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Dominance and recessiveness at loci for virulence against potato and tomato in *Phytophthora infestans*

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Summary. In this study we investigated the genetic control of virulence in the diploid fungal pathogen, *Phytophthora infestans*, against host resistance genes *R1*, *R2*, *R3*, and *R4* (potato) and *Ph1* (tomato). For four of these virulence traits, the presence or absence of segregation indicated conclusively which phenotype was dominant. We observed a 3:1 (virulent:avirulent) segregation on *R2* in the progeny of parents which were both virulent, suggesting that virulence is dominant and both parents are heterozygous. In a cross in which one parent was virulent and the other avirulent on potato gene *R3*, all progeny tested were avirulent, so avirulence against *R3* is dominant. The same virulent parent crossed with a different avirulent parent produced virulent and avirulent progeny in a 1:3 ratio, indicating that a second locus may be involved. The progeny of two parents virulent on *R4* segregated for virulence and avirulence, so virulence against *R4* is dominant. For *Ph1*, a 1:3 segregation in the progeny of two avirulent parents showed that the avirulent phenotype is dominant, and a 3:1 ration in a second cross suggested the involvement of a second locus. The segregations for virulence against *R1* did not indicate which phenotype was dominant, but did suggest single-locus control.

Key words: *Phytophthora infestans* – Virulence – Late blight – Gene-for-gene

Introduction

Phytophthora infestans (Mont.) de Bary, the cause of late blight of potato and tomato, is an important pathogen with a worldwide distribution. The late-blight pathosystem is believed to exhibit gene-for-gene interactions similar to those found in rust and smut diseases. Gene-for-gene systems are characterized by the interaction of pathogen virulence genes with host resistance genes which determine compatibility and incompatibility relationships between host and pathogen.

Phytophthora infestans is a good model system for studies of gene-for-gene systems because the fungus is diploid and heterothallic, and sexual crosses yielding large numbers of progeny can be performed between parents of opposite mating type (Shattock et al. 1986; L.J. Spielman, J.A. Sweigard, R.C. Shattock and W.E. Fry, unpublished results).

There are 11 major genes for resistance to *P. infestans* in potato (Black et al. 1953; Malcomson and Black 1966), which represent 11 potential gene-for-gene relationships. Segregation of pathogen virulence toward potato resistance genes *R1*, *R2*, *R3*, and *R4* was reported in several studies (Laviola and Gallegly 1983; Le Grand-Pernot 1988; Romero 1972; Romero and Erwin 1969; Sweigard et al. 1987; L.J. Spielman, J.A. Sweigard, R.C. Shattock and W.E. Fry, unpublished results). However, because of the nature of the crosses in these studies, the dominant alleles and the number of loci controlling each phenotype remained unknown. Al-Kherb (1988) obtained results for virulence against potato genes *R1–R4* which indicated single-locus control with dominant avirulence in each case. At least one late blight resistance gene (*Ph1*) has been isolated in tomato (Gallegly and Marvel 1955), and its interaction with *P. infestans* appears to be independent of those in potato (Wilson and Gallegly 1955). However, genetic studies of virulence toward tomato have not been done.

The specific interactions between *P. infestans* and its hosts are clearly expressed in field settings (Toxopeus 1964), but most virulence testing for race surveys and genetic studies has been done in vitro with detached

leaves or leaflets. The precise methods differ among laboratories (Tooley et al. 1986; Le Grand-Pernot 1988). Valid genetic studies, at both the classical and molecular levels, require methods for virulence testing which accurately reflect the interactions between host and pathogen in their natural setting.

The aims of our work were (1) to determine which phenotype was dominant at loci controlling several virulence phenotypes, (2) to determine how many loci controlled these phenotypes, and (3) to test the validity of our detached-leaflet method of virulence testing.

