Temporal and Spatial Patterns of Genetic Structure of *Phytophthora infestans* from Tomato and Potato in the Del Fuerte Valley

R. Jaime-Garcia, R. Trinidad-Correa, R. Felix-Gastelum, T. V. Orum, C. C. Wasmann, and M. R. Nelson

First, fourth, fifth, and sixth authors: Department of Plant Pathology, University of Arizona, Tucson 85721; second author: Alimentos Del Fuerte, Los Mochis, Sinaloa, Mexico; and third author: Agridiagnosticos, Los Mochis, Sinaloa, Mexico.

Current address of R. Jaime-Garcia: USDA-ARS-SRRC, 1100 Robert E. Lee Boulevard, New Orleans, LA 70124.

Current address of R. Trinidad-Correa: Agrobo S.A. de C.V. Los Mochis, Sinaloa C.P. 81200, Mexico.

Current address of R. Felix-Gastelum: Depto. de Biología, Universidad de Occidente, Unidad Los Mochis, Sinaloa, CP 81223, Mexico.

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ABSTRACT

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The temporal and spatial patterns of *Phytophthora infestans* population genetic structure were analyzed in the Del Fuerte Valley, Sinaloa, Mexico, during the crop seasons of 1994 to 1995, 1995 to 1996, and 1996 to 1997 by geographical information systems. Isolates of *P. infestans* were obtained from infected tissue of tomato and potato collected from two areas: (i) where both potatoes and tomatoes are grown, and (ii) where only tomatoes are grown. The isolates were characterized by mating type, allozymes at the *glucose-6-phosphate isomerase* and *peptidase* loci, restriction fragment length polymorphism (RFLP) fingerprint with probe RG57, metalaxyl sensitivity, and aggressiveness to tomato and potato. The results suggest presence of an asexual population with frequent immigrations from outside the valley. There was a shift of mating type in the population from predominantly A2 to completely A1 in this period. The co-occurrence of mating types was restricted to very

few fields in the area around Los Mochis where tomato and potato crops are grown. Genotype variation based on allozyme analysis and mating type was low with only one genotype affecting both crops each year. The genotypes affecting both crops were the only genotypes highly aggressive to both tomato and potato in laboratory aggressiveness tests and the only genotypes widespread on both the tomato and potato crops in the valley each year. These predominant genotypes were highly resistant to the fungicide metalaxyl. Data on metalaxyl sensitivity indicate that allozyme analysis can discriminate between sensitive and resistant isolates in the Del Fuerte Valley. RFLP analysis with the probe RG57 gives further discrimination of genotypes within an allozyme genotype. In the 1995 to 1996 season, four different RFLP genotypes were found within an allozyme genotype. However, there were five other *dilocus* allozyme genotypes that could not be further split by RFLP analysis in 1995 to 1996 and 1996 to 1997 seasons. Spatial analysis of genotypes suggests that each season individual fields near Los Mochis became infected with one or more genotypes, but only a single genotype, aggressive on both potato and tomato, occurred south and east to the Guasave area.

Potato and tomato late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is one of the most economically important and destructive diseases affecting potato crops worldwide (14,15,20,40,48,52). Late blight is also a destructive disease on tomatoes (29,46,48). Late blight is the most important disease of both potato and tomato crops in the Del Fuerte Valley, in the state of Sinaloa in northwestern Mexico, and can be severe when the weather is favorable for the disease. Even in years with suboptimal weather conditions, late blight can cause damage to both of these crops, and in particular to tomatoes, if proper management practices are not followed. Both tomato and potato are grown in winter in the Del Fuerte Valley from October to April, and it is common to have adjacent crops of tomato and potato in Los Mochis area.

Late blight epidemics have become more difficult to manage in recent years due to increased genetic diversity of the causal agent in many parts of the world (16,17,21,22). Late blight management strategies and forecasting systems in areas with clonal populations of its causal agent *P. infestans* depend on an understanding of its population genetics because of the relationship of genotypes to epidemiologically important features such as host specificity,

cultivar response, and fungicide sensitivity (34,36). Mating type and selectively neutral genetic markers are used in studies of *P. infestans* because they can help discriminate between sexual and asexual populations. The lack of the A2 mating type outside of central Mexico before the 1980s indicated the presence of an asexual population of *P. infestans* worldwide (3,17). With the recent widespread occurrence of the A2 mating type, evidence of sexual reproduction of *P. infestans* has been found in other areas (3,10,17,27,50). This suggests that significant genetic changes have occurred in *P. infestans* populations outside of central Mexico (17).

Goodwin et al. (26) reported a probable clonal population for *P. infestans* in the Del Fuerte Valley in the late 1980s and early 1990s. They also reported for the first time the presence of the A2 mating type occurring in the area in low frequency and only on potato (26). This opened the possibility for an increase in the A2 mating type that would lead to sexual reproduction. The present study, therefore, looked for evidence that a sexual population might become established. As long as the population of *P. infestans* in the Del Fuerte Valley is clonal, genotype information can be related to epidemiologically important phenotypes (36) such as fungicide sensitivity and aggressiveness. The fungicide metalaxyl was the most effective systemic fungicide for late blight control before nonsensitive genotypes were developed (34). Matuszak et al. (30) reported that most of the isolates for the Del Fuerte Valley in the late 1980s were metalaxyl nonsensitive. However, the effectiveness of metalaxyl in the Del Fuerte Valley

Corresponding author: R. Jaime-Garcia; E-mail address: rjaime@srre.ars.usda.gov

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has been inconsistent after that report. Some growers contend that the fungicide is useless, whereas others contend that it is still effective. Differences in aggressiveness of *P. infestans* genotypes to tomato and potato have been documented (29). This situation could happen in the Del Fuerte Valley as shown by previous studies in the area (26). Goodwin et al. (26) found that some of the genotypes present in the Del Fuerte Valley were restricted to tomato whereas others were restricted to potato. Metalaxyl sensitivity and aggressiveness are examples of selectable genetic markers.

The genetic structure of populations of fungal pathogens can best be determined by selectively neutral genetic markers (32). Allozymes provided the first selectively neutral marker useful for analysis of population structure of *P. infestans* (17,53). Allozymes are markers that are relatively inexpensive and easy to use (24,33). *Glucose-6-phosphate isomerase* (*Gpi*) and *peptidase* (*Pep*) have been most widely used for *P. infestans* population genetics studies because they provide sufficient polymorphism and clear resolution (24,45,47,53). Restriction fragment length polymorphism (RFLP) is another selectively neutral genetic marker method that is capable of detecting more variation in both coding and noncoding regions of DNA (31,33). The properties of multiple alleles make RFLP markers advantageous for most studies in population genetics. Moderately repetitive nuclear DNA probes developed for *P. infestans* have been used successfully in population genetics studies of this organism (17,23,26).

