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Phytoprotection, vol. 80, n° 2, 1999, p. 85-95.

Symposium / Symposium – Saint-Jean-sur-Richelieu (Québec), Canada
9 juin / 9 June 1999
Ravageurs nouveaux et en ré-émergence / New and re-emerging pests

Molecular tracking new migrations of an old pathogen : the re-emergence of potato late blight

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PHYTOPROTECTION 80 : 85-95

Late blight of potato is caused by the oomycete *Phytophthora infestans* (Mont.) de Bary. Although often considered to be fungi, oomycetes are phylogenetically much more closely related to certain groups of algae and are now considered part of a separate kingdom, the *Stramenopila*. Thus, in an evolutionary context *P. infestans* is more like an alga that has lost its chlorophyll rather than a fungus. However, *P. infestans* is very similar to a fungus morphologically and in the ecological niche it fills.

This organism is diploid at all stages in its life cycle and requires two mating types, designated A1 and A2, for sexual reproduction. The result of sexual reproduction is an oospore, which is resistant to environmental extremes (e.g., freezing, drying) and can survive for many years in soil. Asexual reproduction is by sporangia, which are produced in huge quantities on the surface of infected tissue. The sporangia are easily detached and highly adapted for aerial dispersal. However, they do not survive freezing or drying so are not persistent in the environment. Sporangia can germinate and penetrate plant parts directly, but if exposed to water and chilled they germinate indirectly by releasing zoospores. As implied by their name, zoospores are motile and can swim towards a suitable host with the aid of two flagella. Each sporangium can release many zoospores.

Late blight is one of the most destructive plant diseases known and can decimate entire fields of potatoes or tomatoes in only a few weeks. In addition to destroying the stems and foliage, the pathogen attacks tubers and fruits and can cause 100% yield loss. After being controlled for decades in most of the developed countries, late blight re-appeared during the 1980s and 1990s. The purpose of this paper is to summarize what is known about the re-emergence of late blight and to suggest ways to minimize similar problems with this and other diseases in the future.

MOLECULAR MARKERS FOR PATHOGEN TRACKING

More is known about the biology and past history of *P. infestans* than almost any other plant pathogen. Much of this information was derived from the application of molecular marker technologies. Two types of molecular marker have provided the majority of information: the allozyme loci *glucose-6-phosphate isomerase* (*Gpi*) and *peptidase* (*Pep*); and DNA fingerprinting with probe RG57.

Note du rédacteur : le texte ci-dessus est présenté tel que soumis / Editor's note : the above text is presented as submitted.

Allozymes are genetic variants at the same enzyme locus. Each allozyme has a slightly different amino acid composition that alters its mobility in an electric field but does not affect its catalytic ability. These amino acid variants are detected as different bands on electrophoretic gels. The bands are visualized by soaking the gels in a solution containing the substrate for each particular enzyme. When the enzyme performs its catalytic function, it generally causes one of the chemicals in the staining solution to form a colored precipitate which accumulates in a band corresponding to the region of enzyme activity. So far, there are at least seven known alleles for *Gpi* in *P. infestans* and five for *Pep*.

DNA fingerprinting uses probe RG57 (Goodwin *et al.* 1992a). This is a moderately repetitive DNA fragment that was cloned from the nuclear genome of *P. infestans*. When total nuclear DNA of *P. infestans* is digested with the restriction enzyme *Eco* RI, size fractionated on an electrophoretic gel, blotted to a membrane and hybridized with RG57, each isolate generally shows from 5-15 bands which are highly variable among isolates. Scoring all of the bands together provides a specific DNA "fingerprint" for each isolate. Genetic analyses of the fingerprint patterns have shown that each band behaves as a separate Mendelian locus, and most of the loci are unlinked (Goodwin *et al.* 1992a). This provides a powerful tool for analyzing the population biology of *P. infestans*.

