Survival of Sporangia of New Clonal Lineages of *Phytophthora infestans* in Soil Under Semiarid Conditions

L. D. Porter, USDA-ARS, Vegetable and Forage Crops Research Unit, Prosser, WA 99350; and D. A. Johnson, Department of Plant Pathology, Washington State University, Pullman 99164-6430

ABSTRACT

Porter, L. D., and Johnson, D. A. 2007. Survival of sporangia of new clonal lineages of *Phytophthora infestans* in soil under semiarid conditions. Plant Dis. 91:835-841.

Currently, there is no information on the viability of sporangia in soil of the new metalaxyl-resistant genotypes of *Phytophthora infestans* in the semiarid Columbia Basin of Washington and potato-growing regions throughout the world. Sporangia of metalaxyl-resistant US-8 and US-11 clonal lineages of *P. infestans* survived a maximum of 23 to 30 days in a Shano silt loam and a Quincy loamy fine sand. There were no significant differences between soil types in area under the spore survival curve (AUSSC) in two trials, however, sporangia of *P. infestans* in the Quincy sand had a significantly greater mean maximum days of sporangia survival (MDSS) than did the Shano silt loam in one of two trials. AUSSC and MDSS were significantly greater ($P < 0.05$) for sporangia in wet soil than in dry soil under shaded conditions. Mean AUSSC and MDSS significantly decreased ($P < 0.01$) under nonshaded conditions versus shaded conditions. Three metalaxyl-resistant isolates (two US-8 and one US-11) of *P. infestans* did not significantly differ ($P < 0.05$) in AUSSC and MDSS.

Additional keywords: late blight, solar radiation

The Columbia Basin of south-central Washington and north-central Oregon is a major potato-growing region in North America where more than 65,000 ha of potatoes are grown annually. The environment is semiarid (mean rainfall is between 15 and 22.5 cm), and the potato crop is irrigated mostly by center-pivot systems. Although the Columbia Basin is semiarid, potato production is annually threatened by late blight caused by *Phytophthora infestans*, a devastating disease of potato throughout North America and the world (8). Late blight develops on potato foliage under conditions when ambient relative humidity is above 90% and temperatures range from 7 to 21°C (19,31). Tubers become infected in the field when zoospores or sporangia of *P. infestans* are washed from infected foliage and come in contact with tubers (18,19), resulting in infections through buds, lenticels, or wounds (15,36).

Beginning in 1991, metalaxyl-resistant genotypes of *P. infestans* were detected in the Columbia Basin (6) and started to replace the metalaxyl-sensitive US-1 strain

that was believed to have dominated the region since 1947 (4,23,24). Previous research on the survival of sporangia of the US-1 genotype of *P. infestans* under controlled conditions found that sporangia survived for 42 days in vitro in nonsterile soil (36) and 70 to 80 days in vitro in sterile soil (16,20,32,36). Under natural environmental conditions, sporangia of *P. infestans* in naturally infested soil and artificially infested soil in pots survived for 21 and 32 days, respectively (18,26). However, none of these environmental conditions assessing sporangia survival were under semiarid conditions.

Viability of sporangia of new clonal lineages of *P. infestans* was previously reported in water (29) and when directly exposed to the environment (33) under semiarid growing conditions that prevail in the Columbia Basin, but currently, there is no published information on sporangia viability in soil of the new metalaxyl-resistant genotypes of *P. infestans* in any potato-growing region throughout the world and particularly in the Columbia Basin. The development of fungicide resistance by plant pathogens on the basis of previous research has often been correlated with a loss in total fitness by the resistant pathogen (7), so determining the survival of metalaxyl-resistant genotypes of *P. infestans* is important in modeling the epidemiology of the pathogen.

Therefore, the purpose of this study was to assess the effects of solar irradiance, soil moisture, and soil type on the survival of sporangia of new metalaxyl-

resistant clonal lineages of *P. infestans* under semiarid environmental conditions.

