Pathogenic Specialization in *Phytophthora infestans*: Aggressiveness on Tomato

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ABSTRACT

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Isolates of *Phytophthora infestans* from the USA, Canada, Mexico, and the Netherlands were evaluated for aggressiveness on tomato in three types of growth chamber experiments and one field experiment. In the first type of growth chamber experiment, fitness components (lesion area and sporangia per lesion) of the four predominant clonal lineages in the USA were measured in growth chamber detached leaflet assays. Isolates of two of the lineages, predominantly associated with potatoes, produced lesions on tomato that were significantly smaller with less sporulation than isolates from two lineages that were found associated with both potato and tomato. The second growth chamber experiment involved isolates from northwest Mexico, as well as those from the USA and Canada; and the third experiment involved isolates from central Mexico and the Netherlands, in addition to those from the USA. Again, two categories were confirmed: genotypes that produced larger lesions with more abundant sporulation on potato than tomato (tomato-nonaggressive genotypes) and genotypes that were equally aggressive on potato and tomato (tomato-aggressive genotypes). With few exceptions, isolates with the same allozyme genotype had similar host reactions. Central Mexican isolates from potato and wild *Solanum* spp. were all tomato-nonaggressive. Since this is the center of origin for the pathogen, aggressiveness to tomato may be a recently acquired trait. A field experiment with one tomato-aggressive isolate and one tomato-nonaggressive isolate was conducted on one potato and two tomato cultivars. The tomato-nonaggressive isolate caused significantly less disease on tomato than potato. The tomato-aggressive isolate caused severe disease on both potato and tomato, but there was significantly more disease on tomato than potato. These results support the hypothesis that host specialization to tomato occurs in *P. infestans*.

Late blight of tomato, caused by *Phytophthora infestans* (Mont.) de Bary, was first reported in 1847 (21,22) only 2 years after Montagne's (19) initial description of potato late blight. Subsequently, late blight of tomato is reported in most regions where tomatoes are grown (1,11,14,15,23,25). Because late blight often appears to spread from potato to tomato plantings (16,25) and the disease is more severe on susceptible varieties of potato than on tomato (17), it was believed that the same isolates caused both diseases.

Other researchers found host range differences among isolates (1,3,7,13,18,25,30), but their results are typically based on relatively few isolates and the genetic relatedness among isolates is unknown. Giddings and Berg (7) reported that isolates of *P. infestans* from potato cause atypical lesions on tomato when compared to those caused by a tomato isolate. Small (25) observed that isolates from tomato infect potato, but isolates from potato do not always infect tomato. Kishi (13) found that all isolates, regardless of origin, infect potato, but they have different levels of virulence on tomato. Most potato isolates are weakly pathogenic on tomato, whereas none of the tomato isolates are weakly pathogenic on tomato (13). Berg (1) claimed that isolates that produce late blight on tomato differ biologically from isolates that produce blight on potato and found that potato isolates infect tomato, but to a lesser degree than tomato isolates. Mills (18) observed that two potato strains produce limited lesions on tomato, whereas a tomato isolate produces heavily sporulating lesions on tomato. A 1985 study of California isolates of *P. infestans* found that all 10 isolates obtained from tomato are pathogenic on potato and three of four isolates from potato are pathogenic on tomato (30).

The availability of molecular and biochemical markers in *P. infestans* now makes it possible to accurately characterize genotypes of isolates, and their use in population studies reveals an association between genotype and host specialization. Host specificity has been hypothesized to explain the occurrence of a genotype of the pathogen on one host in a region, but not on the other host. In northwest Mexico, 88 isolates were collected from 16 tomato fields and five potato fields within a 50-km-diameter area. Although tomato fields and potato fields occurred in the same region, all the isolates from tomato had the same mating type, allozyme genotype, and DNA fingerprint (9) and were genetically distinct from the potato isolates. In Brazil, Brommonschenkel (2) found only two allozyme genotypes of *P. infestans*. One genotype was recovered only from potato and the other only from tomato. In a collection of *P. infestans* from the Philippines, three isolates from tomato have different mitochondrial haplotypes than 25 potato isolates (14). In one set of community gardens in central Netherlands, isolates of *P. infestans* from tomato constitute a population genetically distinct from potato isolates (6). However, at another site in the Netherlands, the genotype found on tomato is also the predominant genotype recovered from potato. Recent work has found that certain genotypes are recovered from both potato and tomato in the USA, whereas other genotypes are only recovered from potato (8,10).

