Populations of *Phytophthora infestans* in Israel Underwent Three Major Genetic Changes During 1983 to 2000

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ABSTRACT

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In this survey, 799 isolates of *Phytophthora infestans* collected from potato crops in Israel during 1983 to 2000 were analyzed for mating type and sensitivity to metalaxyl, and 324 were analyzed for race structure. The A_2 mating type, first recorded in 1983, fully dominated the pathogen population from 1983 until 1991 (9 years). It was thereafter replaced by the A_1 mating type, which dominated the population during 1993 to 2000. Metalaxyl-resistant isolates were first recorded in 1982. During 1983 to 1991, the majority of the isolates were resistant. Isolates with intermediate sensitivity (I) to this fungicide were first observed in 1993, when both A_1 and A_2 mating types occurred in the population. The proportion of I isolates gradually increased, reaching 39 to 41% in 1997 to 1998, and then declined to ≈15% in 1999 to 2000. Pathogenicity to nine potato differential cultivars was determined for 80 potato isolates collected in 1983 to 1991, to 11 potato differentials in 173 isolates collected in 1993 to 1998, and in 71 potato isolates collected in 1999 to 2000. The first population was composed of 5 races with race 1,3,4,7,8,10 predominating (76%), the second population was composed of 19 races with race 1,3,4,7,8,10,11 predominating (63%), and the third population exhibited 42 (34 new) races with no single predominating race. RG-57 DNA fingerprinting and allozymes loci assays of 23 isolates revealed that isolates collected during 1984 to 1986 belonged to the PO-57 lineage, whereas those collected during 1997 to 1999 belonged to the RFO-39 lineage. Among isolates collected during 1993 to 1995, two unreported DNA fingerprinting patterns were found. Severe late blight epidemics occurred in tomato crops during 1998 to 2000. Of 35 tomato isolates, 28 were A_1 and only 7 were A_2. Of these tomato isolates, 94% were sensitive to metalaxyl. Almost every isolate had a different race structure on the 11 potato differentials. When inoculated onto three tomato differential cultivars, tomato isolates showed a virulence much more enhanced than potato isolates. The data suggest the Israeli population of *P. infestans* has passed through three major genetic changes during the past 18 years: in 1983, 1993, and 1999. The recent change included host specialization to tomato.

Additional keywords: epidemiology, oospores.

Late blight caused by the oomycete pathogen *Phytophthora infestans* is a devastating disease of potato and tomato worldwide (1). Severe epidemics of late blight occur in potato in Israel in both the autumn-planted crops and the spring-planted crops, especially under rainy conditions. In recent years, disease has become more severe, especially in tomato crops. Tomatoes are grown all year around, especially in net and plastic houses. *P. infestans* is heterothallic, requiring both A_1 and A_2 mating types for the production of sexual oospores. While sporangia, produced vegetatively, are the sole source of inoculum during the epidemic phase of the disease, oospores produced by mating in the field (2) may serve as an overwintering soilborne inoculum (1,33). Oospores are not only a long-lasting source of inoculum but also may supply new recombinant genotypes of the pathogen (6,7,38), with possibly altered virulence, host specialization (10,16,28), and response to phenylamide fungicides (e.g., metalaxyl) (11).

A_2 mating type isolates were first detected in Israel in 1983 (17), a few years after being recorded in Europe (18). In a previous survey in Israel by Grinberger et al. (17) during 1983 to 1989, it was found that A_2 isolates predominated the fungal population; that is, 37 out of 38 potato isolates collected from an area of ≈800 km^2 belonged to the A_2 mating type. Another survey conducted in my laboratory by Kadish (20) during 1983 to 1991 revealed that out of 544 single-lesion isolates, 69% were resistant to metalaxyl. Earlier studies made during 1954 to 1970 in Israel showed the occurrence of races 0, 1, 3, 4, 1.4, and 1.3.4, with race 4 being most frequent (25,31,36). Race structure analysis conducted in 1995 for isolates collected from potato (11) showed that about half of the isolates carried the virulence factors 1.3.4.7.8.10.11.

The aim of the present survey was to monitor some of the genetic changes (mating type, race structure, and sensitivity to metalaxyl) in the population of *P. infestans* in commercial potato and tomato crops and to possibly relate these changes to the severe epidemics recently caused by the pathogen in nature.

MATERIALS AND METHODS

Sample collection. This survey reports on samples collected during October 1983 to December 2000 from commercial fields distributed over an area of ≈3,000 km^2, from Bet-She'an County in the northeast to the Bsor County in the southwest. Blighted foliage of potato or tomato were collected by us or county agents and shipped overnight to the laboratory at Bar-Ilan University. Infected leaves were placed in moistened petri dishes at 15°C for 20 h in the dark to enhance sporulation of the pathogen. Sporangia were collected from a single leaflet into ice-cold glass-distilled water and used for inoculation. Samples were collected during the epidemic season, namely October through May. No samples were obtained during June through September except for a single case in July from tomato.