Other molecular markers also have been used for *P. infestans*. These include single-copy nuclear and mitochondrial DNA restriction fragment length polymorphisms (Carter *et al.* 1990; Goodwin 1991), random amplified polymorphic DNA (Goodwin *et al.* 1999), and amplified fragment length polymorphisms. Although these provide additional information, they have mostly just confirmed what was already known from analyses of allozymes and DNA fingerprints so will not be discussed further here.

In addition to the molecular markers, a number of phenotypic traits also are analyzed for *P. infestans*. These include mating type (A1 or A2), virulence to a number of potato and tomato resistance genes, and resistance to the systemic fungicide metalaxyl.

THE ORIGIN OF POTATO LATE BLIGHT

Analyses with all of the above markers revealed that *P. infestans* probably evolved in a limited area in the highlands of central Mexico. This is the area in which both mating types were first found in approximately equal frequency (Niederhauser 1991). It also has the highest diversity for almost every molecular and phenotypic marker analyzed, including virulence (Goodwin *et al.* 1995b), allozymes (Goodwin *et al.* 1992b; Tooley *et al.* 1985) and DNA fingerprints (Goodwin 1996; Goodwin *et al.* 1992b). Molecular markers in this geographic area are in Hardy-Weinberg equilibrium (Goodwin *et al.* 1992b; Tooley *et al.* 1985), an indication of regular sexual recombination.

The original hosts for *P. infestans* were probably wild species of *Solanum*. Mexico is a secondary center of diversity for the genus *Solanum* (Correll 1962), with many endemic species. Some of these species, particularly *S. demissum*, contain numerous specific genes for resistance against *P. infestans* that probably developed during a long period of coevolution.

Cultivated potatoes evolved in the Andes region of South America and probably were not exposed to *P. infestans* until the 1800s. This probably explains the high susceptibility of cultivated potatoes to *P. infestans* (there was no selection for resistance) and also may indicate why it took more than 250 years after the introduction of potatoes to Europe before late blight appeared (Goodwin 1996). In a sense, late blight of potato and tomato as we know it today is a man-made disease created by the movement of host and pathogen to new locations.

POTATO LATE BLIGHT : 1843-1976

The first epidemic of late blight outside Mexico probably occurred in the eastern United States during 1843 (Stevens 1933). An analysis of reports of the new potato disease indicated a probable focal point near New York or New Jersey. From its point of introduction, the pathogen spread rapidly and by the end of 1845 late blight was known throughout the northeastern United States, from North Carolina in the south, west to Illinois, and north into the Maritime provinces of Canada and the southern portions of Ontario and Québec (Stevens 1933). Most of this rapid spread probably was from field to field as airborne sporangia, although some movement of infected potato tubers also may have occurred.

A similar pattern occurred in Europe. The disease was first reported in Belgium during late June 1845, and by the end of the year it had spread east into Germany and northern Poland, south through France and possibly northern Italy, north into Scandinavia, and west across Britain and Ireland (Bourke 1964). Most of this spread probably was by airborne sporangia. The disease destroyed the potato crop in Ireland during 1845 and subsequent years leading to the great Irish potato famine, which reduced the human population of that island by more than half due to starvation and emigration.

Recent analyses with molecular markers have shown that these initial migrations probably occurred in three steps (Goodwin 1997; Goodwin *et al.* 1994b). The first step probably was from Mexico to the USA during or shortly before 1843. Transport most likely was in tubers of wild *Solanum* species (Goodwin 1997). This migration may have included a number of genotypes, although it probably did not include the A2 mating type (Goodwin *et al.* 1994a). The second step probably was from the USA to Europe in cultivated potato tubers during 1844 or 1845 and may have included only a single genotype, US-1 (Goodwin *et al.* 1994b). The third step probably was from Europe to the rest of the world in seed or ware potatoes beginning after 1845 (summarized in Goodwin 1997).

The result of these early migrations is that a single clonal lineage, designated US-1, was spread throughout the world. All the members of this lineage may be descended from a single individual that was transported from Mexico to the United States during 1843.

