsin, 1915.

Murphy, P. & McKay, R. (1924) The development of blight in potatoes subsequent to digging. *J Dep Lands Agric Dublin,* **24:**103-116.

Murphy, P. & McKay, R. (1925) Further experiments on the sources and development of blight infection in potato tubers. *J Dep Lands Agric Dublin,* **25:**10-21.

Niemira, B., Kirk, W. & Stein, J. (1999) Screening for late blight susceptibility in potato tubers by digital analysis of cut tuber surfaces. *Plant Dis.,* **83:**469-473.

Olanya, O., Ojiambo, P., Nyankanga, R.*, et al.* (2009) Recent developments and challenges in the mansgement of tuber blight of potato (*Solanum tuberosum*) caused by *Phytophthora infestans*. *Can. J. Plant Pathol.,* **31:**280-289.

Park, T.-H., Vleeshouwers, V. G. A. A., Kim, J.-B.*, et al.* (2005) Dissection of foliage and tuber late blight resistance in mapping populations of potato. *Euphytica,* **143:**75-83.

Pathak, N. & Clarke, D. D. (1987) Studies on the resistance of the outer cortical tissues of the tubers of some potato cultivars to *Phytophthora infestans*. *Physiol. Mol. Plant Pathol.,* **31:**123-132.

Platt, H., Peters, R., Medina, M.*, et al.* (1999) Impact of seed potatoes infected with *Phytophthora infestans* (US-1 or US-8 genotypes) on crop growth and disease risk. *Am. J. Potato Res.,* **76:**67-73.

Platt, H. & Tai, G. (1998) Relationship between resistance to late blight in potato foliage and tubers of cultivars and breeding selections with different resistance levels. *Am. J. Pot Res,* **75:**173-178.

Porter, L. D., Dasgupta, N. & Johnson, D. A. (2005) Effects of tuber depth and soil moisture on infection of potato tubers in soil by *Phytophthora infestans*. *Plant Dis.,* **89:**146-152.

Stevenson, W., Kirk, W. & Atallah, Z. Management of foliar disease, early blight, late blight and white mold. In: Johnson, D. A. (ed), Potato health management. St Paul, APS Press, 2007, pp. 209-222.

Young, G. K., Cooke, L. R., Kirk, W. W.*, et al.* (2009) Influence of competition and host plant resistance on selection in *Phytophthora infestans* populations in Michigan, USA and in Northern Ireland. *Plant Pathol.,* **58:**703-714.

Chapter III: Phenotypic and genotypic variation in Michigan *Phytophthora infestans* populations from 2008 to 2010

Abstract

Isolates of *Phytophthora infestans* characterized as the new genotype US-22 collected from potatoes and tomatoes from 2008-10 in Michigan, USA differed in virulence after cross-inoculation studies over the two crops and in other characteristics. The appearance of this new clonal lineage, which started in tomato plants, was sudden and has displaced the US-8 clonal lineage in Michigan at least in potato crops. This study focused on the analysis of *P. infestans* isolates obtained during 2008 to 2010 in Michigan, and compared them to reference isolates. Characterization included mating type, *Gpi* allozymes, virulence, resistance to mefenoxam, mitochondrial DNA haplotype (mtDNA) and DNA fingerprinting based on simple sequence repeats (SSRs). Most of the isolates were mating type A2, 100/122 *Gpi* profile and Ia mtDNA haplotype. These characteristics were attributed to a new clonal lineage US-22, recently found in the US. Resistance to mefenoxam (EC_{50}) ranged from <0.1 – 91 $\mu g \cdot mL^{-1}$, where most of the isolates were classified as intermediate. Race composition and tuber virulence were also variable among isolates, but those obtained from tomato were less pathogenic in tubers than those obtained from potato. To further characterize the population, SSRs were used and revealed different genotypes within the US-22 designation from isolates collected from tomato and potato. Despite the different virulence races observed, the genotypic diversity observed was low. The continuous tracking of changes within *P. infestans* population could yield evidence of genetic shifting due to introduction of new genotypes to the region or due to variability generated by management, environmental conditions and cultivars.

Introduction

Potato late blight (PLB) is caused by the oomycete *Phytophthora infestans*, and is considered the most important disease affecting potato worldwide and is also a major pathogen of tomatoes (Fry and Goodwin, 1997b; Guenthner *et al.*, 2001). PLB has re-emerged since the mid 1970's because of the increased impact on potato and tomato production particularly in the developed world where modern techniques such as improved fungicides and application technology, seed certification systems and host resistance have been overcome, all largely attributed to changes in *P. infestans* populations during this time (Goodwin *et al.*, 1996; Smart and Fry, 2001; Fry, 2008; Kirk *et al.*, 2009). The most important changes worldwide, especially in the US, have been the occurrence of A2 mating type (Deahl *et al.*, 1991); the resistance to phenylamide fungicides in the field (Shattock, 1988); and the appearance of increasingly aggressive genotypes (Fry and Goodwin, 1997a; Kirk *et al.*, 2001b).

Populations of *P. infestans* are usually characterized by mating type, allozyme genotype of the *glucose-6-phosphate isomerase* (*Gpi*) locus, R-gene differentials, and fungicide resistance (Malcolmson and Black, 1966; Shattock, 1988; Goodwin *et al.*, 1995a). In addition, molecular detection of variation has been used to infer population structure of this pathogen; analyses have included mitochondrial DNA (mtDNA) haplotyping and DNA fingerprinting, which use the probe RG57 (Goodwin *et al.*, 1992b; Griffith and Shaw, 1998). The combination of these phenotypic and genotypic markers has established the existence of new clonal lineages, such as US-8, which replaced the previously dominant US-1 (Fry and Goodwin, 1997a). Since US-8 occurrence was recognized, several other clonal lineages have been reported in the US and Canada (Hu *et al.*, 2010; Kawchuk *et al.*, 2011). One of the most recently reported is the clonal

lineage US-22, which has been associated with recent epiphytotic events in 2009 and 2010 in the Eastern US (Hu *et al.*, 2010).

The total planted area of potatoes in Michigan is about 18,000 ha and in a recent survey of pesticide use growers rated PLB as the most important disease (Kirk and Szendrei, 2011). Populations of *P. infestans* in the northeastern US have been extensively characterized, but Michigan populations have never been comprehensively sampled and described although some recent studies have published characteristics of some isolates collected in MI (Young *et al.*, 2009; Catal *et al.*, 2010). The US-1 genotype of *P. infestans* was present before 1991 and a population shift to clonal lineage US-8 occurred sometime around 1995 (Lacy and Hammerschmidt, 1995). In addition, Goodwin *et al.* (1998a) included Michigan *P. infestans* isolates in a survey in 1994, showing that both clonal lineages were present, but were geographically isolated (US-1 in northern MI and US-8 in central MI). Since then, no further extensive population studies have been done in Michigan. From 1995 to 2008, late blight epidemics have occurred sporadically throughout Michigan in several counties with concomitant economic impact on potato and tomato growers and the predominant genotype isolated in the state was US-8 and occasionally US-14 (Young *et al.*, 2009; Catal *et al.*, 2010). In 2009, late blight epidemics affected potato and tomato crops across eastern US and in several counties of Michigan. These isolates corresponded genotypically to descriptions of the isolates of *P. infestans* responsible for the devastating late blight epidemic of eastern US in 2009 (Ristaino, 2010), which emphasized the importance of describing the composition of populations of *P. infestans* collected from tomatoes and potatoes in Michigan from 2008 to 2010.

Epidemics of *Phytophthora infestans* are often linked to introduction of foreign genotypes that result in shifts in the population structure of local populations (Goodwin, 1997) as

occurred in the late 20th century when US-1 was displaced by US-8 in much of North America (Fry, 2008). However, the mechanisms of genetic variability remain an important issue, since they are the cause of virulence patterns and variations in host specificity.

The aim of the present study was to combine phenotypic and genotypic markers to characterize *P. infestans* isolates obtained in Michigan from 2008 to 2010. The study included tuber virulence and cross-inoculations in tomato and potato to evaluate the aggressiveness and host specificity of the current population; this information is relevant to disease dynamics of both crops in the region. In addition to the traditional genetic and phenotypic identification markers, the study integrated the use of microsatellite or single sequence repeats (SSRs) markers that were recently used in different *P. infestans* epidemic studies that focused on the description of the population composition and the understanding of the dynamics of the pathogen (Lees *et al.*, 2006; Gisi *et al.*, 2010; Montarry *et al.*, 2010a; Brurberg *et al.*, 2011b). Seven *Phytophthora*-specific SSRs markers were used to describe the different genotypes present in the region. The tracking of genetic change and its variance will help not only to understand the composition of populations of *P. infestans* collected from tomatoes and potatoes in Michigan from 2008 to 2010, but will also help in management decisions and cultivar breeding programs to effectively control the disease.

Materials and Methods

Sample collection and maintenance

Isolates were recovered from infected potato and tomato leaves, stems and fruits and potato tubers with visible symptoms of late blight from potato and tomato producing regions in Michigan from 2008 to 2010 (Fig. 3.1). The samples were from commercial fields, home

gardens or research plots. Samples were placed in ventilated plastic bags and stored at 4˚C in the dark before processing. Isolates were obtained from fresh sporangia produced on the tissue and transferred to Rye B media (Caten and Jinks, 1968) amended with ampicillin (100 $mg \cdot mL^{-1}$), nystatin (100 $mg \cdot mL^{-1}$),and rifampicin (50 $mg \cdot mL^{-1}$). If the lesions were not sporulating, the infected tissue was placed in a humid chamber at 18˚C for 24 to 72 hours to induce sporulation. Plates were incubated in the dark at 18˚C until mycelia were observed. Hyphal tipping or single spore isolation was utilized in pure cultures but if contamination was observed, transfers were made on clean-up media (Forbes, 1997). A total of 120 isolates were obtained (potato n=82 and tomato n=38). After isolation, cultures were grown and maintained on rye media.

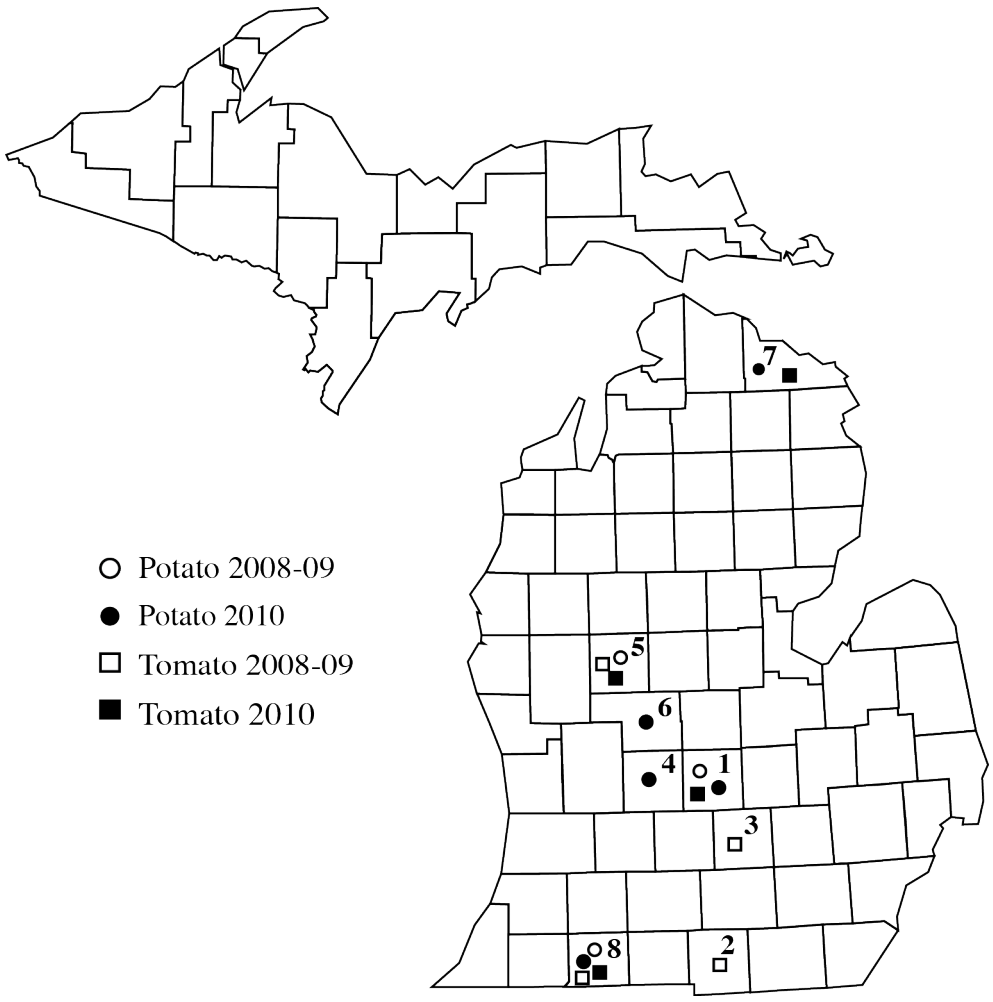

Figure 3.1. Locations and periods from which isolates of *Phytophthora infestans* were collected from 2008 to 2010.

Mating type determination

Mating type was determined in V8 agar by transferring 5 mm mycelial plugs of the unknown isolates and reference isolates A1 and A2 mating type. The plugs were placed 4 cm apart from each other. The plates were incubated at 18˚C for 14 days and evaluated for the presence or absence of oospores in the contact interface between the isolates and reference strains. Three replications were made for isolate.

A second approach including two different DNA markers was used to evaluate mating type in the isolates obtained during the study. First, a cleaved amplified polymorphic (CAPs) sequence assay derived from the study done by Judelson *et al.* (1995), using the primers W16-1 (5'-AACACGCACAAGGCATATAAATGTA-3') and W16-2 (5'-GCGTAATGTAGCGTA-ACAGCTCTC-3'). The PCR conditions were 2 min at 94˚C followed by 30 cycles of 45 s at 94˚C, 45 s at 56˚C and 45 s at 72˚C, and a final amplification fro 10 min at 72˚C. PCR products were cleaved by restriction enzyme *HaeIII* and evaluated with 1.5% agarose gel electrophoresis. The CAP marker showed a 550 bp band for A2 mating type and 600 bp band for mating type A1.

The second marker was an AFLP derived marker designed by Kim and Lee (2002) that was amplified using the primers PHYB-1 (5'-GATCGGATTAGTCAGACGAG-3') and PHYB-2 (5'-GCGTCTGCAAGGCGCATTTT-3'). Amplification was done by the following conditions; 2 min at 94˚C followed by 30 cycles of 45 s at 94˚C, 45 s at 59˚C and 45 s at 72˚C, and a final amplification fro 10 min at 72˚C. The result was evaluated in (1%) agarose gel electrophoresis. The second marker derived from AFLPs, denoted as PHYB, is rated based on the presence (A2) or absence (A1) of a 347 bp band.

Allozyme analysis

Genotype was established by allozyme analysis at the glucose-6-phosphate isomerase (*Gpi*) locus. Proteins were extracted from either fresh sporangia on leaf lesions or 10 d-old pure cultures by grinding in sterile distilled water. Supernatants were recovered by centrifugation and stored at –20˚C prior to use. Cellulose acetate gel electrophoresis (Helena Laboratories, Beaumont, TX) was done as reported by Goodwin *et al.* (1995a), resolving by overlay. Five control isolates were used, representing the genotypes US–1, US–8, US-10, US–11 and US-14. Allozyme alleles were scored according to the procedures established by Goodwin *et al.* (1995a).

Mefenoxam sensitivity *in vitro*

Sensitivity of the isolates to the phenylamide fungicide mefenoxam was determined by transfer of mycelial plugs (5 mm diameter) onto rye media amended with 0, 0.1, 1, 5, 10, 100 $\mu g \cdot L^{-1}$ of active ingredient of mefenoxam fungicide (Subdue MAXX; Syngenta Crop Protection, Inc., Greensboro, NC). Plates were incubated in the dark at 18˚C. Three replications were made for each isolate. Colony diameter was measured at 12 d after inoculation. Responses to mefenoxam were determined based on 5 and 100 ppm amended media in relation to non-amended media. Isolates rated as sensitive had growth less than 40% at 5 and 100 ppm; intermediate isolates had growth 40% or more on 5 ppm amended media, but less than 40% on 100 ppm; and resistant isolates had growth more than 40% on 5 and 100 ppm amended media (Therrien *et al.*, 1993). The distributions of responses (resistant, intermediate and sensitive isolates) were evaluated by test of response homogeneity to determine if there was an effect of mefenoxam sensitivity by host, sampling period or two-way interaction.

In addition, the effective inhibitory concentration that reduced growth of the mycelium of the isolate on amended media by 50% (EC_{50}) was determined for every isolate by transforming to probits the percent of inhibition of each isolate, defined as inhibition = 1- (colony diameter on amended media divided colony diameter on non–amended media). The probits were plotted against the $\log_{10}$ of the mefenoxam concentration. The EC_{50} for each isolate was calculated by regression analysis. The point on the regression line at which 50% of the isolate growth was inhibited is the EC_{50} value. Statistical analysis was conducted with the JMP program version 7.0 (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA).

Virulence determination

Each isolate was challenged against a R-gene differential set of potato clones, each carrying a single resistance gene (R1 – R11), in order to determine virulence and race of *P. infestans* isolates (Malcolmson and Black, 1966). Four detached leaflets of each differential were placed abaxial side up in moist chambers and inoculated with 20 µL of sporangial suspension (aprox. 20 000 sporangia·mL^{-1}) of each isolate. The leaflets were incubated in growth chambers at 18˚C, 85% relative humidity and 14 h light. Seven days after inoculation, leaflets were evaluated for presence of sporulation and rated as compatible or incompatible (Flier and Turkensteen, 1999). The complexity of races was also evaluated with contingency tables to determine if there was an effect of by host, sampling period, or location.

Tuber blight evaluation

Tuber late blight was assessed for all *P. infestans* isolates on the cv. Dark Red Norland. All tubers were washed in distilled H_2O to remove soil. The tubers were then surface sterilized by soaking in 2% sodium hypochlorite solution for 30 min. Tubers were dried at 18^{o}C for 24 h prior to use in a non-forced air environment. The washed, surface-sterilized tubers were inoculated by inserting a mycelial plug (5 mm diameter) at the apical end of the tuber to about 1 cm maximum depth. Fiver tubers were inoculated with each of the isolates of *P. infestans* and five control tubers were inoculated with plugs of non-inoculated rye media. After inoculation, tubers were placed in the dark in sterilized covered plastic crates and returned to controlled environment chambers [Percival Incubator (Model I-36LLVL, Geneva Scientific, LLC, PO Box 408, Fontana, WI)]. The chambers were set at 10^{o}C and 95% humidity and the sample tubers were incubated in the dark for 30 d until evaluation.

A digital image analysis technique was used to assess tuber tissue infection. The method was previously used and standardized (Niemira *et al.*, 1999; Kirk *et al.*, 2001c). The image files were analyzed using SigmaScan V3.0 (Jandel Scientific, San Rafael, CA). The area selection cut-off threshold was set to 40 light intensity units, limiting the determination to the non–dark 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).

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

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

RARI (%) has minimum value of zero (no symptoms) and maximum value of hundred (completely dark tuber surface). Data for all experiments were analyzed by analysis of variance (least squares method) using the JMP program version 7.0 (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA).

DNA extraction

Isolates were grown for 10 to 14 days at 18°C in clear pea broth or V8 broth (Forbes, 1997). The mycelium was filtered, lyophilized, and ground with a mortar and pestle. DNA was extracted according to method of Goodwin *et al.* (1992b). DNA concentration and purity were estimated using a NanoDrop® ND-3300 fluorometer (NanoDrop Technologies, Wilmington, DE, USA).