Bioassays. Sporangia from each sample (isolate) were used for three bioassays: mating type determination, compatibility with differential cultivars (virulence phenotype), and sensitivity to metalaxyl. Mating type assay was conducted as described by Cohen et al. (3). Sensitivity to metalaxyl was conducted as described by Kadish et al. (21). Pathogenicity assays were done with

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the 12 standard potato differentials, R_0 to R_{11}, obtained from U. Gisi (Syngenta Crop Protection, Stein, Switzerland). Plants were clonally reproduced in vitro from stem segments and then grown in 2-liter pots in the greenhouse. Leaflets were detached, placed in moistened petri dishes, lower side uppermost, and inoculated with six 10-µl droplets per leaflet (5,000 sporangia per ml), two leaflets per potato genotype. Plates were incubated in 15°C growth chambers with a 12-h photoperiod for 10 days. Reaction to the pathogen was assessed as either compatible (expanded lesions with sporulation) or incompatible (hypersensitive response). Virulence factors of an isolate are given as a series of numbers, representing the R-gene potato genotypes that produce compatible interaction with that isolate. In all tests, tomato leaflets (inbred ZH or cv. Baby carrying no resistance genes) were also included. For some isolates compatibility with three tomato genotypes was also determined: ZH (own inbred, no genes for resistance, *Ph-0*), New-Yorker (carrying *Ph-1*), and Pieraline (carrying *Ph-2*). The following index was used to visually assess compatibility: HR = hypersensitive response, dark-brown lesions with no sporulation; 1 = lesions, 5 to 10 mm in diameter, with scarce sporulation; 2 = lesions, 10 to 15 mm in diameter, with moderate sporulation; 3 = lesions, 15 to 20 mm in diameter, with abundant sporulation; and 4 = lesions, 20 to 25 mm in diameter, with abundant sporulation.

Allozymes and DNA fingerprinting. A subset of 23 isolates of *P. infestans* were grown in liquid rye medium. Mycelia were used for allozymes loci assays as described by Goodwin et al. (15) and for analyzing nuclear DNA fingerprint by the highly polymorphic, moderately repetitive DNA probe RG-57 (14). The octal nomenclature system was used to summarize the DNA banding data as described by Goodwin et al. (14).

RESULTS

Mating type. In all, 799 isolates obtained from commercial potato fields during 1983 to 2000 (except 1992) were analyzed for mating type (Fig. 1A) and sensitivity to metalaxyl (Fig. 1B). From its appearance in 1983 until 1991 (9 years), the A_2 mating type dominated the population of *P. infestans* in the country (Fig. 1A), composing 98 to 100% of all isolates collected (547 isolates). A sharp decline in the proportion of the A_2 mating type (to 29%) occurred in 1993 (no data available for 1992). This decline continued until 1996, when no A_2 isolates were detected in the population. In 1997 to 2000, the frequency of A_2 remained very low (0 to 4%) except for 1999 (28%). Thirteen isolates collected during January to April 2001 were all A_1 (data not shown).

Sensitivity to metalaxyl. The fungal population during 1983 to 1991 (9 years) was composed of either sensitive (S) or resistant (R) isolates (Fig. 1B). In 7 out of 9 years, R isolates predominated (58 to 83%) in the population; whereas, in the other 2 years (1984 and 1985), approximately equal proportions of S and R occurred. In 1993, two events took place: isolates with intermediate (I) resistance to metalaxyl were detected (14%) for the first time and the proportion of R declined (from 72% in 1991) to 43%. The frequency of I isolates increased until 1998 (41%) and declined toward 2000. The frequency of R isolates continued to drop until 1995 to its lowest level (7%), and then reached another peak (57%) in 1997. During 1999 to 2000, S isolates composed ≈60% of the population and I and R isolates ≈20% each. Of 13 isolates collected during January to April 2001, 6 were S and 7 were I (data not shown), with no R isolates detected.

Severe epiphytotics of *P. infestans* occurred in tomato crops during 1998 to 2000. Seven isolates were collected from tomato in 1998. All were sensitive to metalaxyl, 5 belonged to A_1, and 2 belonged to A_2 mating type. Out of 18 isolates collected in 1999, 13 were A_1 and 5 were A_2, with 16, 1, and 1 showing an S, I, and R response, respectively, to metalaxyl. In 2000, 10 isolates were collected; all were A_1 and sensitive to metalaxyl. Two isolates collected in January 2001 were also A_1 and S (data not shown).

Race structure (virulence phenotypes) of potato isolates. Whereas most isolates collected during 1993 to 2000 were tested for virulence factors, only a small number of the isolates collected during 1983 to 1991 were similarly tested. The race structure of 324 potato isolates collected during 1983 to 2000 is shown in Table 1. Data for 1983 to 1986 and 1987 to 1991 are taken from Kadish and Cohen (23) and Kadish (20), respectively. Data for each of these two periods were pooled together because year-by-year analyses were not available. No data are available for 1992. The compatibility or incompatibility of the isolates collected during 1993 to 2000 was tested on the R_0 to R_{11} potato differential lines, whereas that of the isolates collected during 1983 to 1991 was tested on only nine of the potato differentials (excluding R_5, R_6, and R_{11}).