Most epidemiological studies on potato and tomato late blight disease in recent years have focused on the population genetics of the pathogen (9,16–18,20–22,26,30,34,50,51) and refinements of our understanding of the effect of environmental parameters on epidemic development (4,11–13,40,41,43). Researchers have not given sufficient attention to spatial patterns of pathogens and their susceptible hosts at the regional scale (≈ 150 km). Even though few studies with potato and tomato late blight deal with spatial analysis, they are mostly at the country or continental level (14, 15,27). Spatial analysis (38) can strengthen our understanding of late blight epidemiology. Geographic information systems (GIS) allow us to obtain regional maps of the genotypes and their related features. These maps provide the extent of spread of particular genotypes and the likelihood of those genotypes infecting particular potato or tomato fields (34,42).

The main objective of this study was to determine the temporal and spatial variation in genetic structure of populations of *P. infestans* in the Del Fuerte Valley. Specific objectives were to determine: (i) the possibility of sexual reproduction of *P. infestans* in the area; (ii) the possible main sources of genetic diversity of *P. infestans*; (iii) the relationship between tomato and potato late blight epidemics; and (iv) the sensitivity to metalaxyl of the genotypes in the area.

MATERIALS AND METHODS

Description of the study area. The Del Fuerte Valley is located in the state of Sinaloa in northwestern Mexico between 25.40 and 26.25° north latitude and 108.25 and 109.35° west longitude (Fig. 1). Large-scale production agriculture occurs in the valley, which is on a coastal plain adjacent to the sea of Cortez (Gulf of California) located to the south and west. The topography is flat, and the elevation is just above sea level. There are low mountains at a distance ≈ 25 km to the north and ≈ 50 to 100 km to the east. Two main rivers pass through the valley; the Del Fuerte River in the north runs from northeast to west, and the Sinaloa River in the southeast runs south.

The Del Fuerte Valley can be divided into two areas where late blight occurs depending on the host crops grown. In the northwestern area around the city of Los Mochis, both tomato and potato crops are grown. To the east of this area there is a subarea where mainly potatoes are grown (Fig. 1). In the southeastern area around the city of Guasave, only tomatoes are grown (Fig. 1). The

tomatoes grown in this area are for industry and fresh market use, and they are grown in large fields in contrast to the Los Mochis area where there is a mixture of large and small tomato fields.

Sampling procedure. Isolates were collected by an opportunistic procedure that was based in part on industry scouting reports of disease incidence in tomato and potato fields throughout the region in three consecutive seasons from 1994 to 1995, 1995 to 1996, and 1996 to 1997. The total number of isolates obtained for each season were 128, 70, and 159 for the season of 1994 to 1995, 1995 to 1996, and 1996 to 1997, respectively. Sampling started when an outbreak of late blight was first noticed. This usually occurred between the second and third week of December. Samples were collected from both potato and tomato fields. Each sample site was geographically referenced by the Universal Transverse Mercator (UTM) coordinate system (49) with a hand-held global positioning system (GPS) instrument. The instruments used were the Magellan (Model 69001; Magellan Systems Corp., San Dimas, CA) or the Garmin Personal Navigator (Model 45; Garmin International, Lenexa, KS). All fields surveyed for late blight were geographically referenced, whether the disease was present or not. From each positive sample site, several infected leaves or stems were obtained. The size of the sample site varied according to the presence and severity of late blight. In sites where late blight was very rare only a few lesions within an approximate radius of 20 m were collected. In sites where late blight was severe, samples were collected from several neighboring plants. Infected tomato fruits were sometimes collected. From each sample site, one or more isolates were obtained.

Isolation and maintenance of isolates. To reduce the possibility of isolating mixed genotypes only single, young, small lesions of late blight from tomato and potato were collected. Iso-

Fig. 1. Feature map of the Del Fuerte Valley in northwestern Mexico showing the spatial distribution of tomato and potato crops.

lates of *P. infestans* were clean-cultured by plating the diseased tissue on corn meal agar (17 g/liter) amended with 10 mg of pimaricin, 250 mg of ampicillin, 10 mg of rifampin, and 50 mg of *p*-chloro-nitrobenzene (28). Five to seven pieces from different lesions collected in the same sample location were plated in each petri dish. Each piece represented a different isolate from the same sample point. The plates were incubated at 18 to 22°C in Los Mochis and brought to the University of Arizona, in Tucson, where they were maintained at 15 to 18°C. As soon as a tissue sample showed mycelial growth, a sample of the mycelium was transferred to rye A agar (5) or clarified V8 agar (23) and was maintained as a pure culture for further characterization. Isolates that did not grow on rye A or V8 media (rye A and V8 shy isolates) were maintained on pea agar. Pea agar was prepared by adding 15 g/liter of agar to a pea broth (23).

Genotype characterization. Genotypes were characterized by mating type, allozymes, and RFLP. Mating type was determined by growing the sample isolates together with tester isolates of known mating type in a petri dish containing 10% clarified V8 agar amended with 50 mg of β -sitosterol (23). The tester genotypes were US930258 (US1), US920159 (US6), US920210 (US7), and US930287 (US8), provided by W. Fry, Cornell University. Each plate was scored for oospore formation 10 to 15 days after the test started. Isolates that formed oospores with both known mating types were considered to be self-fertile. For allozyme characterization of isolates, the identity of *Gpi* and *Pep* allozymes were determined. Cellulose acetate gel electrophoresis (Helena Laboratories, Beaumont, TX) was used as described in Goodwin et al. (24) with the overlay protocol. Tissue used for analysis consisted of either sporangia washed directly from a fresh lesion or mycelium taken from a pure culture. Sporangia were collected in Los Mochis on the same day the sample was obtained, and were frozen immediately and kept frozen until used. This procedure worked well for analysis of the *Gpi* locus. The *Pep* locus was not well resolved by sporangia alone. Thus, *Pep* allozyme analysis relied exclusively on cultured material. Allozyme alleles were scored according to the standard procedure described by Goodwin et al. (24). The tester genotypes used were US930258 (US1), US920159 (US6), US920210 (US7), and US930287 (US8), provided by W. Fry. RFLP analysis of *P. infestans* DNA was performed with the RG57 probe provided by W. Fry. DNA extraction followed the protocol described in Goodwin et al. (23). DNA was restricted with *Eco*RI adding spermidine to each reaction buffer to a final concentration of 4 mM (23). The restricted

DNA was separated by electrophoresis in 0.9% agarose gels and transferred to a nylon membrane (Hybond N; Amersham Life Science, Arlington Heights, IL) in 10 $\times$ SSC (1 $\times$ SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate, pH 7.0) buffer (44). The DNA was detected by chemiluminescence according to the method of Wasmann and VanEtten (54), but with either of the substrates CSPD or CDP Star (Tropix, Bedford, MA) instead. The RG57 probe was labeled by the nonradioactive enzyme-linked immunoassay of digoxigenin-conjugated dUTP incorporated by random hexanucleotide priming of the large subunit of *Escherichia coli* DNA polymerase (54).