This study was undertaken to address the controversy surrounding tomato/potato specialization in *P. infestans*. Previous associations between clonal lineages and host of origin caused us to test the hypothesis that certain lineages are more aggressive on tomato. We used growth chamber and field experiments to inves-

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tigate a diverse collection of isolates from the USA, Canada, the Netherlands, and Mexico for noncompetitive fitness differences among isolates on potato and tomato. The isolates had been previously characterized using molecular markers and grouped into specific genotypes. This work provided the groundwork for genetic analysis of the control of tomato pathogenicity in *P. infestans*.

MATERIALS AND METHODS

Selection and culture of isolates. USA and Canadian isolates selected for this study were representatives of the four predominant lineages during the 1980s and early 1990s (8,10). These isolated strains were recently obtained and stored in our culture collection either in liquid nitrogen at −130°C or under oil at 18°C. Isolates from the Netherlands and Mexico were retrieved from our culture collection. All isolates were maintained on rye A medium (4) at 18°C until needed.

Detached leaflet experiment. Inoculations were conducted using greenhouse-grown potato cultivar Norchip (no *R* genes) and tomato cultivar Vendor (indeterminant habit with no *Ph* genes) plants. Fully or near-fully expanded leaves were collected from the middle of the canopy and nonterminal leaflets placed in an inverted 150 × 15-mm petri dish mini moist chamber, which contained a layer of water agar on the top to provide moisture (29). Inoculations were done with sporangia from cultures grown on rye A medium (4) for about 14 days in the dark at 18°C. Sporangial concentrations were determined with a hemacytometer and adjusted to 40,000 to 100,000 sporangia per milliliter. Harvested sporangia were kept at 4°C until used (1 to 2 h) and applied in a drop (50 µl) to the lower (abaxial) leaflet surface. Four or five leaflets in a single petri dish were inoculated with the same isolate, and both hosts were inoculated at the same time with the same sporangial suspension. After the leaflets were inoculated, the petri dish was sealed with parafilm and incubated at 18°C in continuous diffuse light. Aggressiveness of each isolate was determined at the same time for both hosts as described below for each experiment.

The first experiment included three representative isolates of US-1, three of US-6, three of US-8, two of US-7 (as described by Goodwin et al. [8,10]), and one isolate from a new lineage. These named lineages have been the most common during the 1990s in the USA and Canada (8,10). Two were A2 mating types (US-7 and US-8) and two were A1 mating types (US-1 and US-6). Each isolate was inoculated onto five detached leaflets of each host in a single, large petri plate, and each experiment was replicated four times. The analyses were conducted on averages per petri plate. Replications were typically done on different days. Five days after inoculation, four leaflets with typical lesions were selected and lesion areas were estimated using standard diagrams. The diagrams described lesions ranging in size from 1 to 13 cm^2. Lesion areas were recorded to the nearest whole square centimeter, except that lesions less than 1 cm^2 in size were recorded as 0.5 cm^2 in area. Subsequently, the number of sporangia produced in each lesion was determined. Individual lesions (with excess nondiseased tissue removed) were placed into 14-ml disposable polypropylene culture tubes with 2 to 8 ml of sterile distilled water (2 ml for very small lesions, 4 ml for medium sized lesions, and 8 ml for large lesions). The tubes were then vortexed for 10 s to dislodge and suspend sporangia, and an aliquot counted with a hemacytometer. The total number of sporangia per lesion was determined by multiplying hemacytometer counts (i.e., sporangia per milliliter) by the total volume of the suspension (2 to 8 ml). The significance of differences between isolates for sporangia production and lesion size within each host was determined by Fisher's least significant difference after one factor analysis of variance (27), and isolate × host interactions were tested.

