Identification of Late Blight, Colorado Potato Beetle, and Blackleg Resistance in Three Mexican and Two South American Wild 2x (1EBN) *Solanum* Species

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ABSTRACT

Wild potatoes are important sources of genes for resistance to disease and insect pests. A collection of wild Mexican and South American *Solanum* species from the US potato Genebank was evaluated under laboratory and/or field conditions for their reaction to late blight (*Phytophthora infestans*), Colorado potato beetle (CPB, *Leptinotarsa decemlineata* Say), and blackleg (*Erwinia carotovora* subsp. *atroseptica* (van Hall) Dye) in order to identify individual genotypes with multiple resistance genes. Late blight inoculations using aggressive isolates (US-8/A2 and US-11/A1 mating types) of *P. infestans* revealed a wide range of variation for resistance between and within the accessions of the wild species tested. For late blight, susceptible as well as moderately to highly resistant genotypes were observed in all the species tested. However, at least one accession from the three Mexican and one South American wild diploid species tested showed a relatively uniform high level of resistance to *P. infestans*. These included *S. bulbocastanum*, *S. pinnatisectum*, *S. cardiophyllum*, and *S. circaeifolium*. Two accessions from South American species *S. commersonii* were highly susceptible to late blight. For the Colorado potato beetle test, only one species, *S. pinnatisectum* appeared uniformly resistant to CPB under field conditions. Results of screening for blackleg resistance showed that there were major differences between genotypes in the wild species. Accessions of *S. circaeifolium* PI 498119 and *S. bulbocastanum* PI 243504 were identified as having significantly higher blackleg resistance than cultivated potato and the other wild species tested. However, genotypes from these two accessions were more susceptible to late blight and CPB. Characterization of the *P. infestans* isolate P1801C.16 used for late blight evaluation and multi-locus isolate tests using US-8/A2 and US-11/A1 races revealed that the resistance in *S. pinnatisectum* genotypes tested corresponded to a race-non-specific genetic system, which was different from any existing R genes. *Solanum pinnatisectum* genotypes with both high levels of late blight and CPB resistance as well as blackleg resistance genotypes identified in the present study represent a diverse gene pool that may be useful for development of new potato cultivars with multiple disease and insect resistance. The potential utilization of these valuable sources for improvement of cultivated potato is discussed.

RESUMEN

Las papas silvestres son la fuente importante de genes de resistencia a las enfermedades y plagas. Una colección de especies silvestres mexicanas y sudamericanas de *Solanum* del banco de genes de papa de EUA fue evaluada bajo condiciones de laboratorio y campo para reacción al tizón tardío (*Phytophthora infestans*), escarabajo colorado de la papa (CPB, *Leptinotarsa decemlineata* Say) y pierna negra (*Erwinia carotovora* subsp. *atroseptica* (van Hall) Dye) con el objeto de

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Abbreviations: CPB: Colorado potato beetle; DSV: disease severity values; EBN: Endosperm Balance Number; LRC: Lethbridge Research Centre

identificar genotipos individuales con genes múltiples de resistencia. Inoculaciones hechas usando aislamientos agresivos de *P. infestans* (tipos de apareamiento US-8/A2 y US-11/A1) revelaron un amplio rango de variación para resistencia entre y dentro de las accesiones de las especies silvestres probadas. Para tizón tardío se observaron tanto genotipos susceptibles como moderados a altamente resistentes en todas las especies probadas. Sin embargo, por lo menos una accesión de las especies silvestres diploides de las tres mexicanas y una sudamericana mostraron un alto nivel de resistencia relativamente uniforme a *P. infestans*. Estos incluyeron *S. bulbocastanum, S. pinnatisectum, S. cardiophyllum* y *S. circaeifolium*. Dos accesiones de la especie sudamericana *S. commersonii* fueron altamente susceptible al tizón tardío. Solamente la especie *S. pinnatisectum* mostró resistencia uniforme al escarabajo colorado bajo condiciones de campo. Los resultados del tamizado para resistencia a la pierna negra demostraron la existencia de diferencias mayores en las especies silvestres. Las accesiones de *S. circaeifolium* PI 498119 y *S. bulbocastanum* PI 243504 se identificaron como poseedoras de una resistencia significativamente alta a la pierna negra en comparación con las especies cultivadas de papa, al igual que otras especies silvestres probadas. Sin embargo, los genotipos de estas dos accesiones fueron más susceptibles al tizón tardío y al escarabajo colorado. La caracterización del aislamiento PI801C.16 usado para la evaluación de tizón tardío y la prueba de aislamiento multi-locus utilizando las razas US-8/A2 y US-11/A1 revelaron que la resistencia en los genotipos probados de *S. pinnatisectum*, corresponden a una raza no específica del sistema genético, la cual era diferente de cualquiera de los genes R existentes. Los genotipos *S. pinnatisectum* con altos niveles de resistencia a tizón tardío y al escarabajo colorado, lo mismo que los genotipos de resistencia a la pierna negra, identificados en el presente estudio representan un conjunto de genes que pueden ser útiles para el desarrollo de cultivares nuevos de papa con multiple resistencia a enfermedades y plagas. Se discute la utilización potencial de estas valiosas fuentes de resistencia para el mejoramiento de papa cultivada.