Mitochondrial haplotype

Mitochondrial (mtDNA) haplotypes were determined by RFLP–PCR (Griffith and Shaw, 1998). All isolates were analyzed for mitochondrial haplotypes using the following primer pairs for specific mitochondrial DNA regions: P2 and P4, using the primers P2F 5′-TTCCCTTTGTCCTCTACCGAT-3′, P2R 5′-TTACGGCGGTTTAGCACATACA-3′, P4F 5′-TGGTCATCCAGAGGTTTATGTT-3′ and P4R 5′-CCGATACCGATACCAGCACCAA-3′, respectively. PCR was carried out as follows for all primer combinations in a final volume of 25 µl: 1× PCR buffer, 0.5 mM dNTPs (each), 2.5 mM MgCl2, 0.2 mM each primer, and 0.2 µl of *Taq* DNA polymerase (5 $U \cdot \mu L^{-1}$). The PCR conditions were 1 cycle of 94°C for 4 min and 35 cycles of 94°C for 60 s, 60°C for 45 s, and 72°C for 120 s. PCR products for region P2 and P4 were digested with the restriction enzymes *Msp*I and *Eco*RI, respectively. The digestion products were separated on 2% agarose gels and visualized by staining with ethidium bromide and scored as Griffith and Shaw (1998).

Microsatellite Analysis

Seven polymorphic microsatellite loci were chosen for analysis. The markers used were Pi4B and PiG11 (Knapova and Gisi, 2002) and PiD13, Pi02, Pi26, Pi33 and Pi70 (Lees *et al.*, 2006), sequences were given in Table 3.1. Microsatellite polymerase reactions were performed in 25-µl reaction volumes. Each reaction tube contained 1× PCR buffer; 0.2 mM (each) of dNTPs, 1 mM MgCl2, 0.2 mM each forward and reverse primers, and 0.2 µl of Taq (5 $U \cdot \mu L^{-1}$). The thermal cycling parameters were initial denaturation at 94°C for 2 min followed by 35 cycles consisting of denaturation at 94°C for 1 min, annealing at 56°C for 45 s, and extension at 72°C for 2 min. A final extension at 72°C for 7 min was done at the end of the amplification.

PCR products were examined by agarose gel electrophoresis using 3% (wt/vol) Metaphor agarose (FMC, Rockland, Maine), containing 0.5 $\mu g \cdot mL^{-1}$ ethidium bromide, and the remainder was stored at 4˚C for later use. The electrophoretic buffer was 0.5X TBE (89 $mmol \cdot L^{-1}$ Tris–HCl, pH 7.8; 89 $mmol \cdot L^{-1}$ boric acid, 2 $mmol \cdot L^{-1}$ EDTA). After electrophoresis at 98 V for 1.5 h, the image was acquired using the Bio-Rad ChemiDoc XRS imaging system (Bio-Rad, Hercules, CA, USA). Reference isolates were included in the study to compare against the Michigan *P. infestans* isolates. These include 3 isolates from UK kindly provided by Dr. David Cooke, 8 from Colombia isolates kindly provided by Dr. Silvia Restrepo and US-22 isolates isolated in Florida and New York kindly provided by Dr. Pam Roberts and Dr. Bill Fry, respectively.

Table 3.1. Summary of the microsatellite (SSRs) markers used during this study.

Marker		SSR primer sequence	Repeat	Reference
G11	F	TGCTATTTATCAAGCGTGGG	(TC)26	(Knapova and Gisi, 2002)
	R	TACAATCTGCAGCCGTAAGA		
Pi02	F	CAGCCTCCGTGCAAGA	(TG)11	(Lees *et al.*, 2006)
	R	AAGGTGCGCGAAGACC		
4B	F	AAAATAAAGCCTTTGGTTCA	(TC)34	(Knapova and Gisi, 2002)
	R	GCAAGCGAGGTTTGTAGATT		
D13	F	TGCCCCCTGCTCACTC	(CT)27	(Lees *et al.*, 2006)
	R	GCTCGAATTCATTTTACAGA		
Pi70	F	ATGAAAATACGTCAATGCTCG	(AAG)8	(Lees *et al.*, 2006)
	R	CGTTGGATATTTCTATTTCTTCG		
Pi33	F	TGCCGACGACAAGGAA	(CAG)5	(Lees *et al.*, 2006)
	R	CGGTCTGCTGCTGCTC		
Pi26	F	GCAGTAGCCGTAGTCCTCAG	(GT)9	(Lees *et al.*, 2006)

Data Analysis

Score microsatellites for 135 isolates were organized in matrix for the seven loci evaluated, a matrix was constructed using Microsatellite Analyzer MSA 4.02 (Dieringer and Schlötterer, 2003) and alleles frequencies were obtained. Distance matrices were calculated using populations 1.2.30 (Langella, 1999) using Roger's distance (1972) for host-sampling period combination and host, sampling period and location. The resulting matrices were used to infer UPGMA dendograms; bootstrapping of data was done using 1000 permutations. The resulting tree was visualized using Figtree v1.3.1 (Rambaut, 2010). Clonal diversity was examined by location and host – year combination with Genodive (Meirmans and Van Tienderen, 2004) between populations estimating the effective number of genotypes, Nei's genetic diversity, and evenness. The software package Genalex v6.4 (Peakall and Smouse, 2006) was used to conduct analysis of effective alleles, fixation index, information index and observed and expected heterozygosity. The analysis was also conducted for the structure resulting clusters (See below).

A principal component analysis (PCA) was conducted on individual multilocus genotypes and population level using Genodive with the standardized covariance method; the method does not require an underlying model and the resulted axis with the higher covariance were graphed with SigmaPlot v11.0 (Systat Software Inc.). Second, we used the Bayesian clustering algorithm implemented in Structure 2.3.3 (Pritchard *et al.*, 2000), which relies on Bayesian Monte Carlo Markov Chain (MCMC) approach to cluster individuals into *K* distinct populations. The data was analyzed under the admixture model with correlated allele frequencies and without previous population information. Based on preliminary runs, analyses were done with a burn-in period of 500,000 generations and 750,000 MCMC replications and

lambda equal to 1. The number of clusters *K* was varied from 1 to 8 and replicated 20 times. The method of Evanno *et al.* (2005) was used to estimate the most likely K given the data. The ad hoc statistic Δ*K* was computed using Structure harvester (http://taylor0.biology.ucla.edu/struct_harvest/). Results were aligned and the average cluster membership was determined using CLUMPP v1.1.2 (Jakobsson and Rosenberg, 2007), and plotted using the software Distruct v1.1 (Rosenberg, 2004), sorting data by location, period collected, host, mefenoxam response and number of R-gene differentials defeated.

Results

Mating type, allozyme analysis and mitochondrial DNA haplotype

Isolates of *P. infestans* were analyzed using reference isolates and both CAP and RFLP derived-markers were mating type A2 (n = 120; Table 3.2). In addition, most of the isolates during the study had the 100/122 *Gpi* genotype, but there were just two different *Gpi* genotypes were observed in 2008, these are 100/100/111 and 100/111/122 genotypes on cellulose acetate. The summary of allozyme profiles, including 100/100/111 and 100/122 that were found during the study for the *Gpi* locus by location are shown in Table 3.2. All isolates from 2010 had the *Gpi* genotype 100/122, which marked the reduced variability among isolates using this population indicator.

Mitochondrial DNA haplotyping of *P. infestans* isolates by PCR-RFLP yielded two fragments (720 and 350 bp) for P2 region and three fragments (394, 361, and 209 bp) for P4 region. These results restricted the mtDNA haplotype of the isolates sampled (n=120) to the Ia haplotype (Table 3.2).

Table 3.2. Summary of isolates of *Phytophthora infestans* were obtained from 2008 to 2010 by county, host and period collected.

Location	Host	Period	Isolates	Mating Type	mtDNA Haplotype	*Gpi* allozyme	Clonal Lineage
Clinton	Potato	2008-09	17	A2	Ia	100/100/111	US-11
						100/111/122	US-8
						100/122	US-22
		2010	26	A2	Ia	100/122	US-22
	Tomato	2010	6	A2	Ia	100/122	US-22
Hillsdale	Tomato	2008-09	1	A2	Ia	100/122	US-22
Ingham	Tomato	2008-09	2	A2	Ia	100/122	US-22
Ionia	Potato	2010	3	A2	Ia	100/122	US-22
Mecosta	Potato	2008-09	11	A2	Ia	100/122	US-22
	Tomato	2008-09	2	A2	Ia	100/122	US-22
		2010	23	A2	Ia	100/122	US-22
Montcalm	Potato	2010	17	A2	Ia	100/122	US-22
Presque Isle	Potato	2010	5	A2	Ia	100/122	US-22
	Tomato	2010	1	A2	Ia	100/122	US-22
St Joseph	Potato	2008-09	2	A2	Ia	100/122	US-22
		2010	1	A2	Ia	100/122	US-22
	Tomato	2008-09	1	A2	Ia	100/122	US-22
		2010	2	A2	Ia	100/122	US-22

Mefenoxam sensitivity *in vitro*

Mefenoxam *in vitro* assays on amended media showed different percentages for sensitive, intermediate and resistance isolates from 2008 to 2010 (Fig. 3.2). In 2008-09, the EC_{50} values ranged from <0.1 to 91 $\mu g \cdot mL^{-1}$. The frequency of sensitive, intermediate and resistant isolates of *P. infestans* were 28.9% , 65.8% and 5.3%, respectively in 2008-09 (Fig. 3.2A). In 2010, the EC_{50} values ranged from <0.1 to 65.8 $\mu g \cdot mL^{-1}$ and the frequency of sensitive, intermediate and resistant isolates of *P. infestans* were 44.6%, 51.8% and 3.6% respectively in 2010 (Fig. 3.2B). The distribution frequency of EC_{50} values for 2008-09 and 2010 were not significantly different and there was a trend showing a change from a unimodal distribution to a bimodal distribution (Fig. 3.3).

The variation of the response to mefenoxam in relation to host shows that most of potato isolates were intermediate (64.3%), followed by sensitive isolates (31%); while tomato isolates were mostly sensitive (60.5%), followed by intermediate (36.8%). The percentage of resistant isolates for both hosts was low, but potato isolates (4.8%) were nearly twofold to tomato isolates (2.6%). Potato and tomato isolates EC_{50} median value were 4.99 and 1.38 $\mu g \cdot mL^{-1}$, respectively (Table 3.3). This confirms the slightly higher tolerance of potato isolates to mefenoxam than tomato isolates. The tomato resistant isolates were observed only in 2010, while resistant isolates obtained from potato were observed for both periods. The median population EC_{50} for tomato isolates in 2010 was 1.22 $\mu g \cdot mL^{-1}$, which was lower than the median value for the 2008-09 period (2.8 $\mu g \cdot mL^{-1}$).

Across locations the responses for mefenoxam sensitivity were similar, where most of the locations accounted between 65 to 100% percentage of sensitive and intermediate isolates. There are just two out six counties that had resistant isolates in low frequencies. The higher median of observed EC_{50} was for St Joseph county followed by Ionia. The categorical responses of mefenoxam sensitivity were analyzed by test response homogeneity, which shows that there are only significant differences between mefenoxam responses by host (Table 3.4), but not for year or the two-way interaction.

Figure 3.2. Range of distribution and of effective concentration for 50% (EC_{50}) as logEC_{50} ($\mu g \cdot mL^{-1}$) of mycelial growth inhibition of *P. infestans* isolates obtained during (A) 2008-09 and (B) 2010.

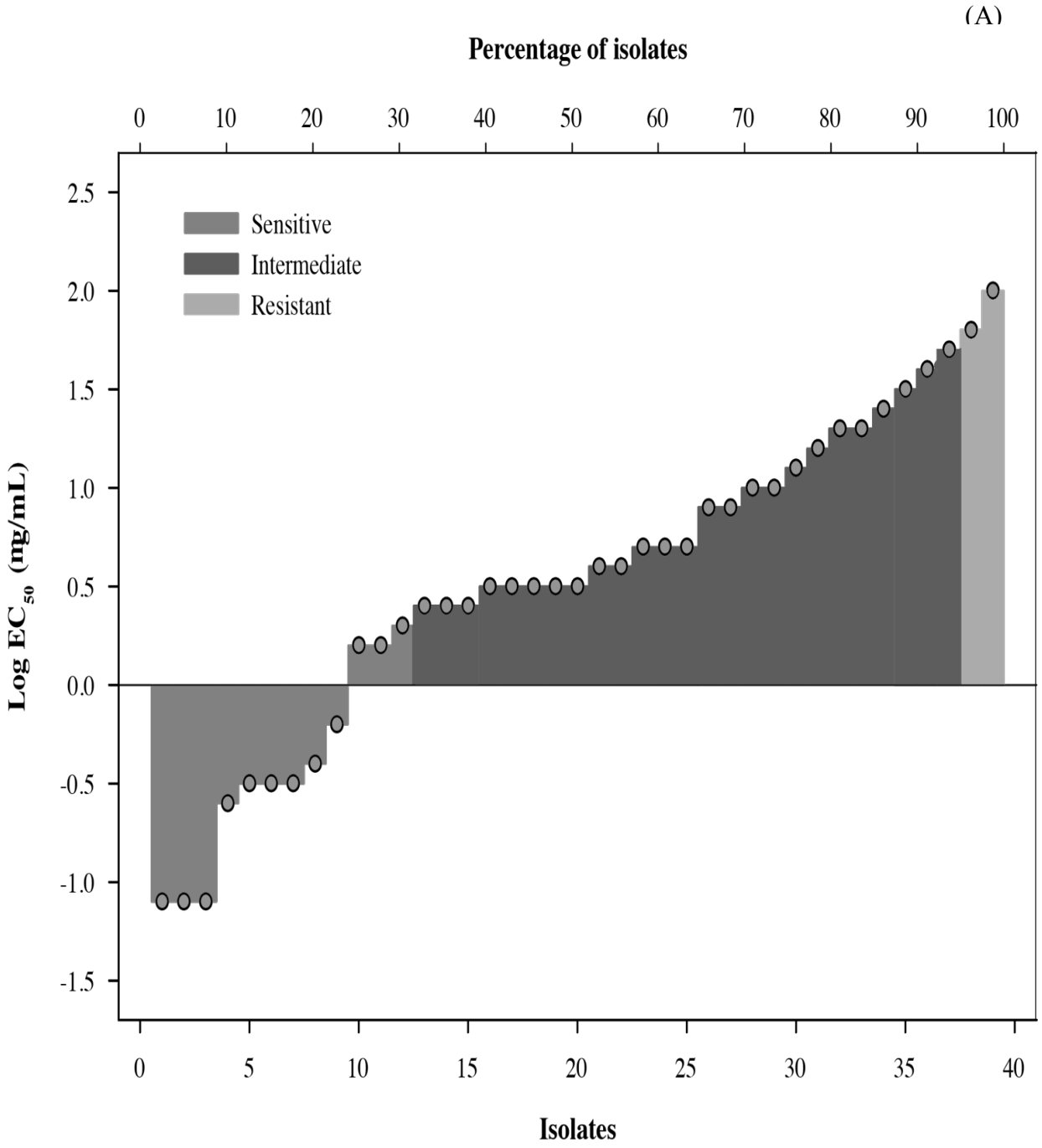

Figure 3.2 (cont'd)

(B)

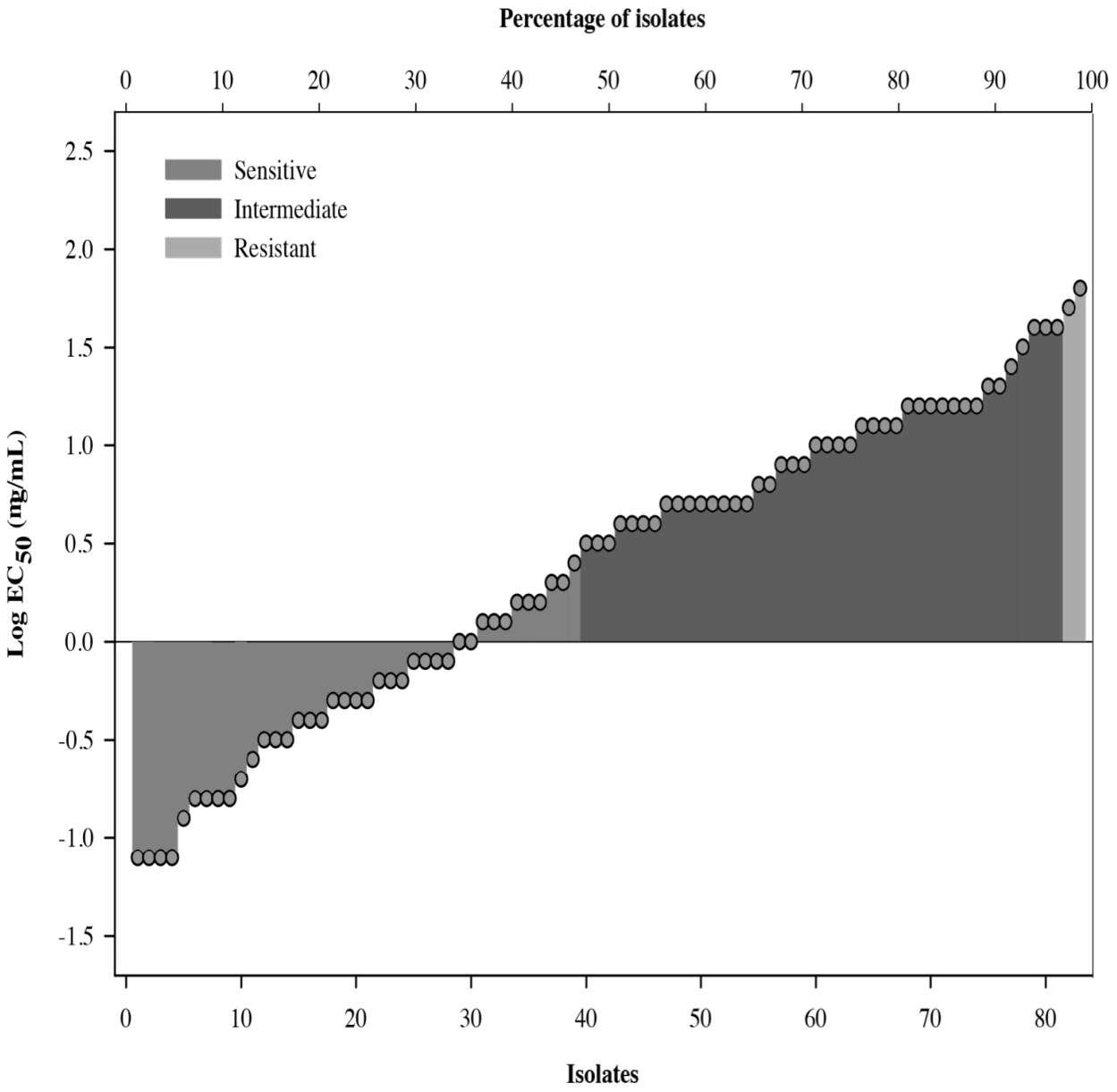

Table 3.3. *In vitro* mefenoxam sensitivities (EC_{50}) and responses of *P. infestans* isolates collected organized by host, sampling period, period – host combination and location (county).

			EC_{50} (µg·mL^{-1})			Mefenoxam responses (%)[c]		
Host		**n[a]**	**Mean**	**Range[b]**	**Median**	**Sensitive**	**Intermediate**	**Resistant**
Potato		84	10.48	0.09 - 91	4.99	31.0	64.3	4.8
Tomato		38	6.64	0.09 - 65.82	1.38	60.5	36.8	2.6
Sampling period								
2008-09		39	11.77	0.09 - 91	3.46	30.8	64.1	5.1
2010		83	8.12	0.09 - 65.82	3.38	44.6	51.8	3.6
Host - Sampling Period								
2008-09	Potato	33	12.70	0.09 - 91	3.60	27.3	66.7	6.1
2008-09	Tomato	6	6.62	0.3 - 24.5	2.89	50.0	50.0	0.0
2010	Potato	51	9.05	0.09 - 53.01	5.25	33.3	62.7	3.9
2010	Tomato	32	6.64	0.09 - 65.82	1.22	62.5	34.4	3.1
Location								
Clinton		49	11.49	0.09 - 60.3	8.00	24.5	73.5	2.0
Hillsdale		1	0.30	0.3	0.30	100.0	0.0	0.0
Ingham		2	13.60	2.7 - 24.5	13.60	0.0	100.0	0.0
Ionia		3	20.15	0.84 - 42.57	17.05	33.3	33.3	33.3
Mecosta		36	5.97	0.09 - 65.82	1.26	61.1	33.3	5.6
Montcalm		17	3.76	0.31 - 10.11	3.65	41.2	58.8	0.0
Presque Isle		6	4.59	0.09 - 13.24	3.83	50.0	50.0	0.0
St Joseph		6	31.89	2.36 - 91	21.19	16.7	50.0	33.3

[a] Isolates number (n).