MATERIALS AND METHODS

Preparation of microplots. A Quincy loamy fine sand from Paterson, WA (30) and a Shano silt loam from Othello, WA (22) with a pH of 6.6 to 7.3 and 7.4 to 9.0, respectively, were used to evaluate the survival of sporangia of *P. infestans*. The Quincy loamy fine sand was used for experiments when only a single soil type was used. The soils were removed from native rangeland (sagebrush scrub) that had never been cropped with potatoes. Soil for each soil type was air dried and sifted through a 0.5-cm² mesh screen to remove rocks and debris and homogenized to minimize differences in pH. Plastic pots that were 35 cm in diameter by 25 cm deep were filled with gravel to a depth of 5 cm. The mean diameter of the gravel measured 0.63 cm. Sand was placed on top of the gravel to a depth of 19 cm. Gravel was also placed below the pots to a depth of 5 cm to promote drainage. Pots were buried to the upper rim in sandy soil and filled with the respective soil. Research plots were maintained free of weeds. Plots were located near the Washington State University Campus at Pullman, located approximately 109 km from the border of the Columbia Basin of Washington. This location was selected because sporangia of *P. infestans* could not be released in the potato-growing areas in the Columbia Basin and it was considered to be downwind from the basin while still maintaining similar climatic characteristics.

Shaded or nonshaded conditions for all trials were created by either covering or not covering microplots with black, non-transparent polyethylene attached to a 47- × 47-cm frame held 10 cm above the soil surface. The polyethylene completely blocked all direct sunlight. The microplots were only shaded from the top, but the area shaded was larger than the microplot area to prevent direct exposure from the sun at all times during the day. The frame was made with 1.3-cm-diameter pvc pipe. In addition to polyethylene covers, nylon screens were also used to filter the sunlight to varying shade levels in two trials assessing the effect of solar irradiance on sporangia survival (see Effect of solar irradiance on survival).

Environmental data. Soil water potential, soil temperature, and solar irradiance

Corresponding author: L. D. Porter
E-mail: lporter@pars.ars.usda.gov

Accepted for publication 5 February 2007.

doi:10.1094/PDIS-91-7-0835

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2007.

data was collected for each trial by placing sensors in a single replication of each treatment per trial. Watermark water potential sensors (Spectrum Technologies Inc., Plainsfield, IL), 2.2×8 cm were buried at a 45° angle with the top of the sensor 1 cm below the soil surface. Sensors were placed in the center of each pot. Water potential was recorded twice daily. Soil temperature was recorded every 30 min using a model 450 Watch Dog Data Logger connected to an external soil temperature sensor (Spectrum Technologies, Inc.). Temperatures were measured at soil depths of 1 cm. The mean water potential and soil temperature for treatments were calculated from replicate values. Water potential values between 0 and -10 centibars represented saturated soil. "Wet" soil was kept moist to just beginning to appear dry at the surface as determined by visual observation. Approximately 400 to 800 ml of distilled water was applied as needed to the soil surface to maintain the desired soil moisture level.

Solar irradiance (SI) in watts per square meter was measured every 15 min in the shade and nonshade using pyranometers (2000SA; Li-Cor, Lincoln, NE) placed horizontally at ground level. A daily mean SI value was calculated from the SI values during the time when nonshaded treatments were exposed to direct sunlight.

Isolates and sporangia production. Two US-8 and one US-11 (12) clonal lineage of *P. infestans* were used in these studies. US-8 isolates "02" and GE-7 were collected from central Washington in 2002 and 2003, respectively and a single US-11 isolate, 110B, was collected from western Washington in 1997. Isolates were maintained and increased on excised leaflets of cvs. Ranger Russet or Russet Burbank. Sporangia from lesions on leaflets were rinsed with distilled water into a beaker to a concentration between 5 and 6.5×10^4 sporangia/ml. The spore suspension was incubated for 2 h at 4°C to induce zoospore formation. A 1-cm² Whatman No. 2 filter paper was then immersed for approximately 1 s in the suspension and placed in the center of the adaxial surface of a freshly cut leaflet. The leaflets were then placed with the adaxial surface downward on a fiberglass screen over moistened paper towels in plastic containers. Leaflets were incubated at 15°C for 6 days with an 18-h photoperiod. Sporangia that formed were rinsed from leaflets with distilled water into a 4-liter flask and the concentration of sporangia was adjusted to 10,000 sporangia/ml of distilled water using a hemacytometer.

Application of inoculum to soil. Soil in each microplot was wetted with 70 ml of distilled water followed by 100 ml of a spore suspension containing 1×10^6 sporangia of *P. infestans* of a given isolate. Spore suspensions were applied using a graduated cylinder. The inoculum formed

an approximate 5 cm wide band of surface infested soil, centered around a 20-cm diameter within each microplot.