INTRODUCTION

Worldwide, late blight, caused by the fungus *Phytophthora infestans* (Mont.) de Bary, and the Colorado potato beetle (CPB, *Leptinotarsa decemlineata* Say) are the most important disease and insect pests of potato (*Solanum tuberosum* L.) (Niederhauser 1993; Guenthner et al. 2001). They can rapidly devastate potato foliage in the field and reduce potato yield and quality. CPB can lower yield by 30% to 50% (Stemeroff and George 1983; McLeod and Tolman 1987), while late blight spreads rapidly under cool and wet conditions and has a significant economic impact on potato production (Guenthner et al. 2001). Blackleg, caused by the bacterium *Erwinia carotovora* subsp. *atroseptica* (van Hall) Dye, is another important potato pathogen, which also can result in significant economic losses during plant growth and tuber storage (Perombelon and Kelman 1980).

Control of late blight and CPB mainly depends on the extensive use of chemical pesticides (Ferro and Boiteau 1993; Stevenson 1993). However, commercially effective bactericides do not exist for blackleg control. Sanitation and the use of disease-free seed are recommended to control this disease. For several decades, late blight has been effectively controlled in North America through either race-specific resistance R genes derived from *S. demissum* Lindl. or the use of systemic and residual fungicides (Ross 1986; Deahl et al. 1993). However, the new aggressive US-8 genotype of *P. infestans* detected recently in North America and other places in the world has developed resistance to the most widely used, systemic fungicide metalaxyl (Fry et al. 1993; Goodwin et al. 1994; Hamm et al. 1994; Platt 1999). The aggressive US-8 genotype of *P. infestans* also limits the effectiveness of R gene-based resistance (Fry and Goodwin 1997; Lambert and Currier 1997). At the same time, with wide-scale use of pesticides, insecticide tolerance in CPB also occurs for all major classes of insecticides. These situations make control of these pests increasingly difficult and costly (Kennedy and French 1994). On the other hand, concerns regarding environmental, health, and sustainability issues are increasing, and the continued use of pesticides is encountering growing public opposition and is gradually being restricted by regulatory agencies.

The widespread occurrence of fungicide and pesticide resistance has also increased interest in alternative disease- and insect-control methods (Kennedy and French 1994). Plant resistance is one of the best solutions for minimizing problems

with these pests and is considered an important part of an integrated pest management system, which will reduce the need for chemical pesticides (Tingey and Yencho 1994). Unfortunately, all currently available commercial varieties are highly susceptible to *P. infestans* and the bacterium *E. carotovora* subsp. *atroseptica*, and no commercial potato cultivars have a significant level of resistance to CPB (Ferro and Boiteau 1993; Inglis et al. 1996; Douches et al. 1997). The development of resistant potato cultivars or even a cultivar with moderate resistance to these pests would significantly benefit potato production and reduce reliance on pesticides.

Solanum is a diverse genus with about 228 wild species growing naturally from the Southwestern United States to Central Chile (Hawkes 1994; Spooner and Hijmans 2001). Among them, the Mexican wild species are considered important donors of genes to potato for improving late blight, CPB, and blackleg resistance (Van Soest et al. 1984; Bamberg et al. 1994; Carputo et al. 1996; Lees et al. 2000; Douches et al. 2001). Mexico is one of the centers of origin/diversity of wild potato species and also the original home and center of variation of different pathogens and insects (Niederhauser 1999). Many races of *P. infestans*, both extremely virulent and highly aggressive, exist in Mexico, both in wild habitats and cultivated fields. These include all of the 11 known virulence factors in the most complex races (Rivera-Pena 1990a). Mexican wild species are protected by a high level of general resistance, which was naturally selected for during exposure to continuous intense infection pressure.

Recent molecular evidence suggests that natural populations such as potato wild species express their resistance to pathogens through deployment of multiple resistance R genes (Bergelson et al. 2001; Dangl and Jones 2001). It was hoped that a very complex and durable resistance could be identified in the gene pool of the wild Mexican species (Rivera-Pena 1990b). Recent changes in *P. infestans* populations have increased the need for new sources of genetic resistance. Thus, the identification and transfer of disease and insect resistance in the wild species will greatly broaden the genetic basis of resistance for potato breeding. However, only a very small proportion of the existing germplasm in the wild Mexican gene pools is being utilized (Watanabe 1991; Kuhl et al. 2001). To date, there has been little research to systematically and simultaneously evaluate the resistance to *P. infestans*, CPB, and blackleg in the same genotypes of Mexican wild species. Moreover, since most *Solanum* species are outcrossers, such populations are often heterogeneous for traits of interest. Great variation in resistance to diseases has been observed within and between accessions and species (Bamberg et al. 1994; Douches et al. 2001). To continue advances in potato production while reducing the need for chemical control, new sources of disease resistance genes must be exploited. It is necessary to conduct genotype screening to identify individuals with high levels of resistance from *Solanum* species accessions before starting a program to transfer genes from wild species.