[b] Mefenoxam concentration range evaluated was from 0.1 to 100 µg·mL^{-1}.

[c] Mefenoxam responses were designated based on Therrien *et al.* (1993). Sensitive (S), Intermediate (I) and Resistant (R).

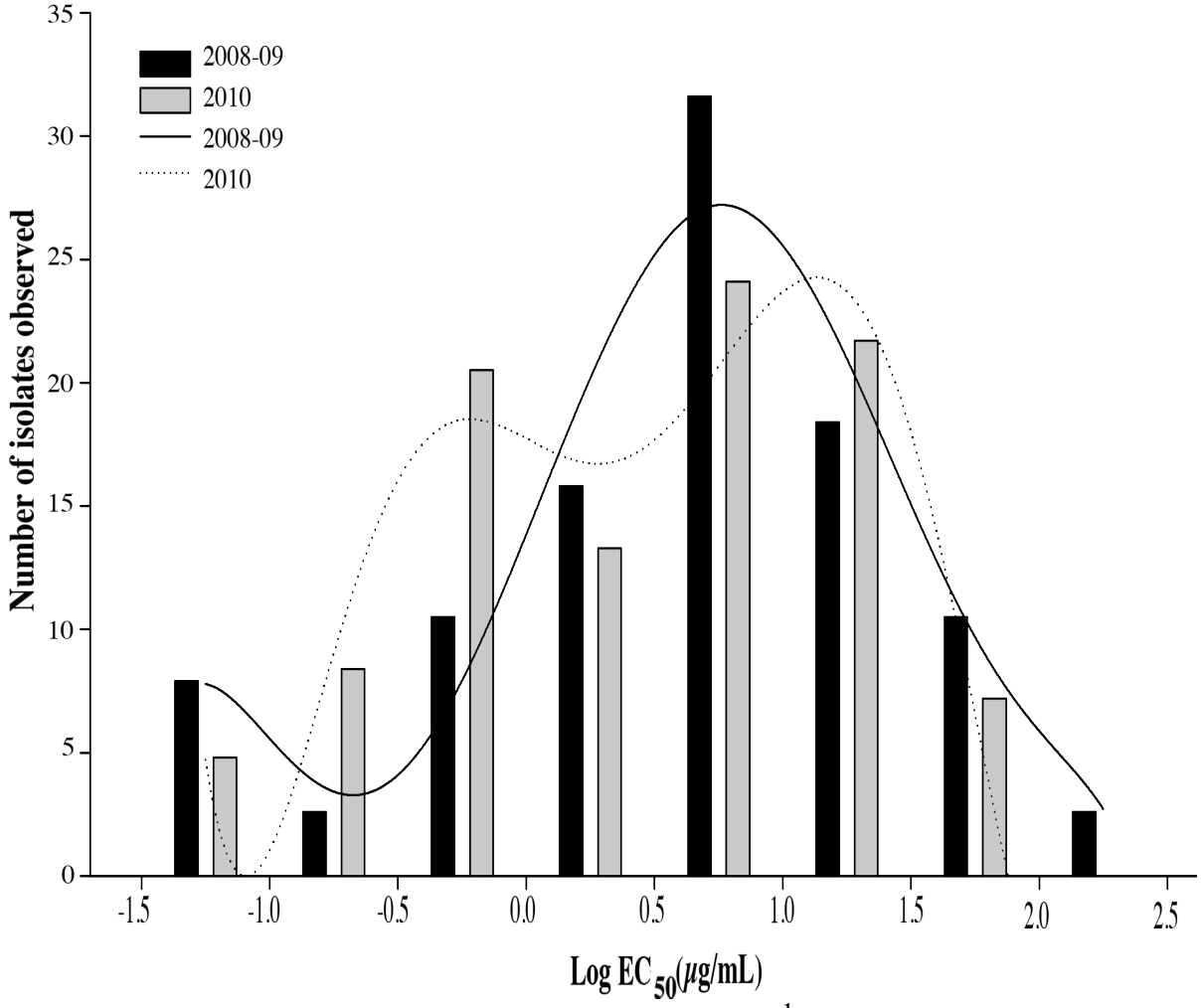

Figure 3.3. Histogram of distribution log-EC_{50} ($\mu g \cdot mL^{-1}$) of populations of *P. infestans* during 2008-09 and 2010. Trend lines were plotted as regression analysis using polynomial equations.

Table 3.4. Test response homogeneity values for mefenoxam responses of *P. infestans* isolates obtained during 2008 – 2010 by host, year and host-year.

	Year	Host	Year-Host
	2008-09 vs. **2010**	**Potato** vs. **Tomato**	**Two-way combination**
Likelihood	0.3384	0.0089*	0.0976
Pearson	0.3451	0.0086*	0.1043

* Significantly different at P=0.05.

Race and tuber blight virulence

The virulence among isolates evaluated was very variable, but there were no isolates able to infect all 11 potato differentials. A total of 72 different races were observed for the complete population, 52 of them corresponded to potato and 25 to tomato. Only three pathotypes were common on the two hosts, including the race 0. Analyzed by period, 2010 had the most diverse virulence composition accounting for 49 different races in comparison to 26 races observed during 2008-09. For both hosts from 2008-09 to 2010, there was an expansion of races observed in the population. However, potato isolates increased from 21 to 33 different races and tomato isolates races increased from 5 to 20. The isolates were more frequently virulent on differentials R10, followed by R5, R8 and R6. However, the frequencies of most of the differentials defeated were lower than 0.4, except R10. The differentials R9 and R11 were less common among the isolates. During the 2008-09 period, the differential R5 was the most commonly infected, followed by R10 and R4. In 2010, the frequencies shifted to R10, followed by R8 and R6 (Fig. 3.4A). In relation to host, R10 was frequently infected by isolates from tomato and potato, followed by R8 and R5. In general, the less frequently infected differentials were R9 and R11 (Fig. 3.4B).

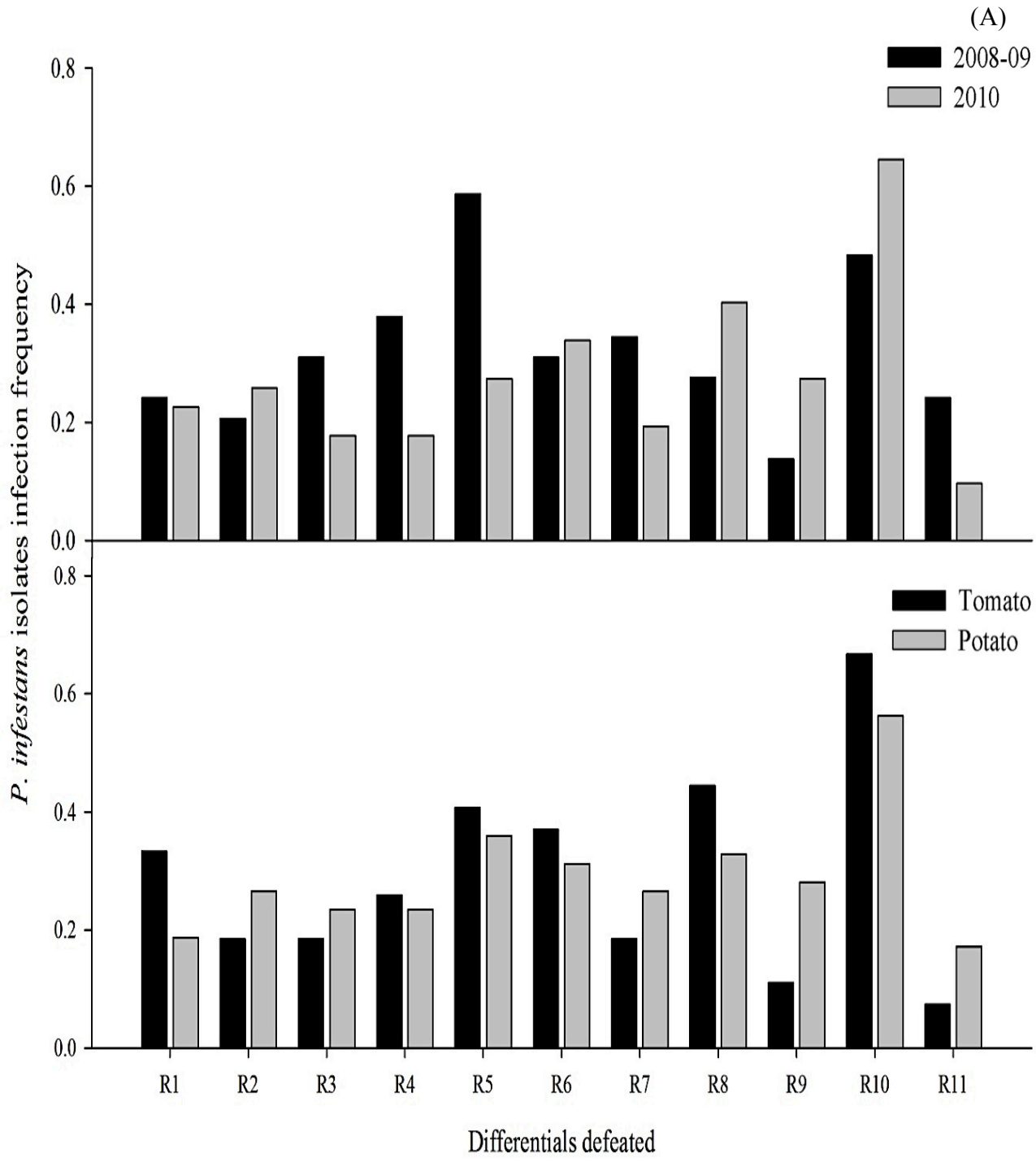

Figure 3.4. Virulence frequency of isolates of *Phytophthora infestans* from Michigan (A) by year; 2008-09 (n= 39) and 2010 (n=81). (B) by host; tomato (n=38) and potato (n=82).

The complexity of the races observed tended to be low, since most of the isolates overcome between 2 to 4 R-genes, the most complex race that was virulent to 9 differentials, and it was isolated from potato (Fig. 3.5A and 3.6). There is no correlation of race complexity and host origin, sampling period or location of *P. infestans* isolates (data not shown). The race most frequent of isolates from tomatoes included two and three differentials defeated; for instance race 2.10 was the most common observed in tomato isolates. In contrast, three and four differential defeated were the most common race complexity observed in potato isolates, but the combinations were variable. Over the two periods evaluated, most of the isolates of 2008-09 were able to infect three different differentials, while in 2010, the isolates ranged from two to four infected differentials (Fig. 3.5B). Although, more complex races occurred in both tomato or potato isolates the complex races were slightly more frequent on those recovered from potatoes.

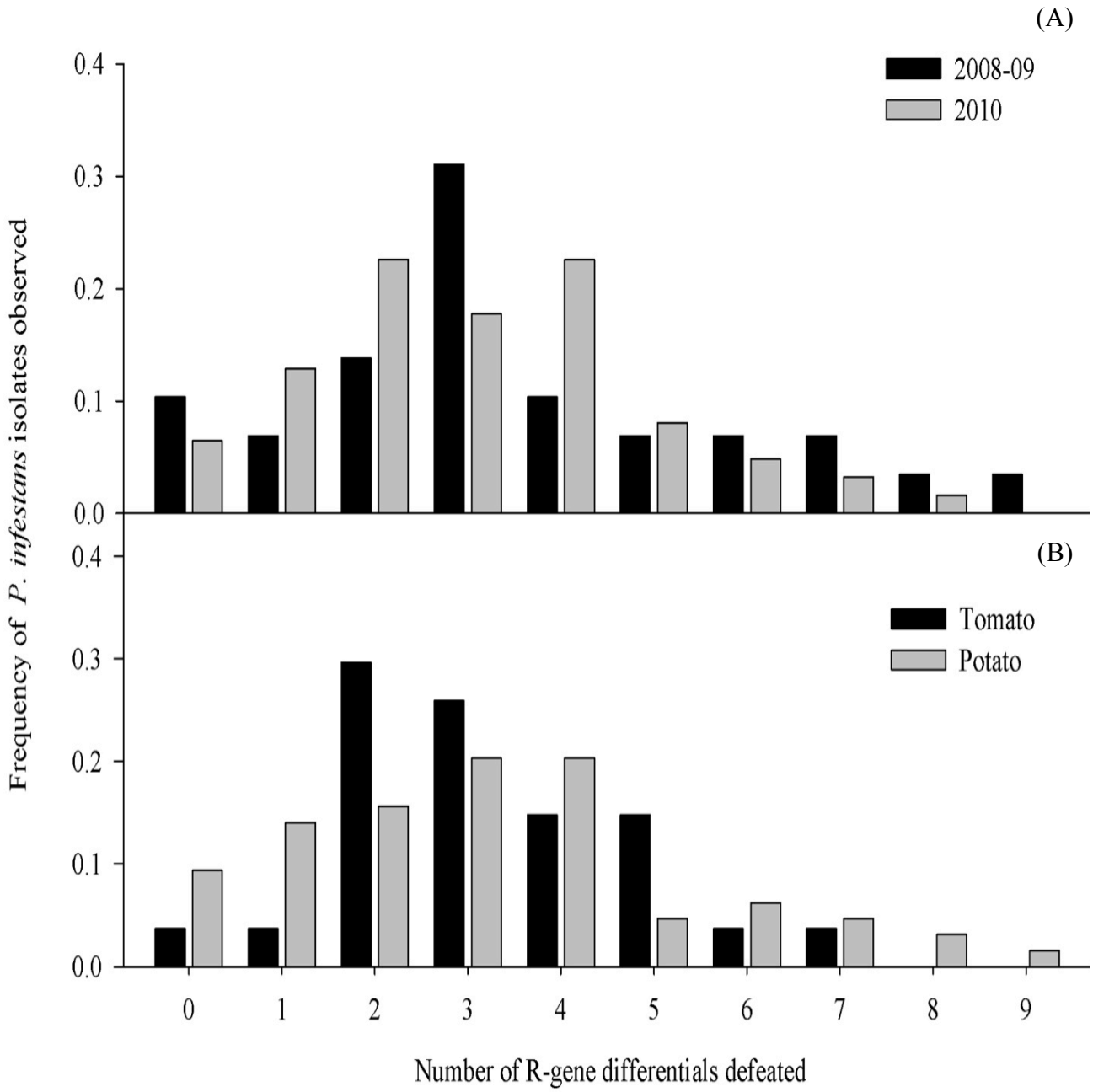

Figure 3.5. Race complexity of *Phytophthora infestans* isolates from from Michigan (A) by year; 2008-09 (n= 39) and 2010 (n=81). (B) by host; tomato (n=38) and potato (n=82).

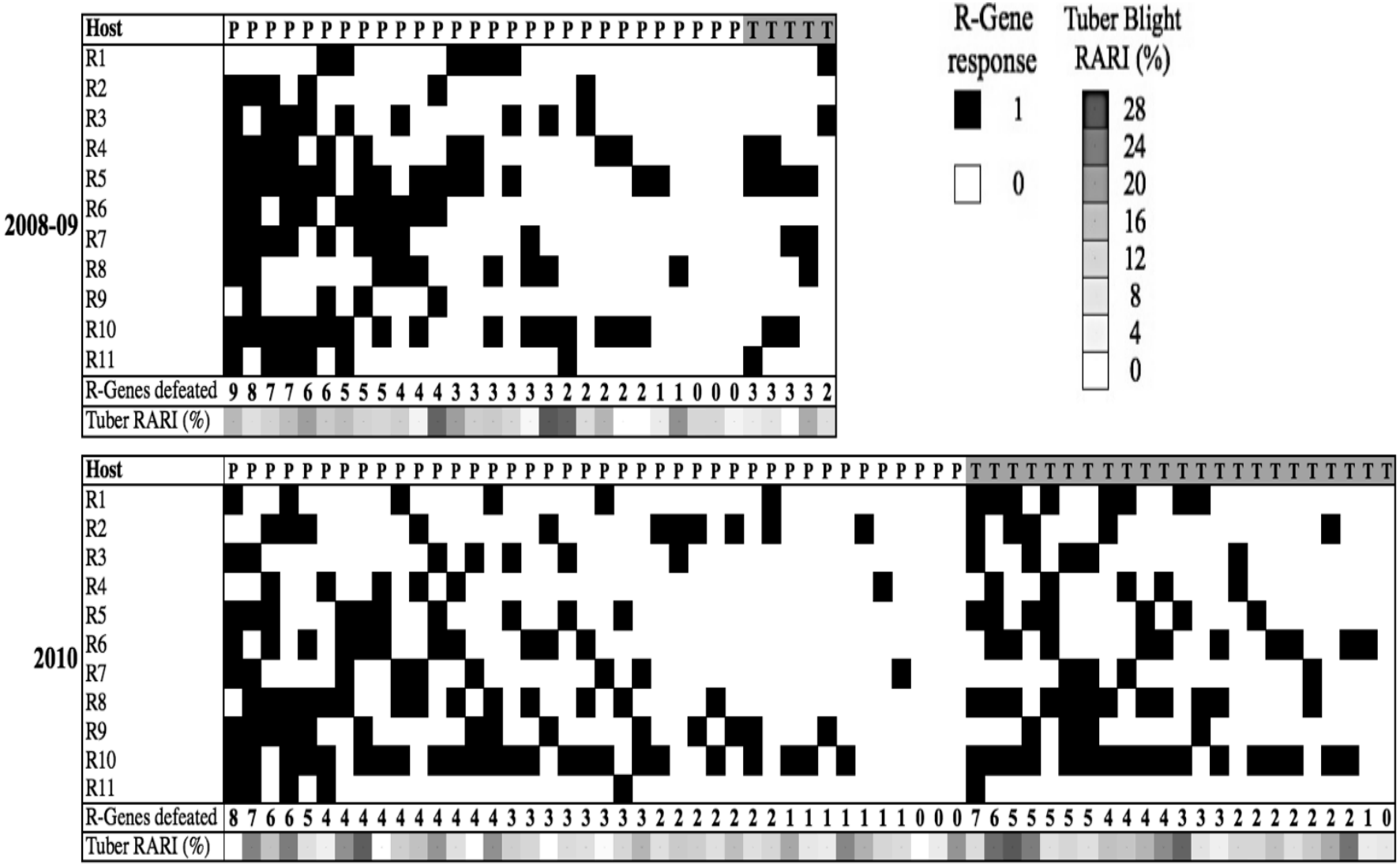

Figure 3.6. Color map for virulence and tuber aggressiveness of *P. infestans* isolates from Michigan. RARI (%) are in gray color scale from white (0%) to black (28%); R1- R11 differentials are colored by disease reaction incompatible (white) or compatible (black). P= Potato and T = Tomato.

Table 3.5. Two-way factorial ANOVA of the effect of sampling period and host of *Phytophthora infestans* isolates on tuber tissue late blight as mean Relative Average Reflection Intensity [RARI (%)]. Variance ratio (F), degrees of freedom (df), and P–Value.

Variables and Interactions	F Ratio	df	P-Value
Sampling period	6.9512	1	0.0086*
Host	0.0021	1	0.9638
Sampling period X Host	4.5242	1	0.0338*

* Significant at P=0.05

The degree of virulence on potato tubers was also evaluated for *P. infestans* isolates, which ranged from low-virulent (US-1) to virulent (US-8). The RARI (%) values ranged from 3.19 to 29.09% (Figs. 3.6 and 3.7); there were no significant differences of isolates from potato in comparison with tomato (Table 3.5). There were significant differences by periods, where 2010 isolates were more virulent on tubers than isolates from 2008-09. However, interaction year-host showed that isolates from potato were more pathogenic on tubers than tomato isolates in 2008-09; whilst, in 2010, isolates from tomato were pathogenic than potato isolates. Tuber virulence was highly variable among isolates, 34 isolates caused tuber tissue darkening similar to that caused by US-8 whether isolated from tomato or potato (Fig. 3.7). The variability of virulence among isolates evaluated was high either on tuber or differentials, and there was no pattern for virulence that related the race complexity to tuber virulence (Fig. 3.6).

