Oospore Survival and Pathogenicity of Single Oospore Recombinant Progeny from a Cross Involving US-17 and US-8 Genotypes of *Phytophthora infestans*

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ABSTRACT

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Oospores of *Phytophthora infestans* produced in vitro and in planta, from a cross between US-17 and US-8 genotypes, were exposed to a variety of environments and their survival was assessed. Additionally, the pathogenic characteristics of some resultant progeny isolates were assessed. Viability of oospores as measured by plasmolysis declined slightly over a period of 18 months whether they were stored in water at 4°C, in soil at 18°C, or in soil under natural field conditions. In comparison, viability as measured by germination was lower overall but appeared to increase after storage in soil. Oospores produced in planta were buried in the field in the fall of 1998, and were capable of infecting both tomato and potato leaflets when recovered in May 1999. Single oospore progeny ($n = 53$) from the in vitro cross were analyzed individually for genetic and pathogenicity characteristics. All 53 progeny tested for restriction fragment length polymorphisms with probe RG57 were hybrids. All but one progeny produced sporulating lesions on detached potato or tomato leaflets in growth chamber tests, but most lesions were smaller and developed more slowly than those produced by either parental isolate. In a further test of pathogenicity, under field conditions, none of a subset of 10 A2 progeny was capable of initiating a detectable epidemic in small plots of either potatoes or tomatoes.

Recent introductions of *Phytophthora infestans* strains into the United States and Canada have created the opportunity for sexual reproduction to become an important component of the life cycle. Prior to 1992, only A1 strains were common, but by the end of the 1990s, strains of both A1 and A2 mating types had become widely distributed (9). Most isolates have been individuals representing a small number of clonal lineages, as determined by neutral markers (15). A clonal lineage contains the asexual descendants of a single genotype, and individuals differ from each other primarily as a result of genetic changes originating by mutation (1). Thus, individuals within a lineage are more similar to each other than to individuals in other lineages in terms of many ecologically important characteristics. For *P. infestans* in the United States and Canada, epidemiological characteristics predictable from knowledge

of clonal lineages have included metalaxyl sensitivity, probable pathogenic specialization, and germination response to temperature (17,18,22,24,28,33).

Recently introduced lineages replaced the predominant US-1 lineage (A1 mating type) in the 1990s in the United States and Canada (9). The US-8 lineage (A2 mating type) achieved a continental distribution within only a few years of its initial detection in 1992 (10). A few lineages of A1 mating type also became widely distributed and problematic in the United States during the 1990s, including US-6, US-11, and US-17 genotypes (9). In newly dominant strains, the absence of genetic markers associated with the US-1 clonal lineage (86 allele for the *Glucose-6-phosphate isomerase* (*Gpi*) locus, 92 allele for the *Peptidase* locus, and mitochondrial haplotype Ib) indicate that the new strains have probably not arisen via sexual reproduction involving the US-1 lineage (9). However, newly dominant lineages appear to be fertile with each other (23), so the opportunity for sexual reproduction appears to be very much greater at the end of the 1990s than it was previously.

Sexual reproduction involves oospore production, which could create change in the ecology/epidemiology of *P. infestans* in the United States and Canada. Oospores are thick-walled spores produced by many *Phytophthora* species. They are an important source of inoculum for diseases caused by soilborne *Phytophthoras* and have the

longest survival period for all infective propagules of that genus (6). Oospores of *P. infestans* survived a range of temperatures, from freezing temperatures to 40°C in short-term laboratory experiments (8). In the United Kingdom (31), oospores remained viable but did not germinate when recovered from field soil after 10 months of incubation. In other studies, oospores survived and could germinate after a winter in the field in both the Netherlands and Canada (5,26). Thus, production of oospores probably creates additional opportunities for between-season survival of this pathogen. As an asexual organism, *P. infestans* is essentially an obligate parasite in natural or agricultural systems. If oospores of *P. infestans* survive in soil, they may serve as a source of initial inoculum—perhaps even some years after a susceptible host had been grown in that soil. There has been a suggestion that for at least one field in Sweden, the soil served as the source of *P. infestans* (2).

