Sensitivity to Protectant Fungicides and Pathogenic Fitness of Clonal Lineages of *Phytophthora infestans* in the United States

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ABSTRACT

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Since 1991, dramatic changes have occurred in the genetic composition of populations of *Phytophthora infestans* in the United States. Clonal lineages recently introduced into the United States (US-7 and US-8) are more common now than the previously dominant lineage (US-1). To help determine why these changes occurred, four clonal lineages of *P. infestans* common during the early 1990s in the United States and Canada were evaluated for sensitivity to the protectant fungicides mancozeb and chlorothalonil using amended agar assays for isolates collected from 1990 to 1994. No isolate or lineage was resistant to either mancozeb or chlorothalonil. There were significant differences among isolates for degree of sensitivity to one fungicide individually, but there were no significant ($P = 0.05$) differences among the US-1, US-6, US-7, and US-8 clonal lineages for degree of sensitivity to both fungicides. Therefore, resistance to protectant fungicides cannot explain the rapid increase in frequency of the US-7 and US-8 clonal lineages. Three components of pathogenic fitness (latent period, lesion area, and sporulation after 96 h) were tested for the three clonal lineages that were detected most commonly during 1994 (US-1, US-7, and US-8). All but one of the isolates in this analysis were collected during 1994 and evaluated within 10 months of collection by inoculating detached leaflets of the susceptible potato cultivar Norchip. There were significant differences between the US-1 and US-8 clonal lineages for lesion area and sporulation, and between US-1 and US-7 for latent period. The US-6 clonal lineage was excluded from the pathogenic fitness experiments, because no isolates of this lineage were collected during 1994. Compared with US-7 and US-8, US-1 had the longest latent period and the smallest lesions with the least sporulation. Incorporation of the differences between US-1 and US-8 in computer simulation experiments revealed that significantly more protectant fungicide (e.g., 25%) would be required to suppress epidemics caused by the US-8 clonal lineage compared with US-1. These differences in pathogenic fitness components probably contribute to the general predominance of the "new" clonal lineages (especially US-8) relative to the "old" US-1 lineage.

Additional keywords: fungicide resistance, late blight, pathogen migration, population genetics.

Prior to the late 1970s, populations of the potato late blight pathogen, *Phytophthora infestans*, throughout most of the world were dominated by a single clonal lineage, named US-1 by Goodwin et al. (17) and PO-1 by Sujkowski et al. (47). Because this clonal lineage was A1 mating type, populations outside the highlands of central Mexico were largely limited to asexual reproduction, probably for more than 120 years. This changed during the late 1970s, when novel genotypes of *P. infestans* were introduced to Europe (11,13,14,16,17,21,33,44). New genotypes now dominate populations in most locations analyzed, including most of Europe, East Asia, and South America (4,8,12,23,27,47). New genotypes were detected in the United States by the early 1990s (5,12,16).

These recent introductions apparently involved several independent migrations from Mexico. The first probably was from Mexico to Europe during 1976 to 1977 (35). The new European populations founded during this migration apparently served as the source for subsequent migrations to South America (8) and Africa (17). Introduction of a novel genotype to Japan and Korea (27,33) apparently was unrelated to the European migrations (15,17). At least two different migrations probably brought new genotypes from northwestern Mexico into the United States during the late 1970s to early 1990s (16,21) that were distinct from the European and Japanese/Korean introductions (11).

The concept of a clonal lineage is crucial to understanding the population biology of *P. infestans* outside central Mexico. A clonal lineage includes the asexual derivatives of a given genotype (1,15). Variation within a clonal lineage can arise by mutation and (in diploids) mitotic recombination; otherwise, all members of the same clonal lineage should be identical and easy to distinguish from individuals in other clonal lineages. Clonal lineages of *P. infestans* have been described on the basis of mating type, allozyme genotype at the glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*) loci, and DNA fingerprint as determined with DNA probe RG57 (17). This large number of markers (including at least 28 independent loci, two with multiple alleles) provides a high ability to identify genotypes and, within a given asexual population, we can be quite certain that isolates with the same multilocus genotype are clonal descendants from the same ancestor. Lineages that have existed for many generations contain more variation (due to mutation and mitotic recombination) than young lineages. For example, in independent studies on different continents, Goodwin et al. (22) and Sujkowski et al. (48) both demonstrated that there was greater diversity for pathogenicity within an old clonal lineage than in a young one. However, there was much less variation within than among lineages (22).