Materials and methods

Cultural manipulations

The parental strains used in this study (510, 543, and 562) were collected in Mexico in 1983 and 1984 (Tooley et al. 1985) and are now stored cryogenically as part of the *P. infestans* culture collection at Cornell University. Isolate 510 is A2 mating type, and 543 and 562 are A1 mating type. Strains derived from single zoospores were crossed by pairing on 15% unclarified V-8 agar (Ribeiro 1978) containing 50 ppm beta-sitosterol. Oospores were treated with NovoZym (Novo Industries, Bagsvaerd, Denmark) following a modification of the procedure developed by A.M. Fyfe (University College of North Wales, United Kingdom, personal communication). Agar containing oospores was aseptically macerated in sterile water with a Brinkmann Homogenizer (Brinkmann Instruments, USA), and a filter-sterilized solution of NovoZym was added to make a final concentration of 0.4%. The oospore suspension was incubated at 18 °C in the dark for 20–24 h (until no intact mycelium or sporangia remained). The NovoZym was removed by three cycles of centrifuging at low speed (3,000 rpm), discarding the supernatant, and resuspending in sterile water. Oospore suspensions were spread on 0.7% water agar and incubated at 18 °C under cool blue fluorescent light, as described in Shattock et al. (1986), and germlings were transferred to rye B agar (Caten and Jinks 1968). Rye A agar (Caten and Jinks 1968) was used for short-term maintenance, and progeny and parents were cryogenically preserved with the method of Tooley (1988). Mating type was determined by pairing unknown strains with known A1 and A2 testers on 10% clarified V-8 agar with 50 ppm beta-sitosterol and by scoring for the presence of oospores. For electrophoresis, cultures were grown in the dark in 10% unclarified V-8 broth (Ribeiro 1978) at 18 °C. Inoculum for virulence testing was produced by washing sporangia from 10 to 14-day-old cultures grown on 10% unclarified V-8 agar. All cultural manipulations were done under containment conditions following USDA requirements.

Allozyme markers

For electrophoresis, V-8 broth cultures were vacuum filtered and macerated in a small amount of TRIS-citrate gel buffer, pH 7 (Shaw and Prasad 1970, buffer system I) with a Brinkmann Homogenizer. The homogenate was centrifuged at 28,000 g for 10 min, and the supernatant was decanted and stored at −80 °C for later use. Starch-gel electrophoresis and enzyme staining were carried out using the standard methods described in Micales et al. (1986). For glucose-phosphate isomerase (GPI, EC 5.3.1.9) the gel buffer was 0.01 *M* histidine-HCl, pH 7, and the electrode buffer was TRIS-citrate, pH 7 (Shaw and Prasad 1970, buffer system I, electrode). Peptidase (PEP, EC 3.4.3.1) gels were run in a TRIS-borate-EDTA buffer system at pH 8.7 (Micales et al. 1986).

Virulence testing

Differential plants carrying single resistance genes were used to determine the virulence phenotypes of parents and progeny. These included potato selections PI 203905 (*R2*), PI 203902 (*R3*), and PI 203900 (*R4*) (all obtained from the USDA Potato Introduction Station, Sturgeon Bay/WI), potato cultivar Rosa (*R1*), and tomato cultivar New Yorker (*Ph1*). The potato cultivar Norchip and the tomato cultivar Vendor were used as universal suscepts. The plants were grown for 8–12 weeks in a greenhouse with natural sunlight augmented by 40 W sodium vapor lighting on a 14-h light, 10-h dark cycle. Virulence phenotypes were determined by inoculating detached leaflets following a modification of the method of Tooley et al. (1986). Large plastic petri dishes (14 cm diameter) were prepared by pouring about 25 ml of 1.5% water agar into each one and inverting them after they cooled. For each differential, leaves were collected from several different plants, and four randomly selected leaflets were placed in a single dish. A 50 µl droplet of sporangial suspension (40,000/ml) was placed on the upper surface of each leaflet, and the dishes were incubated under low light (0.02–0.03 µE/m^2) at 18 °C. Each dish received inoculum of only one strain. Disease interactions were evaluated after 6 (potato) or 7 (tomato) days.

Disease interactions were scored as either virulent, avirulent, or inconclusive. If at least two of the four inoculated leaflets had large sporulating lesions (at least 1 cm diameter for potato, and 0.5 cm diameter for tomato), the phenotype was considered virulent. Non-sporulating lesions 1 cm in diameter or less, hypersensitive flecks, or either in combination with up to two large (greater than 1 cm in diameter for potato or 0.5 cm for tomato) non-sporulating lesions indicated an avirulent interaction. Other combinations (such as one large sporulating lesion, or three or more large non-sporulating lesions) were considered inconclusive. All tests were repeated, and only those progeny which gave consistent results in two different tests were included in the analysis.