Sexual recombination will also produce new genetic combinations, creating the possibility of unusually fit or pathogenic individuals or individuals with novel ecological or epidemiological characteristics. This possibility has been termed the “Red Queen” hypothesis (3,34) and is particularly important for organisms that reproduce both asexually and sexually. The US-11 clonal lineage may have been produced by sexual recombination in the United States or Canada, and it has been problematic in many potato and tomato production systems in the United States (11,12,15). While it seems probable that some epidemiologically important genotypes (such as the US-11 genotype) are produced via sexual reproduction, it also seems likely that some progeny will not be aggressive plant pathogens. However, we do not now know what proportion of progeny are likely to be important, aggressive pathogens. Finally, in some agro-ecosystems where both mating types are known to occur, sexual reproduction is apparently not a factor in the life history of *P. infestans* (29,30).

The potential importance of sexual reproduction in *P. infestans* populations in the United States stimulated our investigation of the ecological characteristics of this aspect of the life history. We tested three individual hypotheses: that two isolates of *P. infestans* currently widespread in New York State could produce viable sexual progeny; that their oospores could survive

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under field conditions; and that the oospore progeny were generally as pathogenic as the parental isolates.

MATERIALS AND METHODS

Oospore production in vitro and in planta. Oospores of *P. infestans* were produced in vitro by pairing two isolates of different mating types on Rye B agar (4). All *P. infestans* isolates were obtained from the culture collection at Cornell University and the isolate numbers relate to that collection. The A1 isolate was US970001, and is a representative of the US-17 clonal lineage. This lineage is characteristically highly resistant to metalaxyl and highly pathogenic to tomatoes (15). The A2 isolate was US940480, a representative of the US-8 clonal lineage. This lineage is characteristically highly pathogenic to potatoes and resistant to metalaxyl (16). These isolates were selected as parents because they have both been detected in New York State, and may have had opportunity for sexual reproduction in our region. Progeny from the in vitro mating, designated as cross 82, were obtained into pure culture and subsequently stored in the Cornell University culture collection. After 4 weeks incubation in the dark at 18°C, oospores were extracted from agar medium by blending the mycelium and agar in water using a polytron homogenizer (Brinkmann Instruments, Westbury, NY). The oospore suspension was then treated with Novozyme 234 (Interspec Products Inc., Foster City, CA) at a concentration of 5g/liter to remove sporangia and mycelial debris. Oospore suspensions then were washed with sterile distilled water to remove the Novozyme, and distributed aseptically on water agar (0.7%, wt/vol) in 100 × 15 mm Petri plates. Oospores were incubated at 18°C in diffuse light and observed for germination almost daily during the subsequent 6 to 8 weeks. Germinating oospores were transferred aseptically to Petri plates containing Rye A agar (4) and maintained on Rye B agar.

Oospores were also produced in both tomato (cv. Sunrise) and potato tissue (cv. Superior) to assess survival of in planta-produced oospores under field conditions. The A1 and A2 strains were US970001 (US-17) and US940480 (US-8), respectively, as described above. Plant tissues (stems, fruits, and tubers) were inoculated with sporangia of US-17 alone, sporangia of US-8 alone, or with a 1:1 mixture of US-17 and US-8 sporangia. The inoculated tissue was then incubated in a plastic box containing moistened paper towels. After 3 weeks, the inoculated tissues were examined for oospores by visual inspection under a compound microscope.

Genetic analysis of progeny. Single oospore progeny that were obtained from the in vitro cross were analyzed for mating type, allozyme genotype at the *Gpi* locus, and genomic fingerprint as detected by

probe RG57. Mating type was assessed as described by Lee et al. (23). Techniques for allozyme analysis utilized cellulose acetate electrophoresis as described by Goodwin et al. (14) and techniques for DNA fingerprinting were those described by Goodwin et al. (13). Because both parental strains had the same (I-a) mitochondrial haplotype (11), the progeny were not assayed for this characteristic.

