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Wild tuber-bearing species of *Solanum* and incidence of *Phytophthora infestans* (Mont.) de Bary on the Western slopes of the volcano Nevado de Toluca. 3. Physiological races of *Phytophthora infestans*

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Summary

A survey of races of *Phytophthora infestans* was made in 1985 and 1986 on the western slopes of the volcano Nevado de Toluca, Mexico. Isolates were collected from infected plants of wild *Solanum* species found in five long (7–10 km) and some short (300–500 m) transects. The study are of 15000 ha was between 2900 and 3900 m altitude. Isolates were collected from *Solanum demissum* (35 isolates), *verrucosum* (11), *iopetalum* (2), *brachycarpum* (7), *stoloniferum* (2), r-genotype (9) and from differential hosts (11).

No isolate was identified as race 0. Ten virulence genes were identified with a set of differential hosts and the most common were numbers 2 and 4. Isolates with eight virulence genes were the most common and those with ten were found on eight occasions in the natural habitat and on three in the experimental fields.

Introduction

Although physiological races of *Phytophthora infestans* (Mont.) de Bary have been studied extensively in agriculture (Graham, 1955; Doling, 1956; Gallegly & Eichenmuller, 1959; Malcolmson, 1969 and 1979; Shattock et al., 1977; Schöber, 1983; Huerta-Miranda, 1977) few studies have been made of their occurrence in natural environments.

The first observation of *P. infestans* attacking wild tuber-bearing species of *Solanum* (WSS) was made by Reddick (1932). Later Niederhauser & Mills (1953) and Mills & Niederhauser (1953) suggested that *P. infestans* in the Toluca area of Mexico might have a level of virulence unequalled elsewhere in the world. Studies since made on race differentiation in WSS in this area also indicated a high level of complexity (Graham et al., 1959; Fernandez, 1985). The potential for genetic recombination between the compatibility types A1 and A2, as demonstrated by Romero & Erwin (1959) and Galindo & Gallegly (1960), partly explains the readiness of the fungus to form physiological races.

The purpose of the present investigation was to identify virulence genes and physiological races of the fungus found in WSS in an area little influenced by agriculture.

The work forms part of a study to investigate how WSS survives with the fungus in their natural habitat (Rivera-Peña 8 Molina Galan, 1989; Rivera-peña, 1990a).

Materials and methods

The area surveyed was described by Rivera-Peña & Molina Galan (1989) and the incidence of late blight investigated by Rivera-Peña (1990a). Isolates were collected during 1985 and 1986 and all tests were made at the Experimental Station of Toluca Valley (CIDAGEM) in Mexico.

Infected plant parts were washed with demineralized water, incubated in Petri dishes or plastic bags for 24 h at room temperature, and sporangia collected by rinsing sporulating lesions with demineralized water; zoospore liberation was enhanced by incubating 4 – 5 °C for 75 min. Each isolation was made from a single small lesion on a leaflet or stem by selecting only those lesions believed to have originated from one primary infection. Most isolates were subcultured onto detached leaves of cv. Lopez. Virulence genes were identified using a set of differential hosts representing resistance genes R1 to R11 (excluding R6) and using cv. Lopez as susceptible check (r). Detached leaflets were placed in plastic boxes on a sheet of moistened cotton and plastic net, and each genotype was represented by five leaflets with two or three replications. If necessary a second test was made by inoculum from R-genotypes. Identification of races was made according to the phenotypic reaction of R-genotypes with single R-genes or combinations (Black et al., 1953).

For short term storage, isolates were maintained on leaflets of cv. Lopez. For longer storage cultures were maintained on V8-agar (Galindo & Gallegly, 1960) covered with mineral oil.

In separate experiments, in 1986 at four sites, differential host plants grown in the glasshouse were planted in the open adjacent to colonies of WSS.

Results

Among the differential host plants grown at three sites on the slopes of the volcano in 1986, the susceptible (r) check was infected by late blight at all sites. Also, most genotypes with a single R-gene were infected at all sites; R11 was attacked only at two sites, and R8 and R9 were not infected. All genotypes containing two or more of the genes R1 – R4 were found to be infected at least in one site. The first symptoms were noted in the (r) genotype on May 5 at Mezon Viejo (MEZ) (2700 m), on June 6 at San Juan (3000 m) and June 25 at Raices (3500 m). At the Toluca Experimental Station (TOL) symptoms appeared in the (r) genotype on June 20. Infections were first observed on July 7 in the most complex genotype R1R2R3R4. Readings were not complete because plants were often damaged by animals.