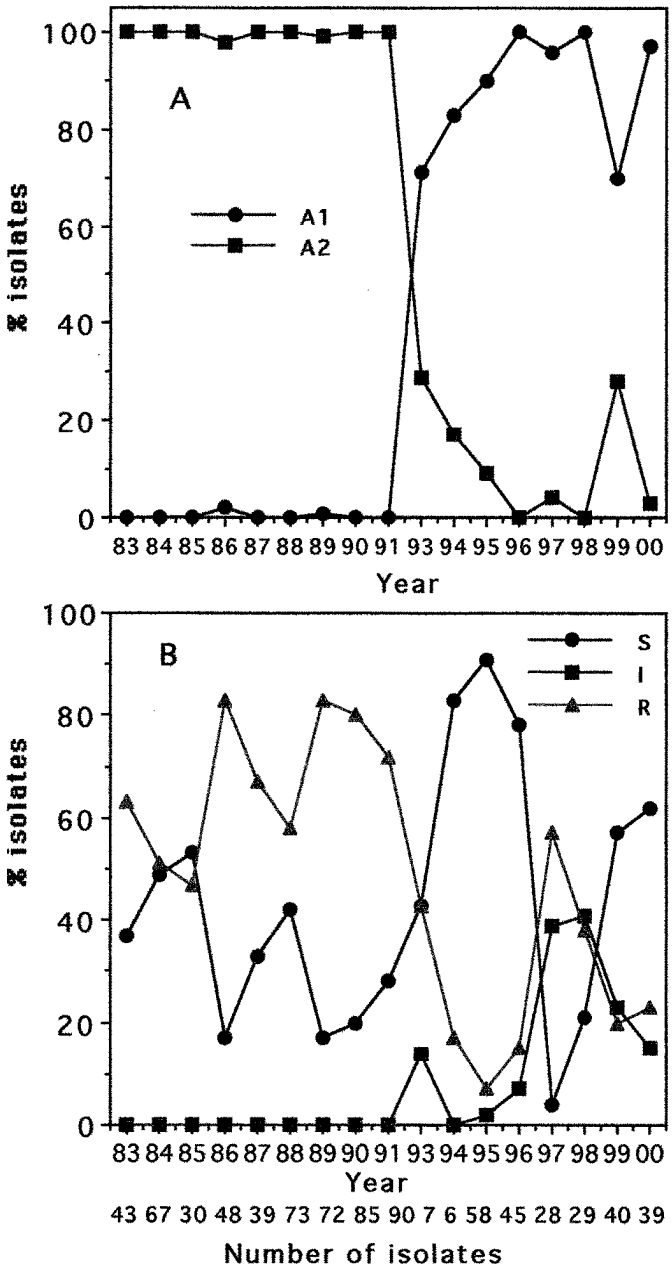

Fig. 1. Genetic changes in potato isolates of *Phytophthora infestans* during 1983 to 2000. **A,** Frequency of A_1 and A_2 mating types among 799 isolates. Data for 1983 to 1988 are from Grinberger et al. (17). **B,** Frequency of metalaxyl-sensitive isolates (S), metalaxyl-resistant isolates (R), and isolates with intermediate (I) response to metalaxyl among 799 isolates. Data for 1983 to 1991 are from Kadish and Cohen (23).

Only six isolates were tested for 1983 to 1986. Three belonged to race 1,3,4,7,8 and three to race 1,3,4,7,8,10. Among the 74 isolates collected during 1987 to 1991, only five races were detected, with 78% being 1,3,4,7,8,10 and 9% race 1,3,4,7,8. The population collected during 1993 to 1998 was mostly composed of race 1,3,4,7,8,10,11 (112 of 173 isolates, 63%). In 1999, a major change occurred in the race structure of the population: the frequency of race 1,3,4,7,8,10,11, which predominated during 1993

TABLE 1. Race structure of *Phytophthora infestans* isolates collected from potato crops during 1983 to 2000