Metalaxyl sensitivity. The metalaxyl sensitivity of the single-oospore progeny and parental isolates was assessed as described by Matuszak et al. (25). Each isolate was grown in Rye B agar without metalaxyl (control) or media containing 5 or 100 µg/ml. Cultures were started with a 7-mm disc of agar containing mycelia taken from the edge of a culture growing on Rye B medium. Horizontal and vertical measurements of the colony on control and test media in two replicate plates were obtained over time. The growth of each individual culture at 5 and 100 µg/ml metalaxyl was compared to growth in the absence of metalaxyl. The trial was done twice and the data were averaged. An isolate of the US-1 clonal lineage (US940507) was included as a metalaxyl sensitive control.

Oospore survival. Survival of oospores produced in vitro was assessed in an experiment beginning in the fall of 1997. Oospores in an agar-water slurry were placed in three different environments: (i) in Falcon tubes (VWR, Rochester, NY) in an incubator at 4°C; (ii) in soil (Honeoye silt-loam soil obtained from the experimental field site) in the greenhouse at 18°C; and (iii) buried at a depth of 32 cm in the field. For burial in the field and in field soil in the greenhouse, subsamples were placed in nylon bags constructed from 10 µm mesh nylon (Tetko Inc., Kansas City, MO). The bags were used to prevent escape of any of the oospores. In the field, soil moisture and temperature were monitored at a depth of 32 cm. Soil moisture was measured with a model CS615 water content reflectometer, and soil temperature was recorded using a model 107B soil probe (Campbell Scientific, Logan, UT). Data were collected and stored twice a day (noon and midnight) with a CR10 Datalogger (Campbell Scientific, Logan, UT) from September 1997 through May 1999. Individual bags containing oospores were harvested from the field soil and from soil in the greenhouse at 3 to 5 month intervals from January 1998 to May 1999. The oospores were then analyzed for viability as described in the section below.

Survival of oospores produced in planta was also assessed after a winter season. Tomato stems and fruits and potato stems and tubers containing oospores were buried at a depth of 30 cm in nylon mesh bags (as described above) in November 1998 at three different sites in central New York State. Additionally, tissues that had been inoculated with only the A1 or A2 parent

were also buried in nylon mesh bags at those sites. The bags were retrieved from the field in May 1999.

Viability, germination, and infectivity of oospores. Viability of oospores was evaluated by the plasmolysis method reported by Jiang and Erwin (20) and also by germination. Oospores were recovered and suspended in water. An aliquot (50-150 µl) was placed in a 35 × 10 mm Petri plate; 2× the volume of 4M NaCl (Sigma) was added and oospores (300 in total) were assessed after 30 min, using a compound microscope at 20 or 40×, for whether or not they had plasmolyzed. To assess germination, a second aliquot of each oospore suspension was spread on 1.5% water agar plates containing ampicillin (100 mg/liter, Sigma, St. Louis, MO). The plates were incubated under blue fluorescent light at 22°C (8). After 20 days of incubation, 300 oospores were observed microscopically for evidence of germination.

At the conclusion of the storage period, both in vitro- and in planta-produced oospores were assayed for their ability to initiate infection on both tomato and potato leaflets. Oospore samples were placed individually in 150 × 15 mm Petri plates with 10 to 25 ml distilled water and were maintained at 18°C. After 2 days at 18°C, both tomato and potato leaflets were added to each Petri plate. Leaflets were examined for infection after 5 to 10 days. Lesions that appeared on the detached leaflets were excised and the *Gpi* genotype, mating type, and RG57 genomic fingerprint of each isolate were determined.

