Novel Genotypes of *Phytophthora infestans* in Canada during 1994 and 1995

R. D. Peters[1]*, H. Förster[3], H. W. (Bud) Platt[1], R. Hall[2], and M. D. Coffey[3]

[1]Agriculture and Agri-Food Canada, Crops and Livestock Research Centre, P.O. Box 1210, Charlottetown, PEI C1A 7M8.
[2]University of Guelph, Department of Environmental Biology, Guelph, ON N1G 2W1.
[3]Department of Plant Pathology, University of California, Riverside, CA 92521-0122.
*Corresponding author: Tel: 902-566-6846; Fax: 902-566-6821, E-mail: petersr@em.agr.ca.

ABSTRACT

Populations of *Phytophthora infestans*, causing late blight of potato, changed significantly in Canada in the mid-1990s. The US-1 genotype (A1, metalaxyl-sensitive [MS]) was displaced by novel genotypes (mainly US-8) in much of the country in 1994 and 1995. Many of the new genotypes were insensitive to metalaxyl and were of the A2 mating type, although metalaxyl-insensitive (MI) A1 isolates of the g11 (or US-11) genotype were found in British Columbia. A total of 14 unique multilocus genotypes were described over the two years of the study based on mating type (A1 or A2), metalaxyl sensitivity (MS or MI), allozyme banding patterns (*Gpi* and *Pep*), and random amplified polymorphic DNA (RAPD) markers. Six of these genotypes could be distinguished by allozyme banding patterns at the *Gpi* locus alone; RAPD analysis revealed additional variation within allozyme groupings and defined additional genotypes. Among 82 isolates collected from various Canadian provinces in 1994, US-1 and US-8 constituted 30% and 49% of isolates, respectively; other genotypes occurred in minor frequency. Among 50 isolates collected in 1995, US-1 and US-8 constituted 10% and 74%, respectively. Isolates of g11 (US-11) were found in British Columbia in 1995. Diversity of genotypes was greater in 1994 (11 genotypes) than in 1995 (six genotypes). We hypothesize that sexual reproduction may have been responsible for generating some of the genetic variability in *P. infestans* populations, during a time period in which A2 isolates first migrated into many provinces in Canada.

INTRODUCTION

The increased incidence and severity of late blight, caused by *Phytophthora infestans* (Mont.) de Bary, observed in Canada and the United States in recent years has been attributed to new, immigrant strains of *P. infestans* that have displaced previous forms of the pathogen (Fry *et al.* 1991, 1993; Goodwin *et al.* 1994, 1995b). The new genotypes are often insensitive to metalaxyl (Deahl *et al.* 1993a, 1993b, 1995) and highly aggressive (Kato *et al.* 1997; Lambert and Currier 1997; Peters 1998). Surveys conducted in Canada from 1994 to 1996 revealed that A1, metalaxyl-sensitive (MS) isolates of the US-1 genotype were displaced by A2, metalaxyl-insensitive (MI) isolates of the US-8 genotype in most of Canada during this time period (Peters *et al.* 1998b, 1999b). In British Columbia, an A1, MI population of the pathogen, designated as g11 (or US-11) by allozyme (*glucose-6-phosphate isomerase*) genotyping (Goodwin *et al.* 1998; Peters *et al.* 1999b), became predominant by 1996 (Peters *et al.* 1998b, 1999b). In total, 8 distinct genotypes were described in a collection of 726 isolates from across Canada during 1994 and 1996 using mating type, metalaxyl sensitivity and allozyme (*glucose-6-phosphate isomerase* and *peptidase*) markers (Peters *et al.* 1999b).

The use of additional markers (other than mating type, metalaxyl sensitivity, and allozymes) to characterize the isolates in our collection would provide more detailed information on the genetic variation in populations of *P. infestans* in Canada. The purpose of this study was to assess Canadian isolates of *P. infestans* collected during 1994 and 1995 using RAPD (random amplified polymorphic DNA) analysis to detect additional genetic variation in populations of the pathogen.

Accepted for publication May 1, 2000
ADDITIONAL KEY WORDS: Late blight, potato, tomato.

