Variation in Aggressiveness of Canadian Isolates of *Phytophthora infestans* as Indicated by Their Relative Abilities to Cause Potato Tuber Rot

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ABSTRACT

Peters, R. D., Platt, H. W., Hall, R., and Medina, M. 1999. 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.

Studies were conducted in 1995, 1996, and 1997 to determine the ability of Canadian isolates of *Phytophthora infestans* to cause tuber disease in a variety of potato cultivars. Most isolates of recently introduced multilocus genotypes (US-7, US-8, g11, g26, g29, and g40) were more aggressive on tuber tissue than isolates of the traditional US-1 genotype, based on surface necrosis (SN), lesion depth (LD), and compound aggressiveness index (CAI = SN $\times$ LD) components. Other multilocus genotypes (g30, g41, g42, and UN-3) were similar in aggressiveness to US-1. The g11 (US-11) genotype consistently displayed aggressiveness that was intermediate to that of the US-8 and US-1 genotypes. Variation also occurred within a multilocus genotype, and an isolate of the US-8 genotype from New Brunswick was consistently less aggressive than other US-8 isolates. Cvs. Dorita and Island Sunshine were consistently the most resistant to infection, regardless of isolate, relative to the more susceptible responses of Green Mountain, Russet Burbank, Kennebec, Superior, Shepody, Red Pontiac, Sebago, and Bintje. Even so, isolates of the newly introduced US-8 genotype were able to cause significantly more disease on these resistant cultivars than isolates of the US-1 genotype. The predominant genotypes currently found in potato production areas in Canada (US-8 and g11) have higher fitness on tuber tissue than displaced, preexisting strains (US-1).

Additional keywords: horizontal resistance, late blight, *Solanum tuberosum*, vertical resistance

The oomycete *Phytophthora infestans* (Mont.) deBary, the cause of late blight of potato, is an important pathogen of foliar and tuber tissues of potato. The epidemiology of the foliar phase of pathogenesis is correlated to infection in the tuber phase. Tubers generally are infected by inoculum produced in aboveground plant parts that is subsequently washed down to the soil by precipitation events (22,38,66). Soil temperature (65), moisture (35,38), composition (2), competing microorganisms (34), and a variety of management practices, including host resistance (38) and haulm destruction (48), can influence incidence and severity of tuber infection. Because tubers represent the marketable portion of

the potato plant, factors that affect the severity of tuber infection are important and need to be studied.

The influx of novel genotypes of *P. infestans* into the United States and Canada has coincided with an increase in the incidence and severity of late blight (9,18). Many of the new genotypes are of the A2 mating type and insensitive to the fungicide metalaxyl (13). In addition, introduced genotypes are often more aggressive (cause a more severe disease reaction) than the older US-1 (A1) genotype. Recent studies (30,31,45,47) have shown that US-8 isolates are more aggressive on potato foliage than US-1 isolates. Although studies on the responses of tuber tissues to recently introduced genotypes have been few, Lambert and Currier (36) found that visible tuber rot developed substantially faster with most isolates of the US-6, -7, and -8 genotypes compared with isolates of the US-1 genotype. Grinberger et al. (20) noted that isolates of *P. infestans* resistant to metalaxyl produced significantly larger and deeper lesions in potato tubers than isolates sensitive to metalaxyl. In Canada, the US-8 genotype has displaced US-1 in most potato production areas outside British Columbia (53,55,57). Increased fitness of new genotypes relative to preexisting types is likely involved in the displacement phenomenon.

Resistance to infection of host tissues by *P. infestans* is commonly referred to as vertical or horizontal (6,58,73). Vertical resistance (race-specific resistance) is conferred by specific R genes that have been introduced into *Solanum tuberosum* from wild sources, such as *S. demissum*, and results in a typical hypersensitive response in an incompatible reaction (70). Vertical resistance generally is unstable, because resistance genes are quickly overcome by compatible races in the pathogen population, and too short-lived to be considered durable (72).

Horizontal resistance (partial, field, or nonrace-specific resistance) is controlled by minor genes and tends to impact the rate of disease development (rate reducing) rather than produce a disease-free plant (6). The environment also plays a significant role in expression of horizontal resistance (32,33). Horizontal resistance generally is more stable and equally active against many races of *P. infestans* (6,73) and, thus, has been the focus of most modern breeding programs (42). In Canada, the cultivars that have gained the widest acceptance based on their processing qualities have low levels of resistance to *P. infestans* (3). It is only recently that some cultivars with higher field resistance have been produced in appreciable quantities.

The objectives of our study were to compare the aggressiveness of new and old genotypes of *P. infestans* found in Canada on potato tuber tissue, from which estimations of variability within and among multilocus genotypes could be made. In addition, the responses of potato cultivars commonly grown in Canada to newly introduced genotypes could be assessed to determine the potential impact of a new pathogen population on potato production.

MATERIALS AND METHODS

Tubers. Tuber rot studies, using isolates collected from across Canada, during 1994, 1995, and 1996 were performed in three experiments (experiments 1, 2, and 3) in 1995; two experiments (experiments 4 and 5) were performed in 1996; and one experiment (experiment 6) was performed in 1997. Tubers used in the experiments were harvested from propagation plots at the Harrington Research Station, Prince Edward Island, Canada, during October of the year prior to each year's studies (i.e., tubers used in 1995 studies were harvested during October 1994,

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AAFC contribution 909.

Accepted for publication 19 March 1999.

Publication no. D-1999-0426-01R
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etc.). Tubers of all potato cultivars were stored at 4°C in a potato storage facility until used.

Preparation of inoculum. Isolates of *P. infestans* obtained from potato leaf tissue in 1994, 1995, and 1996 were cultured on clarified rye agar slants (54) and stored at 4°C. Prior to tuber inoculation, isolates were subcultured on petri dishes (100 × 15 mm) containing clarified rye extract agar (54) and allowed to grow in the dark for 2 weeks at 15°C. Agar cultures (agar, mycelium, and sporangia) were immersed in distilled water and homogenized for 2 min with a homogenizer (Polytron 110V; Brinkmann Instruments, Rexdale, ON, Canada). Inoculum was diluted with distilled water to a final concentration of 10,000 sporangia per ml in the 1995 and 1997 experiments and 20,000 sporangia per ml in the 1996 experiments, as determined with a hemacytometer (Bright-Line Improved, Neubauer, 1/10-mm deep, Spencer, Buffalo, NY).

Only in the 1995 experiments, potato leaves (cv. Green Mountain) infected with individual isolates of *P. infestans* and kept

at 15°C in a dew chamber (100% relative humidity, 16-h photoperiod) for 6 days were immersed in distilled water and swirled to dislodge sporangia. The resulting sporangial suspension obtained for each isolate was diluted to 10,000 sporangia per ml with distilled water and mixed (1:1, vol/vol) with the inoculum produced in agar culture.

In each year of study, inoculum was transferred to 4-liter plastic containers (2 liters of inoculum per container) and allowed to sit at 10°C for 1 h to encourage release of zoospores prior to use in tuber inoculations. In 1997, where possible, isolates representing a particular multilocus genotype were combined (up to three isolates per genotype). In all cases of inoculum produced on agar, isolates were subcultured only once after initial isolation from plant tissues to prevent loss of pathogenicity, which can occur with continuous subculturing (23).

