Characteristics of Populations of *Phytophthora infestans* from Potato in British Columbia and Other Regions of Canada During 1993 to 1995

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ABSTRACT

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The incidence and distribution of A1 and A2 mating types of *Phytophthora infestans* in Canada and of metalaxyl-sensitive (MS) and -insensitive (MI) strains were monitored during 1993, 1994, and 1995. Diseased leaves from about 1,600 plants were collected from 36 cultivars in 168 potato fields from five provinces at various times (June to September) during the growing season. The most extensive sampling (88% of total) was conducted in British Columbia (B.C.). About 500 isolates of *P. infestans* were characterized from over 1,000 collected. In 1993, the A1 mating type was found in all provinces sampled, while western Canada (B.C.) was the only region in which the A2 mating type was detected. In 1994, the A2 mating type was also found in eastern Canada (New Brunswick [N.B.]). In B.C., 23 fields sampled in 1993 had both A1 and A2 mating types, while in N.B. in 1994, both mating types originated from the same plant in seven different fields. When lesions from two of these fields were examined by microscope, sporangia of *P. infestans* and a single oospore were seen in leaf tissues, demonstrating the potential for oospore production in naturally infected leaves in western and eastern Canada if both mating types are present. Metalaxyl sensitivity tests measuring relative growth of isolates with metalaxyl at 0 and 50 µg/ml revealed that all isolates collected in 1993 from Alberta, Manitoba, N.B., Nova Scotia, Ontario, Prince Edward Island, and Quebec were MS. In B.C., isolates showed a range of variation in growth with and without metalaxyl, and 76% of the isolates were MI. There was no correlation between recovery of these MI strains and use or nonuse of metalaxyl during the same growing season. Both MS and MI strains were present together in six fields. A low frequency of MS strains of both mating types was always recovered in B.C. When the frequency of occurrence of MS and MI strains throughout the growing season was examined, a high proportion of isolates collected early (June to July) from B.C. and N.B. in 1993 and 1994 was found to be MI. At the end of the season (September), MI isolates also occurred at a higher frequency in both locations and years. When the frequency of mating types was examined, the A2 type occurred at a higher proportion throughout the season in B.C. during 1993 and 1994. However, in 1995, the A2 mating type was rarely recovered and the A1 type predominated. In N.B. in 1994, the A1 mating type was recovered at a higher frequency than the A2 type in July, but most collections later were of mixed mating type. When the isolates were grouped into A1, A2, MS, and MI categories and growth rates were compared, isolates from populations in B.C. and N.B. in 1994 of A2 mating type grew significantly faster than A1; isolates that were MI from populations in B.C. in 1993 grew significantly faster than MS isolates. However, no differences could be detected in the extent of leaf colonization or sporulation between isolates representing A1, A2, MS, or MI. A preliminary survival study showed that *P. infestans* could overwinter under B.C. conditions on artificially inoculated tubers. These results illustrate the dynamic nature of populations of *P. infestans* within and between growing seasons in western and eastern Canada, and demonstrate higher variation in the population in B.C.

Additional keywords: epidemiology, fungicide resistance, late blight, sexual recombination, *Solanum tuberosum*

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is an important disease of potato (*Solanum tuberosum* L.) worldwide (13). In recent years, the incidence and severity of the disease has increased in many potato-growing regions of the United States (8,13,14). The increased prevalence of late blight may be attributed to a combination of several factors: (i) the occurrence of recently introduced or immigrant genotypes that have displaced original populations (13,15,33); (ii) an increase in the prevalence of strains of the A2 mating type, first detected in the United States in 1987 (6,13); (iii) the occurrence of insensitivity of strains of the pathogen to the widely used systemic phenylamide fungicide metalaxyl (8,13); and (iv) more conducive weather conditions.

In Canada, the prevalence of late blight has also increased in recent years similar to the situation in the United States. In 1989, an isolate of *P. infestans* originating from Richmond, British Columbia (B.C.), was found to be of the A2 mating type and represented the first report of this mating type in Canada (7). An additional four isolates collected from potato fields in B.C. during 1991 to 1992 were found to represent unique genotypes of *P. infestans* (15,18). Insensitivity of the pathogen to metalaxyl was documented for the first time in B.C. in 1993 (6). Except for these reports, little else is known about the characteristics of populations of *P. infestans* in B.C. and throughout Canada. For example, the response of isolates to metalaxyl and the changes that may occur in the sensitivity of isolates to metalaxyl during a growing season are not known. Furthermore, the extent to which the A2 mating type is distributed in B.C. and in other parts of Canada has not been established. This type of information is critical to the development of disease control strategies for late blight on potatoes in Canada.

The objectives of this research were to: (i) characterize isolates of *P. infestans* from leaf samples collected from several provinces in Canada for sensitivity to metalaxyl in vitro; (ii) determine the mating type of isolates of the pathogen; (iii) monitor changes in the ratio of metalaxyl sensitive:insensitive isolates and of A1:A2 mating types during the growing season; (iv) determine the influence of metalaxyl application, potato cultivar, and time of sampling on population characteristics of the pathogen; and (v) compare isolates for growth, colonization of leaf tissues, sporulation, and survival on potato tissues.

MATERIALS AND METHODS

Source of isolates. During the 1993 and 1994 growing seasons, late blight-infected potato leaf samples from field grown plants were obtained from Alberta (provided by R. Howard, Alberta Agriculture, Brooks, and V. Bisht, Alberta Tree Nursery and Horticulture Centre, Edmonton), British Columbia (collected by the authors and also provided by C. Duff, P. Froese,

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and B. Vernon, Agriculture Canada; D. Henderson, E.S. Cropconsult Ltd., Vancouver; D. Ormrod, B.C. Ministry of Agriculture, Cloverdale; B. Peterson, Coast-Agri Crop Products, Cloverdale; and B. Warner, B.C. Ministry of Agriculture, Victoria), Manitoba (provided by L. MacDonald and G. McKenzie, Ciba Canada, Warren), New Brunswick (provided by G. Bernard, Department of Agriculture, Florenceville), and Prince Edward Island (provided by M. Drake, Island Crop Care Services, Charlottetown). During 1995, leaf samples originated from B.C. Samples were collected during the months of May to September in each year (Table 1). In addition, cultures of *P. infestans* originating from Nova Scotia, Ontario, Prince Edward Island, and Quebec (provided by G. White, Agriculture Canada, Ottawa) were included in the study.

The geographic locations within each province from which samples were obtained are indicated in Table 2. The sampling areas in British Columbia were located in four geographically separated regions (Fig. 1). The Okanagan Valley, the Pemberton Valley, and the Fraser Valley are each separated by a distance of about 140 km, while Vancouver Island is about 50 km to the west of the Fraser Valley (Fig. 1). The Fraser Valley, where the most intensive sampling was done, included the rural municipalities of Chilliwack, Cloverdale, Delta, Langley, Ladner, and Richmond. The agricultural area of Cloverdale is about 25 km from Delta, Ladner, and Richmond. In Alberta in 1993, all samples were from the southern irrigation district near Brooks. In 1994, the samples were from the north-central region near Edmonton (Table 2). In New Brunswick (N.B.) in 1994, samples originated from various parts of the province.

Sampling procedure and isolation methods. The potato fields sampled in B.C. ranged from 0.2 to 24 ha, with an average of 4 ha. When late blight lesions first appeared in a field, an average of 12 plants per field were sampled at evenly spaced locations close to the field perimeter. If diseased plants were aggregated, every effort was made to sample different plants at individual locations in the field. During subsequent resampling of the same field, an additional four diseased plants were also sampled 10 m inward from the perimeter. From each plant, a stem or compound leaf with lesions was collected and comprised one sample. A total of 12 or 16 plants per field was represented at each of the sampling dates, which varied from one to four, depending on the field. Fields were resampled at 15- to 55-day intervals depending on whether the disease was present. The total number of fields sampled and the months when samples were obtained are shown in Table 1. From each field, information was obtained on the cultivar grown and whether or not metalaxyl had been applied, and the overall incidence of disease in the field was estimated.