MATERIALS AND METHODS

Source of Isolates

Samples of potato leaves, stems, and tubers and some tomato leaves, stems, and fruits with symptoms of late blight were received from across Canada during July to December, 1994 and 1995. These samples yielded a collection of 299 isolates of *P. infestans* obtained from 138 fields (138 isolates) and 161 fields (161 isolates) in 1994 and 1995, respectively. The provinces from which samples were obtained and the sampling and isolation procedures have been described (Peters *et al.* 1998b). Isolates were stored at 4 C in glass vials containing a liquid medium of organic rye extract (Peters *et al.* 1998a). Based on results of mating type, metalaxyl sensitivity, and allozyme genotype (Peters *et al.* 1998b), a subset of 82 isolates in 1994 and 50 isolates in 1995 were chosen for RAPD analysis. For the purposes of this study, all isolates chosen for RAPD analysis were retested for mating type, metalaxyl sensitivity and allozyme genotype, to ensure the stability of isolate characteristics following storage. The isolates were plated onto clarified rye agar medium (Peters *et al.* 1998a) and kept at 15 C in the dark for a period of two weeks to allow growth of the pathogen prior to harvesting mycelium for DNA extraction.

Mating Type Tests

Isolates from the 1994 and 1995 surveys chosen for RAPD analysis were tested for mating type by growing the isolates together with known mating types (A1 and A2) on clarified rye extract agar in the dark at 15 C for 10 days, after which plates were scored for the production of oospores (Peters *et al.* 1998b).

Response to Metalaxyl

The metalaxyl sensitivity of isolates was determined using protocols described by Peters *et al.* (1998b). In summary, agar plugs (5 mm diameter) taken from the margins of two-week-old cultures of *P. infestans* were transferred to petri plates containing clarified rye extract agar (Peters *et al.* 1998a) amended with 0 and 100 µg metalaxyl/ml. Growth was measured using Vernier calipers (dial-type, Bel-Art Products, Pequannock, NJ) after incubation for 7 days in the dark at 15 C. Two categories of sensitivity, expressed as mean growth (colony diameter) in the presence of 100 µg metalaxyl/ml as a percentage of mean growth in the absence of metalaxyl, were recognized: metalaxyl-sensitive (MS) = growth <10% of control and metalaxyl-insensitive (MI) = growth ≥ 10% of control.

Allozyme Genotype

Allozyme analysis was carried out according to the protocol of Goodwin *et al.* (1995a) and as described by Peters *et al.* (1999b). Briefly, samples for allozyme analysis were prepared by adding 1 ml of sterile distilled water to each colony and scraping sporangia and mycelium from the agar surface with a sterilized rubber policeman. The resulting mixture (water, sporangia, and mycelium) was then pipetted into a labeled, sterile microcentrifuge tube (1.5 ml, polypropolyene, flat top, Fisher Scientific Co., Ottawa, ON). The samples were centrifuged (Model 235C Micro Centrifuge, Fisher Scientific Co., Ottawa, ON) at 13,000 rpm for 1 min. The majority of the supernatant was then decanted and discarded leaving approximately 100 µl in each microcentrifuge tube. Samples were ground for 1 min with a sterile pestle attached to a motorized tissue grinder (VWR Canlab, Dartmouth, NS). The samples were centrifuged again at 13,000 rpm for 1 min, leaving a supernatant containing enzymes for use in electrophoresis.

Gel electrophoresis of samples was carried out on cellulose acetate as described by Goodwin *et al.* (1995a). Four reference isolates representing US-1, US-6, US-7, and US-8 genotypes (courtesy of S.B. Goodwin and W.E. Fry, Cornell U., Ithaca, NY) were always included in each run. Staining for *Gpi* and *Pep* allozymes was accomplished using agar overlays (Goodwin *et al.* 1995a).

RAPD Analysis of Isolates

Extraction of DNA from mycelium grown in agar culture was carried out using the procedure of Judelson (1996). Polymerase chain reaction (PCR) protocols and primer descriptions are described elsewhere (Punja *et al.* 1998). Of 320 primers screened initially, nine were chosen that exhibited polymorphisms and provided consistent, clear banding patterns among isolates of *P. infestans* previously determined to be polymorphic at allozyme loci (Punja *et al.* 1998). Genotypes were described by the electrophoretic patterns revealed by the nine primers and were compared with reference genotypes from the US series (Forbes *et al.* 1998; Goodwin *et al.* 1998). Novel genotypes exhibiting a minimum of a single band difference were coded as described by Punja *et al.* (1998).