All isolates were characterized according to mating type, metalaxyl sensitivity, and allozyme banding patterns (Table 1) as described previously (13,18,53,55,57). Some minor additional variation within allozyme

genotypes was determined by random amplified polymorphic DNA (RAPD) analysis (H. Förster and M. D. Coffey, University of California, Riverside) following protocols described by Punja et al. (63). The nomenclature for genotypes adopted in the current study (Table 1) is based on the systems of Goodwin et al. (18,19) and M. D. Coffey, as described by Punja et al. (63).

Inoculation of tubers. One day prior to inoculation, tubers were washed with water, using a commercial, roller-table potato washer (Haines Equipment, Avoca, NY), and allowed to air-dry for 24 h. In the 1995 experiments, each tuber was wounded at four sites (stolon attachment end, rose end, and one wound on either side of the tuber) with a steel nail (3 mm wide, 2 cm deep). In subsequent experiments, tubers were not wounded, to allow natural infection of tuber tissues. Prior to inoculation, tubers were immersed in sterile distilled water for 2 min.

Tubers were inoculated by dipping in a 4-liter plastic bucket containing 2 liters of inoculum (various isolates per treatments) and transferred to labeled paper bags (no. 10 hardware) for storage. Tubers of each cultivar also were dipped in distilled water as a control. Paper bags with tubers (three tubers per bag in 1995; one tuber per bag in 1996 and 1997) were placed in plastic crates (21 × 36 × 55 cm) or on shelves in the storage facility (1997) in a randomized complete block design (five replications; each crate or shelf represented a block in the design) and covered with soaked burlap bags (55 × 100 cm) to maintain humidity. In 1995, tubers were stored at 15°C for 2 weeks followed by storage at 5°C for 2 weeks; in 1996 and 1997, tubers were stored at 10°C for 3 weeks in a potato storage facility. Relative humidity (100%) was maintained by daily watering of burlap bags surrounding tubers. Tubers were inoculated on 17 January 1995 (experiments 1 and 2), 31 March 1995 (experiment 3), 18 January 1996 (experiment 4), 14 May 1996 (experiment 5), and 15 January 1997 (experiment 6).

Experiment 1 compared the response of seven potato cultivars (Superior, Shepody, Green Mountain, Kennebec, Island Sunshine, Red Pontiac, and Russet Burbank) to inoculation with three isolates (P4A [US-1], P73A-9 [g29], and P76A-16 [US-8]) of *P. infestans*. Experiment 2 compared the response of three potato cultivars (Green Mountain, Kennebec, and Russet Burbank) to inoculation with four isolates (P4A [US-1], P54A-1 [US-8], P73A-9 [g29], and P76A-16 [US-8]) of *P. infestans* and three isolate combinations. Experiment 3 compared the response of three potato cultivars (Green Mountain, Kennebec, and Russet Burbank) to inoculation with four isolates (P4A [US-1], P54A-1 [US-8], P73A-9 [g29], and P76A-16 [US-8]) of *P. infestans*. Experiments 4 and 5 were identical in design and compared the response of seven potato cultivars (Shepody, Green Mountain, Island Sunshine, Russet Burbank, Dorita, Sebago,

Table 1. Canadian isolates of *Phytophthora infestans* collected in 1994, 1995, and 1996 used for tuber rot studies

CLRC Isolate ^a	Canadian province	Year collected	Mating type	Metalaxyl sensitivity ^y	Allozyme (<i>Gpi</i>) banding pattern ^w	Genotype ^x
P3B	PQ	1994	A1	MS	86/100/100	US-1
P4A	PE	1994	A1	MS	86/100/100	US-1
P8A-1	NB	1994	A2	MI	100/111/122	g42
P11A-2	NB	1994	A2	MI	100/111/122	g42
P16A-8	NB	1994	A2	MI	100/111/122	g40
P30A-1	NB	1994	A2	MI	100/111/122	g41
P46A-1	ON	1994	A1	MS	86/100/100	g30
P54A-1	PQ	1994	A2	MI	100/111/122	US-8
P73A-9	ON	1994	A2	MI	100/111/122	g29
P76A-16	NB	1994	A2	MI	100/111/122	US-8
P119A-3	AB	1994	A1	MS	86/100/100	US-1
P200A-7	MB	1994	A2	MI	100/111/122	g29
P200A-9	MB	1994	A1	MS	86/100/100	g30
P207A	AB	1994	A2	MI	100/111	US-7
P229A	AB	1994	A2	MI	100/111	US-7
P230A	AB	1994	A2	MI	100/111	US-7
P259A-5	NS	1995	A2	MI	100/111/122	US-8
P272C-4	PE	1995	A2	MI	100/111/122	US-8
P419A-7	BC	1995	A1	MI	100/100/111	g11 ^y
P451B-17	MB	1995	A2	MI	100/111/122	US-8
P455A-36	SK	1995	A2	MI	100/111/122	US-8
P539A	PQ	1995	A2	MI	100/122	g26
P901B-17	ON	1996	A1	MI	100/100/111	g11 ^y
P904A-12	BC	1996	A2	MS	111/111	UN-3 ^z
P904A-15	BC	1996	A2	MS	111/111	UN-3 ^z
P904A-16	BC	1996	A2	MS	111/111	UN-3 ^z
P907A-2	BC	1996	A1	MI	100/100/111	g11 ^y

^a Designation in the collection of the Crops and Livestock Research Centre (CLRC), Charlottetown, PEI, Canada.

^y Metalaxyl sensitivity rating reflects the comparison of diameter of growth of isolates at 0 and 100 µg of metalaxyl per ml. Metalaxyl sensitive (MS) = <10% growth of control; metalaxyl insensitive (MI) = >10% growth of control.

^w Numbers indicate distance of migration of bands staining for *glucose-6-phosphate isomerase* (*Gpi*) allozymes.

^x Designation of US-1, -6, -7, and -8 multilocus allozyme genotypes according to the method described by Goodwin et al. (18) and g11, g26, g29, g30, g40, g41, and g42 according to the method of M. D. Coffey, University of California, Riverside, as described by Punja et al. (63). Genotypes g29, g40, g41, and g42 are rare variants of the US-8 multilocus allozyme genotype, and g30 is a rare variant of the US-1 multilocus allozyme genotype based on random amplified polymorphic DNA analysis (M. D. Coffey).

^y Also known as US-11, according to Goodwin et al. (19).

^z Genotypes with no designation to date.

and Bintje) to inoculation with nine isolates (P4A [US-1], P54A-1 [US-8], P73A-9 [g29], P76A-16 [US-8], P259A-5 [US-8], P272C-4 [US-8], P419A-7 [g11], P451B-17 [US-8], and P455A-36 [US-8]) of *P. infestans*. Experiment 6 compared the response of five potato cultivars (Green Mountain, Island Sunshine, Dorita, Sebago, and Bintje) to inoculation with 23 isolates (P4A, P8A-1, P11A-2, P16A-8, P30A-1, P46A-1, P73A-9, P76A-16, P119A-3, P200A-7, P200A-9, P207A, P229A, P230A, P259A-5, P272C-4, P419A-7, P539A, P901B-17, P904A-12, P904A-15, P904A-16, and P907A-2) representing 11 multilocus genotypes (US-1, US-7, US-8, g11, g26, g29, g30, g40, g41, g42, and UN-3).