Tissue pieces from lesioned areas were usually plated within 24 to 48 h after collection; however, for leaf samples originating from out-of-province that were shipped, a delay of 5 to 7 days often occurred. All leaf samples were placed in plastic bags after collection and transported over ice in a cooler. Tissue pieces from the margin of lesions were plated onto rye agar (boiled extract from 60 g of rye grains in 1 liter of distilled water, 20 g of sucrose, and 15 g of water agar) amended with nystatin at 25 µg/ml, vancomycin at 100 µg/ml, and rifampicin at 20 µg/ml (3,4,30). An average of six to eight tissue pieces per plant were plated on one petri dish. Occasionally, leaves were placed on freshly cut halves of potato tubers and incubated for 5 to 7 days at 16°C before isolation was attempted. In 1994 and 1995, isolations were also made onto 2% V8 agar (20 ml of V8 juice per liter of water) amended with the same antibiotics as above. All petri dishes were incubated in the dark at 16 to 18°C for 7 to 14 days and then examined for colonies of *P. infestans*. Subcultures (mass transfers of mycelium) were made onto fresh rye agar or V8 agar containing antibiotics and incubated for 2 to 8 weeks, at which time a third subculture was made. Penicillin G (200 µg/ml) was added to minimize growth of contaminant bacteria in some cultures. From each field sampled, an average of one to four isolates was maintained, with each isolate originating from a different plant within the field.

Response to metalaxyl. *Agar assay.* From each sampled field at each of the different sampling dates, one to four isolates were evaluated for growth on agar containing metalaxyl. The medium used in 1993 was rye agar without antibiotics, and in 1994 and 1995, 2% V8 agar was used. Initially, colony growth on metalaxyl (Ridomil 25 WP) at 0, 5, 30, 50, and 100 µg a.i./ml was measured; in all subsequent experiments, growth on 0 and 50 µg/ml was used to assess whether an isolate was sensitive (MS) or insensitive (MI). Colonies were initiated from 0.5-cm-diameter mycelial plugs taken from 14- to 28-day-old cultures growing on rye agar or 2% V8 agar. After 10 to 14 days of incubation at 20°C, colony diameter was measured on

Table 1. Recovery of isolates of *Phytophthora infestans* during 1993 to 1995 with regard to time of season, potato cultivars, and fields

Year Location	Time of collection	Potato cultivars	Fields sampled	Isolates obtained
1993				
Alberta	July, Sept.	Norchip, Russet Burbank	7	8
British Columbia:				
Fraser Valley	June, July, Aug., Sept.	Epicure, Eramosa, Krantz, Norchip, Norland, Red Lasoda, , Russet Norkotah, Russet Burbank, Shepody, Sunrise, Warba, Yukon Gold	53	472
Okanagan Valley	Aug., Sept.	Nooksack, Russet Burbank, Russet Norkotah, Shepody	7	28
Pemberton Valley	July, Aug.	Chieftain, Pontiac, Ranger Russet, Russet Burbank, Russet Norkotah, White Rose	10	24
Vancouver Island	Aug.	Epicure, Nooksack, Red Lasoda, Shepody, Russet Burbank, Russet Norkotah, Yukon Gold	10	38
New Brunswick	Aug.	Atlantic, Russet Norkotah, Superior	3	7
1994				
Alberta	Sept.	All Blue, Banana, Russet Burbank	7	10
British Columbia:				
Fraser Valley	June, Aug., Sept.	Eramosa, Hilite Russet, Nooksack, Norchip, Russet Burbank, Sunrise, Warba	14	92
Pemberton Valley	Sept.	Red Lasoda, Russet Burbank	3	5
Vancouver Island	Sept.	Russet Burbank	1	3
Manitoba	Aug.	Russet Burbank	2	4
New Brunswick	July, Aug.	A.C. Chaleur, Chieftain, Frontier Russet, Norris, Ranger Russet, Russet Burbank, Russet Norkotah, Shepody, Superior	18	75
Prince Edward Island	Aug.	Hilite Russet	1	4
1995				
Alberta	Aug.	Niska	1	2
British Columbia:				
Fraser Valley	June, July, Aug., Sept.	Butte, Cherokee, Gems, Hilite, Island Sunshine, Kennebec, Nooksack, Norchip, Norkotah, Norqueen, Ranger Russet, Shepody, Sunchip, Tolaas, Warba, Yukon Gold	29	261

two replicate plates by taking three measurements per plate. Initially, up to four plates per isolate were used, but the variation among plates was sufficiently low to warrant using two plates. Isolates with known responses to this assay method were included: CG 96 (sensitive) and CG 215 (insensitive) (provided by C. Steden, Ciba, Basel), B.C. 4.1 (sensitive), B.C. 5.2 (sensitive), and B.C. 7.1 (insensitive) (provided by S. B. Goodwin, Cornell University, Ithaca). The response of these tester isolates to the in vitro assay method (on rye agar) is illustrated in Figure 2A. In most trials, CG 96 and CG 215 were included as standards. Isolates were considered to be insensitive if the ratio of growth at 50:0 µg/ml exceeded 0.3, i.e., if isolates grew at 30% of the controls in the presence of metalaxyl at 50 µg/ml (5). In experiments where isolates grew poorly or the testers were inconsistent, the trials were repeated.

Leaf disk assay. Colonies of nine *P. infestans* isolates, including the two tester strains (CG96 and CG215), were grown on 2% V8 agar without antibiotics for 2 to 4 weeks at 16°C in the dark. Each colony was then flooded with 5 to 10 ml of sterile distilled water and gently agitated to release sporangia. The sporangial concentration was adjusted to 1 to 2 × 10^4 per ml (average of four counts in a hemacytometer). Potato cv. Norchip leaf disks (15 mm diameter) were taken from fully expanded leaves from plants grown in a room provided with supplemental halogen lighting (16-h photoperiod). The leaf disks were misted with sterile distilled water and floated adaxial side up in petri dishes containing 20 ml of either sterile distilled water or metalaxyl (Ridomil 25 WP) at 50 µg/ml. The sporangial suspension (a 30-µl droplet) was placed on each leaf disk; there were eight disks per dish and two dishes per isolate. The dishes were incubated at 16°C and a 12-h photoperiod until the control disks were completely colonized (usually 7 to 9 days). The percent area of the leaf disk covered with sporangia was then determined. The isolate was considered to be sensitive if the lesioned area was ≤25%. The experiment was conducted twice.

Mating type determination. Tester isolates of known mating type, which included WE 9 (A1) and B.C. 5.2. (A2) (provided by W. E. Fry, Cornell University, Ithaca) and B.C. 8.1.1. and B.C. 8.1.8. (both A2) collected in this study were used. Unknown field isolates (an average of one to four per sampled field, in most cases the same isolates as those used in the metalaxyl test) were paired against each of the A1 and A2 tester isolates. Pairings were made either on agar-coated microscope slides or in 60 × 15 mm petri dishes containing clarified 20 or 2% V8 agar. Mycelial plugs (0.5 cm diameter) were placed 1 cm apart, and the slides or dishes were incubated within a sealed container at 20°C in the dark for 2 to 3 weeks. In some pairings, it was necessary to place the unknown isolate on the agar 8 to 10 days prior to the tester due to its slow growth. The presence or absence of oospores was recorded by viewing merged colonies directly with an inverted microscope. Oospore formation in colonies paired against one of the tester strains denoted the opposite mating type in the unknown. In some isolates, oospore formation was observed

Table 2. Characterization of isolates of *Phytophthora infestans* from Canada during 1993 to 1995 with regard to both mating type and response to metalaxyl in vitro