Within the United States, only four clonal lineages of *P. infestans* (US-1, US-6, US-7, and US-8) were detected commonly during the early 1990s (12), and each contained specific ecologically important traits. For example, US-6 and US-7 have caused

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severe disease on potatoes and tomatoes, whereas US-8 was associated primarily with potatoes (21,30). US-6, US-7, and US-8 are resistant to metalaxyl, and US-1 is sensitive (23). The association of a unique *Gpi* genotype with each of the four predominant clonal lineages and knowledge that most epidemic populations have been homogeneous for lineage (16,21,32) enabled investigators to use *Gpi* genotype to rapidly predict epidemiologically important characteristics of sample populations (19).

There are several reasons that could explain the rapid replacement of US-1 by the "new" strains in the United States and Canada. Resistance to metalaxyl is one possibility. The most common immigrant strains (US-6, US-7, and US-8) were resistant to metalaxyl (23,32), and this, presumably, gave them a large selective advantage where metalaxyl was used. However, resistance to metalaxyl alone cannot explain the predominance of new genotypes in all locations, because resistant strains often dominated populations even in agro-ecosystems where metalaxyl was not used (23). A second reason could be enhanced pathogenicity to tomato. The US-6 and US-7 lineages reproduce rapidly on tomatoes (21,29,30), which would give them a selective advantage in agro-ecosystems that included tomatoes. However, this does not explain the predominance of new genotypes in potato-only agro-ecosystems such as those in Maine and North Dakota. A third possibility is increased pathogenic fitness on potatoes. Because epidemics during the early 1990s appeared to be especially rapid and severe (25), it seems possible that at least some of the immigrant strains may have greater pathogenic fitness on potatoes than did the previous strains (primarily US-1). Increased pathogenic fitness of immigrant strains has been a factor in the rapid turnover of genotypes in the United Kingdom (4). Greater pathogenicity to potato tubers (28) may explain the apparent greater severity of tuber rot and the rapid distribution of the US-8 lineage. A final reason may be tolerance to protectant fungicides. The speed and severity of epidemics during the early 1990s in the United States and Canada would be explained if the recently introduced strains were less sensitive to standard protectant fungicides. Disease management strategies will need to be adjusted if exotic strains have greater pathogenic fitness or have less sensitivity to protectant fungicides.

The objective of this study was to determine whether decreased sensitivity to protectant fungicides, higher pathogenic fitness, or both could have contributed to the rapid increase of recent immigrant strains relative to the previously dominant US-1 strain. The specific hypotheses tested were that (i) isolates in all lineages were sensitive to protectant fungicides, and (ii) all lineages had similar pathogenic fitness as detected by latent period, lesion area, and sporulation. The approaches were to assess sensitivity to two commonly used protectant fungicides and to measure selected pathogenic fitness attributes of individuals within clonal lineages of *P. infestans* that were common during the early 1990s in the United States. These hypotheses would be rejected if the new lineages (i) were resistant or consistently less sensitive to common protectant fungicides than US-1; or (ii) had greater pathogenic fitness than US-1. An abstract reporting some aspects of this study has been published (26).

TABLE 1. Isolates of *Phytophthora infestans* used in pathogenic fitness studies

Isolate no.	Date of collection	State or province	Mating type	*Gpi* genotype	Lineage
940499[a]	October 1994	MI	A1	86/100	US-1
940500	October 1994	MI	A1	86/100	US-1
940501	October 1994	MI	A1	86/100	US-1
940486	September 1994	TN	A2	100/111	US-7
940488	August 1994	NY	A2	100/111	US-7
930234	June 1993	CA	A2	100/111	US-7
940472	August 1994	PA	A2	100/111/122	US-8
940473	July 1994	ME	A2	100/111/122	US-8
940474	October 1994	NB[b]	A2	100/111/122	US-8
940476	August 1994	NY	A2	100/111/122	US-8
940489	September 1994	NY	A2	100/111/122	US-8
940495	September 1994	NY	A2	100/111/122	US-8

[a] Accession number of the isolate in the *Phytophthora infestans* culture collection at Cornell University.
[b] New Brunswick, Canada.