A second experiment involved 44 isolates representative of recent collections made in the USA, Canada, and northwest Mexico. Inoculations and incubations were conducted as for the first experiment, except that some comparisons had only three replications. Also, lesion area was measured by tracing the lesion outline on paper and determining the area with a planimeter (26). Lesion sporulation was determined for a subset of isolates representative of the group subset. Sporangia were collected from the upper surface (adaxial) of the lesion using a cyclone spore collector (E.R.I. Machine Shop, Iowa State University, Ames) (26). Sporangia from each lesion were collected into a single Eppendorf tube containing a solution of 0.04 M copper sulfate and 0.2 M sodium acetate-acetic acid (pH 5.4) and stored at 4°C. Within 48 h, sporangia numbers were determined using a hemacytometer. Each number was the average of six independent aliquots. The significance of pairwise differences between host reactions on tomato and potato was determined using a paired *t* test ($P \leq 0.05$) with unequal variances (27).

In a third experiment, 83 isolates (from central Mexico, the Netherlands, and the USA) were inoculated onto detached leaflets and incubated. Inoculations and incubations were again conducted as for first experiment, and there were four replications typically done at different times. The isolates were then scored as tomato-aggressive or tomato-nonaggressive based on a visual comparison of lesion sizes on potato and tomato leaflets. An isolate was considered tomato-nonaggressive if lesions on potato leaflets were more than twice as large as on tomato leaflets. However, if lesions were similar sizes (± 30%) on tomato and potato leaflets, the isolate was considered tomato-aggressive. For those few isolates that produced lesions between these two categories, sporangia were counted from these lesions as described in the second growth chamber experiment. In all cases, there were > 10 times more sporangia on potato than tomato; so all of these isolates were characterized as tomato-nonaggressive.

Field experiments. Two experiments were conducted to assess the ability of a tomato-nonaggressive and a tomato-aggressive isolate to cause disease on potato and tomato in the field in 1993. In one experiment, isolate NDFC-2 (described as US-1 and tomato-nonaggressive in growth chamber tests) was used. In the other experiment, isolate Pasco 92-2 (US-6, tomato-aggressive) was used. Both experiments were conducted in adjacent areas at the Homer C. Thompson Research Farm at Freeville, NY. Each experiment contained both potatoes and tomatoes. Certified potato seed of the highly susceptible cultivar Norchip were machine planted on June 2 in plots consisting of four rows, each 4 m long and 90 cm apart, and plants approximately 0.23 m apart within rows. A preemergence herbicide (linuron as a 50WP, at 1.7 kg a.i./ha) was used and fertilizer (168 kg of N, 168 kg of P, and 168 kg of K per hectare) was applied at planting. Five-week-old transplants of tomato cultivars Sunrise and Pik Rite were hand-planted on June 4 in plots consisting of four rows, each containing six plants. These determinant cultivars were selected because they are common commercial cultivars grown in and adapted to upstate New York and possess no known *Ph* genes. Plants were approximately 60 cm apart within rows, and rows were 90 cm apart. Plots were separated in all directions by a 4-m-wide fallow strip. Tomatoes received the same fertilization as potatoes, but no herbicide. Each experiment contained four replicates of each cultivar in a completely randomized design.

On the nights of July 12 and 13, the appropriate sporangial suspension (75,000 sporangia in 50 ml of water) was applied with a backpack sprayer onto one plant in the center of each plot. Plots were inoculated with the US-6 isolate (Pasco 92-2, tomato-aggressive) or with the US-1 isolate (NDFC-2, tomato-nonaggressive). Conditions favorable to late blight were maintained by periodic sprinkler irrigation. Plots were irrigated in the evening for 2 to 4 h, three times per week, at the rate of 0.2 to 0.4 in. per irrigation, except during rain periods. At the time of inoculation, there had been no other late blight detected in the region.

Disease severity was assessed visually using a modification of a blight assessment key published by the British Mycological Society (5). Assessments were conducted on July 28, August 1, 4, 7, and 11. Area under the disease progress curve (AUDPC) was calculated using the method of Shaner and Finney (24).