The objective of this study was to assess resistance to late blight and blackleg pathogens and CPB in three wild Mexican and two South American 2x (1EBN) *Solanum* species to find the best sources of resistance genes for their exploitation in potato breeding. Our ultimate goal is to transfer high levels of resistance to *P. infestans*, CPB, and blackleg that occurs naturally in wild species into cultivated potato.

MATERIALS AND METHODS

Plant Materials

Late blight, CPB, and blackleg resistance were evaluated on 20 accessions representing three wild Mexican 2x (1EBN) *Solanum* species and two South American species (Table 1). All these wild *Solanum* species were obtained as botanical seeds from the NRSP-6 Inter Regional Potato Introduction Station at Sturgeon Bay, Wisconsin. Seeds were germinated and random samples of 10 to 15 seedlings from each accession were selected and transferred to 1-gal pots filled with Cornell mix and grown in a greenhouse, and/or maintained as micropropagated plants on Murashige and Skoog (1962) medium with 1% sucrose and 0.8% agar. Plants were placed in a growth chamber under 18-h light and 6-h dark with temperatures of 18-20 C. Apical tip cuttings from the plants were grown in plastic inoculation containers (41 cm x 52 cm x 9 cm) in Cornell mix or maintained and replicated through tissue culture micropropagation. Late blight differential plants possessing resistance R gene of R1 to R11 (Malcolmson and Black 1966) were also obtained from the NRSP-6 Inter Regional Potato Introduction Station at Sturgeon Bay, Wisconsin.

Late Blight Test

Inoculum was prepared from a single, aggressive isolate P1801C.16 of *P. infestans* (US-8/A2 mating type), which was kindly provided by Dr. H.A. (Bud). Platt, Agriculture and Agri-

Food Canada (Charlottetown, Prince Edward Island, Canada). To produce sporangia the isolate was cultured on Rye-A medium (Caten and Jinks 1968) in the dark for 12 days at 10°C. Then sterile distilled water was added onto the agar surface, and mycelium was stirred using a sterilized glass rod or rubber and filtered through two layers of cheesecloth. The sporangial suspension was placed at 8-12 C for 2-3 h to induce the release of zoospores. The inoculum was then diluted with distilled water to a final concentration of 30,000 sporangia per ml using a hemacytometer at 100× magnification.

TABLE 1—*Late blight resistance evaluation of wild diploid Mexican and South American* Solanum *species with an aggressive US-8/A2 isolate P1801C.16 of* P. infestans.

Solanum spp	Accession	Ploidy	EBN	No. of genotypes tested	Phenotype[a]		
					R	I	S
Mexican wild species							
S. bulbocastanum							
Dunal	PI 243510	2×	1	12	11	1	
	PI 275184	2×	1	12	10	2	
	PI 275191	2×	1	12	8	1	3
	PI 243504	2×	1	6	3	1	2
	PI 275193	2×	1	6	4	2	
	PI 498223	2×	1	12	11	1	
S. cardiophyllum							
Lindl.	PI 347759	2×	1	12	5	4	3
	PI 283062	2×	1	12	7	3	2
	PI 186548	2×	1	12	0	1	11
	PI 283063	2×	1	8	6	2	
S. pinnatisectum							
Dunal	PI 253214	2×	1	12	8	4	
	PI 275236	2×	1	12	8	4	
	PI 275233	2×	1	12	10	2	
	PI 275234	2×	1	6	5	1	
	PI 275232	2×	1	12	2	7	3
	PI 184764	2×	1	12	5	5	2
South American wild species							
S. commersonii							
Dunal	PI 320267	2×	1	6	0	0	6
	PI 320266	2×	1	6	0	0	6
S. circaeifolium sub. *quimense*							
Hawkes & Hjert	PI 498119	2×	1	6	1	0	5
	PI 498116	2×	1	6	5	1	
Cultivated potato							
S. tuberosum L.	Russet Burbank	4×	4	12	0	0	12
	Shepody	4×	4	12	0	0	12
	V0379-2	4×	4	6	0	0	6
	V0123-25	4×	4	6	0	0	6

[a]R: Resistant (Disease severity value (DSV) = 0-1.5); I: Intermediate (DSV = 1.6-3.5); S: Susceptible (DSV = 3.6-5).