MATERIALS AND METHODS

Isolates. Isolates of *P. infestans* were obtained from samples submitted to our lab during the early 1990s from throughout the United States and Canada. Isolates received before 1994 have been described (16,21), and a selection of these had been stored at –130°C using a technique described by Tooley (49). During 1994 (April through October), we received 136 samples (most as infected foliage/tubers, but some as pure cultures). Isolations were attempted on all plant samples using techniques described previously (10), and pure cultures were maintained at 18°C on rye A agar (3). The resulting isolates were analyzed for mating type and for genotype at the two allozyme loci *Gpi* and *Pep* (47). Isolates were obtained from one Canadian province (NB) and 15 states (CA, FL, GA, ME, MI, NC, ND, NE, NY, OR, PA, SC, TN, VA, and WA). Most of the isolates (>75%) were A2 mating type and had a dilocus allozyme genotype like the US-8 clonal lineage (*Gpi* 100/111/122 and *Pep* 100/100); approximately 8% had genotypes consistent with the US-7 clonal lineage (A2, *Gpi* 100/111 and *Pep* 100/100); and 9% had the US-1 genotype (A1, *Gpi* 86/100 and *Pep* 92/100). Other new lineages were much less frequent. Approximately 3% were US-11 (A1, *Gpi* 100/111 and *Pep* 100/100), 1% were US-13 (A2, *Gpi* 100/100 and *Pep* 100/100), and less than 0.5% were US-14 (A2, *Gpi* 100/122 and *Pep* 100/100). No US-6 isolates had been detected during 1994, when the isolates for this study were chosen.

A subset of the 1994 isolates was analyzed further for DNA fingerprint as determined by probe RG57 (18,21). These included three isolates that had been tentatively described as US-1, two that were tentatively identified as US-7, and four that were tentatively identified as US-8. The DNA fingerprints confirmed the tentative designations, and these plus three additional isolates (Table 1) then were used for fitness assessments.

Fungicide sensitivity. Sensitivities to the protectant fungicides mancozeb (Dithane M-45 80WP; Rohm and Haas Co., Philadelphia) and chlorothalonil (Bravo-720; ISK BioSciences, Mentor, OH) were determined as described by Sujkowski et al. (46). Isolates were chosen from the four most commonly occurring lineages collected during the early 1990s for a total of 40 isolates, 10 isolates each of US-1, US-6, US-7, and US-8. Fungicides as formulated commercial products were suspended in sterile water. These suspensions were then added to pea agar (20) at 10 concentrations ranging from 0.2 to 50.0 µg a.i./ml. Pea agar without fungicide was used as the control medium. Rye agar discs (9 mm in diameter) containing mycelium of *P. infestans* were obtained from the perimeter of an actively growing colony and were placed at the center of a 9-cm petri plate containing pea agar with or without fungicide. Diameters were measured after 6 to 8 days of incubation at 18°C. Growth was measured as the colony diameter less the diameter of the inoculum disc. The effect of each fungicide concentration was assessed twice for each isolate. Data were obtained as the proportion (percent) of growth on fungicide-amended medium relative to growth on the no-fungicide control. The fungicide concentration that inhibited growth by 50% (EC_{50}) was estimated for each isolate from a regression of relative mycelial growth versus fungicide concentration.

Fitness assessments. Isolates for pathogenic fitness comparisons were selected from those collected within a year of initiating the experiments. Stored isolates have been reported to have lower fitness than recently collected isolates (7). Therefore, no isolate in the US-6 clonal lineage was included, because we had not detec-

ted these in the 10 months prior to beginning the experiment. For other isolates, all except one US-7 isolate were evaluated within 10 months of collection.

