Five Reasons to consider *Phytophthora infestans* a re-emerging pathogen

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Abstract

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Phytophthora infestans has been a named pathogen for well over 150 years and yet it continues to "emerge", with thousands of articles published each year on it and the late blight disease that it causes. This review explores five attributes of this oomycete pathogen that maintain this constant attention. First, the historical tragedy associated with this disease (Irish potato famine) causes many people to be fascinated with the pathogen. Current technology now enables investigators to answer some questions of historical significance. Second, the devastation caused by the pathogen continues to appear in surprising new locations and/or with surprising new intensity. Third, populations of *P. infestans* worldwide are in flux, with changes that have major implications to disease management. Fourth, the genomics revolution has enabled investigators

to make tremendous progress in terms of understanding the molecular biology (especially the pathogenicity) of *P. infestans.* Fifth, there remain many compelling unanswered questions.

Introduction

The late blight disease caused by *Phytophthora infestans* is regarded as one of the most devastating of plant diseases and certainly the most devastating disease of potatoes (Agrios 2005). For potato, the disease has been estimated to cause more than $6 billion in losses and management costs annually (Haverkort et al. 2008). Not only is potato foliage destroyed (Fig. 1A), but potato tubers can also become infected (Fig. 1B). The disease is at least as destructive on tomato as it is on potato (Fig. 2). It can destroy plants rapidly, and is sometimes reported to kill plants in a matter of hours (see below). The tomato plants depicted in Figure 2 have been nearly completely destroyed by late blight, and were destined to be removed shortly after this picture was taken. Much fungicide is used to protect potatoes and tomatoes; for example in the USA in 2001 alone, more than 2000 tons of fungicides were used on potatoes to suppress this disease (Anonymous 2004).

Asexual reproductive cycles (Fig. 3) are responsible for devastating epidemics. As an oomycete, *P. infestans* produces sporangia (Fig. 3C) which can germinate directly (to produce a germ tube) or indirectly to produce zoospores (Fig. 3D). After a short period of motility (minutes to hours), the zoospores encyst and germinate via a germ tube. If the zoospores are on host tissue the germ tube can penetrate the host and initiate infections (Fig. 3E). Sporulation occurs from lesions and is stimulated by moist conditions at moderate temperatures (15-22˚C). A single lesion can produce several hundred thousand sporangia (Fig. 3 A) which are aerially

dispersed (Fig. 3C). Asexual reproduction can also lead to the development of clonal lineages. The individuals in a clonal lineage are all derived from a single recombination event, and differ from each other only by mutation and/or mitotic recombination. Members of the same clonal lineage are generally phenotypically similar to each other.

Given the devastating potential of this pathogen, it's easy to understand the attention it receives. However, the pathogen and disease have emerged and re-emerged so many times, that it might be logical to conclude that nothing new could be said about this disease. And yet, much continues to be said (and written). A search on Google Scholar for "late blight of potato" returned 61,100 articles with 16,700 since 2010. Obviously, the world continues to devote much attention to this pathogen and disease.

We think there are several attributes that maintain the visibility of this pathogen (and its disease), thus causing it to be always "re-emerging". Because this review cannot be totally comprehensive, we have identified five attributes that we believe are responsible for the fact that this pathogen and its disease remain "emerging", and thus of intense interest to growers, home gardeners, historians and scientists.

1. The historical tragedy associated with this disease (Irish potato famine) causes many people to be fascinated with the pathogen. Current technology now enables investigators to answer some questions of historical significance.
2. The devastation caused by the pathogen continues to appear in surprising new locations and/or with surprising new intensity.
3. Populations of *P. infestans* worldwide are in flux, with changes that have major implications to disease management.

4. The genomics revolution has enabled investigators to make tremendous progress in terms of understanding the host-pathogen interactions.
5. There are many compelling unanswered questions.

Current questions of historical significance.

The availability of current genomic and next generation sequencing resources, culture collections, and herbarium collections have converged to enable investigators to apply additional data and insight to controversies concerning the center of origin of *P. infestans* and to resolving the identity of the genotype(s) of *P. infestans* responsible for the Irish potato famine.

Center of origin. The disease appeared very suddenly in the mid-19th century. Where did the pathogen come from? The initial assumption was that *P. infestans* originated in the Andes of South America along with the potato (Berkeley, 1846; Jones et al. 1912). However, there were some doubters – among them was Donald Reddick of Cornell University (Reddick 1928). Reddick felt that if *P. infestans* had been endemic to South America, it would have been observed there by European botanists, but he found no such reports. Eleven years later, and based partially on the fact that the native species of *Solanum* in Mexico are largely resistant to *P. infestans,* Reddick was willing to suggest that Mexico was the center of origin of *P. infestans* (Reddick 1939).

The idea that central Mexico might be the center of origin for *P. infestans* gained much momentum when it was discovered in the 1950s that the *P. infestans* population in the Toluca Valley in central Mexico was sexual, containing both A1 and A2 mating types (Niederhauser

1956; Gallegly and Galindo 1958; Galindo and Gallegly 1960). Prior to that time, *P. infestans* had been thought to be exclusively asexual (de Bary 1863; Reddick 1939). Demonstration that the population in central Mexico was very diverse genotypically (Grunwald et al 2001; and reviewed in Grunwald and Flier (2005)] further coalesced opinion that central Mexico was the center of origin of this species.

This hypothesis prevailed until the early 21st century when a study by Gomez-Alpizar et al. (2007) on mitochondrial and nuclear gene genealogies of isolates from several locations worldwide caused these authors to conclude that *P. infestans* had a South American origin. Their report refueled the controversy, but a subsequent study (Goss et al. 2014), using a wider set of isolates and including more close relatives of *P. infestans*, again led to a conclusion that the highlands of central Mexico are the center of origin. This latter study reconciles previous observations about genetic diversity, host range, and the natural history of the pathogen.

Irish famine strain? We have also long been interested to know the identity of the specific strain(s) of *P. infestans* that caused the Irish potato famine. Goodwin et al (1994b) suggested that the famine might have been caused by the US1 clonal lineage of *P. infestans*. They based their suggestion on the worldwide dominance of this clonal lineage in the mid-late 20th century (Goodwin et al. 1994b). However, analyses of herbarium specimens indicated that US1 was not present in 1845 (Ristaino et al. 2001). Further evidence on this topic was obtained by two groups of investigators who used shotgun sequencing of herbarium samples that had been collected between 1845 and 1896 and comparison of these to modern strains (Martin et al. 2013; Yoshida et al. 2013). This analysis confirmed that US1 was not present in 1845, but instead

populations were dominated by a single genotype named HERB-1 (Yoshida et al. 2013). HERB-1 apparently dominated for 50 years, but was subsequently replaced by the closely related US1 clone (Yoshida et al. 2013). As further evidence of the generality of interest in these historical questions, the name HERB-1 was even the subject of television comedy in the USA (see http://thecolbertreport.cc.com/videos/7fm2v2/irish-potato-famine-pathogen).

Surprisingly severe epidemics

Another reason that late blight seems to be continually emerging is that there have been repeated occurrences worldwide where the disease has become unexpectedly serious. These events are surprising because they are not explained by unusual weather. Instead, changes in the pathogen population are frequently associated with such situations. We describe here several such events.

USA/Canada. During the past four decades in the United States and Canada, late blight has been particularly severe twice over large regions – once in 1994/1995) (Fry and Goodwin 1997) and again in 2009 (Fry et al. 2013). In the early-1990s, exotic strains from Mexico (US6, US7, and US8) that were particularly aggressive and resistant to the fungicide, metalaxyl (now known as mefenoxam) were introduced (Goodwin et al. 1994a). These strains caused severe epidemics throughout both the USA and Canada in 1994 and 1995 (Fry and Goodwin 1997). Losses quantified in the 1995 epidemic in the Columbia basin in the Pacific Northwest were estimated at $30 million (Johnson et al. 1997). To manage these new strains, it was predicted that about 25% more fungicide applications would be required than for the previously dominant strains (Kato et al. 1997). Growers have indicated that this prediction was correct.

In 2009, a tomato late blight pandemic in the eastern USA unfortunately introduced many organic growers and home gardeners to the late blight disease (Fry et al. 2013; Hu et al. 2012). It seems likely (at least for the USA) that this epidemic introduced more non-plant pathologists to the disease and pathogen than any other single recent event. The emotional and economic effect on a home gardener is illustrated in the following email to WEF.

> Date: Fri, 24 Jul 2009 18:03:47 -0700
> "FYI: I am an organic gardener in Amsterdam, NY with 63 heirloom tomato plants of 23 different varieties, all gone. I was growing very rare vareities, blacks, greens, oranges, whites. All purchased from a very reputable grower in Schoharie. Tonight I had to leave home as my husband is pulling and bagging all 63 plants. I have 100% loss. We live on ten acres. I inspected every day and it seems the blight took my plants in matter of hours. I was hoping to sell them for additional income."

During the 2009 pandemic, there were many articles in the popular press – and much digital communication. The interest was chronicled via "Google Trends" (described in (Scherm et al. 2014). Based on these data, it's clear that there is an annual interest in the summer but this was greatly magnified in 2009 (Fig. 4).