A whole-plant assay under laboratory conditions was used for the detection of late blight resistance. Young plants 10 cm high from tissue culture were inoculated with a suspension of zoospores and sporangia obtained from 12-day-old *P. infestans* cultures grown on Rye-A agar plates or potato leaves. Plantlets were dipped in an inoculum solution (2 to 4×10^4 sporangia/mL) and then planted in an inoculation container with moist vermiculite. The container was sprayed using a hand-held sprayer with sterile distilled water, covered with a plastic lid, wrapped in parafilm and placed in a growth incubator at 17-18 C with a 12-h day/night light cycle. Six to12 genotypes of each accession were inoculated. Each genotype was replicated three to five times. In each experiment, plants in containers were arranged in a randomized design. Five plants of 'Russet Burbank' and 'Shepody' from tissue culture tubes were included in each replication as the susceptible check. At 7, 14, and 21 days after inoculation, disease severity was estimated using disease severity values (DSV) based on the percentage of stem and leaf area with symptoms of late blight. Severity values were scored using a scale of 0-5, where 0 = no disease-< 3%, 1 = 3%-24%, 2 = 25%-49%, 3 = 50%-74%, 4 = 75%-94%, and 5 = 95%-100% infection. For the characterization of the avirulence (avr) genes in the *P. infestans* isolate P1801C.16 that was used in the study, a complete set of differential potato plants possessing resistance R gene from R1 to R11 (Malcolmson and Black 1966) was used in inoculation. The experiment was conduced twice using the same method and same *P. infestans* isolate.

To confirm the nature of resistance in the wild Mexican diploid species, two other isolates of *P. infestans*, LRC 10.01 and P1621A. 27, which are virulent on R8 and R9 were used to inoculate the resistant wild Mexican species and differential plants carrying the R8 or R9 gene. Isolate LRC 10.01 of *P. infestans* is a US-11/A1 mating type and was virulent on R8, but avirulent to R9. The second isolate P1621A. 27 of *P. infestans* is a US-8/A2 mating type. It was avirulent to R8, but was virulent to R9.

Colorado Potato Beetle Test

A modified fine-screening method described by Bamberg et al. (1996) was applied to examine CPB resistance under field conditions at the Lethbridge Research Centre (LRC), Lethbridge, Alberta, Canada. A total of 30 genotypes from 13 accessions belonging to the four wild species that had been tested for late blight was chosen to screen for CPB resistance. Three to five plants from each genotype were prepared from

apical tip cuttings of plants grown during winter in a greenhouse at LRC to replicate the individual genotypes.

In early June 2000, plants were transplanted to a field site at LRC. The plots were arranged in a randomized complete block design, with 20 plants per single plot row. Each genotype was replicated three to five times. Row length and row spacing were 10 m and 1 m, respectively. Cultivated potatoes grown directly from tubers were transplanted to the field with the wild species at the same time. The experiment was exposed to a naturally occurring population of adult beetles from a nearby potato field that had not been treated with insecticide. All plants were rated three times at 7-day intervals beginning the last week of July. Counts were made of the number of adult beetles and the percentage of defoliation on each plant. Individual whole plants were evaluated at each observation date. The percentage defoliation was averaged over three sampling dates to determine the mean injury per plant. The test was repeated again in 2001 using the same method and design, but with different clones.

Blackleg Test

Erwinia carotovora subsp. *atroseptica* BL2 was grown to the logarithmic phase in nutrient broth yeast extract liquid medium (NBY; Vidaver 1967) on shake culture at 250 rpm at 28 C. The bacterial density was adjusted to ca 1×10^6 cfu mL^{-1}, which was used to inoculate plants. Inoculum density was verified through dilution plating on NBY plates. Phosphate buffer served as the negative control.

The blackleg assay was based on the detached leaf procedure of Bisht et al. (1993) and modified by Bains et al. (1999) to screen germplasm against *E. carotovora* subsp. *atroseptica* BL2. Plantlets from tissue culture were grown in small pots for about 2-3 wk, and then transferred to 1-gal pots and allowed to grow for approximately 2-4 wk, depending on the species. Each replication consisted of four leaves from one plant and individual plants were assayed at least twice over time. For the assay the 3rd leaf from the apical meristems were taken from the plants. Russet Burbank was used as the moderately resistant control and 'Sangre' was used as the susceptible control in the blackleg assays. Detached leaves were incubated in bacterial inoculum for 48 h in a growth room under the conditions specified above for plant growth conditions. Then, the length of stem rot was measured in mm. Plants of the wild species exhibiting the greatest blackleg resistance were multiplied through micropropagation on MS medium and were tested again with a larger sample size (16 leaves of each genotype) and two replications over time.

Data from the same genotype were pooled and statistical analyses were carried out using ANOVA (SAS, Version 6; SAS Institute Inc., Cary, North Carolina) according Least Significant Difference (LSD) tests to compare the disease resistance among the different genotypes (P=0.05) within accession.