Year	Mating type and response to metalaxyl[a]					
	A1		A2		Mix (A1 and A2)	
Origin	S	I	S	I	S	I
1993						
Alberta						
Brooks	4	1	...	...	...	...
Duchess	1	1	...	...	...	...
Lethbridge	1	...	...	...	...	...
Taber	1	...	...	...	...	...
British Columbia						
Chilliwack	...	4	...	6	...	...
Cloverdale	1	8	11	93	1	1
Delta	...	4	17	12	...	...
Ladner	...	2	2	12	...	1
Okanagan	1	2	6	4	...	...
Pemberton	...	...	3	3	...	...
Richmond	3	2	1	1	...	...
Vancouver Island	1	...	3	8	1	...
Manitoba						
Bruxelle	1	...	...	...	...	...
Plum Coulee	1	...	...	...	...	...
Portage La Prairie	3	...	...	...	...	...
New Brunswick						
Grand Falls	3	...	...	...	...	...
Hartland	3	...	...	...	...	...
Undine	1	...	...	...	...	...
Nova Scotia	20	...	...	...	...	...
Ontario	4	...	...	...	...	...
Prince Edward Isl.	12	...	...	...	...	...
Quebec	1	...	...	...	...	...
1994						
Alberta						
Edmonton	5	1	...	...	...	...
Spruce Grove	6	2	...	...	...	...
British Columbia						
Cloverdale	...	6	1	27	...	...
Courtenay	...	...	...	1	...	1
Delta	...	2	...	4	...	...
Pemberton	...	...	1	...	...	...
Richmond	1	9	...	...	...	...
Manitoba						
Portage La Prairie	3	...	...	...	...	...
New Brunswick						
Bon Accord	...	...	...	4	...	...
Charleston	...	...	...	...	...	1
Drummond	...	3	...	1	...	4
Four Falls	...	1	...	...	...	3
Glassville	...	1	...	...	...	...
Greenfield	...	...	...	...	1	4
Holmesville	...	...	...	2	1	2
Knoxford	1	...	...	1	...	1
Limestone	...	1	1	3	...	...
New Denmark	1	1	2	2	...	5
Upper Kent	...	...	...	1	...	2
Woodstock	1	...	...	1	...	...
Prince Edward Isl.	1	...	...	...	...	...
1995						
Alberta						
Edmonton	2	...	...	...	...	...
British Columbia						
Cloverdale	3	38	...	1	...	...
Delta	2	31	...	4	...	...
Langley	...	10	...	...	...	...
Richmond	...	8	...	...	...	...

[a] S = metalaxyl sensitive; I = metalaxyl insensitive.

against both of the tester strains; these isolates were subsequently determined to be of mixed composition and were usually also found to produce oospores in unpaired cultures.

Oospore formation in vivo. From four to five leaf samples collected from British Columbia and New Brunswick in 1994 that yielded colonies of mixed mating type (Table 2), small pieces (ca. 0.5 cm^2) of the lesioned area were fixed and cleared in 95% ethanol:glacial acetic acid (1:1) for 4 to 5 days, transferred to fresh fixative, and stored at room temperature. The cleared tissues were examined under phase contrast in the microscope to determine if sporangia, oogonia, and oospores were present.

Survival of isolates under field conditions. Four isolates of *P. infestans*, collected during the 1993 growing season from the Fraser Valley of B.C. and which represented A1 MS, A1 MR, A2 MS, and A2 MR, were grown on rye agar for 10 days. Potato cv. Norchip tubers were dipped in 95% EtOH; then a core (7 mm diameter) was removed, a 5-mm-diameter mycelial plug (one isolate per tuber) was placed inside the cavity, and the potato plug was replaced. The tubers were wrapped in moist paper towel and incubated in the dark at 18°C for 1 week. A few tubers were cut open to ensure that growth of *P. infestans* had occurred. The inoculated tubers were placed individually in nylon mesh bags which were filled with soil and buried to a depth of 10 cm in a field in Cloverdale, B.C., which had not been planted to potatoes in 1993. For each isolate, there were six to eight replicate tubers for each of two sampling dates, and the tubers were randomized prior to burial (in December 1993). At each sampling date, the tubers were recovered and isolation of the pathogen was attempted following surface sterilization in sodium hypochlorite (0.625%) and plating of tuber pieces onto rye agar containing antibiotics.

Growth and sporulation of isolates in vivo. Eight isolates of *P. infestans,* which were representative of A1 MS, A1 MI, A2 MS, and A2 MI were compared for rate and degree of colonization of potato leaf tissues. Colonies were grown on 2% V8 agar for 2 to 4 weeks, and a sporangial suspension was prepared (1 to 2 × 10^4 per ml). A 20-µl drop of spore suspension was inoculated onto fully expanded leaflets of potato cv. Norchip, which were placed on the surface of water agar in 100 × 15 mm petri dishes. The dishes (four to six replications per isolate) were sealed with Parafilm and incubated at 16°C for 7 days, at which time the lesioned areas were traced onto clear acetate film and measured. The leaf disks were then placed in a test tube containing 4 ml of water with a drop of Tween 20 and shaken vigorously, and the number of sporangia was determined with a hemacytometer. The data were expressed as numbers of sporangia (average of four counts) per mm^2 of lesioned area. The experiment was conducted twice.

Fig. 1. Geographic locations within British Columbia from which diseased potato leaf samples were obtained during 1993 to 1995.

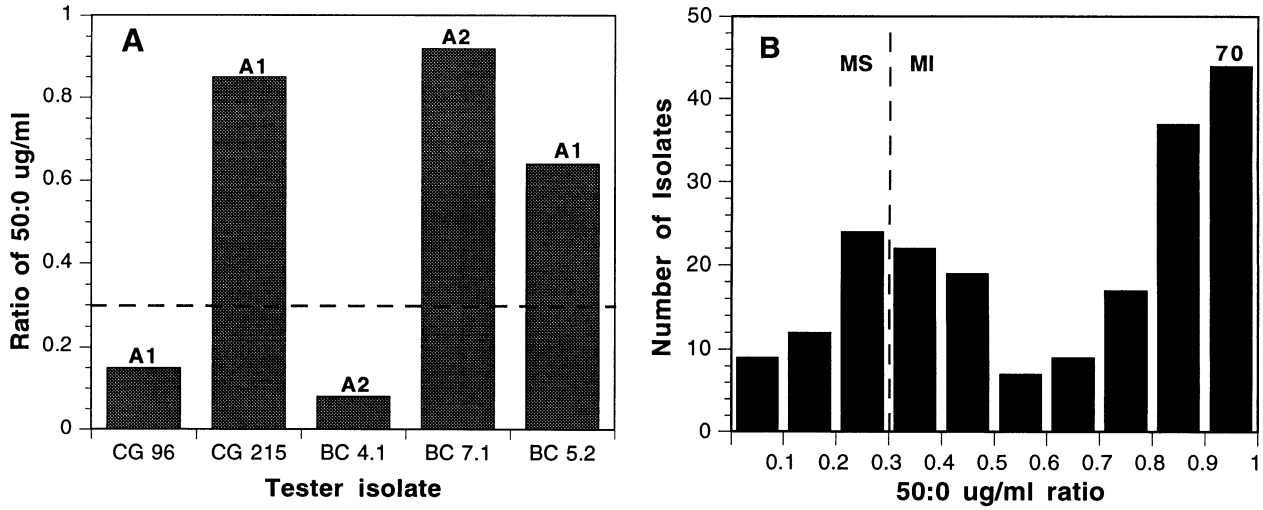

Fig. 2. Response of isolates of *Phytophthora infestans* to metalaxyl using an in vitro agar assay method that measures relative growth with metalaxyl at 50 and 0 µg/ml. **(A)** Response of five tester isolates showing the clear differentiation between sensitive (CG 96 and B.C. 4.1) and insensitive (CG 215, B.C. 5.2, B.C. 7.1) isolates. **(B)** Frequency distribution of isolates collected from British Columbia during 1993 in different metalaxyl growth ratio (50:0 µg/ml) categories.