Tuber ratings. After 3 to 4 weeks in storage, tubers were rated for disease by estimating the percentage of visible necrosis on the tuber surface. In addition, tubers were cut in two (along the longitudinal axis), and the depth of penetrating lesions (visible necrosis) was measured with calipers (Vernier, dial-type, Bel-Art Products, Pequannock, NJ) at each of the four wound sites (1995 experiments) or at the rose end, stolon attachment end, and one side of the tuber (1996 and 1997 experiments).

Statistical analysis. Values for lesion depth (depth of pathogen penetration) at the rose, side, and stolon end of each tuber were combined and averaged to obtain a measurement of mean lesion depth (LD). The compound aggressiveness index (CAI) was calculated by multiplying surface necrosis (SN) by LD for each tuber. Analysis of randomized complete block design experiments was performed (release 6.12, SAS Institute, Inc., Cary, NC), using the GLM procedure, and when a significant treatment effect was found, the least significant difference

(LSD, $P = 0.05$) test was used to separate means.

Although significant cultivar-isolate interactions occurred, their contribution to total variation was very low compared with variation among cultivars and isolates, and therefore, main effects also were compared (in addition to simple effects). Correlations between SN and LD variables were performed, using the CORR procedure to determine Pearson's correlation coefficients. Because no disease developed in uninoculated tubers, control treatments were excluded from the analyses.

RESULTS

Incubation of inoculated tubers at 10°C resulted in significant infection and necrosis within 3 to 4 weeks. However, uninoculated controls remained free of disease. Significant differences ($P \leq 0.0001$ to 0.002 depending on variable) were found among cultivars, isolates, and cultivar-isolate interactions for all variables (SN, LD, and CAI) in experiments 1, 4, 5, and 6. In experiments 2 and 3 (1995), significant differences ($P \leq 0.0001$ to 0.05 depending on variable) also were found among cultivars, isolates, and cultivar-isolate interactions for all variables (SN, LD, and CAI), with the exception of cultivar response to SN and cultivar-isolate interactions for LD in experiment 2 and cultivar response to LD and CAI in experiment 3. Correlations (r) comparing SN and LD were strong and highly significant ($P \leq 0.0001$ to 0.05 depending on variable) in all experiments (with the exception of the response of cv. Shepody in experiment 1; isolate P4A [US-1] in experiment 2; and cv. Bintje in experiment 5). An increase in the percentage of tuber sur-

face covered by necrotic lesions tended to coincide with an increase in pathogen penetration into the interior of the tuber, which led to calculation of CAI by multiplying correlated components of SN and LD, which estimated fitness of isolates and host genotypes with respect to tuber tissue.

Experiments, 1995. Significant differences among isolates and cultivars were found in experiments 1 and 2 (Tables 2 and 3, respectively). All isolates produced significantly ($P = 0.05$) more disease than a water control treatment. Isolates P73A-9 (genotype g29 from Ontario is a variant of the US-8 multilocus allozyme genotype based on RAPD analysis) and P4A (US-1 from Prince Edward Island) were significantly more aggressive on tuber tissue than isolate P76A-16 (an isolate of the US-8 genotype from New Brunswick; Tables 2 and 3). Isolate P54A-1 (US-8 from Quebec) was similar in aggressiveness to P73A-9 and P4A in experiment 2 (Table 3). In addition, treatments composed of A1 and A2 combinations tended to produce less disease than single isolate inoculations (Table 3). Potato cvs. Kennebec, Red Pontiac, Shepody, Green Mountain, and Russet Burbank were the most severely affected, whereas cvs. Superior and Island Sunshine were the least affected in experiment 1. Tubers of cvs. Kennebec and Green Mountain were more severely diseased than Russet Burbank tubers, although all cultivars showed significant levels of disease in experiment 2. Significant ($P = 0.05$) cultivar-isolate interactions occurred in both experiments.

Experiment 3 represented the final examination in 1995 of the response of three potato cultivars to tuber infection by four Canadian isolates of *P. infestans* (Table 4).

Table 2. Response of seven commercial potato cultivars to infection by A1 and A2 isolates of *Phytophthora infestans* collected in 1994 (experiment 1, 1995)

Isolate (C/G/P) ^w	Cultivar							Mean ^x
	Russet Burbank	Kennebec	Green Mountain	Superior	Shepody	Island Sunshine	Red Pontiac	
Surface necrosis (%) ^y								
P73A-9/g29/ON	40.3	67.3	50.1	39.3	66.0	22.3	77.3	51.8 a
P76A-16/US-8/NB	26.0	50.3	42.3	16.7	15.3	12.7	69.0	33.2 b
P4A/US-1/PE	76.0	41.3	45.0	51.3	46.7	6.3	58.7	46.5 a
Mean ^z	47.4 bc	53.0 b	45.8 bc	35.8 d	42.7 cd	13.8 e	68.3 a	
Lesion depth (mm) ^y								
P73A-9/g29/ON	7.1	13.2	12.1	5.8	11.5	7.5	8.1	9.3 b
P76A-16/US-8/NB	6.0	15.1	7.6	4.9	10.4	2.2	8.0	7.8 c
P4A/US-1/PE	13.4	13.6	13.3	10.2	18.3	3.6	13.2	12.2 a
Mean ^z	8.8 bc	14.0 a	11.0 b	7.0 c	13.4 a	4.4 d	9.8 b	
Compound aggressiveness index (CAI) ^y								
P73A-9/g29/ON	318.6	909.4	805.0	320.6	769.2	177.3	647.6	564.0 a
P76A-16/US-8/NB	206.3	856.2	400.5	115.1	223.4	61.0	639.8	357.5 b
P4A/US-1/PE	1,036.4	571.4	614.3	650.0	845.2	37.5	815.2	652.9 a
Mean ^z	520.4 cd	779.0 a	606.6 bc	361.9 d	612.6 bc	91.9 e	700.8 ab	

^w CLRC isolate number/genotype/Canadian province.

^x Means followed by the same letter are not significantly ($P = 0.05$) different according to the least significant difference test (LSD = 5.7 [surface necrosis], 1.4 [lesion depth], 108.2 [CAI]).

^y Observations are based on a mean of five repetitions (three observations per repetition). Values are significantly ($P = 0.05$) different from other values if they differ by LSD = 15.0 (surface necrosis), 3.8 (lesion depth), or 286.4 (CAI). Tubers in uninoculated controls did not develop disease symptoms. CAI = surface necrosis × lesion depth.

^z Means followed by the same letter are not significantly ($P = 0.05$) different according to the LSD test (LSD = 8.7 [surface necrosis], 2.2 [lesion depth], 165.3 [CAI]).

As before, P76A-16 (US-8 from New Brunswick) was less aggressive on tuber tissue than P73A-9 (g29 from Ontario) and P4A (US-1 from Prince Edward Island). However, P54A-1 (US-8 from Quebec) was similar to P76A-16 in aggressiveness. Again, cvs. Green Mountain and Kennebec were more severely diseased than Russet Burbank, although the distinction was less noticeable for LD and CAI variables.