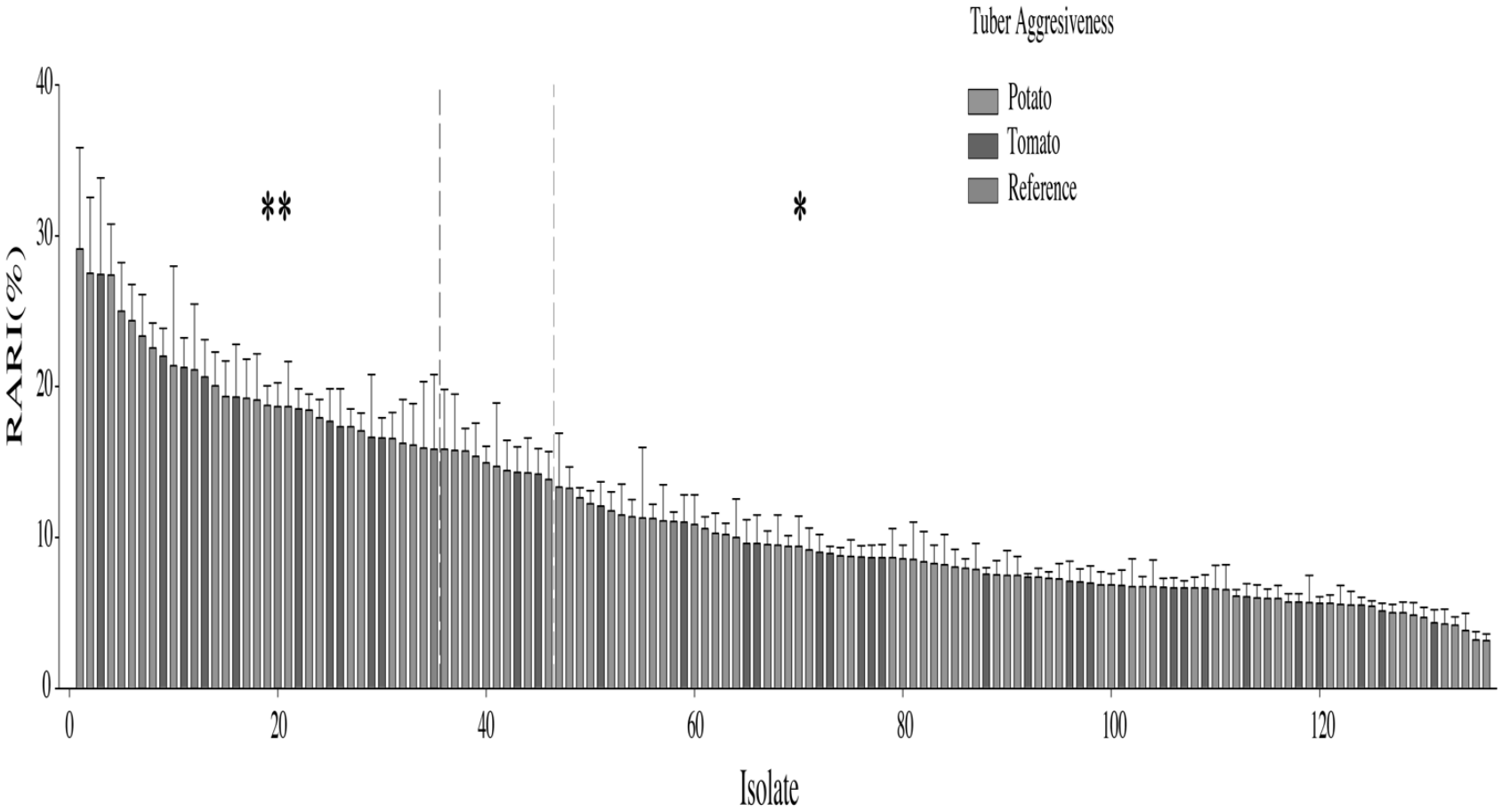

Figure 3.7. Tuber late blight measured as tissue-darkening [RARI (%)] 30 d after inoculation with isolates of *Phytophthora infestans* collected from 2008 to 2010. Tested in tubers of the susceptible cv. Dark Red Norland. (RARI: Relative Area of Reflection Index). ** Isolates before dashed line are not significantly different from reference US-8 isolate; * Isolates after dashed line are not significantly different from US-1 isolate (P=0.05).

Microsatellite

A total of 120 isolates were analyzed for SSRs genotyping, producing 22 different multilocus genotypes that were used to analyze population structure. Across the microsatellite markers evaluated in this study number of alleles ranged from 2 to 7. Three of the seven loci had reduced number of alleles (D13, Pi70 and Pi33), but the number of effective alleles was low for most of the genotypes observed (Table 3.6). Some of the loci had lower values of observed heterozygosity than the expected, which indicates a lack of polymorphism. For instance, the locus D13 was the less effective because of its reduced heterozygosity and it is also due to the null alleles on isolates for 2010 sampling period. The resolving power of markers is also evident on the information index and the number of effective alleles, where G11 was the most informative locus across the seven markers used for the study.

The genetic diversity of the genotypes observed during the study varied across locations sampled, where counties with substantial production of potato or tomato presented the higher number of genotypes observed. Nonetheless, sample sizes for different locations were limited, e.g. one isolate obtained from home garden or plot sample (Table 3.7). Locations with multiple isolates presented as well multiple genotypes, but the diversity is still low possibly due to bias of sample size. Nei's genetic diversity index (1987) for the locations sampled showed a low diversity in comparison to reference population, which includes different clonal lineages (US-8, US-14, US-10, US-1, Blue-13) in order to compare. The location with the highest diversity was St. Joseph, where isolates from tomato and potato were obtained. Evenness for some of the locations was relative low indicating the dominance of some genotypes, but it was not the case for St Joseph.

Table 3.6. Allelic diversity observed at seven loci in the total population and cluster obtained by Bayesian clustering analysis of isolates of *P. infestans* in Michigan.

Marker		G11	Pi02	Pi4B	PiD13	Pi70	Pi33	Pi26
	Allele Range	142-166	152-176	205-217	108-142	189-195	203-209	193-228
Cluster A	**Number Alleles**	6	2	4	3	3	3	4
	Effective Alleles	2.952	1.017	3.307	3.242	2.044	2.142	2.478
	Information Index	1.440	0.048	1.353	1.353	0.819	0.831	1.076
	Ho[a]	0.683	0.017	0.283	0.192	0.383	0.467	0.200
	He[b]	0.661	0.017	0.698	0.692	0.511	0.533	0.597
Cluster B	**Number Alleles**	2	2	3	1	3	2	2
	Effective Alleles	1.994	2.000	2.083	1.000	2.049	1.994	1.057
	Information Index	0.692	0.693	0.780	0.000	0.755	0.692	0.127
	Ho	0.944	1.000	1.000	0.000	0.958	0.944	0.000
	He	0.498	0.500	0.520	0.000	0.512	0.498	0.054
Total Population	**Number Alleles**	7	2	5	3	3	3	4
	Effective Alleles	2.473	1.508	2.695	2.121	2.047	2.068	1.768
	Information Index	1.066	0.371	1.066	0.677	0.787	0.761	0.602
	Ho	0.814	0.508	0.642	0.096	0.671	0.706	0.100
	He	0.580	0.258	0.609	0.346	0.511	0.516	0.325

[a] Ho observed heterozygosity

[b] He Expected heterozygosity

Analyzing the isolates by sampling period – host combination, the subpopulation of isolates of potato during 2008-09 was more diverse in comparison to the other sub populations (Table 3.7). Tomato isolates from 2008-09 was next most diverse subpopulation, but it is important to remark that the sample size is still small. In general, isolates obtained during 2010 showed a reduced diversity, where just two genotypes were common across both hosts. Genetic distances estimated for the subpopulations by host-year and location-host-year combination were estimated and represented in UPGMA dendograms, which confirms the division of the population over time. Isolates from 2010 were grouped together in both cases, and some isolates obtained from 2008-09 were relative close to some of the isolates obtained in 2010 (Fig. 3.8).

Table 3.7. Summary of genetic diversity of *P. infestans* isolates by location.

	Population	N	Genotypes	Effective Genotypes[a]	Nei's index[b]	Evenness
Location						
Michigan Counties	US reference	19	13	8.395	0.930	0.646
	Clinton	54	7	2.089	0.531	0.298
	Hillsdale	1	1	1.000	-	-
	Ingham	1	1	1.000	-	-
	Ionia	2	1	1.000	-	-
	Mecosta	28	6	2.042	0.529	0.340
	Montcalm	8	1	1.000	-	-
	Presque Isle	3	1	1.000	-	-
	St Joseph	5	4	3.571	0.900	0.893
Host - Year						
	Reference	19	10	5.918	0.877	0.592
	Potato 2008-09	28	6	2.240	0.574	0.373
	Tomato 2008-09	3	2	1.800	0.667	0.900
	Potato 2010	45	2	1.093	0.087	0.546
	Tomato 2010	30	2	1.301	0.239	0.650

[a] Effective number of genotypes

[b] Nei's genetic diversity corrected for sample size (1987).

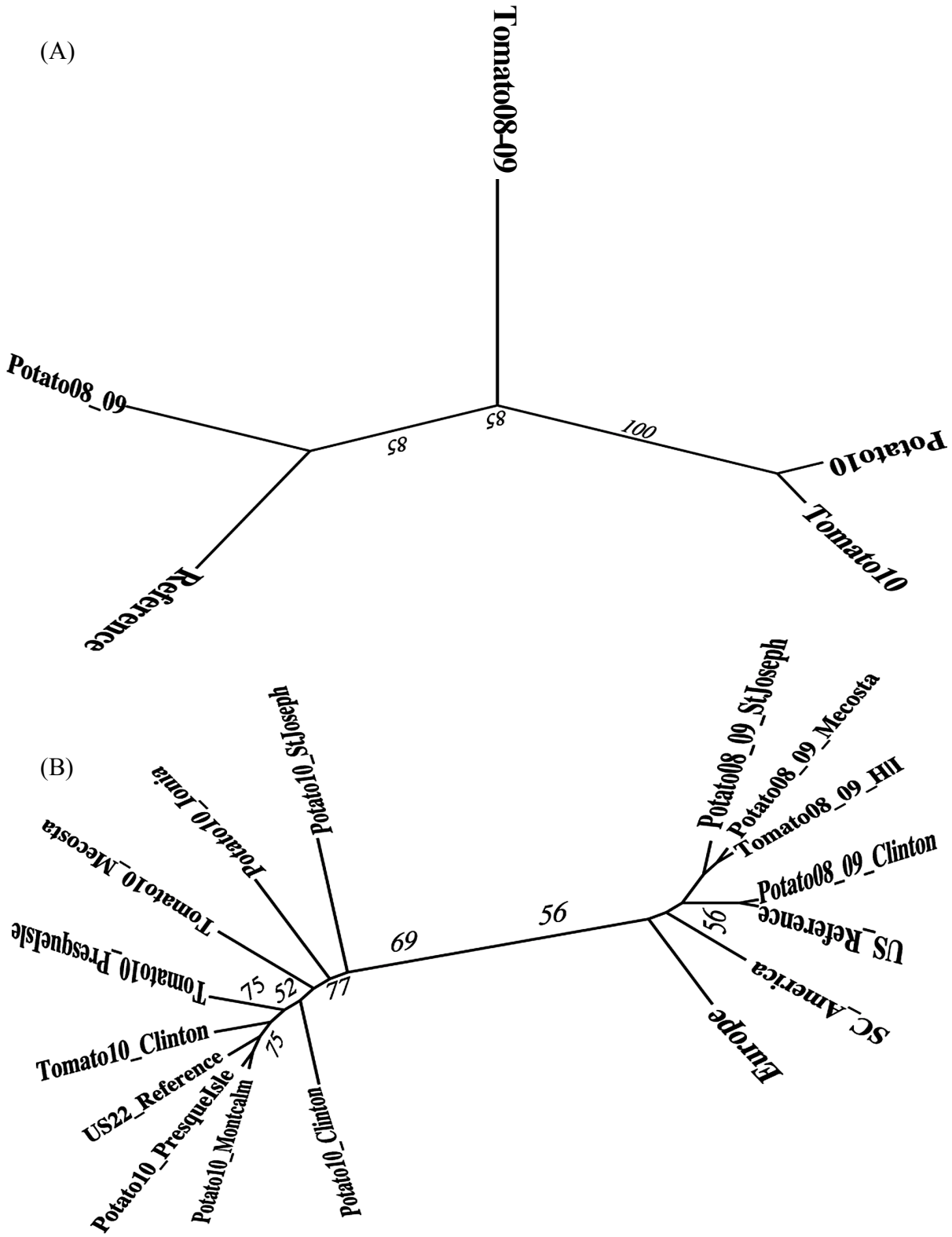

Figure 3.8. UPGMA dendograms of genetic distances among subpopulations established by host – sampling period (A) and host-sampling period-location (B). Support values greater than 50% using 1000 bootstrap permutations are shown.

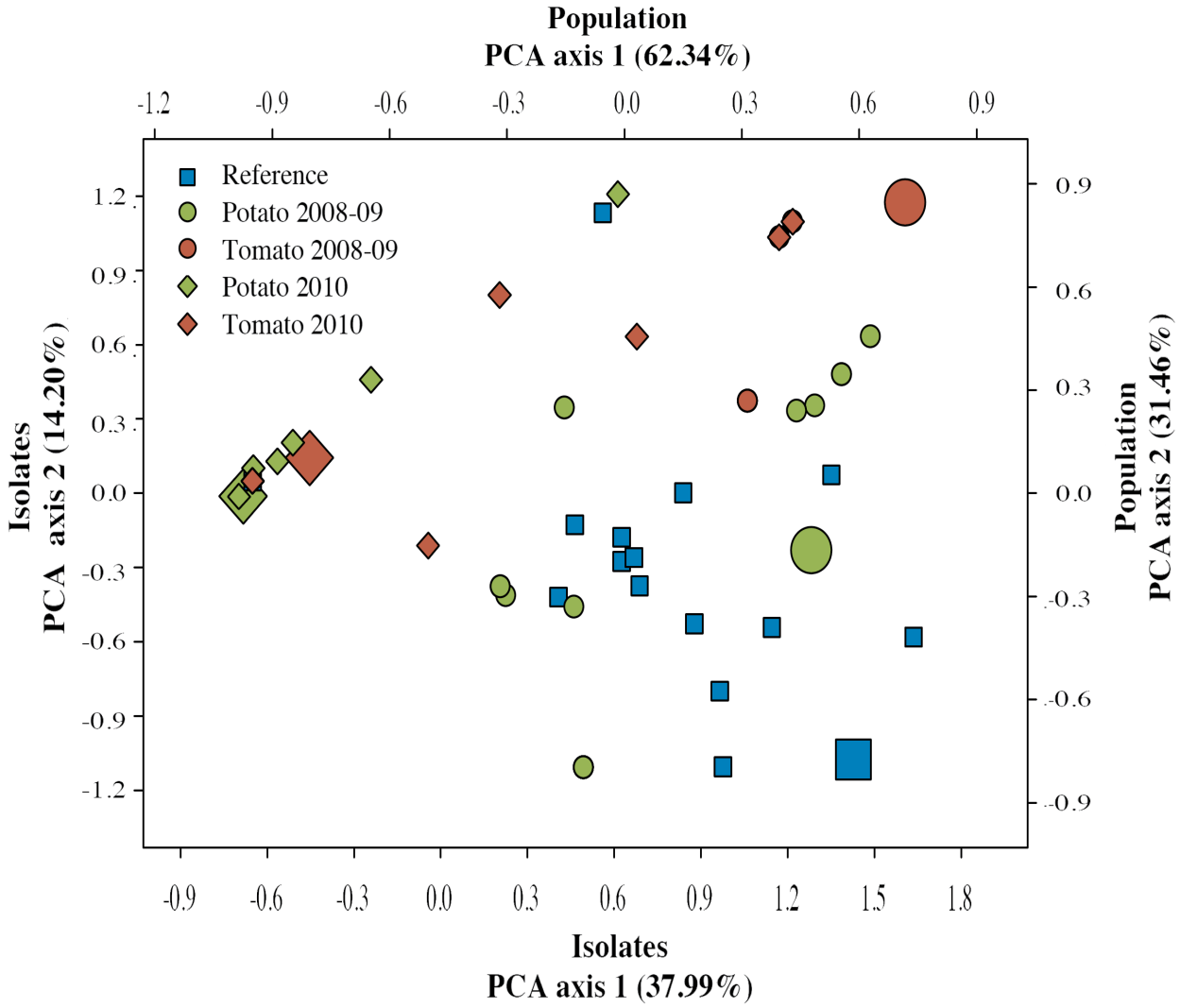

Figure 3.9. Principal component analysis (PCA) performed on 135 isolates of *Phytophthora infestans* from Michigan and reference isolates based on seven microsatellite markers. Small markers represent isolates (main axis) and larger markers represent populations (secondary axis).

Principal component analysis (PCA) done on the different genotypes observed as individuals and populations defined by sampling period and host showed some pattern. The Axis of PCA for individuals accounted respectively for 37.99% and 14.20% of the total genetic variability (Fig. 3.9). Populations PCA axis 1 and 2 accounted for 62.34% and 31.46% respectively. The cluster observed in the multivariate analysis reflected the observed pattern on UPGMA dendograms, despite that the populations are differentiated, many isolates were overlapped or grouped together due to to reduced variability within the subpopulations. For instance, isolates of tomato and potato from 2010 were grouped together in the PCA analysis.

On the other hand, Bayesian clustering done by Structure using the microsatellite data revealed highest likelihood for two clusters (K=2), and then sorted by prior population information revealed little evidence of admixture; just three isolates were slightly admixed. The differentiation among the clusters is evident, but it is statistically significant by AMOVA analysis (Table 3.8); variation is mainly explained by diversity within the isolates, followed by variation among the clusters. Cluster 1 (Blue color) is composed by 48 isolates and cluster 2 (Green color). Some structure was observed, mainly attributed by sampling period, where most of isolates from 2008-09 belonged to cluster 1 and likewise for cluster 2 with isolates for 2010 (Fig. 3.10).

Analyzing the genetic features of the clusters obtained reveal a higher number of clones in 2008-09 than 2010, which is also noticed on Nei's genetic diversity index and Shannon corrected index. The increased number of genotypes on cluster 1 reduces the evenness within the population. Cluster 2 tends to be more clonal with reduced number of observed genotypes (Table 3.9 and Fig. 3.10, Green bars). Fis were variable for both clusters among loci, ranging

from -0.277 to 0.947 for cluster 1 and from -1 to 1for cluster 2 (Table 3.9). Fixation index (Fis) multilocus indicates a deficit of heterozygotes on cluster 2, but not in cluster 1.

Table 3.8. Analysis of molecular variance (AMOVA) of *Phytophthora infestans* isolates arranged in two clusters result of Bayesian analysis.

Source of Variation	d.f.[a]	Sum of Squares	Percentage of Variation	P-value[b]	Fixation Indices
Among clusters	1	71.369	23%	0.001	0.255
Among isolates within clusters	130	171.931	0%	1.000	-0.154
Within isolates	132	238.000	77%	0.002	0.141

[a] Degrees of freedom.

[b] Statistically significant at P<0.05.