Out of necessity, fitness was assessed only on detached leaflets in petri plate moist chambers. It was not possible to compare US-1 (A1 mating type) with the A2 mating types (US-7 and US-8) in the field, because of the high probability for cross-plot contamination and subsequent oospore formation. Comparison of US-1 with US-6 (also A1) was also not possible in the field, because the commonly detected strains near our field site had been only A2 mating type since 1993.

Fitness was measured on detached leaflets of the highly susceptible potato cultivar Norchip that had been grown in a greenhouse (18°C night temperature, 25°C day temperature). The photoperiod was extended to 14 h with 400-W GE Lucalox lamps (General Electric Lighting, Cleveland). Leaflets for experimental purposes were obtained randomly from nonsenescent, fully expanded leaves of plants at or before flowering.

Inoculations were accomplished in moist chambers constructed of 15-cm-diameter petri plates. High relative humidity was maintained by a layer of water agar formed by pouring molten water agar (about 25 ml of 1.5% water agar) into the bottom of each plate. After the agar solidified, the plate was inverted so that the agar layer was on top. Leaves were collected from several different plants, and five randomly selected leaflets were placed in a single dish, with the layer of water agar above the leaflets.

Inoculations were done with sporangia from 10- to 14-day-old cultures of each isolate grown on rye B agar (3) at 18°C. Sporangia were washed from the culture with distilled water and adjusted to 20,000/ml with a hemacytometer. Each sporangial suspension was prepared as rapidly as possible, so that no sporangia were in water for more than 120 min before inoculation. Each of the five leaflets in a single petri dish was inoculated with a 40-µl drop of the sporangial suspension of a single isolate. Thus, each leaflet received approximately 800 sporangia. Inoculated leaves in moist chambers were placed in a lighted growth chamber with weak light (0.02 to 0.03 µE m^{-2} s^{-1}) at 18°C as described previously (22,30,45).

Latent period was defined as the time between inoculation and first sporulation. Leaflets were observed at 54, 66, 72, 78, 90, and 96 h after inoculation with a dissecting microscope. An isolate was considered sporulating when sporangia were observed on at least three of the five inoculated leaflets. The experiment was done a total of five times, and there was one petri plate per isolate in each experiment.

Lesion area was measured using leaflets inoculated as described above. Because there were occasional leaflets without lesions, data were recorded from four representative leaflets in each moist chamber. Lesion area was assessed visually 96 h after inoculation using a set of standard diagrams ranging in size from 1 to 13 cm^2. The inoculation was performed four times for each isolate, with each trial treated as a block in the statistical analysis.

Sporulation was measured on the same four leaflets/moist chamber that had been measured for lesion area. Each leaflet containing a single lesion was vortexed individually with 4 ml of 10% ethanol in a 14-ml disposable polyethylene culture tube for 10 s. The number of sporangia was counted with a hemacytometer (three independent counts). The entire procedure (inoculation, incubation, and measurement) was performed four separate times (trials), with each trial treated as a block in the statistical analysis.

Simulation analyses. To estimate the potential epidemiological impact of the differences in pathogenic fitness among lineages, we employed a mechanistic simulation model of the late blight disease. The model describes disease development in small plots of potatoes (4 × 4 m) and reflects differences in weather, host resistance, fungicide (chlorothalonil, metalaxyl) application, and pathogen attributes (2,31). The model has been in development since the late 1970s and has been validated extensively (6). Simulation experiments conducted with the model have led to hypotheses that have been verified in subsequent field experiments (39–42).

Epidemics caused by the US-1 and US-8 clonal lineages were simulated using adjusted values for lesion expansion rates in the simulator. Lesion expansion rates directly affect lesion area, indirectly affect sporulation, and might be an extension of latent period. The lesion expansion factor in the current version of the model (as used by Shtienberg and Fry [40]) was set at 1.0 for the US-1 clonal lineage, because the model was constructed from experiments using US-1. The adjustment factor for US-8 was set at 1.29 from data presented in Results below.

