Late blight-resistant tuber-bearing *Solanum* species in field and laboratory trials

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SUMMARY
The effects of a current *Phytophthora infestans* population in Russia on genotype of Mexican tuber-bearing *Solanum* species were assessed in field and laboratory trials. Two-year experiments documented disease progress in 78 accessions of 19 species under high infection pressure. Detached leaf tests conducted independently in laboratories of two scientific institutions identified the response of 19 species to isolates collected in different regions of Russia. As a whole, we characterized *Solanum* genotypes with a broad range of resistance to late blight.

KEYWORDS
Phytophthora infestans, late blight, Solanum species.

INTRODUCTION
Wild *potato* collection at VIR (N.I.Vavilov Institute of Plant Industry) is a source of useful breeding characteristics, in particular the resistance to pests and diseases. To describe the breeding potential of wild species the results of evaluation of potato germplasm preserved at VIR has been provided by many authors (Bukasov & Kameraz, 1972, Budin *et al.*, 1984, Zoteeva *et al.*, 2004). Since most evaluation data on wild potato comes from screening populations, such information provides only a general guide for selecting prospective germplasms (Hoekstra *et al.*, 1997). The reaction of potato germplasm to late blight (LB) must be additionally verified due to the changes in *Phytophthora infestans* populations in Russia (Elansky *et al.*, 2001). Mexican wild potatoes have been recognized as an outstanding source of late blight resistance. Here we report the evidence on 78 accessions of Mexican tuber-bearing *Solanum* species assessed for LB resistance to the current *P. infestans* population in the field and to a complex race of *P. infestans* in the laboratory. The well-characterized

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Solanum genotypes manifesting the broad range of resistances can be further employed in breeding programs and concurrent molecular-genetic studies.

MATERIALS AND METHODS

Plants

The plant genotypes used in this study belong to 78 accessions representing 19 species of eight series as listed in Table 1. To compare the behavior of Mexican species to a cultivated potato, the plants of *S. chilotanum* were included into the experiment. Each accession grown from greenhouse seedlings was presented by one genotype selected because of abundant tubers and lack of virus symptoms. The tubers of each individual genotype were harvested in the greenhouse. The clones were propagated in the greenhouse annually and used for field and laboratory tests.

Table 1. *Accessions of Solanum tuber-bearing species tested for LB resistance.*

Series	Species	Accession numbers in the VIR collection
Demissa	*S. brachycarpum*	k-2830
	S. demissum	k-15173, k-18521
	S. hougasii	k-8818
Longipedicellata	*S. fendleri*	k-5747, k-5751, k-23335, k-23841, k-24218, k-24221
	S. hjertingii	k-15194, k-19276, k-21409, k-24223, k-24387
	S. papita	k-16889, k-24417
	S. polytrichon	k-7426, k-16905, k-18142, k-19164, k-23561, k-23556, k-23563, k-24298, k-24462, k-24463
	S. stoloniferum	k-3336, k-19196, k-20106, k-21547, k-21618, k-23652, k-24420
Borealia	*S. wightianum*	k-24250
Polyadenia	*S. polyadenium*	k-23553
Pinnatisecta	*S. brachystotrichium*	k-22919, k-23200, k-24197
	S. jamesii	k-15203, k-22619, k-23397, k-23398, k-23399, k-24395, k-24397
	S. pinnatisectum	k-21955, k-23569, k-24239, k-24243
	S. stenophyllidium	k-20105, k-24255
	S. tarnii	k-23936
Cardiophylla	*S. cardiophyllum*	k-3319, k-4464, k-10456, k-16827, k-16828, k-17370, k-18086, k-24203, k-24206, k-24207, k-24209, k-24375
	S. ehrenbergii	k-18085, k-18225, k-19059, k-19061, k-19257, k-21300, k-23277, k-23279, k-24279, k-24373
Bulbocastana	*S. bulbocastanum*	k-19981, k- 21266
Tuberosa	Control - *S. chilotanum*	k-1671
Total	19	78

Field test

Field assessments were carried out during the 2008 and 2009 plant growth periods at the Pushkin Experimental station of VIR situated 20 km south of St-Petersburg in the Leningrad region, the North-Western part of Russia. Each year 2-5 plants per genotype were grown in the field. The experimental plots were positioned between adjacent border rows of potato cultivars. Foliage LB assessments were taken on each plant weekly for 5 weeks in 2008 and for 4 weeks in 2009 using the 1 to 9-score scale, where 1 corresponds to 100% necrotic tissue and 9, to no visible lesions. These scores were converted into mean percent defoliation for the corresponding range (i.e., 1=0%, 5=50%, and 9=100%) and used to calculate the area under the disease progress curve (AUDPC) for each individual accession. To compare AUDPC across two-year experiments the relative AUDPC (RAUDPC) was calculated using Microsoft Excel (Anonymous, 2007).