RESULTS

Recovery of *P. infestans* from potato tissues. The pathogen was not always readily isolated from the leaf samples collected. It was intended that an average of 12 to 16 individual plants would be sampled per field, with six to eight tissue pieces plated per plant, to yield a minimum of four to eight isolates per field. However, not all of the fields sampled had active lesions from which *P. infestans* could be recovered; furthermore, many of the colonies that developed were contaminated and could not be subcultured. It was usually necessary to subculture twice to obtain contaminant-free colonies. On average, the recovery rate of *P. infestans* from lesions was 50%, yielding one to four isolates per field (Table 1). Over 1,000 isolates of the pathogen were recovered from 36 different potato cultivars during 3 years of sampling (Table 1). The differences in sampling frequency between 1993 and 1994 reflect the lower incidence of late blight in B.C. during 1994 due to hot and dry weather conditions. In 1993, 562 isolates were obtained from 80 different fields in B.C., and samples originated at different times during the growing season. In 1994, 100 isolates were obtained from 18 different fields in B.C., and 75 isolates originated from N.B. In 1995, 261 isolates were collected from B.C. (Table 1).

Response to metalaxyl. With the in vitro agar assay that compared growth of *P. infestans* on metalaxyl at 50 µg/ml to the unamended control, a clear differentiation between known sensitive and insensitive isolates was obtained (Fig. 2A). When the agar assay was compared to the leaf disk assay, seven of the nine isolates tested showed identical responses to metalaxyl; two isolates that were insensitive in the agar assay were sensitive in the leaf disk assay. For the agar assay, a cutoff ratio of 0.3 was subsequently used to distinguish between MS and MI isolates. The frequency distribution of isolates from B.C. in 1993 in response to metalaxyl was bimodal, with a cluster of isolates at 0.3 (sensitive) and a cluster at 0.9 to 1.0 (highly insensitive) (Fig. 2B). About 76% of the isolates collected from throughout the province were MI. The frequency distribution of isolates collected in 1994 from B.C. and N.B. is shown in Figure 3. About 92% of the isolates from B.C. and 86% of the isolates from N.B. were MI. The geographic origins of MS and MI isolates collected from B.C. in 1993 are indicated in Figure 4 and Table 2. Most of the MI isolates in B.C. were recovered from Cloverdale, and all of the regions sampled (with the exception of Chilliwack) had both MS and MI isolates present (Fig. 4). When the samples were considered with regard to time of collection in the season, the majority of isolates in 1993 were recovered during July, when late blight was most severe (Fig. 5). During June to September of 1993 and 1994, the population of MI was always higher than MS (Fig. 5).

Distribution of mating types in B.C. The geographic origins of A1 and A2 mating types and the cultivars from which they were isolated during 1993 in B.C. are illustrated in Figure 6. The region with the highest frequency of A2 was Cloverdale, and all of the regions sampled (with the exception of the Pemberton Valley) had combinations of both A1 and A2 (Fig. 6A). Only one area (Richmond) had a higher frequency of A1 than of A2. The A2 mating type was recovered from a wider range of cultivars than was A1 (Fig. 6B), with Russet Burbank and Russet Norkotah yielding the most isolates. When the samples were considered with regard to the time of collection in the season, the majority of A2 isolates in 1993 was recovered in June and July, and the relative proportion of A2 was always higher than A1 throughout the entire growing season (Fig. 7). In 1994, the trend was similar except for a peak in the number of A1 isolates in July, and the total number of isolates collected was lower (Fig. 7).

Characteristics of the population from B.C. *Colony growth.* A comparison of colony diameters of isolates grouped in A1, A2, MS, and MI categories in the absence of metalaxyl showed there were some significant differences (Table 3). In 1993, MI isolates grew significantly ($P < 0.05$) faster than MS isolates; while in 1994, isolates of the A2 mating type grew significantly ($P < 0.05$) faster than those of the A1 mating type (Table 3). The colony growth of isolates collected in 1993 on rye agar was variable, with the A2 isolates displaying a wider range of growth (Fig. 8A). Most of the A2 colonies grew between 3.0 and 6.0 cm in 10 days, but some reached diameters of up to 7.5 cm. The A2 isolates collected from B.C. in 1994 had a lower mean colony diameter compared to 1993 but displayed a similar range of growth (Fig. 8B). When the morphology of isolates of the A1 and A2 mating type of *P. infestans* in culture were compared, there were no specific characteristics that were unique to either mating type. Some isolates had dense fluffy mycelium, while others had thin patchy mycelium, and linear growth was variable.

During the sample collection period, information was obtained on whether meta-

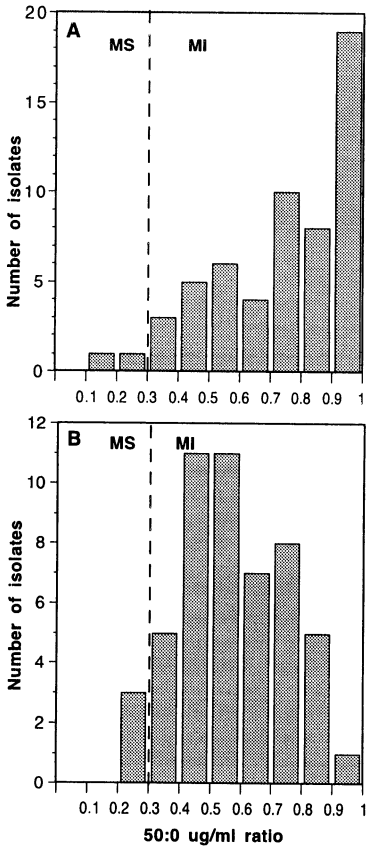

Fig. 3. Frequency distribution of isolates of *Phytophthora infestans* in different metalaxyl growth ratio (50:0 µg/ml) categories. **(A)** Isolates from British Columbia in 1994. **(B)** Isolates from New Brunswick in 1994.

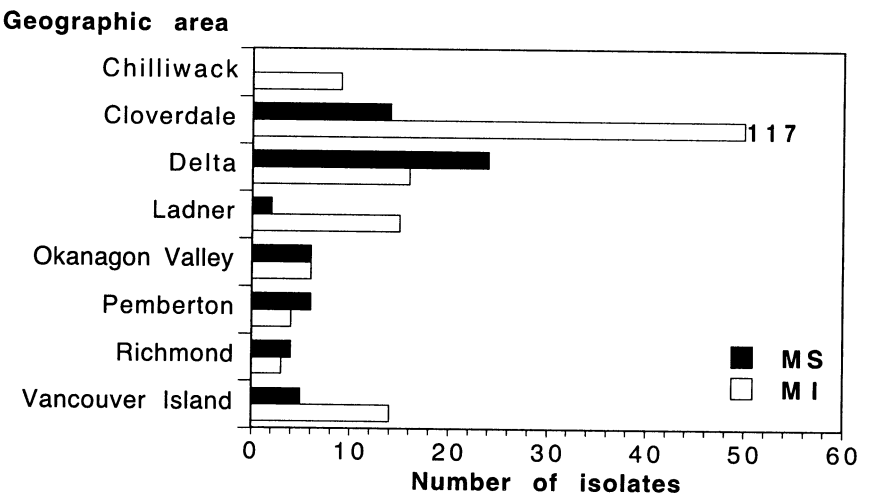

Fig. 4. Geographic areas within British Columbia from which metalaxyl-sensitive (MS) and metalaxyl-insensitive (MI) isolates of *Phytophthora infestans* were recovered during 1993.