The widespread distribution of US-1 is important for understanding the biology and epidemiology of late blight. If the above scenario is correct, then for more than 130 years late blight in most of the world was caused exclusively by US-1 and its clonal descendants. Almost all of the genetic variation within the species remained behind in Mexico. The US-1 clonal lineage is A1 mating type and, by itself, is limited to asexual reproduction. Clonal populations of US-1 must survive winter cold and summer drought as mycelium inside living tissue, mostly potato tubers but also possibly in tomato fruits (Vartanian and Endo 1985). Because epidemics were caused by the same clone from year to year, growers knew what to expect and plant pathologists could devise effective strategies for disease management. During this time, epidemics were rare and sporadic and mostly controlled with a combination of crop hygiene (planting disease-free seed, removing cull piles) and judicial use of fungicides.

THE RESURGENCE OF LATE BLIGHT IN EUROPE : 1976-1990

This changed during the early 1980s in Europe, when late blight suddenly became much more difficult to control. It was soon realized that this was due at least in part to the development of resistance to the most effective fungicide, metalaxyl. This fungicide provided almost complete protection against epidemics caused by US-1. However, resistance developed rapidly during the 1980s. This stimulated an increase in research, and it was soon discovered

that, in addition to fungicide resistance, the A2 mating type also was present. Analyses of stored cultures showed that the A2 mating type had been present in western Europe at least since 1981 (Hohl and Iselin 1984).

When these populations were analyzed with molecular markers, it was soon discovered that the increased disease problems were due to the migration of new genotypes of *P. infestans* from Mexico (Fry *et al.* 1993; Goodwin *et al.* 1994b; Spielman *et al.* 1991). These new populations were different for mating type, allozymes, DNA fingerprints and mitochondrial DNA markers (Fry *et al.* 1993), and also had a higher diversity for virulence (Drenth *et al.* 1994; Sujkowski *et al.* 1996).

In addition, they must have had a much higher fitness, because they rapidly replaced US-1 in all locations analyzed. Total replacement of US-1 usually occurred within 2-4 years of the first detection of new genotypes (Fry *et al.* 1993). Similar replacements of US-1 by new genotypes also began to occur in Africa, Asia and South America in addition to Europe.

THE RESURGENCE OF POTATO (AND TOMATO) LATE BLIGHT IN THE UNITED STATES AND CANADA : 1990-1998

Those of us in northern North America sat by smugly as the new populations swept through Europe. We were confident that the same things would not happen on our side of the Atlantic. Some speculated that the rapid development of fungicide resistance in Europe must have been due to inappropriate applications, not in accordance with the label instructions. Others thought the problems must be the result of careless crop sanitation, or to the importation of potatoes from North Africa or Asia. Such problems would never occur in North America; we were much too careful for that. We also had an unseen ally. In most years in the potato-growing regions of North America, the weather is a little warmer and drier than in Europe and, therefore, not as favorable for *P. infestans*.

This all changed beginning in 1992. Late blight epidemics suddenly appeared in many of the potato-growing regions of the USA and Canada (Fry and Goodwin 1997b; Goodwin *et al.* 1995a). In contrast to previous years, these epidemics caused much more damage and were not easily controlled with metalaxyl. Similar problems occurred on tomatoes. Molecular marker analyses soon revealed the cause of the epidemics: new genotypes that were A2 mating type and highly resistant to metalaxyl (Goodwin *et al.* 1995a). Surprisingly, these genotypes were not related to the new genotypes in Europe. Instead, they were identical to genotypes identified a few years earlier in northwestern Mexico (Goodwin *et al.* 1995a).