Laboratory test

Detached leaf tests (see Eucablight protocol – Detached leaf test for foliage blight resistance: www. euroblight. net/) were conducted independently in two laboratories: at the Institute of Phytopathology (IP) and the Institute of Plant Protection (VIZR). Leaflets were collected from 30-55-day-old greenhouse plants. Single pathogen isolates collected in the Leningrad and Moscow regions (both containing the race 1,2,3,4,5,6,7,8,9,10,11) were used in this study. Three to five leaflets per each tested accession were inoculated with zoospore suspension at the concentration of 30-40 × 1000 per ml according to standard protocol. The leaflets of cvs.: Alpha, Bintje, Eersteling, Escort, Robijn, Gloria, Sapro Mira and Sante (Eucablight standard set) were used as the control in IP and cvs. Latona and Elizaveta, in VIZR. Disease assessments were carried out for six days following inoculation. Both the leaf area affected by pathogen and the extent of sporulation were scored.

Statistical analysis

Data were analyzed by ANOVA to compare late blight damage in the accessions of wild potato species across two-year field tests. The correlation between area under disease progress curve (AUDPC) and the resistance of the wild potato infected in the laboratory was estimated with the Spearman's coefficient. *STATISTICA* 6 1 package (StatSoft Inc. /StatSoft Russia) was used for processing the data.

RESULTS AND DISCUSSION

Field trials

Epidemic performance of *P. infestans* was recorded for both years of field trials. Isolates of *P. infestans* from those fields were very complex and virulent to all 11 *R*-gene differentials tested, except R9. In both years, the diseases emerged at the same period, 55-60 days after planting, but the time-course of disease progress varied substantially. The AUDPC scores and their variations for individuals of 19 *Solanum* species in the two years are given in Table 2.

Table 2. *AUDPC indices in 19 Solanum species in two-year field evaluations.*

Species name	Number of individual genotypes	2008 year mean	2008 year ±SD	2008 year min	2008 year max	2009 year mean	2009 year ±SD	2009 year min	2009 year max
S. chilotanum	1	0.575				0.370			
S. brachycarpum	1	0.350				0.000			
S. demissum	2	0.612	0.159	0.500	0.725	0.240	0.311	0.020	0.460
S. hougasii	1	0.500				0.270			
S. fendleri	6	0.729	0.090	0.550	0.800	0.715	0.172	0.440	0.900
S. hjertingii	5	0.570	0.186	0.350	0.775	0.454	0.312	0.150	0.890
S. papita	2	0.375	0.000	0.375	0.375	0.310	0.155	0.200	0.420
S. polytrichon	10	0.725	0.171	0.350	0.950	0.483	0.223	0.250	0.880
S. stoloniferum	7	0.571	0.266	0.175	0.925	0.344	0.242	0.000	0.650
S. wightianum	1	0.625				0.080			
S. polyadenium	1	0.600				0.040			
S. brachystotrichium	3	0.775	0.225	0.550	1.000	0.670	0.370	0.260	0.980
S. jamesii	7	0.782	0.087	0.625	0.925	0.281	0.207	0.000	0.630
S. pinnatisectum	4	0.406	0.096	0.350	0.550	0.185	0.194	0.000	0.450
S. stenophyllidium	2	0.837	0.053	0.800	0.875	0.685	0.021	0.670	0.700
S. tarnii	1	0.400				0.090			
S. cardiophyllum	12	0.679	0.150	0.475	1.000	0.293	0.215	0.080	0.710
S. ehrenbergii	10	0.719	0.096	0.525	0.800	0.565	0.237	0.200	0.910
S. bulbocastanum	2	0.137	0.053	0.100	0.175	0.100	0.141	0.000	0.200
Total	78	0.642	0.197	0.100	1.000	0.398	0.271	0.000	0.980