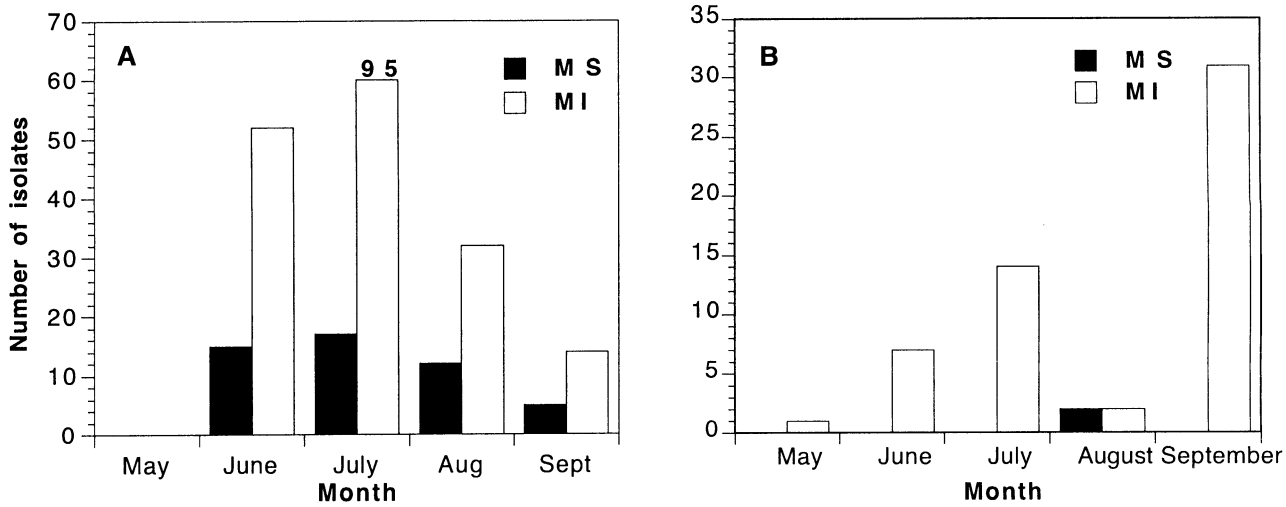

Fig. 5. Proportion of metalaxyl-sensitive (MS) and metalaxyl-insensitive (MI) isolates of *Phytophthora infestans* recovered at monthly intervals in British Columbia. **(A)** 1993 growing season. **(B)** 1994 growing season.

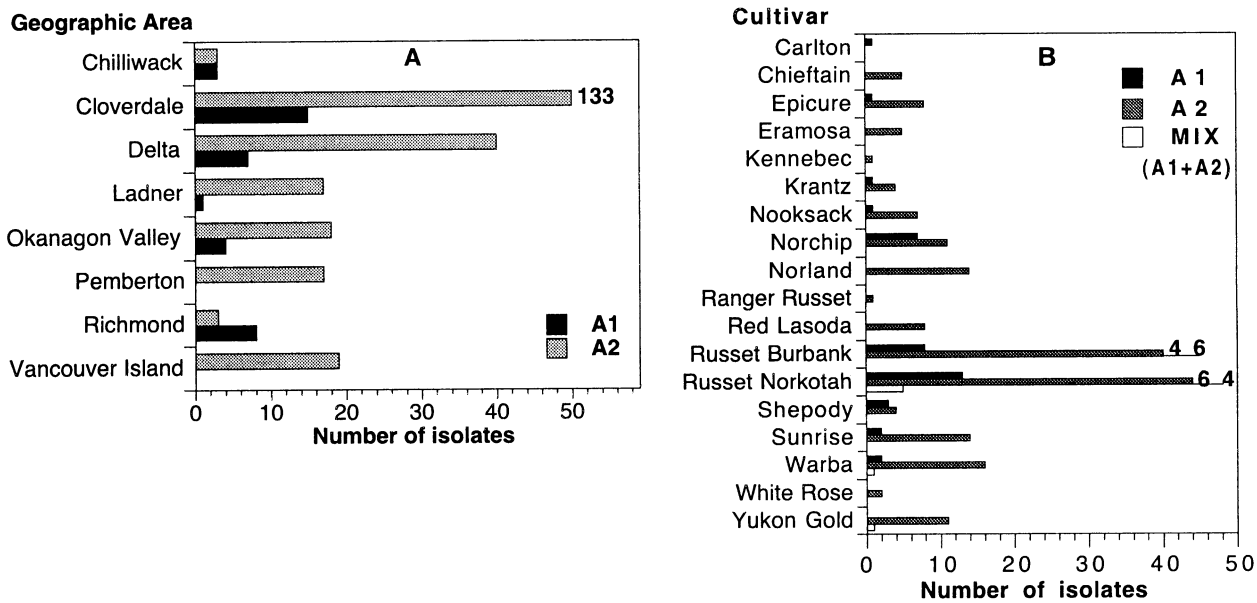

Fig. 6. Recovery of A1 and A2 mating types of *Phytophthora infestans* from British Columbia during 1993. **(A)** Geographic areas from which the two mating types were recovered. **(B)** Potato cultivars from which the mating types were isolated.

laxyl had been applied at least once to individual fields prior to the time of sampling, and the recovery of MS and MI isolates from these fields was compared (Table 4). Fields that had been treated with metalaxyl had a lower frequency of MS strains than untreated fields. However, fields in which metalaxyl had not been used were found to have the highest frequency of MI strains (Table 4). When the recovery of MI strains throughout the growing season was examined, the frequency of isolates increased in fields receiving metalaxyl and decreased in fields not treated with metalaxyl (Table 5). Most of the MI isolates were of the A2 mating type (Table 6).

Survival and fitness. The recovery of isolates from buried tubers after 72 days was comparable for A1 strains (2.5 to 4.0%) and was similar to that for the A2 strains (6.0 to 11.0%). These differences were not significant (P = 0.05, Bonferroni's t test). The measurements of sporangial numbers per mm^2 of lesioned area for isolates representing A1 MS, A1 MI, A2 MS, and A2 MI revealed a high degree of variability between replications and repetitions of the experiment. Therefore, no significant differences could be detected between the isolate categories (data not shown).

Characteristics of isolates collected throughout Canada. All isolates collected in 1993 from Alberta, Manitoba, New Brunswick, Nova Scotia, Ontario, Quebec, and Prince Edward Island were of the A1 mating type, and all (except one from Brooks, AB) were found to be MS (Table 2). During 1994, the A2 mating type was found in N.B., where late blight was very severe. A high proportion of isolates recovered from N.B. in 1994 were from mixed fields, in which both A1 and A2, or mixed mating type isolates, were found. The mixed cultures originated from single lesions on individual leaves collected from different fields (Table 2). The cultivars from which A1, A2, and mixed cultures were recovered are illustrated in Figure 9. A majority of the mixed cultures were insensitive to metalaxyl. When cleared leaf samples from two fields that yielded mixed cultures (one in B.C. and the other in N.B.) were examined in the microscope, sporangia of *P. infestans* were abundant and a single oospore was observed. When the samples were considered with regard to the time of season when they were collected, the majority were recovered in July and were MI (Fig. 10A) and of mixed mating type (Fig. 9B). The proportion of A1 was higher than that of