In contrast to the majority of situations described below, the pandemic in 2009 was <u>not</u> caused by the introduction of particularly aggressive strains (Danies et al. 2013), or by particularly favorable weather (Fry et al. 2013). Instead, the pandemic was caused by the

183 massive distribution of a particular strain (US22) via infected tomato transplants from a single
184 national supplier sold in large retail stores over large regions of the USA (Fry et al. 2013).
185 The pandemic of 2009 stimulated an interest in generating more accurate data concerning
186 populations of *P. infestans* in the USA on a near real-time basis. Fortunately, a USDA AFRI
187 grant enabled such analyses. Microsatellite markers developed by Lees et al. (2006) were used
188 to identify the clonal lineage of *P. infestans* in each sample that was submitted to a central
189 laboratory for analysis. The specimens were sent via overnight courier and in the vast majority of
190 cases the results were returned to the submitter within one or two days of receipt, and also
191 reported on a national website (USAblight.org), which also contains a map illustrating the
192 location (county). This information was valuable to the submitter because each clonal lineage
193 had reasonably consistent and unique fungicide resistance and host preference characteristics,
194 which could help growers develop their management plans (Table 1) (Danies et al. 2013).
195 Figure 5 provides a chronological description of the reports obtained from 2009 through 2014.
196 Most reports came from the eastern part of the USA. While there were certainly additional
197 occurrences of late blight in the country, the samples submitted to the central lab resulted in the
198 most extensive and comprehensive assessment of *P. infestans* in the USA in history. Summaries
199 of these reports were recorded on usablight.org.
200 There were just a few dominant clonal lineages in the USA during 2009-2014 (Fig. 6). This
201 is consistent with the situation for the previous decade as well – a small number of clonal
202 lineages dominated the population of *P. infestans* (Fig. 6) in any particular year.
203 A feature of *P. infestans* in the USA has been that the population structure is typically very
204 simple, often with only a single lineage in a region (Fig. 5), or with only a single lineage on
205 potatoes and sometimes a different lineage on tomatoes (Fig. 7). For example, in 2009 only US8

and US22 were widely reported (Fig. 6). US22 was reported on potato and tomato, but US8 was reported only on potato (Fig. 7). US23 and US24 were reported in 2009, but at low frequency (Hu et al. 2012; Fig. 5). US11 was very important in Florida in 2012 (Figs. 5 and 6). Interestingly, there has been some regional sub-structuring, with US11 and US24 being the most common in the western USA (Fig. 5). Since 2011, US23 has become increasingly dominant (Figs. 6 and 7) and has been the only lineage reported in many states in 2012, 2013 and 2014 (Fig. 5). US23 has recently expanded its range westward (Fig. 5)

The simplicity of the population structure has been useful to the management of late blight in the USA. This is because the phenotype of most individuals within a lineage is relatively conserved. Characterizing the phenotype of an isolate can require weeks to months – particularly if one needs to work with the isolate in pure culture. However, determining the genotype of the pathogen from a sporulating lesion using simple sequence repeats [SSRs, or microsatellites] can be done in less than 24 hours. Thus, from knowledge of the phenotype of individuals in a lineage, one can typically predict the impact of certain management actions. For example if tomato growers are aware of potato late blight in the area, and if they also know that the lineage causing potato late blight is US8 or US24, then they can safely conclude that their tomato crop is not at immediate risk. In contrast, if the lineage on potato is US23 they need to take immediate precautions, because US23 is very aggressive on tomatoes. Growers would also know that mefenoxam could be used to help protect their tomato crop because US23 has been largely sensitive to mefenoxam in the USA. Finally, if US11 was on potato then immediate action would be necessary because US11 has been consistently highly pathogenic to both tomato and potato and resistant to mefenoxam (Saville et al. 2015). Of course it is necessary to

continually monitor the phenotypes of diverse strains to learn if mutants with new epidemiologically important traits have appeared.

Southwest India . In southwest India (Karnataka state, with 46,000 ha of tomatoes for fresh market), late blight of tomato had not been reported as a particularly important disease prior to 2007 even though the disease had been reported there on potato since 1953 (Chowdappa et al. 2013). However, in 2009 and 2010 there were severe tomato late blight epidemics with crop losses of up to 100% (Chowdappa et al. 2013). The genotypes of 19 isolates obtained from diseased tomatoes in different locations in Karnataka were assayed using molecular markers (SSR and RG57). All assays were consistent with these isolates being members of the 13_A2 genotype (Blue_13) of *P. infestans* (Chowdappa et al. 2013) – the particularly aggressive genotype that dominated Great Britain in 2005-2008 (Cooke et al. 2012). It seems likely that migration of 13_A2 into India was responsible for the increased disease severity. Mechanisms for such migration events exist. There were importations of tons of seed potatoes from Great Britain and Europe prior to 2009 (Chowdappa et al. 2013) where this lineage had been dominant. Subsequent collections revealed that 13_A2 was also detected on potatoes in 2010, 2011, and 2012 (Chowdappa et al. 2015). Because 13_A2 is pathogenic on tomatoes as well as on potatoes, and because it is more aggressive than the previously dominant strains, there has been a dramatic 5-to-7-fold increase in the number of fungicide sprays applied to tomatoes in Karnataka (Chowdappa et al. 2013). Late blight is certainly a re-emerging disease in Karnataka India.

Tunisia. A North African example of unexpected late blight severity occurred in Tunisia in the first decade of the 21st century (Harbaoui et al. 2014). This was coincident with the first report

of an A2 mating type isolate in Tunisia (cited in (Harbaoui et al. 2014)), which raised the possibility that there have been changes to the *P. infestans* population there. One clonal lineage (North Africa – 01 = NA-01) was dominant, particularly on tomatoes (Harbaoui et al. 2014). However, a group of diverse strains (containing both A1 and A2 mating types) was found in a region in which late blight was particularly difficult to manage. It is not yet determined if there is or is not a residential sexual population of *P. infestans* in Tunisia (Harbaoui et al. 2014). In contrast to the situation in southwest India, the diverse isolates on potatoes in Tunisia appear unrelated to current European strains (Harbaoui et al. 2014).

Other locations. In addition to unexpected occurrences of severe late blight in the USA/Canada, India, and Tunisia, we are aware of similar events in Chile, China, Oman, and Nigeria. In Chile, late blight was first reported in the 1950s, but it has become very serious since 2005 (Acuna et al. 2012). Before 2005, the mitochondrial haplotype was Ib (Acuna et al. 2012) – suggestive of the US1 clonal lineage. However, the current population is characterized by the Ia mitochondrial haplotype and is resistant to mefenoxam (Acuna et al. 2012). This population retains a strongly clonal structure with only A1 mating types being reported (Acuna et al. 2012). China leads the world in potato production (Li et al. 2013). Prior to 1996, only A1 mating type strains had been reported (Li et al. 2013). Strains of the A2 mating type were first detected in 1996 (Zhang 1996). A dominant clonal lineage (SIB-1) was found widely throughout China and was identical to that lineage found in Siberia – suggesting migration between Russia and China (Guo et al, 2010). Interestingly, the 13_A2 lineage is now well established in China in the Sichuan province – having been detected as early as 2007 (Li et al. 2013). Some variants of the lineage were also detected in Sichuan (Li et al. 2013), but as of 2013 there was not yet evidence for a residential

sexual population. (Li et al. 2013). Very recent reports (2012 and 2013) of unexpectedly severe late blight in Oman (personal communication from Ali Obaid Al-Adawi) and in Nigeria (personal communication from Ranajit Bandyopadhyay) are so far observational and populations there have not yet been characterized. It is logical to conclude that the total number of unexpected occurrences is unknown, but in each of the locations above, late blight is indeed re-emerging.

Changes in populations of *P. infestans* worldwide

For the first century or more of its existence in Europe, USA and Canada, Africa and Asia, populations of *P. infestans* appear to have been highly clonal with domination first by HERB-1 (Yoshida et al. 2013), and later by US1 (Goodwin et al. 1994b), although HERB-1 might be part of a larger US1 metapopulation. US1 is of the A1 mating type. The mating type of HERB-1 is not known, but we hypothesize that it was also A1, because the occurrence of two A1 mating types explains the absence until the mid-20th century of documented sexual reproduction of *P. infestans*. Obviously, the situation in central Mexico was entirely different with a very diverse and sexual population (Grünwald and Flier 2005).

The absence of sexual reproduction in most parts of the world meant that *P. infestans* was essentially an obligate parasite (requiring a living host for its long term survival) everywhere except in central Mexico. In potato agro-ecosystems in the temperate zone, infected tubers from storage or from the field (as volunteers) provide a mechanism for survival between seasons. In the absence of living host tissue and as an asexual organism, survival is much shorter. Sporangia can survive for weeks in soil (Mayton 2006). In contrast, oospores can survive for years in soil

296 (Drenth et al. 1995; Mayton et al. 2000), and they can also survive drying while in soil
297 (Fernandez-Pavia et al. 2004).
298 The presence or absence of sexual recombination is thus a huge factor in the epidemiology of
299 late blight. Additionally, sexual reproduction generates new genotypes of the pathogen with
300 unexpected traits. Finally, there was the fear that a soil source of the pathogen might lead to
301 more common and earlier epidemics. It is for these several reasons that most countries did not
302 allow imports of potatoes from central Mexico. Any introduction of A2 mating type strains had
303 been highly feared.
304 Thus, the first detection of A2 mating type strains outside of Mexico in Switzerland in 1984,
305 (Hohl and Iselin 1984) was enormous news. This first report stimulated other investigators to
306 search locally for A2 mating type strains, and these searches detected some A2 strains, first in
307 Europe (Shaw et al. 1985) and then in the USA and Canada (Deahl et al. 1991), and subsequently
308 in Asia (Nishimura et al. 1999; Singh et al. 1994; Zhang 1996;).
309
310 In Europe, the detection of A2 strains in the 1980s was the initial indication of a major
311 migration event and subsequent population shift. The US1 strain that had dominated non-
312 Mexican populations worldwide prior to the 1980s was displaced by a diverse population
313 containing both A1 and A2 strains (Fry and Goodwin 1997; Spielman et al. 1991).These exotic
314 strains were quickly associated with more severe late blight outbreaks (Leary 1993).
315 A major concern was that sexual populations would occur in locations where these new
316 strains now were dominant. Certainly there was considerable diversity present in such
317 populations in northern Europe. There were reports of diverse populations in the Netherlands
318 (Fry et al. 1991; Zwankhuizen et al. 2000), Poland (Sujkowski et al. 1994), Estonia (Runno-

Paurson et al. 2009) and the Nordic countries (Lehtinen et al. 2008). In these locations both A2 and A1 mating type individuals were present – often in the same field in high proportion. Fortunately, the mere occurrence of both A1 and A2 strains in a region is not alone sufficient to create a residential sexual population. For example, both A1 and A2 strains have been in the USA for over 20 years, but a residential sexual population has not been detected (see below).