Metalaxyl sensitivity. The reaction of *P. infestans* isolates to the phenylamide fungicide metalaxyl was evaluated by transferring each isolate to V8 agar or pea agar medium (23) amended with 0, 1, 10, and 100 mg/liter of active ingredient of metalaxyl fungicide (Ridomil 2E; Novartis Crop Protection Inc., Greensboro, NC). Four plugs (9-mm diameter) were placed equidistant on the edge of each plate. The plates were incubated at dark at 15°C. Radial growth of each plug was recorded 5 and 10 days after inoculation. Five days for the fast growing isolates and 10 days for slow growing isolates. The relative growth of each isolate growing in fungicide-amended media was obtained by dividing the radial growth of the isolate growing in metalaxyl-amended media by the radial growth of the same isolate growing in media without fungicide. Isolates were recorded as sensitive if they did not grow even at the lowest concentration of 1 mg/liter, moderately sensitive if the relative radial growth at 10 mg/liter was less than 10%, moderately resistant if the relative radial growth at 10 mg/liter was greater than 10%, and resistant if their growth was similar (>50%) to the control even at the highest concentration of 100 mg/liter (7).

Aggressiveness on potato and tomato. The aggressiveness of *P. infestans* isolates was assessed by inoculating detached leaflets from cultivars of potato and tomato in vitro. Two different detached leaflet procedures were used. In the first, the leaflets were floated on water in a 9-cm petri dish as described by Cohen et al. (6). In the second procedure, the leaflets were placed in a 20-cm petri dish with a layer of water agar on the top as described in Legard et al. (29). Two potato cultivars were used for this assay: cv. Norchip which is very sensitive to late blight and cv. Alpha which possesses field resistance (19) and is one of the most common cultivars grown in the Del Fuerte Valley. In tomato, the assay was performed with cvs. Brigadier, 49er, and Sinaloa which are all commonly used in the Del Fuerte Valley. Inoculations were

TABLE 1. Number and frequency of *Phytophthora infestans* isolates characterized by allozymes and mating type in the Del Fuerte Valley, Sinaloa, Mexico, during the 1994 to 1995, 1995 to 1996, and 1996 to 1997 seasons

Genotype	Allozymes ^a		Mating type ^b	Tomato number (%)	Potato number (%)	Total number (%)
	<i>Gpi</i>	<i>Pep</i>				
1994–1995						
LM2	100/111	100/100	A1	...	6 (32)	6 (5)
LM3	100/100	100/100	A1	...	1 (5)	1 (0.5)
LM3SF	100/100	100/100	SF	...	1 (5)	1 (0.5)
LM4	100/111	100/100	A2	109 (100)	11 (58)	120 (94)
Total	...	...	...	109 (100)	19 (100)	128 (100)
1995–1996						
LM4	100/111	100/100	A2	9 (16)	...	9 (13)
LM5	100/111	100/100	SF	1 (2)	...	1 (01)
LM6	100/122	100/100	A1	31(56)	15 (100)	46 (66)
LM7	111/122	92/100	A1	14 (25)	...	14 (20)
Total	...	...	...	55 (100)	15 (100)	70 (100)
1996–1997						
LM3	100/100	100/100	A1	34 (33)	...	34 (21)
LM6	100/122	100/100	A1	68 (67)	54 (95)	122 (77)
LM8	100/122	92/100	A1	...	3 (05)	3 (02)
Total	...	...	...	102 (100)	57 (100)	159 (100)

^a *Pep* indicates peptidase and *Gpi* indicates glucose-6-phosphate isomerase.

^b SF = self-fertile isolates.

made with sporangia washed from a fresh lesion (≈ 5 days old) and taken from in vitro inoculated tomato leaflets. The sporangia were adjusted to a concentration of 40,000 sporangia per ml and placed in the refrigerator for at least 2 h before inoculation (29). A drop of 40 μ l of the sporangial suspension was placed on the abaxial side of the leaflet. Inoculated leaflets were incubated at 18°C in the dark until they showed symptoms. The aggressiveness of the isolates was recorded on an arbitrary scale from 0 to 4, where 0 represents no infection, 1 represents small lesions with almost no sporulation, 2 represents small lesions with sparse sporulation, 3 represents small to medium size lesions with medium sporulation, and 4 represents large lesions with abundant sporulation.

Mapping and data display. Two commercially available programs, ArcInfo (PC version 3.4) and ArcView (PC version 3.0; ESRI, Redlands, CA), were used for GIS analysis. A base map of the Del Fuerte Valley digitized for a previous study in the area (37) contained the features roads, canals, cities, rivers, and coastline. The features were geographically referenced in the UTM coordinate system so that sample locations in the present study referenced by GPS and represented by a dot could be overlaid on them.

Maps of mating type, allozymes at two loci (*Gpi* and *Pep*), and RFLP fingerprint were developed for each season. These spatial patterns of genotype distribution were analyzed together and separately for tomato and potato fields.

RESULTS

Mating type. The *P. infestans* mating types shifted from dominance by A2 in 1994 to 1995 to presence of only A1 in 1996 to 1997 (Table 1; Fig. 2). Co-occurrence of both mating types was restricted to very few fields in the area around Los Mochis where both tomato and potato crops are grown. In 1994 to 1995, A1 was only found in four potato fields (Fig. 2A). In three of the four fields, both mating types were isolated from the same part of the field. One self-fertile isolate was also found in one of the fields where both mating types were found. In 1995 to 1996, the prevalent mating type was the A1, but A2 was found in four tomato fields (Fig. 2B). Both mating types co-occurred in only one field. A self-fertile isolate was also found in one tomato field in 1995 to 1996 (Fig. 2B).