In order to confirm the accuracy of our virulence assay, we compared the detached-leaflet method with inoculations of whole, field-grown plants. Two U.S. isolates were used, one of which gave negative reactions on *R1, R2, R3,* and *R4* in earlier detached-leaflet tests (F163), and one of which gave positive reactions (F118). Leaflets were collected from field-grown plants in mid-July, 45 days after planting, and four leaflets were inoculated with each isolate, using the detached-leaflet method described above. The same sporangial suspension was used to inoculate leaflets on whole plants in the field. Two leaflets on each of four leaves were inoculated, giving a total of eight leaflets per isolate per trial. Optimal conditions for infection were achieved by inoculating just before dark, after the plants had been sprayed with water until runoff. Disease reactions were evaluated after 7 days, early in the morning so that sporulation could be observed. A second trial was carried out 48 days after planting.

Genetic nomenclature

In developing a nomenclature for *P. infestans* virulence genes, we considered convenience of use, ability to convey the necessary information, and ease of expansion to cover knowledge acquired in the future. Symbols for loci consist of two letters, indicating the host on which the specific interaction was expressed (St for *Solanum tuberosum* and Le for *Lycopersicon esculentum*), followed by a number, indicating the corresponding host resistance gene (in italic type with only the first letter

capitalized). Alleles at these loci are represented by the same symbols, also italicized, followed by *V* or *v* (if virulence is dominant) or by *A* or *a* (if avirulence is dominant). The dominant allele is capitalized and the recessive allele is uncapitalized. Thus, *St2* symbolizes the pathogen locus for virulence against the *R2* gene of potato, while *ST2V* and *st2v* represent the dominant and recessive alleles at this locus. Where the locus is clearly understood, the alleles are symbolized by single, italicized letters (*V, v* or *A, a*). Alleles at hypothesized loci which may be interacting with virulence loci are represented by single, italicized letters (*B, b* and *I, i*). Symbols for allozyme loci are *Gpi1* (glucose phosphate isomerase) and *Pep1* (peptidase), and their alleles are indicated by numbers representing relative mobility.

Results and preliminary discussion

Crosses

The two crosses reported here involved two different A1 parents and a common A2 parent. Cross no. 62 (562 × 510) yielded 142 progeny, of which 61 were A1, 67 were A2, and 14 were self fertile (SF). The oospore germination rate was 11.2%, while the overall viability rate (oospore germination rate × percentage of germinating oospores which were successfully established) was 7.5%. After this cross was performed, we learned that isolate 562 may be triploid or aneuploid (based on cytophotometric measurements of nuclear DNA content by Tooley and Therrien 1987), in contrast to the apparent diploid DNA content of 510 and 543. However, the progeny of 562 × 510 segregated as expected for both *Gpi1* and *Pep1*, with the exception of two *Pep1 83/83* genotypes (Table 1). These two exceptions were not expected and were probably self-matings of 562. Both were nonpathogenic. The virulence phenotypes of the progeny are presented in Table 2. Cross no. 63 (543 × 510) produced 155 progeny, with a mating type ratio of 61:60:3 (A1:A2:SF). The oospores germinated at a rate of 13.5%, and the overall viability rate was 8.2%. Both parents in this cross had the genotype, *Gpi1 100/100 Pep1 100/100*, and, as expected, all of the progeny had the same genotype. The virulence phenotypes of the progeny are presented in Table 3.

Table 1. Segregation at the allozyme loci, *Gpi1* and *Pep1*, in cross no. 62 (562 × 510)

Parental strains	Allozyme genotypes		Observed no. of progeny	Expected ratio
	Gpi1	*Pep1*		
562	86/100	83/100		
510	100/100	100/100		
	86/86		0	0
	86/100		59	1
	100/100		73	1
				($P=0.23$)
		83/83	1	0
		83/100	61	1
		100/100	68	1
				($P=0.55$)

Testing of virulence phenotypes

From each of the two crosses, a subset of progeny was tested for virulence, and only those which were clearly pathogenic on the universal suscept (potato cultivar Norchip or tomato cultivar Vendor) were analyzed for segregation on potato or tomato differentials, respectively.