In northern Europe, there is now convincing evidence that there are residential sexual populations of *P. infestans* , particularly in the Nordic countries (Yuen and Andersson 2013). One of the first indications occurred in a field experiment conducted in Sweden in 1996 (Andersson et al. 1998). In that experiment, late blight was associated with particular locations in the field where late blight in 1994 had been severe (Andersson et al. 1998). Cereals were planted in 1995 and there had been no potatoes in the field in 1995 (Andersson et al. 1998). Both mating types were detected among isolates obtained from that field in 1996 and oospores were observed in infected tissue (Andersson et al. 1998). Unfortunately, the reported genetic diversity in that research field turned out to be a predictor of a now common situation in the Nordic countries (Brurberg et al. 2011): very high genetic diversity among *P. infestans* isolates. In a study involving 200 isolates from Denmark, Finland, Norway and Sweden 75% of individuals were unique as determined by analysis using only 9 SSR loci (Brurberg et al. 2011).

The epidemiological consequences of a residential sexual population have been reported by Hannukkala et al. (2007). These authors studied late blight epidemics in Finland from 1933 to 1962 and from 1983 to 2002. They found that the risk of a late blight outbreak was 17-fold greater in 1998-2002 than in two previous periods (1933-1962 and 1983-1997) and occurred 2-4 weeks earlier than before (Figure 8) (Hannukkala et al. 2007). Weather probably contributed in only a minor way to the increased intensity of late blight because the number of rainy days had

increased only slightly (Hannukkala et al. 2007). Interestingly, once epidemics started they did not differ in intensity than those from the earlier period. Nonetheless, the earlier start to epidemics caused Finnish farmers to apply fungicides more frequently – almost doubling the number of annual applications from the early 1990s to the 1997-2002 period (Figure 9) (Hannukkala et al. 2007).

It seems clear that the fears of plant pathologists concerning the introduction of a diverse population containing individuals with both mating types were well-founded. The evidence clearly leads to the conclusion that in the Nordic countries and probably also in other parts of northern Europe, the introduction of a diverse population has established residential sexual populations in agricultural fields. The newly formed residential sexual populations are responsible for generating high genetic diversity in the pathogen population. The soil has become a source of inoculum and epidemics are now starting earlier.

Fortunately, in other parts of the world, recent studies have detected populations that are largely clonal with no evidence for sexual reproduction. For example, Montarry et al. (2010) found two admixed clonal populations of *P. infestans* in 220 isolates collected from 20 commercial fields in 2004 and 2005 in France. They concluded that this population structure resulted from limited or no sexual reproduction in the French *P. infestans* population (Montarry et al. 2010). In China and India, recent populations were strongly clonal with no strong evidence for sexual reproduction (Chowdappa et al. 2013; Chowdappa et al. 2015; Li et al. 2013).

In the USA/Canada, reported populations of *P. infestans* remain strongly clonal with little evidence of residential sexual populations that contribute significantly to the ecology and epidemiology of this pathogen (Fry et al. 2013; Hu et al. 2012). However, there are two reports

of ephemeral populations that were apparently recombinants. These populations are ephemeral because after the initial detection, there has not been further production of recombinant individuals and most strains were not detected subsequently – probably because most of the recombinants were not as fit as the dominant genotypes. The first such population was detected in the Columbia basin of the Pacific Northwest in 1993 (Gavino et al. 2000). The diversity characterizing these isolates was dramatically different from collections from other parts of the USA in the 1980s, 1990s and the 2000s. The population contained both mating types, and many combinations of alleles in isolates collected in rather close geographical proximity (Gavino et al. 2000). The authors postulated that the parents of this population were US6 and US7, and that one of the progeny was US11 (Gavino et al. 2000), a lineage that has been very troublesome for more than 20 years. However, other progeny of this recombination event have not been detected for many years, so most of the progeny appeared only ephemerally. Nonetheless, this progeny provides an example that recombination can produce individuals that are particularly troublesome.

The second ephemeral recombinant population has been reported recently from the northeastern part of the USA (Danies et al. 2014). The majority of isolates were detected in central/western New York State. These isolates were detected in 2010 and 2011, but not subsequently. As in the Pacific Northwest in 1993, this population contained diverse individuals in a somewhat localized region and had great diversity for allele combinations based on analysis of allozymes, mating type, RFLPs, and microsatellites (Danies et al. 2014). The parents for this population were postulated to be US22 (A2) and at least two other genotypes. Using a recent protocol that identifies at least 36 mitochondrial haplotypes, these individuals all had the same mitochondrial haplotype (H-20), the same haplotype as US22 (Danies et al. 2014). As with the

1993 population, this 2010/2011 population appears to have been ephemeral, because these individuals have not been detected since 2011 (Danies et al. 2014). However, these two reports of recombinant progeny in the USA demonstrate that sexual reproduction is possible and may happen again.

Contributions of genomics to enhancing our understanding of host-pathogen interactions.

Evolution from "nightmare" to model. A key challenge to the scientific community in trying to combat late blight can be encapsulated by the phrase 'know your enemy'. Over more than a decade late blight researchers have embraced the genomics era, providing a molecular framework within which to tease out the details of infection processes. The subsequent progress has caused *P. infestans* to be considered as the most important oomycete in molecular plant pathology (Kamoun et al. 2014). How does *P. infestans* evade, manipulate or overcome the immune system of major crop hosts such as potato and tomato? Why have so many efforts to breed for resistance been dismissed with apparent disdain by this pathogen? The legendary capacity for *P. infestans* to adapt to environmental diversity, or to overcome almost all obstacles that breeders have laid before it, has created a 'nightmare' disease that cannot be stopped except through the copious application of agrochemicals. The genomics *era* is starting to provide insight into the mechanisms and processes underlying *P. infestans* pathogenicity. With such understanding, our ideas about how to prevent late blight are becoming more sophisticated.

Large-scale studies of expressed sequence tags (ESTs) (Kamoun et al. 1999; Randall et al. 2005) followed by the genome sequence (Haas et al. 2009) have provided the entire genetic blueprint with which to discover the molecular components of *P. infestans* pathogenicity. These

411 resources have been combined with bioinformatic algorithms to predict genes encoding proteins
412 with secretion signal peptides (Torto et al. 2003). This has revealed many candidate proteins that
413 are exported from the pathogen, and which thus may directly interact with plant cells. Amongst
414 the central players that dictate whether microbial infection results in plant disease or disease
415 resistance are effector proteins. Effectors may act outside of plant cells (so-called apoplastic
416 effectors), or be delivered to the inside of living plant cells (cytoplasmic effectors), to suppress
417 immunity and alter host processes in favor of the invading microbe. In contrast, effectors are
418 themselves 'targets' for recognition by host resistance proteins and their detection activates the
419 hypersensitive response (HR), including programmed cell death (PCD); a process more recently
420 referred to as effector-triggered immunity (ETI) (Jones and Dangl 2006).
421 Apoplastic *P. infestans* effectors include a number of inhibitors of secreted, defense-
422 associated host enzymes. Inhibitors of either cysteine (Tian et al. 2007) or serine proteases (Tian
423 et al. 2004), and of secreted glucanases (Damasceno et al. 2008) have been characterized. The
424 effectors are exquisitely specific to the host enzymes that they target. The cysteine protease
425 inhibitor EPIC1 targets the tomato protease RCR3, which is also a target of fungal *Passalora*
426 *fulva (Cladosporium fulvum*) effector CfAVR2 (Song et al. 2009), and of the nematode effector
427 Gr-VAP1 (Lozano-Torres JL et al. 2012), indicating that pathogens from diverse kingdoms need
428 to disable the same host proteins to undermine plant immunity. Interestingly, perturbations to
429 RCR3 by CfAVR2 and Gr-VAP1 are detected by the tomato resistance protein Cf2 (Lozano-
430 Torres JL et al. 2012), revealing that monitoring, or guarding, host proteins can provide
431 resistance to multiple pathogens. Recently, diversifying selection of the EPIC1 protease
432 inhibitor orthologues from *P. infestans* and the closely-related *P. mirabilis* was shown to be

433 required for inhibition of the equivalent protease targets within their respective hosts, tomato and
434 *Mirabilis jalapa* (Dong et al. 2014).

435 Cytoplasmic effectors from *P. infestans* include the RXLR class, named for the conserved
436 Arg-any amino acid-Leu-Arg motif that is required for their translocation inside plant cells
437 (Whisson et al. 2007), and the crinkler (CRN) candidate effector class, which has been shown to
438 be translocated inside living plant cells (Schornak et al. 2010). All *P. infestans* avirulence
439 proteins detected by cytoplasmic nucleotide-binding, leucine-rich repeat (NB-LRR) resistance
440 (R) proteins are members of this class of effectors (Rodewald J and Trognitz 2013;
441 Vleeshouwers et al. 2011). Our understanding of what *P. infestans* RXLR effectors target in host
442 plant cells, and how they act collectively to promote pathogenicity, is in its infancy.
443 Nevertheless, each effector can be regarded as an experimental 'probe' to explore host regulatory
444 and mechanistic processes that are disabled or altered to cause disease. The RXLR effector
445 PiAVR3a stabilizes the host ubiquitin E3 ligase CMPG1 to prevent PCD triggered by perception
446 of elicitors, such as the secreted elicitin INF1 (Bos JI et al. 2010). PiAVR-blb2 has been shown
447 to prevent secretion of defense-associated proteases (Bozkurt et al. 2011). PiAVR2 interacts
448 with BSL1, a putative phosphatase implicated in brassinosteroid signal transduction. It is not
449 known why BSL1 is apparently targeted, but this interaction is detected by the resistance protein
450 R2, resulting in ETI (Saunders et al. 2012). The RXLR effector Pi03192 prevents re-localization
451 of two host NAC transcription factors from the endoplasmic reticulum to the nucleus, thus
452 presumably attenuating their normal activity (McLellan et al. 2013). More recently, PexRD2 has
453 been shown to interact with and inhibit MAP3Kε, which is required for signal transduction
454 leading to PCD following activation of the immune receptor Cf4 (King et al. 2014). In addition,
455 a range of *P. infestans* RXLRs act redundantly to suppress the activation of a different MAP

kinase pathway and subsequent host early gene expression following perception of the elicitor flg22 by receptor FLS2 (Zheng et al. 2014). Given that potentially hundreds of RXLR effectors are encoded by the *P. infestans* genome (Haas et al. 2009), many more effector targets and alternative modes of action to manipulate host immunity are likely to emerge in the coming years. However, our preliminary studies, driven initially by genomics, provide a model of a sophisticated pathogen with effectors acting inside or outside of host cells to disable or manipulate many host processes for its benefit.