Epidemics with different fungicide treatments were simulated using a mid-season cultivar of moderate susceptibility. Disease was initiated at a level of 1% severity at 30 days after emergence. The season length was 85 days. Epidemics were simulated using 50 years of weather data from upstate New York (36). Each treatment (= each data point in Fig. 1) had 50 replications (for each year of weather data). Fungicide applications (chlorothalonil) were simulated at 1.3 kg a.i./ha, beginning at 32 days after emergence. There were nine fungicide treatments that differed in application frequency (ranging from every 4 days to every 14 days). Each data point resulted from a unique fungicide application schedule, with a unique clonal lineage replicated over 50 seasons. Each clonal lineage received each fungicide treatment. Area under the disease progress curve (AUDPC) was calculated as described by Shaner and Finney (38) at 85 days after emergence. AUDPC incorporates earliness, speed, and severity into a single summary statistic and has been demonstrated to be a particularly robust parameter for detecting differences among treatments (9).

Statistical analysis. All data were analyzed using analysis of variance. The fungicide sensitivity experiments were set in a completely randomized design, and the fitness component experiments were set in a randomized complete block design. For both sets of experiments, a linear model for nested design was used. The model for fungicide sensitivity assays was $Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + e_{ijk}$, in which μ is the overall mean, α_i is the fixed effect of clonal lineage, $\beta_{j(i)}$ is the fixed effect of isolates nested within clonal lineages, and e_{ijk} is the random experimental error. For the fitness component experiments, the model was slightly modified to $Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \gamma_k + e_{ijk}$, in which μ is the overall mean, α_i is the fixed effect of clonal lineage, $\beta_{j(i)}$ is the fixed effect of isolates nested within clonal lineages, γ_k is the fixed effects of blocks, and e_{ijk} is the random experimental error. Lineages or groupings of lineages were compared using orthogonal contrasts (43).

RESULTS

Sensitivity to chlorothalonil and mancozeb. No isolate or lineage was resistant to either protectant fungicide. However, there were some differences in degree of sensitivity to a particular fungicide (Tables 2 and 3). The EC_{50} for US-1 for chlorothalonil appeared greater (less sensitive) than for the other lineages (Table 2), and this difference was marginally significant ($P = 0.061$). The chlorothalonil EC_{50} for US-1 was significantly greater ($P = 0.014$) than the EC_{50} for US-8. The results were somewhat different for sensitivity to mancozeb, in which US-7 tended to be less sensitive than US-1 ($P = 0.057$). There were no consistent differences among lineages in regard to degree of sensitivity to both protectant fungicides (Tables 2 and 3).

There were some generalities regarding individual fungicides. The isolates were sensitive to lower concentrations, and there was less variance for chlorothalonil than for mancozeb (Table 2). The mean EC_{50}s for chlorothalonil ranged from 0.47 to 0.73 µg/ml, and the mean EC_{50}s for mancozeb ranged from 1.61 to 4.22 µg/ml.

Latent period. The latent period was longer than 54 h at 18°C for all isolates, but was longer for US-1 than for US-7 or US-8 isolates (Tables 4 and 5). The mean latent period for US-1 isolates was significantly longer than for US-7 isolates ($P = 0.019$) and for the combined sample of US-7 and US-8 isolates ($P = 0.023$). The difference between US-1 and US-8 was nearly significant (P =

0.081). There also were highly significant differences among isolates within lineages ($P = 0.005$).

Lesion area. The lesion sizes produced by the isolates at 96 h after inoculation differed as a function of clonal lineage (Tables 4 and 5). Lesions produced by US-1 were smallest (4.1 cm^2), followed by US-7 (4.7 cm^2) and US-8 (5.2 cm^2). The difference between US-1 and US-7 was not significant ($P = 0.223$), but the US-1 isolates did produce significantly smaller lesions than the US-8 isolates ($P = 0.009$) (Table 5). Again, there were highly significant differences among isolates within lineages ($P = 0.003$) (Table 5).

Sporulation. In general, the larger lesions produced more sporangia than the smaller lesions. There were no significant differences between US-1 and US-7 ($P = 0.553$), but the difference between US-1 and US-8 was significant ($P = 0.043$). There were highly significant differences among isolates ($P = 0.0001$). There were no significant differences among lineages in terms of sporulation per unit of lesion area ($P = 0.257$).