RESULTS

The results of RAPD analysis confirmed the genotype identity obtained by allozyme analysis. However, some additional variation was revealed by the RAPD markers. Of the

14 multilocus genotypes described (Table 1), six could be distinguished based on allozyme banding patterns at the *Gpi* locus alone. The other genotypes were variants of US-1 and US-8 allozyme genotypes as described by RAPD analysis. In 1994, of 28 isolates banding as *Gpi* 86/100/100, 89% were confirmed as US-1 genotypes with 11% variants (denoted g28 and g30 in the coding system of M.D. Coffey, U. of California, Riverside, CA), and of 47 isolates banding as *Gpi* 100/111/122, 85% were confirmed as US-8 genotypes with 15% variants (denoted g29, g40, g41, and g42 in the coding system of M.D. Coffey). Of 38 isolates collected in 1995 and banding as *Gpi* 100/111/122, 97% were confirmed as US-8 by RAPD analysis. Results of allozyme and RAPD analysis were correlated with mating type and metalaxyl sensitivity data (Table 1).

Of the 82 isolates of *P. infestans* chosen for RAPD analysis in 1994, the majority of isolates from Alberta (73%), Manitoba (70%), Saskatchewan (100%), and Prince Edward Island (100%) were US-1 (Figure 1A). Other genotypes were found in low frequency in Alberta and Manitoba (Figure 1A). Of isolates examined from Ontario, Quebec and New Brunswick, the US-8 genotype was most prevalent (56%, 60%, and 77% frequency, respectively), although US-1 isolates were also found (22%, 25%, and 3% frequency, respectively). Other genotypes were found in minor frequency in Ontario, Quebec, and New Brunswick (Figure 1A). No isolates were obtained from British Columbia, Nova Scotia, or Newfoundland in 1994. Isolates of the US-8 and US-1 genotypes constituted 49% and 30%, respectively, of the isolates chosen for RAPD analysis in 1994. A total of 11 unique multilocus genotypes were described in 1994 (Figure 1A).

Of the 50 isolates of *P. infestans* chosen for RAPD analysis in 1995, the majority of isolates from Saskatchewan (100%), Manitoba (75%), Ontario (100%), Quebec (85%), New Brunswick (100%), Nova Scotia (75%), and Prince Edward Island (64%) were US-8 (Figure 1B). Isolates of US-1 were found in minor frequency in Manitoba, Quebec, and Prince Edward Island. One isolate of g26 was recovered from Quebec and several isolates of UN-1 were recovered from Nova Scotia and Prince Edward Island. In British Columbia, only the g11 (or US-11) genotype was recovered in 1995 (Figure 1B). No isolates were obtained from Alberta or Newfoundland in 1995. Isolates of the US-8 and US-1 genotypes constituted 74% and 10%, respectively, of the isolates chosen for RAPD analysis in 1995. A total of six unique multilocus genotypes were found in 1995 (Figure 1B).

TABLE 1.—*Characteristics of genotypes of* Phytophthora infestans *found in Canada in 1994 and 1995.*

Genotype designation[1]	Mating type	Metalaxyl sensitivity[2]	Allozyme banding pattern *Gpi*[3]	*Pep*[4]
US-1	A1	MS	86/100/100	92/100
US-6	A1	MI	100/100	92/100
US-7	A2	MI	100/111	100/100
US-8	A2	MI	100/111/122	100/100
g11*	A1	MI	100/100/111	100/100
g26	A2	MI	100/122	100/100
g28	A1	MS	86/100/100	92/100
g29	A2	MI	100/111/122	100/100
g30	A1	MS	86/100/100	92/100
g40	A2	MI	100/111/122	100/100
g41	A2	MI	100/111/122	100/100
g42	A2	MI	100/111/122	100/100
UN-1**	A2	MI	100/122	100/100
UN-2**	A1	MI	100/111/122	100/100

[1]Designation of US-1,6,7, and 8 genotypes according to Goodwin *et al.* (1998). Designation of other genotypes according to the scheme of M.D. Coffey, University of California, Riverside, CA.