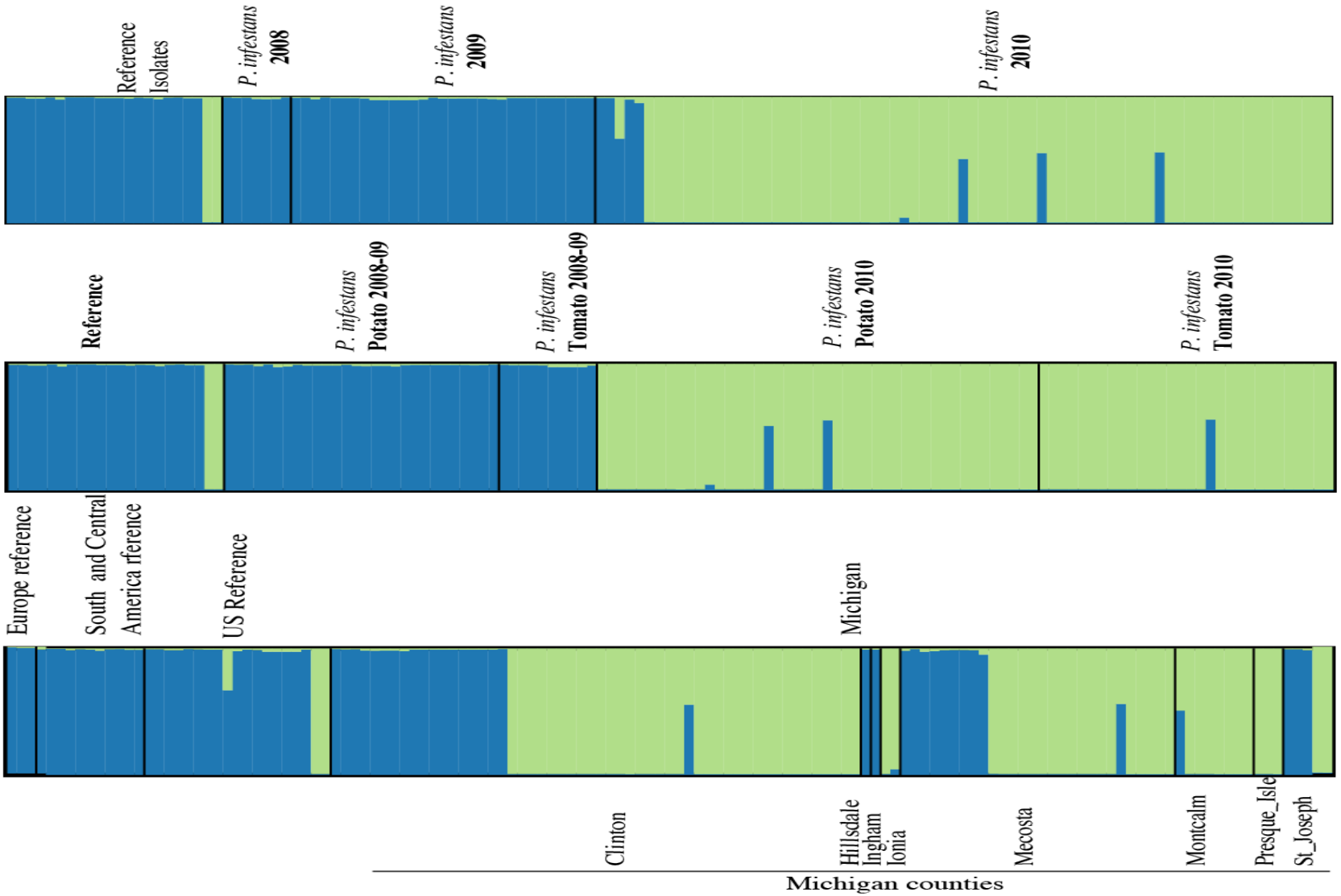

Figure 3.10. Clustering of isolates by Structure 2.3.3 into two underlying groups. For each year *P. infestans* isolates are ordered by their probability of membership and grouped (A) by sampling period, (B) sampling period-host combination and (C) by location sampled. The height of each color represents the probability of membership.

Location and sampling period defined the population structure among the isolates. By period, there was a drastic change from 2008-09 to 2010, where the late blight epidemics occurred; 2010 was mostly defined by just one cluster (Cluster 2, green color) of isolates not found in the other periods (Fig. 3.10A). The late blight epidemic was caused by the genotype US-22, which was the genotype represented by the green cluster. The structure observed by year remains true when data was sorted by host-year combination, where a drastic change occurred from 2009 to 2010 (Fig. 3.10B). The grouping by location indicated that individuals from both clusters were present in more than one location, but Presque Isle, Montcalm and Mecosta counties were mostly represented by US-22 clonal lineage (Fig. 3.10C). Additional analysis using pairwise differentiation among isolates grouped by host-sampling period proved that isolates were moderately differentiated between sampling periods, but potato and tomato isolates from 2008-09 were negligibly differentiated, and it was likewise for tomato and potato isolates on 2010 (Table 3.10).

Table 3.9. Genetic features of the two clusters of *P. infestans* isolates revealed by Structure analysis.

Statistic	Cluster 1	Cluster 2	Total Population
	(N=48)	(N=72)	(N=120)
Number of genotypes	12	2	13
Effective genotype number	3.927	1.058	2.188
Nei's corrected index [a]	0.767	0.056	0.548
Evenness	0.327	0.529	0.168
Shannon corrected index	0.969	0.062	0.648
Ho [b]	0.313	0.696	0.505
Hs [c]	0.477	0.364	0.427
Fis [d] **per locus**			
G11	-0.127	-0.919	-0.579
Pi02	-0.014	-1.000	-0.973
Pi4B	0.765	-0.947	-0.234
PiD13	1.000	ND	1.000*
Pi70	-0.277	-0.894	-0.708
Pi33	-0.222	-0.919	-0.672
Pi26	0.947	1.000	0.953*
Fis multilocus	0.296	-0.613	-0.386

[a] Nei's genetic diversity corrected for sample size (1987).

[b] Ho: observed heterozygosity

[c] Hs: Heterozygosity within populations corrected for sample bias.

[d] Fis: Inbreeding coefficient.

Table 3.10. Pairwise comparison of grouped of *Phytophthora infestans* isolates grouped by sampling period– host combination using fixation index.

Sampling period-Host					
Fixation index (Fst)	**Reference**	**Potato 2008-09**	**Tomato 2008-09**	**Potato 2010**	**Tomato 2010**
Reference	--				
Potato 2008-09	0.062*	--			
Tomato 2008-09	0.162*	0.077	--		
Potato 2010	0.278*	0.241*	0.412*	--	
Tomato 2010	0.25*	0.204*	0.331*	0.008*	--

* Significant at P<0.05

Discussion

Potato late blight has been a continuous concern for growers over time, likewise for tomato crop. The pathogen has managed to cause epidemics from time to time, where changes in some of the traits are observed like fungicide resistance, more virulent strains or different mating type (Shattock, 1988; Spielman *et al.*, 1991). In the US, clonal lineage US-8 prevail as the dominant lineage for last 10 years, characterized as mating type A2, resistant to mefenoxam and virulent on potato (Goodwin *et al.*, 1998a; Platt *et al.*, 1999; Kirk *et al.*, 2001b); despite of that other lineages have been also described, but their impact was moderate. The epidemics occurred on 2009 and 2010 were important because of the introduction of new clonal lineages that expanded quickly over the Eastern Coast of US (Hu *et al.*, 2010), the genotypes that caused this epidemic were US-22 (mating type A2), US-23 (mating type A1) and US-24 (mating type A1), all of them affecting potato and tomato crops.

The population structure analysis of *P. infestans* isolates from Michigan has changed between 2008 and 2010. Young *et al.* (2004) demonstrated that US-8 clonal lineage was the dominant genotype on the region, and it has been since was initially reported on 1995 (Lacy and Hammerschmidt, 1995). This clonal lineage was characterized as mefenoxam resistant and the most aggressive on foliage and tuber, causing devastating effects. Our study demonstrated that several traits of this pathogen have been changing since 2008. The initial assessment of the isolates obtained showed that the mating type dominant on the region is A2, and mitochondrial haplotype is Ia, which do not indicate any disturbance and agrees with the traditional findings in US (Goodwin *et al.*, 1998a; Fry, 2008). Rather, *Gpi* profiles suggested a reduced genotype from what is expected for US-8 clonal lineage, which has a 100/111/122 *Gpi* genotype. The observed genotype was 100/122 that has been also associated to US-8 isolates, which could be a result of a

asexual variation (Abu-El Samen *et al.*, 2003). However, the frequency of the isolates with the genotype 100/122 suggests a change in the population, and this was later confirmed.

Mefenoxam response of *P. infestans* isolates varied among sampling period and it is not a result of use of mefenoxam on the field, since this fungicide has been widely recommended on potato application programs in Michigan. During 2008 and 2009, *P. infestans* isolates tended to have an intermediate response to mefenoxam, but during 2010 the pattern change to more sensitive isolates. The sensitivity to mefenoxam was previously observed on recent clonal lineages (US-20 and US-21) described that affected potato and tomato crops on Florida (Schultz *et al.*, 2010). Similarly, US-22 was reported as sensitive to mefenoxam, which suggested that isolates in Michigan shifted their responses to this phenylamide fungicide displacing the clonal lineage US-8. Nonetheless, the response among isolates from 2010 varied in their response from sensitive to intermediate, similar to reports elsewhere (Wangsomboondee *et al.*, 2002; Schultz *et al.*, 2010)There were not differences across locations, but by host tomato isolates tended to be more sensitive. Mefenoxam is usually not recommended for tomato application programs due to widespread resistance in US (Goodwin *et al.*, 1996), also the outbreak was related to tomato plants for home gardens, which usually do not receive any application and served as source of inoculum.

On the other hand virulence against R-gene differentials and tuber seem to be quite diverse, despite of the fact genetically the population seem to be pretty clonal. Although, the number of differentials defeated by different isolates seems to be close, isolates originated from potato had broader range than isolates from tomato. The virulence variation has been normally observed in different studies (Knapova and Gisi, 2002; Abu-El Samen *et al.*, 2003; Guo *et al.*, 2009), but *P. infestans* isolates virulence variation could be originated by asexual variation

among isolates, which has been documented as chromosome loss or aneuploidy or other mechanisms of genetic variation apart from sexual reproduction (Lee *et al.*, 2001; Catal *et al.*, 2010). Indeed, Haas *et al.* (2009) described *Phytophthora infestans* genome had a particular organization, where conserved genes across other *Phytophthora* spp. where separated by sparse repeat-rich regions that contains genes related to virulence, which could facilitate recombination and virulence diversity. The second reason is the introduction of a new clonal lineage, which is supported by microsatellite data that establish a change in the population and genetic structure in Michigan. During the sampling period of 2008-09, US-22 started to show in the field, but it was just until 2010 that the genotype was all over Michigan, since it was a recent introduction and it was alternating from tomato to potato, and the pathogen was not limited to a single cultivar or host. In fact, Guo *et al.* (Guo et al., 2009) described a population of *P. infestans* in China with reduce genotype diversity, but high virulence diversity; similar to what we found on Michigan *P. infestans* isolates, where most of the markers that we used in this study revealed a low genotypic diversity, but tuber blight and races revealed an increased variability. It is important to mention that there were isolates race 0, not able to infect any of the differentials, but the control cv. Craigs royal. This suggests that all R-gene differentials were able to recognize all *avr* genes on these isolates, but this is just for potato physiological races.

The degree of tuber virulence across isolates was broad, but those isolates related to the clonal lineage US-22 tend to have significant reduced virulence on tubers in comparison to the virulent control US-8. This is an important fact since the effects on potato crops could be less severe, as it has been documented for US-8 clonal lineage (Kirk *et al.*, 2001b; Kirk *et al.*, 2009; Kirk *et al.*, 2010). Nonetheless, it does not discard the fact that US-22 is able to infect tuber and

overwinter favoring the dominance of this new clonal lineage on the region. In this case, management will play an important role to contain the expansion and survival of US-22.

Microsatellite data did showed some moderate diversity among the *P. infestans* isolates during 2008 to 2010. However, clustering and PCA analysis showed that Michigan late blight populations were divided in two clusters. The structure was defined over time that means that isolates obtained from 2008-09 drastically changed from isolates obtained in 2010. It is related to the time line of the epidemics, during 2009 multiple outbreaks occurred and diversity was slightly higher. The year after the outbreaks occurred, the population changed and it was dominated by US-22, which was characterized to mefenoxam sensitive, pathogenic to potato and tomato, different degrees of virulence observed by race composition and tuber blight.

The observed genetic structure unveil by the microsatellite data is supported but some of the other genotypic and phenotypic traits, like the only existence of a mating type A2, which agrees with the clonal population. Therefore, variability could be only explained by mitotic recombination, heterokaryosis observed previously in *Phytophthora* (Goodwin, 1997). The genotype flow rather than the gene flow could explain the drastic change occurred from 2008-09 to 2010. Genotype flow would successfully explain the introduction of virulent genotypes among different fields, where pathogens like *P. infestans* that produces long-distance dispersal propagules could have an increased fitness, since the genotype is a package that has been selected (McDonald and Linde, 2002). This implies a higher risk in the field because the pathogen is more difficult to manage and drastic measures need to be taken to limit the spread of the new genotype to avoid what already happened with US-8 (Goodwin *et al.*, 1998a). Our hypothesis of the displacement of US-8 in Michigan by a new genotype is supported by different evidence that is presented here, the appearance of US-22 in Michigan impose a new landscape

for potato and tomato growers, emphasizing the importance of integrate management, forecasting and surveying. The last one requires special attention since microsatellite genotyping provides a fast and reliable tool to track *P. infestans* population changes, as it has been used in Europe (Hansen *et al.*, 2007; Cooke *et al.*, 2008; Gisi *et al.*, 2010). Continued genotyping of *P. infestans* will be fundamental to define the fitness and definitely establishment of US-22 or the appearance of new genotypes.

References

References

Abu-El Samen, F., Secor, G. & Gudmestad, N. (2003) Variability in virulence among asexual progenies of *Phytophthora infestans*. *Phytopathology,* **93:**293-304.

Brurberg, M. B., Elameen, A., Le, V. H.*, et al.* (2011b) Genetic analysis of *Phytophthora infestans* populations in the Nordic European countries reveals high genetic variability. *Fungal Biology,* **115:**335-342.

Catal, M., King, L., Tumbalam, P.*, et al.* (2010) Heterokaryotic nuclear conditions and a heterogeneous nuclear population are observed by flow cytometry in *Phytophthora infestans*. *Cytometry. Part A : the journal of the International Society for Analytical Cytology,* **77:**769-775.

Caten, C. & Jinks, J. (1968) Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. *Can. J. Bot,* **46:**329-348.

Cooke, D., Lees, A., Grønbech Hansen, J.*, et al.* From a European to a global database of *Phytophthora infestans* genetic diversity: examining the nature and significance of population change. In., 2008, pp. 19-26.

Deahl, K. L., Goth, R. W., Young, R.*, et al.* (1991) Occurrence of the A2 mating type of *Phytophthora infestans* in potato fields in the United States and Canada. *Am. J. Potato Res.,* **68:**717-725.

Dieringer, D. & Schlötterer, C. (2003) Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Mol. Ecol. Notes,* **3:**167-169.

Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. *Mol. Ecol.,* **14:**2611-2620.

Flier, W. & Turkensteen, L. (1999) Foliar aggressiveness of *Phytophthora infestans* in three potato growing regions in the Netherlands. *Eur. J. Plant Pathol.,* **105:**381-388.

Forbes, G. Manual for laboratory work on *Phytophthora infestans*, 1997.

Fry, W. (2008) *Phytophthora infestans*: the plant (and R gene) destroyer. *Mol. Plant Pathol.,* **9:**385-402.

Fry, W. & Goodwin, S. (1997a) Re-emergence of potato and tomato late blight in the United States. *Plant Dis.,* **81:**1349-1357.

Fry, W. E. & Goodwin, S. B. (1997b) Resurgence of the Irish potato famine fungus. *Bioscience,* **47:**363-371.

Gilmour, J. (1973) Octal notation for designating physiologic races of plant pathogens. **242:**620-620.

Gisi, U., Walder, F., Resheat-Eini, Z.*, et al.* (2010) Changes of genotype, sensitivity and aggressiveness in *Phytophthora infestans* isolates collected in European countries in 1997, 2006 and 2007. *J. Phytopathol.,* **159:**223-232.

Goodwin, S., Schneider, R. & Fry, W. (1995a) Use of cellulose-acetate electrophoresis for rapid identification of allozyme genotypes of *Phytophthora infestans*. *Plant Dis.,* **79:**1181-1185.

Goodwin, S., Smart, Sandrock, R.*, et al.* (1998a) Genetic change within populations of *Phytophthora infestans* in the United States and Canada during 1994 to 1996: Role of migration and recombination. *Phytopathology,* **88:**939-949.

Goodwin, S., Sujikowski, L. & Fry, W. (1995b) Rapid evolution of pathogenicity within clonal lineages of the potato late blight disease fungus. *Phytopathology,* **85:**669-676.

Goodwin, S., Sujkowski, L. & Fry, W. (1996) Widespread distribution and probable origin of resistance to metalaxyl in clonal genotypes of *Phytophthora infestans* in the United States and western Canada. *Phytopathology,* **86:**793-800.

Goodwin, S. B. (1997) The population genetics of *Phytophthora*. *Phytopathology,* **87:**462-473.

Goodwin, S. B., Drenth, A. & Fry, W. E. (1992b) Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. *Curr. Genet.,* **22:**107-115.

Griffith, G. & Shaw, D. (1998) Polymorphisms in *Phytophthora infestans*: Four mitochondrial haplotypes are detected after PCR amplification of DNA from pure cultures or from host lesions. *Appl. Environ. Microbiol.,* **64:**4007-4014.

Guenthner, J., Michael, K. & Nolte, P. (2001) The economic impact of potato late blight on US growers. *Pot. Res.,* **44:**121-125.

Guo, J., van der Lee, T., Qu, D.*, et al.* (2009) *Phytophthora infestans* isolates from Northern China show high virulence diversity but low genotypic diversity. *Plant Biol.,* **11:**57-67.

Haas, B. J., Kamoun, S., Zody, M. C.*, et al.* (2009) Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature,* **461:**393-398.

Hansen, J. G., Colon, L. T., Cooke, D. E. L.*, et al.* (2007) Eucablight – collating and analysing pathogenicity and resistance data on a European scale. *EPPO Bulletin,* **37:**383-390.

Hu, C., Perez, F., Donahoo, R.*, et al.* (2010) Genetic structure of *Phytophthora infestans* population in eastern North America, 2002-2009. *Phytopathology,* **100:**S52.

Jakobsson, M. & Rosenberg, N. A. (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics,* **23:**1801-1806.

Judelson, H. S., Spielman, L. J. & Shattock, R. C. (1995) Genetic mapping and non-Mendelian segregation of mating type loci in the oomycete, *Phytophthora infestans*. *Genetics,* **141:**503-512.

Kawchuk, L. M., Howard, R. J., Peters, R. D.*, et al.* (2011) First Report of *Phytophthora infestans* Genotype US-23 Causing Late Blight in Canada. *Plant Dis.,* **95:**873.

Kim, K. & Lee, Y. (2002) Genetic DNA marker for A2 mating type in *Phytophthora infestans*. *J. Microbiol,* **40:**254-259.

Kirk, W., Abu-El Samen, F., Tumbalam, P.*, et al.* (2009) Impact of different US genotypes of *Phytophthora infestans* on potato seed tuber rot and plant emergence in a range of cultivars and advanced breeding lines. *Pot. Res.,* **52:**121-140.

Kirk, W., Felcher, K., Douches, D.*, et al.* (2001b) Susceptibility of potato (*Solanum tuberosum* L.) foliage and tubers to the US-8 genotype of *Phytophthora infestans*. *Am. J. Pot Res,* **78:**319-322.

Kirk, W., Niemira, B. & Stein, J. (2001c) Influence of storage temperature on rate of potato tuber tissue infection caused by *Phytophthora infestans* (Mont.) de Bary estimated by digital image analysis. *Pot. Res.,* **44:**87-96.

Kirk, W. & Szendrei, Z. (2011) Potato diseases survey report. *Michigan Potato Newsline,* **22:**4-6.

Kirk, W. W., Rojas, A., Tumbalam, P. G.*, et al.* (2010) Effect of different genotypes of *Phytophthora infestans* (Mont. de Bary) and temperature on tuber disease development. *Am. J. Pot Res,* **87:**509-520.