Simulation analyses. The differences in lesion growth rates led to epidemics caused by US-8 that were significantly more severe (larger AUDPC) than those caused by US-1 at all fungicide application regimes (Fig. 1). Frequency of application was negatively correlated with epidemic development for both lineages. For US-8, the regression equation was AUDPC = $–2.50 + 0.90x$ ($R^2 = 0.970$) and, for US-1, the regression equation was AUDPC = $–2.51 + 0.67x$ ($R^2 = 0.962$) (in which x = the application frequency in terms of days between application). Using indicator variables to represent the lineages (34), the slopes of the two regressions were significantly different ($P = 0.026$).

DISCUSSION

The detection of significant differences in pathogenic fitness among clonal lineages of *P. infestans* provides additional demonstration of the utility of lineages as predictors of biological attributes. The predictive power of clonal lineages exists, in part, because of their small number currently in most locations in the United States and Canada, and will diminish if the population becomes more diverse. A larger number of lineages of *P. infestans* would (i) make it more difficult to identify individual lineages; (ii) decrease the probability that lineages would occur singly in fields; and (iii) greatly reduce accuracy of neutral markers to predict epidemiologically important attributes.

No isolate and, consequently, no lineage was resistant to either mancozeb or chlorothalonil. Therefore, we cannot reject our first hypothesis that all isolates/lineages were sensitive to these two protectant fungicides. Although all isolates were sensitive, there were differences in degree of sensitivity. However, the only significant difference between US-1 (the previous dominant lineage) and US-8 (the current dominant lineage) occurred with chlorothalonil, in which US-1 had a higher EC_{50}. This change is opposite of that expected if this difference in fungicide sensitivity was a factor in the current predominance by US-8. Overall, the reactions of the United States isolates to chlorothalonil were similar to those of isolates collected in different places and in different years in Mexico (46). Mancozeb was not included in the Mexican study, so comparisons with that fungicide are not possible. We

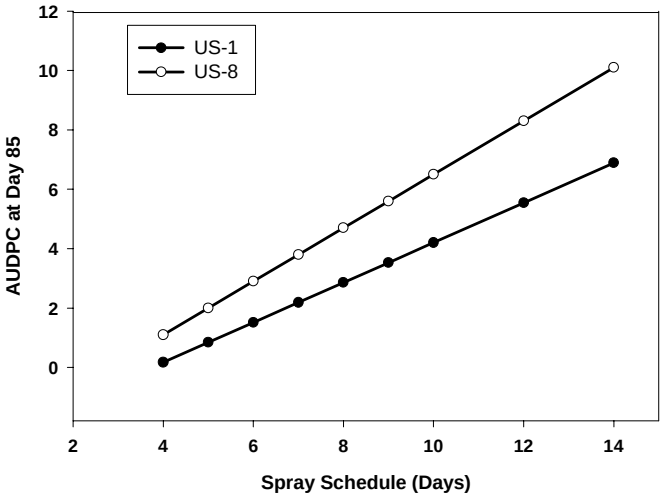

Fig. 1. Simulation model predictions of the effect of different fungicide application frequencies to suppress epidemics of late blight caused by the US-1 and US-8 clonal lineages. The application frequencies are described according to the number of days between applications. Thus, application every 2 days involved the greatest amount of fungicide and application every 14 days involved the least. The lineages differed only in terms of the lesion expansion rate in the simulation model as described in the text. All other factors were the same for each lineage and are described in the text. Each data point resulted from 50 independent simulations—one for each of 50 years of weather data from upstate New York. Linear regressions for the lineages were US-1, AUDPC = $–2.51 + 0.67x$, $R^2 = 0.962$; and US-8, AUDPC = $–2.50 + 0.90x$, $R^2 = 0.970$. Using indicator variables to represent the lineages (34), the difference between the slopes of the lines was significant ($P = 0.026$).