The same pattern continued during 1993 as the new genotypes spread to additional areas. By 1996 it was clear that late blight in the United States and Canada was caused primarily by a handful of genotypes (Table 1). It was also clear that US-1 was disappearing rapidly (Fig. 1). The replacement of US-1

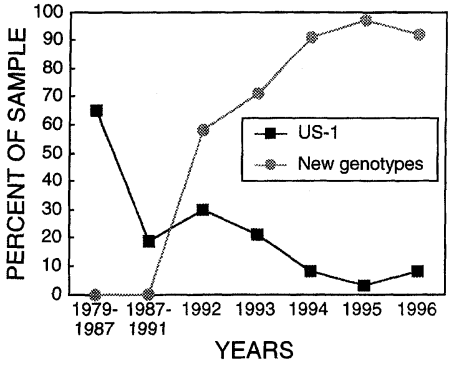

Figure 1. Replacement of the US-1 clonal lineage of *Phytophthora infestans* in the United States and Canada by new genotypes that were first detected during 1992 (genotypes US-7 and above). This analysis is based on data from 1112 isolates. The US-6 clonal lineage probably migrated into the United States and Canada during the late 1970s or early 1980s so was excluded from this analysis.

Table 1. The major genotypes of *Phytophthora infestans* detected in the United States and Canada from 1979 through 1996

Genotype	Mating type	Allozyme genotype		RG57 DNA fingerprint[c]	Metalaxyl sensitivity[d]	Primary host(s)	Comments
		Gpi[a]	Pep[b]				
US-1	A1	86/100	92/100	10111010110011010000110011	S	potato, tomato	Old clonal lineage found throughout the world
US-6	A1	100/100	92/100	10111110010011000010110011	R or S	tomato, potato	Introduced from northwestern Mexico during the late 1970s or early 1980s. Replaced by US-7
US-7	A2	100/111	100/100	10011000010011010010110011	R	tomato	Introduced from northwestern Mexico during the early 1990s. Replaced US-1 and US-6 on tomato
US-8	A2	100/111/122	100/100	10011000010011010000110111	R	potato	Introduced from northwestern Mexico during the early 1990s. Replaced US-1 and US-6 on potato
US-11	A1	100/100/111	100/100	10101110010011010010110011	R	potato	Possibly generated by recombination in the Pacific northwest. May be a competitor for US-8
US-17	A1	100/122	100/100	10100010000011010010110011	R	tomato	Probably a new sexual recombinant. Replacing US-7

[a] Glucose-6-phosphate isomerase.
[b] Peptidase.
[c] Presence (1) or absence (0) of RG57 fingerprint bands 1 - 25 (Goodwin *et al.* 1992a) are indicated from left to right. DNA fingerprint band 4 is not reproducible and should not be used for genotype identification.
[d] R = resistant; S = sensitive.

began during the late 1970s or early 1980s with the introduction of the US-6 genotype from northwestern Mexico (Goodwin *et al.* 1994a). It accelerated rapidly during the 1990s when the US-7 and US-8 genotypes began replacing both US-1 and US-6. By 1996 the replacement of US-1 by new genotypes was almost complete (Fig. 1). US-1 is still found occasionally, but it makes up a very small proportion of the total *P. infestans* population (Goodwin *et al.* 1998).

In addition to replacement, there was some evidence for possible sexual reproduction. The previously dominant genotypes (US-1, US-6) were A1 mating type, but the new migrants were mostly A2. Thus, for the first time in the USA and Canada there was the possibility of sexual recombination. The first evidence for probable recombinant genotypes in the field was in British Columbia during 1992 (Goodwin *et al.* 1995a). Other evidence for possible recombinant genotypes has been found in the Columbia Basin of Washington and Oregon (Miller *et al.* 1997) and in New York (Goodwin *et al.* 1998). Sexual reproduction could make late blight much more difficult to control by providing a long-lived source of inoculum in soil and by generating a succession of new genotypes with potentially higher fitness.

The consequences of these migrations were devastating to many potato and tomato growers. The northeastern United States was particularly hard hit, and many potato growers lost their family farms (Fry and Goodwin 1997a; Table 2). The problem was not only lost production, but also the destruction of crops after harvest (Fry and Goodwin 1997b). Late blight in stored potatoes facilitates entry of many other organisms, including the bacteria that cause soft rot. Stored potatoes from infected fields often "melted" during storage, creating a horrible, smelly mess.

