

Variability in Virulence Among Asexual Progenies of *Phytophthora infestans*

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ABSTRACT

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One hundred two single zoospore isolates of *Phytophthora infestans*, derived asexually from four parental isolates of US-8 genotype and one isolate of US-1 genotype, were characterized for their virulence phenotypes to determine changes in virulence during asexual reproduction. Potato differentials, each containing a major gene for resistance to *P. infestans* (R1 to R11), were used to characterize the virulence patterns. Isolates were also characterized for mating type, *glucose-6-phosphate isomerase* (*Gpi*) banding pattern, and DNA fingerprints using probe RG57 to determine any genotypic changes in the single zoospore isolates. A subset of these single zoospore isolates was tested for response to mefenoxam to determine any shifts in sensitivity. Results showed that single zoospore isolates derived from parent PI-1 (US-8, 11 isolates)

were identical to their parental virulence. Isolates derived from parent PI-191 (US-8, 29 isolates) showed some differences in virulence, mainly toward R8 and R9. Isolates derived from parent PI-126 (US-8, 14 isolates) demonstrated a higher level of virulence diversity. Isolates derived from parents PI-52 (US-1, 28 isolates) and PI-105 (US-8, 20 isolates) showed the highest level of virulence variability among the single zoospore isolates. Mating type, *Gpi* banding pattern, and DNA fingerprints for the single zoospore isolates were, in most cases, identical to the parental isolates. Single zoospore isolates showed different levels of sensitivity to mefenoxam. Virulence and other genetic changes during asexual reproduction are likely to play a major role in changing the race structure of *P. infestans* populations. This continuous change in the race structure is a serious problem and now poses a new challenge for utilization of race-specific resistance to manage late blight of potato.

Additional keywords: phenylamide fungicides, *Solanum tuberosum*.

The Chromista organism *Phytophthora infestans* (Mont.) de Bary, the cause of potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* Mill) late blight, is the most important foliar and tuber pathogen of potato worldwide. Central Mexico is believed to be the center of origin of *P. infestans* (9). Until relatively recently, this was the only location where both mating types of *P. infestans* occurred (8). Until recently, *P. infestans* populations outside Mexico were confined to the A1 mating type (8). The A2 mating type was introduced to North America and Europe in a recent migration from Mexico, probably in the late 1970s (9). Prior to that, populations of *P. infestans* throughout the world were dominated by a single clonal lineage, designated US-1 by Goodwin et al. (13). Because this clonal lineage was an A1 mating type, populations of *P. infestans* outside Mexico were limited to asexual reproduction.

Sexual reproduction of *P. infestans*, associated with genetic recombination during meiosis in the antheridium or the oogonium, is a major mechanism of genetic variation in this diploid organism. However, other mechanisms of genetic variability may have a significant role in creating new variants of this pathogen. Mutation, mitotic recombination, and parasexual recombination are the most common mechanisms of genetic variability in the absence of sexual reproduction (12). The most important aspect of genetic variability in plant pathogens is the variability in pathogenicity and virulence toward the host. Virulence variability in *P. infestans* populations is recognized as a major reason for failure of race specific genes for resistance in cultivated potato as a disease management strategy (40).

Variability in virulence was recognized in the old populations of *P. infestans*, which were confined to asexual reproduction due to the absence of the A2 mating type (10,37,39,41). Variation among single zoospore isolates of *P. infestans* in other characteristics such as aggressiveness, growth rate, and colony morphology was also reported (3–5,41). Variability in virulence among single zoospore strains has also been reported for some oomycetes including *P. sojae* (30) and *Aphanomyces euteiches* (22). The same phenomenon of intraspecific variability has been observed in many plant pathogens belonging to the kingdom Mycota. Pederson and Kiesling (25) noted recurrent changes in host range upon repeated selfing of sporidia arising from a single teliospore of the smut fungus *Ustilago hordei* (Pers.) Lagerh. These shifts led to progeny capable of infecting a broader range of barley (*Hordeum vulgare* L.) cultivars.

During the last decade, much emphasis has been placed on the role of sexual reproduction in *P. infestans*, for generation of new races of the pathogen and the role of the oospores as a survival mechanism (6). On the other hand, most recent surveys of *P. infestans* populations in the United States and Canada (16,26,27) have shown that most of the pathogen populations are dominated by the US-8 genotypes of the A2 mating type.

The race concept as applied to *P. infestans* refers to possession of certain virulence factors. Isolates sharing the same virulence factors are considered to be a race that can be distinguished from other races possessing other groups of virulence factors (7). Characterization of isolates to different races is based on their interaction with major genes for resistance in potato. Major resistance genes in *Solanum* spp. and their corresponding avirulence genes in *P. infestans* were first characterized by Black et al. (2), who based their analysis on the gene-for-gene concept of Flor (7). So far 11 major genes for resistance have been identified in *Solanum* spp. These R genes have shown Mendelian inheritance as a single dominant factor. The race concept in *P. infestans* has been

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confused with other terms and concepts. Most recent population genetic studies of *P. infestans* were focused on identifying and characterizing these genotypes, with less emphasis placed on identifying the race structure of these populations.

This investigation addresses the extent of variation in virulence and genotypes using different markers and sensitivity to mefenoxam in two clonal lineages of *P. infestans* (US-8 and US-1) during asexual reproduction. Single zoospore progenies from four parental isolates of US-8 genotype and one isolate from US-1 genotype of *P. infestans* were studied. Virulence phenotypes were assessed on all known resistance genes in potato (R1 to R11) using different inoculum concentrations and two culturing methods of the zoospores. Genotypic variation within each progeny was investigated with allozyme and DNA markers (restriction fragment length polymorphism [RFLP] using probe RG57). Variation for mefenoxam sensitivity among single zoospore isolate progenies was evaluated using in vitro radial growth inhibition. The main objective of this study was to determine if the new populations of *P. infestans* (the US-8 genotype) generate pathogenic and genotypic variation during production of zoospores.

MATERIALS AND METHODS

***P. infestans* isolates.** Five parental isolates of *P. infestans*, collected from Minnesota and North Dakota in 1994 to 1996, were chosen to represent different race structures (Table 1). These parental isolates were hyphal tip isolates from potato leaves or tubers, purified by repeated hyphal tip culturing, and characterized for their virulence and genotypes in 1997 (Table 1). All isolates were maintained on rye B agar slants (5) at 15°C during the course of the study. Single zoospore progenies were generated from each of the parental strains by inducing zoospore production as described by Caten and Jink (5). Single germinating zoospores were removed on a disk of agar, transferred to plates of rye B agar, and incubated at 20°C in the dark for 4 days. Well-developed colonies of single zoospore isolates were transferred into plates or slopes of rye B agar and maintained at 15°C.

Inoculum for pathogenicity and assessment of virulence structure of the single zoospore isolates was prepared by washing sporangia with sterile distilled water from 10- to 12-day-old cultures growing on rye B agar. The sporangial suspension was chilled at 4 to 6°C for 2 h to induce zoospore release. Inoculum concentration was adjusted with sterile distilled water to 2.0×10^4 zoospores per ml with the aid of a hemacytometer. For some isolates that showed lower than expected virulence, inoculations were repeated with higher levels of inoculum (3.0 to 4.0×10^4 zoospores per ml). In some cases, inoculum was produced by inoculating potato leaves of cv. Craig's Royal, with no known R genes, and harvesting sporangia from infected leaves.

Pathogenicity and virulence assessment. All parental and single zoospore progeny strains of *P. infestans* were tested for their virulence on a set of differential potato plants, each carrying a single gene for resistance against *P. infestans* (R1 to R11; U.S. Department of Agriculture–Potato Introduction Station, Sturgeon Bay, WI; differential set). Potato cv. Craig's Royal was used as a universal susceptible. The plants were grown for 4 to 6 weeks in the greenhouse with natural sunlight augmented by 400 W sodium vapor lighting on a 14-h light, 10-h dark cycle. Detached leaf

assay tests were used to assess the virulence of the single zoospore isolates. Leaves were detached from each test plant and leaflets of similar age and size were selected. From each leaf, one fully expanded leaflet was detached. Three to four detached leaflets were used for each isolate. Leaflets were sprayed with sterile distilled water by a hand sprayer and were placed abaxial side up on a moistened filter paper in glass petri dishes (15 × 100 mm). Each leaflet was inoculated with two 25- μ l drops of zoospore suspension adjusted to a concentration of 2.0×10^4 zoospores per ml. A single leaflet was used as an experimental unit, and three to four replications were conducted per test. Petri plates were placed on damp tissue in transparent boxes and covered with transparent plastic sheets. Leaflets were incubated at 18°C, with a 16-h photoperiod, and inverted 24 h after inoculation. The reaction of each leaflet was evaluated at 4 days postinoculation, and then every 24 h until 7 days postinoculation. Leaflets were examined visually and under a stereoscope for the presence of sporulating lesions. Disease was scored according to the methods described by Spielman et al. (36). Isolate interactions were scored as virulent, avirulent, or inconclusive. All tests were repeated at least twice, and for those isolates that showed non-parental virulence patterns, the test was repeated several times, up to 10 times for some isolates. The effect of inoculum concentration on the expression of virulence was evaluated by inoculating the leaflets with different levels of inoculum in the same test using 1.0 , 2.0 , and 4.0×10^4 zoospores per ml of suspension. Culturing methods of the single zoospore subcultures were evaluated to examine any effects on the virulence expression. Production of inoculum on potato leaflets with no resistance genes was compared with subculturing the isolates on rye B media.