RESULTS

Evaluation of Wild Species for Late Blight Resistance

Diverse reactions to *P. infestans* (US-8/A2) were observed between and within species and even between genotypes of the same accession. Several clones exhibited a wide range of response to late blight. However, these represented only 7% of the genotypes tested; 93% of the genotypes exhibited a relatively uniform level of response. Four out of six accessions of *S. bulbocastanum* and four out of six accessions of *S. pinnatisectum* showed high levels of resistance to late blight (Table 1). One of four accessions of *S. cardiophyllum* and one of two accessions of *S. circaeifolium* were resistant to late blight. Two accessions of *S. commersonii* were susceptible to late blight. Genetic diversity within the same accession in terms of resistance was also detected. Resistant, intermediate, and/or susceptible individuals were found in all 20 accessions tested from the Mexican and South American *Solanum* species (Table 1 and 2). *Solanum bulbocastanum* and *S. pinnatisectum* were predominantly resistant (47 out of 60 genotypes and 38 out of 66 genotypes, respectively) and most of the remaining individuals from these two species showed intermediate resistance. The accessions of *S. cardiophyllum* were composed of more susceptible individuals (26 of 44 genotypes), although most genotypes in accession PI 283063 were predominantly resistant (six out of eight genotypes). Two accessions of *S. commersonii* produced only susceptible individuals. *S. circaeifolium* displayed a mixture of resistant (6), susceptible (5), and intermediate (1) genotypes.

Evaluation for Race Specificity of Late Blight Isolate P1801C.16

To determine the virulence spectrum of P1801C.16, a complete set of differential potato plants was inoculated with the late blight isolate P1801C.16. The result of the inoculation showed that all but R8 and R9 differential plants were highly

TABLE 2—*Disease and insect screening of individual genotypes from different accessions of wild diploid Mexican and South American* Solanum *species.*

Solanum spp *accession*	Genotypes	Late Blight Mean DSV (Range)	CPB % of Defoliation	Blackleg Lesion (mm)[1]
S. bulbocastanum				
PI 275191	003-10	0.0 (0-0)	33.3	16.6 def
PI 243504	013-3	2.0 (1-3)	45.0	5.1 b
	013-4	4.0 (3-5)	20.0	4.1 ab*
PI 498223	015-10	2.5 (2-3)	53.3	9.1 bc*
	015-11	1.0 (1-1)	37.7	13.5 cd
	015-12	0.5 (0-1)	20.0	19.5 defg
S. cardiophyllum				
PI 347759	004-1	0.3 (0-1)	63.3	18.6 defg
	004-2	1.3 (0-2)	26.6	22.6*
	004-3	3.7 (3-5)	46.6	18.1 defg
	004-5	2.7 (1-4)	33.3	14.5 cd
PI 283062	016-9	0.0 (0-0)	30.0	31.6*
	016-10	0.5 (0-1)	30.0	18.9 defg*
PI 283063	018-5	2.3 (1-5)	28.3	21.1 defg
	018-9	0.5 (0-1)	18.3	18.6 defg
S. pinnatisectum				
PI 253214	005-3	0.7 (0-1)	0.0	22.1 fg
	005-4	1.5 (1-2)	0.0	21.1 def
	005-6	1.5 (1-2)	0.0	22.6 g
	005-9	0.3 (0-1)	0.0	17.6 de
PI 275236	006-4	0.3 (0-1)	0.0	19.6 defg
	006-5	2.0 (1-3)	3.3	21.6 efg
	006-7	1.3 (1-2)	0.0	20.6 defg
PI 275233	007-2	0.7 (0-1)	0.0	23.1 g
	007-4	0.3 (0-1)	0.0	19.6 defg
	007-7	1.3 (1-2)	0.0	20.6 defg
PI 275232	019-7	4.3 (4-5)	1.7	15.6*
	019-8	2.7 (1-4)	0.0	20.1 defg
PI 184764	020-6	3.5 (3-4)	0.0	15.1 cde
	020-7	1.0 (1-1)	0.0	17.1 def
S. circaeifolium				
PI 498119	009-3	5.0 (5-5)	36.7	0.1 a
	009-4	3.3 (3-4)	33.3	3.6 ab*
S. tuberosum				
Russet Burbank	042	5.0 (5-5)	100.0	17.1 def*
Sangre	LR375	–	–	22.4 g*

[1]Mean values followed by the same letter are not significantly different from each other at P=0.05 according LSD tests. 0-10 mm rot = resistant; 10.5-20 mm rot = moderately resistant; 20.5-30 mm rot = moderately susceptible; 30.5-40 mm rot = susceptible.

* Lines used for second blackleg test.

susceptible to the race P1801C.16 (Table 3). The R8 and R9 differentials had intermediate mean disease severity values (DSV) of 2.2 each for the first test and 2.5 and 2.1 for the second test, respectively. In both tests, the high levels of DSV 4 (75%-95% of leaf disks were infected by the late blight isolate) were scored in more than half of the leaf disks of R8 and R9

TABLE 3—*Mean disease severity values (DSV) of late blight differential plants after inoculation with isolate P1801C.16 of* Phytophthora infestans.