The use of effectoromics in the search for durable disease resistance. Breeding for late blight disease resistance has a long history with little sustained success. Cycles of 'boom and bust' are well documented as dominant R genes, introgressed through lengthy programs of breeding, have been deployed only to be overcome within a few growing seasons by virulent genotypes emerging from rapidly changing pathogen populations. This has led to *P. infestans* being described as the plant and R gene destroyer (Fry 2008). To get a grip on how the pathogen overcomes these R genes, it is important to identify the avirulence effectors that they recognize.

A number of *P. infestans* avirulence (AVR) genes have been identified, all of which encode RXLR effectors. They include *AVR3a* (Armstrong et al. 2005) *AVR4* (van Poppel et al. 2008), *AVR-blb1* (Champouret et al. 2009; Vleeshouwers et al. 2008), *AVR-blb2* (Oh S-K et al. 2009) and *AVR2* (Gilroy et al. 2011). Multiple mechanisms have been revealed by which *P. infestans* has evaded recognition by the corresponding R proteins. *AVR4* can simply be lost from the pathogen effector repertoire; isolates that are virulent on R4 potato plants contain truncated or mutated, non-functional copies of *AVR4* (van Poppel et al. 2008; van Poppel et al. 2009). This indicates that not all effectors are required by the pathogen for infection. One way in which *P.*

479 *infestans* may be able to readily lose an effector is if others perform a similar role. Functional
480 redundancy has been shown recently; a number of effectors are able to block FLS2-mediated
481 MAP kinase signaling in tomato, suggesting that loss of any one of these effectors can be
482 compensated for (Zheng et al. 2014). Loss of an effector, or silencing of its expression, is
483 implicated in evasion of detection by the potato *R2* gene (Gilroy et al. 2011). Nevertheless,
484 virulent isolates possess a related effector, AVR2-like (A2L), containing amino acid
485 polymorphisms that evade recognition by R2, but presumably retain pathogenicity function
486 similar to AVR2 (Gilroy et al. 2011; Saunders et al. 2012). In addition to loss of an effector to
487 evade recognition, it has been proposed that additional effectors may evolve to suppress the
488 recognition of an avirulence protein. It has been reported that the effector variant *IpiO4* is able
489 to suppress recognition of *AvrBlb1* (*ipiO1*) (Halterman et al. 2010).
490 The genome sequence of *P. infestans* contains potentially >500 RXLR genes, many of which
491 fall into families of related sequences (Haas et al. 2009). Some of the effectors within each
492 family perhaps have similar functions. RXLR genes generally occupy repeat-rich, gene-sparse
493 regions of the genome; locations at which higher rates of mutation and possibly transcriptional
494 silencing may occur in order to reduce or control the expression of transposable elements which
495 reside with the effectors (Haas et al. 2009). Indeed, small RNAs associated with silencing of
496 RXLR effector genes have been observed in *P. infestans* following deep sequencing of sRNAs
497 from isolates that differ in pathogenicity (Vetukuri 2012). The genome sequence has thus
498 revealed a high potential for evolutionary adaptation. Effector genes, in particular, can be
499 readily duplicated and mutated, and copies can be silenced without compromising the overall
500 infection efficiency of the pathogen. This further emphasizes the 'nightmare' of controlling *P.*
501 *infestans*, and explains why it is regarded as constantly re-emerging as a threat to food security.

However, genomic and transcriptomic studies of *P. infestans* are also providing potential solutions. The transcriptome of the genotype 13_A2 (also known as Blue_13), an aggressive genotype that has emerged as the predominant form of *P. infestans* in Europe in the past decade, revealed that the expression of 45 RXLR effectors was conserved with two other genotypes tested (Cooke et al. 2012). This raises the possibility that some effectors may be essential for potato infection, and thus cannot be readily lost to evade detection. The 13_A2 genome lacks functional copies of *AVR1* and *AVR4*, and possesses *AVR2-like* rather than *AVR2*, explaining why the corresponding R1, R4 and R2 resistances are overcome by this genotype. However, it expresses *AVR-blb1, AVR-blb2* and *AVR-vnt1*, and all three of the resistances Blb1, Blb2 and Vnt1 provide effective resistance to 13_A2 (Cooke et al. 2012). Therefore, genome-wide knowledge of the effectors that are expressed in different *P. infestans* genotypes may highlight a core set of key effectors for which corresponding resistances may be durable.

There is one additional consideration in prioritizing effectors as 'good' targets for potentially durable *R* genes: whether those effectors are essential for infection. AVR3a is a good example. Two alleles of *AVR3a* have been reported within pathogen populations worldwide. They encode proteins differing in two amino acids (K80E and I103M); AVR3aKI is recognized by R3a, whereas AVR3aEM evades detection (Armstrong et al. 2005). Interestingly, historic lineages of *P. infestans* lack the virulent form of *Avr3a* and several other effectors, suggesting that modern plant breeding may have driven expansion of effectors in the pathogen (Martin et al. 2013). The genotype 13_A2 overcomes R3a as it only possesses AVR3aEM (Cooke et al. 2012). As AVR3a is an essential pathogenicity determinant (Bos et al., 2010), deployment of an R gene that targets AVR3aEM, in combination with *R3a* itself, could impose strong selection pressure on the pathogen population.

525 Many programs to breed for late blight resistance have thus been re-shaped by our knowledge
526 of the *P. infestans* genome. An understanding of which RXLRs are universally expressed, which
527 are essential for infection and whether they can be mutated to evade recognition whilst retaining
528 their function, is focusing searches for specific R genes that may provide durability (Birch et al.
529 2008; Vleeshouwers et al. 2008). Studies of effector diversity and function, and transient
530 expression screens of key effectors within wild potato germplasm to seek corresponding R genes,
531 has now been termed 'effectoromics' (Vleeshouwers et al. 2011; Vleeshouwers and Oliver
532 2013). The principles of effectoromics, applied initially to late blight, are being adopted for
533 many other crop diseases.
534 Effectoromics has revolutionized our search for durable disease resistance, positioning our
535 understanding of effector function, expression and sequence diversity as central to finding R
536 genes with the potential to stand the test of time. Nevertheless, given the phenomenal capacity
537 for *P. infestans* to evolve, it is likely that many such R genes would need to be combined to
538 provide a durable barrier to infection (Vleeshouwers and Oliver 2013). However, such durable
539 barriers do exist in nature. Within the Solanaceae, whereas *P. infestans* infects potato, tomato
540 and eggplant, it is not reported to infect pepper or tobacco. Recently, *P. infestans* effectoromics
541 was applied to pepper in order to determine whether this non-host crop could provide a new
542 source of R genes (Lee et al. 2014). Recognition of multiple *P. infestans* RXLR effectors was
543 revealed, suggesting that pepper has already 'stacked' R genes to provide a durable barrier to late
544 blight disease.
545 In addition to R genes that recognize effectors, non-host resistance may also be achieved by
546 evolution of the targets of effectors, so that they may no longer either physically interact with an
547 RXLR or be appropriately inhibited or manipulated by it. The recent studies by Dong et al.

(2014) and Zheng et al. (2014) support this. As stated above, Dong and co-workers (Dong et al. 2014) showed that orthologues of the secreted protease inhibitor EPIC1 from *P. infestans* and *P. mirabilis* possessed amino acid differences that tailored them to their job in their respective host plants. In addition, Zheng et al. (2014) showed that many of the *P. infestans* effectors that suppress early immune responses in the host plant tomato are unable to do the same in the non-host plant Arabidopsis. The big question for the future is whether we can move effector targets from non-host plants into host plants to see if they still function to promote immunity, whilst no longer being disabled by *P. infestans* effectors. The most durable barrier to late blight infection would be to convert potato and tomato into non-host plants.

Compelling unanswered questions

What are the relationships among strains of P. infestans? Different locations seem to have diverse answers to this question, and some locations have no defined answers. However, common to all locations is the apparent diversity among individuals. That mutation is the major source of significant variation is well evident in terms of the numbers of Single Nucleotide Polymorphisms (SNPs) when just a few diverse lineages of *P. infestans* are compared. When using genotyping by sequencing (GBS) to compare just a few dozen individuals within a recombinant progeny (Danies et al 2015; Myers et al unpublished) or within a lineage (Hansen et al, unpublished) it is not uncommon to find $10^5 – 10^6$ SNPs. There are also rapid changes within clonal lineages. For example, using SSR analysis, at least three different variants have been detected within the US23 clonal lineage in the USA (Myers et al, unpublished).

571 Preliminary studies on within-lineage variation using GBS have identified a larger number of
572 SNPs in older lineages (US8) compared to a newer lineage such as US23 (Hansen et al
573 unpublished). In a very early study, pathotypic analysis also revealed large differences within
574 clonal lineages (Goodwin et al. 1995). Similar to what is now being observed with GBS, the
575 earlier authors suggested the older lineages had more diversity than new lineages (Goodwin et al.
576 1995).