Pathogenicity of single oospore progeny. All oospore progeny recovered from the in vitro mating were tested for pathogenicity on detached leaflets of both potato and tomato in moist chambers. Whatman No. 1 filter paper was placed in the bottom of a Petri plate (150 × 15 mm), and 1 to 2 ml of distilled water was added to the filter paper. Four potato leaflets (cv. Superior) or four tomato leaflets (cv. Sunrise) were placed in each moist chamber. Leaflets were obtained from the middle third of plants grown in the greenhouse. Sporangia from 2- to 3-week-old cultures grown on Rye B medium were used as inoculum. Sporangia were washed from the medium and adjusted to a concentration of 3,000 to 7,000/ml. Sporangia were stimulated to release zoospores by incubating the suspension at 4°C for 2 h prior to inoculation. A droplet (40 µl) of the suspension was placed on the abaxial surface of the tomato or potato leaflet. Inoculated leaflets were maintained in the moist chambers for 7 to 10 days at 18°C in diffuse light. Pathogenicity was scored on the basis of lesion size and the degree of sporulation as a result of each inoculation. A pathogenicity score of (+++) was given if inoculation with a progeny isolate consistently resulted in a sporulating lesion >3 cm in diameter. An isolate was rated as (++) for patho-

genicity if a sporulating lesion was consistently 1 to 3 cm in diameter. Last, a score of (+) was given if inoculation with a progeny isolate resulted in a lesion of 0.1 to 1 cm in diameter. Assessments were made at least twice for each progeny isolate. Isolates were considered to be pathogenic if a visible sporulating lesion was observed. The progeny isolates were assayed in several groups. Parental isolates were included as controls with each group.

Each progeny isolate was assayed independently at least twice.

Because detached leaflet assays indicated that most of the progeny were less aggressive than the parents (see Results), a subset of progeny isolates was analyzed for pathogenicity in a field test. The assay plants were tomatoes (cv. Sunrise) and potatoes (cv. Superior). Neither cultivar is known to have R-genes. A randomized complete block design with three replicates

was used. Each replicate contained experimental units of both potato and tomatoes. The trial was conducted at the Homer C. Thompson Experimental Farm, located in Freeville, NY. Potato tubers were planted 19 June 1998. Each experimental unit consisted of three rows with five plants per row, spaced 23 cm apart within the row. Tomatoes, started from seed in May, were transplanted 19 June 1998. Each experimental unit consisted of three rows

Table 1. Genetic and pathogenic characteristics of progeny and parental isolates of *Phytophthora infestans*