Mating type, glucose-6-phosphate isomerase genotype, DNA fingerprints with probe RG57, and response to mefenoxam.

Single zoospore isolates were tested for mating type to determine if changes in mating type could occur during asexual reproduction. Mating type was determined by pairing a pure culture of each isolate with reference A1 and A2 isolates on 10% clarified V8-juice agar or rye B agar (5) and incubating at 15°C in darkness. Tester genotypes US940480 (US-8), US940486 (US-7), and US940501 (US-1) were provided by W. Fry, Cornell University. Each plate was scored for oospore formation after 2 weeks from the pairing date. If oospores formed with the A1 pairing, the isolate was considered A2; if oospores formed with the A2 pairing, the isolate was considered A1. In cases where the isolate produced oospores in both pairings, it was considered self-fertile.

The identity of *glucose-6-phosphate isomerase* (*Gpi*) allozyme was determined for all single zoospore isolates. Cellulose acetate gel electrophoresis (Helena Laboratories, Beaumont, TX) was used as described by Goodwin et al. (15) with the underlay protocol. Tissue used for the analysis consisted of mycelium taken from a pure culture growing on rye B or V8-juice agar plates. Allozyme alleles were scored according to the standard procedures described by Goodwin et al. (15). The tester genotypes used were US-940480 (US-8), US-940486 (US-7), and US-940501 (US-1), supplied by W. Fry, Cornell University, and PI-449 (US-6), PI-284 (US-11), and PI-97000 (US-17) from N. C. Gudmestad at North Dakota State University.

Parental and single zoospore isolate progenies were characterized for their DNA fingerprints using the moderately repetitive

TABLE 1. *Phytophthora infestans* parental isolates used in the study

Parental isolate	Origin	Year collected	Genotype	Mating type	Virulence phenotype ^a
PI-105	Fosston, MN	1995	US-8	A2	0,1,2,3,4,5,6,7,10,11
PI-191	Page, ND	1996	US-8	A2	0,1,2,3,4,5,6,7,10,11
PI-52	Norman, MN	1995	US-1	A1	0, 3,4,7,8,10,11
PI-126	Sullivan, MN	1995	US-8	A2	0,1,2,3,4,5,6,7,10,11
PI-1	Glasston, MN	1994	US-8	A2	0,1,2,3,4,5,6,7,10,11

^a Indicates R gene differential that allowed infection and sporulation by *P. infestans* isolate.

probe RG57. The purpose of using RG57 fingerprinting in this study was to make sure that none of the single zoospore isolates obtained was the result of isolates mix, heterokaryosis, or parasexuality and to exclude the possibility of ploidy variations among the single zoospore isolates. DNA extraction, digestion with restriction enzyme *EcoRI*, and Southern analysis with the DNA probe RG57 were performed as described previously (14).

Mefenoxam (Ridomil Gold EC; Syngenta Crop Protection Inc., Greensboro, NC) was used to assess sensitivity of a subset of *P. infestans* single zoospore isolates ($n = 89$) to phenylamide fungicides. Responses to mefenoxam were determined by growing *P. infestans* isolates on rye B agar plates containing 0, 0.1, 1.0, 10, and 100 $\mu\text{g ml}^{-1}$ (active ingredient) of mefenoxam. The assay was performed by placing 0.48-mm agar plugs taken from margins of actively expanding cultures growing on rye B medium in petri plates. Plates were incubated in the dark at 18°C. Evaluations

were made after 7 to 10 days of incubation when the control colonies (no mefenoxam) were at least 3.0 cm in diameter (23). Radial growth as a percentage of the control was determined for each isolate at each concentration. There were two replications at each concentration, and two perpendicular measurements of colony diameter were made for each replicate. The trial was repeated twice for each isolate. EC_{50} (concentration at which 50% growth reduction occurred) values were determined by plotting the percent growth reduction ratings against concentration of mefenoxam for each isolate tested. The EC_{50} values were determined using a simple linear interpolation between the two concentrations bracketing 50% sensitivity. Statistical Analysis System version 6.0 (SAS Institute, Cary, NC) was used to estimate the EC_{50} values.

Data analysis. A final disease rating of 1.0 for compatible interactions, and 0 for incompatible interactions, was given to each cultivar-isolate interaction. Interactions with inconclusive results

TABLE 2. Virulence structure and race names of the isolates characterized in the study