Seventyone of 77 isolates obtained in 1985 and 1986 from 81 samples of the infected plants of WSS, differential host plants, and cultivars (Rivera-Peña, 1990a), were examined in the laboratory. Most of the isolates had been obtained from the predominant species, *S. demissum (dms)* but none of those examined came from *S.* × *edinense (edn).* Six isolates came from lesions obtained from the experimental fields at Toluca.

The incidence of the different virulence genes and the complexity of races were similar in 1985 and 1986 (Tables 1 and 2) and data given in Tables 3 and 4 are means over those two years.

Table 1. Incidence of different virulence genes in 1985 and 1986.

	Virulence genes									
	1	2	3	4	5	6	7	8	9	10
1985 (28 isolates)										
Number of isolates	16	23	19	23	17	22	9	3	16	17
Relative frequency	0.57	0.82	0.68	0.82	0.61	0.79	0.32	0.11	0.57	0.61
1986 (49 isolates)										
Number of isolates	37	37	33	39	32	36	24	17	36	35
Relative frequency	0.75	0.75	0.67	0.80	0.65	0.73	0.49	0.35	0.73	0.71

Table 2. Relative frequencies (f) of the genotypes of 48 physiological races of 78 isolates of *Phytophthora infestans* 1985 and 1986.

Race	*f*	Race	*f*
1	0.013	1.2.3.5.7.11	0.013
4	0.013	2.3.4.7.8.11	0.026
5	0.013	2.3.4.7.10.11	0.026
8	0.013	1.2.3.4.5.7	0.013
11	0.013	1.2.4.5.10.11	0.013
1.5	0.013	1.2.4.5.7.11	0.013
2.4	0.026	1.2.3.4.9.10	0.013
2.7	0.013	1.2.4.5.7.10.11	0.026
4.7	0.013	1.2.3.4.5.10.11	0.013
5.10	0.013	1.2.3.4.8.10.11	0.013
1.2.7	0.013	2.3.4.7.8.10.11	0.013
2.4.7	0.013	1.3.4.5.7.8.10	0.013
2.10.11	0.013	1.2.3.4.5.7.10.11	0.150
7.10.11	0.013	1.2.3.4.5.7.8.10	0.013
4.8.9	0.013	1.2.3.5.7.8.10.11	0.013
2.3.4.7	0.013	1.2.3.4.5.8.10.11	0.013
5.7.10.11	0.013	1.3.4.5.7.8.10.11	0.013
7.9.10.11	0.026	1.2.3.4.8.9.10.11	0.013
1.3.4.7	0.013	1.2.3.4.5.7.8.10.11	0.060
1.2.3.4.7	0.013	1.2.3.4.5.7.89.10	0.013
1.2.3.4.8	0.013	1.2.4.5.7.8.9.10.11	0.013
1.2.4.5.7	0.013	1.2.3.4.5.7.8.9.11	0.013
1.2.3.4.10	0.013	1.2.3.4.5.7.8.9.10.11	0.140
2.3.4.5.7	0.013		
5.7.8.9.11	0.013	Total 48 races	1.000

No isolate was identified as race 0 but all other virulence genes that could be identified with the set of differentials used (1 to 11, excluding 6) were present both on the slopes of the volcano and in the experimental fields (Table 3). Most virulence genes identified were present in 60–70% of the isolates. Virulence genes 2 and 4 were the

Table 3. Incidence of different virulence genes in 1985 and 1986 as related to altitudes.

Altitude (m)	Number of isolates	Virulence genes									
		1	2	3	4	5	7	8	9	10	11
3500	13	6	11	6	11	4	8	3	3	7	6
3400	0	0	0	0	0	0	0	0	0	0	0
3300	13	8	10	9	11	8	10	3	1	9	10
3200	1	0	1	1	1	0	1	1	0	0	1
3100	12	5	6	6	6	8	9	6	5	6	6
3000	17	16	15	15	16	16	15	9	4	16	15
2900	13	11	11	8	10	9	9	7	3	9	8
2800	1	1	1	1	1	0	1	0	0	0	0
2700	1	0	0	0	0	0	0	0	0	0	1
Total	71	47	55	46	56	45	53	29	16	47	47
Relative frequency		0.66	0.77	0.65	0.79	0.63	0.75	0.40	0.22	0.66	0.66
2600 (TOL)	6	6	5	6	6	4	5	4	4	5	5

Table 4. Incidence of different virulence genes in 1985 and 1986 as related to host species/genotypes.