Experiments, 1996. In 1996, two experiments identical in design were conducted during 2 months (experiments 4 and 5). Both experiments examined the interaction of nine isolates of *P. infestans* from across Canada with tubers of seven potato cultivars commonly grown in Canada. Analysis of variance for the combined data sets of both experiments revealed significant ($P \leq 0.0001$) differences between the data sets. In addition, month-cultivar, month-isolate, and month-cultivar-isolate interactions also were significant ($P \leq 0.0001$ to 0.0389 depending on variable). Therefore, the data sets were analyzed separately.

Across cultivars, the most aggressive isolates were P73A-9, P54A-1, P259A-5, and P451B-17, representing g29 from Ontario and US-8 from Quebec, Nova Scotia, and Manitoba, respectively. The least aggressive isolates were P4A and P76A-16, representing US-1 from Prince Edward Island and US-8 from New Brunswick, respectively (Tables 5 and 6). Isolates P272C-4 (US-8 from Prince Edward Island), P455A-36 (US-8 from Saskatchewan), and P419A-7 (g11 or US-11 from British Columbia) also were highly aggressive in experiment 5 (Table 6) but showed a more intermediate response in experiment 4 (Table 5). Across isolates,

tubers of cvs. Shepody, Green Mountain, and Russet Burbank were the most severely affected in experiment 5 (Table 6); tubers of cvs. Sebago and Bintje also were severely affected in experiment 4 (Table 5). Tubers of cvs. Island Sunshine and Dorita were the most resistant to surface colonization and penetration into medullary tissues.

Differences were apparent between specific components of aggressiveness among culti-

vars. For example, Green Mountain had more severe surface necrosis than the other cultivars, whereas Sebago had significantly ($P = 0.05$) higher lesion depth across isolates than the other cultivars in experiment 4 (Table 5). Significant ($P = 0.05$) cultivar-isolate interactions occurred. For example, in experiment 4, P73A-9 (g29 from Ontario) produced significantly more surface necrosis on Island Sunshine tubers than on most

Table 4. Response of three commercial potato cultivars to infection by A1 and A2 isolates of *Phytophthora infestans* collected in 1994 (experiment 3, 1995)

Cultivar	Isolates of <i>P. infestans</i> (C/G/P) ^w				Mean ^x
	P73A-9/ g29/ON	P54A-1/ US-8/PQ	P76A-16/ US-8/NB	P4A/ US-1/PE	
Surface necrosis (%) ^y					
Russet Burbank	31.0	27.7	21.3	45.0	31.3 b
Kennebec	51.7	28.0	35.3	48.7	40.9 a
Green Mountain	49.3	31.3	44.0	40.7	41.4 a
Mean ^z	44.0 a	29.0 b	33.6 b	44.9 a	
Lesion depth (mm) ^y					
Russet Burbank	10.1	12.4	11.4	20.2	13.5 a
Kennebec	16.4	14.1	11.7	14.3	14.1 a
Green Mountain	16.7	11.1	14.3	15.6	14.4 a
Mean ^z	14.4 ab	12.5 b	12.4 b	16.8 a	
Compound aggressiveness index (CAI) ^y					
Russet Burbank	314.2	487.7	290.5	966.2	514.6 b
Kennebec	859.4	465.1	546.7	706.5	644.4 ab
Green Mountain	849.8	415.7	764.3	655.5	671.6 a
Mean ^z	674.4 ab	456.2 c	533.8 bc	778.8 a	

^w CLRC isolate number/genotype/Canadian province.

^x Means followed by the same letter are not significantly ($P = 0.05$) different according to the least significant difference test (LSD = 6.7 [surface necrosis], 2.3 [lesion depth], 155.4 [CAI]).

^y Observations are based on a mean of five repetitions (three observations per repetition). Values are significantly ($P = 0.05$) different from other values if they differ by LSD = 13.5 (surface necrosis), 4.5 (lesion depth), or 310.8 (CAI). Tubers in uninoculated controls did not develop disease symptoms. CAI = surface necrosis $\times$ lesion depth.

^z Means followed by the same letter are not significantly ($P = 0.05$) different according to the LSD test (LSD = 7.8 [surface necrosis], 2.6 [lesion depth], 179.4 [CAI]).

Table 3. Response of three commercial potato cultivars to infection by A1, A2, and combinations of A1/A2 isolates of *Phytophthora infestans* collected in 1994 (experiment 2, 1995)

Cultivar	Isolate of <i>P. infestans</i> (C/G/P) ^w							Mean ^x
	P73A-9/ g29/ON	P54A-1/ US-8/PQ	P76A-16/ US-8/NB	P4A/ US-1/PE	P4A + P73A-9	P4A + P54A-1	P4A + P76A-16	
Surface necrosis (%) ^y								
Russet Burbank	40.3	34.7	26.0	76.0	35.1	35.7	8.7	36.6 b
Kennebec	67.3	54.3	50.3	41.3	36.7	45.7	8.5	43.4 a
Green Mountain	50.1	45.0	42.3	45.0	27.5	45.0	21.5	39.5 ab
Mean ^z	52.6 a	44.7 ab	39.6 bc	54.1 a	33.1 c	42.1 bc	12.9 d	
Lesion depth (mm) ^y								
Russet Burbank	7.1	12.0	6.0	13.4	7.7	8.1	2.7	8.1 b
Kennebec	13.2	15.2	15.1	13.6	8.4	11.2	2.3	11.3 a
Green Mountain	12.1	10.6	7.6	13.3	7.7	10.6	4.3	9.5 b
Mean ^z	10.8 bc	12.6 ab	9.6 cd	13.4 a	8.0 d	9.9 cd	3.1 e	
Compound aggressiveness index (CAI) ^y								
Russet Burbank	318.6	443.3	206.3	1,036.4	443.2	301.7	65.6	402.2 b
Kennebec	909.4	914.3	856.2	571.4	512.0	615.6	106.3	640.7 a
Green Mountain	805.0	635.7	400.5	614.3	467.8	565.4	232.0	531.5 a
Mean ^z	677.7 ab	664.4 a-c	487.7 cd	740.7 a	474.3 d	494.2 b-d	134.6 e	

^w CLRC isolate number/genotype/Canadian province.

^x Means followed by the same letter are not significantly ($P = 0.05$) different according to the least significant difference test (LSD = 6.4 [surface necrosis], 1.7 [lesion depth], 121.1 [CAI]).

^y Observations are based on a mean of five repetitions (three observations per repetition). Values are significantly ($P = 0.05$) different from other values if they differ by LSD = 17.0 (surface necrosis), 4.5 (lesion depth), or 320.3 (CAI). Tubers in uninoculated controls did not develop disease symptoms. CAI = surface necrosis $\times$ lesion depth.

^z Means followed by the same letter are not significantly ($P = 0.05$) different according to the LSD test (LSD = 9.8 [surface necrosis], 2.6 [lesion depth], 184.9 [CAI]).

of the other isolates. Similarly, P259A-5 (US-8 from Nova Scotia) caused significantly higher surface necrosis on tubers of Dorita than many of the other isolates (Table 5). Of particular note in experiment 5 (Table 6), cv. Shepody produced severe internal necrosis in response to many isolates, and cv. Russet Burbank had significant surface necrosis yet relatively lower lesion penetration.