	Years[a]										
Pathotype no., structure[b]	1983–86	1987–91	1993	1994	1995	1996	1997	1998	1999	2000	Total
3											
1 3 4	…	1	…	…	…	…	…	…	…	1	2
4											
1 3 4 7	…	2	1	…	2	…	…	…	2	…	7
1 3 4 9	…	…	…	…	…	…	…	…	…	2	2
1 3 4 11	…	…	…	…	…	…	…	…	1	…	1
1 3 7 11	…	…	…	…	…	…	…	…	1	…	1
5											
1 3 4 5 7	…	…	…	…	…	…	…	…	2	…	2
1 3 4 5 8	…	…	…	…	…	…	…	…	1	…	1
1 3 4 5 11	…	…	…	…	…	…	…	…	1	…	1
1 3 4 6 7	…	…	…	1	2	…	…	…	…	…	3
1 3 4 7 8	3	7	…	1	4	2	1	…	…	…	18
1 3 4 7 9	…	…	…	…	…	…	…	…	…	3	3
1 3 4 7 10	…	6	…	…	…	…	…	…	1	…	7
1 3 4 7 11	…	…	1	…	1	…	…	…	2	…	4
1 3 4 8 9	…	…	…	…	…	…	…	…	…	1	1
1 4 7 9 11	…	…	…	…	…	…	…	…	1	…	1
1 4 7 10 11	…	…	…	…	…	…	…	…	1	…	1
1 6 7 8 9	…	…	…	…	…	…	…	…	1	…	1
6											
1 2 3 4 8 11	…	…	…	…	…	…	…	…	…	1	1
1 3 4 5 6 7	…	…	…	…	1	…	…	…	…	…	1
1 3 4 5 7 10	…	…	…	…	…	…	…	…	2	…	2
1 3 4 5 7 11	…	…	…	…	…	…	…	…	3	…	3
1 3 4 6 7 8	…	…	1	…	1	…	…	…	…	…	2
1 3 4 6 7 9	…	…	…	…	…	…	…	…	…	1	1
1 3 4 6 7 10	…	…	1	…	…	…	…	…	…	…	1
1 3 4 6 7 11	…	…	…	…	1	…	…	…	…	…	1
1 3 4 7 8 10	3	58	…	…	…	…	…	…	…	…	61
1 3 4 7 8 11	…	…	2	1	6	…	…	…	…	…	9
1 3 4 7 10 11	…	…	…	…	5	1	…	…	7	…	13
1 3 4 8 9 11	…	…	…	…	…	…	…	…	…	1	1
1 3 4 9 10 11	…	…	…	…	…	…	…	…	…	3	3
1 4 5 6 7 9	…	…	…	…	…	…	…	…	…	1	1
7											
1 2 3 4 7 8 11	…	…	…	…	1	…	…	…	…	…	1
1 2 4 6 7 8 9	…	…	…	…	…	…	…	…	1	…	1
1 3 4 5 6 7 9	…	…	…	…	…	…	…	…	…	2	2
1 3 4 5 7 10 11	…	…	…	…	…	…	…	…	3	…	3
1 3 4 6 7 8 10	…	…	…	1	…	…	…	…	…	…	1
1 3 4 6 7 8 11	…	…	…	1	1	…	…	…	…	…	2
1 3 4 6 7 9 11	…	…	…	…	…	…	…	…	1	…	1
1 3 4 6 8 10 11	…	…	…	…	1	…	…	…	…	…	1
1 3 4 7 8 9 11	…	…	…	…	…	…	…	…	…	2	2
1 3 4 7 8 10 11	…	…	1	1	26	34	19	28	3	…	112
1 3 4 7 9 10 11	…	…	…	…	…	…	…	…	1	…	1
1 3 4 8 9 10 11	…	…	…	…	…	…	…	…	…	2	2
1 3 7 8 9 10 11	…	…	…	…	…	…	…	…	…	1	1
1 4 6 7 8 10 11	…	…	…	…	…	…	1	…	…	…	1
1 6 7 8 9 10 11	…	…	…	…	…	…	…	…	…	1	1
8											
1 2 3 4 6 7 9 11	…	…	…	…	…	…	…	…	…	1	1
1 2 3 4 6 7 10 11	…	…	…	…	1	…	…	…	…	…	1
1 2 3 4 7 8 9 10	…	…	…	…	…	…	…	…	1	…	1
1 3 4 5 6 7 8 9	…	…	…	…	…	…	…	…	…	1	1
1 3 4 5 7 8 10 11	…	…	…	…	…	…	…	…	1	…	1
1 3 4 6 7 8 10 11	…	…	…	…	4	8	7	1	1	…	21
1 3 4 7 8 9 10 11	…	…	…	…	…	…	…	…	1	2	3
9											
1 2 3 4 6 7 8 9 11	…	…	…	…	…	…	…	…	…	1	1
1 2 3 4 6 7 8 10 11	…	…	…	…	1	…	…	…	…	1	2
1 2 3 4 7 8 9 10 11	…	…	…	…	…	…	…	…	…	4	4
Isolates per year (period)	6	74	7	6	58	45	28	29	39	32	324
Virulence phenotypes	2	5	6	6	16	4	4	2	23	20	…
Isolates/phenotype	…	14.8	…	…	3.6	11.3	7.0	14.5	1.7	1.6	…

[a] Data for 1983 to 1986 were taken from Kadish and Cohen (23) and for 1987 to 1991 from Kadish (20).
[b] Virulence phenotype number and structure.

to 1998 (6 years), sharply declined (to 8%) and 17 new races appeared, indicating the evolution of a new population. Furthermore, the population of 2000 was composed of 20 races, of which 17 were new (not detected in the past 17 years, 1983 to 1999). Race 1,3,4,7,8,10,11 was not detected in 2000 nor in the additional 13 isolates collected during January to April 2001 (data not shown). Almost every second isolate collected in 1999 to 2000 belonged to a different race, whereas 3.6 to 14.8 isolates per race were found in the populations of 1983 to 1998 (Table 1).

The 324 isolates of 1983 to 2000 were grouped according to the number of virulence factors they contained (and number of combinations), their mating type (A_1 or A_2), and response to metalaxyl (S, I, or R) (Table 2).

The 1983 to 1991 population (80 isolates) carried three to six virulence factors which were added to only five combinations. Of these isolates, ≈73% carried six virulence factors. Mean number of virulence factors per isolate was 5.68. All isolates were A_2 and segregated ≈1:1 into sensitive or resistant to metalaxyl.

The 1993 to 1998 population (173 isolates) carried four to nine virulence factors in 19 different combinations. The majority of the isolates (66.5%) carried seven virulence factors and only one isolate carried nine factors. Mean number of virulence factors per isolate was 6.82. The vast majority of the isolates belonged to the A_1 mating type (95%). Of these, ≈60, 16, and 24% were S, I, and R, respectively, to metalaxyl. No A_2 I isolates were detected in this population.

The 1999 to 2000 population (71 isolates) had three to nine virulence factors which were combined into as many as 42 races, with approximately every second isolate presenting a different race (Table 1). The mean number of virulence factors per isolate was 6.31. Of the isolates, ≈80% belonged to the A_1 mating type, with ≈55, 20, and 25% responding as S, I, and R, respectively, to metalaxyl. Interestingly, only 11 of these 42 races also were detected in the tomato population, meaning that 31 races were specific to potato.

Data collected during 1987 to 1991 showed that potato tubers imported to Israel from Europe carried 11, mostly simple, races of *P. infestans* (20). Out of 29 isolates collected (14 sensitive and 15 resistant to metalaxyl), 22 represented five races and carried two to three virulence factors, 6 had four to five factors, and only 1 had the race structure 1,3,4,7,8,10 (20).