Isolate confirmation. The identity of the isolate of *P. infestans* responsible for causing lesions in field plots was determined by analysis of the glucose-6-phosphate isomerase (*Gpi*) genotypes of the fungus directly from lesions using the technique of Sujkowski et al. (28), but modified as follows. Whole lesions or portions of discrete lesions (with an area of approximately 1.5 to 2.5 cm^2) were collected from potato and tomato plants and placed in 1.6-ml microcentrifuge tubes. Samples were stored at 4°C (up to 24 h) or at –20°C for 24 to 72 h. Subsequently, samples were ground in 50 to 125 µl of 0.1 M Tris-HCl (pH 8.0) (the larger buffer volumes were used with larger lesions) for approximately 30 s with a polypropylene tissue grinder (19921-0001; Bel-Art Products, Pequannock, NJ), and microcentrifuged at 12,000 rpm for 30 s. Samples were then electrophoresed overnight on 12% starch gels or stored for a few days at –20°C prior to electrophoresis, then stained and scored as described by Sujkowski et al. (28).

RESULTS

Detached leaflet experiments. In the first experiment comparing the four predominant clonal lineages (US-1, US-6, US-7, and US-8) and one new lineage, isolates formed two groups based on their aggressiveness on tomato (Table 1). Isolates of US-1 and US-8 lineages all produced significantly smaller lesions and significantly fewer sporangia on tomato (tomato-nonaggressive) than the US-6 and US-7 isolates (tomato-aggressive) (Table 1). Typically, the tomato-nonaggressive isolates (US-1 and US-8) produced lesions on tomato that were ≤ 1 cm^2 (Table 1). In contrast, isolates from the tomato-aggressive lineages (US-6 and US-7) produced lesions on tomato that were 3 to 5 cm^2. Sporulation differences were even more dramatic. The tomato-aggressive lineages produced 100 to 1,000 times more sporangia on tomato than did the tomato-nonaggressive lineages. On potato there were no such differences associated with clonal lineages (Table 1). The very few significant isolate × host interaction terms indicated no consistent trend.

In the second experiment, isolates also formed two groups based on the detached leaflet assay: tomato-nonaggressive or tomato-aggressive (Table 2). In the tomato-nonaggressive group, lesions were, on average, 5.6 times larger (1.9 to 19.7 times) and produced 95 times more sporangia (1.5 to 390 times) on potato than tomato. In the tomato-aggressive group of isolates, lesions on potato and tomato were not significantly different in size, and sporulation on potato was not significantly different when compared to tomato. In general, isolates with the same allozyme genotype had the same pathogenic specialization. However, there were three exceptions among the 44 isolates. Isolates TF1 and TF3 (with the US-1 genotype) were aggressive on tomato as well as potato, whereas 19 other isolates of this genotype were nonaggressive on tomato. Isolate LM 92-4 (with the 100/100 *Gpi* and 100/100 Peptidase [*Pep*] genotype) also infected potato and tomato equally well, whereas two other isolates of this genotype infected tomato poorly. Although all isolates from tomato were tomato-aggressive, some isolates from potato (US-6) were also tomato-aggressive.

In the third experiment involving detached leaflet assays, all central Mexican isolates (19 from potato and 24 from wild *Solanum* spp.) were tomato-nonaggressive (Table 3). For the Netherlands community gardens isolates, all four isolates from tomato were tomato-aggressive and all 11 isolates from potato were tomato-nonaggressive. The additional set of USA isolates of the four predominant lineages produced host reactions as in the previous experiments (compare Table 3 with Tables 1 and 2). Isolates of clonal lineages US-6 and US-7 were tomato-aggressive, whether they came from tomato (US-7) or potato (US-6 and US-7). The US-8 isolate was tomato-nonaggressive. Within the US-1 lineage, isolates from potato were nonaggressive on tomato, whereas isolates from tomato were aggressive on tomato.

