Fitness and Virulence of *Phytophthora infestans* Isolates from Sexual and Asexual Populations

P. W. Tooley, J. A. Sweigard, and W. E. Fry

Department of Plant Pathology, Cornell University, Ithaca, NY 14853.
Current address of first author: U.S. Department of Agriculture, Agricultural Research Service, Ft. Detrick Building 1301, Frederick, MD 21701.
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ABSTRACT

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Isolates of A1 mating type of *Phytophthora infestans* from Mexico (sexual population) were compared in growth chamber experiments with A1 isolates from the United States and Wales (asexual population). Fitness components assessed included the proportion of inoculated leaflets on which lesions developed, lesion areas per infected leaflet, and sporulation capacity. In addition, a composite fitness index was calculated as the product of the three fitness components. Virulence was assessed as the ability to produce sporulating lesions on detached leaflets of nine differential (*R*-gene) potato genotypes. The mean number of virulence factors per isolate was seven for isolates from the sexual population and three for isolates from the asexual population. Although isolates from the sexual population infected significantly fewer leaflets than did those from the asexual population, the resulting lesions were larger for isolates from the sexual than the asexual population. No significant population differences in sporulation capacity were observed, nor were isolates from one population more fit than those from the other on the basis of the composite fitness index. The number of virulence factors per isolate was not significantly correlated with the fitness of the isolates.

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is perhaps the most destructive disease of potato (*Solanum tuberosum* L.). Until recently (5,12), only a single mating type (A1) of *P. infestans* had been found in the United States, Canada, Britain, and Europe; thus, reproduction had been exclusively asexual. In Mexico, both mating types (A1 and A2) are commonly found (1,2) and sexual recombination may occur. Concern that the presence of sexual recombination might result in isolates more pathogenically variable or with higher fitness than those reproducing strictly asexually has resulted in efforts to prevent the importation of *P. infestans* from Mexico.

Because *P. infestans* isolates from Mexico have never been directly compared with those from other areas, it is unclear whether large differences in virulence and/or fitness exist between isolates from the two populations or whether environmental factors are in part responsible for the more severe late blight observed in Mexico. Temperature and rainfall conditions occurring during the growing season in the Toluca Valley of Mexico are consistently ideal for development of the disease (7,8). Growing season conditions in the United States and Europe are more variable and often unfavorable for late blight development. Resistance in potato cultivars adapted for growth in the United States may not be fully expressed under Mexican conditions of rainfall, temperature, and day length, making them appear more susceptible when grown in Mexico.

Studies comparing *P. infestans* isolates from the sexual and asexual populations in a constant environment are required to determine the magnitude of hazard to U.S. agriculture posed by isolates from the sexual population. If the sexual population is substantially more virulent or fit than the asexual population, restrictions on importation of cultures from Mexico should be continued and perhaps strengthened. Even if sexual and asexual *P. infestans* populations are equal in virulence and fitness, the A2 mating type poses a threat because it introduces the ability to produce oospores. Oospores survive in soil for undetermined time periods and thus would introduce a source of inoculum not now present in the United States.

The objectives of this study were to determine under constant environmental conditions whether isolates of *P. infestans* from the sexual population have a greater number of virulence factors or have higher fitness than those from the asexual population.

MATERIALS AND METHODS

Sources of *P. infestans*. Isolates sampled from the sexual population were from Mexico, whereas those sampled from the asexual population were from the United States and Wales. Sources of the isolates are listed by Tooley et al (16) (Tables 1 and 2), except for isolate 160, which we collected at Freeville, NY, in August 1984. We collected all but one of the Mexican isolates during early September 1983 and August 1984 from potato research plots and commercial potato fields in and around Toluca and Chapingo, Mexico. The other Mexican isolate (561), provided by J. Galindo, was isolated in 1983 from *Solanum cardiophyllum* growing at Chapingo. Ten of the isolates from the asexual population originated from New York State and were isolated between 1979 and 1984. Little other than the geographic source is known about the other six isolates from the asexual population, because we received these isolates from other workers. All isolates used in this study from both populations were of A1 mating type.