Allozymes. In the 1994 to 1995 season, four genotypes were found in the Del Fuerte Valley based on allozyme markers and mating type (Table 1). The genotype designated as LM4 with allozyme pattern of 100/111 on *Gpi*, 100/100 on *Pep*, and A2 mating type was the most common found (94% of all isolates). This genotype was the only one found on tomato and was also the most common on potato (Table 1). The other three genotypes were restricted to only potatoes. LM2 has an allozyme pattern similar to the LM4, but with the A1 mating type. This genotype accounted for 32% of the isolates found on potato (Table 1). The other two genotypes have an allozyme pattern of 100/100 *Gpi* and 100/100 *Pep*, but one was A1 mating type whereas the other was self-fertile. These genotypes were found in the same potato field and in only one sample each.

In the 1995 to 1996 season four genotypes were found in the area, but the high frequency genotype (LM6) differed in allozyme pattern and mating type from the high frequency genotype (LM4) of the previous season (Table 1). LM6 (100/122 *Gpi*, 100/100 *Pep*, and A1 mating type) was the only genotype found on both crops and in all the samples from potato and in 56% of the samples from tomato. In contrast to the previous season, the other genotypes were found only on tomato samples (Table 1). The genotype LM4, which was the most common in the previous season, was found in 16% of the isolates from tomato. Genotype LM7 (111/122 *Gpi*, 92/100 *Pep*, and A1 mating type) was found late in the season and accounted for 25% of the isolates from tomato.

In the 1996 to 1997 season, three genotypes were found with only LM6 appearing at high frequency on both crops (Table 1).

This same genotype was also found in one tomato field in the Culiacan, Sinaloa area, located 150 km southeast of the Del Fuerte Valley. LM3 was found only on tomato and LM8 (100/122 *Gpi*, 92/100 *Pep*, and A1 mating type) only on potato. The tomato genotype LM3, was the first genotype observed in this season and had been found previously in 1994 to 1995, but in only one potato sample.

Fig. 2. Spatial distribution of mating types of *Phytophthora infestans* in the Del Fuerte Valley, Sinaloa, Mexico, during the seasons of **A**, 1994 to 1995; **B**, 1995 to 1996; and **C**, 1996 to 1997. Mating types are A1, A2, and self-fertile (SF). Insets represent areas where different mating types occur together. Squares and triangles represent isolates.

The spatial patterns of isolates characterized by the *Gpi* allozyme system show that only one genotype of *P. infestans* was widespread on both the tomato and potato crops in the Del Fuerte Valley area each year (Fig. 3). The patterns also show geographic and host substructuring of the *P. infestans* population in the Del Fuerte Valley (Fig. 3). In the 1994 to 1995 season, *Gpi* 100/111 was the only broadly dispersed *Gpi* genotype (Fig. 3A and B). *Gpi* 100/100 was also observed in that season, but was restricted to one potato field (Fig. 3A). In this season only *Pep* 100/100 was found (Table 1). In 1995 to 1996, a different *Gpi* genotype, namely 100/122, was the only one widespread on both tomato and potato (Fig. 3C and D). *Gpi* 100/111, dominant the previous season, was present in restricted areas. *Gpi* 111/122 genotype, not previously observed, was spatially and host restricted (Fig. 3D). *Gpi* 100/111 and *Gpi* 111/122 genotypes were restricted to the area around Los Mochis, where both the tomato and potato crops are grown. They were found only on tomato crops, with the exception of one *Gpi* 111/122 isolate appearing on potato (Fig. 3C and D). In 1995 to 1996, the most broadly spread *Pep* genotype was the *Pep* 100/100, found in association with *Gpi* 100/111 and 100/122. The newly found *Pep* 92/100 was associated with *Gpi* 111/122 (Table 1). All *Gpi* 111/122 and *Pep* 92/100 isolates were found late in the season and were extremely sensitive to the fun-

gicide metalaxyl. In 1996 to 1997, the *Gpi* 100/122 was the only widespread genotype appearing on tomato and potato (Fig. 3E and F). The spatial pattern of *Gpi* 100/100 spanned the study area from the northeast to the southeast, but was restricted to fields at least 30 km inland and occurred only on tomatoes (Fig. 3F). *Gpi* 100/100 was the first genotype detected in the season (22 December 1996) in an isolated tomato field to the northeast part of the valley. This same *Gpi* genotype had also previously been found in 1994 to 1995 restricted to only one potato field (Fig. 3A). As in previous seasons, the most widely spread *Pep* genotype was 100/100. The *Pep* genotype 92/100 was also found in this season, but was restricted to only one potato field.

RFLP. RFLP analysis was able to differentiate more genotypes within allozyme genotypes (Table 2). In the 1994 to 1995 season only five isolates belonging to the most common genotype LM4 were tested. Two isolates had the RFLP pattern designated as LM4-A, which matched the RFLP pattern of genotype US7, and three had the RFLP pattern LM4-E (Table 2). The pattern A (US7) was found in the northeastern area close to Los Mochis on both the tomato and potato crops, whereas pattern E was found only on tomato crops. In the 1995 to 1996 season six different RFLP patterns were found in the 17 isolates tested. In this season all genotypes based on allozyme and mating type, except for LM5, were represented. Four different RFLP patterns were found within genotype LM6, whereas LM4 and LM7 had only one. Within genotype LM6, the RFLP pattern B, which matched the genotype US17, was the most common representing 54% of the isolates tested. This pattern was found on isolates from both the tomato and potato crop. Pattern D was found in 31% of the isolates and all were from tomato. Each tomato RFLP patterns was localized to a particular area. However, there was an area between Los Mochis and Guasave where RFLP patterns B, C, and D were found in close proximity. Patterns F and G were found only in one potato isolate each and from different potato fields. In the 1996 to 1997 season, only three RFLP patterns were found among 84 isolates tested, with only one RFLP pattern within each genotype based on allozyme and mating type. LM6 genotype showed pattern B (US17), the most common pattern for this genotype found in the previous season. Two new RFLP patterns were found this season. Pattern H was found broadly distributed, but only on tomato crops and mainly in the area inland beyond 30 km from the coastline. Pattern I was found only in one potato field.

Fungicide response. No sensitivity to metalaxyl was detected among the 1994 to 1995 isolates tested (Table 3). However, the metalaxyl sensitivity assay for this season was performed with very few genotypes and no isolates belonging to the LM3 genotype were tested. For the 1995 to 1996 season, although most of the isolates tested for metalaxyl sensitivity were moderately resistant or resistant, all six isolates tested within the LM7 genotype were moderately sensitive or sensitive. The results for metalaxyl sensitivity for 1996 to 1997 were very similar to those from the previous season, with most of the isolates moderately resistant or resistant, but with all six isolates within the genotype LM3 being completely sensitive.