Significant month-cultivar ($P \leq 0.0001$) and month-isolate ($P \leq 0.01$ to 0.04 depending on variable) interactions were apparent when the data were examined. Cvs. Shepody, Green Mountain, and Island Sunshine experienced relatively more severe disease in experiment 5 than in experiment 4, whereas cvs. Dorita, Sebago, and Bintje experienced relatively less disease. Tubers of cv. Russet Burbank seemed to respond similarly in both experiments. Although similar isolates were ranked as the most and least aggressive in both experiments, the difference between isolates responding intermediately and those responding very aggressively was smaller in experiment 5 than in

experiment 4. In addition, isolate P451B-17 (US-8 from Manitoba) responded more aggressively relative to the other isolates in experiment 5 than it did in experiment 4. Although variability (including variability in month-cultivar-isolate interactions) existed between the two experiments, overall clustering or ranking of isolates and cultivars was similar.

Experiments, 1997. In experiment 6, the interaction of 11 multilocus genotypes (composed of more than one isolate when possible) with five potato cultivars was examined (Table 7). Across cultivars, the US-7 and -8 genotypes were the most aggressive in terms of colonization and penetration of tuber tissue. Genotypes US-1, g30, g41, g42, and UN-3 (*Gpi III/III*) were the least aggressive. The other genotypes (g11, g26, g29, and g40) were intermediate in aggressiveness based on surface necrosis and lesion depth. Across isolates, cv. Sebago was the most susceptible to infection, followed by Bintje and Green Mountain. Island Sunshine and Dorita were the most resistant to sur-

face necrosis and pathogen penetration. Cultivar-genotype interactions were significant (Table 7), but as in the other experiments, their contribution to total variation was very low.

DISCUSSION

Canadian populations of *P. infestans* changed rapidly during the 1990s. Pathogen populations formerly were composed of a clonal population of the US-1 (A1) genotype (17). By 1996, the US-8 (A2) genotype of *P. infestans* predominated outside British Columbia (53,55,57). In British Columbia, the A1, g11 (US-11) multilocus allozyme genotype of *P. infestans* was the most frequently recovered genotype in 1995 and 1996 (53,55,57). These dramatic population shifts have been accompanied by increased disease management problems due to the increased aggressiveness and metalaxyl insensitivity of many of the new genotypes.

Results from three successive years of study on infection of tubers by Canadian isolates of *P. infestans* revealed significant

Table 5. Response of seven commercial potato cultivars to infection by A1 and A2 isolates of *Phytophthora infestans* collected in 1994 and 1995 (experiment 4, 1996)

Isolate (C/G/P) ^w	Cultivar							Mean ^x
	Shepody	Green Mountain	Island Sunshine	Russet Burbank	Dorita	Sebago	Bintje	
Surface necrosis (%) ^y								
P73A-9/g29/ON	68.0	53.0	26.0	60.0	2.2	56.0	34.0	42.7 a
P54A-1/US-8/PQ	61.0	55.0	5.4	62.0	0.4	55.0	43.0	40.3 a
P76A-16/US-8/NB	0.0	5.0	0.0	0.0	0.0	0.0	1.0	0.9 e
P4A/US-1/PE	8.0	37.0	0.0	14.0	0.0	10.0	11.4	11.5 d
P451B-17/US-8/MB	21.6	57.0	5.6	39.0	8.4	45.0	46.0	31.8 b
P272C-4/US-8/PE	37.0	40.0	6.4	45.0	6.0	43.0	24.0	28.8 b
P455A-36/US-8/SK	23.0	60.0	13.0	48.0	6.0	43.0	36.0	32.7 b
P419A-7/g11/BC	33.0	35.0	5.0	32.0	0.0	28.0	17.4	21.5 c
P259A-5/US-8/NS	27.0	76.0	14.6	57.0	19.0	39.0	60.0	41.8 a
Mean ^z	31.0 c	46.4 a	8.4 d	39.7 b	4.7 d	35.4 bc	30.3 c	
Lesion depth (mm) ^y								
P73A-9/g29/ON	13.7	7.4	3.4	10.1	1.5	14.9	7.5	8.3 b
P54A-1/US-8/PQ	22.4	7.4	2.2	13.9	0.7	15.1	12.2	10.6 a
P76A-16/US-8/NB	0.0	1.9	0.0	0.0	0.0	0.0	1.1	0.4 f
P4A/US-1/PE	2.1	6.1	0.0	5.2	0.0	3.2	2.5	2.7 e
P451B-17/US-8/MB	7.2	10.2	2.6	7.4	4.0	15.6	12.4	8.5 b
P272C-4/US-8/PE	4.4	5.9	1.5	4.5	1.8	11.9	7.0	5.3 d
P455A-36/US-8/SK	6.2	6.3	2.9	10.9	4.0	12.4	9.4	7.4 bc
P419A-7/g11/BC	10.5	12.3	2.4	4.8	0.0	7.4	5.2	6.1 cd
P259A-5/US-8/NS	7.3	8.3	3.9	10.0	2.4	11.5	14.4	8.2 b
Mean ^z	8.2 b	7.3 b	2.1 c	7.4 b	1.6 c	10.2 a	8.0 b	
Compound aggressiveness index (CAI) ^y								
P73A-9/g29/ON	1,005.1	413.0	95.3	601.8	13.2	785.8	290.7	457.8 b
P54A-1/US-8/PQ	1,384.9	510.8	17.5	880.0	0.7	845.4	530.8	595.7 a
P76A-16/US-8/NB	0.0	47.5	0.0	0.0	0.0	0.0	5.5	7.8 f
P4A/US-1/PE	24.8	239.5	0.0	103.2	0.0	65.2	61.4	70.6 ef
P451B-17/US-8/MB	281.4	598.8	17.5	313.0	52.6	715.2	554.6	361.9 bc
P272C-4/US-8/PE	174.2	233.9	17.5	241.2	26.3	562.0	199.5	207.8 d
P455A-36/US-8/SK	166.8	414.1	43.3	496.3	31.5	558.8	429.6	305.8 cd
P419A-7/g11/BC	371.3	440.4	21.7	184.1	0.0	250.6	109.3	196.8 de
P259A-5/US-8/NS	272.4	641.4	60.5	624.0	60.2	491.8	890.9	434.5 b
Mean ^z	409.0 ab	393.3 ab	30.4 c	382.6 ab	20.5 c	475.0 a	341.4 b	

* CLRC isolate number/genotype/Canadian province.

^x Means followed by the same letter are not significantly ($P = 0.05$) different according to the least significant difference test (LSD = 6.6 [surface necrosis], 2.0 [lesion depth], 128.6 [CAI]).

^y Observations are based on a mean of five repetitions (one observation per repetition). Values are significantly ($P = 0.05$) different from other values if they differ by LSD = 17.4 (surface necrosis), 5.3 (lesion depth), or 340.3 (CAI). Tubers in uninoculated controls did not develop disease symptoms. CAI = surface necrosis $\times$ lesion depth.

^z Means followed by the same letter are not significantly ($P = 0.05$) different according to the LSD test (LSD = 5.8 [surface necrosis], 1.8 [lesion depth], 113.4 [CAI]).

differences in cultivar response and aggressiveness of pathogen isolates. In general, isolates of new multilocus genotypes were more aggressive than isolates of the traditional US-1 (A1) genotype based on surface necrosis of tuber tissue and penetration of the pathogen into the tuber interior. Isolates of the US-7 and -8 genotypes were significantly more aggressive than other multilocus genotypes (across cultivars) in a study conducted in 1997. In addition, isolates of the g11 (US-11), g26, g29, and g40 genotypes were more aggressive on tuber tissue than US-1 isolates in the same study, although they were not as aggressive as US-7 and -8 isolates. The majority of US-8 genotype isolates tested in 1996 were significantly more aggressive on tuber tissue than a US-1 genotype isolate. Similarly, g29 and g11 isolates, which displayed intermediate aggressiveness, were more aggressive than US-1 isolates. Calculations of the CAI revealed the enhanced fitness of many of the new genotypes compared with the old genotype with respect to tuber colonization.