Isolate ^a	No. of times tested	Virulence ^b /Avirulence phenotype	Race ^c	Isolate ^a	No. of times tested	Virulence ^b /Avirulence phenotype	Race ^c
PI-105P	6	0,1,2,3,4,5,6,7,8,10,11/9	7766	PI-191C29	2	0,1,2,3,4,5,6,7,10,11/8,9	7746
PI-105C1	6	0,1,2,3,4,5,6,10,11/7,8,9,10	7702	PI-191C30	2	0,1,2,3,4,5,6,7,10,11/8,9	7746
PI-105C2	5	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-52P	4	0,3,4,7,8,10,11/1,2,5,6,9	1466
PI-105C3	5	0,3,10,11/1,2,4,5,6,7,8,9	1006	PI-52C1	3	0,3,7,10,11/1,2,4,5,6,8,9	1046
PI-105C5	3	0,3/1,2,4,5,6,7,8,9,10,11	1000	PI-52C2	3	0,10,11/1,2,3,4,5,6,7,8,9	0006
PI-105C8	3	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-52C4	5	0,1,3,7,10,11/2,4,5,6,8,9	7046
PI-105C10	4	0,3,4,11/1,2,5,6,7,8,9,10	1402	PI-52C5	6	0,3,10,11/1,2,4,5,6,7,8,9	1006
PI-105C11	2	0/1,2,3,4,5,6,7,8,9,10,11	0000	PI-52C6	2	0,3,7,10,11/1,2,4,5,6,8,9	1046
PI-105C12	4	0,1,3,4,6,10/2,5,7,8,9,11	5504	PI-52C7	4	0,10,11/1,2,3,4,5,6,7,8,9	0006
PI-105C13	4	0,2,3,4/1,5,6,7,8,9,10,11	3400	PI-52C8	5	0,3,7,10,11/1,2,4,5,6,8,9	1046
PI-105C14	5	0,1,2,3,4,6,7,10,11/5,8,9	7546	PI-52C9	4	0,1,2,3,10,11/4,5,6,7,8,9	7006
PI-105C15	5	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-52C10	4	0,2,4,10,11/1,3,5,6,7,8,9	2406
PI-105C16	8	0,1,2,3,4,5,6,7,8,10,11/9	7766	PI-52C11	4	0,3,10,11/1,2,4,5,6,7,8,9	1006
PI-105C17	5	0,3/1,2,4,5,6,7,8,9,10,11	1000	PI-52C12	2	0,1,10,11/2,3,4,5,6,7,8,9	4006
PI-105C18	7	0,3,11/1,2,4,5,6,7,8,9,10	1002	PI-52C13	2	0,7,11/1,2,3,4,5,6,8,9,10	0042
PI-105C19	7	0,3,6,7/1,2,4,5,8,9,10,11	1140	PI-52C14	2	0,3,7,10,11/1,2,4,5,6,8,9	1042
PI-105C20	10	0,1,2,3,4,7,10,11/5,6,8,9	7446	PI-52C15	3	0,3,10,11/1,2,4,5,6,7,8,9	1006
PI-105C22	9	1,2,3,4,5,6,7,8,10,11/9	7766	PI-52C16	4	0,10,11/1,2,3,4,5,6,7,8,9	0006
PI-105C23	8	0,1,2,3,4,6,7,8,10,11/5,9	7566	PI-52C17	4	0,7,10,11/1,2,3,4,5,6,8,9	0046
PI-105C25	6	0,1,2,3,4,6,7,8,10,11/5,9	7566	PI-52C18	4	0,1,3,4,7,10,11/2,5,6,8,9	5446
PI-105C26	4	0/1,2,3,4,5,6,7,8,9,10,11	0000	PI-52C19	2	0,10,11/1,2,3,4,5,6,7,8,9	0006
PI-191P	4	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-52C20	4	0,3,10,11/1,2,4,5,6,7,8,9	1006
PI-191C1	3	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-52C21	3	0,3,10,11/1,2,4,5,6,7,8,9	1006
PI-191C2	3	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-52C22	4	0,3,10,11/1,2,4,5,6,7,8,9	1006
PI-191C3	3	0,1,2,3,11/4,5,6,7,8,9,10	7002	PI-52C23	3	0,10,11/1,2,3,4,5,6,7,8,9	0006
PI-191C4	5	0,1,2,3,4,5,6,7,8,9,10,11/---	7776	PI-52C24	3	0,3,10,11/1,2,4,5,6,7,8,9	1006
PI-191C5	2	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-52C25	4	0,7,10,11/1,2,3,4,5,6,8,9	0046
PI-191C6	2	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-52C27	3	0,10,11/1,2,3,4,5,6,7,8,9	0006
PI-191C7	2	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-52C28	4	0,3,7,10,11/1,2,4,5,6,8,9	1046
PI-191C8	6	0,1,2,3,4,5,6,7,8,9,10,11/---	7776	PI-52C29	3	0,3,10,11/1,2,4,5,6,7,8,9	1006
PI-191C9	3	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-52C31	4	0,2,3,4,10,11/1,5,6,7,8,9	3406
PI-191C10	3	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-126P	2	0,1,2,3,4,5,6,7,10,11/8,9	7746
PI-191C11	3	0,1,3,4,5,6,7,10,11/2,8,9	5746	PI-126C3	2	0,1,2,3,4,5,6,7,8,9,10,11/---	7776
PI-191C12	5	0,1,2,3,4,5,6,7,8,9,10,11/---	7776	PI-126C4	2	0,1,2,3,4,5,6,7,9,10,11/8	7756
PI-191C13	3	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-126C5	2	0,1,2,3,4,5,6,7,9,10,11/8	7756
PI-191C14	3	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-126C7	2	0,1,2,3,4,5,6,7,8,10,11/9	7766
PI-191C15	3	0,1,2,3,4,5,6,7,11/8,9,10	7742	PI-126C8	2	0,1,2,3,4,5,6,7,8,10,11/9	7766
PI-191C16	4	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-126C9	2	0,1,2,3,4,5,6,7,8,9,10,11/---	7776
PI-191C17	4	0,1,2,3,4,5,6,7,9,10,11/8	7756	PI-126C10	2	0,1,2,3,4,5,6,7,9,10,11/8	7756
PI-191C18	2	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-126C12	2	0,1,2,3,4,5,6,7,10,11/8,9	7746
PI-191C19	2	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-126C15	2	0,1,2,3,4,5,6,7,8,10,11/9	7746
PI-191C20	2	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-126C16	2	0,1,2,3,4,5,6,7,10,11/8,9	7766
PI-191C21	2	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-126C17	2	0,1,2,3,4,5,6,7,9,10,11/8	7756
PI-191C22	2	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-126C18	2	0,1,2,3,4,5,6,7,8,9,10,11/---	7776
PI-191C23	2	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-126C21	2	0,1,2,3,4,5,6,7,9,10,11/8	7756
PI-191C24	4	0,1,2,3,4,5,6,7,8,9,10,11/---	7776	PI-126C23	2	0,1,2,3,4,5,6,7,9,10,11/8	7756
PI-191C25	2	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-1P	2	0,1,2,3,4,5,6,7,10,11/8,9	7746
PI-191C26	3	0,1,2,3,4,5,6,7,10,11/8,9	7746	PI-1C1 to PI-1C11 ^d	2	0,1,2,3,4,5,6,7,10,11/8,9	7746
PI-191C27	3	0,1,2,3,4,5,6,7,10,11/8,9	7746				

^a The letter P after the isolate number denotes the parental isolate, and the letter C denotes the single zoospore progeny isolates derived from that parental isolate.

^b R genes on which isolates were virulent.

^c Race name using the reverse octal/binary system (17).

^d Ten isolates were analyzed. All single zoospore isolates were identical in virulence to parental isolate PI-1P.

were repeated until consistent results were obtained, otherwise they were considered an incompatible interaction. The octal nomenclature of races, described by Gilmour (11), was used as an efficient method to identify differences in virulence between single zoospore isolates. According to Gilmour's method of nomenclature, differentials were assigned to groups of three, and each group of three differentials has one octal digit. The 11 known potato resistance genes (R1 to R11) formed four octal digits. The first digit on the left has information about potato differentials R1, R2, and R3; second digit, R4, R5, and R6; third digit, R7, R8, and R9; and fourth digit, R10 and R11. The susceptible control (potato cv. Craig's Royal) is not included in the race nomenclature. The use of Gilmour's nomenclature system required that the isolate-differential interaction be expressed as a series of zeros and ones, where 0 represents an incompatible interaction and 1.0 represents a compatible interaction. The reverse octal system is used here to keep the order of cultivars from left to right (17). Gilmour's system of race nomenclature required the conversion of the binary values to an octal value. The zeros and ones are transformed into an octal number by taking each group of three and converting it to its octal equivalent so that 000 = 0; 001 = 1; 010 = 2; 011 = 3; 100 = 4; 101 = 5; 110 = 6; and 111 = 7 (17).

Virulence difference. Virulence difference was defined as the number of disagreements in pathogenicity between race i and the sample's most frequent (dominant) race i_0 (19). For a dichotomous scale, such as the one used here, 0 represents the incompatible interaction and 1 represents the compatible interaction. The corresponding virulence difference (V_i) can be calculated using the following formula: $V_i = \sum |p_\delta - q_\delta|$ (19), where p_δ and q_δ , with $\delta = 1, 2, \dots, D$, denote components of the pathotype vectors p and q of race i and i_0 , respectively. Virulence difference was calculated for each single zoospore progeny sample using the above-mentioned formula.

Pathogenicity index. Pathogenicity index as described by Zhang et al. (42) was calculated for each differential- zoospore progeny combination. This pathogenicity index or disease index provides invaluable information about the effectiveness of each resistance gene in providing protection against *P. infestans*. For each differential δ (with $\delta = 1, 2, 3, \dots, D$), the corresponding pathogenicity index (PI%) is calculated using the following formula: $PI_\delta = \sum a\delta_j / (\text{MAX} \times N)$, where $a\delta_j$ is the number of isolates that infect the differential δ_j , divided by the total number of isolates tested (N), multiplied by the upper limit of the assessment scale.

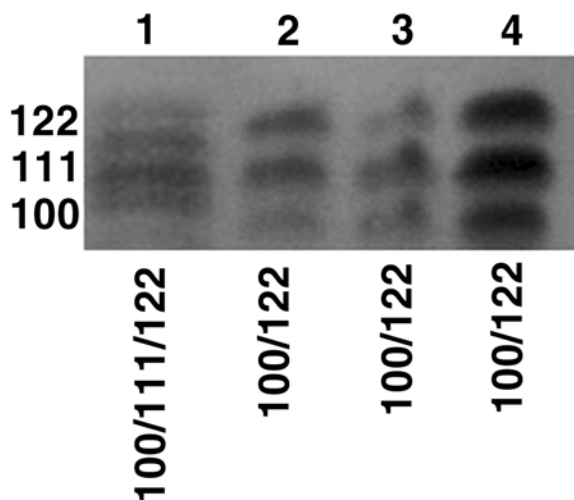


Fig. 1. Cellulose acetate gel banding patterns at the *glucose-6-phosphate isomerase* locus for single zoospore isolates derived from the parental isolate PI-126. Lane 1: PI-126P; Lane 2: PI-126C3; Lane 3: PI-126C5; Lane 4: PI-97000 (US-17).

Measurements of genetic variability within the single zoospore isolate progenies. Diversity was estimated for each population using Shannon's diversity index (H_S) (28), Simpson's index (S_i) (35), simple index of diversity (Q) (28), and Gleason index (H_G) (31). Shannon's index is calculated as

$$H_S = -\sum_{j=1}^n P_j \ln P_j, \quad j = 1 \dots N_p$$

where P_j is the frequency of the j th race in the population. Values of Shannon's index range between zero and $\ln N_p$, where zero indicates no diversity, i.e., when all isolates in the population belong to the same race, and $\ln N_p$ indicates the highest level of diversity, i.e., each isolate in the population is a distinct race. A correction factor is desirable when the size of a sample drawn from a population is relatively small (less than 100 individuals) as is the case of this study, because Shannon's index, as calculated above, is a biased estimate of the actual diversity, because frequencies are only estimated from samples. This correction factor is called relative Shannon index (H_{SR}) (34), $H_{SR} = H_S / H_{S_{max}}$, where $H_{S_{max}}$ is the greatest possible value of H_S in a sample of N_i individuals. This maximum value is reached when $N_p = N_i$ (hence, $P_j = 1/N_i$, then $H_{SR} = H_S / \ln N_p$), where N_p is the total number of phenotypes (races) in a sample. H_{SR} value ranges between zero and one, where zero indicates no diversity and one indicates the highest level of diversity. Simpson's index (S_i) is calculated as

$$S_i = 1 - \sum_{i=1}^n P_i^2$$

(35) where P_i is the frequency of the i th phenotype, $P_i = n_i / N$, where n_i is the total number of distinct phenotypes, and N is the total number of isolates in the sample, i.e., the probability of obtaining two distinct phenotypes when randomly drawing two isolates from the sample. Simpson's index can take a value between zero and one.