MECHANISMS OF MIGRATION

How could such problems develop? Clearly, the difficulties were caused by the migration of new genotypes. However, the means of long-distance transport were not known. Molecular markers provided some of the answers.

Once within the United States and Canada, long-distance transport occurred in infected potato tubers. Proof of this came from Florida. Late blight in two fields in southern Florida during 1993 was caused by US-1 (Goodwin *et al.* 1995a). This was the only region in Florida in which US-1 was detected that year, and the area had essentially no late blight for several years prior to 1993. The seed tubers for those fields came from Maine and North Dakota, and US-1 was known to be present in those states during 1992, the year the seed was produced (Goodwin *et al.* 1995a).

Table 2. Economic impact of late blight on one farm in the northeastern United States during 1994 (data courtesy of W. E. Fry, Cornell University)

Potato acreage	196 ha
Cost of production	$836 000
Cost of pesticide spray, 1994	$136 000
Average cost of pesticide spray, 1992-1993	$72 500
Average (historical) yield	6360 tons
1994 yield	2360 tons
1994 sales	1180 tons
Total value after harvest	$212 000
Net loss	$760 000

The next year, fields on one of the same farms were infected with US-8. That year, the seed was purchased from Maine, and US-8 was confirmed in samples from the same seed lot. US-1 was not found in Florida that year (Goodwin *et al.* 1998). The only likely source of inoculum for US-1 in southern Florida during 1993 and for US-8 during 1994 was infected seed potato tubers.

Movement of infected potato seed explains most of the long-distance migration of *P. infestans* within the United States and Canada, but does not explain how the new strains arrived in northern North America originally. Importation of potato tubers, especially from Mexico, is prohibited, so it seems highly unlikely that infected tubers could have been imported from Mexico into the United States or Canada. The production areas of northwestern Mexico are surrounded by arid mountains and deserts which preclude airborne movement of sporangia over long distances. Escape from culture collections in the USA or Canada seems remote, because the initial occurrences of new strains were distant from research centers and those cultures are maintained under strict quarantine.

This leaves the most likely culprit as infected tomato fruits. Tomatoes are imported from Mexico into the United States for fresh consumption. They probably are not screened as carefully for late blight, because it is usually considered only a potato disease. The area of northwestern Mexico that the DNA fingerprint data indicated as the probable source of the US-7 and US-8 genotypes also produces tomatoes.

Proof of the tomato-fruit connection finally came during 1995 when infected tomatoes from northwestern Mexico were intercepted in South Carolina (Goodwin 1997). Infection by *P. infestans* was confirmed, although no isolations were made, so it was not possible to confirm the genotype. However, the US-1, US-7 and US-8 genotypes have been recovered from infected tomato fruits. Therefore, movement of *P. infestans* in infected tomato fruits probably is a highly effective mechanism for long-distance dispersal.

Another potential method for long-distance transport of *P. infestans* is in tomato transplants. Most of the evidence for this is anecdotal. There have been rumors of movement of infected tomato transplants from Florida into New York, and from California into Washington and even British Columbia (Fry and Goodwin 1997a, 1997b). The pathogen has been isolated from tomatoes in greenhouses. However, as far as I am aware, *P. infestans* has never been confirmed on tomato transplants. The hypothesis of movement in tomato transplants needs to be tested by thorough checking in the future.

Over short distances, *P. infestans* can disperse by airborne sporangia. Nearby fields are no longer safe once *P. infestans* has been detected within a region. This was shown dramatically on home garden tomatoes in New York during 1993. From an initial focus in one county (possibly started by infected transplants), the disease spread out over six counties in less than 4 weeks (Fry and Goodwin 1997b). This spread probably was by aerial dispersal of sporangia and indicates how rapid asexual reproduction can facilitate migration by increasing the number of infective propagules available for transport.

MECHANISMS OF HIGHER FITNESS IN NEW POPULATIONS

There are many potential mechanisms to explain the higher fitness of the migrating genotypes. The most obvious one is resistance to the fungicide metalaxyl. The new genotypes US-7 and US-8 are highly resistant to metalaxyl, whereas all isolates of US-1 from the United States and Canada tested to date have been sensitive (Goodwin *et al.* 1996). Metalaxyl was still widely used at the time of the 1990s migrations and would have applied tremendous selection pressure in favor of the migrant genotypes.