Table 2. Segregation of virulence phenotypes in cross no. 62 (562 × 510)

Strain no.	Phenotypes of parents for virulence loci[a]				
	St1	*St2*	*St3*	*St4*	*Le1*
562	−	+	−	+	−
510	+	+	+	+	−
	Phenotypes of progeny				
	9+ 14−	6+ 17−	0+ 23−	6+ 17−	6+ 16−
Expected ratio (+:−)	1:1	3:1	0:1	3:1	1:3
χ^2	1.1	29.3		29.3	0.1
P	0.32	<0.005		<0.005	0.75

[a] + indicates a virulent phenotype and − an avirulent phenotype against the corresponding host resistance gene

Table 3. Segregation of virulence phenotypes in cross no. 63 (543 × 510)

Strain no.	Phenotypes of parents at virulence loci[a]				
	St1	*St2*	*St3*	*St4*	*Le1*
543	+	+	−	−	+
510	+	+	+	+	−
	Phenotypes of progeny				
	61+ 0−	41+ 18−	17+ 40−	20+ 37−	44+ 14−
Expected ratio (+:−)	1:0	3:1	1:1	1:1	1:1
χ^2		0.95	9.3	5.1	15.5
P		0.36	<0.005	0.025	<0.005
Expected ratio (+:−)[b]			1:3	1:3	3:1
χ^2			0.71	3.1	0.02
P			0.43	0.08	0.9

[a] + indicates a virulent phenotype and − an avirulent phenotype against the corresponding host resistance gene
[b] Additional χ^2 tests are given for alternate hypotheses

Disease reactions on the differentials were generally similar or slightly less intense than those of the same strain on the universal suscepts, which are known to be very susceptible to *P. infestans*. The nonpathogenic fraction of progeny found in each cross probably resulted from the segregation of polygenic factors, and the lack of pathogenicity would have masked the expression of any virulence genes which were present. Of the 44 progeny of cross no. 62 (562 × 510) which were tested for virulence, 20 were not pathogenic on either of the susceptible cultivars. All of the remaining 24 caused disease lesions on Vendor, and 23 of these caused disease lesions on Norchip. Two isolates pathogenic on tomato were excluded because they gave intermediate reactions on New Yorker (*Ph1*). Therefore, the analysis of segregation for virulence in cross no. 62 included 23 progeny for potato and 22 for tomato. From cross no. 63 (543 × 510), 94 progeny were tested on the differential series, and 27 of these were nonpathogenic. Of the remainder, 62 and 67 were pathogenic on Norchip and Vendor, respectively. The number of progeny excluded because of intermediate reactions on particular differentials ranged from one (*R1*) to nine (*Ph1*). The low incidence of intermediate reactions indicates that dominance was complete at the loci investigated. The few intermediates observed were probably caused by segregation at minor loci affecting pathogenicity, and were excluded in order to avoid misclassification.

Virulence against R1

Segregation for virulence/avirulence on *R1* occurred in cross no. 62, while all progeny of cross no. 63 were virulent (Tables 2 and 3). These results suggest control at a single locus (*St1*), but there are two equally probable hypotheses: recessive virulence with 562 heterozygous and 510 and 543 homozygous recessive (Table 4, first alternative), or dominant virulence with 510 heterozygous, 543 homozygous dominant, and 562 homozygous recessive (Table 4, second alternative). Both of these hypotheses lead to an expected ratio of 1:1 in cross no. 62, and the Chi-square test indicates no significant deviation. However, other ratios cannot be ruled out because of the small number of progeny tested.

Virulence against R2

Both crosses nos. 62 and 63 involved parents which were virulent on *R2*, so segregation in the two progenies (Tables 2 and 3) indicates that virulence against *R2* is dominant and that all three parents are heterozygous (*V/v*) at the locus controlling this trait, *St2*. In cross no. 63, the numbers of virulent and avirulent progeny conform closely ($P=0.36$) to the 3:1 ratio expected for segregation at a single locus (Table 3). However cross no. 62 shows a serious departure from 3:1 (Table 2), with an excess of avirulent progeny which is difficult to explain with one- or two-locus models. We present some possible explanations in the general discussion.

Table 4. Hypothesized virulence genotypes of parents of cross nos. 62 (562 × 510) and 63 (543 × 510)

Strain no.	Virulence loci				
	St1	*St2*	*St3*	*St4*	*Le1*
510					
Phenotype[a]	+	+	+	+	−
Genotype	*a/a* or *V/v*	*V/v*	*a/a* (*b/b*)[b]	*V/v*	*A/a* (*i/i*)[b]
543					
Phenotype	+	+	−	−	+
Genotype	*a/a* or *V/v*	*V/v*	*A/a* (*B/b*)	*v/v*	*a/a* (*I/i*)
562					
Phenotype	−	+	−	+	−
Genotype	*A/a* or *v/v*	*V/v*	*A/A* (or *B/B*)	*V/v*	*A/a* (*i/i*)