Field experiments. In the experiment inoculated with Pasco 92-2 (US-6, tomato-aggressive), significantly more disease developed on both tomato cultivars than on the potato cultivar Norchip (Table 4). Tomato cultivar Sunrise developed significantly more disease than cultivar Pik Rite (Table 4). Analysis of *Gpi* genotypes of lesions from all plots revealed that primary lesions collected within this experiment were all caused by the inoculated isolate (US-6, tomato-aggressive). Subsequent sampling during the epidemic confirmed that only the US-6 isolate was causing disease in this experiment.

TABLE 1. Size of lesion and sporulation on tomato and potato leaflets caused by *Phytophthora infestans* isolates representative of the predominant clonal lineages in the USA

			Tomato cv. Vendor[x]		Potato cv. Norchip[x]	
Clonal lineage[v]	Isolate	Original host[w]	Sporangia per lesion	Lesion size (sq. cm)	Sporangia per lesion	Lesion size (sq. cm)
US-1	US92-148 (ME-7)	Potato	2,875 a	0.56 a	252,500 ab	5.63 ab
	Harvey FL-10	Potato	12,375 a	1.06 a	461,500 cde	7.13 def
	Troyer FL-1	Potato	13,875 a	1.00 a	387,500 bc	5.94 abcd
US-8	138 Stockton Potato 3	Potato	2,875 a	0.63 a	394,000 bcd	6.13 abcde
	106 Dillard Tomato	Tomato	5,625 a	0.75 a	374,500 bc	6.38 bcde
	146 Troyer Pa	Potato	10,750 a	1.06 a	606,500 e	7.38 ef
US-6	US91-73 (L1)	Tomato	145,500 b	3.63 b	195,750 a	4.94 a
	FL Potato-4	Potato	258,250 cd	5.50 d	407,500 bcd	7.06 cdef
	MV92-13	Tomato	271,750 d	5.25 cd	353,000 abc	6.38 bcde
US-7	103 Steve Garden	Tomato	192,750 bc	4.50 c	368,500 abc	7.75 f
	Ca Home 2-4	Tomato	202,000 bcd	4.63 c	322,000 abc	5.81 abc
New	137- Maclean Tom	Tomato	258,000 cd	5.19 cd	567,000 de	8.13 f
		F[y]	22.4*	63.7*	3.6*	4.6*
		LSD[z]	70,417	0.76	175,243	1.27

[v] Clonal lineages are as indicated in Goodwin et al. (8,10).
[w] Host isolate was originally recovered from.
[x] Lesion sporulation and area are the means of 16 detached leaflets in four replicated experiments of four leaflets each.
[y] F value as determined by one factor analysis of variance; * = values are significant ($P \leq 0.01$).
[z] Means followed by different letters within columns are significantly different as determined using Fisher's least significant difference (LSD) ($P \leq 0.05$).

In the experiment inoculated with NDFC-2 (US-1, tomato-nonaggressive), significantly more disease developed on the potato cultivar Norchip than on either tomato cultivar (Table 4). Analysis of hundreds of *Gpi* genotypes of lesions revealed that the primary lesions collected from both tomato cultivars and potato cultivar Norchip were caused by the US-1 isolate. However, the US-6 isolate (tomato-aggressive) was recovered from the majority of lesions on tomato cultivars Sunrise and Pik Rite at 18 and 21 days after inoculation, respectively. Thus, a majority of disease on the tomato cultivars was caused by a contaminating isolate. However, despite this contamination, the severity of disease on tomato was still significantly lower than on potato cultivar Norchip (Table 4).