Comparative data on the frequency of appearance of the 11 virulence factors in the three populations collected from potato during 1983 to 1991, 1993 to 1998, and 1999 to 2000 is shown in Table 3. The frequency of virulence factors 2, 5, and 9 increased, whereas that of 7, 8, 10, and 11 decreased.

DNA-fingerprinting and allozymes of potato isolates. Twenty-three isolates were examined for fingerprinting with the DNA RG-57 probe (14) and allozymes loci of peptidase (*Pep*) and glucose-1,6-phospho-isomerase (*Gpi*) (15). Nine isolates collected during 1984 to 1986 belonged to the PO-57 lineage found in Poland and Russia (8,13) (Table 4). Four isolates sampled during 1993 to 1995 retained their allozymes loci but three exhibited two different patterns of RG-57 banding which do not occur elsewhere (8). Still another change occurred in the 10 isolates collected during 1997 to 1999. They all belonged to lineage RFO-39 found in the United Kingdom and Western Europe (D. Shaw, *personal communication*).

Race structure of tomato isolates. Late blight in tomato was a minor disease in Israel until recently. Two A_1 isolates were obtained in 1997, and five A_1 and two A_2 isolates in 1998. In 1999 and 2000, severe epiphytotics developed in tomato crops grown in plastic houses and net houses, especially in the northwestern Negev (Bsor County). Of the 28 isolates collected during 1999 to 2000, 23 belonged to the A_1 and 5 to the A_2 mating type (Table 5). All except two isolates from tomato were sensitive to metalaxyl

TABLE 3. The frequency of appearance of 11 virulence factors in potato and tomato isolates of *Phytophthora infestans* collected during 1983 to 2000

	Frequency of appearance (%)[a]			
	Potato			Tomato
Virulence factor	1983–1991 (n = 80)	1993–1998 (n = 173)	1999–2000 (n = 71)	1998–2000 (n = 32)
1	100	100	100	100
2	0	1.7	12.3	15.6
3	100	99.4	86.3	93.8
4	100	100	89	90.6
5	nt	0.6	23.3	21.9
6	nt	20.2	17.8	25.0
7	98.8	99.4	65.7	84.3
8	74.4	89.6	39.7	15.6
9	0	0	54.8	62.5
10	78.0	81.5	50.7	34.4
11	nt	60.2	5.5	53.1

[a] nt = not tested.

TABLE 2. Race structure of 324 potato isolates of *Phytophthora infestans* collected during 1983 to 2000 grouped according to mating type (A_1 or A_2) and response to metalaxyl

	Virulence phenotype		Number of isolates[a]						
Period	Number	Structures	A_1S	A_1I	A_1R	A_2S	A_2I	A_2R	Total
1983–91	3	1	…	…	…	1	…	…	1
	4	1	…	…	…	1	…	…	2
	5	2	…	…	…	10	…	9	19
	6	1	…	…	…	26	…	32	58
Total		5	…	…	…	38	…	42	80
1993–98	4	1	1	…	1	1	…	…	3
	5	3	10	…	1	2	…	…	13
	6	6	12	2	3	2	…	1	20
	7	6	62	24	27	1	…	1	115
	8	2	11	2	7	…	…	1	21
	9	1	1	…	…	…	…	…	1
Total		19	97	28	39	6	…	3	173
1999–2000	3	1	1	…	…	…	…	…	1
	4	4	3	…	2	…	…	1	6
	5	10	7	2	1	1	…	3	14
	6	8	6	3	3	2	3	2	19
	7	10	9	3	4	…	1	…	17
	8	6	6	…	1	1	…	…	8
	9	3	3	2	1	…	…	…	6
Total	…	42	35	10	12	4	4	6	71

[a] S = sensitive, I = intermediate, R = resistant to metalaxyl.

(Table 5). Virulence analysis of these isolates on potato differentials showed (Table 5) that only six isolates were simple races carrying three to four virulence factors. The number of isolates carrying five, six, seven, eight, and nine virulence factors was nine, four, seven, four, and two, respectively, with a mean of 5.97 virulence factors per isolate. More than half of the isolates were compatible with the R-9 differential potato. Isolates varied greatly in race structure, with 32 of them belonging to 25 different races (Table 5), thus presenting a mean of 1.3 isolates per race. The frequency of appearance of the 11 virulence factors in the tomato population is given in Table 3. Most frequent were factors 1, 3, 4, 7, and 9 and least frequent were 2, 5, 6, and 8. Relative to the 1999 to 2000 potato population, a lower frequency of factor 8 and a higher frequency of factor 11 was observed (Table 3) in the tomato population. Of the 25 tomato races, 11 also were common to the potato population, whereas 14 races were specific to tomato.

Compatibility of potato and tomato isolates with tomato. Three tomato differential cultivars, ZH, New-Yorker, and Piera-

TABLE 4. RG-57 DNA fingerprints and allozymes loci of 23 *Phytophthora infestans* isolates collected from potato crops during 1984 to 1999