Isolate ^x	Mating type	Gpi genotype	Genotype at RG57 bands ^y						Pathogenicity ^z	
			3	5	7	10	18	23	Potato	Tomato
US940480	A2	100/111/122	0	1	0	1	0	1	+++	+++
US970001	A1	100/122	1	0	1	0	1	0	+++	+++
CS82001	A1	100/100/111	1	1	1	1	0	0	+	+
CS82002	A1	100/100	1	1	1	1	0	0	+	+
CS82003	A2	100/111	1	1	1	1	1	1	+++	+++
CS82004	A1	100/122	1	1	1	1	1	0	++	++
CS82005	A1	111/122	1	1	1	1	0	0	++	++
CS82006	A1	100/111/122	1	1	1	1	0	0	+++	+++
CS82007	A1	100/122	1	0	1	1	1	1	+	+
CS82008	A1	100/111	1	1	1	1	0	1	+	+
CS82009	A2	111/122	1	1	1	1	0	0	+	+
CS82010	A1	122/122	1	1	1	1	0	0	++	++
CS82011	A2	100/122	1	1	1	1	1	1	+	+
CS82012	A1	100/122	1	1	1	1	0	0	++	++
CS82013	A1	100/122	1	1	1	1	0	1	++	++
CS82014	A1	122/122	1	0	1	1	1	1	++	++
CS82015	A1	100/122	1	1	1	1	1	1	+	+
CS82016	A1	100/100	1	1	1	0	1	0	+	+
CS82017	A2	100/100	0	1	0	1	1	1	+	–
CS82018	A1	100/111	1	1	1	0	0	0	++	+
CS82019	A1		1	0	1	0	0	1	+	+
CS82021	A1	100/122	1	1	1	1	0	0	+	+
CS82022	A1	100/100	1	1	1	1	0	1	++	++
CS82023	A1	111/122	1	1	1	1	1	1	++	++
CS82024	A1	100/122	1	1	1	1	1	1	++	++
CS82025	A1	100/122	1	1	1	0	1	0	+	+
CS82026	A2	100/122	0	1	1	1	0	1	+++	+++
CS82027	A1	100/122	1	1	1	1	1	0	++	++
CS82028	A1	100/122	1	1	1	1	0	0	++	++
CS82029	A1	122/122	1	1	1	0	1	1	++	+
CS82030	A2	100/122	1	1	1	1	1	0	++	++
CS82031	A1	111/122	1	1	1	1	1	1	++	++
CS82032	A1	100/122	1	1	1	0	0	1	+	+
CS82033	A2	100/122	0	1	0	1	1	1	+	+
CS82034	A2	100/122	1	1	1	1	0	1	+	+
CS82035	A1	100/122	1	0	1	1	1	0	+	+
CS82036	A2	100/100	0	1	0	1	1	0	+	+
CS82037	A1	111/122	1	1	1	1	1	1	++	+
CS82038	A2	100/111	1	1	1	1	0	0	++	++
CS82039	A2	100/122	1	1	1	1	0	1	++	++
CS82040	A1	100/100/122	1	0	1	1	1	1	+	+
CS82041	A2	100/100	0	1	0	1	1	1	+	++
CS82042	A2	122/122	1	1	1	0	1	1	+	+
CS82043	A1	100/111	1	1	1	0	0	0	++	++
CS82044	A1	100/122	1	1	1	1	0	0	+	+
CS82045	A2	100/100	1	1	1	1	1	1	+	–
CS82046	A1	100/122	1	1	1	1	0	0	+	+
CS82047	A1	100/111	1	1	1	1	1	1	+	+
CS82048	A2	100/111	1	1	1	1	0	1	+	+
CS82049	A2	100/122	1	1	1	1	0	1	+	+
CS82050	A2	100/111/122	1	0	1	0	1	1	+	+
CS82051	A1	100/122	0	1	0	0	0	1	+	+
CS82052	A1	100/122	1	1	1	1	0	0	–	+
CS82053	A1	111/122	1	1	1	1	0	1	+	+
CS82054	A2	100/122	1	1	1	1	0	0	–	–

^x US970001 + US940480 are parental isolates of cross 82. Other isolates are the progeny from this cross.

^y Bands 3,5,7,10,18 and 23 as described by Goodwin et al. (13) were scored as present (1) or absent (0) for the parental and progeny isolates.

^z Pathogenicity was scored as follows: (+++) = large (>3 cm in diameter) lesions with sporulation consistently resulting from each inoculation; (++) = intermediate (1 to 3 cm in diameter) lesions produced at most inoculations; (+) = small (0.1 to 1 cm in diameter) lesions resulting from only some inoculations; (–) = no sporulating lesions.

with three plants per row, spaced 23 cm apart.

The field trial included 26 treatments which consisted of 10 single-oospore progeny, a water control, the US-8 A2 parent, and a US-7 (US940486) A2 tomato aggressive isolate (24) on potato plots and tomato plots. Because our field experiments involved field isolates of A2 mating types, we restricted our analysis to only A2 progeny to avoid the possibility of sexual reproduction among isolates and resultant oospore contamination of soil at our field test site. The replicated field test was conducted only in 1998 and not repeated because results confirmed those from in vitro pathogenicity tests.