Aggressiveness on potato and tomato. All isolates tested were equally highly aggressive to potato cv. Norchip. However, there was a differential response in aggressiveness among isolates tested on the potato cv. Alpha, a common cultivar planted in the area (Table 3). The isolates within the genotype LM6 with RFLP pattern D, F, and G showed very low aggressiveness, and isolates with RFLP pattern B showed moderate aggressiveness. The aggressiveness among the isolates within the genotype LM7 was highly variable on potato (Table 3). Results from the three tomato cultivars are combined because all tomato cultivars reacted similarly to all isolates tested. There was a differential reaction to tomato among the isolates tested. In the 1994 to 1995 season, the isolates were highly aggressive to tomato, except the genotype LM2 (LM3 was not studied) (Table 3). In the 1995 to 1996

Fig. 3. Spatial distribution of Glucose-6-phosphate isomerase allozyme genotypes of *Phytophthora infestans* on A, C, and E, potato and B, D, and F, tomato crops in the Del Fuerte Valley, Sinaloa, Mexico, during the seasons of A and B, 1994 to 1995; C and D, 1995 to 1996; and E and F, 1996 to 1997.

season, all isolates, except LM6-G (with RFLP pattern G), were highly aggressive to tomato.

DISCUSSION

To understand the epidemiology of tomato and potato late blight in the Del Fuerte Valley, it is important to know whether the population is predominantly sexual or asexual (clonal). Sexual populations, such as that of *P. infestans* in the Toluca Valley in central Mexico, usually have a great diversity of genotypes with almost each isolate being of a different genotype (16,26,50). Asexual populations, however, have a low genotypic diversity with one or a few genotypes dominating the population (16,17, 26,50). Sexual populations make management of disease more difficult due to the constant appearance of new genotypes, that increase the variability of features like fungicide resistance, higher aggressiveness, and better fitness in the population. On the other hand, in asexual populations these features are more constant. This allows us to find relationships between genotypes and their particular features (34). Goodwin et al. (26) reported the occurrence of the A2 mating type in the Del Fuerte Valley for the first time in 1989. Although they concluded the population was probably clonal, the observation of the A2 mating type made sexual reproduction a possibility. Theoretically, sexual reproduction in the Del Fuerte Valley should be rare, because 7 days of constant leaf wetness are required for oospore production (6) and this condition almost never occurs there. The low genotypic diversity 8 years after the initial observation of the A2 mating type (Tables 1 through 3) suggests that the population of *P. infestans* in this area continues to be asexual. This conclusion is supported by the predominance of only one mating type in each year (Fig. 2). In fact, even though mating type A2 was ubiquitous in 1995, in the final season sampled (1997), it was not found at all in the Del Fuerte Valley. If sexual reproduction were epidemiologically important, one would expect to find both mating types at approximately the same frequencies.

The change in genotypes from one season to the next (Tables 1 through 3) can be explained by the fact that *P. infestans* cannot easily survive vegetatively in the hot summer, where temperatures above 37°C are common. The most likely source of genetic diversity is outside the Del Fuerte Valley because summer temperatures should prevent both vegetative and oospore survival. Each year potato seed tubers are introduced from areas where late blight is prevalent (26,39). Potato seed tubers are well documented as a

TABLE 2. Characterization of *Phytophthora infestans* genotypes by restriction fragment length polymorphism (RFLP) with the probe RG57 in the Del Fuerte Valley during the 1994 to 1995, 1995 to 1996, and 1996 to 1997 seasons

Genotype ^a	MT ^b	Allozyme genotype ^c		RFLP pattern ^d
		<i>Gpi</i>	<i>Pep</i>	
US1	A1	86/100	92/100	10101010110011010001100110
US6	A1	100/100	92/100	10101110010011000101100110
US8	A2	100/111/122	100/100	10001010010011010001101110
LM4-A	A2	100/111	100/100	10001000010011010101100110
LM4-E	A2	100/111	100/100	10001001010011010101100111
LM6-B	A1	100/122	100/100	1010001000011010101100110
LM6-D	A1	100/122	100/100	10100011010011010101100111
LM6-F	A1	100/122	100/100	10100011010111010001100111
LM6-G	A1	100/122	100/100	10100011000011010101100100
LM7-C	A1	111/122	92/100	10101010010011010111100011
LM3-H	A1	100/100	100/100	1000100000011010001100110
LM8-I	A1	100/122	92/100	10101010010011010101100110

^a The letter after the genotype represent the type of RFLP pattern.

^b Mating type.

^c *Pep* indicates *peptidase* and *Gpi* indicates *glucose-6-phosphate isomerase*.

^d 1 = presence and 0 = absence of a band. The last column represents bands 9b, a band found between bands 9 and 10. Underlined numbers in the RFLP pattern indicate bands used to differentiate isolates with the same *Gpi*, *Pep*, and mating type characters.

source of inoculum for late blight disease (1,31,55). Some of these potato seed tubers from outside the valley are likely to be infected and carry the inoculum for new populations in the Del Fuerte Valley (20,31). Although the most widespread genotype for a particular season was present in the next season (Table 1), we think that they were reintroduced in potato seed tubers from outside sources rather than that they survived the Del Fuerte Valley summer. It is unlikely that potatoes from the Del Fuerte Valley used for seed are a source of the occasional repeating genotypes, because late blight very rarely infects tubers in the Del Fuerte Valley. On the other hand, potato seed tubers are introduced to the valley each season from areas where conditions permit vegetative survival of clones or sexual reproduction is possible (26) such as Saltillo, Coahuila and Tapalpa, Jalisco.

Surprisingly, the repeating genotypes (LM4-A and LM6-B) correspond to the most common genotypes found in the United States in the same season or in the recent previous or subsequent seasons (genotypes US7 from 1994 to 1995 and 1995 to 1996 and US17 from 1995 to 1996 and 1996 to 1997) (25). This strengthens the hypothesis that migration is the cause of genetic variation of *P. infestans* populations in North America (25). The clonal lineage US17 was found in northwestern Mexico in the season before it was found in the United States (25). In northwestern Mexico there were four different RG57 patterns within LM6, the dilocus allozyme and mating type corresponding to US17 (Tables 1 and 2). Two of these four patterns were found only on potato, one only on tomato, and one on both potato and tomato. Only pattern LM6-B found on both the tomato and potato crops, which corresponds to the US17, was found the next season (Fig. 4). This suggests that this genotype probably originated in Mexico.