TABLE 2. Mean EC_{50}s (µg a.i./ml) of chlorothalonil and mancozeb for four clonal lineages of *Phytophthora infestans* (10 isolates each) grown in amended agar medium

	Chlorothalonil			Mancozeb		
Lineage	Mean	SD[a]	Range	Mean	SD	Range
US-1	0.73	0.31	0.44–1.47	1.61	0.53	0.03–2.62
US-6	0.60	0.14	0.36–0.88	3.71	1.44	1.68–5.97
US-7	0.64	0.29	0.21–1.33	4.22	5.17	0.63–18.5
US-8	0.47	0.12	0.30–0.84	1.76	1.14	0.72–4.13

[a] Standard deviation. None of the differences among lineages are significant at $P = 0.05$.

TABLE 3. Analysis of variance and mean comparisons of EC_{50}s (µg a.i./ml) for sensitivity to chlorothalonil and mancozeb among four clonal lineages of *Phytophthora infestans*

	Fungicide							
	Chlorothalonil				Mancozeb			
Source	df	MS[a]	*F*[a]	*P* value	df	MS	*F*	*P* value
Lineage[b]	3	0.2388	2.30	0.096	3	33.166	1.91[b]	0.147
Contrasts								
US-1 vs. US-6	1	0.1435	1.38	0.249	1	33.007	1.90	0.177
US-1 vs. US-7	1	0.0939	0.90	0.349	1	67.756	3.91	0.057
US-1 vs. US-8	1	0.697	6.71	0.014	1	0.215	0.01	0.912
US-1 vs. (US-6 + US-7 + US-8)	1	0.394	3.79	0.061	1	37.099	2.14	0.153
Isolates (Lineage)	32	0.1039	5.59	0.0001	32	17.336	91.20	0.0001
Error	36	0.0186			36	0.190		
Total	71				71			

[a] MS and *F* = mean squares and *F* statistic, respectively.
[b] The lineage effect and contrasts were tested with isolate (lineage) as error term.

conclude that differential sensitivity to these protectant fungicides has not contributed to the dominance by new lineages in the United States.

In contrast to the situation for fungicide sensitivity, there were significant differences among the clonal lineages for the three fitness attributes measured. In all comparisons with significant differences, the US-1 clonal lineage had lower fitness. We, thus, reject our second hypothesis that lineages had the same pathogenic fitness attributes. The new clonal lineages (US-7 and US-8) had greater pathogenic fitness than did the old US-1 lineage. Thus, it seems likely that differential pathogenic fitness has contributed to the current predominance of the new clonal lineages.

The simulation results predict that epidemics caused by the US-8 clonal lineage are likely to be more severe than those caused by US-1, and that more frequent application of protectant fungicide is required to suppress epidemics caused by US-8 (compared with epidemics caused by US-1) to a given level. These results are consistent with the experience of growers and practitioners that more protectant fungicide is necessary to suppress epidemics caused by the new clonal lineages than those caused by US-1. Although weather and cultivar resistances influence the needed intensity of fungicide application, clonal lineage also should be considered. For example, from Figure 1, one can predict that to achieve the same average AUDPC applications will be required every 7 days for epidemics caused by US-8 compared with applications only every 9 to 10 days for US-1. This prediction could be tested in field experiments in Mexico or other locations where both mating types are established.

Although the most accurate and meaningful assessment of fitness is via polycyclic experiments in the field, there were several reasons that those experiments were not possible. Because of aerial dispersal of *P. infestans*, assessments of epidemiological fitness (ability to induce an epidemic within one season) are confounded by migration among plots (29). If plots are separated sufficiently so that migration is not a factor, then environmental differences preclude comparisons. Competitive fitness experiments in field plots might have been possible, but because we currently cannot distinguish isolates within a lineage after they are mixed together, there would be data only from clonal lineages and not from individuals within lineages. Additionally, mixture experiments involving A1 and A2 isolates in the field at our research site (Tompkins County, NY) would be unwise, because there currently is not a residential population of oospores at that site. Any comparison in the field of isolates involving the current predominant lineages (US-7 and US-8, both A2 mating types) and the clonal lineage that predominated during the 1980s (US-1, A1 mating type) would create the very high probability for sexual recombination, production of oospores, and creation of a residential oospore population at our field site. In addition to creating a hazard for future experiments with late blight, a resident population of oospores could be hazardous for potato and tomato crops in subsequent years, because of unknown gene combinations resulting from sexual reproduction. For these reasons, field experiments of fitness were not permissible, so all fitness assessments were limited to the laboratory.