Plants	R genes	Mean DSV (Range)		Phenotype
		First test[a]	Second test[b]	
PI 423651	R1	4.0 (4-4)	4.0 (4-4)	S
PI 423652	R2	4.0 (4-4)	4.0 (4-4)	S
PI 423653	R3	3.1 (1-4)	4.0 (4-4)	S
PI 203900	R4	3.7 (1-4)	4.0 (4-4)	S
PI 303146	R5	3.1 (0-4)	3.9 (3-4)	S
PI 587059	R6	3.4 (1-4)	4.0 (4-4)	S
PI 303148	R7	3.6 (1-4)	4.0 (4-4)	S
PI 303149	R8	2.2 (1-4)	2.5 (2-3)	MS
LB1	R9	2.2 (1-4)	2.1 (1-4)	MS
PI 423656	R10	3.7 (1-4)	4.0 (4-4)	S
PI 587060	R11	3.7 (1-4)	4.0 (4-4)	S
Shepody	R0	–	4.0 (4-4)	S
Stirling	–	–	3.9 (3-4)	S

[a]Mean DSV determined at 9 days after inoculation.

[b]Mean DSV determined at 7 days after inoculation.

differential plants, indicating isolate P1801C.16 is virulent on R8 and R9. The results clearly indicate that isolate P1801C.16 is highly virulent to most of the R genes.

To confirm resistance in the wild species, two other late blight isolates LRC 10.01 (virulent on R8 and avirulent on R9) and P1621A. 27 (avirulent on R8 and virulent on R9) were used to inoculate the two wild Mexican species *S. pinnatisectum* and *S. bulbocastanum* as well as the R8 and R9 differentials (Table 4). Mean disease severity values on R8 and R9 differential plants for the test with LRC 10.01 isolate were 2.2 and 3.0, respectively, while the mean DSV for two genotypes of *S. pinnatisectum* was only 1.2 (Table 4). These results indicate that the LRC 10.01 isolate of *P. infestans* was virulent to genes R8 and R9 in differential plants R8 and R9, but genotypes of *S. pinnatisectum* were resistant to this isolate (Table 4). Similar results were obtained from the test with the P1621A. 27 isolate. In the same test, one of the two accessions of *S. bulbocastanum* was susceptible to the LRC 10.01 and P1621A. 27 isolates (Table 4), indicating that the susceptibility in one genotype of *S. bulbocastanum* was not related to that in *S. pinnatisectum*. These results establish that *S. pinnatisectum* tested in the present study carries a new resistance that is not controlled by any existing R genes and is different from the resistance present in another Mexican wild species *S. bulbocastanum*. The responses of *S. pinnatisectum* genotypes to the late blight pathogens produced mean DSVs that were very

TABLE 4—*Mean disease severity values (DSV) of late blight in differential plants R8 and R9 and two Mexican wild species with isolate LRC 10.01 and P1621A.27 of* Phytophthora infestans.

Materials	R gene & Accessions	Mean DSV (Range)*	Phenotype
Isolate LRC 10.01			
Differential plant	PI 303149 R8	2.2 (1-3)	MS
Differential plant	LB 1 R9	3.0 (3-3)	S
S. bulbocastanum	PI 243510 001-5	2.3 (2-3)	MS
S. bulbocastanum	PI 275191 003-1	5.0 (5-5)	S
S. pinnatisectum	PI 275236 006-4	1.2 (1-2)	R
S. pinnatisectum	PI 275233 007-9	1.2 (1-2)	R
Isolate P1621A.27			
Differential plant	PI 303149 R8	1.4 (1-2)	R
Differential plant	LB 1 R9	3.0 (3-3)	S
S. bulbocastanum	PI 243510 001-5	1.8 (1-2)	R
S. bulbocastanum	PI 275191 003-1	1.6 (1-2)	R
S. pinnatisectum	PI 275236 006-4	0.4 (0-1)	R
S. pinnatisectum	PI 275233 007-9	1.2 (0-2)	R

Mean DSV determined at 14 days after inoculation.

often between 0.5 to 2 rather than zero (immune reaction) (Tables 2 and 4). These results indicate that *S. pinnatisectum* reduced the rate of disease development rather than producing a disease-free or hypersensitive reaction to the pathogen.

Response of the Wild Species to Colorado Potato Beetle

All accessions were more resistant to CPB defoliation at an early stage of growth than the commercial variety Russet Burbank (Table 2). However, defoliation was observed among genotypes of different accessions for three out of the four wild species (*S. bulbocastanum, S. cardiophyllum*, and *S. circaeifolium*). Defoliation on average in the most susceptible genotype in these wild species was less than 63.3%. Defoliation ratings ranged between 18.3% and 63.3% in all the genotypes of seven accessions from three species. Only one species, *S. pinnatisectum* appeared uniformly resistant to CPB among all accessions tested under field conditions (Table 2), with only 1.7%-3.3% defoliation observed on a few plants of this species.

In the second year of the study, the defoliation in the wild species was still low in comparison to the severe defoliation of Russet Burbank. Differences in defoliation were noted between genotypes within accessions for three of the four species. *Solanum pinnatisectum* still showed consistently uniform resistance to CPB in the field (data not shown).