The epidemics on potato crops are likely to start from infected potato seed tubers. Epidemics on tomato crops, however, could start from infected potato tubers or infected tomato fruits. First, genotypes found infecting only tomatoes (Table 1) had variable aggressiveness on potato foliage (Table 3), however, they still

TABLE 3. Characterization of genotypes of *Phytophthora infestans* in the Del Fuerte Valley, Sinaloa, Mexico, during the 1994 to 1995, 1995 to 1996, and 1996 to 1997 seasons

Genotype ^a	Allozyme genotype ^b				Aggressiveness ^c	
	<i>Gpi</i>	<i>Pep</i>	MT ^c	Resistance ^d	Tomato	Potato
1994–1995						
LM2	100/111	100/100	A1	MR	1	4
LM3	100/100	100/100	A1	ND	ND	ND
LM3SF	100/100	100/100	SF	MR	4	4
LM4-A	100/111	100/100	A2	MR	4	4
LM4-E	100/111	100/100	A2	MR-R	4	4
1995–1996						
LM4-A	100/111	100/100	A2	MR	4	4
LM5	100/111	100/100	SF	ND	ND	ND
LM6-B	100/122	100/100	A1	R	4	2–4
LM6-D	100/122	100/100	A1	MR	4	1
LM6-F	100/122	100/100	A1	ND	4	1
LM6-G	100/122	100/100	A1	MR	2	1
LM7-C	111/122	92/100	A1	S-MS	4	1–4
1996–1997						
LM6-B	100/122	100/100	A1	MR-R	4	2–4
LM3-H	100/100	100/100	A1	S	4	1–2
LM8-I	100/122	92/100	A1	MR	4	1

^a The letter after the genotype indicates the type of restriction fragment length polymorphism pattern.

^b *Pep* indicates *peptidase* and *Gpi* indicates *glucose-6-phosphate isomerase*.

^c Mating type. SF = self-fertile.

^d According to reaction of the isolates to the fungicide metalaxyl, where S is sensitive, MS is moderately sensitive, MR is moderately resistant, and R is resistant. ND = not determined.

^e Arbitrary scale from 0 to 4, where 0 indicates no infection or no sporulation, and 4 indicates large lesions with abundant sporulation. Aggressiveness on potato are based on the reaction to cv. Alpha. All isolates were equally and highly aggressive to potato cv. Norchip.

could infect potato tubers, considering that no direct relationship between foliage epidemic and tuber epidemic have been documented (8,55). We speculate that infected potato tubers give rise to infected stems, which produce just enough sporangia to spread to the more susceptible tomato crops nearby. However, more research is needed to prove this. A possible source of infected tomato fruit could be fruit purchased in the local markets (20) brought into the Del Fuerte Valley in summer and early fall when local tomatoes are not yet produced. These tomatoes could come from areas in Mexico where late blight is a problem on tomatoes. With small villages scattered throughout the tomato and potato growing areas of the Del Fuerte Valley, there is a reasonable possibility of infected fruit being discarded in a trash heap near a tomato field. This could be an explanation of the spatial pattern of the late blight epidemic observed in 1996 to 1997, because the first outbreak of the disease was in a tomato field in the northeast most part of the valley upwind from potato fields (Fig. 3F). The *P. infestans* genotype occurring in this field was found only in tomato fields and was not very aggressive to the commonly grown potato cv. Alpha. However, in general, we think that potato seed tubers could be the main source of inoculum and *P. infestans* genetic variability even for tomato. Supporting this is the observation that late blight is not as big of a problem in the Culiacan Valley as it is in the Del Fuerte Valley. The Culiacan Valley, 150 km to the southeast, is separated from the Del Fuerte Valley by an area where no commercial potatoes or tomatoes are grown. Neither potatoes nor processing tomatoes are grown in the Culiacan Valley, but there are large areas devoted to fresh market tomatoes. In the Culiacan area, late blight, when present, occurs $\approx$ 1 month later than in the Los Mochis area. The genotypes found in the Culiacan area present the most common genotype found in the Los Mochis area, similar to what is happening in the Guasave area, in the southeastern part of the Del Fuerte Valley, where only tomatoes are grown.

The spatial pattern of genotypes showed geographic substructuring in the north and northwest and a convergence or mixing of most of the genotypes in the central part of the valley. It also shows the spreading of only one or two genotypes further to the southeastern part of the valley. The genotypes that spread to the southeast were mainly those that were found infecting both potato and tomato crops. This suggests that the genotypes that are able to reach tomato crops are more likely to spread further. *P. infestans* can be disseminated by air up to 50 km (18,46). However, this dissemination could only be efficient enough to start a new infection on isolated plants in a few random fields. Under these circumstances, nonaggressive genotypes, which produce fewer sporangia, will not spread far from the initial area. This is true if we consider that sporangia carried in the air are very sensitive to desiccation and to solar and ultraviolet radiation (20,35,52).

After Matuszak et al. (30) reported that only isolates nonsensitive to the fungicide metalaxyl were found in the Del Fuerte Valley, a great debate occurred among farm advisors on the effectiveness of metalaxyl to control late blight. Some contended the fungicide was useless, whereas others contended it was still effective. The results presented here, with those reported previously (26,30), show that even though the *P. infestans* populations are clonal, the genetic structure changes from year to year, indicating frequent migrations of genotypes into the area. The discrepancy in opinion and experience among farm advisors on metalaxyl efficacy, might be due, in part, to the migration of new genotypes, some which are sensitive to metalaxyl (Table 3).

Our data and others (30) indicate that allozyme analysis frequently can discriminate between metalaxyl sensitive and non-sensitive isolates in areas with clonal populations. Allozyme genotypes can also be associated with host specificity. RFLP analysis can discriminate genotypes that cannot be detected by allozyme analysis (Table 2), but the costs and technical requirements, as well as the time ($\approx$ 2 weeks) required for processing

samples, make this technique unsuitable for management purposes. On the other hand, cellulose acetate electrophoresis method for allozyme analysis identifies genotypes within 2 to 3 h (24). Each year genotypes discovered early in the season can be characterized for the phenotypes of fungicide resistance and aggressiveness on potato and tomato. Because the populations of *P. infestans* in the Del Fuerte Valley are clonal, the easily acquired genotype information can be used to predict phenotypes and support management decisions the rest of the season.