Variability for infestation by *P. infestans* was found both among and within *Solanum* species and in both years of trial. The rate of increase of infected leaf area across the tested material was greater in 2008 than in 2009. The average AUDPC value for the Mexican species was 0. 64 (from 0. 10 to 1. 00) in 2008 and 0. 39 (from 0 to 0. 98) in 2009 as compared to 0.58 and 0.37 respectively, in the control of *S. chilotanum*. In both years we observed a highly significant species effect. The most resistant were *S. bulbocastanum* plants, its final defoliations were not more than 20%. Some individuals of ssp. *S. brachycarpum* (k-2830), *S. demissum* (k-15173), *S. hougasii* (k-8818), *S. tarnii* (k-23936) and *S. cardiophyllum* (k-24207) were significantly resistant to the local population of *P. infestans*. Their final defoliations did not exceed 50% in both years of trial. Plants of *S. pinnatisectum* and *S. papita* displayed a different level of LB resistance. Their AUDPC values were either below or occasionally equal to those of control *S. chilotanum* plants. Plants of *S. brachystotrichium, S. stenophyllidium* and *S. ehrenbergii* were extremely susceptible and their final defoliation was almost 100%. Most individuals of those species were damaged even more than *S. chilotanum* (Table 2). All other Mexican tuber-bearing *Solanum* species showed variable degrees of damage depending on the year. Two-year observations indicated a wide-range response of *S. stoloniferum* plants LB infection. For instance, the accession k- 21618 was resistant in 2008 (AUDPC 0.175) and showed medium susceptibility in 2009 (AUDPC 0.48). On the other hand, the accession k-3336 was resistant in 2009 (AUDPC 0.00) and showed medium susceptibility in 2008 (AUDPC 0.48). Thus, the response of *S. stoloniferum* individuals under natural infection was not consistent over years. Similarly, the performance of individuals of *S. fendleri, S. polytrichon, S. hjertingii, S. jamesii* was depended on the year. Factorial ANOVA indicated that plant species and the year of testing significantly affected the final defoliation of tested accessions, whereas the interaction of these two factors was insignificant.

Laboratory trial

Classification of accessions of 19 *Solanum* species according to the results of detached leaf tests (DLT) is presented in Table 3. Of 78 accessions tested in the field trial, 64 were evaluated for resistance to the *P. infestans* isolate collected in the Leningrad region in the test performed at VIZR and 24 were evaluated at IP using the isolate collected in the Moscow region.

Table 3. *Number of individuals from 19 Solanum species in resistance categories (detached leaf tests)*

Species	Number of individual genotypes *	Resistance category **			
		R	MR	MS	S
S. chilotanum	1\				1\
S. brachycarpum	1\	1\			
S. demissum	2\1	2 \1			
S. hougasii	1\	1\			
S. fendleri	6 \3	1\	\1		5 \ 2
S. hjertingii	2\	1\			1\
S. papita	2\1		\1		2\
S. polytrichon	10\5	4\			6\5
S. stoloniferum	6\2	2\			4\2
S. wightianum	1\	1\			
S. polyadenium	1\1	1\1			
S. brachystotrichium	2\1				2\1
S. jamesii	5\1			2\	3\1
S. pinnatisectum	4\3	2\1	\1	1\1	1\
S. stenophyllidium	2\1				2\1
S. tarnii	1\1		1\1		
S.cardiophyllum	7\2	5\2			2\
S.ehrenbergii	8\1			\1	8\
S.bulbocastanum	2\1	2\1			
Total	64\24	23\6	1\4	3\2	37\12

* *VIZR\IP data*

** *R-resistant, MR-medium resistant, MS-medium susceptible, S- susceptible*

Detached leaf tests conducted at two scientific institutions produced a similar pattern. In general, the proportion of resistant and susceptible individuals greatly exceeded that of the intermediate type of reaction. The proportion of susceptible individuals in both tests was approximately equal (58 and 50 %, respectively). Similar to field trials, variation for infestation by *P. infestans* was found both among and within *Solanum* species in laboratory tests. All individuals of *S. demissum* and *S. bulbocastanum* species as well as in single accessions of *S. brachycarpum, S. hougasii, S. polyadenium* ssp. were highly resistant to infestation. All tested genotypes of *S. brachystotrichium* and *S. stenophyllidium* species as well as all controls plants were susceptible. A broader distribution of response to *P. infestans* inoculums was observed in *S. fendleri* and *S. pinnatisectum* (Table 3). The individuals of *S. demissum* (k-18521), *S. polyadenium* (k-23553), *S. pinnatisectum* (k-24239), *S. cardiophyllum* (k-16828, 24375) and *S. bulbocastanum* (k-19981) were recognized as resistant to both *P. infestans* isolates.