Inoculation was done on the evening of 14 August 1998. Plants were irrigated via overhead sprinklers prior to inoculation so that the soil and foliage were wet. Inoculum for each individual unit consisted of a sporangial suspension (1,700 sporangia/ml) of the appropriate isolate. Sporangial suspensions were cooled at 4°C for 2 h prior to inoculation to induce zoospore formation. The center of each experimental potato/tomato unit was inoculated by atomizing 5 ml of the appropriate sporangial suspension onto leaves in the middle of the canopy. The plants were monitored daily so that any aggressive strains could be detected and destroyed before significant sporulation and spread to adjacent field plots occurred. The incidence of primary and secondary lesions was monitored almost daily and the *Gpi* genotype of the isolates sporulating from a subset of individual lesions was determined.

Statistical analysis. Statistical analyses were performed on some of the data. Analysis of variance (General Linear Model Procedure) was used to analyze sensitivity data of isolates to metalaxyl. Regression analysis was used to examine the effect of environment and time on oospore germination and viability. These analyses were computed using Statistical Analysis System 6.12 (SAS Institute, Cary, NC). The genotypic data using neutral markers and the pathogenicity data were not subjected to statistical analysis.

RESULTS

Production and genetic characterization of in vitro produced oospore progeny.

A total of 53 single-oospore progeny was obtained from the in vitro cross between US-17 and US-8 (Table 1). Thirty-five of the 53 were A1 mating type and 18 were A2 mating type. All of the progeny examined were products of recombination as determined by segregation of allozyme alleles and mating type (Table 1). Eight of the ten theoretically possible *Gpi* genotypes were observed in the progeny (Table 1).

All of the 53 progeny isolates were characterized for DNA fingerprint with probe RG57. The parental strains were polymorphic at loci for bands 3, 5, 7, 10,

18, and 23. Therefore, these bands were instructive in determining if the progeny were hybrids as opposed to selfs. All 53 progeny isolates had genotypes that could only be indicative of hybrids (Table 1).

All of the progeny tested were insensitive to metalaxyl (growth at 5 µg/ml metalaxyl > 40 % of the growth in the absence of metalaxyl) (Fig. 1). However, the relative growth rates differed significantly among the progeny; at 5 µg/ml, growth ranged from 40% to well above 100% of the controls. Growth of some progeny was apparently stimulated by the presence of metalaxyl. Some progeny were more sensitive than either parental isolate, while others were more resistant than either parental isolate.

Pathogenicity of progeny. All but one of the progeny were capable of infecting

tomato and/or potato in the in vitro detached leaf assays. Most isolates produced smaller lesions than the parental strains, but several produced lesions that were similar to those produced by the parental isolates (Table 1).

In the field test, the progeny isolates had much lower pathogenicity than either of the control field isolates. Only half of the progeny tested produced primary lesions as a result of the initial inoculation; the other half produced no lesions (Table 2). While some primary lesions appeared, they did not expand rapidly and infected leaflets did not die within 2 weeks of the original inoculation. In those plots containing primary lesions caused by progeny isolates, there were no secondary lesions near the primary lesions (in the center of the plot) before the plots were destroyed. In con-

Fig. 1. Sensitivity of US-17 × US-8 progeny isolates to metalaxyl at (A) 5 µg/ml and (B) 100 µg/ml. Error bars represent the standard deviation of the mean growth of two replicates of each isolate.

trast, leaflets with primary lesions died within 5 to 6 days after lesions appeared and there were many secondary lesions surrounding the primary lesions in plots

inoculated with either of the control field isolates. In some of the plots inoculated with progeny, there were a few lesions not near the initial inoculation site, and these

Table 2. Incidence of primary (P) and secondary (S) late blight lesions in small field plots of potatoes and tomatoes inoculated with progeny isolates, US-8 parent and a US-7 field isolate

Isolate	Potato		Tomato	
	P	S	P	S
US940480 (US-8)	+	+	+	+
US940486 (US-7)	+	+	+	+
CS82003	+	-	+	-
CS82009	+	-	+	-
CS82017	-	-	-	-
CS82026	+	-	+	-
CS82030	+	-	+	-
CS82038	+	-	+	-
CS82039	-	-	-	-
CS82041	-	-	-	-
CS82048	-	-	-	-
CS82050	-	-	-	-
Water	-	-	-	-