Improved management of late blight is possible at the regional scale in the Del Fuerte Valley, in part, because three companies control almost all of the processing tomato crop and a potato farmers association controls almost all of the potato crop. The essential recurring pattern in this system is that new genotypes of *P. infestans* appear first in the northwest part of the valley and only a subset of those are subsequently found throughout the valley. By characterizing the phenotypes of genotypes collected in the northwest early in the season, genotype data on samples collected throughout the season can be used to appraise risk, select fungicide treatments, and schedule the fungicides sprays. For example, none of the genotypes occurring in the Del Fuerte Valley in the seasons of 1995 to 1996 and 1996 to 1997 were very aggressive on potato. With this knowledge potato farmers could have used lower rates of fungicides or longer schedules between spraying to control the disease during these seasons. This genotype risk assessment can be coupled with disease forecasting methods based on weather monitoring (2) to improve crop protection regionally. The work presented here provides an example of the thesis that there are recurring spatial patterns at the regional scale that can guide disease management programs (38).

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LITERATURE CITED

- Andrison, D. 1995. Biology, ecology, and epidemiology of the potato late blight pathogen *Phytophthora infestans* in soil. *Phytopathology* 85:1053-1056.
- Bolkan, H. A., and Reinert, W. R. 1994. Developing and implementing IPM strategies to assist farmers: An industry approach. *Plant Dis.* 78: 545-550.
- Brown, J. K. M. 1996. The choice of molecular markers methods for population genetics studies of plant pathogens. *New Phytol.* 133:183-195.
- Bruhn, J. A., and Fry, W. E. 1981. Analysis of late blight epidemiology by simulation modeling. *Phytopathology* 71:612-616.
- Caten, C. E., and Jinks, J. L. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. *Can. J. Bot.* 46: 329-348.
- Cohen, Y., Farkash, S., Reshit, Z., and Baider, A. 1997. Oospore production of *Phytophthora infestans* in potato and tomato leaves. *Phytopathology* 87:191-196.
- Deahl, K. L., Inglis, D. A., and DeMuth, S. P. 1993. Testing for resistance to metalaxyl in *Phytophthora infestans* isolates from northwestern Washington. *Am. Potato J.* 70:779-795.
- Dowley, L. J., O'Sullivan, E., and Kehoe, H. W. 1991. Development and evaluation of blight resistant potato cultivars. Pages 373-382 in: *Phytophthora*. J. A. Lucas, R. C. Shattock, D. S. Shaw, and L. R. Cooke, eds. Br. Mycol. Soc./Cambridge University Press, Cambridge.
- Drenth, A., Goodwin, S. B., Fry, W. E., and Davidse, L. C. 1993. Genotypic diversity of *Phytophthora infestans* in the Netherlands revealed by DNA polymorphisms. *Phytopathology* 83:1087-1092.
- Drenth, A., Tas, I. C. Q., Govers, F. 1994. DNA fingerprinting uncovers a new sexual reproducing population of *Phytophthora infestans* in the Netherlands. *Eur. J. Plant Pathol.* 100:97-107.
- Fry, W. E., Apple, A. E., and Bruhn, J. A. 1983. Evaluation of potato late blight forecasts modified to incorporate host resistant and fungicidal weathering. *Phytopathology* 73:1054-1059.
- Fry, W. E., and Doster, M. A. 1991. Potato late blight: forecasts and dis-