				DNA bands[a]										
Year	Isolate	Type[b]	*n*	1 2 3	4 5 6	7 8 9	10 11 12	13 14 15	16 17 18	19 20 21	22 23 24 25	Octal	*Pep*	*Gpi*	Lineage
1984–86	…	A_2 S	4	100	110	000	100	110	100	011	001 1	130, 131, 641[c]	100/100	100/100	PO-57
1984–86	…	A_2 R	5	100	110	000	100	110	100	011	001 1	130, 131, 641	100/100	100/100	PO-57
1993	KS	A_1 S	1	101	011	111	100	110	100	111	101 1	567, 131, 751[d]	100/100	100/100	RFO-39
1993	RE	A_2 R	1	101	010	000	100	110	100	111	101 1	520, 131, 751	100/100	100/100	Unknown
1994	EH	A_2 R	1	100	011	000	000	110	100	000	10 11	160, 031, 051	100/100	100/100	Unknown
1995	EHF	A_2 S	1	100	011	000	000	110	100	001	1011	160, 031, 451	100/100	100/100	Unknown
1997	GED	A_1 R	1	101	011	111	100	110	100	111	1011	567, 131, 751	92/100	100/100	RFO-39
1998	BIU	A_1 I	7	101	011	111	100	110	100	111	1011	567, 131, 751	92/100	100/100	RFO-39
1999	BIU	A_1 I	1	101	011	111	100	110	100	111	1011	567, 131, 751	nd[e]	nd	RFO-39
1999	BIU	A_2 I	1	101	011	111	100	110	100	111	1011	567, 131, 751	nd	nd	RFO-39

[a] 0 = negative (no band); 1 = positive.
[b] S = sensitive, R = resistant, I = intermediate.
[c] Determined by Goodwin to belong to PO-57 found in Poland and Russia (8,13).
[d] Determined by D. Shaw to belong to RFO-39, common in the United Kingdom and Western Europe (D. Shaw, *personal communication*).
[e] nd = not determined.

TABLE 5. Race structure of *Phytophthora infestans* isolates collected from tomato crops during 1998 to 2000 grouped according to mating type and response to metalaxyl[a]

	1998		1999				2000	
Phenotype no., structure[b]	A_1S	A_2S	A_1S	A_1I	A_1R	A_2S	A_1S	Total
Unknown	…	2	1	…	…	…	…	3
3								
1 3 4	…	…	…	…	…	1	…	1
4								
1 3 4 7	1	…	…	…	1	…	…	2
1 3 4 9	1	…	…	…	…	…	…	1
1 3 4 10	…	…	…	…	…	…	1	1
1 3 7 9	…	…	…	…	…	…	1	1
5								
1 3 4 5 7	…	…	…	1	…	…	…	1
1 3 4 5 10	…	…	1	…	…	…	…	1
1 3 4 7 9	2	…	1	…	…	…	1	4
1 3 4 7 10	…	…	1	…	…	…	…	1
1 3 4 10 11	…	…	…	…	…	1	…	1
1 3 5 7 9	…	…	…	…	…	…	1	1
6								
1 3 4 7 9 11	1	…	…	…	…	…	2	3
1 3 4 7 10 11	…	…	…	…	…	1	…	1
7								
1 2 3 6 7 9 11	…	…	…	…	…	…	1	1
1 2 4 6 7 9 11	…	…	1	…	…	…	…	1
1 3 4 5 7 10 11	…	…	…	…	…	1	…	1
1 3 4 6 7 8 9	…	…	1	…	…	…	…	1
1 3 4 6 7 9 11	…	…	2	…	…	…	…	2
1 3 4 7 8 9 11	…	…	…	…	…	…	1	1
8								
1 2 3 4 5 7 10 11	…	…	1	…	…	…		1
1 2 3 4 6 7 9 11	…	…	1	…	…		…	1
1 2 4 5 6 7 10 11	…	…	…	…	…	1	…	1
1 3 4 7 8 9 10 11	…	…	…	…	…	…	1	1
9								
1 3 4 5 7 8 9 10 11	…	…	…	…	…	…	1	1
1 3 4 6 7 8 9 10 11	…	…	1	…	…	…	…	1
Total isolates	5	(2)	10 (+1)	1	1	5	10	35
Virulence phenotypes	4	…	10	…	…	5	9	…
Isolates/phenotype	1.2	…	1.0	…	…	1.0	1.1	…

[a] S = sensitive, I = intermediate, R = resistant.
[b] Virulence phenotype number and structure.

line, carrying genes *Ph-0*, *Ph-1*, and *Ph-2*, respectively, for resistance against tomato late blight, were inoculated with each of 25 potato or 22 tomato isolates. Results indicate a large difference between the two populations (Table 6). Whereas one, two, and six potato isolates were incompatible with *Ph-0*, *Ph-1*, and *Ph-2*, respectively (produced hypersensitive necrotic lesions with no sporulation), none of the tomato isolates was incompatible with any of the tomato lines. All tomato isolates were highly compatible with *Ph-0* and *Ph-1*, whereas potato isolates were moderately compatible with the two lines carrying these genes. All isolates had a reduced compatibility with *Ph-2*, much more so for the potato than for the tomato isolates (Table 6).

A summary of the major traits of *P. infestans* in Israel during 1983 to 2000 is given in Table 7.

DISCUSSION

We show here that three major waves of genetic changes have taken place during the past 18 years in the population of *P. infestans* in Israel.

The first major change occurred in 1983, when the A_2 mating type was first detected (17) in the country. The A_2 mating type dominated the pathogen population for the next 9 years. In 1982, the first metalaxyl-resistant (R) isolates were detected (4). The A_2 mating type, naturally occurring in Mexico (29), was first reported in the western Hemisphere in 1980 (18). The appearance of A_2 in Israel has probably resulted from the panglobal distribution of the A_2 mating type after it was released in 1976 (9,13) from Mexico to Europe. Israel has no potato or tomato trade with its neighboring countries, Egypt, Jordan, or Syria, indicating that migration of *P. infestans* from these countries is unlikely. The intensive annual potato seed tuber import to Israel from Western Europe may explain the "landing" of A_2 in Israel. However, whereas the mean frequency of A_2 in European countries ranged between ≈20 and 30% (11), it was constantly stable at a frequency of 100% in Israel (except 98% in 1986) until 1991, indicating that either only the A_2 genotype was transported from Europe or, if both A_1 and A_2 were transported, the A_1 was much inferior to A_2 under the Israeli conditions and did not survive. It seems imperative also to assume that the introduced A_2 genotype had a much higher fitness than the population (probably A_1) occurring in Israel prior to 1983, so as to strongly suppress it.