Knapova, G. & Gisi, U. (2002) Phenotypic and genotypic structure of *Phytophthora infestans* populations on potato and tomato in France and Switzerland. *Plant Pathol.,* **51:**641-653.

Lacy, M. L. & Hammerschmidt, R. (1995) Diseases of Potato: Late Blight. *Michigan State University Extension,* **Extension Bulletin E-1802**.

Langella, O. (1999). Populations 1.2.30: population genetic software (individuals or populations distances, phylogenetic trees). from http://bioinformatics.org/~tryphon/populations/

Lee, T., Testa, A., Klooster, J.*, et al.* (2001) Chromosomal Deletion in Isolates of *Phytophthora infestans* Correlates with Virulence on R3, R10, and R11 Potato Lines. *Mol. Plant-Microbe Interact.,* **14:**1444-1452.

Lees, A., Wattier, R., Shaw, D.*, et al.* (2006) Novel microsatellite markers for the analysis of *Phytophthora infestans* populations. *Plant Pathol.,* **55:**311-319.

Malcolmson, J. & Black, W. (1966) New R genes in *Solanum demissum* lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. *Euphytica,* **15:**199-203.

McDonald, B. A. & Linde, C. (2002) Pathogen population genetics, evolutionary potential, and durable resistance. *Annu. Rev. Phytopathol.,* **40:**349-379.

Meirmans, P. G. & Van Tienderen, P. H. (2004) Genotype and Genodive: two programs for the analysis of genetic diversity of asexual organisms. *Mol. Ecol. Notes,* **4:**792-794.

Montarry, J., Andrivon, D., Glais, I.*, et al.* (2010a) Microsatellite markers reveal two admixed genetic groups and an ongoing displacement within the French population of the invasive plant pathogen *Phytophthora infestans*. *Mol. Ecol.,* **19:**1965-1977.

Nei, M. Molecular Evolutionary Genetics, Columbia University Press, 1987.

Niemira, B., Kirk, W. & Stein, J. (1999) Screening for late blight susceptibility in potato tubers by digital analysis of cut tuber surfaces. *Plant Dis.,* **83:**469-473.

Peakall, R. & Smouse, P. E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes,* **6:**288-295.

Platt, H., Peters, R., Medina, M.*, et al.* (1999) Impact of seed potatoes infected with *Phytophthora infestans* (US-1 or US-8 genotypes) on crop growth and disease risk. *Am. J. Potato Res.,* **76:**67-73.

Pritchard, J. K., Stephens, M. & Donnelly, P. (2000) Inference of Population Structure Using Multilocus Genotype Data. *Genetics,* **155:**945-959.

Rambaut, A. (2010). FigTree v1. 3.1. Institute of Evolutionary Biology, University of Edinburgh. from http://tree.bio.ed.ac.uk/software/figtree/

Ristaino, J. (2010) The 2009 potato and tomato late blight epidemics: Genealogical history, multiple sources and migration events. *Phytopathology,* **100:**S161.

Rogers, J. S. (1972) Measures of genetic similarity and genetic distance. *Univ. Texas Publ,* **7213:**145-153.

Rosenberg, N. A. (2004) Distruct: a program for the graphical display of population structure. *Mol. Ecol. Notes,* **4:**137-138.

Schultz, D., Donahoo, R., Perez, F.*, et al.* (2010) A survey of tomato and potato fields in florida reveals unique genotypes of *Phytophthora infestans* between 2005 and 2007. *Hortscience*.

Shattock, R. (1988) Studies on the inheritance of resistance to metalaxyl in *Phytophthora infestans*. *Plant Pathol.,* **37:**4-11.

Smart, C. & Fry, W. (2001) Invasions by the late blight pathogen: renewed sex and enhanced fitness. *Biol. Invasions,* **3:**235-243.

Spielman, L., Drenth, A., Davidse, L.*, et al.* (1991) A second world-wide migration and population displacement of *Phytophthora infestans*? *Plant Pathol.,* **40:**422-430.

Therrien, C., Tooley, P., Spielman, L.*, et al.* (1993) Nuclear DNA content, allozyme phenotypes and metalaxyl sensitivity of *Phytophthora infestans* from Japan. *Mycol. Res.,* **97:**945-950.

Wangsomboondee, T., Trout-Groves, C., Shoemaker, P.*, et al.* (2002) *Phytophthora infestans* Populations from Tomato and Potato in North Carolina Differ in Genetic Diversity and Structure. *Phytopathology,* **92:**1189-1195.

Young, G. K., Cooke, L. R., Kirk, W. W.*, et al.* (2004) Competitive selection of *Phytophthora infestans* in the US and Northern Ireland. *Proceedings of the eighth workshop of an European network for development of an integrated control strategy of potato late blight,* **European Journal of Plant Pathology Special Report No. 10:**271 - 275.

Young, G. K., Cooke, L. R., Kirk, W. W.*, et al.* (2009) Influence of competition and host plant resistance on selection in *Phytophthora infestans* populations in Michigan, USA and in Northern Ireland. *Plant Pathol.,* **58:**703-714.

Supplemental table 3.1. *Phytophthora infestans* isolates analyzed in this study.

Sampling period	Sampling location	Isolate	Host	Mating Type	Mt Hap[a]	*Gpi*[b]	MS EC_{50}[c]	MS[d]	Race	Tuber Blight[e]
2008-09	Clinton	Pi09-0111	P	A2	Ia	100/111/122	3.60	I	2.3.4.5.7.10.11	11.31
2008-09	Clinton	Pi09-012	P	A2	Ia	100/122	8.00	I	10.11	27.49
2008-09	Clinton	Pi09-013	P	A2	Ia	100/100/111	15.20	I	2.3	10.25
2008-09	Clinton	Pi09-014	P	A2	Ia	100/122	60.30	I	3.4.5.6.7.10.11	14.94
2008-09	Clinton	Pi09-015	P	A2	Ia	100/122	3.46	I	2.3.4.5.6.7.8.10.11	15.36
2008-09	Clinton	Pi09-016	P	A2	Ia	100/100/111	13.40	I	3.6.7.8	12.63
2008-09	Clinton	Pi09-017	P	A2	Ia	100/122	5.37	I	2.3.5.6.10.11	19.09
2008-09	Clinton	Pi09-018	P	A2	Ia	100/122	1.55	S	2.4.5.6.7.8.9.10	9.18
2008-09	Clinton	Pi09-019	P	A2	Ia	100/122	2.83	I	5.7.8	8.03
2008-09	Clinton	Pi09-0110	P	A2	Ia	100/122	4.70	I	4.10	15.75
2008-09	Clinton	Pi09-0123	P	A2	Ia	100/122	8.16	I	0	10.85
2008-09	Clinton	Pi09-	P	A2	Ia	100/	0.40	S	1.4.5.7.9.10.	13.32

Supplemental Table 3.1 (cont'd)

		0112				122				
2008-09	Clinton	Pi09-0113	P	A2	Ia	100/122	52.48	I	8	21.38
2008-09	Clinton	Pi09-0114	P	A2	Ia	100/122	20.50	I	2.5.6.9	27.38
2008-09	Clinton	Pi09-0115	P	A2	Ia	100/122	10.14	R	5.6.7.8	7.46
2008-09	Clinton	Pi09-0116	P	A2	Ia	100/122	5.20	I	7.8.10	4.68
2008-09	Clinton	Pi09-0117	P	A2	Ia	100/111/122	3.22	I	-	18.45
2008-09	Mecosta	Pi09-0118	P	A2	Ia	100/122	3.60	I	1.3.6.10.11	14.70
2008-09	Mecosta	Pi09-0119	P	A2	Ia	100/122	0.32	S	1.3.6.10.11	5.99
2008-09	Mecosta	Pi09-0120	P	A2	Ia	100/122	19.75	I	1.4.5	19.34
2008-09	Mecosta	Pi09-0121	P	A2	Ia	100/122	1.65	I	5.8.11	24.87
2008-09	Mecosta	Pi09-0122	P	A2	Ia	100/122	0.09	S	-	11.75
2008-09	Mecosta	Pi09-011	P	A2	Ia	100/122	1.99	I	1.8.10	13.24
2008-09	Mecosta	Pi09-0124	P	A2	Ia	100/122	0.09	S	-	8.77
2008-09	Mecosta	Pi09-0125	P	A2	Ia	100/122	2.80	S	5	7.04
2008-09	St Joseph	Pi09-	P	A2	Ia	100/	2.36	I	1.3.5	10.59

Supplemental Table 3.1 (cont'd)

		0126				122				
2008-09	St Joseph	Pi09-0127	P	A2	Ia	100/122	2.60	I	4.5.6.7.9	11.26
2008-09	Hillsdale	Pi09-023	T	A2	Ia	100/122	0.30	S	5.7.10	4.24
2008-09	Ingham	Pi09-022	T	A2	Ia	100/122	2.70	I	4.5.11	6.80
2008-09	Ingham	Pi09-021	T	A2	Ia	100/122	24.50	I	1.3	9.00
2008-09	Mecosta	Pi09-024	T	A2	Ia	100/122	8.85	I	-	4.24
2008-09	Mecosta	Pi09-025	T	A2	Ia	100/122	0.30	S	4.5.10	8.66
2008-09	St Joseph	Pi09-021	T	A2	Ia	100/122	3.08	S	5.7.8	17.68
2010	Clinton	Pi10-0156	P	A2	Ia	100/122	4.88	I	3.5.7.8.9.10.11	16.13
2010	Clinton	Pi10-013	P	A2	Ia	100/122	0.15	S	2.3	8.57
2010	Clinton	Pi10-014	P	A2	Ia	100/122	0.17	S	2.9	8.26
2010	Clinton	Pi10-015	P	A2	Ia	100/122	5.55	I	-	7.93
2010	Clinton	Pi10-016	P	A2	Ia	100/122	11.20	I	-	9.40
2010	Clinton	Pi10-017	P	A2	Ia	100/122	5.25	I	1.3.5.6.7.9.10.11	
2010	Clinton	Pi10-018	P	A2	Ia	100/122	5.11	I	2.6.8.9.10	6.58

Supplemental Table 3.1 (cont'd)

2010	Clinton	Pi10-019	P	A2	Ia	100/122	16.78	I	8.10	6.73
2010	Clinton	Pi10-0110	P	A2	Ia	100/122	21.45	I	5.6.7.8	14.72
2010	Clinton	Pi10-0111	P	A2	Ia	100/122	14.86	I	-	7.87
2010	Clinton	Pi10-0112	P	A2	Ia	100/122	9.48	I	-	18.01
2010	Clinton	Pi10-0113	P	A2	Ia	100/122	0.32	S	2.9	9.99
2010	Clinton	Pi10-0114	P	A2	Ia	100/122	0.09	S	5.6.9.10	20.05
2010	Clinton	Pi10-0115	P	A2	Ia	100/122	8.78	I	10	6.09
2010	Clinton	Pi10-0116	P	A2	Ia	100/122	0.35	S	9.10	6.55
2010	Clinton	Pi10-0117	P	A2	Ia	100/122	33.41	I	1.2	7.52
2010	Clinton	Pi10-0118	P	A2	Ia	100/122	40.90	I	1.3.6.10.11	6.74
2010	Clinton	Pi10-0119	P	A2	Ia	100/122	12.74	I	4.5.6.10	3.83
2010	Clinton	Pi10-0120	P	A2	Ia	100/122	5.46	I	1.7.8.10	8.37
2010	Clinton	Pi10-0121	P	A2	Ia	100/122	0.39	S	2.4.5.6.8.9	9.47
2010	Clinton	Pi10-0122	P	A2	Ia	100/122	22.79	I	0	5.52
2010	Clinton	Pi10-	P	A2	Ia	100/	8.36	I	9	5.66

Supplemental Table 3.1 (cont'd)

		0123				122				
2010	Clinton	Pi10-0124	P	A2	Ia	100/122	2.16	I	-	-
2010	Clinton	Pi10-0125	P	A2	Ia	100/122	0.54	S	10	15.71
2010	Clinton	Pi10-0126	P	A2	Ia	100/122	13.59	I	5.8.11	7.50
2010	Clinton	Pi10-0127	P	A2	Ia	100/122	18.77	I	2	11.50
2010	Ionia	Pi10-0128	P	A2	Ia	100/122	17.05	I	4.8.10.11	5.00
2010	Ionia	Pi10-0129	P	A2	Ia	100/122	42.57	R	3.5.10	8.17
2010	Ionia	Pi10-0130	P	A2	Ia	100/122	0.84	S	6.10	-
2010	Mecosta	Pi10-0131	P	A2	Ia	100/122	0.70	S	4.10	3.19
2010	Mecosta	Pi10-0132	P	A2	Ia	100/122	0.09	S	5.6.7.8.10	-
2010	Mecosta	Pi10-0133	P	A2	Ia	100/122	0.27	S	-	24.35
2010	Montcalm	Pi10-0134	P	A2	Ia	100/122	91.00	R	3.8.10	29.10
2010	Montcalm	Pi10-0135	P	A2	Ia	100/122	10.11	I	2.10	6.86
2010	Montcalm	Pi10-0136	P	A2	Ia	100/122	0.41	S	1.3.6.10.11	-
2010	Montcalm	Pi10-0137	P	A2	Ia	100/122	0.31	S	5.8.11	10.19

Supplemental Table 3.1 (cont'd)

2010	Montcalm	Pi10-0138	P	A2	Ia	100/122	5.56	I	7.9.10	11.10
2010	Montcalm	Pi10-0139	P	A2	Ia	100/122	5.10	S	2.4.7.8	9.40
2010	Montcalm	Pi10-0140	P	A2	Ia	100/122	0.39	S	3.5.6.10	14.44
2010	Montcalm	Pi10-0141	P	A2	Ia	100/122	0.65	I	2.10	5.63
2010	Montcalm	Pi10-0142	P	A2	Ia	100/122	5.34	I	4.6.8.10	6.67
2010	Montcalm	Pi10-0143	P	A2	Ia	100/122	0.88	S	1.2.8.9.10.11	16.57
2010	Montcalm	Pi10-0144	P	A2	Ia	100/122	3.91	I	0	14.27
2010	Montcalm	Pi10-0145	P	A2	Ia	100/122	1.85	I	4	-
2010	Montcalm	Pi10-0146	P	A2	Ia	100/122	0.58	S	-	7.35
2010	Montcalm	Pi10-0147	P	A2	Ia	100/122	4.15	I	3.7.9.10	-
2010	Montcalm	Pi10-0148	P	A2	Ia	100/122	7.88	I	7	7.23
2010	Montcalm	Pi10-0149	P	A2	Ia	100/122	9.60	I	1.4.5	-
2010	Montcalm	Pi10-0150	P	A2	Ia	100/122	3.38	I	3.5.6.10	5.42
2010	Presque Isle	Pi10-0151	P	A2	Ia	100/122	0.16	S	10	12.23
2010	Presque	Pi10-	P	A2	Ia	100/	0.09	S	0	-

Supplemental Table 3.1 (cont'd)

	Isle	0152				122				
2010	Presque Isle	Pi10-0153	P	A2	Ia	100/122	6.36	I	6.8.10	6.84
2010	Presque Isle	Pi10-0154	P	A2	Ia	100/122	13.24	I	1.7.10	5.73
2010	Presque Isle	Pi10-0155	P	A2	Ia	100/122	4.54	S	1.7.10	4.20
2010	St Joseph	Pi10-012	P	A2	Ia	100/122	53.01	R	1.8.9.10	14.97
2010	Clinton	Pi10-021	T	A2	Ia	100/122	14.79	I	1.4.5.6.8.10	18.51
2010	Clinton	Pi10-022	T	A2	Ia	100/122	17.42	I	7.8	8.71
2010	Clinton	Pi10-0219	T	A2	Ia	100/122	0.99	S	6.8.10	5.10
2010	Clinton	Pi10-024	T	A2	Ia	100/122	0.09	S	1.4.5.6.8	6.65
2010	Clinton	Pi10-025	T	A2	Ia	100/122	16.34	I	5.6.8.10	11.01
2010	Clinton	Pi10-026	T	A2	Ia	100/122	0.45	S	2.10	12.08
2010	Mecosta	Pi10-027	T	A2	Ia	100/122	14.96	I	3.4	7.54
2010	Mecosta	Pi10-028	T	A2	Ia	100/122	1.08	S	5.10	-
2010	Mecosta	Pi10-029	T	A2	Ia	100/122	1.65	S	6.10	9.61
2010	Mecosta	Pi10-0210	T	A2	Ia	100/122	0.47	S	1.5.10	19.29

Supplemental Table 3.1 (cont'd)

2010	Mecosta	Pi10-0211	T	A2	Ia	100/122	1.26	S	1.2.6.8.10	20.64
2010	Mecosta	Pi10-0212	T	A2	Ia	100/122	1.18	I	1.2.3.5.8.10.11	6.95
2010	Mecosta	Pi10-0213	T	A2	Ia	100/122	1.50	S	1.5.10	4.32
2010	Mecosta	Pi10-0214	T	A2	Ia	100/122	0.16	I	-	14.31
2010	Mecosta	Pi10-0215	T	A2	Ia	100/122	40.81	I	1.8.9	6.24
2010	Mecosta	Pi10-0216	T	A2	Ia	100/122	1.12	S	2.3.5.9.10	16.79
2010	Mecosta	Pi10-0217	T	A2	Ia	100/122	0.83	S	3.5.6.10	7.05
2010	Mecosta	Pi10-0218	T	A2	Ia	100/122	2.88	S	6.10	6.71
2010	Mecosta	Pi10-023	T	A2	Ia	100/122	10.97	I	1.2.8.10	9.60
2010	Mecosta	Pi10-0220	T	A2	Ia	100/122	4.12	I	-	7.37
2010	Mecosta	Pi10-0221	T	A2	Ia	100/122	0.22	S	0	6.05
2010	Mecosta	Pi10-0222	T	A2	Ia	100/122	65.82	R	2.10	6.59
2010	Mecosta	Pi10-0223	T	A2	Ia	100/122	2.30	I	1.4.7.10	8.94
2010	Mecosta	Pi10-0224	T	A2	Ia	100/122	0.09	S	1.4.5	22.01
2010	Mecosta	Pi10-	T	A2	Ia	100/	3.78	S	-	16.61

Supplemental Table 3.1 (cont'd)

		0225				122				
2010	Mecosta	Pi10-0226	T	A2	Ia	100/122	0.66	S	4.6.8.10	15.31
2010	Mecosta	Pi10-0227	T	A2	Ia	100/122	0.54	S	6.10	17.89
2010	Mecosta	Pi10-0228	T	A2	Ia	100/122	0.90	S	6	5.71
2010	Mecosta	Pi10-0229	T	A2	Ia	100/122	0.23	S	1.5.10	27.43
2010	Presque Isle	Pi10-0230	T	A2	Ia	100/122	3.12	I	3.5.10	7.08
2010	St Joseph	Pi10-0231	T	A2	Ia	100/100	39.30	I	5.10	-
2010	St Joseph	Pi10-0232	T	A2	Ia	100/122	0.12	S	3.7.8.9.10	-

[a] Mitochondrial haplotype.

[b] *Glucose-6-phosphate isomerase* profile.

[c] Mefenoxam effective concentration 50% (EC_{50})

[d] Mefenoxam response based on Therrien *et al.* (1993); Sensitive (S), intermediate (I), and resistant (R).

[e] Tuber blight as mean Relative Area Reflective Intesity [Mean RARI(%)].

Chapter IV: Differences in tuber blight development for different genotypes of *Phytophthora infestans*

Abstract

Potato late blight, cause by the oomycete *Phytophthora infestans* is one of the most devastating diseases in this crop causing significant loses. New migrations or introduction of new genotypes to a specific region impose a different panorama for potato growers and breeders. During 2009 and 2010, North Eastern US suffered a late blight epidemic that quickly spread through several states. The epidemic was characterized by the appearance on a new clonal lineage designated as US-22, which was isolated from tomato and potato. Since tubers are an essential component of epidemics, responses of different cultivars were challenged against different genotypes of *P. infestans*, including different isolates of the clonal lineage US-22. Tuber blight development was characterized in terms of tissue darkening as [mean Relative Average Reflectance Intensity (RARI %)] and lenticel infection over time expressed as area under the disease progress curve (AUDPC). Tuber blight measured as RARI (%) showed that US-8 was more aggressive than the recent introduced US-22 in general, but US-22 isolates obtained from potato were more virulent than isolates obtained from tomato. Foreign clonal lineages like Blue-13 had similar tuber blight values to the aggressive US-8. The periderm responses to infection tended to be low, but still US-8 was more likely to infect than US-22. The cultivar Jacqueline Lee showed a consistent tuber blight resistance. Although, US-22 was not highly virulent, it can still infect tuber and it should be considered in breeding programs. This screening provided useful information of a new genotype in the region for breeders and growers of the risk that implies the appearance of clonal lineage US-22.