The difference in aggressiveness between old and new genotypes was not revealed in tuber rot studies conducted in 1995 (Tables 2 through 4). The US-1 isolate was consistently rated as aggressive or more aggressive than other isolates in the experiments. However, tubers tested in 1995 were wounded prior to inoculation to simulate previous studies (14,36,37,39,71,76), whereas studies in 1996 and 1997 relied on inoculation of intact tubers. In studies involving inoculation of tubers, wounding bypasses natural resistance mechanisms found in the epidermal, outer cortical, and vascular regions of the tuber, allowing pathogen colonization to occur within inner storage tissues (12,14,29,39,43,51). Toxopeus (71) noted that although mycelia develop best in susceptible tubers, once the tuber is penetrated (wounded) the pathogen flourishes in many highly field-resistant cultivars. Therefore, it is likely that wounding tubers in the 1995 experiments bypassed natural resistance mechanisms in the tubers and obscured real differences in aggressiveness between new

and old genotypes that were revealed by subsequent experiments.

Not all recently introduced genotypes of *P. infestans* were highly aggressive on tuber tissue. In the 1997 tuber rot study, multilocus genotypes g30, g41, g42, and UN-3 (*Gpi III/III*) were no more aggressive than the US-1 genotype. In addition, genotypes g11 (US-11), g26, g29, and g40 displayed aggressiveness that was intermediate between US-1 and -8; g11 also showed an intermediate response in 1996 experiments, and g29 tended to be more aggressive in 1996. It is interesting that the majority of the multilocus genotypes (with the exception of g11 and UN-3) were minor genotypes that were variants (based on RAPD analysis [H. Förster and M. D. Coffey]) of US-1 and -8 populations isolated in Canada in 1994 (53). These genotypes were never recovered in the subsequent sampling seasons of 1995 and 1996 (53). It is possible that their reduced fitness relative to the US-8 genotype was at least partially responsible for their demise and the pre-

Table 6. Response of seven commercial potato cultivars to infection by A1 and A2 isolates of *Phytophthora infestans* collected in 1994 and 1995 (experiment 5, 1996)

Isolate(C/G/P) ^w	Cultivar							Mean ^x
	Shepody	Green Mountain	Island Sunshine	Russet Burbank	Dorita	Sebago	Bintje	
Surface necrosis (%) ^y								
P73A-9/g29/ON	76.0	70.0	22.0	73.0	0.4	33.0	17.0	41.6 a–c
P54A-1/US-8/PQ	65.0	69.0	37.0	76.0	0.2	44.0	15.4	43.8 ab
P76A-16/US-8/NB	12.0	9.0	2.0	4.0	0.0	1.0	4.0	4.6 e
P4A/US-1/PE	29.0	23.0	0.0	12.0	0.0	0.2	8.0	10.3 e
P451B-17/US-8/MB	67.0	72.0	38.0	73.0	2.0	51.0	14.0	45.3 a
P272C-4/US-8/PE	51.0	80.0	7.6	62.0	1.2	35.0	13.0	35.7 cd
P455A-36/US-8/SK	62.0	84.0	19.0	60.0	5.0	13.0	21.0	37.7 bc
P419A-7/g11/BC	46.0	56.0	6.0	42.0	0.0	41.0	26.0	31.0 d
P259A-5/US-8/NS	71.0	79.0	28.0	81.0	3.0	38.0	31.0	47.3 a
Mean ^z	53.2 b	60.2 a	17.7 d	53.7 b	1.3 e	28.5 c	16.6 d	
Lesion depth (mm) ^y								
P73A-9/g29/ON	20.1	7.1	3.1	7.1	0.1	13.1	4.2	7.8 ab
P54A-1/US-8/PQ	25.8	12.2	3.3	6.9	0.0	12.0	6.7	9.6 a
P76A-16/US-8/NB	6.2	4.1	1.2	0.9	0.0	1.7	1.7	2.2 c
P4A/US-1/PE	11.4	5.9	0.0	3.3	0.0	1.3	6.4	4.0 c
P451B-17/US-8/MB	25.5	9.6	6.1	5.1	1.4	12.5	6.2	9.5 a
P272C-4/US-8/PE	21.8	10.2	2.9	7.0	0.6	12.2	10.9	9.4 a
P455A-36/US-8/SK	19.5	7.7	2.8	5.9	0.4	5.2	5.6	6.7 b
P419A-7/g11/BC	19.6	11.3	1.0	11.1	0.0	10.2	6.4	8.5 ab
P259A-5/US-8/NS	18.8	9.9	2.0	8.0	0.3	8.9	7.0	7.9 ab
Mean ^z	18.7 a	8.7 b	2.5 d	6.2 c	0.3 e	8.6 b	6.1 c	
Compound aggressiveness index (CAI) ^y								
P73A-9/g29/ON	1,528.3	479.9	64.4	514.1	0.3	463.3	81.8	447.4 ab
P54A-1/US-8/PQ	1,644.1	874.0	120.7	509.1	0.0	500.0	105.8	536.2 a
P76A-16/US-8/NB	136.5	68.5	5.9	17.3	0.0	8.4	33.3	38.6 c
P4A/US-1/PE	326.6	146.2	0.0	55.4	0.0	1.3	63.8	84.8 c
P451B-17/US-8/MB	1,713.8	713.0	214.3	376.5	7.2	636.9	105.1	538.1 a
P272C-4/US-8/PE	1,133.9	827.8	27.7	548.8	1.6	384.7	136.7	437.3 ab
P455A-36/US-8/SK	1,273.5	642.6	72.8	502.7	6.0	113.6	124.4	390.8 b
P419A-7/g11/BC	956.7	648.0	13.0	504.8	0.0	454.3	146.9	389.1 b
P259A-5/US-8/NS	1,382.5	779.3	94.7	698.7	5.0	342.7	247.7	507.2 ab
Mean ^z	1,121.8 a	575.5 b	68.2 d	414.2 c	2.2 d	322.8 c	116.2 d	

^w CLRC isolate number/genotype/Canadian province.

^x Means followed by the same letter are not significantly ($P = 0.05$) different according to the least significant difference test (LSD = 6.3 [surface necrosis], 2.0 [lesion depth], 134.2 [CAI]).

^y Observations are based on a mean of five repetitions (one observation per repetition). Values are significantly ($P = 0.05$) different from other values if they differ by LSD = 16.7 (surface necrosis), 5.4 (lesion depth), or 355.1 (CAI). Tubers in uninoculated controls did not develop disease symptoms. CAI = surface necrosis $\times$ lesion depth.

^z Means followed by the same letter are not significantly ($P = 0.05$) different according to the LSD test (LSD = 5.6 [surface necrosis], 1.8 [lesion depth], 118.4 [CAI]).

dominance of US-8 populations outside British Columbia.