*Also known as US-11 according to Goodwin *et al.* (1998).

**Isolates currently not coded in a classification scheme. Isolates banded as US-8 via RAPD analysis, but in the case of UN-1, with an allozyme banding pattern of 100/122 at the *Gpi* locus (possibly US-14 according to Goodwin et al. [1998]) or, in the case of UN-2, of the A1 mating type.

[2]Metalaxyl sensitivity rating reflects the comparison of diameter of growth of isolates on a clarified rye agar medium amended with 0 and 100 µg metalaxyl/ml. Metalaxyl sensitive (MS) = growth <10% of control; metalaxyl insensitive (MI) = growth ≥ 10% of control.

[3]*Glucose-6-phosphate isomerase.*

[4]*Peptidase.*

DISCUSSION

Results from this study and previous studies (Goodwin *et al.* 1994, 1998; Peters *et al.* 1999b) clearly document the displacement of the US-1 genotype of *P. infestans* by novel genotypes in Canada during 1994 and 1995. Although g11 (or US-11) occurred in British Columbia and US-8 predominated in the rest of Canada, other novel genotypes were described in each year of the study. Isolates of both mating types that were either sensitive or insensitive to metalaxyl were found. This variability in the pathogen may have implications for the successful management of late blight in Canada.

The prevalence of US-8 and g11 (or US-11) populations of *P. infestans* in Canada (Peters *et al.* 1999b) probably reflects an enhanced fitness of these genotypes over preexisting forms (US-1). Kato *et al.* (1997) found that isolates of the US-8 clonal lineage produced significantly larger lesions