The new genotypes also generally had higher virulence compared to the old US-1 clonal lineage (Goodwin *et al.* 1995b; Fig. 2). Virulence probably was

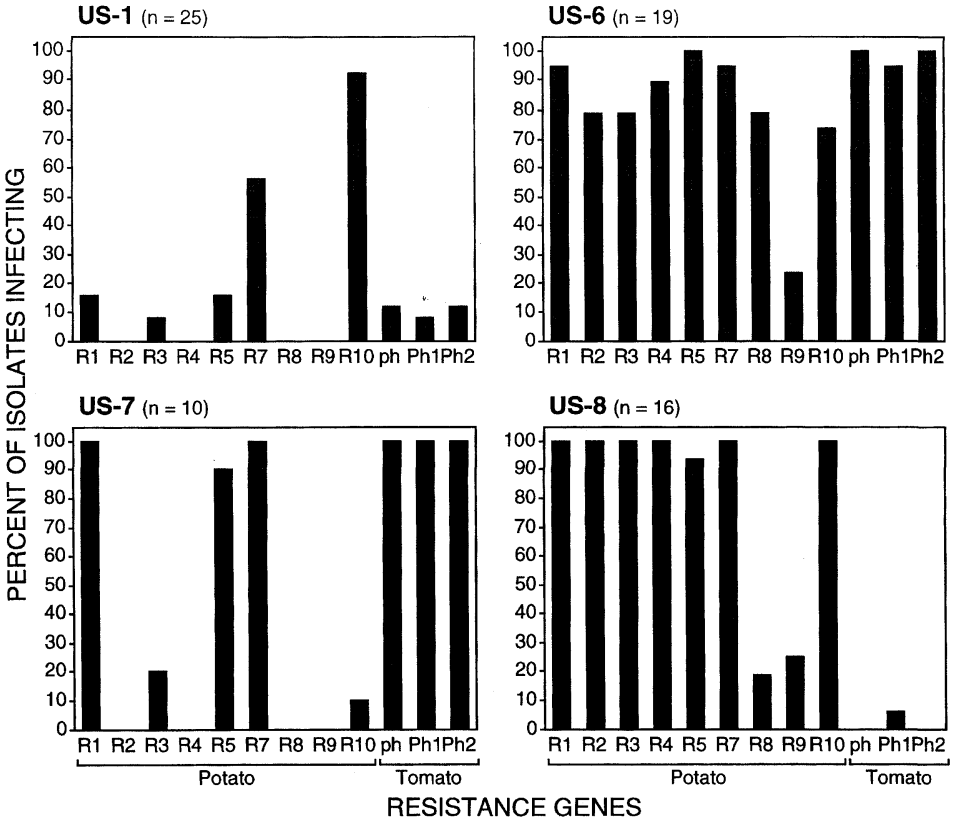

Figure 2. Virulence of isolates of *Phytophthora infestans* from the United States and Canada to potato and tomato resistance genes analyzed according to clonal lineage.

not a major factor in the higher fitness of new genotypes, because most potato and tomato cultivars in the United States and Canada do not have resistance genes. However, higher virulence may have helped with infection of the rare cultivars that did have one or more resistance genes, and may indicate that the new genotypes were from populations that were exposed to resistance genes (e.g., from wild hosts) in the recent past.

Evidence is beginning to accumulate that the new genotypes are more aggressive. They may cause greater infection of potato tubers (Lambert and Currier 1997), have shorter latent periods, and cause larger lesions with more sporulation (Kato *et al.* 1997; Miller *et al.* 1998). Greater aggressiveness would have allowed the new genotypes to replace US-1 in the absence of other contributing factors.