Before the 1980s, worldwide populations of *P. infestans* were dominated by a single clonal lineage, the US-1 genotype or Ib mitochondrial DNA (mtDNA) haplotype (30). This lineage has since been displaced by the other known modern haplotypes Ia, IIa, and IIb (30). A subset of 27 Israeli isolates collected during 1984 to 2000 were found to belong to Ia (41%), IIa (56%), and IIb (3%) (data not shown), thus reaffirming the possible displacement of the Israeli population during the late 1980s.

In spite of its high fitness, the frequency of isolates belonging to the A_2 mating type sharply declined in 1992 to 1993, until it was totally undetectable by 1996. A partial reemergence of A_2 isolates occurred in 1997 to 2000. Isolates collected during January to April 2001 were all A_1 (data not shown). A similar shift from A_2 to A_1 was reported in Sinaloa, Mexico (19).

The reemergence of A_1 in 1992 to 1993 may have been another new introduction from Europe, unless we assume that the local A_2 changed by selfing to A_1 (26). The new A_1 must have had a higher fitness than the predominating A_2 it had replaced. Indeed, as mentioned above, although A_1 and A_2 cosurvived in Europe since the 1980s, the A_2 frequency ranged between 20 and 30% and never exceeded 44% (in Poland in 1989) (11,34,37) and declined toward 1995 in most European countries (11).

Goodwin et al. found that nine A_2 potato isolates he obtained from our 1984 to 1986 potato collection (12) belonged to the PO-57 lineage prevailing in Poland and Russia (13). PO-57 is A_2, has DNA fingerprinting (octal 130, 131, 641) and allozymes identical to our isolates, but was first observed in Poland much later (in 1990) than in Israel (34). Interestingly, isolates collected in Estonia (34) in 1983 (lineage PO-4) show DNA fingerprinting similar to our 1984 to 1986 isolates (except one band difference) but are A_1.

Parallel changes were observed in the response of *P. infestans* to metalaxyl. Resistance was first observed in 1982 (4). During 1983 to 1991, when all isolates were A_2, the mean frequency of metalaxyl-resistant versus metalaxyl-sensitive isolates was 67 and 33%, respectively, with resistant isolates being much fitter to potato than sensitive ones (22,24). The first appearance of metalaxyl-intermediate isolates occurred in 1993 (no data available for 1992), concurrent with the reemergence of A_1 isolates.

Although oospores may develop in potato crops in Israel (2), it is not clear if this appearance of I isolates was a consequence of local mating (I isolates are often offspring of sexual mating

TABLE 6. Compatibility of potato and tomato isolates of *Phytophthora infestans* with tomato differential cultivars carrying genes for resistance against late blight[a]

	Proportion of isolates showing compatibility index[b]									
	Potato isolates (n = 25)[c]					Tomato isolates (n = 22)[d]				
Tomato differential	HR	1	2	3	4	HR	1	2	3	4
Ph-0 (ZH)	4	8	16	16	56	0	0	0	0	100
Ph-1 (NewYorker)	8	8	16	16	52	0	0	0	0	100
Ph-2 (Pieraline)	24	16	40	16	0	0	18	68	14	0

[a] Tests were conducted with detached leaflets floating on water at 17°C.
[b] The 1-to-4 scale represents the lesion size and abundance of sporangial production at 7 days postinoculation, where HR = hypersensitive response, dark brown lesions with no sporulation; 1 = lesions, 5 to 10 mm in diameter, with scarce sporulation; 2 = lesions, 10 to 15 mm in diameter, with moderate sporulation; 3 = lesions, 15 to 20 mm in diameter, with abundant sporulation; and 4 = lesions, 20 to 25 mm in diameter, with abundant sporulation.
[c] Isolates collected during 1993 to 2000.
[d] Isolates collected during 1997 to 2000.

TABLE 7. Summary of the characteristic of *Phytophthora infestans* in Israel during 1983 to 2000[a]

				Allozyme loci			Races	
Period	Major hosts	Mating type	Response to metalaxyl	*Pep*	*Gpi*	Lineage	Number of	Predominant
1983–1991	Potato	A_2	R (+S)	100/100	100/100	P0-57	5	1 3 4 7 8 10
1993–1997	Potato	$A_1 + A_2$	S (+R)	100/100	100/100	New + R0F-39	19	1 3 4 7 8 10 11
1998–2000	Potato + tomato	A_1 ($+A_2$)	S + I (+R)	92/100	100/100	R0F-39	42	None

[a] Figures in parenthesis indicate a low proportion in the population. S, I, and R = sensitive, intermediately resistant, and resistant to metalaxyl, respectively.

between S and R isolates) (11,20,32) because little A_1 occurred in the country to enable such mating (unless we assume that A_1 had already reemerged in 1992). Intermediate isolates which were rare in Switzerland, for example, in 1988 to 1990 were doubled in 1992 to 1993 and further increased to 30% in 1994 to 1995 (11). It seems, therefore, that the A_1, which was reintroduced to Israel in 1992 to 1993, was either metalaxyl-sensitive, -intermediate, or both. S, I, and R subpopulations probably derived from mating between A_1 and A_2 isolates whose proportions fluctuated during 1993 to 2000 (Fig. 1B).