Tuber disk bioassay. A previously established tuber disk bioassay was used to assess the area under the spore survival curve (AUSSC) (29) and the mean maximum days of sporangia survival (MDSS). Briefly, tuber disks used to assay viability of spores were cut from certified seed tubers of cv. Ranger Russet. Tubers were washed thoroughly in running tap water and dried. Tuber disks with a 4- to 5-cm diameter were cut with a flamed knife to a thickness of 0.5 cm. Tuber disks were then placed on a fiberglass screen over moistened Whatman No. 2 filter paper in 9-cm diameter by 1.5-cm deep petri dishes.

Two soil samples per microplot were taken during each assessment of spore viability by dividing the soil surface in a pot in half and selecting a location within each half where spores had been applied. A soil bore was used to take a 1-cm diameter by 1-cm deep soil plug. The soil from each plug was placed in the center of a freshly cut potato disk contained in petri dishes as previously described. One milliliter of sterile distilled water was applied on top of the soil to spread the soil evenly on the tuber disk surface. Tuber disks in petri dishes were randomly arranged in an 18.5-liter plastic container with wetted paper towels in the bottom and incubated for 6 days at 15°C with an 18-h photoperiod. The percentage of tuber surface area producing sporangiophores and sporangia was determined on the tuber disks by visual observation after incubation for 6 days and the AUSSC was calculated (29).

Effect of soil type, solar irradiance, and soil moisture on survival. The effect of soil type, solar irradiance, and soil moisture on sporangia survival was determined in two trials during August through September (Trial 1) and September through October (Trial 2) of 2003. The experiments were arranged in a one isolate, by two soil types, by two solar irradiances, by two soil moistures, factorial design with three replications. The isolate used was US-8 isolate 02. Soil in the pots were either shaded as previously described or not shaded. Soil was either kept moist by adding distilled water when the soil surface appeared dry to the sight or not watered following the initial application of sporangia to the soil. Sporangia viability was determined 10, 16, 23, and 30 days after infesting the soil in Trial 1 and 7, 17, 22, and 27 days in Trial 2.

Effect of isolate on survival. The effect of isolates on sporangia survival was determined in three trials during April (Trial 1) and May (Trials 2 and 3) of 2004. The experiment was arranged in a two or three isolates, by two solar irradiances, factorial design with three replications. Soil surfaces in all microplots were kept moist with equal applications of water as previ-

ously described. The two solar irradiances were classified as shaded or nonshaded. The three isolates tested were 02 (US-8), GE-7 (US-8), and 110B (US-11). All isolates were metalaxyl resistant. Sporangia viability was determined 14, 21, and 26 days after infesting the soil for both trials.

Effect of moisture level on survival. The effect of moisture level on sporangia survival was determined in two trials during June of 2005. The experiment was arranged in a one isolate (isolate 02), by two light intensities, by four water levels, factorial design with three replications. The two light intensities were shaded or nonshaded. The four water levels were: level 1 = watered to maintain moist soil at the soil surface; level 2 = watered every other time that level 1 was watered; level 3 = watered every third time level 1 was watered; and level 4 = not watered after the initial water was applied when the sporangia were applied to soil. Sporangia viability was determined 5, 8, 12, and 16 days after soil infestation for both trials.

Effect of solar irradiance on survival. The effect of solar irradiance on sporangia survival was determined in two trials during August of 2004. The experiment was arranged in a one isolate, by four solar irradiances, by one soil moisture, factorial design with three replications. The four solar irradiances were: i) complete shade created using the black, nontransparent plastic covers as used in other experiments; ii) partial shade using six layers of light brown, nylon screen; iii) limited shade using four layers of nylon screen; and iv) no shade provided (nonshaded). Sporangia viability was determined 8, 11, and 15 days after soil infestation.

Timing of experiments. The time of year when survival experiments were conducted were all within the potato-growing season in the Columbia Basin. Survival of sporangia of *P. infestans* in soil is a concern from planting, because of survival of sporangia on contaminated seed and sporulation of the pathogen from mycelium overwintering in tubers, until harvest. Potatoes are planted in early March in the Columbia Basin and continue to be harvested into November.

Statistics. A tuber disk in an individual petri dish was considered an experimental unit. Data for AUSSC and MDSS were analyzed by analysis of variance (ANOVA) using Proc GLM procedure in SAS (SAS Institute, Cary, NC). Mean pair-wise comparison of data were analyzed using a Fisher LSD procedure. Regression analysis using the REG procedure of SAS (SAS Release 8.0, SAS Institute) was used to examine the relationship of solar irradiance on sporangial survival. Dependent variables were either AUSSC or MDSS and the independent variable was mean solar irradiance. Regression analysis was not used to examine the relationship of water potential on sporangial survival be-

cause distinct, quantitative separations in water potential values were not observed among the four soil water treatments within all the shaded and nonshaded plots. All statistical designs were balanced and arranged using a randomized complete block design.