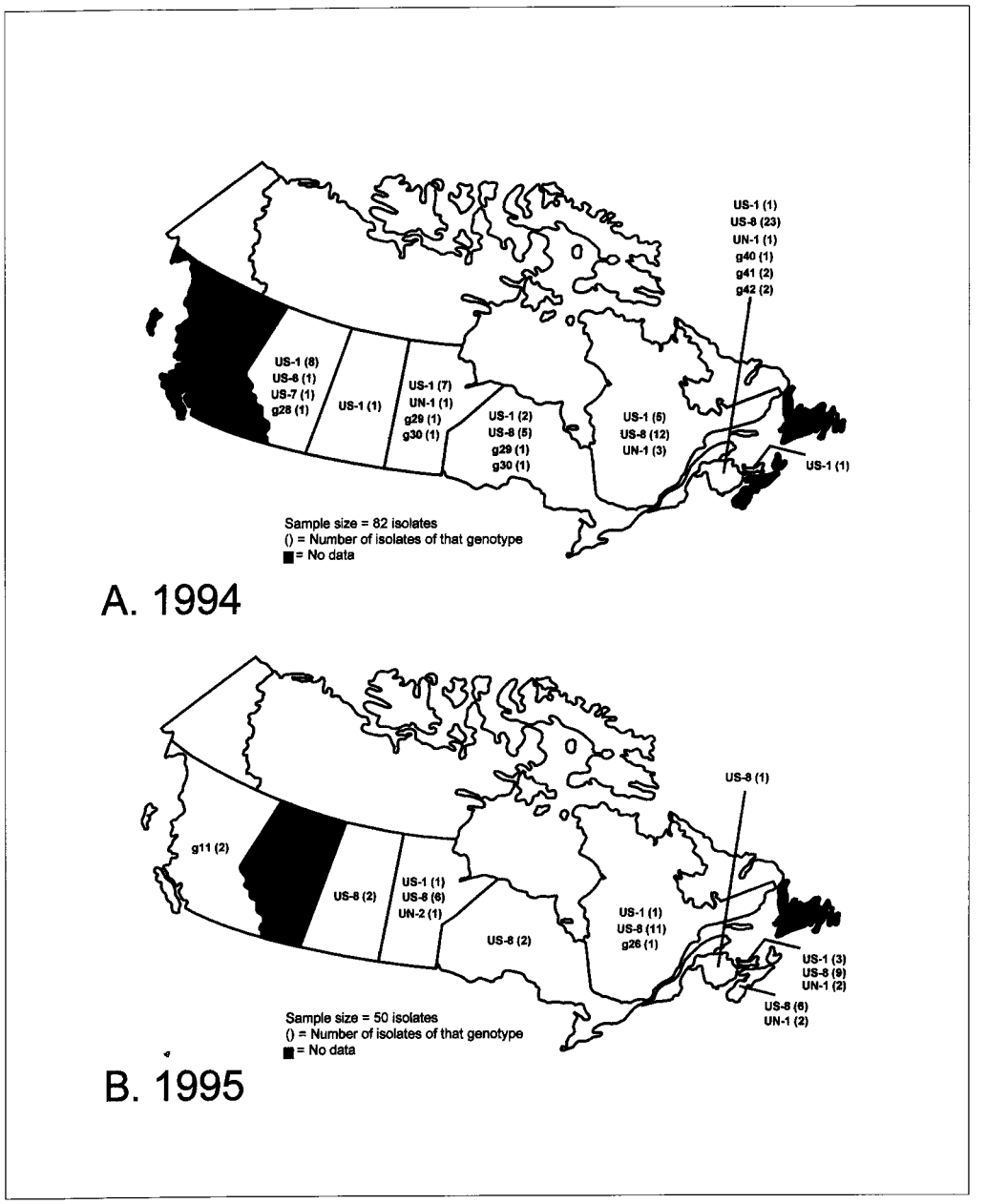

FIGURE 1.
Location and number of isolates (brackets) of genotypes of *Phytophthora infestans* recovered from various Canadian provinces in 1994 (A) and 1995 (B).

with greater sporulation on detached leaves than isolates of the US-1 clonal lineage. Miller and Johnson (1997) found an increase in incidence and severity of stem lesions on greenhouse potatoes inoculated with US-8 isolates compared with plants inoculated with US-1 isolates. Lambert and Currier (1997) noted that isolates of the US-8 genotype produced faster visible rot on tubers than US-1 isolates. Tubers inoculated with US-8 isolates also produce more diseased sprouts (19.4% compared to 1.9%) than those inoculated with US-1 isolates (Marshall and Stevenson 1996). Isolates of the g11 (or US-11) genotype have been found to be more aggressive on tuber tissue than US-1 isolates, but less aggressive than US-8 isolates (Peters *et al.* 1999c). Isolates of minor genotypes (g30, g41, g42) were not more aggressive than US-1 (Peters *et al.* 1999c), which may indicate reduced fitness, explaining their disappearance in 1995.

The use of RAPD markers showed variability in the pathogen that was not detected by allozyme analysis. It is possible that the more sensitive RAPD assay detected minor genetic variation within existing clonal lineages. However, some of the minor genotypes identified by RAPD analysis (g26, g28, g29, g30, g40, g41, g42) also varied in specific virulence and aggressiveness compared to isolates of defined clonal lineages (Peters 1998; Peters *et al.* 1999c), indicating more substantial genetic differences were present. Unfortunately, the RAPDs do not allow direct comparison with isolates characterized by restriction fragment length polymorphism (RFLP) using probe RG57 (Goodwin *et al.* 1994, 1995b, 1998) and described in a global database (Forbes *et al.* 1998).

In addition to migration, new genotypes also may appear in a region as the result of mutation and other asexual processes, or by sexual reproduction. These processes prob-

ably generated some of the novel genotypes found in this study. Variation was revealed within US-1 and US-8 allozyme genotypes. Some variation was also revealed within the *Gpi* 100/122 allozyme genotype (RAPD analysis described one isolate in this group as g26, others as US-8). Miller *et al.* (1997) postulated that the A2, *Gpi* 100/122 genotype could have arisen by the loss of the *Gpi* 111 allele from the US-8 genotype. Also, Goodwin *et al.* (1992) found an isolate in Mexico with the *Gpi* 100/111 genotype that had the same mating type and DNA fingerprint pattern as one of the isolates with the *Gpi* 100/111/122 genotype. They postulated that *Gpi* 100/111 isolates were derived from *Gpi* 100/111/122 isolates by the loss of the 122 allele. Therefore, loss of the *Gpi* 111 allele from the US-8 genotype seems to be a plausible mechanism in the derivation of the UN-1 genotype. If so, this genotype is probably the same as US-14 described by Goodwin *et al.* (1998). The UN-2 (A1, *Gpi* 100/111/122) genotype could have arisen by the production of selfed oospores by an A2, *Gpi* 100/111/122 isolate as described by Shattock *et al.* (1986) or as the result of sexual processes.