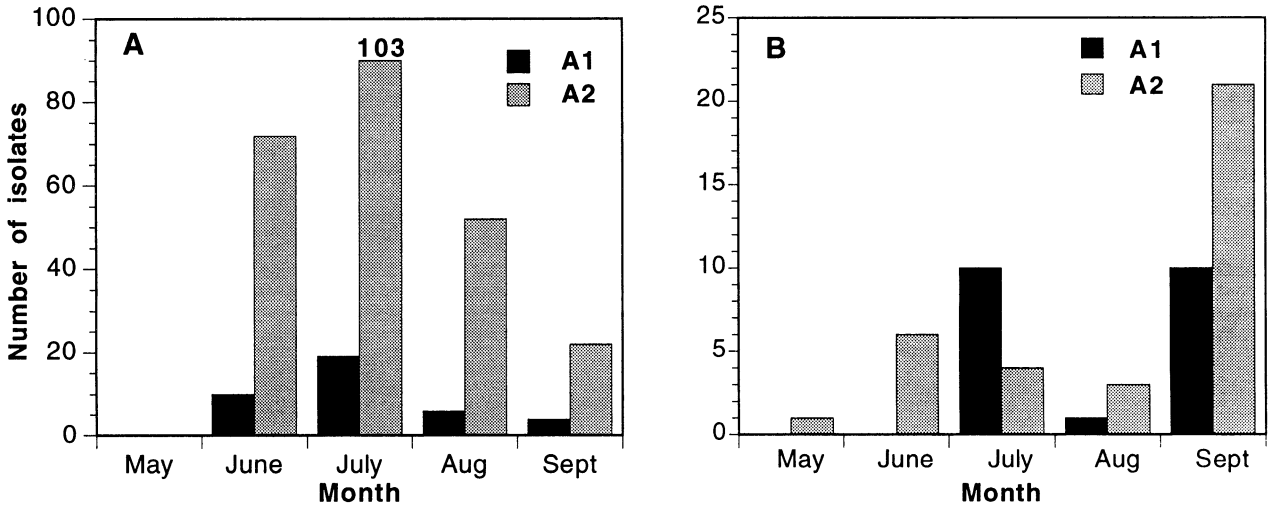

Fig. 7. Proportion of A1 and A2 mating types of *Phytophthora infestans* recovered at monthly intervals in British Columbia. **(A)** 1993 growing season. **(B)** 1994 growing season.

A2 in July (Fig. 10B). The mean colony diameter of A2 strains collected from N.B. in 1994 in the absence of metalaxyl was significantly greater than that of A1 (Fig. 8C), while the average diameter of MI and MS strains was not significantly different (Table 3).

DISCUSSION

The results from this 3-year study have provided information on the nature of populations of *P. infestans* on potatoes in Canada, similar to recent studies conducted in the United States (8,13,15,18) and elsewhere (1,10,11,23,25,29,35). The results have also illustrated the changes that are occurring in the populations from one season to the next in different regions of Canada.

The widespread presence of the A2 mating type was confirmed in B.C. in 1993 following its initial discovery in 1989 (7). Although isolates of the A2 mating type were not detected in any of the other Canadian provinces sampled in 1993, they were subsequently found in N.B. in 1994. The A2 mating type was also reported in 1994 from two additional provinces: Ontario and Quebec (26), illustrating its spread throughout Canada. The prevalence of the A2 mating type within B.C. and its appearance in other provinces within Canada was not unexpected, given the rapid migration patterns elsewhere in the world (13,33). Many potato production fields in Canada are also located in close proximity to fields in the United States where the A2 mating type was recently reported (18), and movement of seed potatoes occurs between the two countries.

The widespread occurrence of the A2 mating type in most potato production fields in B.C. in 1993 and 1994 is likely the outcome of dissemination of diseased tubers, in addition to the spread of inoculum from adjoining fields over the growing season. The Cloverdale area, from which a majority of the samples originated, represents an area of about 30 km^2, making it relatively easy for airborne sporangia to spread from one field to another (13). Although the first report of the A2 mating type was in 1989 (7), it is not known how long it has been present in B.C. nor all of the environmental conditions or cultural practices that may have contributed to its spread. Regardless of the initial source of inoculum, spread has been rapid, similar to that in other regions of the world (13,33).

Despite the predominant occurrence of isolates of the A2 mating type in B.C., a proportion of the isolates were of the A1 mating type (13% in 1993 and 34% in 1994). The large sample size (268 isolates) over a relatively small geographic area (30 km^2) would have enhanced the probability of recovery of this strain. In many regions where sampling for late blight has been conducted, the A2 mating type is reported to have displaced the A1 mating type (13,18,19,23,25,33,36). It is not known if a more intensive sampling of these fields would have revealed a small proportion of A1 isolates. In other regions, including some Canadian provinces, the A2 mating type has not yet been detected and the populations currently are still comprised of the A1 mating type (1,8). In 1995, however, the A2 mating type was rarely found, contrary to expectations of continued widespread occurrence in B.C. The reason for the high incidence of the A1 mating type in 1995 is unknown, and it could represent a new strain.

The A2 mating type was found on numerous potato cultivars grown under B.C. conditions, some of which have been reported to have moderate resistance to late blight, e.g., Atlantic and Nooksack (28,34). This observation suggests that strains with this mating type may have the ability to infect and colonize potato cultivars to a greater extent than previously reported for strains of the A1 mating type. Cultivar evaluations for resistance to late blight should take into consideration the different levels of complexity of the pathogen populations in different potato growing regions in Canada and elsewhere.

A comparison of isolates of the A1 and A2 mating types from B.C. for rate of colonization of leaf tissues and extent of

Table 3. Differences in colony diameter of *Phytophthora infestans* strains from two provinces and years according to mating type and sensitivity to metalaxyl

	British Columbia				New Brunswick	
	1993		1994		1994	
Isolate category	Diameter (cm)[a]	Isolates (no.)	Diameter (cm)	Isolates (no.)	Diameter (cm)	Isolates (no.)
A1	46.31	29	23.14	19	45.95	11
A2	43.36	161	31.03	35	61.18	14
P value (*t* test)	0.4567	...	0.0227*[b]	...	0.0269*	...
MS	38.30	70	27.06	5	59.78	8
MI	46.68	141	27.95	52	50.16	43
P value (*t* test)	0.0001*	...	0.8925	...	0.1631	...

[a] Growth was measured after 10 days of incubation at 20°C on rye agar in 1993 and on 2% V8 agar in 1994. Diameters represent the mean of three measurements from two replicate dishes. Numbers of isolates used are indicated.

[b] * = Analysis with Bonferonni's *t* test indicated that the probability of a greater value was $P < 0.05$.

sporulation did not reveal any differences, due to considerable variation within and between strains of one mating type. Since the genotypic background of these strains can differ (A1 mating type isolates were U.S. 1 and U.S. 6, while A2 mating type isolates were U.S. 7), it would be difficult to attribute any fitness characteristics to a particular mating type unless the isolates were known to be genetically similar. Deahl et al. (7) were also unable to detect any differences in virulence between isolates representing the A1 and A2 mating types. When growth rates (measured as colony diameter) were examined, however, isolates of the A2 mating type from B.C. (genotype U.S. 7) and from N.B. (genotype U.S. 8) (16) grew significantly faster than isolates of the A1 mating type (genotypes U.S. 6 in B.C. and U.S. 1 in N.B.) (15). These results suggest that genotypic differences may impart differences in growth rate. Differences in growth and aggressiveness between metalaxyl sensitive and insensitive strains have also been reported (2,9,21,22), but these traits are not linked to mating type (7,33). In this study, MI strains from the population in B.C. in 1993 grew significantly faster than MS strains.