The simple index of diversity (Q) (28) and Gleason index (H_G) (31) are calculated as $Q = N_p / N_i$ and $H_G = (N_p - 1) / \ln N_i$, where in both indices, N_i is the number of isolates tested and N_p is the number of races identified among these isolates. Q values range between zero and one, where zero indicates no diversity and one indicates the highest level of diversity. Both of these indices are sensitive to the richness aspect of diversity (i.e., the number of different phenotypes present in the sample), but ignore the even-

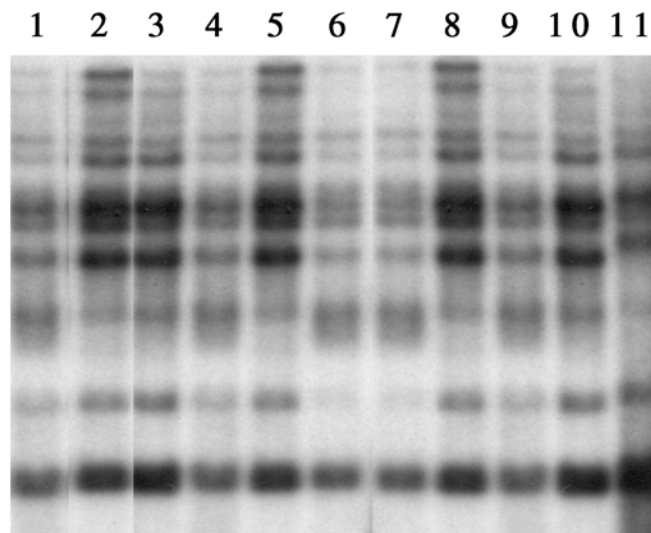


Fig. 2. RG57 fingerprint patterns of 10 single zoospore isolates derived from the parental isolate PI-191P. Lane 1: PI-191P; Lane 2: PI-191C1; Lane 3: PI-191C3; Lane 4: PI-191C4; Lane 5: PI-191C7; Lane 6: PI-191C8; Lane 7: PI-191C11; Lane 8: PI-191C12; Lane 9: PI-191C13; Lane 10: PI-191C15; Lane 11: PI-191C17.

ness aspect (i.e., the similarities in phenotype frequencies). Single zoospore isolates derived from isolate PI-1P (Table 2) did not show any variability in their virulence pattern and were not included in the analysis.

Measurement of genetic diversity among single zoospore populations. Genetic diversity among the five single zoospore populations was quantified using Rogers index of genetic diversity (29). Rogers index is calculated as $HR = 0.5 \sum |P_{j1} - P_{j2}|$, where P_{j1} and P_{j2} represent the frequencies of phenotype j in population 1 and population 2, respectively. Rogers index is an estimate of phenotypic similarity between pairs of populations and can be used to compare race structure either within populations collected in the same geographical region over a period of time or within geographically distinct populations collected simultaneously. Rogers index varies from 0, for two populations with identical race structure, to 1.0, for populations with no phenotypes in common.

RESULTS

Single zoospore isolates. The proportion of zoospores that developed into vegetative colonies varied from 2 to 50% depending on the parental isolate. The parental isolate PI-1 produced very small zoospores and the percent recovery of colonies was very low. Other parental isolates produced larger sized zoospores and showed higher levels of developed colonies. In total, 102 single zoospore isolates were recovered, 20 isolates from isolate PI-105, 29 isolates from PI-191, 28 isolates from PI-52, 14 isolates from PI-126, and 11 isolates from PI-1. The single zoospore isolates (Table 2) were given a tracking number designated by the number of the parental isolate followed by the letter C (for clone). Single zoospore isolates from each parental isolate differed in their growth rate in culture as well as in their aggressiveness on the inoculated leaflets. No difference in the expression of virulence among different inoculum concentrations was observed. No difference was observed in the expression of virulence between the two methods of inoculum production.

Mating type, *Gpi* genotype, DNA fingerprints with RG57 probe, and response to mefenoxam. Results of mating type tests showed that all single zoospore isolates retained the mating type of their parent. One isolate derived from isolate PI-105 (PI-105C20) produced oospores with both A1 and A2 mating type tester isolates as well as in single culture. Attempts to germinate oospores from this isolate several times were unsuccessful. The parental isolate from which this isolate was derived (PI-105) did not produce oospores in single culture after several months of storage on rye B plates.

Parental isolates PI-1, PI-105, PI-191, and PI-126 all have (100/111/122) alleles at the *Gpi* locus, typical of the US-8 genotype. The parental isolate PI-52, an A1 mating type isolate, has (86/100) alleles at *Gpi* locus typical of the US-1 genotype. Genotypes based on *Gpi* locus remained constant in most of the single zoospore isolates derived from the five parental isolates. However, three single zoospore isolates derived from parental isolate PI-126 demonstrated a banding pattern different from that of the parental isolate. In these isolates, three bands (100/122) instead of five bands (100/111/122) occur at the *Gpi* locus (Fig. 1). The banding pattern of these three isolates was similar to the banding pattern of the US-17 (A1 mating type) and US-14 genotype (A2 mating type) (16).

Single zoospore isolates derived from each parental isolate showed no difference in DNA fingerprints based on hybridization to the middle repetitive DNA probe RG57. In all cases, the patterns of the single zoospore progeny were identical to their parent. In all cases, there was no polymorphism in band numbers or sizes among the single zoospore isolates and the parental isolate from which they were derived (Figs. 2 and 3). These results provide strong evidence that the single zoospore isolates from each parent were asexual derivatives and were not a result of isolates mix, heterokaryosis, or parasexuality.

Results from mefenoxam response experiment showed that, in most cases, the EC_{50} values for the single zoospore isolates were different from those of the parental isolates (Fig. 4). Some single zoospore isolates showed higher levels of sensitivity, whereas others showed lower levels when compared with the parental isolates from which they were derived. The lowest levels of variability for mefenoxam sensitivity occurred in the single zoospore isolates derived from parent PI-52 (US-1 genotype; data not shown). The parent PI-52 has an EC_{50} value of less than $0.1 \mu\text{g ml}^{-1}$. Similarly, most of the single zoospore isolates from this parent had an EC_{50} value of less than $0.1 \mu\text{g ml}^{-1}$. Two single zoospore isolates from this parent had an EC_{50} value higher than $0.1 \mu\text{g ml}^{-1}$ (PI-52C21, $EC_{50} = 0.2$; PI-52C25, $EC_{50} = 0.15$). Single zoospore isolates from parent PI-191 ($n = 29$) were variable in their response to mefenoxam. About 50% of the single zoospore isolates were less sensitive to mefenoxam compared with their parent. The other 50% showed similar or higher sensitivity based on the EC_{50} values. Three isolates from this parent have an EC_{50} value of more than $100 \mu\text{g ml}^{-1}$ (this EC_{50} value is indicative of resistance to mefenoxam). In addition, two isolates showed EC_{50} values of less than $0.1 \mu\text{g ml}^{-1}$, indicating high sensitivity to mefenoxam (Fig. 4A). The majority of single zoospore isolates derived from parent PI-126 ($n = 15$) were more sensitive to mefenoxam than the parental