Quarantine precautions. Mexican isolates were imported into the United States under a permit from the USDA, Animal Plant

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TABLE 1. Fitness components and composite fitness index for *Phytophthora infestans* isolates from sexual and asexual populations[a]

Sexual population					Asexual population				
Isolate[b]	IF[c]	LA[d]	SC[e]	CFI[f]	Isolate	IF	LA	SC	CFI
533	0.95	5.0	10,828	56,816	146	0.93	3.2	16,473	49,550
541	0.90	5.0	11,490	55,329	150	0.96	5.0	9,258	44,593
507	0.87	6.3	7,018	45,526	106	0.99	4.3	11,104	43,767
568	0.90	5.7	6,837	41,687	128	0.87	4.3	15,523	42,767
517	0.72	4.6	10,278	36,122	137	0.95	3.6	10,124	37,457
538	0.64	3.2	13,278	33,360	160	0.91	3.0	11,822	28,801
561	0.83	4.7	7,070	30,141	152	0.84	2.5	7,672	16,503
515	0.66	2.3	14,074	23,459	127	0.96	2.4	7,266	15,583
545	0.67	3.8	6,798	20,426	147	0.74	2.8	6,045	13,341
562	0.69	3.0	9,146	20,007	118	0.82	3.0	2,979	7,038
544	0.36	2.7	11,181	10,030	135	0.86	1.8	3,974	6,389
527	0.62	2.8	4,709	8,733	111	0.78	1.5	4,848	3,417
543	0.40	2.2	5,973	4,807	141	0.44	0.9	5,761	2,008
542	0.39	1.7	6,238	4,564	102	0.72	0.9	2,765	1,971
529	0.38	3.1	3,825	4,406	139	0.84	0.5	3,943	1,290
547	0.27	0.9	5,477	1,149	112	0.49	0.3	4,862	648
BLSD[g]	0.27	0.7	6,828	35,004		0.27	0.7	6,828	35,004

[a] The sexual population consisted of *P. infestans* isolates from Mexico, whereas the asexual population consisted of isolates from the United States and Wales.
[b] Isolates are ranked from most to least fit on the basis of the composite fitness index.
[c] IF (infection frequency) = proportion of inoculated leaflets on which lesions developed.
[d] LA (lesion area) = area (cm^2) of lesions produced per leaflet.
[e] SC (sporulation capacity) = number of sporangia produced per square centimeter of lesion.
[f] CFI (composite fitness index) = IF × LA × SC.
[g] Bayes least significant difference, $k = 100$ (13), for comparing means of isolates both within and between sexual and asexual populations.

TABLE 2. Contrasts between means of isolates from sexual and asexual *Phytophthora infestans* populations for fitness components and composite fitness index

	Sexual vs. asexual population[a]		
Fitness measure	Contrast estimate[b]	t^c	$P>t^d$
IF[e]	−0.1781	−5.21	0.0001
LA[f]	1.07	10.18	0.0001
SC[g]	613	0.78	0.4373
CFI[h]	5,090	1.29	0.1978

[a] The sexual population consisted of *P. infestans* isolates from Mexico, whereas the asexual population consisted of isolates from the United States and Wales.
[b] The contrast estimate represents the difference between the means of the sexual and asexual populations.
[c] The t value is calculated by dividing the contrast estimate by its standard error.
[d] Probability of obtaining a larger absolute value of the t value.
[e] IF (infection frequency) = proportion of inoculated leaflets on which lesions developed.
[f] LA (lesion area) = area (cm^2) of lesions produced per leaflet.
[g] SC (sporulation capacity) = number of sporangia produced per square centimeter of lesion.
[h] CFI (composite fitness index) = IF × LA × SC.

Health Inspection Service. All Mexican isolates were transferred in a laminar-flow biosafety cabinet (class II, type A) in a level P2 laboratory. Plant inoculations were performed only with isolates of A1 mating type in a growth chamber from November through March, when living host material does not survive outside. The growth chamber was separated from other host plants that might be growing in the greenhouse. All materials were autoclaved before disposal at the end of the experiments.