The changes that occur in the prevalence of specific strains of the late blight pathogen within a small geographic area over one season have not been previously studied. By conducting early-, mid-, and late-season sampling of the same potato fields in B.C. during 1993 and 1994, the results revealed that the highest proportion of isolates found early in the season (June) were of the A2 mating type. This early-season occurrence may reflect an overwintering component of the pathogen under

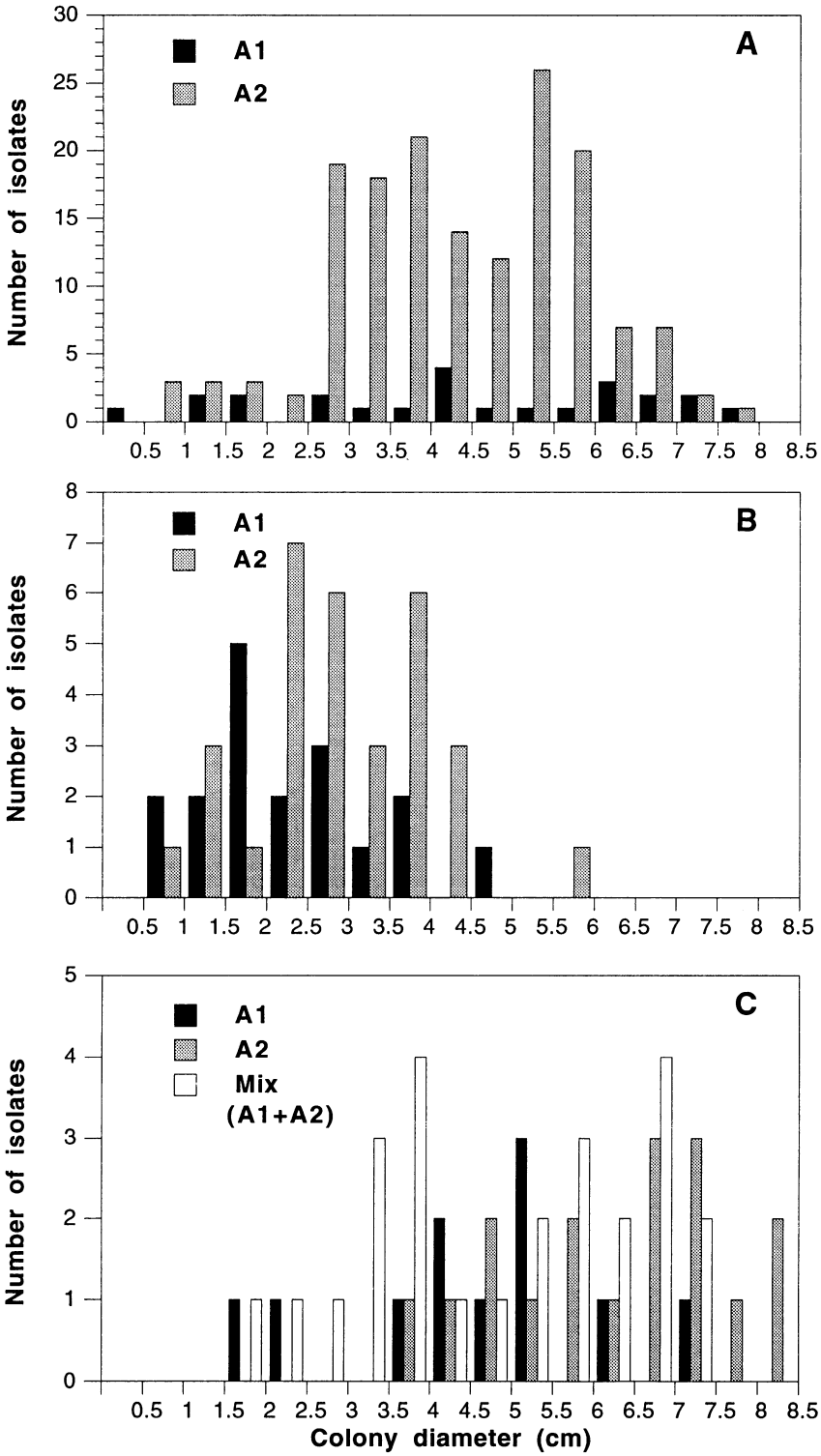

Fig. 8. Colony diameters of isolates of *Phytophthora infestans* representing A1 and A2 mating types grown on agar at 20°C for 14 days. **(A)** Isolates collected from British Columbia during 1993. **(B)** Isolates collected from British Columbia during 1994. **(C)** Isolates collected from New Brunswick during 1994.

Table 4. Number of metalaxyl-sensitive (MS) and metalaxyl-insensitive (MI) isolates of *Phytophthora infestans* recovered from fields with or without metalaxyl application in the same season[a]

	Isolates recovered	
Metalaxyl	**MS**	**MI**
Yes	10	28
No	67	138

[a] Data from 72 fields in the Fraser Valley of British Columbia during 1993.

Table 5. Frequency of recovery of metalaxyl-insensitive (MI) isolates of *Phytophthora infestans* during the growing season from fields with or without metalaxyl application in the same season[a]

	Frequency of recovery of MI isolates	
Sampling time	**With metalaxyl**	**Without metalaxyl**
June	0.80 (n = 10)	0.63 (n = 60)
July	0.63 (n = 16)	0.77 (n = 93)
August	0.78 (n = 9)	0.60 (n = 35)
September	1.00 (n = 3)	0.44 (n = 16)

[a] Data from isolates collected in British Columbia during 1993.

Table 6. Relationship between mating type and sensitivity to metalaxyl of isolates of *Phytophthora infestans*[a]

	Metalaxyl response		
Mating type	**S**	**I**	**Total**
A1	4	28	32
A2	62	123	185
Total	66	151	217

[a] Summary of data for isolates tested for both mating type and metalaxyl sensitivity in 1993 originating from British Columbia. S = sensitive; I = insensitive.

B.C. conditions or may be the result of reintroduction of the pathogen on infected seed tubers or inoculum originating from volunteers or cull piles. The ability of the A2 strain to overwinter on infected tubers was suggested in this study. The detection of a low frequency of A1 strains early in the season may suggest that overwintering of this strain is possible. In 1995, however, most of the isolates were of the A1 mating type, a result that was unexpected in view of the high proportion of A2 isolates in preceding years. It is probable that the 1995 population is a result of the reintroduction of the A1 strain.

Isolates of both the A1 and A2 mating types were recovered from the same field, and in some cases from the same plant or leaf, in both B.C. and N.B. in 1994. In many regions where late blight sampling studies have been recently conducted, it has been reported that fields predominantly contained isolates of one mating type, either the A2 (19,36) or A1 (1,8). However, both A1 and A2 mating types have been reported from the same fields in Switzerland (20), Korea and Japan (23,25), England (29,31), and Poland (35). A large proportion of the isolates originating from N.B. in 1994 were categorized as "mixed" to indicate the presence of both A1 and A2 in one field sample. Furthermore, in fields sampled sequentially over the growing season in N.B., isolates of the A2 mating type appeared to displace the A1 strain during July. Since colony transfers were made using mycelial plugs in this study and not from single sporangia, mycelium from lesions that may have contained both mating types could not be distinguished during isolation. Single field lesions have rarely been found to contain mixtures of two isolates, and self-fertile cultures from either mixtures of A1 and A2 hyphae or from a single genotype are reported only occasionally (14,25,29,31). In most of the mixed cultures, oospore formation was observed without any pairings with tester isolates; or where pairings were done, oospores formed against both A1 and A2 testers. Although *P. infestans* is reported to form oospores in self-fertile cultures (31), this phenomenon is unlikely to explain our observations. Furthermore, in most fields in which mixed colonies were detected, lesions caused by the individual A1 and A2 mating types were also present on other plants. Shattock et al. (29) also reported recovery of self-fertile isolates from potato foliage, which they believed to be intimate mixtures of A1 and A2 isolates. With continued subculture of these isolates in this study, the colonies displayed only one mating type when retested after 14 to 16 months. This suggests that one mating type may have displaced the other in a mixed colony, and in all cases, the faster growing A2 isolates appeared to have displaced the A1 mating type (C. I. Chycoski and Z. K. Punja, *unpublished*).