[a] + indicates a virulent phenotype and − and avirulent phenotype against the corresponding host resistance gene
[b] Genotypes at additional loci hypothesized to explain segregation ratios (see Discussion)

Virulence against R3

All progeny tested from cross no. 62 were avirulent on *R3* (Table 2). This is a strong indication of nonsegregation, since the sample size of 23 has a 0.999 probability of detecting segregation even if only 25% of the progeny were expected to be virulent (Bailey 1961). Nonsegregation indicates that avirulence is dominant and suggests that the avirulent parent (562) is homozygous dominant (*A/A*) at a locus (*St3*) controlling this trait (Table 4). The virulent parent (510) must then be homozygous recessive (*a/a*). Since segregation occurred in cross no. 63, the third parent, 543, must be heterozygous (*A/a*) at this locus (Table 4). The significant departure from the expected 1:1 ratios in the cross no. 63 progeny (Table 3) indicates that another locus may be involved in the control of this phenotype. At this second locus the avirulence allele (*B*) would also be dominant, and the presence of a dominant avirulence allele at either locus would result in an avirulent phenotype. If 543 is heterozygous at both loci (*A/a B/b*) and 510 is homozygous (*a/a b/b*) at both (Table 4), the progeny would segregate in the observed 1:3 ratio. If this hypothesis is true, strain 562 could be homozygous dominant at either one of these loci (*A/A* or *B/B*).

Virulence against R4

Both parents of cross no. 62 were virulent on *R4*, so the segregation in the progeny indicates that virulence is

Table 5. A comparison of disease reactions in assays on detached-leaflets and whole, field-grown plants

Isolates and assay conditions	Disease reactions[a] on potato differentials				
	Norchip	*R1*	*R2*	*R3*	*R4*
Isolate F163[b]					
Detached leaflets					
Trial 1	4+	4−	4−	4−	4−
Trial 2	4+	4−	4−	4−	4−
Whole plants					
Trial 1	8+	7− 1L/ns	8−	8−	8−
Trial 2	8+	8−	8−	8−	8−
Isolate F118[c]					
Detached leaflets					
Trial 1	4+	4+	4+	4+	4+
Trial 2	4+	4+	4+	3+ 1sL/ns	4+
Whole plants					
Trial 1	8+	8+	5+ 1L/ns 2−	6+ 2−	7+ 1L/ns
Trial 2	7+ 1−	7+ 1L/ns	6+ 2−	4+ 1L/ns 3−	8+

[a] The disease reaction for each of four (detached leaflets) or eight (whole plants) inoculated leaflets are indicated as follows: +, sporulating lesion at least 1 cm diameter; −, hypersensitive flecks or no visible reaction; L/ns, non-sporulating lesion at least 1 cm diameter; sL/ns, non-sporulating lesion less than 1 cm diameter

[b] Isolate F163 was avirulent on *R1, R2, R3,* and *R4* in previous tests

[c] Isolate F118 was virulent on *R1, R2, R3,* and *R4* in previous tests

dominant and that 510 and 562 are heterozygous (*V/v*, Table 4) at a locus (*St4*) controlling this phenotype. However, the progeny numbers did not fit the expected 3:1 ratio (Table 2). If virulence is dominant, 543 (the avirulent parent in cross no. 63) should be homozygous recessive (*v/v*, Table 4), but again the progeny numbers do not conform to the expected 1:1 ratio (Table 3). If an inhibitor locus which alters virulence to avirulence is being expressed in cross no. 63, the parental genotypes, *v/v I/i* (543) × *V/v i/i* (510), would give rise to virulent and avirulent progeny in a ratio of 1:3. However, there is a poor fit between this ratio and the observed numbers ($P=0.08$), and the excess of avirulent progeny in cross no. 62 would not be explained by this hypothesis. We present some possible explanations in the general discussion.

Virulence against Ph1

Both parents in cross no. 62 were avirulent on *Ph1*, so the segregation in the progeny (Table 2) shows that avirulence is dominant. The progeny numbers conform to the 1:3 ratio expected from heterozygous (*A/a*) parents ($P=0.75$), suggesting control by a single locus, *Le1* (Table 4). Strain 543, the virulent parent of cross no. 63, must then be homozygous (*a/a*), but the progeny numbers in this cross do not fit the expected 1:1 ratio (Table 3). However, if 543 is also heterozygous at an inhibitor locus whose dominant allele (*I*) alters the avirulent phenotype to virulent, then the cross 543 (*a/a I/i*) × 510 (*A/a, i/i*) would yield virulent and avirulent progeny in the ratio 3:1, as occurred in cross no. 63.