This study contributes to the emerging explanation for the predominance by "new" clonal lineages (12). Predominance alone indicates (by definition) that the "new" clonal lineages have greater fitness (37). Factors that probably contribute to the greater overall fitness of the new clonal lineages include metalaxyl insensitivity (23), enhanced tomato pathogenicity (30), enhanced pathogenicity to potato tubers (28), and enhanced pathogenicity to potato foliage (this study). Factors that either do not contribute or contribute so little that they have not yet been demonstrated include lessened sensitivity to the most common protectant fungicides (this study) and differential aggressiveness to potato cultivars (24). Finally, different responses to abiotic factors also might contribute to the predominance of the new clonal lineages. Explanations such as differential response to temperature, moisture, and solar radiation need to be investigated.

A factor that may become important, but one that will also help destroy the current clonal population structure, is the contribution of oospores to between-season survival. It is now clear that some crosses among common clonal lineages in the United States will produce viable progeny (50) and will survive winter temperatures (7). Oospore survival will contribute to the continuity of alleles, but will necessarily counter dominance by individual lineages.

TABLE 4. Latent period, lesion area, and sporulation per lesion of isolates in the US-1, US-7, and US-8 clonal lineages of *Phytophthora infestans*

		Fitness component		
Lineage	Isolate no.[a]	Latent period[b]	Lesion area[b]	Sporulation[b]
US-1	940499	69	4.16	112
	940500	66	4.53	143
	940501	76.5	3.50	101
Mean		70.5	4.06	119
US-7	940486	66	4.41	128
	940488	63	4.84	143
	930234	60	4.71	161
Mean		63.0	4.65	144
US-8	940472	69	4.69	160
	940473	66	4.72	136
	940474	63	5.28	303
	940476	66	5.47	154
	940489	66	5.53	204
	940495	66	5.72	218
Mean		66.0	5.24	196

[a] Accession number of the isolate in the *Phytophthora infestans* culture collection at Cornell University.

[b] Latent period is measured in hours; lesion area is measured in square centimeters; and sporulation is measured in thousands of sporangia per lesion.

TABLE 5. Analysis of variance for latent period, lesion area, sporulation per lesion, and sporulation per unit of lesion area

	Latent period				Lesion area				Sporulation/lesion				Sporulation/lesion area			
Source	df	MS[a]	*F*[a]	*P* value	df	MS	*F*	*P* value	df	MS	*F*	*P* value	df	MS	*F*	*P* value
Block	4	24.56	1.50	0.220	3	8.37	3.68	0.013	3	49,506.9	8.58	0.0001	3	4,172.1	13.53	0.001
Lineage[b]	2	215.16	4.10	0.054	2	39.10	5.92	0.023	2	116,007.7	3.15	0.092	2	17,873.9	1.58	0.257
Contrasts																
US-1 vs. US-7	1	421.88	8.04	0.019	1	11.34	1.72	0.223	1	15,470.4	0.42	0.533	1	28,136.1	2.49	0.149
US-1 vs. US-8	1	202.50	3.86	0.081	1	74.32	11.25	0.009	1	204,089.4	5.55	0.043	1	655.8	0.06	0.815
US-7 vs. US-8	1	90.00	1.71	0.223	1	22.39	3.39	0.099	1	94,950.8	2.58	0.143	1	28,250.4	2.50	0.148
US-1 vs. (US-7 + US-8)	1	392.73	7.48	0.023	1	42.68	6.46	0.032	1	96,677.0	2.63	0.139	1	13,115.7	1.16	0.309
Isolate (lineage)	9	52.5	3.20	0.005	9	6.61	2.91	0.003	9	36,770.7	6.37	0.0001	9	11,280.2	36.57	0.001
Error	44	16.38			177	2.27			177	5771.9			177	308.5		

[a] MS and *F* = mean squares and *F* statistic, respectively.

[b] The lineage effect and contrasts were tested with isolate (lineage) as error term.

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