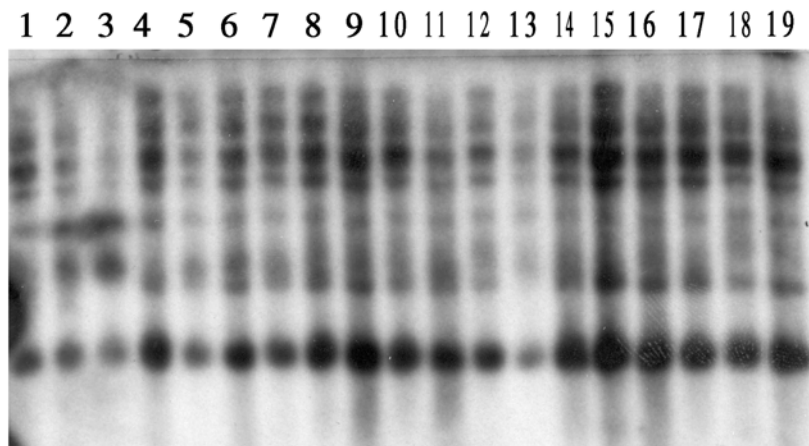


Fig. 3. RG57 fingerprint patterns of 18 single zoospore isolates derived from the parental isolate PI-105P. Lane 1: PI-105P; Lane 2: PI-105C1; Lane 3: PI-105C2; Lane 4: PI-105C3; Lane 5: 105PC5; Lane 6: PI-105C8; Lane 7: PI-105C10; Lane 8: PI-105C11; Lane 9: PI-105C12; Lane 10: PI-105C13; Lane 11: PI-105C14; Lane 12: PI-105C15; Lane 13: PI-105C16; Lane 14: PI-105C18; Lane 15: PI-105C19; Lane 16: PI-105C20; Lane 17: PI-105C22; Lane 18: PI-105C23; Lane 19: PI-105C25.

isolate, except for two isolates (PI-126C21 and PI-126C18) that were less sensitive than the parental isolate (Fig. 4B). Both higher and lower levels of sensitivity were observed in single zoospore isolates derived from parent PI-1 ($n = 8$) (Fig. 4C). Single zoospore isolates from parent PI-105 ($n = 20$) were highly variable in their response to mefenoxam. The parental isolate PI-105 had an EC_{50} value of $6.87 \mu\text{g ml}^{-1}$, and the single zoospore isolates

from this parent showed EC_{50} values ranging from less than $0.1 \mu\text{g ml}^{-1}$ to more than $100 \mu\text{g ml}^{-1}$. Three isolates showed EC_{50} values of more than $100 \mu\text{g ml}^{-1}$ and one isolate showed an EC_{50} value of less than $0.1 \mu\text{g ml}^{-1}$. Shifts toward higher or lower sensitivities occurred in these single zoospore isolates (Fig. 4D).

Race diversity and complexity of single zoospore isolates. Single zoospore isolates derived from the five parental isolates

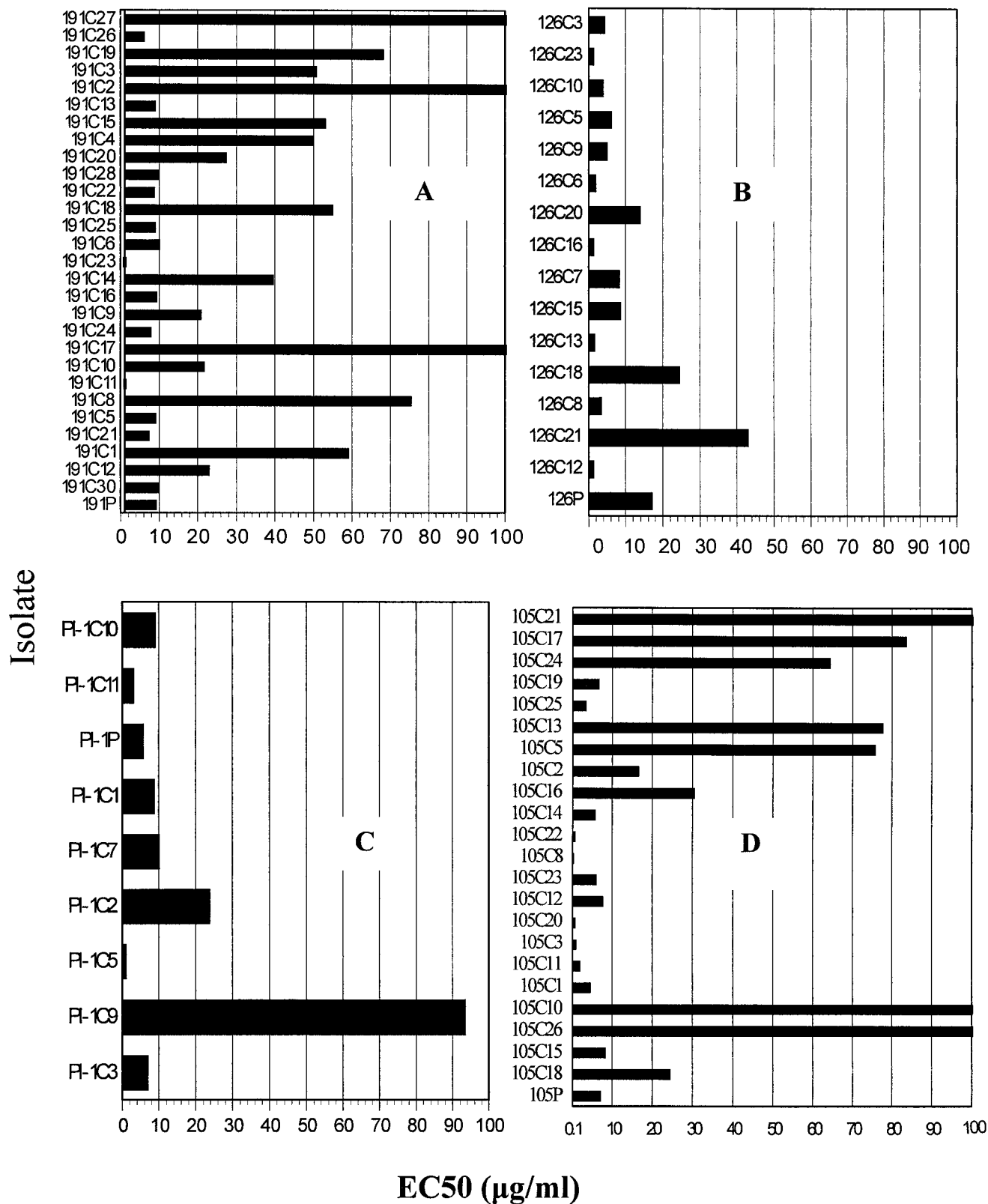


Fig. 4. Mefenoxam at 50% effective dose values for single zoospore isolates derived from the parental isolates A, PI-191P; B, PI-126P; C, PI-1P; and D, PI-105P.

demonstrated different levels of variability for virulence. Although some single zoospore isolates showed the same virulence as their parental isolate, others showed lower or higher virulence than the isolate from which they were derived. Single zoospore isolates derived from isolate PI-1 (11 isolates) were identical in virulence to their parental isolate. All isolates were identified as race 7746 (Table 2). Single zoospore isolates derived from isolate PI-191 (29 isolates) showed low levels of variability for virulence compared with their parental isolate; 73% of these isolates (21 isolates) retained the same virulence pattern as their parent. Four isolates gained additional virulence to R8 and R9 (isolates PI-191C4, PI-191C8, PI-191C12, and PI-191C24). These isolates could be described as a "super race" because they were able to overcome all the known resistance genes for *P. infestans* in potato. Isolate PI-191C17 had additional virulence to R9, which was stable in three tests done in March and June of 1999 using two different sets of plants. The other two isolates (PI-191C15 and PI-191C3) showed lower virulence compared with the parental isolate. Isolate PI-191C15 was avirulent to R10 in three tests compared with all other isolates from this series that were virulent on R10. Isolate PI-191C3 showed the greatest difference in virulence, being avirulent on R4, R5, R6, R7, R8, R9, and R10. This lower virulence was confirmed by repeating the virulence test three times on three different testing dates. Six races were identified from the single zoospore isolates of the parental isolate PI-191 (Fig. 5A).

Single zoospore isolates derived from isolate PI-126 showed higher levels of variability for virulence, despite the small size of the sample studied (14 isolates). Variation in virulence was char-

acterized by an increase in the virulence spectrum. This increase in virulence was specific to R8, R9, or both. Three isolates in this series gained virulence to both R8 and R9 (isolates PI-126C3, PI-126C9, and PI-126C18), three isolates gained additional virulence to R8, six isolates gained additional virulence to R9, and only two isolates retained the same virulence spectrum of the parental isolate. Four races were identified within this series of isolates (Fig. 5B).

Isolates derived from the parental isolate PI-52P, an A1 mating type isolate, were highly variable for virulence (Table 2; Fig. 5C). Among 28 single zoospore isolates studied, none of them showed an identical virulence pattern to their parent. These isolates were studied extensively by repeating the virulence test three to four times, using different plant sets, and conducting the test at different times during the year. The overall trend in this series of isolates was toward lower virulence relative to the parental isolate. In a few cases, there was a gain in virulence to R1 or R2. Isolates PI-52C12 and PI-52C18 showed virulence to R1; isolates PI-52C10 and PI-52C31 to R2; and isolate PI-52C9 was virulent on both R1 and R2. The total number of races identified from this parental isolate was 13 (Fig. 5C).