577 In some situations, global trade is most likely responsible for the intercontinental transport of
578 some strains and migration has had a huge role. The detection of 13_A2 in China and India is not
579 surprising given the global trade in seed potatoes. In addition to migration via seed tubers, we
580 have now seen migration via infected tomato transplants. Some occurrences are still baffling.
581 For example the US8 clonal lineage was first detected in northwest Mexico and then in the USA;
582 this linage could have been imported into the USA on plant tissues. Certainly, infected tomato
583 fruits imported into the USA from Mexico have been observed. However, the pathway by
584 which US8 was moved to Colombia (Vargas et al. 2009), presumably from the USA, remains a
585 mystery. Certainly, migration affects structures of populations of other pathogens. For example,
586 migration has been documented for other pathogens such as *P. ramorum* (Goss et al. 2011;
587 Grünwald et al. 2012).

588 In locations such as northern Europe and central Mexico, where sexual reproduction is
589 common, recombination appears to play a huge role in creating a very diverse population. In
590 such locations the influences of migration on population structure may be overwhelmed.

591 In other locations where sexual reproduction plays a minor role, the rise and fall of dominant
592 lineages remains unexplained. However, in these locations, we now realize the one constant is
593 the continuous turnover in clonal lineages. This turnover may be best exemplified in the USA

(Fig. 6). Emergence of American and European lineages has been documented repeatedly yet we do not have a definitive understanding of the mechanism of emergence (Cooke et al. 2012). When new genotypes were first detected in northern Europe in the 1980s, there were reports that these genotypes were of greater aggressiveness than the previous population (Day and Shattock 1997). When US8 first appeared in the USA, there were also reports that it had greater aggressiveness than previous strains (Kato et al 1997; Lambert and Currier 1997). Yet both US8 and 13_A2 have declined in prominence and we do not yet have good evidence to explain this phenomenon. One wonders if dominant lineages accumulate sufficient deleterious mutations to lose fitness.

For the USA in particular, the simple population structure enables such questions to be at least conceptualized. From whence did the recent lineages (US21, US22, US23 and US24) come? Are these lineages the result of undetected sexual recombination in the USA? Were they imported into the USA from some other location?

What controls the mating biology of P. infestans? While we know much about the mating biology of *P. infestans*, much remains to be learned about what influences the frequency and outcomes of sexual recombination. Oospores form when A1 and A2 strains are co-inoculated on plant tissue, and have been detected in natural infections (Andersson et al. 1998; Fernandez-Pavia et al. 2004; Lehtinen and Hannukkala 2004). Generation of oospores occurs on both tomato and potato leaves over a wide temperature range, but reportedly only during periods of sustained high humidity (Cohen et al. 1997; Drenth et al. 1995). More oospores were produced in sprouting compared to dormant tubers (Levin et al. 2001).

Although A1 and A2 strains of *P. infestans* have been found near each other in many locations, only in some regions are recombinants common. This differs from the situation with some other heterothallic oomycetes such as *P. capsici*, where recombinant progeny arise frequently (Dunn et al. 2014; Lamour et al. 2012). This may be explained in part by the concept of population bottlenecks – where a derived population has a tiny fraction of the diversity in the source population. Sporangia of *P. infestans* are aerially dispersed, but sporangia of *P. capsici* are not aerially dispersed (Granke et al. 2009). Thus a single season epidemic of late blight caused by *P. infestans* can be initiated by a single aerially dispersed sporangium (single mating type) being deposited on a leaf and causing infection – an extreme example of a genetic bottleneck. In contrast, long distance dispersal of *P. capsici* is most likely via infected plant material or via infested soil or water (all of which are likely to have a diverse population of individuals). Other epidemiological and genetic factors may also restrict the occurrence of successful matings in P. infestans. For example, recessive mutations accumulated during long periods of exclusively asexual reproduction may lead to unviable oospores or progeny with reduced fitness. Many isolates of *P. infestans* also vary in ploidy, which may lead to genetic imbalances in their recombinants (Tooley and Therrien 1987). One study demonstrated that 3n x 3n and 2n x 3n crosses yielded fewer viable oospores than 2n x 2n crosses (Hamed and Gisi 2013). Researchers have also noted that the fitness of progeny relative to their parents is often reduced, and decreased fitness is more likely when the parents vary in ploidy (Al-Kherb et al. 1995; Hamed and Gisi 2013; Klarfeld et al. 2009). Another factor that may restrict matings is the observation that some isolates preferentially infect tomato or potato (Danies et al. 2013). Combined with the fact that growing seasons or locations of the two crops may overlap only partially, the likelihood

638 that tomato- and potato-adapted strains of opposite mating type would meet in time or space
639 would be reduced.

640 There is much interest in developing a DNA assay for mating type, but its molecular basis has
641 not yet been determined in any oomycete. Mating type appears to be determined genetically by a
642 single locus, with A1 acting as heterozygote and A2 acting as a homozygote (Fabritius and
643 Judelson 1997). The two mating types are distinguished by their abilities to produce and respond
644 to acyclic diterpene mating hormones named α1 and α2 (Ojika et al. 2011; Qi et al. 2005). The
645 α2 hormone is made from phytol by A2 strains and metabolized to α1 by A1 strains, which is
646 consistent with A1 being a heterozygote if the mating type locus encodes the relevant enzymes.
647 Approaches based on genomics, or focusing on the enzymes that produce the hormones, hold
648 promise for revealing the mechanism and evolution of the mating system. Heterothallism may
649 have evolved from homothallism in oomycetes since the latter predominantly occupies its basal
650 clades (Riethmueller et al. 2002; Thines 2014). Both homothallic and heterothallic species occur
651 within *Phytophthora, Pythium* and downy mildews, which suggests that heterothallism evolved
652 multiple times in oomycetes or can revert to self-fertility.

653 Interestingly, strongly self-fertile strains of *P. infestans* have been described in field
654 populations (Anikina et al. 1997; Fyfe and Shaw 1992) and in progeny of laboratory crosses
655 (Judelson 1996). These appear to be tertiary trisomics that make abundant oospores in single
656 culture, and not heterokaryons. This distinguishes their mating systems from normal strains of
657 *P. infestans*, which sometimes exhibit weak secondary homothallism in response to stresses such
658 as fungicide exposure (Groves and Ristaino 2000). Oosporogenesis during stress appears to
659 represent a temporary breakdown in the regulation of self-incompatibility, as opposed to being a

660 stable genetic change. The epidemiological impact of natural self-fertility or secondary
661 homothallism on late blight is unknown.
662
663 *What factors explain fungicide resistance?* As noted earlier, the re-emergence of late blight in
664 the 1980s and 1990s was due to the appearance of strains that were more aggressive and
665 insensitive to metalaxyl (now usually sold as its active enantiomer, mefenoxam). Recently, the
666 fraction of strains that are resistant has declined, as the vast majority in the USA since 2012 were
667 sensitive (Hu et al. 2012; Saville et al. 2015), while on other continents a mixture of sensitive
668 and resistant strains exist (Chmielarz et al. 2014; Han et al. 2013; Klarfeld et al. 2009; Pule et al.
669 2013). Mefenoxam, which has strong systemic activity, maintains value even though new
670 chemistries have appeared. Understanding the genetic basis of resistance could lead to a fast
671 assay for the trait to aid management decisions, and reveal what processes may cause resistance
672 to emerge against other fungicides in the future.
673 Studies of genetic crosses indicated that a semi dominant major locus determines resistance to
674 metalaxyl, since insensitive and sensitive parents usually yielded progeny with those phenotypes
675 at a 1:1 ratio (Fabritius and Judelson 1997; Judelson and Roberts 1999; Lee et al. 1999). Genes
676 influencing sensitivity to lesser degrees also segregated, hence resistance may be considered a
677 quantitative trait determined by one (or more) major genes plus genes of minor effect. Following
678 a lead that metalaxyl inhibited ribosomal RNA synthesis (Davidse et al. 1988), one group
679 implicated a tyrosine to phenylalanine change at amino acid 382 of the RNA polymerase 1
680 subunit 1 protein as a major factor in resistance (Randall et al. 2014). The existence of other
681 genes determining insensitivity was also suggested, since not all resistant isolates contained the
682 tyr → phe (Y382F) change and the locus did not co-segregate tightly with resistance.

683 Consequently, a full understanding of what causes insensitivity to metalaxyl remains to be
684 learned.

685 Mechanisms that influence the metalaxyl-sensitivity of *P. infestans* may also affect responses
686 to other chemistries. Isolates exhibit 10-fold or more variation in baseline sensitivity to many
687 fungicides including cymoxanil, dithiocarbamates, mandipropamid, and strobilurins (Daayf and
688 Platt 2002; Judelson and Senthil 2006b; Grunwald et al. 2006; Samoucha and Cohen 1984;
689 Saville et al. 2015b). Positive correlations between sensitivities to fungicides in distinct
690 chemical classes are described in natural isolates and strains selected for resistance after UV-
691 mutagenesis (Judelson and Senthil 2006; Ziogas et al. 2006). Genes causing cross-resistance in
692 other species include detoxifying enzymes and efflux pumps such as cytochrome P450s and
693 ABC transporters, respectively (Abou Ammar et al. 2013; Bauer et al. 1999; Leroux et al. 2002).
694 Proof that such genes are responsible for cross-resistance in *P. infestans* is lacking, but strains
695 adapted to growth on metalaxyl were found to express higher levels of two ABC transporters
696 (Childers et al. 2015). In the future, changes in such genes within *P. infestans* populations may
697 not cause total control failures, but may reduce the effectiveness of fungicides or require
698 increases in application rates. Studies in fungi (Cools et al. 2013) suggest that such changes
699 usually have fitness costs. Whether the same is true for oomycetes, with their diploid and more
700 plastic genomes, remains to be tested with rigor.