Introduction

The oomycete *Phytophthora infestans* (Mont.) de Bary is the causal agent of potato late blight, which is the most devastating disease of potato worldwide. Since the disease occurred in the 1840s (de Bary, 1876), outbreaks occurred intermittently with different degrees of impact. However, since the re-emergence of late blight in the 1980s (Fry and Goodwin, 1997a), new and more aggressive genotypes were observed impacting potato and tomato crops. The disease has not lost importance over time. Indeed, Vleeshouwers *et al.* (2010) documented searching trends that peaked during late blight epidemics on US and UK that caused a big impact. For instance, the clonal lineage US-8 has been described as one the most virulent genotypes yet, due to their aggressiveness on foliage and tuber blight (Goodwin *et al.*, 1996; Kirk *et al.*, 2001b).

The introduction of the clonal lineage US-8 meant the appearance of the mating type A2 in the US and resistance to mefenoxam; this genotype advanced and dominated fairly quickly on the potato fields (Goodwin *et al.*, 1998a). It also meant increased severity of tuber blight, where tuber rot symptoms appeared faster than isolates observed previously. In fact, there was a relation between the appearance of US-8 and the displacement of US-1, with the increased severity of tuber blight (Lambert and Currier, 1997). Similar case was observed in Europe in the recent years, the genotype 13_A2, also known as Blue-13, spread from 2006 to 2008 and has become the dominant genotype in different European countries and since its appearance it has shown an increased virulence on foliage and tuber (Lees *et al.*, 2008; Cooke *et al.*, 2011). The introduction of new genotypes into a region implies several changes in epidemics and crop protection, but one key factor is the genetic material available to fight back against late blight.

Traditionally, breeding has been focused on foliage resistance with little effort on tuber blight, but the trend has changed over the time, due to the importance of tuber blight that results

in losses for rotting in storage and transmission from season-to-season (Johnson and Cummings, 2009; Kirk *et al.*, 2009; Kirk *et al.*, 2010). The impact of tuber late blight occurs at different levels: quality of fresh and processing tubers; tuber seed production either being source of new epidemics or reducing yield because of stem blight; and volunteers that can also serve as sources of inoculum (Bonde and Schultz, 1943; Kirk *et al.*, 2009). Latent infection on tubers, like volunteers or seed is also an important mechanism of long-term dispersion and introduction of new genotypes (Abad and Abad, 1997; Nyankanga *et al.*, 2010). The resistance of tuber 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. The tuber has different components involved in resistance: periderm, outer cortical cells, medulla, lenticels and eyes that are involved in this infection process affect resistance restraining the pathogen (Pathak and Clarke, 1987; Flier *et al.*, 2007; Nyankanga *et al.*, 2008). However, different cultivars would have different responses due to the variation of these resistance components, as well as the *P. infestans* genotype variation.

The late blight epidemics of 2009 – 2010 in the Eastern US were characterized by the appearance of new genotype, the clonal lineage US-22. The genotype US-22 was initially reported in Florida on 2007 (Ristaino, 2010), but it was found potato and tomato, using both hosts to spread through the Eastern coast. We previously demonstrated that this new genotype in Michigan is complex and it temporally displaced the US-8 genotype. The change of the genetic structure of *P. infestans* populations imposes the necessity to evaluate the genetic material available to determine variability and response against the new pathogen genotype. Therefore, the aim of this study compares the ability of the new clonal lineage US-22 and other *P. infestans* genotypes to cause rapid tuber breakdown at moderate storage temperatures.

Materials and Methods

Germplasm selection

Six different cultivars of potato were selected for evaluation. The tubers of this study were obtained from the Potato breeding program at Michigan State University and commercial potato fields in Michigan. The cultivars provided by MSU Potato breeding program have identified potato cultivars with different response against the US-8 genotype of *P. infestans* included in this study; the cultivars and its foliar and tuber ratings were Jacqueline Lee [Resistant (R), Susceptible (S); (Douches *et al.*, 2001)]; Missaukee [R,I; (Douches *et al.*, 2010)]. Other varieties used in this study were FL1879 (susceptible), Russet Burbank (moderately susceptible), Red Norland (susceptible) and Monticello (susceptible) (Douches *et al.*, 1997; Porter *et al.*, 2004). Potato tubers from cultivars were stored at 3^{o}C in the dark at 90% relative humidity until used. Tubers were warmed to 15^{o}C in incremental steps of 2^{o}C for 7 d 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 sample was further tested with the ELISA immunodiagnostic Alert Multi-well kit (Alert Multiwell Kit - *Phytophthora sp.* Neogen Corporation, Lansing, MI, USA); *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 then surface sterilized by soaking in 2% sodium hypochlorite (Clorox) solution for 30 min. Tubers were dried in a controlled environment with continuous airflow at 15^{o}C in dry air (30% relative humidity)

for 4 h prior to inoculation. After inoculation tubers were returned to 10^{o}C by decreasing temperature by 2^{o}C decrements over a 2 d for storage treatment.

Isolates and inoculum preparation

Characteristics of the twelve different of *P. infestans* isolates used in this study are summarized in Table 4.1. The selected Michigan isolates were from the collection of W. Kirk (Michigan State University), US-8 and US-22 reference isolates were provided by Dr. Bill Fry (Cornell), Colombian isolates were provided by Dr. Silvia Restrepo (LAMFU, Los Andes University) and UK isolates by Dr. David Cooke (SCRI). For lenticel infection, the isolates US-8M and Pi10-012 were grown to produce inoculum. The isolates were grown in rye B media for 14 days in the dark at 18^{o}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^{o}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 cheesecloth and sporangia concentration was measured with a hemacytometer and adjusted to about 1 x 10^{4} total sporangia·ml^{-1} (discharged and non-discharged). The sporangial suspensions were stored for 4 h at 4^{o}C to encourage zoospore release from the sporangia.

Table 4.1. Characteristics of *Phytophthora infestans* isolates used for tuber blight study.

Origin	Isolate	Host	Location[a]	Mating Type	Mt Hap[b]	*Gpi* [c]	Met[d]	Clonal Lineage
Michigan	Pi09-011	*Potato*	Mecosta	A2	Ia	100/122	I	US-22
Michigan	Pi09-021	*Tomato*	Ingham	A2	Ia	100/122	S	US-22
Michigan	Pi10-023	*Tomato*	Mecosta	A2	Ia	100/122	S	US-22
Michigan	Pi10-012	*Potato*	St Joseph	A2	Ia	100/122	I	US-22
US	US-14	*Potato*	US	A2	Ia	100/122	I	US-14
US	US-8	*Potato*	US	A2	Ia	100/111/122	R	US-8
Colombia	2568	*P. peruviana*	COL	A2	Ia	100/111/122	I	US-8
Colombia	1810	*Potato*	COL	A1	IIa	100/100	I	CO-2
UK	07-39	*Potato*	UK	A2	Ia	100/100	I	Blue-13
UK	3298A	*Potato*	UK	A2	Ia	100/100	I	Blue-13
US	US-8F	*Potato*	US	A2	Ia	100/111/122	R	US-8
US	US-22F	*Potato*	US	A2	Ia	100/122	S	US-22

[a] Michigan location names refer to counties.

[b] Mt Hap corresponds to mitochondrial haplotype.

[c] *Gpi*, Glucose-6-phosphate isomerase profile.

[d] Mefenoxam response: (S) sensitive, (I) intermediate, and (R) resistant.

Whole tuber inoculation with *P. infestans*

Tuber late blight development caused by the different *P. infestans* genotypes on the tuber cultivars were evaluated at 10^{o}C storage temperature using whole tuber sub–peridermal inoculation. The washed, surface-sterilized tubers were inoculated by a sub-peridermal injection of a sporangia suspension of 2×10^{-5} ml (delivering zoospores released from about 20 sporangia inoculation) with a hypodermic syringe and needle at the apical end of the tuber about 1 cm from the dominant sprout to a maximum depth of 1 cm. Ten tubers of each cultivar were inoculated with each *P. infestans* genotype and three replicates were done per treatment. Ten control tubers per cultivar/ABL were inoculated with cold (4^{o}C) sterile distilled H_2O. After inoculation, tubers were placed in the dark in sterilized covered plastic crates and returned to controlled environment chambers [Percival Incubator (Model I-36LLVL, Geneva Scientific, LLC, PO Box 408, Fontana, WI)]. The chamber was set at 10^{o}C and 95% humidity and the sample tubers were incubated for 30 days until evaluation. The tuber tissue inoculation experiments were repeated twice.

Lenticel susceptibility to *P. infestans* genotypes

Tuber for three different cultivars with different responses to *P. infestans* were obtained from MSU potato breeding program to evaluate periderm susceptibility. The three cultivars with different tuber blight ratings were Atlantic (susceptible), Jacqueline Lee (moderate resistant) and Stirling (resistant). Tubers were washed with water to remove soil. The tubers were then surface sterilized by soaking in 2% sodium hypochlorite solution for 30 min. Tubers were dried before inoculation. For each genotype and cultivar combination, 12 tubers were used and the

experiment was repeated three times. Tubers were dipped in about 1 x 10^4 total sporangia·ml^{-1} suspension for 48 h at room temperature. After inoculation time, tubers were placed in the dark in sterilized covered plastic crates with damp towels to maintain high humidity, and then the crates were place into controlled environment chambers [Percival Incubator (Model I-36LLVL, Geneva Scientific, LLC, PO Box 408, Fontana, WI)] at 10^{o}C. Three tubers were chosen arbitrarily and removed from each treatment at 3, 6, 10 and 15 d post inoculation for evaluation. At each time the number of lenticels infected were assessed under the dissecting scope and light microscope when it was needed.

Evaluation of tuber blight

A digital image analysis technique was used to assess tuber tissue infection. The method was previously used and standardized (Niemira *et al.*, 1999; Kirk *et al.*, 2001c). The image files were analyzed using SigmaScan V3.0 (Jandel Scientific, San Rafael, CA). The area selection cut-off threshold was set to 10 light intensity units, limiting the determination to the non–dark 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).

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, centrifuged at 12000 rpm for 5 min, and the 20 µL of supernatant diluted with 80 µL of Tris (pH 8·0). PCR was done 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 1min and 72˚C for 1 min, and final extension at 72˚C for 10 mins. PCR products were visualized in agarose gels (1%) for the detection of the 600 bp band, as 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/ABL. The relative ARI (RARI) was calculated as:

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

RARI (%) has minimum value of zero (no symptoms) and maximum value of hundred (completely dark tuber surface). Data for all experiments were analyzed by analysis of variance (least squares method) using the JMP program version 7.0 (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA). Treatment effects were determined by three-way factorial ANOVA, where the main effects corresponded to: Cultivar and *P. infestans* genotype and the two-factor interaction. Principal component analysis was done to explore variability among cultivars and *P. infestans* isolates.

For lenticel infection, results for *P. infestans* genotypes were reported as area under the disease progress curve values (AUDPC) as described by Shaner and Finney (1977). Two-way

ANOVA was done to evaluate differences among the genotypes of *P. infestans* and cultivars evaluated using the JMP program version 7.0 (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA).

Results

Whole tuber inoculation

The whole tuber inoculation using different genotypes of *Phytophthora infestans* showed significant differences of the two main factors (genotype and cultivar) and the two-way interaction (Table 4.2). Among the cultivars evaluated, Red Norland was the most susceptible, but it was not significant different from Russet Burbank and Monticello. Jacqueline Lee was the least susceptible of the six cultivars evaluated, but it still showed tuber blight. Among the different genotypes of *P. infestans* evaluated several responses were observed. The clonal lineage US-8 remained as the most aggressive genotype, a different isolate also classified as clonal lineage US-8 had lower mean RARI (Table 4.3). The second most aggressive isolate was an isolate designated as clonal lineage US-22, isolated from potato in 2009 from St. Joseph county. 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 genotypes Blue-13, the isolate Pi09-011 also obtained during the epidemics on 2009 from potato was fairly virulent.

The rest of the isolates used in this study caused low impact on the cultivars employed on the study. The isolates US-14 and US-22 had slightly low virulence. Ultimately, Michigan *P. infestans* isolates characterized as US-22 and isolated from tomato were significant different

from the virulence control US-8, and grouped with isolates from Colombia, as low virulent isolates on tuber tissue (Table 4.3).

Table 4.2. Two-way factorial ANOVA of the effect of genotype of *Phytophthora infestans* and cultivars on tuber tissue late blight as mean Relative Average Reflection Intensity [RARI(%)]. Variance ratio (F), and degrees of freedom (df).

Source of Variation	df	Sum of Squares	F Ratio	P-Value
Cultivar	5	8129.21	52.32	<0.0001*
Genotype	11	67168.73	196.49	<0.0001*
Cultivar X Genotype	55	20849.30	12.20	<0.0001*

*Significant at P<0.05

Table 4.3. Effect of different genotypes of *P. infestans* on tuber tissue late blight as mean Relative Average Reflection Intensity [RARI (%)] in different cultivars.

	Tuber tissue darkening caused by different genotypes of *P. infestans*			
Cultivar	**Mean RARI (%)**		**Isolate**	**Mean RARI (%)**
Red Norland	13.56	a[b]	US-8	21.93 a
Russet Burbank	13.18	a	Pi10-012	20.45 a
Monticello	12.82	a	3298A	16.79 b
Missaukee	10.75	b	US-8F	14.16 c
FL 1879	9.67	b	7-39	13.96 c
Jacqueline Lee	7.74	c	Pi09-011	13.04 c
LSD 0.05	2.85		US-14	8.62 d
			US-22F	5.87 e
			2568	5.69 e
			Pi09-023	5.29 e
			Pi09-021	5.22 e
			1810	5.10 e
			LSD 0.05	3.27

[a] Normalized tuber tissue darkening score expressed as RARI (%) = [1- Mean ARI treatment / Mean ARI control] *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). The numbers are derived from the mean average reflective intensity of three surfaces cut latitudinal at 25, 50 and 75% from the apex of n = 10 tubers per treatment combination.

[b] Values followed by the same letter are not significantly different at $p = 0.05$ for comparisons of mean RARI values within different *P. infestans* genotypes of cultivar/ABL combinations and temperature treatments (Based on Fishers protected LSD).

The two-way interaction visualized as principal component analysis showed that for cultivars axis 1 and axis 2 accounted for 56.9 and 14.6% variability respectively. With respect to the *P. infestans* isolates axis 1 and axis 2 accounted for 36.4 and 13.1% variability respectively (Fig. 4.1). Jacqueline lee was the less variable among the cultivars due to its reduced susceptibility to most of the genotypes evaluated. The other cultivars had similar behavior, where Red Norland and Russet Burbank were the least susceptible. In the other hand, *P. infestans* isolates were variable, but isolates assigned as clonal lineage US-22 had reduced variability, which means reduced tuber blight among the different cultivars evaluated (Fig 4.1). However, Pi09-011 and Pi10-012 that came from potato were more variable among the cultivars, implying a higher degree of virulence than US-22 isolated from tomato. Still, US-22 isolated from tomato were less virulent than clonal lineage US-8 and Blue-13. In general, US-8M and Blue-13 (07-39 and 3298) had the largest contribution to variability among isolates and cultivars. Cultivars were highly variable, but Jacqueline Lee introduced less variability, meaning lees tuber blight inflicted by the different genotypes evaluated (Fig. 4.1).

The evaluation of the different genotypes of *P. infestans* by cultivar in Table 4.4 shows a detailed result of the interaction of cultivar and isolate. With respect to the US-22 isolates obtained in Michigan, Pi10-012 is moderately virulent in most of the cultivars evaluated, but the isolates Pi09-21 and Pi10-023 isolated from tomato had consistent low virulence among cultivars, which agrees with the PCA analysis. Overall, the clonal lineage US-8 was the most aggressive and most US-22 isolates were less virulent.

Table 4.4. The effect of different genotypes of *P. infestans* on tuber tissue late blight as mean Relative Average Reflection Intensity [RARI (%)] in different cultivars of potatoes.

	Tuber tissue darkening caused by different isolates of *P. infestans* [Mean RARI (%)[a]]					
P. infestans isolates	FL 1879	Jacqueline Lee	Missaukee	Monticello	Red Norland	Russet Burbank
07-39	8.55 ***l-v***[b]	5.52 ***p-v***	13.39 ***i-o***	17.81 ***e-i***	21.63 ***c-f***	16.86 ***f-j***
1810	4.12 ***t-v***	4.89 ***q-v***	2.70 ***v***	6.59 ***p-v***	6.54 ***p-v***	5.77 ***p-v***
2568	3.19 ***uv***	4.13 ***t-v***	4.57 ***s-v***	5.73 ***q-v***	7.44 ***n-v***	9.08 ***l-u***
3298	12.46 ***i-p***	7.38 ***n-v***	11.38 ***j-q***	18.56 ***e-i***	21.55 ***c-f***	29.41 ***a***
Pi09-021	5.51 ***q-v***	4.68 ***r-v***	5.89 ***p-v***	5.92 ***p-v***	5.86 ***p-v***	3.46 ***uv***
Pi10-023	3.97 ***s-v***	4.86 ***r-v***	4.98 ***q-v***	5.44 ***q-v***	6.57 ***p-v***	5.92 ***p-v***
Pi10-012	28.96 ***ab***	14.13 ***h-m***	21.07 ***c-g***	23.57 ***a-e***	20.30 ***d-h***	14.64 ***h-l***
US-14	6.26 ***p-v***	6.95 ***o-v***	6.88 ***o-v***	10.09 ***k-t***	10.97 ***j-r***	10.54 ***k-s***
US-22F	3.30 ***uv***	8.15 ***m-v***	8.67 ***l-v***	3.73 ***u-v***	6.93 ***p-v***	4.44 ***t-v***
US-8F	5.78 ***p-v***	14.43 ***h-m***	13.59 ***i-n***	20.13 ***d-h***	15.14 ***g-k***	15.89 ***f-k***
US-8M	22.43 ***b-f***	10.83 ***j-s***	25.97 ***a-d***	20.82 ***d-g***	24.54 ***a-d***	27.02 ***a-c***
Pi09-011	15.19 ***g-k***	7.18 ***n-v***	10.14 ***k-t***	14.82 ***g-l***	15.21 ***g-k***	15.69 ***f-k***

[a] Normalized tuber tissue darkening score expressed as RARI (%) = [1- Mean ARI $_{treatment}$ / Mean ARI $_{control}$] *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). The numbers are derived from the mean average reflective intensity of three surfaces cut latitudinal at 25, 50 and 75% from the apex of n = 10 tubers per treatment combination.

[b] Values followed by the same letter are not significantly different at $p = 0.05$ for comparisons of mean RARI values within different *P. infestans* genotypes of cultivar/ABL combinations and temperature treatments (Based on Fishers protected LSD).

Table 4.5. Two-way factorial ANOVA of three different cultivars evaluated against the *Phytophthora infestans* genotypes US-8 and US-22 for lenticel infection as area under disease progress (AUDPC). Variance ratio (F), and degrees of freedom (df).

Source of Variation	df	Sum of Squares	F Ratio	P-Value
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

* Significant at P=0.05.