Mexico is the likely source of some of the new strains of *P. infestans* that have appeared in Canada and the United States in the 1990s (Goodwin and Drenth 1997). The US-8 genotype was the most common genotype on potatoes in northwestern Mexico in 1989 (Goodwin *et al.* 1992). This genotype has subsequently become more common in the U.S. and Canada. However, additional variation in populations of the pathogen (i.e., the presence of other genotypes) may not be the result of migration only. Goodwin *et al.* (1995b) found evidence for the occurrence of sexual reproduction in British Columbia based on RFLP analysis with probe RG57 of several isolates of *P. infestans* from the province. The g11 (US-11) multilocus allozyme genotype, predominant in British Columbia in 1996 (Peters *et al.* 1999b), possibly arrived by migration (a similar genotype was present in the Columbia Basin of Washington and Oregon in 1993 (Miller *et al.* 1997)), but may also be the result of sexual recombination in the previous population given the genotypes present (US-6 and US-7) in 1993 (Chycoski and Punja, 1996). Punja *et al.* (1998) postulated that sexual reproduction was responsible for generating some of the novel genotypes found in collections of isolates of *P. infestans* from British Columbia and New Brunswick obtained in the 1990s and characterized by RAPD analysis. Direct evidence for sexual reproduction in Canada is limited to the discovery of a few oospores in plant tissues obtained from British Columbia, New Brunswick (Chycoski and Punja 1996), and Quebec (Peters *et al.* 1998b).

The increased genotypic diversity in the Canadian *P. infestans* population in 1994 relative to 1995 possibly reflects increased opportunities for sexual reproduction in 1994, the year in which A2 isolates were first found in many Canadian provinces and in which both mating types of the pathogen were isolated from several provinces (Peters *et al.* 1998b, 1999b). New Brunswick yielded several novel genotypes in 1994 (no data were available for British Columbia), which is consistent with other published work (Punja *et al.* 1998) and the documented isolation of both A1 and A2 mating types from single fields in the province (Chycoski and Punja 1996; Peters *et al.* 1998b). The almost complete displacement of US-1 by more aggressive genotypes (particularly US-8) in 1995 probably limited opportunities for sexual reproduction, leading to less variability in populations and the predominance of a few, highly fit forms. This hypothesis is substantiated by surveys conducted in Canada which revealed that most fields were colonized by populations of single mating type demes (Peters *et al.* 1998b). However, results of a more recent study, which assessed genetic variation in a larger collection of Canadian isolates of *P. infestans* using a different RAPD marker system, indicated that haplotypic diversity was high and similar among collections of isolates obtained in 1994, 1995, and 1996 (Mahuku *et al.*, 2000). Although the results of Mahuku *et al.* (2000) differ from those presented in this paper, both studies identified genetic variation in pathogen populations not described by other marker systems and revealed pathogen diversity on a national scale. Efforts to assess the dynamics of populations of *P. infestans* in Canada using a variety of molecular tools are ongoing.

On the large scale, oospore formation probably has limited epidemiological importance (in terms of a major source of inoculum for epidemics) due to the predominance of a single mating type (A2) in most of Canada. However, the potential of sexual reproduction to create new forms has significance regionally, and by dispersal of these forms, nationally as well. Oospores of *P. infestans* have been shown to overwinter in Europe and subsequently incite disease (Drenth *et al.* 1995). Oospores have also survived exposure to a wide range of temperatures in the laboratory (Drenth *et al.* 1995; Fay and Fry 1997) and have overwintered successfully in eastern Canada (Medina and Platt 1999). British Columbia has consistently yielded a diversity of new forms, probably as a result of sexual reproduction (Goodwin *et al.* 1995b; Peters *et al.* 1999b; Punja *et al.* 1998). Although found in low frequency, genotypes other than US-1 or US-8 have been found in Canada outside of British Columbia in surveys