701

702 **Looking to the future**

703 Recent strides in rapid genotyping of *P. infestans* isolates during an epidemic within a season
704 from locations across the USA have improved our ability to make quick and knowledgeable
705 disease management recommendations to tomato and potato growers. Using information that is

now publically available on USAblight.org, it is possible to know where reported late blight outbreaks are occurring and the clonal lineage of the pathogen causing the outbreak. Continued monitoring and genotyping of future outbreaks is critical for advanced warning of pending epidemics. This monitoring will also identify the emergence of novel linages of *P. infestans*.

Monitoring in the fairly near future will be able to take on an evolutionary approach. To date, a new clonal lineage of *P. infestans* has been named in the USA based on polymorphism for RFLP, isozyme and/or SSR markers. The distinguishing feature of an evolutionary approach, incorporating genome wide information, and high density SNP genotyping, will allow determination if a lineage arises by migration or by mutation, recombination or hybridization from one or more existing clonal lineages. An evolutionary framework would allow distinction of identity-by-migration from identity-by-descent and provide new insights into what makes lineages emerge and disappear time and time again (Grünwald and Goss 2011). Another parallel aspect building on the evolutionary framework is use of whole genome sequence data to identify effectors and adaptive genes such as RXLR effectors as described above, mefenoxam resistance and mating type that provide a newly emerging lineage with increased fitness (Cooke et al. 2012). Factors that contribute to the decline in prominence of a clonal lineage will be an interesting question to attack as we move forward.

Finally, the role that a changing environment will play on late blight epidemics is an important consideration. As breeders use genomic approaches to develop durable resistance against late blight in tomato and potato, it will be important to ensure that resistance holds up under a wide range of environmental conditions. Current studies tend to test resistance in a small number of environments over several years (Hansen et al. 2014), although a more powerful approach may be to test breeding lines over a wide geographic area covering temperate to sub-tropical

729 environments with varying regional soil types and growing practices. This genotype by
730 environment approach may help identify a more durable resistance. A changing environment
731 may also modify the timing of initial inoculum of *P. infestans* present in a region, and the
732 development of early detection strategies including methods to detect air-borne sporangia (and
733 optimally detect fungicide sensitivity and mating type of these sporangia) will aid in disease
734 management.

735 **Concluding comments**

736 During the past decade the global community working on the biology and management of *P.*
737 *infestans* has learned a tremendous amount about its genomics, pathogenicity, population
738 genetics, and evolutionary capacity, and thus our respect for this organism as a formidable foe
739 continues to grow. With increased globalization, we have realized that the challenges of one
740 region can readily be transported to other regions. Now management as well as science has close
741 international connections. Because our early hopes of finding a "silver bullet" for management
742 have not yet been realized, and because we have not yet been able to convert potatoes and
743 tomatoes into non-host plants, we need to be alert to the many factors that influence epidemics
744 and employ all appropriate management tactics. We think that enhanced and more rapid
745 diagnostic and genotyping technologies will contribute to better informed management
746 strategies, and we expect these contributions to come on line in the near future. We also fully
747 expect to learn more about how *P. infestans* interferes with plant defenses which could enable
748 the discovery of new approaches to managing this pathogen. As result, we expect that in the
749 next review of emerging pathogens, *Phytophthora infestans* will again be included.

750

751 **Acknowledgements**:

The work reviewed here was supported by many institutions and funding agencies. Acquisition of the new data (Figures 5-7) was supported by our institutions and by the Agriculture and Food Research Initiative Competitive Grants Program (Grant No. 2011-68004-30154) from the USDA.

Figure Legends:

Figure 1. Illustrations of devastation on potatoes caused by *Phytophthora infestans.* (A) Field of potatoes in which all foliage has been destroyed by *P. infestans*. Only weeds are green. Repeated asexual cycles of reproduction lead to very rapid destruction of foliage. (B) Potato tubers infected by *P. infestans.* Sporangia washed through the soil contact tubers and lead to infections.

Figure 2. Devastation of tomatoes by *Phytophthora infestans*. (A) Tomato plants severely affected by late blight on a small farm. Most of the lower foliage had already been killed. This was the only planting of 10 different plantings of tomatoes on this particular farm that had not yet been totally destroyed. (B) Tomato fruits infected by *P. infestans* (photo by T. A. Zitter).

Figure 3. The asexual life cycle of *Phytophthora infestans* on potato tissue. After moist conditions for several hours (at least 6-8 hours) at moderate temperatures (15-22˚ C), the pathogen sporulates from lesions (A). Sporangia are borne on sporangiophores (B), and sporangia are dehiscent (readily removed from sporangiophores) and are aerially dispersed (C). Sporangia can germinate directly (via a germ tube at warmer temperatures (> 18˚C)), or at lower

775 temperatures (<18˚C) via zoospores (D). Within 3-6 days, young lesions appear on after
776 infection on host tissue (E). This image was first published in (Fry 2008), and subsequently in
777 (Fry et al. 2013).
778
779 Figure 4. Search results in "Google Trends" for "tomato blight" or "late blight" from 2008 to
780 2013. The relative results are reported with the maximum being reported during the summer
781 2009. The number of searches on tomato blight + late blight in 2009 was at least triple that in
782 any other year during this period. (The absolute number of searches was not discernable from
783 the website.)
784
785 Figure 5. Reports of diverse genotypes of *P. infestans* by state from 2009 through 2014. The
786 genotype is indicated by the color scheme identified in Figure 6. The number of the samples
787 from a state is identified in the circle and the size of the circle reflects the number of samples
788 reported. In many states in 2012, 2013 and 2014 only US23 was reported.
789
790 Figure 6: Dominant clonal lineages detected in the USA from 1997 through 2014. * The data
791 for 1997-2008 come from the Fry Lab; Hu et al 2012; and Wangsomboondee and Ristaino
792 b2002; the data for 2009-2014 come from the Fry lab, the Ristaino lab and the USAblight
793 consortium. The sample size for each year is indicated in parentheses at the top of each column.
794 Figure 7. Occurrence of different clonal lineages of *Phytophthora infestans* on potatoes (A) or
795 tomatoes (B) from 2009 through 2014. US8 and US24 are restricted mainly to potatoes. The
796 data for 2009-2014 come from the Fry lab, the Ristaino lab and the USAblight consortium.
797

Figure 8. The first late blight observation (in days after planting) in Finnish potatoes from 1991 through 2002 (Hannukkala et al. 2007; redrawn and used with permission of the author and publisher).

Figure 9. The estimated number of fungicide applications made by Finnish farmers to potato crops from 1983 to 2002 (Hannukkala et al. 2007; redrawn and used with permission of the author and publisher).

Table 1. Phenotypic characteristics of the most common clonal lineages detected in the USA 2009-2014. (Data are from Childers et al 2015; Danies et al 2013, and Hu et al 2012)

Lineage	A1/A2	Host Preference	Mefenoxam sensitivity
US8	A2	Potato	moderately resistant
US11	A1	Potato and Tomato	resistant
US22	A2	Potato and Tomato	sensitive
US23	A1	Potato and Tomato	sensitive – moderately sensitive
US24	A1	Potato	moderately sensitive

Figure 1.

Figure 2.

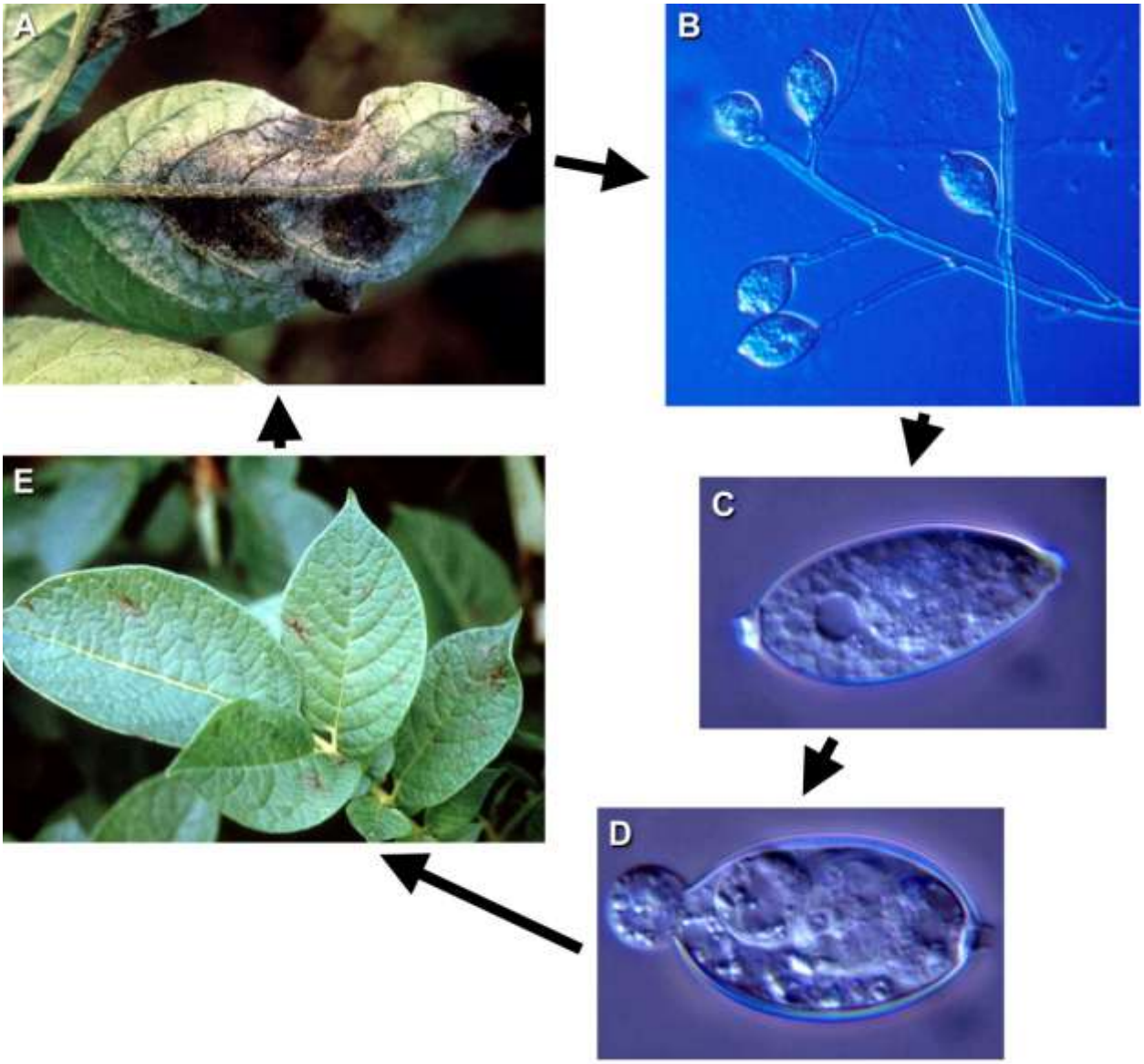


Figure 3.