DISCUSSION

On the basis of all of our lines of evidence (growth chamber tests, field tests, and host of origin), we concluded that host specialization on tomato occurred in *P. infestans*. While all isolates examined in this study were aggressively pathogenic on potato, only some isolates were aggressively pathogenic on tomato. These results clarified previous experimental (1,7,18,30) and ob-

TABLE 2. Size of lesion and sporulation on detached potato or tomato leaflets inoculated with recently acquired isolates of *Phytophthora infestans*

			Allozyme genotype[w]					Potato (cv. Norchip)		Tomato (cv. Vendor)	
Tomato pathogenicity[u]	Isolate	Clonal lineage[v]	Glucose-6-phosphate isomerase	Peptidase	Mating type	Original host	Collection location[x]	Lesion size (sq. cm)	Sporangia per lesion	Lesion size (sq. cm)	Sporangia per lesion
Nonaggressive:											
	Elba 3-5	US-8	100/111/122	100/100	A2	Potato	New York	7.2*[y]		2	
	PF-6	"	100/111/122	100/100	A2	Potato	New York	6.4*		3	
	RS-1	"	100/111/122	100/100	A2	Potato	New York	8.9*	128,000	4	11,750
	BC-9.1	BC-3	100/100	100/100	A2	?	British Columbia	11.5*	578,167*	4	19,208
	LM92 52	nd[z]	100/100	100/100	A2	Potato	Los Mochis	8.0*	60,273*	3	3,792
		nd	100/100	100/100	A2						
	LM92 37	nd	86/122	100/100	A1	Potato	Los Mochis	5.4*		2	
	LM92 53	nd	86/122	100/100	A1	Potato	Los Mochis	7.1*		2	
	Alberta #2	US-1	86/100	92/100	A1	Potato	Alberta, Canada	13.2*	32,667*	1	6,833
	Alberta #9b	"	86/100	92/100	A1	Potato	Alberta, Canada	13.1*	44,250	2	28,583
	Harvey-FL 3	"	86/100	92/100	A1	Potato	Florida	9.1*	148,833*	1	2,250
	Harvey-FL 5	"	86/100	92/100	A1	Potato	Florida	6.4*	84,250*	2	2,417
	Harvey-FL 8	"	86/100	92/100	A1	Potato	Florida	9.4*	181,333*	2	1,708
	Troyer-FL 2	"	86/100	92/100	A1	?	Florida	6.9*		1	
	ME-4	"	86/100	92/100	A1	Potato	Maine	10.3*		1	
	ME-7	"	86/100	92/100	A1	Potato	Maine	6.8*		1	
	ND #8	"	86/100	92/100	A1	Potato	North Dakota	6.6*	97,333*	2	417
	ND #10	"	86/100	92/100	A1	Potato	North Dakota	5.8*	100,250*	2	375
	ND #11	"	86/100	92/100	A1	Potato	North Dakota	7.0*	98,917*	1	1,000
	NDFC-2	"	86/100	92/100	A1	Potato	North Dakota	9.9*	249,417*	2	1,667
	92-56	"	86/100	92/100	A1	Potato	Washington/Oregon	11.8*	335,375*	1	10,944
	92-57	"	86/100	92/100	A1	Potato	Washington/Oregon	6.2*	30,333*	2	500
	92-58	"	86/100	92/100	A1	Potato	Washington/Oregon	7.9*		2	
	92-63	"	86/100	92/100	A1	Potato	Washington/Oregon	9.1*	81,083	2	208
	92-62	"	86/100	92/100	A1	Potato	Washington/Oregon	5.4*	67,542	2	958
	WE-7	"	86/100	92/100	A1	Potato	Washington/Oregon	9.5*		2	
	Lus-2	"	86/100	92/100	A1	Potato	Canada	8.7*	134,750*	2	3,125
							Average for tomato-nonaggressive lineages	**8**	**144,281**	**2**	**5,631**
Aggressive:											
	TF1	US-1	86/100	92/100	A1	Tomato	New York	4	41,500	3	10,042
	TF3	"	86/100	92/100	A1	Tomato	New York	8	111,750	5	115,042
	FL Potato-4	US-6	100/100	92/100	A1	Potato	Florida	10		9	
	FL Potato-7	"	100/100	92/100	A1	Potato	Florida	4		4	
	BC-6.2	"	100/100	92/100	A1	?	British Columbia	5		5	
	BC-7.1	"	100/100	92/100	A1	?	British Columbia	4		4	
	Bin 16	"	100/100	92/100	A1	Potato	Washington/Oregon	9	109,333	10	135,417
	MV92-6		100/100	92/100	A1	Potato	Washington/Oregon	10	92,583	8	
	Pasco 92-2	"	100/100	92/100	A1	Potato	Washington/Oregon	7	83,417	8	102,000
	LM92 12		100/111	100/100	A2	Tomato	Los Mochis	7	17,042	3	6,125
	LM92 27		100/111	100/100	A2	Tomato	Los Mochis	5	10,333	2	11,833
	BG-8		100/111	100/100	A2	Tomato	New York	6	101,250	4	
	SBGC-3	US-7	100/111	100/100	A2	Tomato	New York	10	126,667	9	82,778
	TFNC-1	"	100/111	100/100	A2	Tomato	North Carolina	8	58,917	5	80,750
	LM92 1	nd	111/122	100/100	A2	Tomato	Los Mochis	5		5	
	LM92 81	nd	111/122	100/100	A2	Tomato	Los Mochis	7	17,875	4	30,417
	LM92 95	nd	111/122	100/100	A2	Tomato	Los Mochis	5	35,208	4	26,167
							Average for tomato-aggressive lineages	**7**	**67,156**	**5**	**58,381**