The single zoospore progeny isolates derived from isolate PI-105 were also highly variable for virulence (Table 2; Fig. 5D). As described previously, to assess the virulence spectrum of these isolates, the virulence test was repeated several times using different sets of plants, different testing dates, and different levels of inoculum. Due to this variability in virulence, some isolates were tested as many as eight or nine times to ensure the accuracy of the viru-

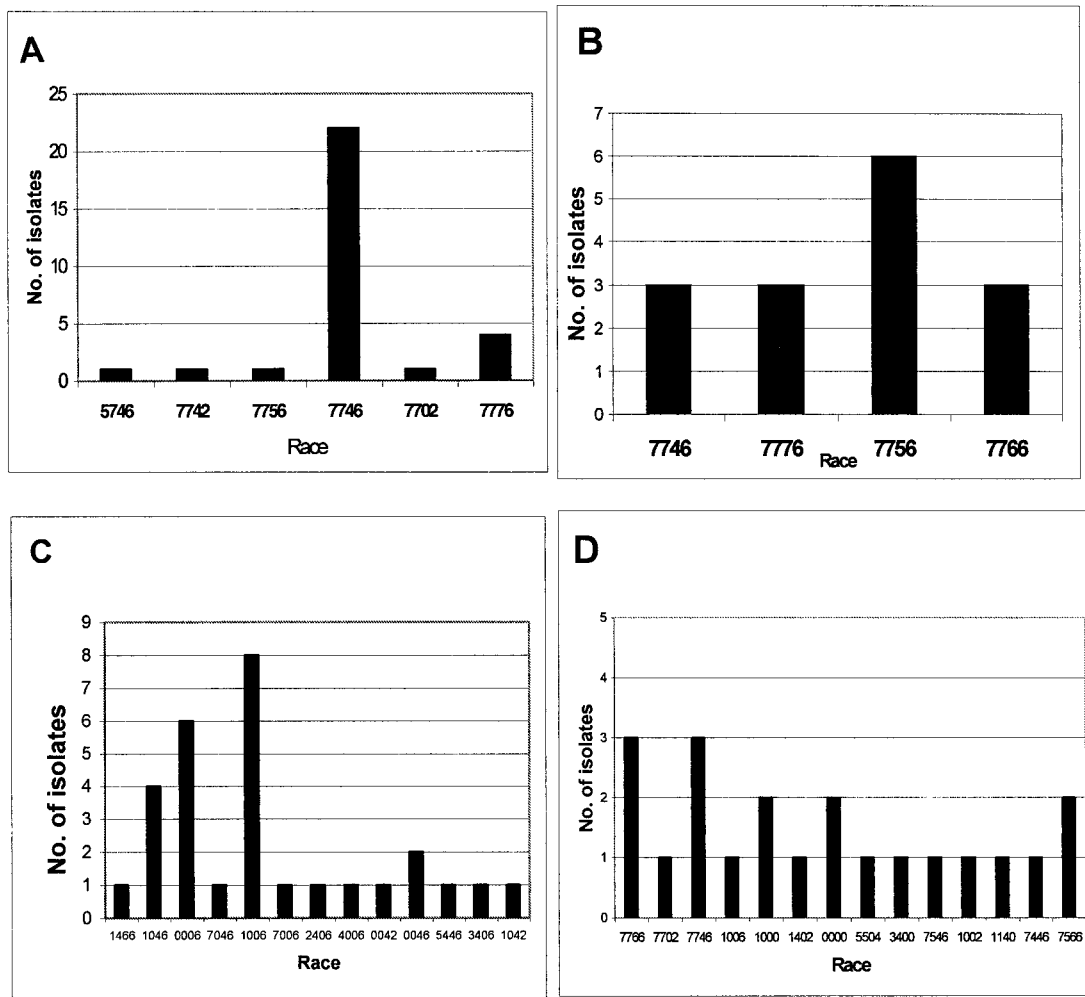


Fig. 5. Frequency distribution of *Phytophthora infestans* races derived from the parental isolates A, PI-191P; B, PI-126P; C, PI-52P; and D, PI-105P.

lence pattern. In this series of isolates there was a tendency for reduced virulence of the single zoospore isolates compared with their parent. Only two isolates from this series retained the same virulence pattern as their parent (isolates PI-105C16 and PI-105C22). Fourteen races were identified from this set of isolates (Fig. 5D).

Virulence difference. Single zoospore isolates from each series showed different levels of virulence difference (Fig. 6). Isolates derived from PI-105 displayed the highest level of virulence difference with eight levels of virulence among the different single zoospore isolates (Fig. 6D). In this series of isolates, only 14% of the isolates showed zero virulence difference to the dominant race. The dominant race in this series was race 7746 (three isolates). In contrast, all the isolates derived from the parental isolate PI-1 have a zero value of virulence difference (data not shown). Single zoospore isolates derived from parental isolates PI-52 and PI-191 showed four levels of virulence difference (Fig. 6A and C, respectively), whereas those from the parental isolate PI-126P showed three levels of virulence difference (Fig. 6B).

Pathogenicity index. The pathogenicity index, which reflects the susceptibility of the differential set used in this study, showed that resistance genes R8 and R9 were most effective in providing resistance against the *P. infestans* isolates used in this study (Fig. 7). In all cases, plants with either gene showed the highest frequency of incompatible interactions. All of the isolates derived from the parental isolates PI-52 and PI-105 had incompatible interactions with R9 plants (Fig. 7C and D, respectively). However, in the case of the parental isolates PI-191 and PI-126, where

both were avirulent on R8 and R9, the change in the virulence in the progeny was toward virulence to R8, R9, or both (Fig. 7A and B, respectively).

Genetic diversity within single zoospore populations. Diversity indices (Q , H_G , H_S , S_i) indicated that single zoospore isolates derived from the parental isolate PI-105 had the highest level of genetic diversity (Table 3). The Shannon relative index placed isolates from PI-105 and PI-126 in the same level of diversity. Both the Shannon and Simpson indices agree that PI-105 isolates are the most diverse followed by PI-52, PI-126, and PI-191 isolates. The Gleason index showed that PI-191 isolates are more diverse than PI-126 isolates (Table 3). Because all single zoospore isolates derived from parent PI-1 were identical in virulence to their parent, all genetic diversity indices values were zero (data not shown).

Genetic diversity among single zoospore populations. Genetic diversity among the five single zoospore populations was estimated using the Rogers index based on virulence data and demonstrated different levels of genetic diversity among these populations. The highest level of genetic diversity was between the population derived from isolate PI-52 (US-1 genotype) and populations from PI-105, PI-126, PI-191, and PI-1, with diversity values of 0.95, 1.0, 1.0, and 1.0, respectively (Table 4). These values suggest that the population derived from isolate PI-52 is genetically distinct from all other populations. Values of genetic diversity between other pairs of populations varied from 0.27 for PI-191 with PI-1 to 0.86 for PI-105 with PI-1.