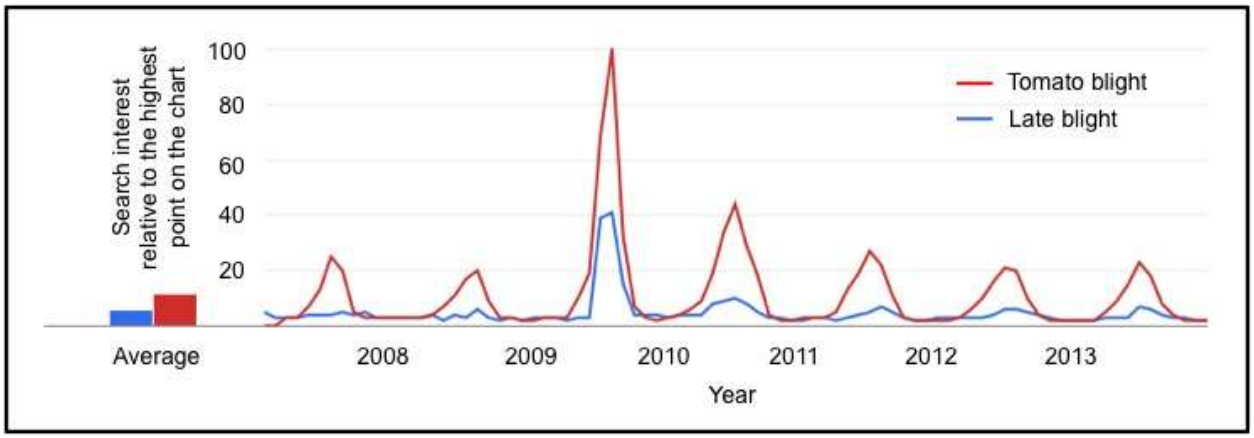

Search interest relative to the highest point on the chart
100
80
60
40
20
Tomato blight
Late blight
Average
2008
2009
2010
2011
2012
2013
Year

Figure 4.

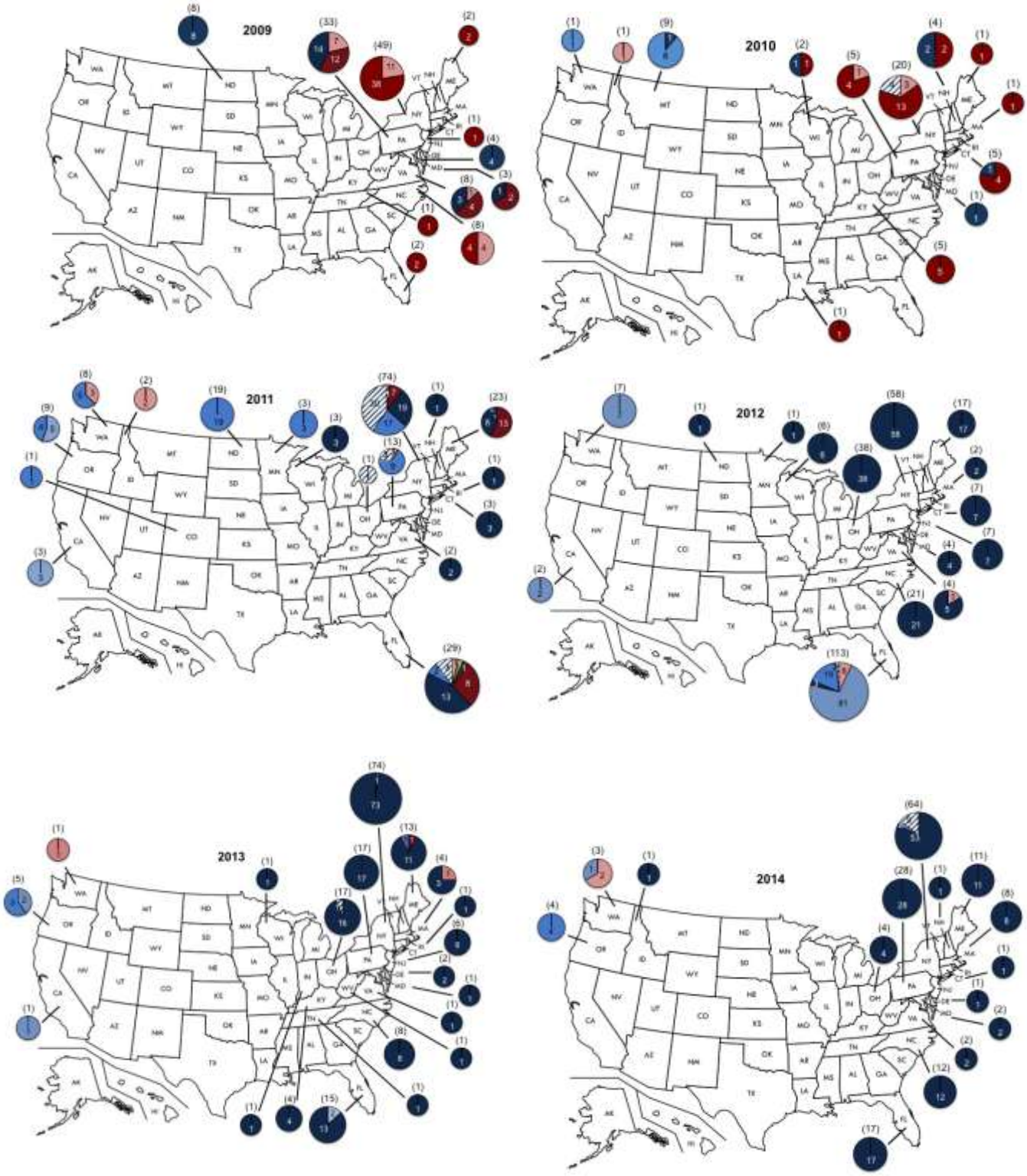


Figure 5.

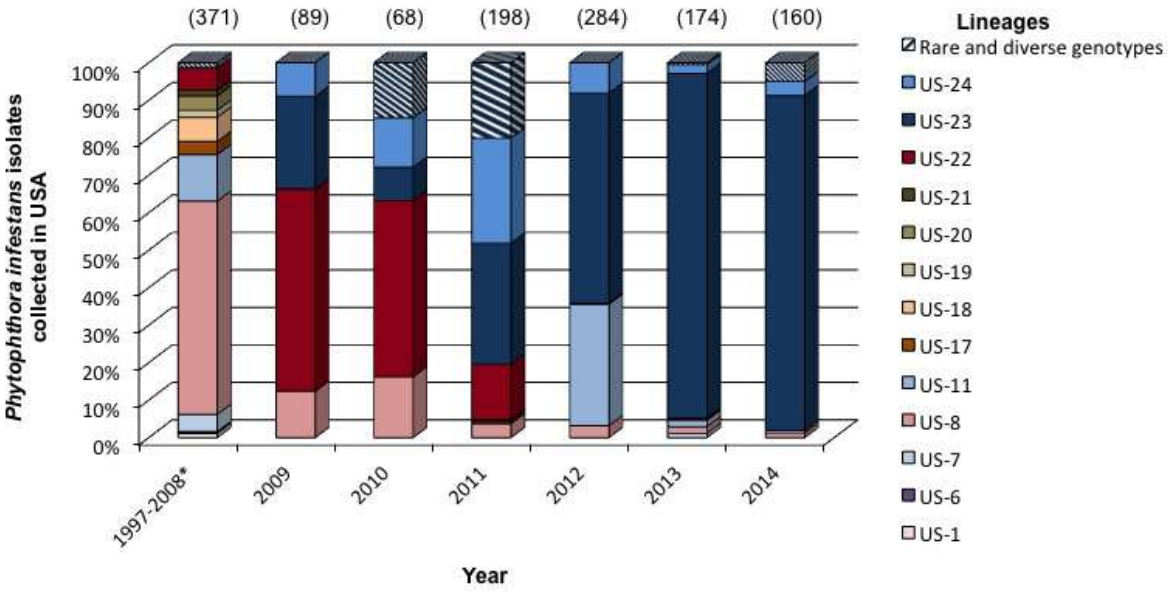
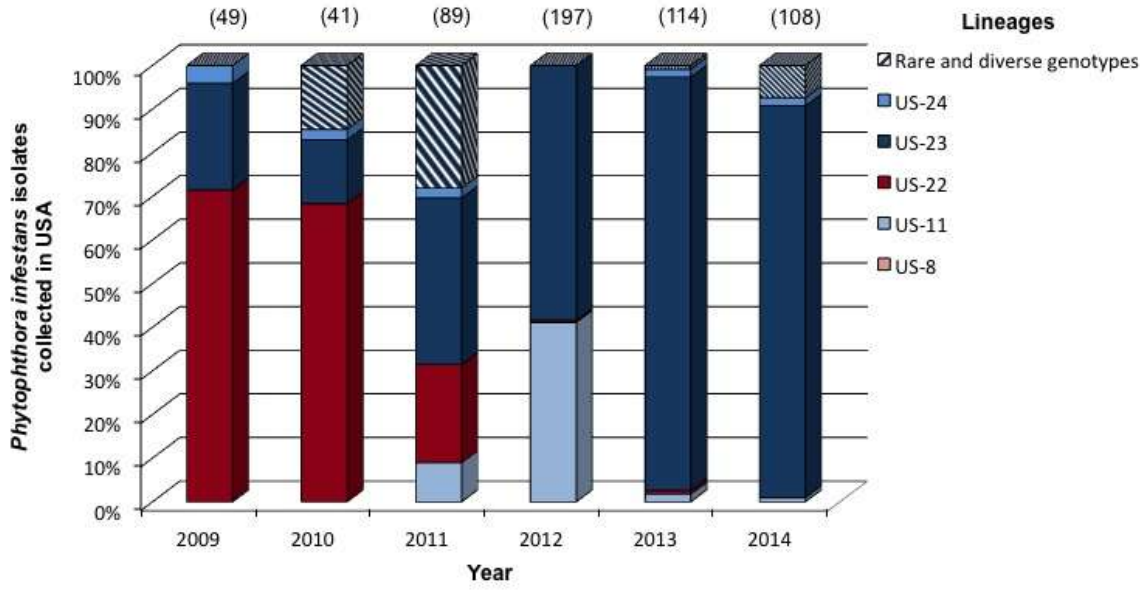