conducted during the 1990s (Peters *et al.* 1999b; Punja *et al.* 1998). For example, the g11 (US-11) genotype was found in a tomato sample from Ontario in 1996 (Peters *et al.* 1999b). We have found novel genotypes of *P. infestans* to be dispersed on potato or tomato tissue and by the winds of storm systems (Peters *et al.* 1999a). The dispersal of new forms can introduce more aggressive genotypes (such as US-8) into a region and also provide localized opportunities for sexual reproduction. Sexual reproduction, even if it is occurring at low frequency, is significant since it can result in the creation of new variants. Continued monitoring of pathogen populations and research on the impact of sexual reproduction on *P. infestans* populations in Canada is required.

ACKNOWLEDGMENTS

The authors would like to thank Agriculture and Agri-Food Canada and all growers, potato industry representatives, extension personnel, and government workers from across Canada for sample submissions and general involvement in Matching Investment Initiative Project #3026. In addition, the outstanding technical assistance of B. Matheson, L. McNally-Shanahan, A. Driscoll, S. Jenkins, E. Connors, S. Powell, A. MacPhail, and V. MacLean is also gratefully acknowledged. AAFC Contribution No. 926.

LITERATURE CITED

Chycoski, C.I. and Z.K. Punja. 1996. Characteristics of populations of *Phytophthora infestans* from potato in British Columbia and other regions of Canada during 1993 to 1995. Plant Dis 80:579-589.

Deahl, K.L., S.P. DeMuth, G. Pelter, and D.J. Ormrod. 1993a. First report of resistance of *Phytophthora infestans* to metalaxyl in eastern Washington and southwestern British Columbia. Plant Dis 77:429.

Deahl, K.L., S.P. DeMuth, S.L. Sinden, and A. Rivera-Peña. 1995. Identification of mating types and metalaxyl resistance in North American populations of *Phytophthora infestans*. Am Potato J 72:35-49.

Deahl, K.L., D.A. Inglis, and S.P. DeMuth. 1993b. Testing for resistance to metalaxyl in *Phytophthora infestans* isolates from northwestern Washington. Am Potato J 70:779-795.

Drenth, A., E.M. Janssen, and F. Govers. 1995. Formation and survival of oospores of *Phytophthora infestans* under natural conditions. Plant Pathol 44:86-94.

Fay, J.C. and W.E. Fry. 1997. Effects of hot and cold temperatures on the survival of oospores produced by United States strains of *Phytophthora infestans*. Am Potato J 74:315-323.

Forbes, G.A., S.B. Goodwin, A. Drenth, P. Oyarzun, M.E. Ordoñez, and W.E. Fry. 1998. A global marker database for *Phytophthora infestans*. Plant Dis 82: 811-818.

Fry, W.E., A. Drenth, L.J. Spielman, B.C. Mantel, L.C. Davidse, and S.B. Goodwin. 1991. Population genetic structure of *Phytophthora infestans* in the Netherlands. Phytopathology 81:1330-1336.

Fry, W.E., S.B. Goodwin, A.T. Dyer, J.M. Matuszak, A. Drenth, P.W. Tooley, L.S. Sujkowski, Y.J. Koh, B.A. Cohen, L.J. Spielman, K.L. Deahl, D.A. Inglis, and K.P. Sandlan. 1993. Historical and recent migrations of *Phytophthora infestans*: Chronology, pathways, and implications. Plant Dis 77:653-661.

Goodwin, S.B., B.A. Cohen, K.L. Deahl, and W.E. Fry. 1994. Migration from northern Mexico as the probable cause of recent genetic changes in populations of *Phytophthora infestans* in the United States and Canada. Phytopathology 84:553-558.

Goodwin, S.B. and A. Drenth. 1997. Origin of the A2 mating type of *Phytophthora infestans* outside Mexico. Phytopathology 87:992-999.