- ease suppression. Pages 326-336 in: *Phytophthora*. J. A. Lucas, R. C. Shattock, D. S. Shaw, and L. R. Cooke, eds. Br. Mycol. Soc./Cambridge University Press, Cambridge.
13. Fry, W. E., and Fohner, G. R. 1985. Construction of predictive models. I. Forecasting disease development. Pages 161-178 in: *Advances in Plant Pathology*. Vol. 3. Mathematical Modeling of Crop Disease. C. A. Gilligan, ed. Academic Press, New York.
 14. Fry, W. E., and Goodwin, S. B. 1997. Re-emergence of potato and tomato late blight in the United States. *Plant Dis.* 81:1349-1357.
 15. Fry, W. E., and Goodwin, S. B. 1997. Resurgence of the Irish potato famine fungus. *BioScience* 47:363-371.
 16. Fry, W. E., Goodwin, S. B., Dyer, A. T., Matuszak, J. M., Drenth, A., Tooley, P. W., Sujkowski, L. S., Koh, Y. J., Cohen, B. A., Spielman, L. J., Deahl, K. L., Inglis, D. A., and Sandlan, K. P. 1993. Historical and recent migrations of *Phytophthora infestans*: Chronology, pathways, and implications. *Plant Dis.* 77:653-661.
 17. Fry, W. E., Goodwin, S. B., Matuszak, J. M., Spielman, L. J., and Drenth, A. 1992. Population genetics and intercontinental migrations of *Phytophthora infestans*. *Annu. Rev. Phytopathol.* 30:107-129.
 18. Fry, W. E., and Spielman, L. J. 1991. Population biology. Pages 171-192 in: *Advances in Plant Pathology*. Vol. 7. *Phytophthora infestans*: The Cause of Late Blight of Potato. D. S. Ingram and P. H. Williams, eds. Academic Press, New York.
 19. Gees, R., and Hohl, H. R. 1988. Cytological comparison of specific (R3) and general resistance to late blight in potato leaf tissue. *Phytopathology* 78:350-357.
 20. Goodwin, S. B. 1997. The population genetics of *Phytophthora*. *Phytopathology* 87:462-473.
 21. Goodwin, S. B., Cohen, B. A., Deahl, K. L., and Fry, W. E. 1994. Migration from northern Mexico as the probable cause of the recent genetics changes in populations of *Phytophthora infestans* in the United States and Canada. *Phytopathology* 84:553-558.
 22. Goodwin, S. B., Cohen, B. A., and Fry, W. E. 1994. Panglobal distribution of a single lineage of the Irish potato famine fungus. *Proc. Natl. Acad. Sci. USA* 91:11591-11595.
 23. Goodwin, S. B., Drenth, A., and Fry, W. E. 1992. Cloning and genetic analysis of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. *Curr. Genet.* 22:107-115.
 24. Goodwin, S. B., Schneider, R. E., and Fry, W. E. 1995. Use of cellulose-acetate electrophoresis for rapid identification of allozyme genotypes of *Phytophthora infestans*. *Plant Dis.* 79:1181-1185.
 25. Goodwin, S. B., Smart, C. D., Sandrock, R. W., Deahl, K. L., Punja, Z. K., and Fry, W. E. 1998. Genetic change within populations of *Phytophthora infestans* in the United States and Canada during 1994 to 1996: Role of migration and recombination. *Phytopathology* 88:939-949.
 26. Goodwin, S. B., Spielman, L. J., Matuszak, J. M., Bergeron, S. N., and Fry, W. E. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. *Phytopathology* 82:955-961.
 27. Goodwin, S. B., Sujkowski, L. S., Dyer, A. T., Fry, B. A., and Fry, W. E. 1995. Direct detection of gene flow and probable sexual reproduction of *Phytophthora infestans* in northern North America. *Phytopathology* 85:473-479.
 28. Kannwischer, M. E., and Mitchell, D. J. 1978. The influence of a fungicide on the epidemiology of black shank of tobacco. *Phytopathology* 68:1760-1765.
 29. Legard, D. E., Lee, T. Y., and Fry, W. E. 1995. Pathogenic specialization in *Phytophthora infestans*: Aggressiveness on tomato. *Phytopathology* 85:1356-1361.
 30. Matuszak, J. M., Fernandez-Elquezabal, J., Gu, W. K., Villarreal-Gonzalez, M., and Fry, W. E. 1994. Sensitivity of *Phytophthora infestans* populations to metalaxyl in Mexico: Distribution and dynamics. *Plant Dis.* 78:911-916.
 31. McDermott, J. M., and McDonald, B. A. 1993. Gene flow in plant pathosystems. *Annu. Rev. Phytopathol.* 31:353-373.
 32. McDonald, B. A. 1997. The population genetics of fungi: Tools and techniques. *Phytopathology* 87:448-453.
 33. McDonald, B. A., and McDermott, J. M. 1993. Population genetics of plant pathogenic fungi: Electrophoretic markers give unprecedented precision to analyses of genetic structures of populations. *BioScience* 43:311-319.
 34. Milgroom, M. G., and Fry, W. E. 1997. Contributions of population genetics to plant disease epidemiology and management. Pages 1-30 in: *Advances in Botanical Research*. Vol. 24. Incorporating Advances in Plant Pathology. J. H. Andrews and I. C. Tommerup, eds. Academic Press, New York.
 35. Mizubuti, E. S. G., Aylor, D. E., and Fry, W. E. 2000. Survival of *Phytophthora infestans* sporangia exposed to solar radiation. *Phytopathology* 90:78-84.
 36. Mizubuti, E. S. G., and Fry, W. E. 1998. Temperature effects on developmental stages of isolates from three clonal lineages of *Phytophthora infestans*. *Phytopathology* 88:837-843.
 37. Nelson, M. R., Felix-Gastelum, R., Orum, T. V., Stowell, L. J., and Myers, D. E. 1994. Geographic information systems and geostatistics in the design and validation of regional plant virus management programs. *Phytopathology* 84:898-905.
 38. Nelson, M. R., Orum, T. V., Jaime-Garcia, R., and Nadeem, A. 1999. Applications of geographic information systems and geostatistics in plant disease epidemiology and management. *Plant Dis.* 83:308-319.
 39. Niederhauser, J. S. 1991. *Phytophthora infestans*: The Mexican connection. Pages 25-45 in: *Phytophthora*. J. A. Lucas, R. C. Shattock, D. S. Shaw, and L. R. Cooke, eds. Br. Mycol. Soc./Cambridge University Press, Cambridge.
 40. Platt, H. W. 1992. Potato Late Blight. Pages 93-123 in: *Plant Diseases of International Importance, Disease of Vegetables and Oil Seed Crops*. Vol. 2. H. S. Chaube, U. S. Singh, A. N. Mukhopadhyay, and J. Kumar, eds. Prentice Hall, Englewood Cliffs, NJ.
 41. Raposo, R., Wilks, D. S., and Fry, W. E. 1993. Evaluation of potato late blight forecasts modified to include weather forecasts: A simulation analysis. *Phytopathology* 83:103-108.
 42. Real, L. A., and McElhany, P. 1996. Spatial patterns and process in plant-pathogen interactions. *Ecology* 77:1011-1025.
 43. Royer, M. H., Russo, J. M., and Kelley, G. W. 1989. Plant disease prediction using a mesoscale weather forecasting technique. *Plant Dis.* 73:618-624.
 44. Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
 45. Shaw, D. S. 1991. Genetics. Pages 131-170 in: *Advances in Plant Pathology*. Vol. 7. *Phytophthora infestans*: the Cause of Late Blight of Potato. D. S. Ingram and P. H. Williams, eds. Academic Press, New York.
 46. Sherf, A. F., and MacNab, A. A. 1986. *Vegetables Disease and their Control*. 2nd ed. John Wiley & Sons, New York.
 47. Spielman, L. J., Sweigard, J. A., Shattock, R. C., and Fry, W. E. 1990. The genetics of *Phytophthora infestans*: Segregation of allozyme markers in F2 and backcross progeny and the inheritance of virulence against potato resistance genes R2 and R4 in F1 progeny. *Exp. Mycol.* 14:57-69.
 48. Stanley, D. 1994. Potato Blight—What was Around Comes Around. U.S. Dep. Agric. Agric. Res. Serv. Rep. 42:4-7.
 49. Star, J., and Estes, J. 1990. *Geographic Information Systems: An Introduction*. Prentice Hall, Englewood Cliffs, NJ.
 50. Sujkowski, L. S., Goodwin, S. B., Dyer, A. T., and Fry, W. E. 1994. Increased genotypic diversity via migration and possible occurrence of sexual reproduction of *Phytophthora infestans* in Poland. *Phytopathology* 84:201-207.
 51. Therrien, C. D., Ritch, D. L., Davidse, L. C., Jespers, A. B. K., and Spielman, L. J. 1989. Nuclear DNA content, mating type and metalaxyl sensitivity of eighty-three isolates of *Phytophthora infestans* from the Netherlands. *Mycol. Res.* 92:140-146.
 52. Thurston, H. D., and Schultz, O. 1981. Late blight. Pages 40-42 in: *Compendium of Potato Diseases*. W. J. Hooker, ed. The American Phytopathological Society, St. Paul, MN.
 53. Tooley, P. W., Fry, W. E., and Villarreal-Gonzalez, M. J. 1985. Isozyme characterization of sexual and asexual *Phytophthora infestans* populations. *J. Hered.* 76:431-435.
 54. Wasmann, C. C., and VanEtten, H. D. 1996. Transformation-mediated chromosome loss and disruption of a gene for pisatin demethylase decrease the virulence of *Nectria haematococca* on pea. *Mol. Plant-Microbe Interact.* 9:793-803.
 55. Wastie, R. L. 1991. Breeding for resistance. Pages 193-224 in: *Advances in Plant Pathology*. Vol. 7. *Phytophthora infestans*: The Cause of Late Blight of Potato. D. S. Ingram and P. H. Williams, eds. Academic Press, New York.