(371)
(89)
(68)
(198)
(284)
(174)
(160)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Phytophthora infestans isolates collected in USA
1997-2008*
2009
2010
2011
2012
2013
2014
Year
Lineages
Rare and diverse genotypes
US-24
US-23
US-22
US-21
US-20
US-19
US-18
US-17
US-11
US-8
US-7
US-6
US-1

Figure 6:

A


(49)
(41)
(89)
(197)
(114)
(108)
Lineages
Rare and diverse genotypes
US-24
US-23
US-22
US-11
US-8
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Phytophthora infestans isolates collected in USA
2009
2010
2011
2012
2013
2014
Year

B

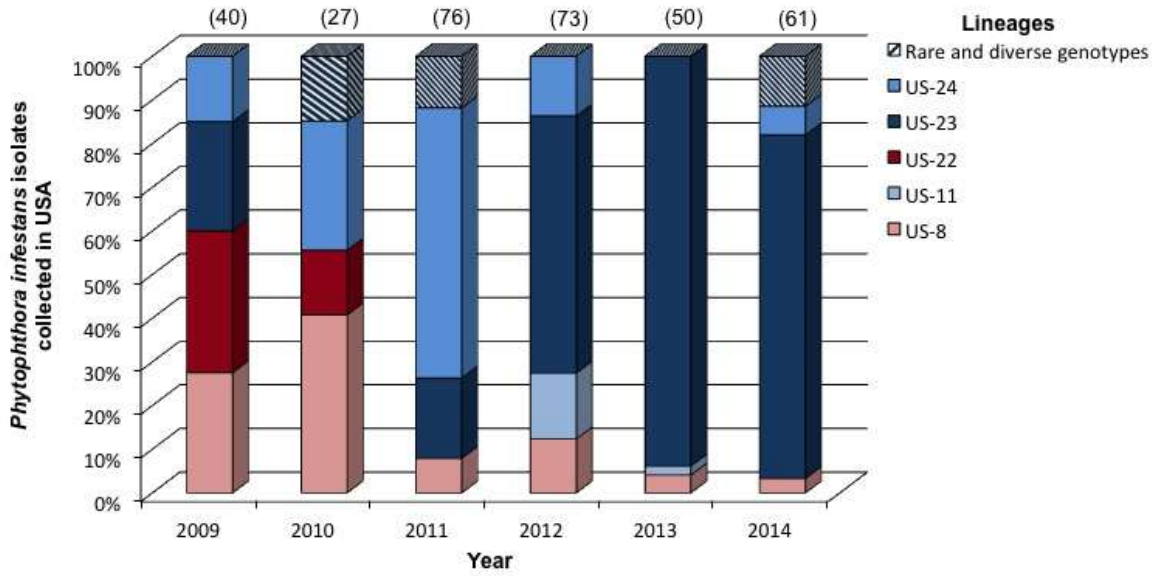

(40)
(27)
(76)
(73)
(50)
(61)
Lineages
Rare and diverse genotypes
US-24
US-23
US-22
US-11
US-8
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Phytophthora infestans isolates collected in USA
2009
2010
2011
2012
2013
2014
Year

Figure 7.

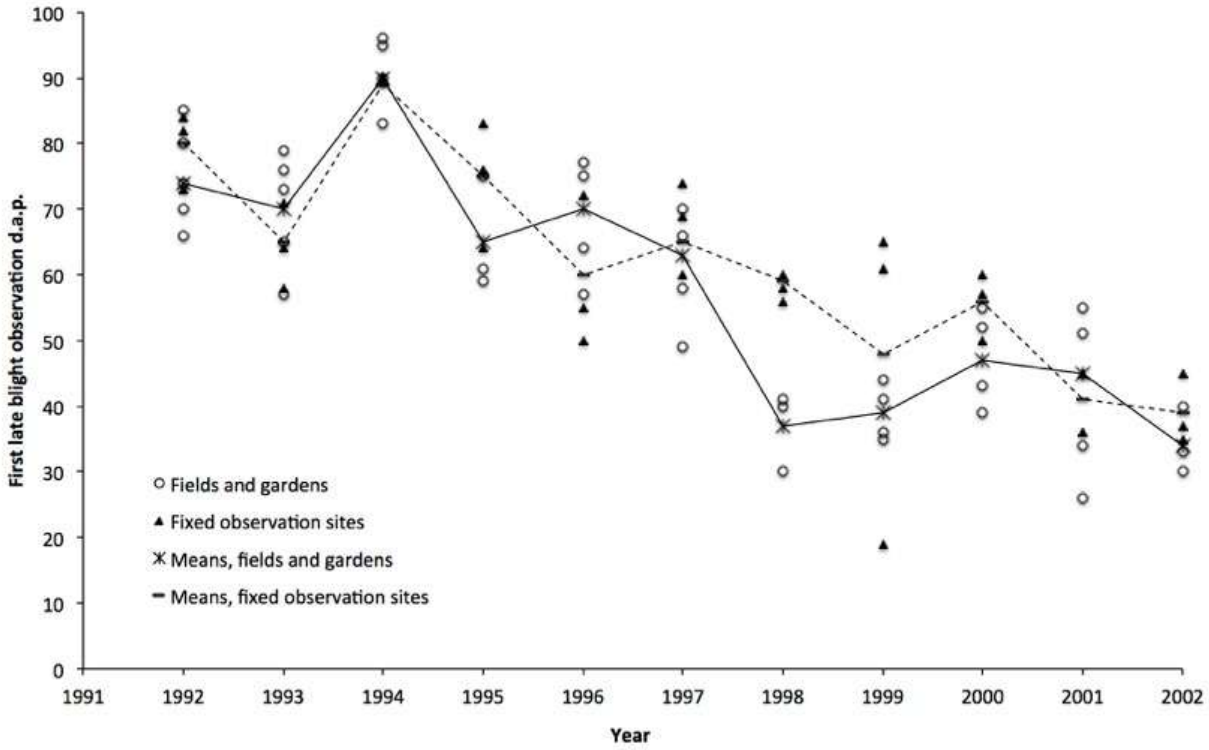

First late blight observation d.a.p.
Year
Fields and gardens
Fixed observation sites
Means, fields and gardens
Means, fixed observation sites
0
10
20
30
40
50
60
70
80
90
100
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002

Figure 8.

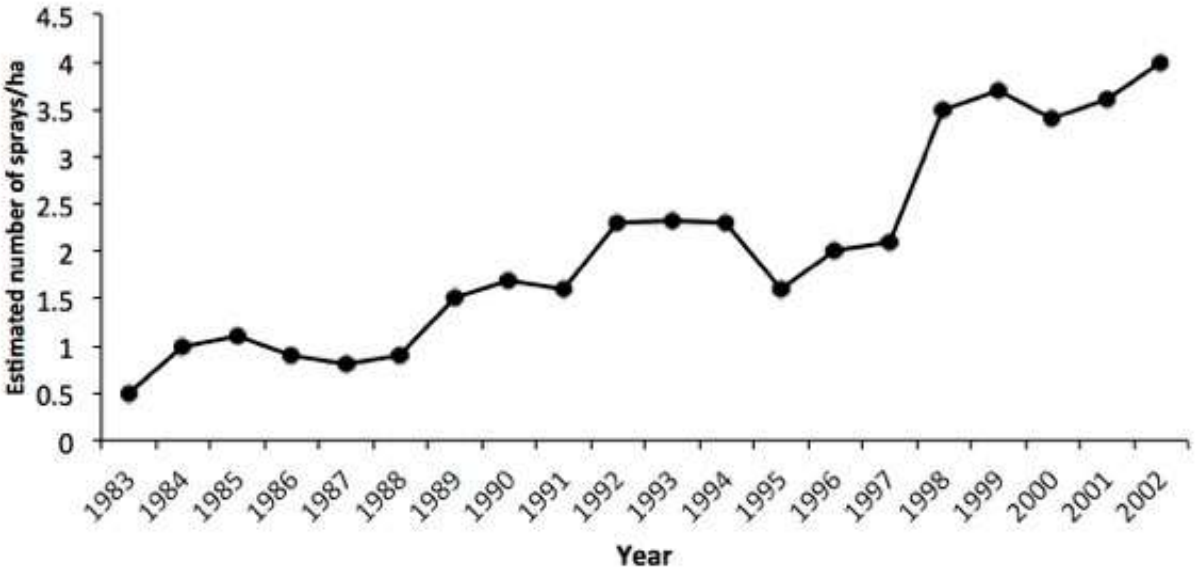

Estimated number of sprays/ha
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
Year

Figure 9.