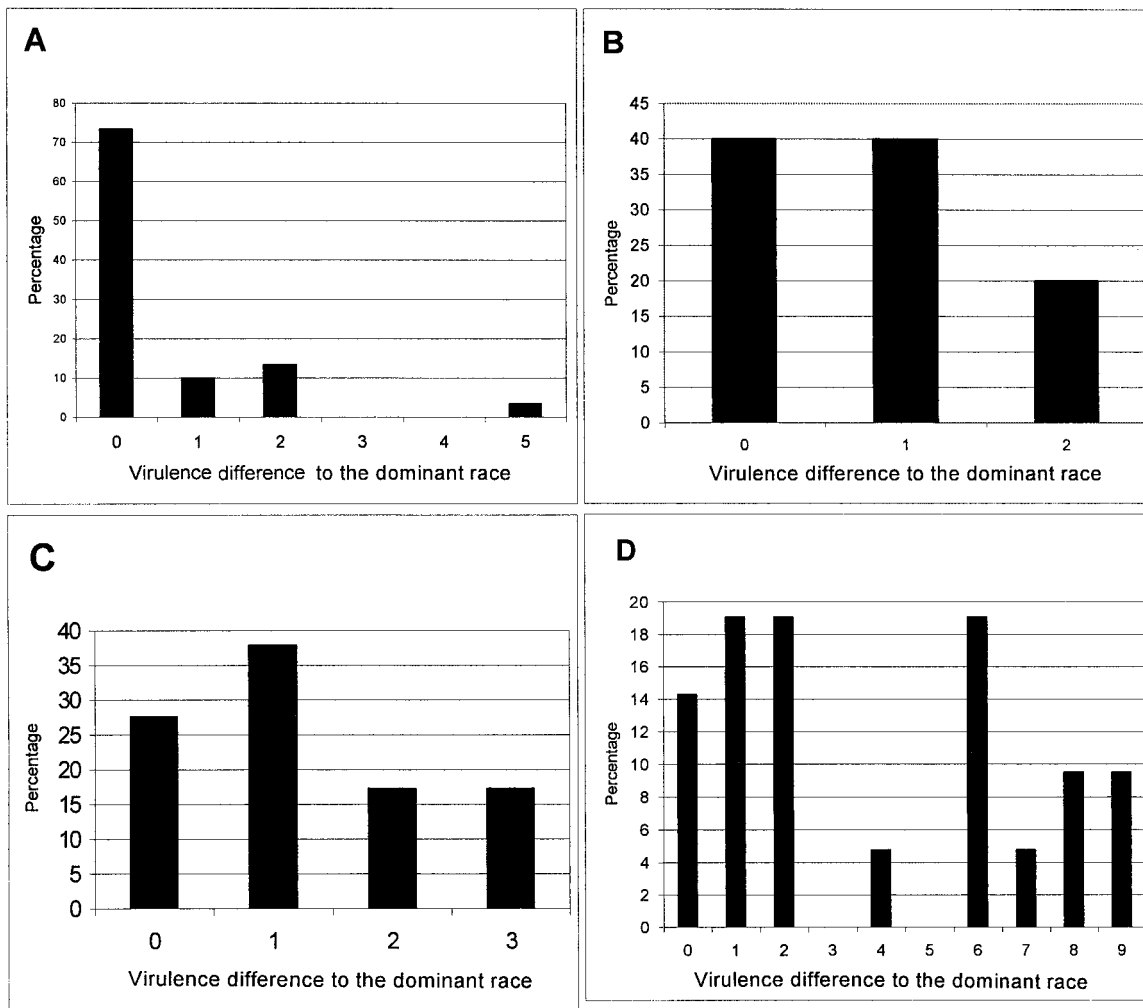


Fig. 6. Frequency distribution of virulence difference to the dominant race for the single zoospore progeny isolates derived from *Phytophthora infestans* parental isolates A, PI-191; B, PI-126; C, PI-52; and D, PI-105.

DISCUSSION

Results from this study support the hypothesis that *P. infestans* has the capability of continuous change in virulence during asexual reproduction. This phenomenon has been previously reported (10,37,39,41) as well as variation in other traits, such as growth rate and colony morphology among single zoospore isolates (3,4,5). Other studies have suggested high levels of stability in virulence phenotypes among single zoospore isolates derived from a single parental isolate (3,18). Variation in pathogenicity and aggressiveness among single zoospore cultures was noticed also in some of these studies (3,4,39,41). Most of these studies were done with the old *P. infestans* population (US-1 genotype), which is characterized by low virulence complexity and none of these studies included all the known resistance genes in *Solanum* spp. as was the case in the studies reported here. The findings of the previous studies (10,37,41) presented limited conclusions and were confused by the probability of having mixed isolates or heterokaryotic isolates that were segregating to different races after isolation of single zoospore isolates, which probably led to the contradictory findings. In our work, these two possibilities were excluded by genotyping the parental isolates and single zoospore isolates using the *Gpi* allozyme marker and the DNA marker RG57, which revealed identical fingerprinting patterns within the parental isolate and the single zoospore isolates derived from them.

Virulence phenotypes of single zoospore isolates studied here varied in a majority of the cases. Levels of virulence variability among single zoospore isolates within each asexual progeny varied greatly from one parent to another, and both extremes were observed. In one extreme, single zoospore isolates showed no variability among themselves and were identical in their virulence to their parent, exemplified by 11 single zoospore isolates derived from parent PI-1. The virulence phenotype stability in this progeny are in agreement with the findings of Caten (3) and Graham (18) who found no differences in virulence among single zoospore isolates studied, except for four isolates that became avirulent in Graham's (18) study. In the other extreme, none of the 28 single zoospore isolates derived from parent PI-52 showed virulence identical to that of the parental isolate. The majority of single zoospore isolates from this progeny were characterized by lower virulence spectrum when compared with the parental isolate. However, six single zoospore isolates from this progeny gained virulence that was specific to plants with R1 or R2 genes or both. Similarly, most single zoospore isolates derived from parent PI-105 were characterized by loss of some virulence factors. Two isolates from this progeny were avirulent and another two were identical to the parental virulence.

The increase in virulence spectrum was observed mainly in single zoospore isolates derived from parent PI-126 and this increase in virulence was specific to R8, R9, or both. In this progeny, three isolates were virulent on the entire differential set. Similarly,

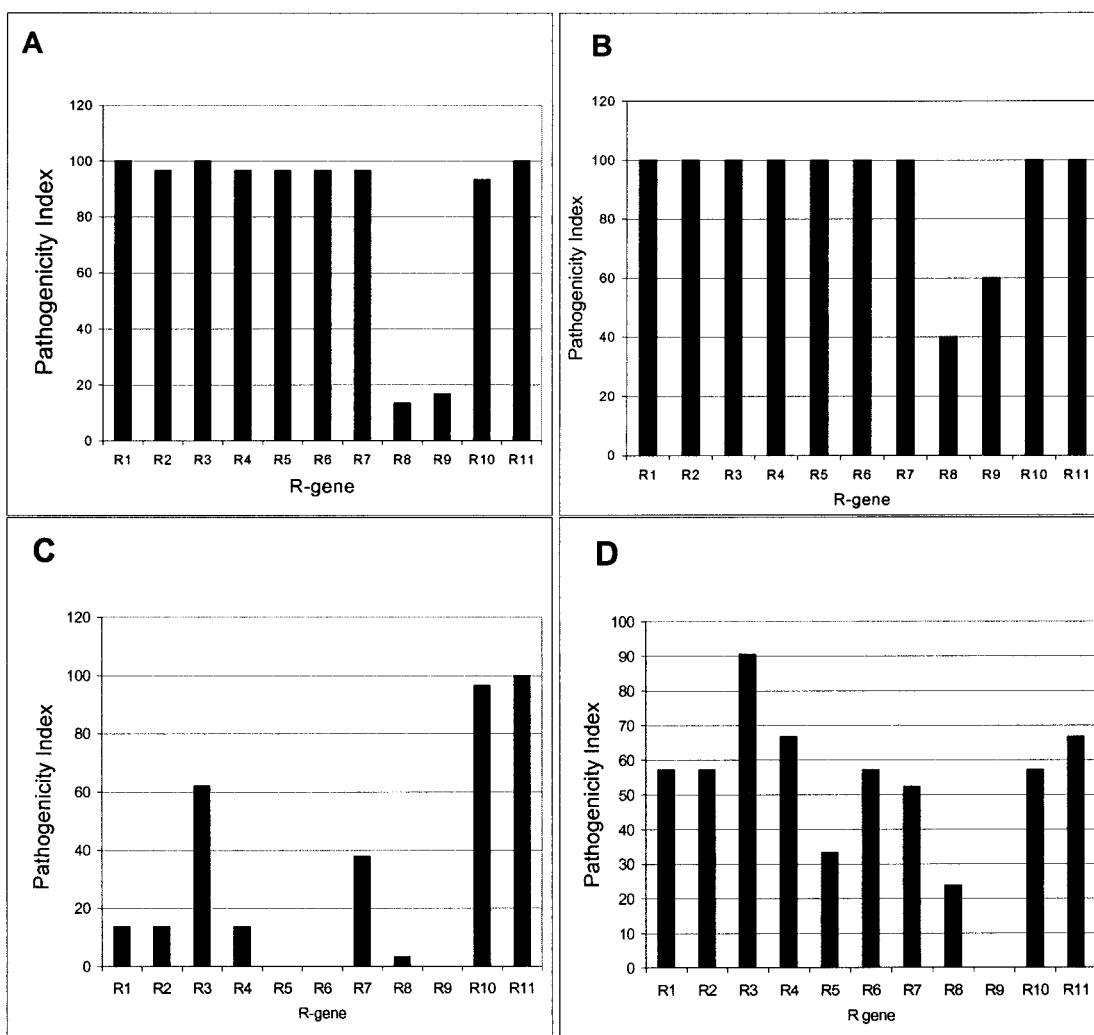


Fig. 7. Susceptibility distribution of the 11 major resistance genes (R1 to R11) against *Phytophthora infestans* for single zoospore progenies derived from *P. infestans* parental isolates **A**, PI-191P; **B**, PI-126P; **C**, PI-52P; and **D**, PI-105P.