Fig. 2. Assessment of viability of in vitro produced oospores from a US-17 × US-8 mating as determined by (A) germination on water agar and (B) ability to plasmolyze in NaCl. Oospores were produced in vitro and subsequently stored in the lab at 4°C, in soil at 18°C, or buried in the field under natural weather conditions. Error bars represent the standard error of the mean of three replicates obtained from each environment at each sample date.

were confirmed by *Gpi* analysis to be caused by either of the control field isolates and thus were contamination. Therefore, there was no evidence of secondary lesions caused by any of the progeny isolates. We destroyed all plots within 2 weeks after inoculation to preclude the population increase of any progeny.

Oospore survival. Oospores produced in vitro on agar plates survived in all environments during the study period (Fig. 2). The plasmolysis technique provided a higher estimate of viability (maximum of 50%) compared to the germination assay (maximum of 9%) (Fig. 2). The plasmolysis technique also provided results that were less variable over time (Fig. 2). Of the oospores recovered, there was no significant change in germination for those stored at 4°C ($P = 0.74$) in the laboratory. However, there was a trend ($P = 0.07$) toward an increased rate of germination for oospores held in soil, in the growth chamber, at 18°C over the 18 months, and a highly significant increase in germination rate ($P = 0.001$) for oospores recovered after field burial. In contrast, there was a significant decline in viability, as determined by plasmolysis, over time for oospores stored at 4°C ($P = 0.001$) and in soil at 18°C ($P = 0.001$), but not in soil under field conditions ($P = 0.115$). The decline in viability was most pronounced at 4°C (Fig. 2).

The soil environment in the field was generally moderate during the assay period. Soil temperatures remained fairly constant during the winter, ranging from 1 to 5°C (Fig. 3). Soil temperatures increased steadily in the spring and early summer months and peaked at 21°C in July and August 1998 (Fig. 3). Soil moisture was high during the winter and early spring, and was lower in May and the soil remained dry during the summer months (Fig. 3).

Oospore infectivity. Some of the oospores produced in vitro and in planta were infectious after several months in the field or after several months in soil at 18°C. In contrast, no oospores produced from the in vitro cross stored at 4°C in the lab over the 18-month interval produced lesions. From two of the three field sites, some in planta produced oospores recovered after overwintering were capable of infecting potato and tomato leaflets. For example oospores from tomato fruits and from stems of both tomatoes and potatoes were infectious, whereas oospores from potato tubers did not cause infection. Allozyme and restriction fragment length polymorphism (RFLP) analyses revealed that all 22 isolates recovered from infected potato and tomato leaflets were recombinant hybrid progeny. Oospores were required for long-term survival, as no viable *P. infestans* was recovered from control tissue (tomato stems and fruits and potato stems and tubers infected with US-8 or US-17 alone)

after long-term storage in any of the three environments.

DISCUSSION

The results of this study provided data concerning the potential significance of sexual reproduction in *P. infestans* in northeastern United States. We determined that the US-17 and US-8 lineages of *P. infestans*, occurring commonly in New York State during the late 1990s, were fertile and could produce sexual hybrid progeny; thus our first hypothesis was not rejected. We further learned that oospores survived for at least 18 months in a variety of environments including in soil in the field, thus preventing us from rejecting our second hypothesis: that oospores can survive in the field. However, while progeny were generally capable of infecting both tomato and potato in detached leaflet tests, most progeny appeared to have lower pathogenicity than parental strains, and of a subset of 10 progeny, none produced secondary lesions in the field. Under the same conditions a parental strain and another field isolate were able to initiate rapid epidemics in the field. Thus we reject our third hypothesis that progeny are generally as pathogenic as field-adapted parental strains.