Another possible mechanism of greater fitness, at least in Europe, is sexual reproduction. There is now good evidence for sexual reproduction of the new genotypes in Poland (Sujkowski *et al.* 1994) and the Netherlands (Drenth *et al.* 1994, 1995). The sexual cycle generates an additional source of inoculum as oospores that could provide a huge advantage compared to asexual reproduction alone. Large numbers of overwintering oospores could overwhelm the low level of survival of asexual lineages in living tissue.

A final potential explanation for the mechanism of greater fitness could be Muller's ratchet. This phenomenon occurs when the most fit genotype

within a clonal lineage fails to survive by chance (genetic drift). When this occurs, the mean fitness of the clonal population decreases (Muller 1964). During many asexual generations, the fitness can ratchet downwards considerably. The speed of the ratchet depends on the rate of mutation and the population size; it proceeds fastest with high mutation rates and small populations. Populations can reach "mutational meltdown" after only 10^3 asexual generations (Lynch *et al.* 1993). The US-1 clonal lineage may have experienced near this number of asexual generations by the 1980s and 1990s (Drenth *et al.* 1993; Goodwin 1997), so its fitness could have been much lower than when it originally escaped from Mexico. This may explain the relatively low fitness of US-1 compared to US-7 and US-8, which probably arose from recent sexual populations. Muller's ratchet could lower all aspects of fitness so most studies that evaluate differences in fitness components between US-1 and the new genotypes may find that US-1 is less fit.

CONCLUSIONS

The global resurgence of late blight was caused by the migration of new genotypes with higher fitness. These new genotypes originated in Mexico, the center of diversity for the pathogen. This underscores the importance of preventing new migrations of old pathogens, particularly from their original centers of diversity which may contain huge reservoirs of genetic variation.

There appear to have been at least three recent migrations out of Mexico that brought different genotypes to Europe, east Asia and northern North America. The new European populations already may have spread to Africa and South America. There is still a great potential for genetic exchange among these regions. The US-7 and US-8 genotypes may be even more damaging in Europe than in North America. Conversely, the European genotypes may cause more problems in North America. Those are questions we do not want answered. Hopefully, quarantine procedures can minimize future intercontinental migrations.

The long-term consequences of these migrations for potato and tomato producers in North America are not known. Two potential outcomes seem likely. One is the development of sexually reproducing populations, which may be occurring in certain parts of Europe. The other is that there will be a gradual replacement of clonal lineages over years, as new clones with higher fitness are generated by occasional sexual recombination or possibly by rare mutations within lineages.

Clonal replacement seems to be occurring in northwestern Mexico (S.B. Goodwin, unpublished), the original source for many of these genotypes, and already seems to be occurring on tomatoes in the United States and Canada. Tomatoes originally were infected by US-1, which gave way to US-6 during the late 1980s and early 1990s (Goodwin *et al.* 1994a). US-7 replaced US-6 from about 1992-1995 (Goodwin *et al.* 1995a, 1998). Since 1996 an even newer genotype, US-17, has been replacing US-1 (Goodwin *et al.* 1998). A similar situation may be occurring on potatoes with the appearance of US-11.

This means that it is not possible to predict which genotypes will occur in a location from year to year. Genotypes in seed tubers provided a much better prediction of what would occur in a field than the genotypes that were present in the same location the year before (Goodwin *et al.* 1998). Planting disease-free seed and minimizing other potential sources of inoculum are of paramount importance with a clonal pathogen population.

Molecular markers provide a powerful tool for tracking pathogen migrations, but are not a substitute for traditional monitoring and quarantine activities. Better communication among growers, scientists and quarantine officials is essential to minimize future migrations and reduce the damage they cause. We must always remain vigilant for new forms of old foes that may be

waiting for transport to pristine agricultural areas.

ACKNOWLEDGMENTS

I thank Bill Fry for many years of postdoctoral support during which most of the work for this paper was performed. I also thank the USDA-Agricultural Research Service and the Department of Botany and Plant Pathology, Purdue University for current support, and Larry Dunkle for helpful comments on a previous draft of the manuscript.

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