[u] Host specialization as determined by growth chamber inoculations. Nonaggressive = isolates that were more aggressive on potato than tomato. Aggressive = isolates that were aggressive on both tomato and potato.
[v] Clonal lineages are as indicated in Goodwin et al. (8,10).
[w] Isolates are grouped according to allozyme genotype and mating type.
[x] Location where isolate was originally collected.
[y] * = are significantly different ($P \leq 0.05$) as determined by paired t test.
[z] nd = DNA fingerprints have not been determined.

servational (2,6,9) findings concerning tomato specialization in *P. infestans*.

In general, isolates from the same clonal lineage (i.e., with the same allozyme genotype and mating type) were similar in their aggressiveness on tomato. However, there were some exceptions to the association between allozyme genotype and tomato aggressiveness. Three of twenty-seven US-1 isolates in this study (two in the second set of experiments and one in the third set) were aggressively pathogenic on tomato. All three isolates had been recovered from tomato, whereas all other US-1 isolates had been recovered from potato. All 27 isolates have the same DNA fingerprint (10; S. Goodwin and W. Fry, *unpublished data*), providing further evidence that they were from the same clonal lineage. Therefore, it seemed likely that aggressiveness to tomato could evolve within a clonal lineage. Another possible exception was isolate LM92-4 that also had been isolated from tomato (Table 2). This isolate had the same isozyme genotype as two tomato-nonaggressive isolates; however, the DNA fingerprints of these isolates have not been determined so it is not yet known if these isolates are of a common clonal lineage.

TABLE 3. Aggressiveness on tomato of *Phytophthora infestans* isolates from the USA, the Netherlands, and Mexico

Location[w]	Genotype(s)[x]	Number of isolates	Original host(s)[y]	Tomato aggressiveness[z]
Central Mexico:				
	Diverse	19	Potato	–
	Diverse	24	Wild *Solanum* spp.	–
USA:				
	US-8	1	Potato	–
	US-1	2	Potato	–
	"	3	Tomato	+
	US-6	3	Potato	+
	US-7	4	Potato	+
	"	12	Tomato	+
The Netherlands:				
	Diverse	4	Tomato	+
	Diverse	11	Potato	–

[w] Location where isolates were collected.
[x] Clonal lineages as described by Goodwin et al. (8,10), if "diverse" = isolates from sexual populations with different genotypes.
[y] Host(s) from which the isolates were originally recovered.
[z] Tomato aggressiveness was determined by visual comparison of lesion sizes on tomato and potato. – = isolates that produced lesions twice as large on potato as tomato. + = isolates that produced lesions of similar size on potato and tomato.