Species	Number of isolates	Virulence genes									
		1	2	3	4	5	7	8	9	10	11
S. demissum	35	24	24	26	27	24	27	22	10	26	26
S. verrucosum	11	1	8	2	8	1	7	2	0	3	5
S. iopetalum	2	2	2	2	2	2	2	2	2	2	1
S. brachycarpum	7	7	7	7	7	5	5	2	3	6	4
S. stoloniferum	2	2	2	2	2	2	2	0	0	2	2
r-host	9	6	6	3	5	4	4	0	0	3	4
R-host	11	11	11	10	11	11	11	5	5	10	10
Total	77	53	60	52	62	49	58	33	20	52	52
Relative frequency		0.69	0.78	0.67	0.80	0.64	0.75	0.43	0.26	0.67	0.67

most frequent and occurred in 77% and 79% of the isolates respectively. Virulence gene 9 was the least frequent and was found in only 22% of the isolates; also gene 8 was infrequent (40% of isolates).

The number of virulence genes in the isolates ranged from 1 to 10, the most complex which could be identified with the differentials available (Table 2). Eight of the isolates were able to infect all the differentials. The most abundant isolates were those with 8, 9 or 10 (or more) virulence genes. Eight virulence genes were found in 15 out of 71 isolates (21%) and 9 or 10 each in 8 (11%) respectively.

No differences in frequency of the different genes were detected in the six isolates obtained late in the season from the Experimental Station but all were complex and three had 10 or more virulence genes.

Of the 15 isolates with 8 virulence genes, 11 had the complexity 1.2.3.4.5.7.10.11 and this was the most common race found on the slopes of the volcano. Eight isolates had the virulence genes 1.2.3.4.5.7.8.9.10.11. There were no differences with altitude in frequency of the virulence genes or of race complexity (Table 3).

All virulence genes were found in isolates from all *Solanum* species (Table 4) and although complex races were found in isolates from all species, those from *S. verrucosum (ver)* tended to be less complex (1 to 7 virulence genes) than those from *dms*. Three of the five simple races identified were found in isolates collected from susceptible *S. tuberosum* cultivars early in the season.

Discussion

Virulence genes appeared to be evenly distributed over the area on the slopes of the volcano where wild *Solanum* species were found. The complexity of races was very high irrespective of altitude although the distribution of most species and plant spacing were influenced by altitude (Rivera-Peña & Molina Galan, 1989). The population of *P. infestans* appeared to be similar and very diverse to the upper limit of its distribution.

There was some indication of a progression from simple to complex races over the growing period but previously Huerta (1977) found no defined pattern in the occurrence of races in Toluca and Chapingo, and Fernandez (1985) reported that the occurrence of races was independent of time during the growing season in *S. cardiophyllum* and cultivated potatoes at Chapingo and Aguascalientes. At the Toluca Experimental Station, investigations by the International Potato Center (Anon., 1976, 1977) showed that races compatible with the less common R-genes (R5, R8, R10) did not appear until late in the season, and that the most complex race 1.2.3.4.5.7.8.10.11 did not appear until halfway through the late blight period.

Race 0 was not found in this survey; Huerta (1977) found it in cultivated potatoes at Toluca whereas Fernandez (1985) did not. The present investigation seems to confirm that race 0 is no longer present in the area. A similar disappearance of the race was reported in Germany (Schöber, 1983) and it has been suggested that the proportion of agricultural land planted with potato cultivars containing specific resistance to late blight has increased. In Mexico, since 1960, there has also been a large increase in the potato acreage.

The frequency of virulence genes found in this survey partly confirms observations made in 1976/1977 at the Experimental Station (Anon., 1976); race 4 was the most common in the natural habitat and races 8 and 9 among the least common.

Genetic recombination and oospores are known to occur in Mexico (Romero & Erwin, 1967) but it is not known what influence oospore formation has on the diversity of races in comparison with inoculum originating from isolates surviving from year to year or with air-borne inoculum of outside origin. Oospore formation was not detected in this survey (Rivera-Peña, 1990a). The complexity of races found early in the season and the similarity in frequencies of virulence genes between the two years of investigation (Table 1) may indicate that inoculum survives in the natural habitat and that there is little selection pressure against particular races.

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