Test of virulence assay

The detached-leaflet assay accurately reflected the disease reactions observed on whole plants in the field, and in fact, showed greater consistency, probably because of more uniform environmental conditions (Table 5). The whole-plant assays tended to produce lower numbers of positive disease reactions than the detached-leaflet assays. The detached-leaflet method is, therefore, a useful bioassay for determining virulence phenotypes.

General discussion and conclusions

Our analysis of five gene-for-gene interactions involving *P. infestans* has led us to hypothesize several different kinds of genetic control: dominant virulence (*St2* and *St4*), dominant avirulence (*Le1*) with an epistatic inhibitor locus, and dominant avirulence (*St3*) with a comple-

mentary locus. Recent results from two other laboratories are contradictory in one case and supportive in the other. Al-Kherb (1988) analyzed crosses between European and Egyptian strains, and concluded that virulence against potato genes *R1, R2, R3,* and *R4* is under single-locus control, with avirulence dominant. Peterson (T. Peterson, unpublished results) found that virulence against R1 and R4 was altered to avirulence after exposure to low concentrations of intercalating mutagens, suggesting that dominant alleles for virulences were originally present. The contradictions between our work and Al-Kherb's may indicate that more than one locus is involved in the control of virulence against *R2* and *R4*. Our genetic material and Al-Kherb's may have been segregating for different loci conditioning the same disease interaction, ours with the virulent phenotype dominant, and Al-Kherb's with avirulence dominant. This hypothesis is supported by the poor fit to a 1:1 ratio for *St4* in Table 3, and some inconsistencies among crosses noted by Al-Kherb (1988).

In the more thoroughly studied rust and powdery mildew pathosystems, dominant avirulence is the most common kind of genetic control, but dominant virulence alleles have been proposed for some rusts and smuts (Christ et al. 1987). Inhibitor loci have also been invoked in other systems (Christ et al. 1987; Jones 1988a, b). In *Bremia lactucae* (the only other Oomycetous pathosystem in which virulence has been studied genetically), avirulence was found to be dominant for all the interactions studied, but additional inhibitory loci were involved in three of the interactions (Michelmore et al. 1984; Norwood and Crute 1984). The epistatic relationships implied by the labels avirulence locus and inhibitor locus can only be observed if the loci are segregating in the same cross. Further investigation is necessary, therefore, to determine whether our *St2* and *St4* loci should actually be termed inhibitory loci.

The hypotheses developed above provide reasonable explanations for all of our observations except the segregations at *St2* in cross no. 62 and *St4* in cross nos. 62 and 63. The fact that the allozyme markers, *Gpi1* and *Pep1*, as well as several of the virulence traits, segregated as expected in cross no. 62 argues against overall abnormalities (such as might be caused by triploidy in isolate 562). However, if some, but not all, chromosomes are duplicated in 562, meiotic irregularities could have occurred which distorted the segregations at *St2* and *St4* but did not affect the two isozyme markers.

Self-mating could also have occurred, especially in cross no. 62 because of the difference in ploidy between 562 and 510. About 6% of the progeny of cross no. 62 may be selfs of 562 (based on the detection of two *Pep1 83/83* genotypes out of 131 progeny tested for segregation at *Pep1*), and undetected selfs of 510 could also have occurred. However, segregation in the selfed progeny of a virulent parent also implies dominant virulence, so selfing of either 562 or 510 would not alter the conclusion that virulence is dominant. The percentage of selfing in previous crosses between diploid Mexican field isolates has ranged from 0% to 1.8% (from the data of Shattock et al. 1986), so cross no. 63 is unlikely to have been affected by a high frequency of self-mating.

The low rates of oospore viability in crosse nos. 62 and 63 (7.5% and 8.2%) could also have affected segregation ratios. However, low frequencies of progeny recovery are characteristic for *P. infestans* (L.J. Spielman, J.A. Shattock, R.C. Sweigard and W.E. Fry, unpublished results), and other crosses between diploid Mexican field isolates (with similar oospore viabilities) have consistently shown normal segregations for known markers (Shattock et al. 1986). Oospore viability was similar in the two crosses, but the segregation at *St2* appeared to be abnormal only in cross no. 62, so viability cannot be the primary factor causing abnormal segregation. However, *St4* may have segregated abnormally in both crosses, and therefore could have been affected by viability.

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