RESULTS

Effect of soil type, soil irradiance, and solar moisture on survival. In the first

trial, the AUSSC was significantly affected by solar irradiance ($P = 0.0004$) and soil moisture ($P = 0.0103$) but not by soil type ($P = 0.0816$). Shaded and nonshaded treatments had mean AUSSC of 1,292 and 678, respectively, and sporangia in wet soil survived significantly longer than those in dry soil, 1,181 to 789, respectively (Table 1). There were no significant interactions ($P < 0.05$) among the treatments for AUSSC. MDSS was significantly ($P =$

0.0286) greater in the Quincy loamy fine sand than in the Shano silt loam soil, 17.8 and 13.9 days, respectively. Sporangia were capable of surviving for 23 days in both soil types but no spore survival was detected after 30 days (Table 1). The interaction between solar irradiance and soil moisture significantly ($P = 0.0154$) affected the MDSS. Spores that were shaded and had low levels of soil moisture survived longer than spores that were nonshaded at the same soil moisture level, 21.8 and 6.7 days, respectively (Footnote "d", Table 1). However, there were no significant differences in MDSS between treatments with levels of high soil moisture that were shaded or nonshaded, 20.7 and 14.2 days, respectively. Moisture level did not significantly impact MDSS ($P < 0.05$) but was notable at $P = 0.0634$.

In the second trial, only solar irradiance significantly affected ($P = 0.0354$) the mean AUSSC. Mean AUSSC for shaded and nonshaded treatments were 906 and 631, respectively (Table 1). In the second trial, there were no significant differences in MDSS between soil type, solar irradiance, and soil moisture or the interactions among these factors. Spores were capable of surviving for 22 days in both soil types, but surviving spores were not detected after 27 days (Table 1).

Mean solar irradiance, water potential, and soil temperature are recorded for both trials in Table 2. Solar irradiance for the nonshaded treatment was less in the second than in the first trial because of cloudy weather.

Effect of isolate on survival. In the first trial, there were no significant differences in AUSSC ($P = 0.3564$) and MDSS ($P =$

Table 1. Mean area under the spore survival curve (AUSSC) and mean maximum days of sporangia survival (MDSS) of *Phytophthora infestans* in two soil types that were either shaded or nonshaded and maintained at two soil moistures^v

Treatment	Trial 1		Trial 2	
	AUSSC ^w	MDSS ^x	AUSSC	MDSS
Soil type				
Quincy loamy fine sand	1,109 a	17.8 (23) a	852 a	20.8 (22)
Shano silt loam	861 a	13.9 (23) b	684 a	16.8 (22)
Solar irradiance				
Shaded	1292 a	21.3 (23) a ^y	906 a	19.9 (22)
Nonshaded	678 b	10.4 (23) b	631 b	17.7 (22)
Moisture level ^z				
Wet	1181 a	17.4 (23) a	687 a	17.3 (22)
Dry	789 b	14.3 (23) a	849 a	20.3 (22)

^v Trial 1 was conducted from August to September and trial 2 from September to October of 2003. See Table 2 for solar irradiance, soil temperature, and soil moisture level data for each trial. Sporangia viability was assessed 10, 16, 23, and 30 days in trial 1 and 7, 17, 22, and 27 days in trial 2, after sporangia were applied to the soil. One million sporangia were applied to the soil of each pot for each treatment. Values followed by the same letter are not significantly different at $P < 0.05$ based on Fisher's least significant difference.

^w AUSSC was determined using the equation given in Porter and Johnson (29).

^x Values in parentheses are the maximum range of detected survival measured in days.

^y There were no significant interactions between main effects for both trials except in the first trial, where there was a significant interaction ($P = 0.0154$) between solar irradiance and moisture level for MDSS. The following interactions: shaded $\times$ dry, shaded $\times$ wet, nonshaded $\times$ dry, and nonshaded $\times$ wet had the following means and standard errors: 21.8 ± 2.86 , 20.7 ± 3.61 , 6.7 ± 5.16 , and 14.2 ± 5.23 , respectively.

^z Wet = soil was kept moist to just beginning to appear dry at the surface as determined visually, 400 to 800 ml of distilled water was added as needed. Dry = no water added beyond that used to apply the sporangia to the soil surface.