The occurrence of both mating types on a single leaf suggested the possibility for oospore production in vivo. When numerous leaf samples originating from fields where mixed populations were found in B.C. and N.B. during 1994 were examined, two samples (one from each province) were found to contain an oospore. This appears to represent the first report of oospore production by the late blight pathogen in naturally infected leaves in North America. Oospore production has been reported in other regions following artificial inoculation with both mating types (7,12,19,27). Although the frequency of oospore formation in diseased leaves in this study was extremely low, it suggests there is the possibility for sexual recombination and increased genetic variation in populations of this pathogen in western and eastern Canada. A report describing four unique genotypes of *P. infestans* originating from B.C. in 1991 to 1992, for example (18), implicates sexual

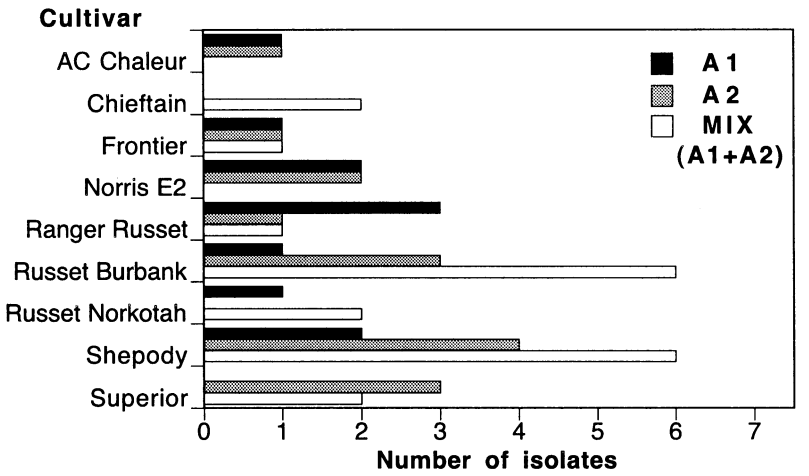

Fig. 9. Recovery of A1 and A2 mating types and mixed colonies from different potato cultivars grown in New Brunswick during 1994.

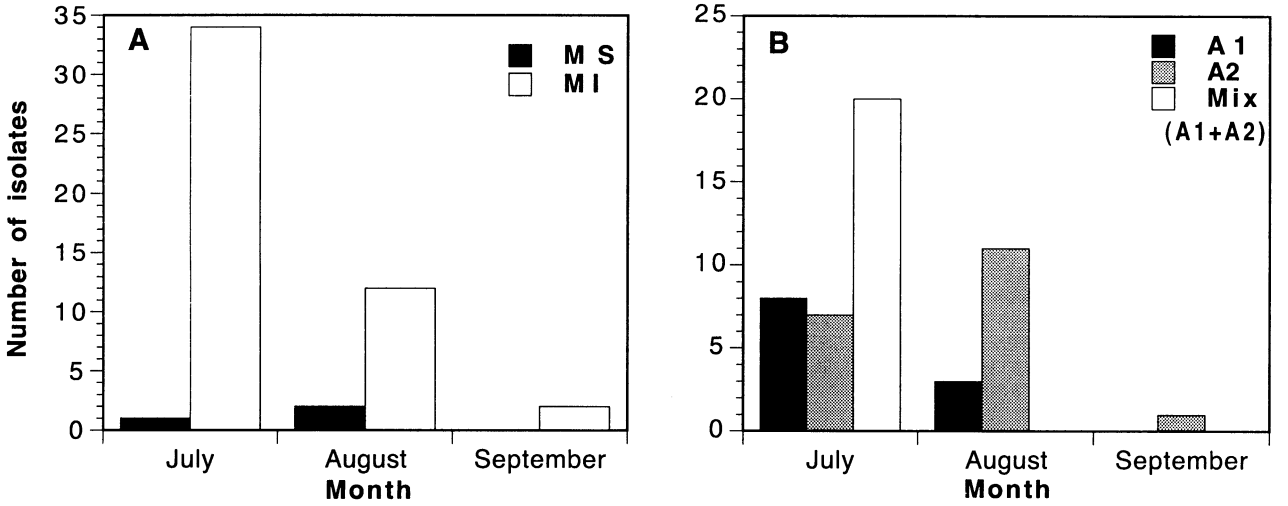

Fig. 10. **(A)** Proportion of metalaxyl-sensitive (MS) and metalaxyl-insensitive (MI) isolates of *Phytophthora infestans* recovered at monthly intervals during the 1994 growing season in New Brunswick. **(B)** Proportion of A1, A2, and mix mating types recovered at monthly intervals during the 1994 growing season in New Brunswick.

recombination as one of the possible origins of these genotypes. Therefore, it is conceivable that oospore production can (or has) given rise to an increase in unique genotypes. Additional molecular studies similar to those reported by Goodwin et al. (18) are needed to confirm the extent to which these genotypes are distributed. Increased genetic diversity in other regions of the world has been attributed to sexual recombination (11,17,24,35,36). It is not clear whether this increased genotypic variation from sexual populations will impart increased fitness and virulence to strains of *P. infestans* (37), since the genetic structure of populations appears to change dramatically from one year to the next (11,18).

The response of isolates of *P. infestans* to metalaxyl using an in vitro agar assay was found to provide data that correlated with the in vivo leaf disk assay. Matuszak et al. (24) also observed a high correlation between the agar assay and the leaf disk assay, with the former being easier to conduct and evaluate. However, the in vivo leaf disk assay provides more precise indications of metalaxyl insensitivity (32) since the agar assay may overestimate metalaxyl insensitivity. In 1993, a high proportion (83%) of isolates collected in B.C. were insensitive, while those from the rest of Canada were sensitive. In 1994, insensitive isolates were also recovered from N.B. and from several other Canadian provinces, including Ontario and Quebec (26). Metalaxyl insensitivity was associated with both mating types in B.C., but a higher association was seen with the A2 mating type. However, metalaxyl sensitive A1 and A2 strains were also recovered at a moderate frequency (19%). Studies on the distribution of metalaxyl insensitive strains in other regions have shown that populations may be almost completely insensitive (8) or comprise a mixture (1,23,24,29,36).

Strains that were MS and MI were recovered from all sampled areas in B.C., and both were found together in six fields. MI isolates appeared early in the season (June), in some cases in fields that had not been treated with the fungicide. This observation suggests that MI isolates may be introduced on infected seed tubers or that a proportion of isolates can overwinter under B.C. conditions. The proportion of MI isolates was higher throughout the season, but there was no correlation between incidence and application of metalaxyl. This apparent lack of correlation suggests that metalaxyl insensitive strains may have previously occurred in the B.C. population and that detectable shifts are not seen during one growing season. Cohen and Samoucha (4) similarly observed that with a high initial frequency of metalaxyl insensitivity in a population, isolates with insensitivity are maintained regardless of fungicide application. However, the recovery of MI strains in the absence of metalaxyl appeared to decline over the 1993 growing season. It is not known when metalaxyl insensitivity first developed in the population in B.C., since the first report was in 1993 (6); based on the high incidence of MI strains in B.C., insensitivity may have been present prior to 1993.

The frequency distribution of isolates from B.C. in relation to growth on metalaxyl at 50 µg/ml was bimodal, with separate peaks of MS and MI isolates. A similar finding was made for a population of isolates from Mexico, in a region where sexual recombination is known to contribute to increased variation (24). The population from B.C. showed a large range of variation in response to metalaxyl, although it is not known whether this is an outcome of sexual recombination. Genotypes U.S. 1, U.S. 6, and U.S. 7 are present in this region (18). The extent to which this variability may persist over successive growing seasons is not known. The growth response to metalaxyl in the population from N.B. in 1994 was more uniform, and the A2 mating type (genotype U.S. 8) and mixed populations were detected for the first time in 1994. Over time, this population may show more variation similar to the situation in B.C. and Mexico. A comparison of colony growth rates of isolates that were MS and MI showed that in 1993, MI isolates grew faster. MI isolates have been reported to display reduced sporulation and survival (9,21) or, conversely, to be better able to infect and sporulate (2,22).

It is not clear whether a decline in the use of metalaxyl would promote a decline in the occurrence of MI strains in B.C., although it is probable. Such a phenomenon has been reported in areas where only one mating type was present and the population was more uniform (10). In B.C., where the population is variable and where both mating types occur, the effect of reducing metalaxyl use on the prevalence of MI strains needs to be determined.

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