Much effort was devoted in this study to determine the virulence phenotypes (race structure) present in the local population of *P. infestans*. In the 1950s and 1960s, race 4 was frequent and, to a lesser extent, races 0, 1, 3, 1.4, and 1.3.4 (25,36). In the 1970s, race 0 was still present (31).

Isolates we collected from potato during 1983 to 1986 (24) were 1.3.4.7.8 or 1.3.4.7.8.10. During 1987 to 1991, only five races were found (on nine potato differentials) among 74 isolates collected from potato. Of these, 78% were 1,3,4,7,8,10 and the rest carried three to five virulence factors. Kadish (20) made parallel tests (1987 to 1991) with isolates he collected from tubers imported to Israel from western Europe. Amongst 29 isolates, 18 carried two virulence factors and only one isolate from Europe was 1,3,4,7,8,10. The predominance of this race in Israel during 1987 to 1998 may be attributed to a local evolvement, import, or both. Although potato R_{11} was not included in 1983 to 1986 or in 1987 to 1991 (24), it may be possible that virulence factor 11 was also present in these populations.

Race structure of the 1993 to 1998 populations from potato was mostly (63%) composed of virulence factors 1, 3, 4, 7, 8, 10, and 11, with the rest (37%) of the population exhibiting 18 other virulence combinations. The predominant race was distributed at a ratio of 2:1:1 among S/I/R isolates of the prevailing A_1 mating type. The population collected during 1999 to 2000 presented 42 race structures among 71 isolates from potato, with almost every second isolate belonged to another race. Similar variability in genetic structure was observed by U. Gisi (*personal communication*) for isolates collected in 1996 to 1997 in France and Switzerland. Our races were highly complex, having up to nine virulence factors. The reasons for this complexity are not clear. The fact that ≈30 different cultivars of potato are annually imported from Europe and commercially grown in the country may have contributed to it. The major changes occurred in factors 2, 5, and 9, whose frequencies were increased, as against factors 7, 8, and 10, which have declined. A different situation occurred in Poland, where factor 9 was not detected among 1,177 isolates collected during 1987 to 1998 and factor 5 was present at a frequency of only 5% (35), or in North America (16), where the older clonal lineages, US-1 and US-6, had much more pathogenic diversity than the more recently immigrated genotypes US-7 and US-8.

A recent study conducted in 1998 to 1999 by Derie and Inglis (5) in western Washington revealed highly complex races in isolates of US-7, US-8, and US-14 genotypes (8 to 9.3 virulence factors). Factor 9 was the rarest (4 of 109 isolates, 3.7%). These authors indicated that unnecessary virulences have been maintained in the population since 1990. Whether a similar persistence of virulences will hold in Israel remains to be studied in the future.

Of special interest are the characteristics of the tomato isolates. Heavy epidemics of late blight occurred in tomato during 1999 to 2000. Heavy epidemics were recently reported in California (10) and Europe (27). Almost all our isolates (80%) were A_1, thus performing a different situation compared with Europe (27) or the United States (28), where A_2 was equally or preferentially isolated from tomatoes. During that period, a similar ratio of 87:13 between A_1 and A_2 also was recorded in our potato isolates.

The 32 tomato isolates examined were composed of 25 races (on potato differentials), of which 14 were specific to tomato. More than half were complex races containing seven to nine virulence factors, unlike tomato isolates in France and Switzerland, which were found to be simple races (U. Gisi, *personal communication*). The tomato and potato populations collected during 1999 to 2000 showed similar frequencies of virulence factors except factor 8 (which was lower in tomato) and factor 11 (which was higher in tomato). Apparently, factor 11 in tomato showed a frequency (≈60%) similar to that of the potato population collected earlier (1993 to 1998) (Table 3). Moreover, all our tomato isolates, but not potato isolates, profusely sporulated on leaves of the tomato differentials carrying the genes *Ph-0* or *Ph-1* for resistance against tomato late blight, with none of them showing HR with *Ph-2*. Such race complexity and aggressiveness may partially explain the severe epidemics caused by these isolates in tomato crops in Israel. Goodwin et al. (16) showed that most US-1 isolates did not infect tomato. With the widespread distribution of the immigrated US-6 and US-7 genotypes, however, late blight epidemics on potato could serve as a source of inoculum for tomato fields (16). Legard et al. (28) suggested that aggressiveness of *P. infestans* to tomato may be a recently acquired trait. Tomato-aggressive isolates caused severe disease on both potato and tomato, but significantly more disease on tomato than on potato (28). We assume that tomato isolates in our country were locally evolved from the potato population and acquired specialization to tomato. Whether this specialization took place via sexual recombinations is not known.

Taken together, the Israeli population of *P. infestans* varied greatly during the 18-year period of this study. Major changes have occurred in 1982 to 1983, when resistance to metalaxyl and A_2 were first introduced; in 1992 to 1993, when the A_2 population was replaced by A_1 while isolates with intermediate sensitivity to metalaxyl first occurred; and in 1999 to 2000, when an A_1-sensitive population with extreme virulence variation and high aggressiveness to tomato dominated. These shifts may partially reflect the changes that occurred in Europe, from which potato tubers are annually imported, but also implicate a rapid local evolution of new genotypes.

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