Genotypes g11 (US-11) and UN-3 were isolated from British Columbia. Genotype g11 (US-11) was found at high frequencies in samples from British Columbia in 1995 and 1996 (53,55,57). Genotype UN-3 (*Gpi 111/111*) was recovered from Vancouver Island and limited locations on the mainland in 1996 (minor frequency). It is possible that geographic isolation has maintained the existence of UN-3 in the face of more aggressive genotypes. Miller et al. (44)

found that in Oregon, *Gpi 100/111* and *100/100/111* genotypes were displaced by US-8. Preliminary results of sampling in British Columbia during the 1997 field season indicate that the US-8 genotype is becoming more common in this province (F. Daayf and H. W. Platt, *unpublished data*). Time will tell whether the more aggressive US-8 genotype also will displace the g11 (US-11) genotype in British Columbia.

Evidence for variation in aggressiveness among multilocus genotypes was accompanied by evidence for variation in aggres-

siveness within a multilocus genotype. An isolate of the US-8 genotype from New Brunswick was consistently less aggressive than other US-8 isolates in all trials. Variation in aggressiveness also was evident among other US-8 isolates in experiment 4 (Table 5), although the differences were less apparent in experiment 5 (Table 6). In similar tuber rot studies, Lambert and Currier (36) noted that although most isolates of the US-7 genotype were highly aggressive certain US-7 isolates were similar to US-1 isolates in aggressiveness. In addition, US-6 isolates displayed a broad range of aggressiveness (36). Miller et al. (46) found significant differences in aggressiveness on foliage among 30 isolates of *P. infestans* and considerable variability among isolates that were A1 metalaxyl resistant, A2 metalaxyl resistant, and A1 metalaxyl sensitive. Metalaxyl-resistant isolates as a group were not consistently more aggressive than metalaxyl-sensitive isolates (46).

Isolates of the US-8 genotype used in our studies were all highly complex and included a minimum of nine known virulence genes (53,56). Therefore, variation in aggressiveness within this multilocus allozyme genotype was not related to specific genes for virulence. Previous studies have found variation in aggressiveness among isolates of *P. infestans* of the same race (8,26,41). Variation, in terms of genes for virulence, did exist among the genotypes used in our study. Although US-8 isolates were complex, many of the other genotypes (US-1 and g11) carried fewer (three to five) genes for specific virulence (53,56). Race composition should not have contributed to variability in aggressiveness among genotypes in most cases, because only one cultivar (Dorita) possessed R genes that were unmatched by virulence genes in some genotypes.

Observation of inoculated tubers revealed that the vast majority of infections occurred through the eyes, which agreed with published research that suggests the intact periderm provides a barrier to *P. infestans* penetration (22,51) and infections develop only through wounds, eyes, and lenticels (in order of preference) and rarely through the stolon attachment end (22,38,51,77).

Many researchers have noted positive correlations between the resistance of potato foliage to infection by *P. infestans* and resistance of tubers (15,39,67,71). However, many exceptions to this rule (foliar resistance-tuber susceptibility and foliar susceptibility-tuber resistance) also have been reported (7,16,24,28,64). Cvs. Green Mountain, Shepody, Red Pontiac, Bintje, and Russet Burbank produced susceptible responses in foliar field trials conducted on Prince Edward Island with A1 (US-1) isolates of *P. infestans* (59,62). Cvs. Kennebec and Sebago were moderately resistant, and Dorita was highly resistant in similar foliar trials (59,62). Foliar field trials conducted on Prince Edward Island in 1995 with an A2 (US-8) iso-

Table 7. Response of five commercial potato cultivars to infection by Canadian genotypes of *Phytophthora infestans* collected in 1994, 1995, and 1996 (experiment 6, 1997)

Genotype ^u	Cultivar					Mean ^v
	Green Mountain	Island Sunshine	Dorita	Sebago	Bintje	
Surface necrosis (%) ^w						
US-1	7.0	0.0	0.0	28.0	4.0	7.8 d
US-7	52.0	1.0	0.0	69.0	52.0	34.8 a
US-8	22.0	21.0	11.4	59.0	51.0	32.9 ab
g11 ^x	11.0	6.2	0.0	40.0	44.0	20.2 c
g26	23.0	1.6	0.2	63.0	29.0	23.4 bc
g29	7.2	0.8	9.0	58.3	39.0	19.8 c
g30	1.0	0.0	0.0	27.0	0.0	5.6 d
g40	23.0	3.0	0.0	50.0	31.0	21.4 c
g41	3.0	0.0	0.0	0.0	0.0	0.6 d
g42	0.2	0.0	0.0	18.0	0.0	3.6 d
UN-3 ^y	4.2	0.0	0.0	12.0	3.0	3.8 d
Mean ^z	14.0 c	3.1 d	1.9 d	38.6 a	23.0 b	
Lesion depth (mm) ^w						
US-1	2.4	0.0	0.0	1.4	1.0	0.9 c
US-7	7.5	0.4	0.0	10.4	6.7	5.0 a
US-8	5.2	3.2	1.2	7.6	8.5	5.1 a
g11 ^x	5.4	0.5	0.0	4.9	8.3	3.8 ab
g26	7.0	1.9	0.1	9.1	6.1	4.8 ab
g29	1.7	0.5	4.9	5.7	4.6	3.3 b
g30	0.7	0.0	0.0	4.0	0.0	0.9 c
g40	4.4	0.3	0.0	9.0	8.2	4.4 ab
g41	0.6	0.0	0.0	0.0	0.0	0.1 c
g42	0.5	0.0	0.0	2.9	0.0	0.7 c
UN-3 ^y	4.4	0.0	0.0	1.8	1.6	1.6 c
Mean ^z	3.6 b	0.6 c	0.6 c	5.2 a	4.1 ab	
Compound aggressiveness index (CAI) ^w						
US-1	27.4	0.0	0.0	71.8	19.3	23.7 e
US-7	434.5	2.0	0.0	698.7	377.1	302.5 a
US-8	173.6	78.4	53.7	430.6	531.7	253.6 ab
g11 ^x	101.2	2.4	0.0	320.3	400.4	164.9 bc
g26	170.3	6.0	0.1	580.1	185.1	188.3 bc
g29	29.0	0.9	83.2	501.1	233.9	140.8 cd
g30	3.7	0.0	0.0	281.7	0.0	57.1 de
g40	146.8	4.0	0.0	450.5	400.1	200.3 a-c
g41	8.8	0.0	0.0	0.0	0.0	1.8 e
g42	0.5	0.0	0.0	127.3	0.0	25.6 e
UN-3 ^y	42.0	0.0	0.0	46.8	23.7	22.5 e
Mean ^z	103.4 c	8.5 d	12.5 d	319.0 a	197.4 b	

^u Designation of US-1, -6, -7, and -8 multilocus allozyme genotypes according to the method described by Goodwin et al. (18) and g11, g26, g29, g30, g40, g41, and g42 according to the method of M. D. Coffey, University of California, Riverside, as described by Punja et al. (63). Genotypes g29, g40, g41, and g42 are rare variants of the US-8 multilocus allozyme genotype, and g30 is a rare variant of the US-1 multilocus allozyme genotype based on random amplified polymorphic DNA analysis (M. D. Coffey).

^v Means followed by the same letter are not significantly ($P = 0.05$) different according to the least significant difference test (LSD = 9.7 [surface necrosis], 1.7 [lesion depth], 106.7 [CAI]).