Galleghy and Eichenmuller (10) observed a gain in virulence factors when they obtained single zoospore isolates virulent on R4 from 10 parental isolates that were avirulent on R4 genotype potatoes. The progeny derived from parent PI-191 exemplifies an intermediate case between the two extremes. In this progeny, the majority of the single zoospore isolates were identical in their virulence to the parental type; approximately one-third of the isolates showed differences in virulence, also gaining virulence to R8, R9, or both.

Variability in sensitivity to mefenoxam (the R-emantiser of metalaxyl) among single zoospore isolates observed in this study supports and strengthens the idea of genetic changes in *P. infestans* during asexual reproduction. The genetics of mefenoxam resistance have been investigated in a number of studies (21, 32). Resistance to mefenoxam is believed to be under genetic control of a major locus (*MEX*) and additional minor loci (21). Genetic analysis of insensitive isolates showed that highly insensitive isolates were homozygous for the *MEX* allele, whereas intermediately sensitive isolates were heterozygotes (21). The recovery of single zoospore isolates that are mefenoxam sensitive or mefenoxam insensitive from the parental isolates of the US-8 clonal lineage, which were all intermediately sensitive, suggests that some genetic change has occurred at the *MEX* locus. This change is probably a change from heterozygous to homozygous at the *MEX* locus. These changes in mefenoxam sensitivity among single zoospore isolates have not been reported previously. However, high levels of variability for metalaxyl sensitivity were observed by Peters et al. (26) among field isolates of the US-8 clonal lineage. The majority of the Canadian US-8 isolates in the Peters et al. (26) study were classified as moderately resistant (intermediately sensitive) with a wide range of EC_{50} values (1 to $100 \mu\text{l ml}^{-1}$). Among those isolates there was also low proportion of metalaxyl highly resistant isolates ($EC_{50} > 100 \mu\text{l ml}^{-1}$) and metalaxyl sensitive isolates ($EC_{50} < 1.0 \mu\text{l ml}^{-1}$). This variability in metalaxyl sensitivity among isolates of the US-8 clonal lineage occurred in an asexual population of *P. infestans* in Canada at the time when the recovery of A1 mating type isolates was rare (26,27).

The race concept as identified in this work might be confusing due to the large number of races that can be identified based on the gene-for-gene concept of Flor (7). The gene-for-gene relationship is predicted for *P. infestans*-potato pathosystem based on genetic analysis of inheritance of virulence/avirulence phenotypes in this pathogen (1,36). Al-Kherb et al. (1) analyzed the inheritance of virulence in F_1 and F_2 progenies of six matings between *P. infestans* isolates. Patterns of inheritance in F_1 and F_2 indicated the presence of gene-for-gene interactions and generally supported the hypothesis that avirulence alleles were dominant and virulence alleles were recessive. However, unexpected segregations also were obtained, such as the recovery of avirulent progeny on a specific R gene from two parents that were both virulent. It was suggested that this change in virulence phenotype could have happened as a result of somatic segregation from virulence to avirulence at the avirulence locus by mechanisms such as mutation, insertion of a transposon, nondisjunction/monosomy, and mitotic crossing over.

Previous genetic studies of *P. infestans* (38) indicated that this organism is vegetatively diploid. The diploid chromosomal sys-

tem, which is similar to that of higher plants, provides for classical allelic interactions of dominance and recessiveness at each locus as well as the effect of homozygosity and heterozygosity on complex gene expression. Shaw (33) discussed the possible mechanisms of genetic variability during asexual reproduction in *Phytophthora* spp. and considered three major mechanisms for this variability: mutation, mitotic crossing over, and extra-chromosomal elements. Mutations, in the broader sense, involve any changes in the nuclear genome that might take place during DNA replication and can have a significant impact on the expression of certain genes. Shaw (33) argued, however, that it is difficult to believe that mutations of chromosomal genes in a diploid organism could result in the high frequency of variability so often observed. Shaw's argument that mutations cannot account for the big difference in virulence appear to be supported in the present study because the change from race 7766 (i.e., isolate PI-105) to race 3400 (i.e., isolate PI-105C13) following zoosporogenesis would require mutations at seven loci and it is highly improbable that this change could result from mutations. Mitotic crossing over as a mechanism for variation during asexual reproduction provides an alternative to the mutations theory. Crossing over during mitotic prophase in heterozygous diploid nuclei in diploid organisms such as the yeast *Saccharomyces cerevisiae* Hansen (20) is well understood. The mechanism of genetic changes would involve formation of diploid segregants if recessive and dominant alleles become homozygous. A cross-over during mitosis causes a change to homozygosity at all heterozygous loci on the same chromosome arm that are distal to the recombination break point. Mitotic recombination does not generate new variation but it can reveal recessive variation that was hidden in heterozygotes. It is well known that mitotic crossing over is not a highly regulated process, it occurs spontaneously, and provides an alternative mechanism for variation in an asexually reproducing organism. It is possible that this mechanism has evolved in *P. infestans* and has become more organized due to the long-time separation of the two mating types. Evidence for mitotic crossing over was provided by the observed allozyme variation within clonal lineages. All the observed allozyme variation within clonal lineages involved changes from heterozygosity to homozygosity (12). Evidence of such an event was observed in this study. Isolates PI-126C3 and PI-126C5 have 100/122 genotype at the *Gpi* locus, and both isolates were asexual derivatives of the parental isolate PI-126P which has a 100/111/122 genotype at the *Gpi* locus. However, we cannot exclude the possibility of a chromosomal loss for the chromosome carrying the (111) allele as suggested by Miller et al. (24). So far, the mitotic recombination theory has not been tested directly to account for the high levels of virulence variability often observed among asexual progenies.

The third possible cause of genetic variability during vegetative growth and asexual reproduction is the influence of extra-chromosomal genes (12,33). Mitochondrial DNA is a prominent feature of the zoospores in *P. infestans* (33); the mtDNA is a strong candidate for the location of mutations showing nonchromosomal behavior, particularly for those induced in the zoospores with

TABLE 3. Diversity indices values for single zoospore progeny isolates derived from the parental *Phytophthora infestans* isolates used in the study

Diversity index	Diversity index value			
	PI-105	PI-52	PI-126	PI-191
Simple (Q)	0.67	0.44	0.27	0.2
Gleason (H_G)	4.24	3.56	1.11	1.47
Shannon (H_S)	2.53	2.0	1.33	0.95
Simpson (S_i)	0.96	0.85	0.77	0.46
Shannon-relative	0.96	0.78	0.96	0.53

TABLE 4. Rogers index calculated for pairs of the single zoospore isolate populations derived from the five parental *Phytophthora infestans* isolates

Population pair	H_R
PI-105 × PI-1	0.86
PI-105 × PI-126	0.71
PI-105 × PI-191	0.82
PI-105 × PI-52	0.95
PI-1 × PI-126	0.80
PI-1 × PI-191	0.27
PI-1 × PI-52	1.00
PI-52 × PI-126	1.00
PI-52 × PI-191	1.00
PI-126 × PI-191	0.63

abundant mitochondria. Caten (4) believed that variations among zoospore cultures have a cytoplasmic basis and suggested that individual mycelia consist of a mosaic of different cytoplasmic types, which are continually available for selection. If some of the genes involved in pathogenicity and virulence are of cytoplasmic origin, it is possible that mutations in these genes would create new variants, however, there is no evidence at this time to support the cytoplasmic factors hypothesis.

The high levels of variability among asexual progenies observed in this study are difficult to resolve by a single mechanism. It is possible that more than one mechanism is involved to generate such variability. Regardless of the mechanisms involved, the implications of this variability are of significance to manage late blight disease as well as to understand the host–pathogen interaction.

The race-specific resistance in potato to *P. infestans*, conferred by single dominant R-genes (2), is short-lived because of rapidly changing pathogen populations. This fact was realized by the early 1960s when the first potato cv. Pentland Dell, which possessed genes R1, R2, and R3, was released in 1961 in the United Kingdom (40). At that time, the prevailing race of *P. infestans* was race 4 (compatible with R4). ‘Pentland Dell’ resistance was soon broken by new races of the pathogen virulent on this cultivar. This happened when *P. infestans* populations were restricted to asexual reproduction (40). Obviously, new races of *P. infestans* differing from their parent cultures in virulence toward specific resistance genes can be obtained by single zoospore isolations. As observed in this work, this can involve either a loss or gain of virulence with respect to individual R genes. This phenomenon is worthwhile to the potato late blight management efforts and should be studied in more depth to understand the causes and the mechanisms involved, particularly in light of the increased efforts worldwide to investigate the role of sexual reproduction in appearance of new pathogenic races of *P. infestans*.

The availability of molecular tools should make this task possible. Codominant molecular markers such as RFLP and simple sequence repeats can be used to investigate if these observed changes in virulence are associated with changes in the genomic DNA. Such an approach could even identify certain markers that are linked to the virulence/avirulence loci in *P. infestans*.

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