Blackleg Test

The results of screening for blackleg resistance in the wild species showed that there were significant differences between genotypes (Table 2). Accessions PI 498119 from *S. circaeifolium* and PI 243504 from *S. bulbocastanum* had significantly higher blackleg resistance than the other wild species accessions and the moderately resistant cultivar Russet Burbank and Sangre. However, these two genotypes were less resistant to late blight and CPB than the other genotypes of *S. bulbocastanum* and *S. pinnatisectum* (Table 2).

Combination of Disease and CPB Resistance

At least one accession from three Mexican and one South American wild diploid species showed a relatively uniform and high level of resistance to *P. infestans*. While only one species, *S. pinnatisectum* was uniformly resistant to CPB among the accessions tested under field conditions with high levels of late blight resistance, it was not blackleg resistant. One accession of each species of *S. circaeifolium* PI 498119 and *S. bulbocastanum* PI 243504 was identified as having significantly higher blackleg resistance than the other wild species, but they were moderately susceptible to late blight. Two genotypes of accession PI 498223 in *S. bulbocastanum* showed a high level of resistance to blackleg and were also resistant to late blight and moderately resistant to CPB.

DISCUSSION

In the present study, screening for late blight, CPB and blackleg resistance was carried out on a genotype-by-genotype basis for the wild Mexican and South American *Solanum* species. Inoculation of the wild *Solanum* species with different isolates of *P. infestans* revealed that there was a mixture of resistant, intermediate, and susceptible phenotypes in different wild potato species. Accessions of *S. bulbocastanum*

and *S. pinnatisectum* were the most resistant Mexican species tested and had the highest frequency of resistance to late blight (Table 1). Another Mexican species *S. cardiophyllum* and the South American species *S. circaeifolium* showed 50%-60% susceptibility to late blight, while two accessions of *S. commersonii* were susceptible to late blight. A wide range of variation for resistance to late blight, CPB, and blackleg occurred among and within accessions of both the Mexican and South American species. Differences in disease and pest severity from susceptible to resistant were noted between different genotypes of the same accession (Table 2). Although 93% of the genotypes showed relatively uniform levels of response to late blight, the remainder exhibited a great variation within a genotype. These results agreed with other reports on late blight resistance in Mexican wild species (Douches et al. 1997; Kuhl et al. 2001). A wide range of responses to late blight within a genotype is difficult to explain. It is possible that factors, such as environment and stage of a clone used in different tests may have had an effect on its reactions to late blight.

Our study demonstrates that it is important to intensively screen species accessions to find the best sources of resistance before beginning a potato germplasm enhancement and breeding program to transfer genes from wild species. Although great variation in late blight resistance existed within and between the accessions observed, at least one accession from the three Mexican and one South American species demonstrated a relatively uniform high level of resistance to *P. infestans* (Table 1). They included *S. bulbocastanum* PI 243510 and PI 275184, *S. cardiophyllum* PI 283063, *S. pinnatisectum* PI 275233 and PI 275234, and *S. circaeifolium* PI 498116 (Table 1). An accession PI 243504 from *S. bulbocastanum* and accession PI 498119 from *S. circaeifolium* were resistant to blackleg. It is interesting to note that two accessions of *S. pinnatisectum* PI 275233 and PI 275236 showed high levels of resistance to both late blight and CPB (Table 2). Some genotypes from accession PI 498223 of *S. bulbocastanum* were also highly or moderately resistant to late blight, blackleg, and CPB. The presence of high levels of resistance to two or more important diseases and insects in one species makes it a very useful source for breeding for potato disease and CPB resistance. These genotypes also possessed some other desirable traits, such as the production of high numbers of tubers and early maturity in the greenhouse, which can be exploited in a breeding program. All of these make *S. pinnatisectum* and *S. bulbocastanum* excellent candidates for the development of new potato cultivars with late blight, blackleg, and CPB resistance.

Evaluation of the race specificity of the US-8/A2 isolate P1801C.16 used in the study revealed that this *P. infestans* isolate is virulent on most of the R genes in the differential series with intermediate disease severity values on R8 and R9 genes (Table 3). Further inoculations using two other late blight isolates LRC 10.01 and P1621A. 27 confirmed that the type of resistance in *S. pinnatisectum* differed from the existing R genes and the resistance in *S. bulbocastanum* (Table 4). Furthermore, the reaction of *S. pinnatisectum* plants to the late blight pathogen was different from the other species and the R gene-based genotypes. For example, the reaction of genotypes of *S. pinnatisectum* to late blight was often rated as DSV 1, rather than as completely disease-free (Black 1970, Table 2). The late blight pathogen affected the leaves of *S. pinnatisectum*, very often starting on the lower leaves, making them turn black or yellow, but the pathogen did not spread and grow on the younger leaves. This type of reaction in *S. pinnatisectum* always killed 10%–20% of lower leaves, but the disease did not develop on the whole plant. These results suggest that resistance in *S. pinnatisectum* might be controlled by a general resistance mechanism, and may correspond to a race- non-specific resistance of a durable nature.