The ability of common strains of U.S. lineages to produce oospores capable of germinating after exposure to field conditions in New York, differs from the data reported from Wales and Japan. Viable oospores were produced (as determined by ability to plasmolyze) by Welsh strains of *P. infestans* but did not germinate after storage in the field for 10 months (31). In Japan, oospores produced in matings between the common strains there did not germinate (29,30). However, our study is consistent with reports from the Netherlands and Canada, where sexually compatible strains occurred and where survival in nature of some progeny has been inferred (5,26).

An important result from our study is that oospores can survive in a variety of environments including in the field, greenhouse, and cold storage. Field survival is potentially very important, therefore we compared long-term monthly averages of soil temperature data obtained from a monitoring station in the northeastern United States with those encountered in the 18 months of our study. Soil temperature in our field experiment, measured at 32 cm, never went below 0°C. Data from the soil monitoring station revealed that soil temperatures at a depth of 30 cm rarely went below 0°C (USDA Forest Service, Durham, NH). Soil moisture levels were typical of soils in the region. The soil moisture level in our field plot was close to saturation during the winter and early spring and became drier during the summer.

Oospore viability as determined by plasmolysis declined with time in several

environments, while storage in soil appeared to promote germination of oospores. A decline in oospore viability as determined by plasmolysis is consistent with the study of Hanson and Shattock (19). Although the large variance in oospore germination limited conclusions, we observed an apparent increase in germination of oospores after storage in soil. This is potentially important. If something in the soil stimulates oospores to germinate, their role in initiating epidemics would be increased. Soil-stimulated germination of oospores is also important if this phenomenon can be utilized to improve oospore germination rates in genetics studies. The low and inconsistent germination percentages that we observed are not unusual for oospores of *P. infestans* (26,31).

We hypothesize that the viability of oospores of *P. infestans* declines slowly over time, but that they could remain viable for extended periods (>18 months). Presumably, the actual germination data are an underestimate of the oospore viability, because dormancy may prevent some oospores from germinating. However, ability to plasmolyze might be an overestimate of viability. It seems appropriate to regard the plasmolysis data as maxima for viability and the germination data as minima for viability.

The segregation for metalaxyl sensitivity is consistent with the model of a single or linked gene(s) of major effect with additional genes of minor effect (7,21,23). The occurrence in the progeny of individuals that were more insensitive than either parent supports this model and indicates that sexual recombination can provide combinations of genes that might produce individuals with adaptability and virulence factors that are different than those of either parent.

Interestingly, all oospore progeny analyzed were found to be recombinant hy-

brids and were not the product of selfing or apomixis. This was true of progeny produced in vitro as well as in vivo. This was somewhat unexpected, as one of the strains used in this study (US940480) has been shown to be self-fertile in agar culture (32).

Despite the potential for new combinations of genes, the generally reduced pathogenic potential of most progeny relative to either parent is of considerable interest and of some potential importance. While several progeny appeared to produce lesions as large as those of either parent in growth chamber assays on detached leaflets, the majority appeared less pathogenic than either parent. Similarly, the entire subset of 10 progeny seemed dramatically less pathogenic than the A2 parental strain or another field isolate in a field test. This seemed counter-intuitive to us for an organism that has essentially no active saprophytic stage in its life history. We had expected genes for pathogenicity to be fixed in an organism such as *P. infestans*. However, this result is consistent with the data of Miller et al. (27) and Gavino et al. (12), who reported the ephemeral appearance of a group of isolates (probable siblings) in the Pacific northwest of the United States, from which only one isolate, giving rise to the US-11 clonal lineage, survived for several seasons.

One conclusion concerning the epidemiology of *P. infestans* is that some progeny (and indeed if our results are generally applicable, then most progeny) are not as pathogenic as the parental strains. Thus, most progeny will not be selected and will not survive as epidemiologically important individuals. However, the rapid asexual reproduction of epidemiologically fit individuals enables even a small number of fit individuals to become important very rapidly.

Fig. 3. Soil moisture and temperature recorded in the field where oospores were buried.

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