Preparation of inoculum. Cultures of *P. infestans* were grown on V-8-lima bean agar medium (10) in 9-cm-diameter petri dishes for 10–14 days at 18 C in darkness. Sporangial suspensions were prepared using a cotton swab and by washing cultures with 10 ml of distilled water. The suspensions were filtered through four layers of cheesecloth to remove mycelial fragments and were adjusted to 25,000 sporangia per milliliter with a hemacytometer.

Assessment of fitness components. Potato plants of cultivar Norchip were grown from certified seed tuber pieces. Plants were grown in a greenhouse (20–24 C) in 15-cm-diameter plastic pots containing a peat and vermiculite mixture (1:1, v/v) with 0.4 kg each of nitrogen, phosphorus, and potassium per cubic meter of mixture. Natural sunlight was augmented with illumination from 40W sodium-vapor lamps on a 14-hr-light/10-hr-dark cycle.

Plants were transferred to a growth chamber for inoculation and subsequent incubation. The chamber was maintained at 18–20 C and illuminated with 40W cool-white fluorescent tubes on a 12-hr-light/12-hr-dark cycle (29 $\mu E\ m^{-2}\ s^{-1}$). The chamber contained a humidifier (model 250, DeVilbiss Co., Somerset, PA 15501) used to maintain 100% relative humidity as determined by hygrothermograph readings.

Two experiments, with five trials each, were performed. Each experiment involved eight isolates from the sexual population and eight from the asexual population. All isolates in the first experiment were different from the isolates in the second. In each trial, 20 plants (4–8 wk old with at least four fully expanded leaves) were used. A 50-μl drop of sporangial suspension (25,000/ml) of a given isolate was placed on the upper surfaces of the four subterminal leaflets of one potato leaf. This treatment was repeated five times per trial in a completely randomized design. Therefore, each isolate was inoculated onto 20 leaflets in each trial and onto 100 leaflets over the five trials of each experiment. Inoculated plants were maintained at 100% relative humidity in darkness for 24 hr to allow infection by *P. infestans*. Lesions were then allowed to expand for 48 hr after which plants were again maintained at 100% relative humidity for 36 hr to induce sporulation of *P. infestans*.

Fitness was defined as the contribution made by a genotype to the gene pool of the next generation (11). Thus, it is a measure of the capacity of a genotype to survive, grow, and reproduce. For *P. infestans*, factors contributing to fitness (fitness components [4]) include the ability to infect the host, colonize host tissue, and sporulate. We assessed three fitness components. First, we recorded the proportion of inoculated leaflets on which lesions developed. This variable was termed infection frequency. Second, we measured areas of lesions produced per leaflet 5 days postinoculation. We termed this variable lesion area and measured it as follows. Leaflets containing lesions were placed on blank paper and circumscribed with a pencil. A sharp pencil point was pressed through each leaflet at points along the lesion margins leaving a series of dots to mark the lesion outline. The leaflet was removed and the dots connected, producing an impression of the lesion. Areas of lesion impressions were then measured with a

planimeter.

Third, we assessed sporulation capacity of the isolates. Sporangia produced by an isolate on the four inoculated leaflets of a single leaf were collected into 1 ml of 10% ethanol with a cyclone spore collector (E.R.I. Machine Shop, Iowa State University, Ames 50011) connected to a vacuum pump. The number of sporangia per sample was estimated by reading absorbance of the samples at 620-nm wavelength with a spectrophotometer. This wavelength was chosen because it is used commonly for bacterial turbidity measurements. Absorbance readings were converted to numbers of sporangia per sample through use of a calibration curve based on hemacytometer counts. Sporulation capacity per square centimeter of lesion was calculated by dividing the combined number of sporangia produced on the four leaflets of a given leaf by the combined areas of lesions produced on those four leaflets.

Finally, a composite fitness index was calculated for each isolate and replicate as the product of infection frequency, lesion area, and sporulation capacity.

Data were analyzed by analysis of variance, and isolate means were separated using Bayes least significant differences (13). Contrasts (14) were used to compare means of sexual and asexual populations.