Recently, using a US-8/A2 isolate MSU96 of *P. infestans*, Kuhl et al. (2001) identified a single dominant late blight resistance locus in accession PI 253214 of *S. pinnatisectum*. Since the MSU96 was avirulent on R9 differential, it was assumed that the resistance from that accession of *S. pinnatisectum* may correspond to R9, which originated from *S. demissum*. Great genetic diversity within populations of the S. *pinnatisectum* species may be responsible for the differences noted in our studies. Most wild potatoes are out-crossing species. Therefore, high genetic variability is expected. Genetic variability in natural populations of wild potato species has also been reported for other agronomic traits by many different authors (Debener et al. 1990; Bamberg et al. 1994, 1996; Demeke et al. 1996; Peters et al. 1999).

For the Colorado potato beetle test, there was no difference in the reactions between and within accessions of *S. pinnatisectum* in terms of visual defoliation scores under field conditions. This indicated that these accessions might be homogenous for the resistance. However, a low level of defoliation was detected on some plants of *S. pinnatisectum*

(Table 2). Differences in the number of larvae and adult beetles in a study by Bamberg et al. (1996) were also significant, indicating some genetic variation exists for CPB resistance in *S. pinnatisectum*. It may be necessary to develop a more effective screening procedure to differentiate the variation between resistant and susceptible genotypes. The uniform resistance in this species may have high breeding value and may be useful for studying the physiological and genetic basis of resistance. It also would help to facilitate the use of the exotic germplasm as superior parents for potato cultivar improvement and germplasm enhancement. However, uniform resistance to CPB in all accessions of *S. pinnatisectum* may result in difficulties for genetic studies on CPB resistance. A lack of susceptibility among the accessions of this species could make mapping and tagging of the gene difficult. However, the susceptible *S. cardiophyllum* accession identified in the present study could be crossed with the resistant *S. pinnatisectum* plants. This CPB susceptible accession may be desirable or helpful in establishing segregating populations from which DNA markers linked with both late blight and CPB resistance can be developed (Kuhl et al. 2001). Development of the DNA markers will be useful in a marker-assisted selection program to introgress the genes conferring resistance to late blight and CPB. These hybrids will facilitate synthesis of even more uniformly resistant populations, and will be valuable in genetic studies.

Carputo et al. (1996) reported that based on a stem bioassay, some accessions from the wild species *S. canasense*, *S. tarijense*, *S. multidissectum*, and *S. tuberosum* haploid SVP11 carried some level of resistance to blackleg. Lees et al. (2000) identified that 22 of 23 clones of *S. phureja* Juz. & Bukasov were as resistant to blackleg as the most resistant control, cultivar Ailsa. Under controlled conditions, 18 of 21 *S. phureja* clones were significantly more resistant to blackleg than the commercial cultivars. In the present study, two accessions from *S. bulbocastanum* and *S. circaeifolium* have been shown to possess high levels of resistance to blackleg. In particular, accession PI 243504 of *S. bulbocastanum* was highly resistant to blackleg and also moderately resistant to late blight and CPB (Table 2). Since most accessions of *S. bulbocastanum* are highly resistant to late blight (Tables 1 and 2), it may be possible to find the genotypes carrying high levels of resistance to late blight, CPB, and blackleg if more accessions are screened.

Identification of new disease and insect resistance genes represents the first step towards the efficient development of new resistant cultivars. To transfer disease and insect resistance from these 2x (1EBN) *Solanum* species to cultivated potato, more elaborate introgression techniques will be necessary for utilizing the resistance in the Mexican germplasm due to two significant barriers: ploidy level and Endosperm Balance Number (EBN) incompatibility. Several approaches have been successfully used in overcoming these barriers including embryo rescue, double pollination (Singsit and Hanneman 1991), chromosome doubling, and 2n gametes (Ehlenfeldt and Hanneman 1984, 1988; Hermsen 1994). Another possible approach is the use of protoplast fusion to directly combine cross-incompatible species (Menke et al. 1996; Thieme et al. 1997; Helgeson et al. 1998). The availability of *S. pinnatisectum* resistance genes, along with other recently identified resistance genes in some North American potato cultivars and breeding lines may enable breeders to pyramid multiple genes for resistance to late blight and CPB into a single cultivar (Platt and Reddin 1994a, 1994b; Douches et al. 1997). Incorporating multiple resistance genes into a single genotype may be accomplished through the use of a combination of different components of late blight resistance from wild species with other sources found in cultivated potato. Because introgression of genes from wild species to cultivated potato requires a great deal of effort, the use of genotypes that have multiple resistance genes as parents for the crossing would be advantageous. The genotypes identified in the present study with multiple resistance to disease and insect constitute a broad genetic base of *Solanum* germplasm and provide the potential to combine alternative sources of late blight resistance, and thereby develop more durable host plant resistance. Genotypes of *S. pinnatisectum* carrying resistance to both late blight and CPB have been selected to cross with other diploid and tetraploid *Solanum* species for further studies on the genetic inheritance and the possible transfer of late blight and CPB resistance in *S. pinnatisectum* to cultivated potato.

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