In general there was a good agreement between the results of the field and laboratory trials for tested genotypes. Correlation coefficients between resistance ratings obtained for genotypes tested in the two-year field experiment and infected with two *P. infestans* isolates are significant (Table 4).

Table 4. *Spearman's coefficients of correlation between field and laboratory test results: significant at P=0.05*

	AUDPC 2009	DLT of VIZR	DLT of IP
AUDPC 2008	0.500	0.391	0.491
AUDPC 2009		0.445	0.527
DLT of VIZR			0.781

The highest correlation (*r*= 0.78) was obtained when two laboratory tests were compared. The Spearman's rank order correlation is lower when comparing two field tests. A medium correlation was obtained between field and laboratory tests for each pair of experimental data (Table 4). These data suggest different levels of resistance in inoculated detached leaves and intact plants. As a whole, the results obtained by both test methods show that only the individual plants of such *Solanum* species as *S. brachycarpum, S. demissum, S. pinnatisectum, S. cardiophyllum* and *S. bulbocastanum* possess high levels of LB resistance. *Solanum* genotypes which belong to *S. brachycarpum* (k-2830), *S. demissum* (k-15173), *S. pinnatisectum* (k-24239), *S. cardiophyllum* (k- 18086, 24206, 24207, 24375) and *S. bulbocastanum* (k-19981, 21266) are resistant to late blight both in inoculated detached leaves and intact plants in the field.

Indeed, in our experiments, some genotypes resistant in the field were infected in the laboratory test: *S. pinnatisectum* (k-21955), *S. polytrichon* (k-24463) and *S. stoloniferum* (k-21547). In contrast, four individuals recognized as resistant to both *P. infestans* isolates, *S. demissum* (k-18521), *S. fendleri* (k-5751), *S. cardiophyllum* (k-16828) and *S. polyadenium* (k-23553), manifested greater resistance to the artificial than natural infection. There is more than one explanation for these phenomena. *Solanum* accessions involved in this study belong to genetically distant groups and vary in many characteristics including plant architecture and physiological status. As plants senesce, they become more susceptible to LB attack than younger plants. A strong pubescence or a widely spaced arrangement of leaves may cause some difference in foliage attack or *P. infestans* penetration in the tissue. On the other hand, in the laboratory trials, the uniform conditions of the experiment minimize the effects of plant age and morphology. However, in the latter case, the short period of plant-pathogen interaction is far from natural. Field tests, in which the observations were carried out over a longer period, reflect the reaction of intact plants and probably provide an assessment of several components of race-nonspecific resistance. However, the laboratory method can determine the race-specific reaction and allow detecting wild potato plants with non-compatible reaction to a complex race of *P. infestans*, crucial for the search for new genes for LB resistance.

Currently, there is rapid progress in mapping and isolating genes from wild potato species that confer resistance to *P. infestans* in. Molecular insight into the complex processes involved in potato-pathogen interactions is believed to be a necessary precondition for breeding for durable LB resistance (Hein *et al.,* 2009). Based on data from the field and the laboratory tests, the genotypes of *Solanum* species with related genome constitutions and contrasting responses to *P. infestans* invasion are identified within series Cardiophylla and Pinnatisecta. These *Solanum* genotypes could be a subject of further study.

CONCLUSIONS

The response of *Solanum* species to LB differed markedly depending on the test to which they were exposed. *Solanum* genotypes which possessed of resistance to late blight both in field and laboratory trials belong to *S. brachycarpum* (k-2830), *S. demissum* (k-15173), *S. pinnatisectum* (k-24239), *S. cardiophyllum* (k- 18086, 24206, 24207, 24375) and *S. bulbocastanum* (k-19981, 21266)..

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