Assessment of number of virulence factors. The number of virulence factors of an isolate was determined from the number of compatible interactions on a set of single *R*-gene differential potato genotypes. The differential potato genotypes were obtained from the USDA Potato Introduction Station (Sturgeon Bay, WI 54235). Differentials were used that contained single resistance genes *R*2, *R*3, *R*4, *R*5, *R*7, *R*8, and *R*10. Potato cultivars Norchip (*R*0) and Rosa (*R*1) were also included.

Detached leaflets of the differentials were placed in inverted 9-cm-diameter petri dishes containing about 15 ml of 1.5% water agar. Two 50-μl drops of sporangial suspension (25,000/ml) of each isolate were placed on the lower surface of a leaflet, which was then incubated at 14 C under low light (0.02–0.03 μE m^{-2} s^{-1}). Seven days after inoculation, the leaflets were examined with a dissecting microscope for sporulation of *P. infestans*. If sporulation was observed, the interaction was rated compatible; if no sporulation was observed, the interaction was rated incompatible. The number of compatible interactions for each isolate was recorded.

RESULTS

Both populations showed substantial variation for fitness components (Table 1). Significant differences ($P < 0.05$) among isolates were observed for infection frequency, lesion area, sporulation capacity, and composite fitness index (Table 1).

Contrasts (Table 2) revealed that isolates from the asexual population were significantly higher in infection frequency than those from the sexual population, whereas the converse was true for lesion area. The populations did not differ significantly in sporulation capacity or composite fitness index (Table 2).

Some isolates from the asexual population had been maintained in culture for long periods of time, and we wished to determine whether this affected their fitness. A highly significant ($P < 0.01$) negative correlation existed between the number of months in culture and infection frequency ($r = -0.65$). Negative but nonsignificant correlations existed between the number of months in culture and lesion area, sporulation capacity, and composite fitness index ($r = -0.47, -0.31$, and -0.42, respectively). Isolate 141 was much older than other isolates from the asexual population and was thus clearly an outlier (148 mo in culture). When this isolate was omitted, no significant correlations ($P = 0.05$) existed between the number of months in culture and the other variables.

Isolates from the sexual population contained more virulence factors than did those from the asexual population (Table 3). Eight isolates from the asexual population were race 0 and able to infect only Norchip. There were no isolates of race 0 in the sexual population. Furthermore, seven isolates from the sexual population were virulent on eight of the nine differentials (Table 3).

We calculated correlation coefficients among the fitness components, composite fitness index, and the number of virulence

TABLE 3. Virulence of 32 isolates of *Phytophthora infestans* from sexual and asexual populations[a]

Sexual population		Asexual population	
Isolate[b]	Race[c]	Isolate[b]	Race[c]
542	0,1,2,3,4,5,7,10	118	0,1,2,3,4,5,8
561	0,1,2,3,4,5,7,10	146	0,1,2,4,5,10
544	0,1,2,3,4,5,7,10	147	0,1,2,3,5,10
538	0,1,2,3,4,5,7,10	150	0,1,2,3,4,10
529	0,1,2,3,4,5,7,10	152	0,2,7,10
515	0,1,2,3,4,5,7,10	137	0,1,5,10
527	0,1,2,3,4,5,7,8	135	0,1,5,10
545	0,1,2,3,4,7,10	160	0,1,5
507	0,1,2,3,4,5,7	141	0
517	0,2,3,4,7,10	139	0
543	0,1,2,5,7,10	128	0
533	0,1,2,5,7,10	127	0
568	0,1,2,4,5,7	112	0
547	0,2,5,7,10	111	0
562	0,2,4,7,10	106	0
541	0,10	102	0

[a] The sexual population consisted of *P. infestans* isolates from Mexico, whereas the asexual population consisted of isolates from the United States and Wales.
[b] Isolates within each population are ranked from most virulent to least virulent.
[c] Numbers in this column alone or separated by commas indicate *R*-gene differentials that allowed infection and sporulation by *P. infestans*.