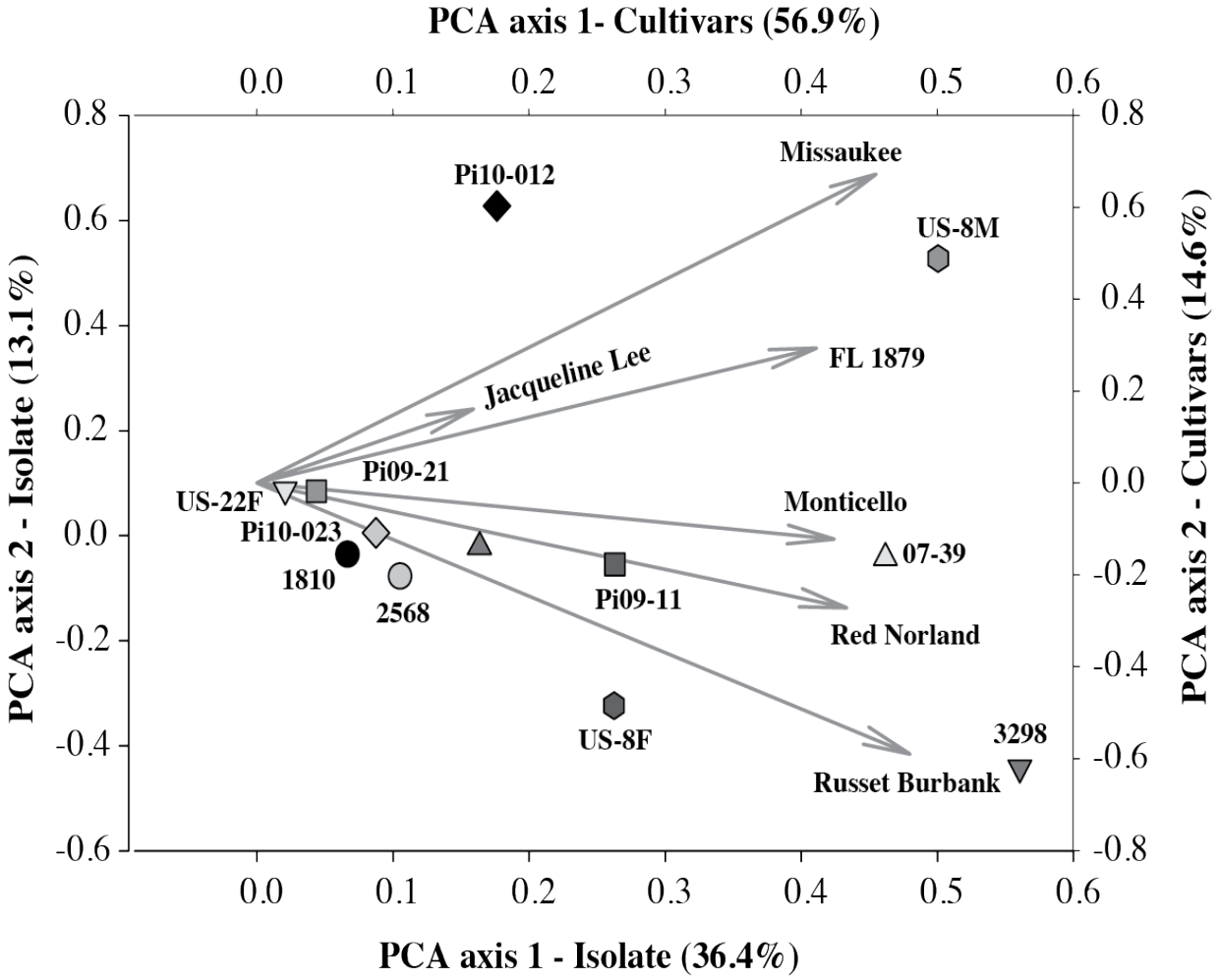

Figure 4.1. Principal component analysis (PCA) of cultivar and genotype of *P. infestans* evaluated for tuber blight as mean RARI (%). Cultivars are represented by lines; isolates by symbols.

Lenticel infection

The infection of periderm was evaluated in terms of lenticel infection using clonal lineages US-8 and US-22 to determine if the new introduced genotype in Michigan was likely to infect tubers through the periderm without wounding. In general moderate lenticel infection was observed, but infected tubers presented mycelia growth on the surface 10 dpi (Fig. 4.2). The ANOVA analysis of the main effects resulted in just significant differences among the clonal lineage 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 4.5). Mean values for AUDPC for clonal lineage US-22 were lower in all cultivars, but the lowest were cvs. Atlantic and Stirling. On the other hand US-8 was more aggressive in Atlantic and less virulent on Stirling (Fig. 4.3).

Figure 4.2. Periderm infection on lenticels and eyes on two different cultivars caused by *P. infestans* clonal lineages US-8 and US-22.

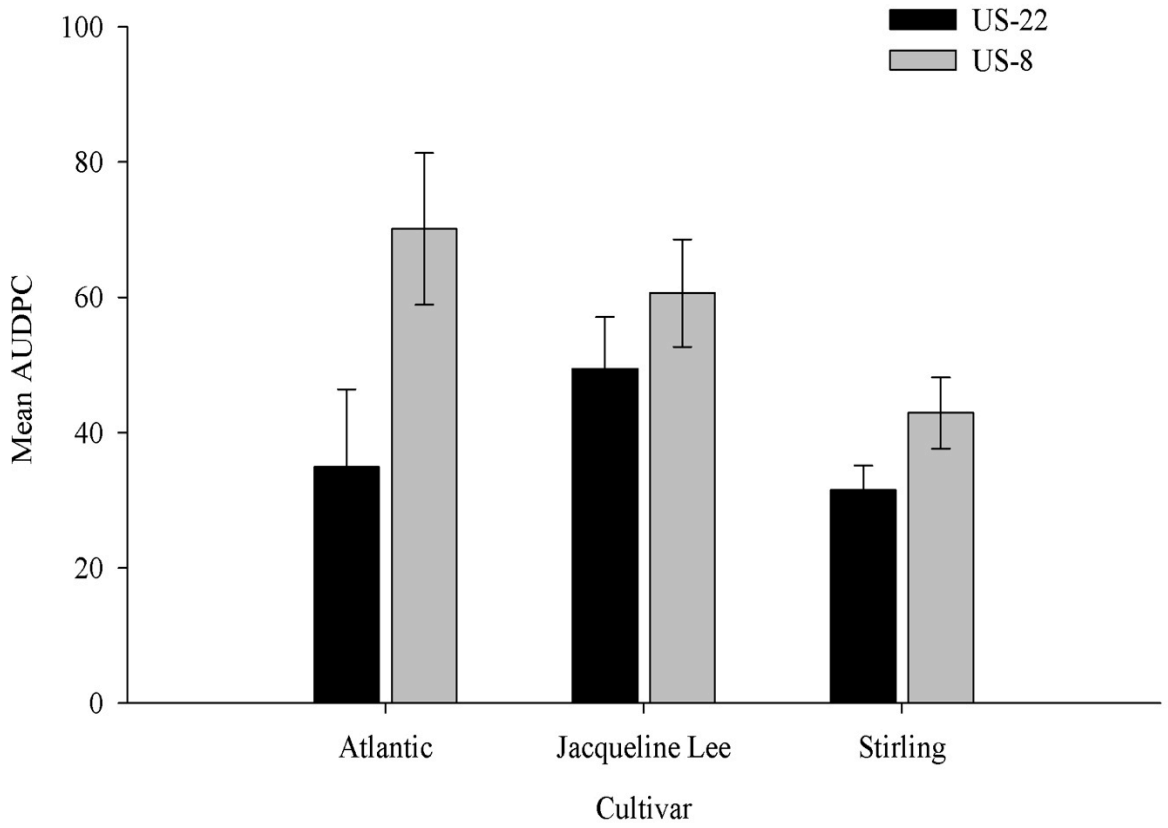

Figure 4.3. Mean area under disease progress curve for lenticel infection caused by *P. infestans* genotypes US-8 and US-22 on three different cultivars of potato. Significant differences were observed among genotypes with a $P<0.05$.

Discussion

The impact of tuber blight on the epidemics of this pathogen remarks the importance to characterize the interaction of different genotypes against different cultivars and the effect that new genotypes could have in the existing breeding material. Tuber blight importance has been previously identified as critical factor in storage and season-to-season transmission (Kirk *et al.*, 2009; Kirk *et al.*, 2010; Nyankanga *et al.*, 2010). Potato tubers have three define barriers against pathogens, which are periderm, cortical and medulla; these can vary among cultivars in response to different genotypes (Pathak and Clarke, 1987; Flier *et al.*, 2001). In our experiment we assessed the resistance of six different cultivars against twelve isolates, where the new identified clonal lineage US-22 was included and compared with other lineages already observed in the field. We focused in the resistance responses in medullar tissue and periderm, to determine what is the risk that implies the new clonal lineage US-22 for potato growers. Large differences were observed in relation to medullar tissue among the different isolates and cultivars.

The evaluation of tuber blight on medullar tissue revealed that US-8 is still the most virulent genotype in tuber in comparison to other genotypes. Colombian isolates were less virulent probably due to the lack of pathogenic fitness to infect tuber, this has been previously observed in other lineages found in South America (Oyarzún *et al.*, 2005). The UK isolates designated as genotype Blue-13 were highly aggressive similar to US-8, these isolates impacted potato crops in Europe during 2007 to 2008 (Gisi *et al.*, 2010; Cooke *et al.*, 2011). The variability observed among the US-22 isolates used could be due to different factors, mainly due to recent introduction of this genotype to the region. However, we observed a slight high virulence from isolates obtained from potato than those obtained from tomato. This could be explained by host specificity, which has been observed in some isolates of *P. infestans* (Cooke *et*

al., 2006). However, the degree of virulence on tuber could be an important factor to consider in terms of survival, it has been described that aggressive isolates have a penalty in survival due to fast decay that suffer the tissue or dead of sprouts before transmission to next season (Montarry *et al.*, 2007). Therefore, isolates like US-22 might survive better with reduced tuber blight, but it has been shown that transmission after overwintering tend to be low and varies according to the season.

On the other hand, periderm responses were similar among cultivars for the two *P. infestans* genotypes evaluated, but US-8 was more virulent and effective in terms of infection. The establishment of infection during the season is an important step on epidemics. Usually, infection of potato tubers by *P. infestans* may occurred when inoculum (sporangia, zoospores or mycelia) are washed from the foliage into the soil (Andrivon, 1995). Unwounded tubers would be only infected through natural openings like lenticels and eyes (Lacey, 1967) that early stages are more likely to be infected. Then tuber age would also be related to resistance on the periderm and its resistance to *P. infestans* (Walmsley-Woodward and Lewis, 1975). We observed that US-8 was more likely to infect through the periderm than US-22, but in general terms the AUDPC fro lenticel infection was low, which could be related to the age of the tubers. The inoculation method used assures that the lenticels were open to promote infection, this has been done previously using high humidity to promote infection (Montarry *et al.*, 2007). It has been observed than lenticel are moderately susceptible, but and intact skin is least susceptible to infection by *P. infestans* than wounds that are more likely to be infected (Darsow, 2004).

In general terms, our aim was evaluate the virulence of US-22 in different cultivars and we observed that tended have a reduced virulence. The reduced virulence disagrees with population changes observed the past years in US and Europe (Lambert and Currier, 1997;

Cooke *et al.*, 2011), which could mean that US-22 could be just a transitory outbreak. Nonetheless, the age of tubers and the absence of gene-for-gene interaction on the tuber could affect the development of the disease (Henderson and Friend, 1979; Pathak and Clarke, 1987). The consistent virulence of US-8 agrees with previous studies and it can be considered as reference isolate for breeding programs to determine tuber resistance. Although, US-22 should not be underestimated since it has recently emerged and it could be become a greater issue for potato growers in the future.

Late blight could be controlled by using different fungicides, but the input could be reduced using tolerant varieties (Kirk *et al.*, 2005) and understanding the mechanisms of the infection could improve the breeding for resistance and enforce better practices for management. The tuber blight caused by newly introduced *P. infestans* genotypes implied a change of breeding efforts to generate more tolerant varieties. However, US-22 do not represent a higher risk on tuber, it does not mean that should be underestimated for selection of resistant genetic material because of its recent introduction. The variability of susceptibility observed among the cultivars to the different isolates of US-22 would have implications for breeding programs.

References

References

Abad, Z. & Abad, J. (1997) Another look at the origin of late blight of potatoes, tomatoes, and pear melon in the Andes of South America. *Plant Dis.,* **81:**682-688.

Andrivon, D. (1995) Biology, ecology, and epidemiology of the potato late blight pathogen *Phytophthora infestans* in soil. *Phytopathology,* **85:**1053-1056.

Bonde, R. & Schultz, E. (1943) Potato cull piles as a source of late-blight infection. *Am. J. Potato Res.,* **20:**112-118.

Cooke, D., Lees, A., Oliva, R.*, et al.* (2006) Is *Phytophthora infestans* a good taxonomist? Host recognition and co-evolution in the *Phytophthora/Solanum* interaction. *VI International Solanaceae Conference: Genomics Meets Biodiversity 745***:**465-471.

Cooke, L., Schepers, H., Hermansen, A.*, et al.* (2011) Epidemiology and Integrated Control of Potato Late Blight in Europe. *Pot. Res.,* **54:**183-222.

Darsow, U. (2004) The use of four different assessment methods to establish relative potato tuber blight resistance for breeding. *Pot. Res.,* **47:**163-174.

de Bary, A. (1876) Researches Into the Nature of the Potato-fungus (*Phytophthora Infestans*). *Journal of the Royal Agricultural Society of England,* **12:**239-269.

Douches, D., Coombs, J., Felcher, K.*, et al.* (2010) Missaukee: A round white potato variety combining chip-processing with resistance to late blight, *Verticillium* wilt and Golden cyst nematode. *Am. J. Potato Res.,* **87:**10-18.

Douches, D., Jastrzebski, K., Coombs, J.*, et al.* (2001) Jacqueline Lee: A late-blight-resistant tablestock variety. *Am. J. Potato Res.,* **78:**413-419.

Douches, D., Kirk, W., Jastrzebski, K.*, et al.* (1997) Susceptibility of potato varieties and advanced breeding lines (*Solanum tuberosum* L.) to *Phytophthora infestans* (Mont.) de Bary in greenhouse screenings. *Am. J. Potato Res.,* **74:**75-86.

Flier, Kroon, L., Hermansen, A.*, et al.* (2007) Genetic structure and pathogenicity of populations of *Phytophthora infestans* from organic potato crops in France, Norway, Switzerland and the United Kingdom. *Plant Pathol.,* **56:**562-572.

Flier, W., Turkensteen, L., Van Den Bosch, G.*, et al.* (2001) Differential interaction of *Phytophthora infestans* on tubers of potato cultivars with different levels of blight resistance. *Plant Pathol.,* **50:**292-301.

Fry, W. & Goodwin, S. (1997a) Re-emergence of potato and tomato late blight in the United States. *Plant Dis.,* **81:**1349-1357.

Gisi, U., Walder, F., Resheat-Eini, Z.*, et al.* (2010) Changes of genotype, sensitivity and aggressiveness in *Phytophthora infestans* isolates collected in European countries in 1997, 2006 and 2007. *J. Phytopathol.,* **159:**223-232.

Goodwin, S., Smart, Sandrock, R.*, et al.* (1998a) Genetic change within populations of *Phytophthora infestans* in the United States and Canada during 1994 to 1996: Role of migration and recombination. *Phytopathology,* **88:**939-949.

Goodwin, S., Sujkowski, L. & Fry, W. (1996) Widespread distribution and probable origin of resistance to metalaxyl in clonal genotypes of *Phytophthora infestans* in the United States and western Canada. *Phytopathology,* **86:**793-800.

Henderson, S. & Friend, J. (1979) Increase in PAL and lignin-like compounds as race-specific resistance responses of potato tubers to *Phytophthora infestans*. *J. Phytopathol.,* **94:**323-334.

Johnson, D. A. & Cummings, T. F. (2009) Latent Infection of Potato Seed Tubers by *Phytophthora infestans* During Long-Term Cold Storage. *Plant Dis.,* **93:**940-946.

Kirk, W., Abu-El Samen, F., Muhinyuza, J.*, et al.* (2005) Evaluation of potato late blight management utilizing host plant resistance and reduced rates and frequencies of fungicide applications. *Crop Prot.,* **24:**961-970.

Kirk, W., Abu-El Samen, F., Tumbalam, P.*, et al.* (2009) Impact of different US genotypes of *Phytophthora infestans* on potato seed tuber rot and plant emergence in a range of cultivars and advanced breeding lines. *Pot. Res.,* **52:**121-140.

Kirk, W., Felcher, K., Douches, D.*, et al.* (2001b) Susceptibility of potato (*Solanum tuberosum* L.) foliage and tubers to the US-8 genotype of *Phytophthora infestans*. *Am. J. Pot Res,* **78:**319-322.

Kirk, W., Niemira, B. & Stein, J. (2001c) Influence of storage temperature on rate of potato tuber tissue infection caused by *Phytophthora infestans* (Mont.) de Bary estimated by digital image analysis. *Pot. Res.,* **44:**87-96.

Kirk, W. W., Rojas, A., Tumbalam, P. G.*, et al.* (2010) Effect of different genotypes of *Phytophthora infestans* (Mont. de Bary) and temperature on tuber disease development. *Am. J. Pot Res,* **87:**509-520.

Lacey, J. (1967) Susceptibility of potato tubers to infection by *Phytophthora infestans*. *Ann. Appl. Biol.,* **59:**257-264.

Lambert, D. & Currier, A. (1997) Differences in tuber rot development for North American clones of *Phytophthora infestans*. *Am. J. Potato Res.,* **74:**39-43.

Lees, A., Cooke, D., Stewart, J.*, et al. Phytophthora infestans* population changes: implications. In: HTAM, S. (ed), Proceedings of the eleventh EuroBlight workshop. PPO-special report 2008, pp. 55-60.

Montarry, J., Corbière, R. & Andrivon, D. (2007) Is there a trade-off between aggressiveness and overwinter survival in *Phytophthora infestans*? *Funct. Ecol.,* **21:**603-610.

Niemira, B., Kirk, W. & Stein, J. (1999) Screening for late blight susceptibility in potato tubers by digital analysis of cut tuber surfaces. *Plant Dis.,* **83:**469-473.

Nyankanga, R., Olanya, O., Wien, H.*, et al.* (2008) Development of tuber blight (*Phytophthora infestans*) on potato cultivars based on *In vitro* assays and field evaluations. *Hortscience,* **43:**1501-1508.

Nyankanga, R. O., Olanya, O. M., Ojiambo, P. S.*, et al.* (2010) Validation of tuber blight (*Phytophthora infestans*) prediction model. *Crop Prot.***:**1-7.

Oyarzún, P., Garzón, C., Leon, D.*, et al.* (2005) Incidence of potato tuber blight in Ecuador. *Amer J of Potato Res* **82:**117-122.

Pathak, N. & Clarke, D. D. (1987) Studies on the resistance of the outer cortical tissues of the tubers of some potato cultivars to *Phytophthora infestans*. *Physiol. Mol. Plant Pathol.,* **31:**123-132.

Porter, G., Lambert, D., Bushway, A.*, et al.* (2004) Monticello: A mid-season variety with good tuber appearance and chipping potential from mid-to long-term storage. *Am. J. Potato Res.,* **81:**143-152.

Ristaino, J. (2010) The 2009 potato and tomato late blight epidemics: Genealogical history, multiple sources and migration events. *Phytopathology,* **100:**S161.

Shaner, G. & Finney, R. (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology,* **67:**1051-1056.

Trout, C. L., Ristaino, J. B., Madritch, M.*, et al.* (1997) Rapid detection of *Phytophthora infestans* in late blight-infected potato and tomato using PCR. *Plant Dis.,* **81:**1042-1048.

Vleeshouwers, V., Raffaele, S., Vossen, J.*, et al.* (2010) Understanding and exploiting late blight resistance in the age of effectors. *Annu. Rev. Phytopathol.*

Walmsley-Woodward, D. & Lewis, B. (1975) Behaviour of *Phytophthora infestans* (Mont.) de Bary on potato tubers in relation to lenticel resistance. *Physiol. Plant Pathol.,* **7:**293-302.

Wang, H., Qi, M. & Cutler, A. J. (1993) A simple method of preparing plant samples for PCR. *Nucleic Acids Res.,* **21:**4153-4154.