TABLE 4. Area under the disease progress curve (AUDPC) for tomato and potato plants inoculated with *Phytophthora infestans* in the two field experiments

		Inoculum	
Host	Cultivar	Tomato-aggressive isolate[v] US-6 (Pasco 92-2)	Tomato-nonaggressive isolate[w] US-1 (NDFC-2)
Potato:			
	Norchip	589 a	665 b
Tomato:			
	Pik Rite	912 b	446 a[x]
	Sunrise	1073 c	413 a[x]
	F[y]	61.0*	6.5*
	LSD[z]	101	172

[v] Inoculated with only the tomato-aggressive isolate (Pasco 92-2), an isolate that equally pathogenic on detached leaflets of potato and tomato.
[w] Inoculated with only the tomato-nonaggressive isolate (NDFC-2), an isolate that was more pathogenic on detached leaflets of potato than tomato.
[x] Most of the lesions in these treatments were caused by Pasco 92-2 (US-6) and not NDFC-2 (US-1).
[y] F value as determine by one factor analysis of variance.
[z] Means followed by different letters within a column are significantly different as determined using Fishers' least significant difference (LSD) ($P \leq .05$).

The detached leaflet assay was found to be a reasonably accurate predictor of isolate behavior under field conditions. The large differences observed between tomato-aggressive and tomato-nonaggressive isolates on detached tomato leaflets were verified in the field, in which the tomato-aggressive isolate caused severe disease on tomato but the tomato-nonaggressive isolate did not. However, interpretations on the relative aggressiveness of isolates on tomato and potato using the detached leaflet assay could be influenced by the greenhouse conditions and cultivar. We had the best success using plants grown from November to March, and had difficulty in interpreting results obtained from plants grown during warmer periods in the year. Detached leaflet assays of the different tomato cultivars used in this study revealed that *P. infestans* caused similar sized lesions on cultivars Vendor and Pik Rite, but produced larger lesions on Sunrise (D. Legard and W. Fry, *unpublished data*). This difference was supported by field data in which late blight was significantly more severe on Sunrise than on Pik Rite (Table 4), although both cultivars were highly susceptible. These results suggested that detached leaflet assays may be useful not only as an estimator of aggressiveness on tomato, but also for ranking the susceptibility of different cultivars to late blight.

The results of these experiments provide information essential for the development of effective disease control strategies. When late blight is found on tomatoes in a region, because of the aggressiveness of isolates from tomato on both potato and tomato, we recommend that protectant applications of fungicides be applied to both tomato and potato fields in the area. However, tomato-nonaggressive isolates on potatoes should present much less risk to nearby tomato plantings even though tomato-nonaggressive isolates can cause limited disease on tomato. The availability of rapid genotype analyses would enable disease management to be adjusted within a season.

Although the tomato-aggressive isolate migrated into the plots inoculated with the tomato-nonaggressive isolate, there was still significantly less disease on tomatoes inoculated with the tomato-nonaggressive strain than the tomato-aggressive strain. The immigration and subsequent pathogen population increase greatly inflated disease severity on tomatoes initially inoculated only with the tomato-nonaggressive isolate. Thus, we were unable to obtain an accurate assessment of the disease that would have been produced on tomatoes by the tomato-nonaggressive isolate. The safest conclusion was that tomato-nonaggressive strains produced considerably less disease on tomatoes than did tomato-aggressive strains.

Our results were consistent with the hypothesis that increased aggressiveness on tomato (*Lycopersicon* spp.) has evolved in isolates already pathogenic to *Solanum* spp. Cultivated forms of tomato are thought to have been domesticated in Mexico from *Lycopersicon* spp. brought into Mexico from South America (12). The center of origin for the pathogen is believed to be the central highlands of Mexico, where it probably evolved on indigenous wild *Solanum* spp. (20). Because all the isolates tested in this study and previous studies were aggressively pathogenic on potato (7,14,30), but only some were aggressive on tomato, we hypothesized that this species first evolved aggressiveness to potato and recently developed aggressiveness to tomato. This hypothesis was supported by detached leaflet assays of the 43 isolates from potato and wild *Solanum* spp. in central Mexico. All isolates were aggressive on potato, but nonaggressive on tomato. The absence of tomato-aggressive isolates in this sample suggested that aggressiveness to tomato is not especially common in central Mexico, the center of origin for *P. infestans*.

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