TABLE 4. Correlations among fitness components, composite fitness index, and number of virulence factors for isolates from sexual and asexual *Phytophthora infestans* populations[a]

	Sexual population				Asexual population			
	IF[b]	LA[c]	SC[d]	CFI[e]	IF[b]	LA[c]	SC[d]	CFI[e]
LA[c]	0.8754	...	...	...	0.7154	...	...	...
	(0.0001)[g]	...	...	...	(0.0018)[g]	...	...	...
SC[d]	0.3406	0.1290	...	...	0.4874	0.6844	...	...
	(0.1967)	(0.6339)	...	...	(0.0555)	(0.0034)	...	...
CFI[e]	0.9207	0.8554	0.5073	...	0.6752	0.8826	0.9037	...
	(0.0001)	(0.0001)	(0.0449)	...	(0.0041)	(0.0001)	(0.0001)	...
NVF[f]	−0.3010	−0.2216	−0.1270	−0.4182	0.3007	0.4656	0.1157	0.3005
	(0.2572)	(0.4094)	(0.6392)	(0.1069)	(0.2578)	(0.0692)	(0.6695)	(0.2580)

[a] The sexual population consisted of isolates from Mexico, whereas the asexual population consisted of isolates from the United States and Wales.
[b] IF (infection frequency) = proportion of inoculated leaflets upon which lesions developed.
[c] LA (lesion area) = area (cm^2) of lesions produced per leaflet.
[d] SC (sporulation capacity) = number of sporangia produced per square centimeter of lesion.
[e] CFI (composite fitness index) = IF × LA × SC.
[f] NVF = the number of virulence factors per isolate.
[g] Probability value associated with the correlation coefficient.

factors present in the isolates (Table 4). In both populations, lesion area and infection frequency were significantly correlated while lesion area and sporulation capacity and infection frequency and sporulation capacity were not correlated. All three fitness components were significantly ($P < 0.05$) correlated with the composite fitness index (Table 4). The number of virulence factors in the isolates was not significantly correlated ($P < 0.05$) with infection frequency, lesion area, sporulation capacity, or composite fitness index in either population, although a marginally significant correlation existed between the number of virulence factors and lesion area in the asexual population (Table 4).

DISCUSSION

We found that *P. infestans* isolates from sexual and asexual populations differed in virulence but not in overall fitness as measured in growth chamber experiments.

The greater number of virulence factors found in the sexual population is consistent with the observation that specific resistance, initially effective in the United States and Europe, is ineffective in Mexico (6,8).

The sexual and asexual populations differed significantly for certain fitness components but not for overall fitness as estimated by the composite fitness index. Isolates from the asexual population were able to infect significantly more leaflets than were isolates from the sexual population. However, isolates from the sexual population were able to produce larger lesions on infected leaflets than were isolates from the asexual population.

Lesion area and infection frequency were highly correlated in both populations and also were correlated with the composite fitness index. Thus, in future studies, it may be possible to estimate fitness by assessing infection frequency or lesion area alone.

It is unknown which of the fitness components we measured has the greatest influence on overall pathogen fitness. Other workers (9) have stated the difficulty of defining the relationship between single fitness components and overall fitness. Thus, the overall fitness of one population relative to another would be difficult to predict solely on the basis of knowledge of fitness components. For this reason, we attempted to combine the fitness components in a manner that would reasonably approximate overall fitness by calculating the composite fitness index. A similar composite index was used by Haymer and Hartl (3), who found it valuable for comparing the fitness of strains of *Drosophila melanogaster*.

We stress that our composite fitness index approximates fitness in a constant, growth chamber environment and does not include other fitness components potentially important under field conditions. Nonetheless, isolate rankings based on composite fitness indices for five isolates from the asexual population showed moderate agreement with those based on fitness estimates previously obtained under field conditions (15) (Tables 3 and 4). Additional comparisons between growth chamber and field fitness estimates should be made using more isolates.

We found no significant correlation among isolates from either population between the number of virulence factors and the fitness of the isolates. Thus, our results do not support the concept of "stabilizing selection" (17), which holds that isolates that contain extra or unnecessary virulence genes are less fit on simple host genotypes (those containing few or no *R*-genes).

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