Goodwin, S.B., R.E. Schneider, and W.E. Fry. 1995a. Use of cellulose acetate electrophoresis for rapid identification of allozyme genotypes of *Phytophthora infestans*. Plant Dis 79:1181-1185.

Goodwin, S.B., C.D. Smart, R.W. Sandrock, K.L. Deahl, Z.K. Punja, and W.E. Fry. 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.

Goodwin, S.B., L.J. Spielman, J.M. Matuszak, S.N. Bergeron, and W.E. Fry. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopathology 82:955-961.

Goodwin, S.B., L.S. Sujkowski, A.T. Dyer, B.A. Fry, and W.E. Fry. 1995b. Direct detection of gene flow and probable sexual reproduction of *Phytophthora infestans* in northern North America. Phytopathology 85:473-479.

Judelson, H.S. 1996. Genetic and physical variability at the mating type locus of the Oomycete, *Phytophthora infestans*. Genetics 144:1005-1013.

Kato, M., E.S. Mizubuti, S.B. Goodwin, and W.E. Fry. 1997. Sensitivity to protectant fungicides and pathogenic fitness of clonal lineages of *Phytophthora infestans* in the United States. Phytopathology 87:973-978.

Lambert, D.H. and A.I. Currier. 1997. Differences in tuber rot development for North American clones of *Phytophthora infestans*. Am Potato J 74:39-43.

Mahuku, G., R.D. Peters, H.W. Platt, and F. Daayf. 2000. Random amplified polymorphic DNA (RAPD) analysis of *Phytophthora infestans* isolates collected in Canada during 1994 to 1996. Plant Pathol 49:252-260.

Marshall, K.D. and W.R. Stevenson. 1996. Transmission of *Phytophthora infestans* from infected seed potato tubers to developing sprouts. Am Potato J 73:370 (abstr).

Medina, M.V. and H.W. (Bud) Platt. 1999. Viability of oospores of *Phytophthora infestans* under field conditions in northeastern North America. Can J Plant Pathol 21:137-143.

Miller, J.S., P.B. Hamm, and D.A. Johnson. 1997. Characterization of the *Phytophthora infestans* populations in the Columbia basin of Oregon and Washington from 1992 to 1995. Phytopathology 87:656-660.

Miller, J.S. and D.A. Johnson. 1997. Aggressiveness of *Phytophthora infestans* genotypes on potato stems and leaves. Phytopathology 87:S66 (abstr).

Peters, R.D. 1998. Characterization of evolving populations of *Phytophthora infestans* causing late blight of potato in Canada. Ph.D. Thesis, University of Guelph, Guelph, ON. 305p.

Peters, R.D., H.W. (Bud) Platt, and R. Hall. 1998a. Long-term survival of *Phytophthora infestans* on liquid media prepared from autoclaved seeds. Can J Plant Pathol 20:165-170.

Peters, R.D., H.W. (Bud) Platt, and R. Hall. 1998b. Characterization of changes in populations of *Phytophthora infestans* in Canada using mating type and metalaxyl sensitivity markers. Can J Plant Pathol 20:259-273.

Peters, R.D., H.W. (Bud) Platt, and R. Hall. 1999a. Hypotheses for the inter-regional movement of new genotypes of *Phytophthora infestans* in Canada. Can J Plant Pathol 21:132-136.

Peters, R.D., H.W. (Bud) Platt, and R. Hall. 1999b. Use of allozyme markers to determine genotypes of *Phytophthora infestans* in Canada. Can J Plant Pathol 21:144-153.

Peters, R.D., H.W. (Bud) Platt, R. Hall, and M. Medina. 1999c. Variation in aggressiveness of Canadian isolates of *Phytophthora infestans* as indicated by their relative abilities to cause potato tuber rot. Plant Dis 83:652-661.

Punja, Z.K., H. Förster, I. Cunningham, and M.D. Coffey. 1998. Genotypes of the late blight pathogen (*Phytophthora infestans*) in British Columbia and other regions of Canada during 1993-1997. Can J Plant Pathol 20: 274-282.

Shattock, R.C., P.W. Tooley, and W.E. Fry. 1986. Genetics of *Phytophthora infestans*: Determination of recombination, segregation, and selfing by isozyme analysis. Phytopathology 76:410-413.