^w Observations are based on a mean of five repetitions (one observation per repetition). Values are significantly ($P = 0.05$) different from other values if they differ by LSD = 21.8 (surface necrosis), 3.8 (lesion depth), or 239.0 (CAI). Tubers in uninoculated controls did not develop disease symptoms. CAI = surface necrosis $\times$ lesion depth.

^x Also known as US-11, according to Goodwin et al. (19).

^y Genotypes with no designation to date.

^z Means followed by the same letter are not significantly ($P=0.05$) different according to the LSD test (LSD = 6.6 [surface necrosis], 1.2 [lesion depth], 71.9 [CAI]).

late ranked Green Mountain as susceptible and Sebago and Dorita as moderately resistant (60,61).

In our study, based on CAI, tubers of cvs. Green Mountain, Kennebec, Red Pontiac, Shepody, and Sebago were highly susceptible to late blight; Russet Burbank, Superior, and Bintje were slightly less susceptible; and Island Sunshine and Dorita generally were resistant across isolates, although some erosion of the resistance of Island Sunshine and Dorita by isolates of the US-8 genotype was evident. The rankings loosely correspond to those for foliar blight, although Kennebec and Sebago were more susceptible to blight in the tuber phase than in the foliar phase. The results underscore the need for potato-breeding programs to assess both foliar and tuber blight during germ plasm screening. Variability occurred in cultivar ranking among trials, indicating the effects of environmental components on cultivar resistance responses (33).

Cultivar-isolate interactions were significant in each year of the study. The only cultivars with major genes for resistance were Kennebec (R1) and Dorita (R1, R3, and R10). Most isolates were able to overcome R1 (53,56), and therefore, race-specific resistance should not have accounted for any of the apparent variability in the host response of Kennebec. However, race-specific resistance in the more complex Dorita, which also is reported to have a high level of field resistance, undoubtedly conferred resistance against several of the less complex isolates (US-1, US-7, g41, and g42). Some researchers have argued that significant cultivar-isolate interactions indicate a breakdown in field resistance of the host by adaptation of the pathogen to a particular host (5,8,40). Other researchers have documented the stability of field resistance (49,73) of cultivars challenged with many or complex isolates of *P. infestans* (11,25, 50,52). Inglis et al. (24) found that cultivar rankings for susceptibility to new genotypes of *P. infestans* in Washington were nearly identical to rankings achieved with US-1 inoculations (using area under the disease progress curve estimations).

Perceived cultivar-isolate interactions can be caused by environmental effects (33,50). Parker et al. (50) found that after inoculation with three isolates of *P. infestans*, cv. Alpha had a dramatically different resistance ranking in Toluca than it did in New York. They concluded that different environments exerted a greater effect on the cultivars and isolates than did the cultivars and isolates on each other (50). Stewart et al. (67) found that the environmental component of variation was greater for tuber blight than for foliage blight. Although the cultivar-isolate interactions were significant in most experiments in our study, they were likely caused by environmental effects and small sample sizes. Their contribution to total variation was very low compared with variation among cultivars and isolates

and did not preclude calculation of main effects. Our conclusion is supported by Jenns et al. (27) who found that significant interactions in trials comparing the response of maize lines to pathogen isolates became nonsignificant when analyses were combined, indicating that host and pathogen components interacted more strongly with changes in condition from trial to trial than with each other.

A comparison of identical experiments conducted in 1996 revealed significant differences ($P \leq 0.0001$) in surface necrosis between the two trials. Month-cultivar interactions for surface necrosis were highly significant ($P \leq 0.0001$), whereas month-isolate interactions for surface necrosis were less significant ($P \leq 0.04$). Similarly, Stewart et al. (69) found cultivar-harvest date and cultivar-year interactions in inoculation experiments with field-grown tubers. Variation in tuber response within a particular cultivar to infection by *P. infestans* over time could be the result of many factors. There are known physiological changes that occur as tubers age, such as accumulation of reducing and nonreducing sugars and sesquiterpenoid stress metabolites (4). Several studies have found an increase in susceptibility of tubers to infection by *P. infestans* with time in storage (1,4,77), whereas others have documented increasing resistance to infection of tubers, in the field or storage, with age (7,68,75,77). Many differences in published studies can be accounted for by the varying reactions of different cultivars used in the studies. In comparing the results of experiments 4 and 5, the susceptibility of Shepody, Green Mountain, Island Sunshine, and Russet Burbank tubers increased with time in storage, whereas the susceptibility of Dorita, Sebago, and Bintje tubers decreased.

Other environmental factors, such as temperature (21) and light (21,74), and biotic factors, such as tuber microflora (10), can influence the susceptibility of potato tissues to infection by *P. infestans*. In our study, the reduced infection of Green Mountain tubers in experiment 6 (Table 7) was probably related to severe infection of tubers with common scab (*Streptomyces scabies*). Therefore, environmental conditions (and possibly the presence of microflora) significantly affected cultivar response.

Repeated transfers can lead to loss of pathogenicity in *P. infestans* (23). However, storage at low temperatures increases the time interval between transfers and reduces the rate at which pathogenicity is lost (23). To ensure pathogenicity in our studies, cultures were maintained at 4°C (in agar slants) immediately after isolation from plant tissues and were subcultured only once to prepare inoculum. In our lab, maintenance of cultures for 1 to 2 years at 4°C in rye agar slants or broth (54) did not result in loss of pathogenicity (R. D. Peters, H. W. Platt, and R. Hall, unpublished data). Results indicate that isolates of *P. infestans* in

our study responded fairly consistently over time. Most isolates of the US-8 genotype were consistently more aggressive than a US-1 genotype isolate in all trials across cultivars. Isolates of the g11 (US-11) genotype were consistently intermediate in aggressiveness, whereas an A2 (US-8) isolate from New Brunswick was consistently low in aggressiveness. These results strongly argue for a genetic component imparting increased fitness to several introduced genotypes relative to the traditional US-1 strain. New, resistant potato cultivars are urgently required to replace cultivars grown in Canada that are susceptible to the aggressive, metalaxyl-insensitive genotypes of *P. infestans* that currently dominate populations of the pathogen in Canadian potato production areas.

Based on 3 years of study, several recommendations can be made for assessing potato tuber resistance in breeding programs. Inoculation of tubers with 10,000 to 20,000 sporangia per ml followed by incubation at 10°C for 3 weeks (in repeated experiments) resulted in disease development that permitted differentiation of cultivar and isolate responses. Wounding seemed to obscure the resistance response and is not recommended. Inoculation with a mixture of locally occurring isolates of *P. infestans* is necessary to eliminate variation in aggressiveness within and among genotypes. Finally, inoculation of tubers of various ages is important in assessing the cultivar-specific impact of physiologic aging on tuber infection.

ACKNOWLEDGMENTS

We thank Agriculture and Agri-Food Canada and all the growers, potato industry representatives, extension personnel, and government workers from across Canada for sample submissions and their general involvement in Matching Investment Initiative Project 3026. We thank A. Driscoll, S. Jenkins, E. Connors, S. Powell, A. MacPhail, R. Reddin, and V. MacLean for their outstanding technical assistance. We also thank H. Förster and M. D. Coffey, University of California, Riverside, for RAPD analysis of isolates.

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