Table 2. Solar irradiance, water potential, and soil temperature when sporangia of *Phytophthora infestans* were applied to the soil surface of two soil types and either shaded or nonshaded and maintained at two soil moistures

Treatment ^v	Trial 1 ^u			Trial 2		
	Solar irradiance ^w	Ψ_w ^x	Temperature ^y (°C)	Solar irradiance	Ψ_w	Temperature (°C)
Quincy fine sand	...	...	...	...	...	...
Shaded	1 ± 0.3	...	...	0.4 ± 0.06	...	...
Wet	...	3 ± 1.3	18.3 ± 0.79 (14,26)	...	8 ± 1.54	13.6 ± 1.23 (5,22)
Dry	...	14 ± 1.5	17.7 ± 0.60 (13,21)	...	13 ± 1.87	14.0 ± 1.30 (5,22)
Nonshaded	244 ± 16.4	...	...	153 ± 11.31	...	...
Wet	...	12 ± 2.7	21.2 ± 1.48 (13,29)	...	11 ± 1.76	16.6 ± 1.59 (6,24)
Dry	...	11 ± 1.7	20.9 ± 1.16 (14,27)	...	12 ± 1.75	16.0 ± 1.55 (5,24)
Shano silt loam	...	...	...	...	...	...
Shaded	1 ± 0.3	...	...	0.4 ± 0.06	...	...
Wet	...	17 ± 1.5	17.7 ± 0.75 (14,23)	...	24 ± 1.55	13.4 ± 1.16 (5,23)
Dry	...	21 ± 1.4	17.8 ± 0.63 (14,24)	...	25 ± 1.41	13.9 ± 1.27 (5,23)
Nonshaded	244 ± 16.4	...	...	153 ± 11.31	...	...
Wet	...	25 ± 1.9	20.1 ± 1.66 (13,26)	...	17 ± 1.62	15.9 ± 1.40 (5,25)
Dry	...	39 ± 2.6	20.8 ± 1.36 (14,28)	...	27 ± 1.77	15.6 ± 1.57 (5,24)

^u Values are the daily means over the duration of the trial plus or minus the standard error.

^v Shaded = sporangia completely shaded from direct sunlight by black nontransparent polyethylene or fiberglass and nonshaded = sporangia not shaded. Wet = watered to maintain a moist soil surface and dry = was not watered after the initial water was applied when the sporangia were applied to the soil.

^w Solar irradiance was measured in Watts/m² using a pyranometer at the soil surface.

^x Water potential was measured in centibars using Watermark water potential sensors (Spectrum Technologies Inc., Plainsfield, IL), located in the center of a microplot and buried 1 cm deep at a 45° angle.

^y Numbers in parentheses represent the minimum and maximum temperature for each treatment. Temperature sensors were placed in the center of a microplot at a 1 cm depth.

^z ... = Not applicable.

0.9220) between two US-8 isolates and one US-11 isolate. Since there were no significant differences between isolates, the AUSSC and the MDSS values from all isolates for shaded and nonshaded conditions were combined, respectively, and compared. The combined AUSSC was significantly greater ($P = 0.0521$) for shaded than for nonshaded solar irradiances (Table 3), but there were no significant differences ($P = 0.2576$) between shaded and nonshaded solar irradiances for MDSS.

In the second trial, there were no significant differences in AUSSC ($P = 0.1887$) and MDSS ($P = 0.4304$) between two US-8 isolates. Therefore, the AUSSC and the MDSS values from both isolates for shaded and nonshaded conditions were combined, respectively, and compared. The combined AUSSC ($P = 0.0001$) and MDSS ($P = 0.0404$) were significantly greater for shaded than for nonshaded solar irradiances (Table 3).

In the third trial, there were no significant differences in AUSSC ($P = 0.0555$) and MDSS ($P = 0.3807$) between a US-8 and a US-11 isolate. Therefore, the AUSSC and the MDSS values from both isolates for shaded and nonshaded conditions were combined, respectively, and compared. The combined AUSSC ($P = 0.2156$) and MDSS ($P = 0.3807$) did not significantly differ between shaded and nonshaded solar irradiances (Table 3). Mean solar irradiance, soil temperature, and water potential are recorded for the three trials in Table 4.

Effect of moisture level on survival. In the first trial, there were no significant interactions between moisture level and solar irradiance for the AUSSC ($P = 0.1331$) and MDSS ($P = 0.0882$). AUSSC values were significantly less ($P < 0.05$) for moisture levels 3 and 4 than for moisture level 1 under shaded conditions, but there were no significant differences among moisture levels under nonshaded conditions (Table 5). The MDSS was sig-

nificantly greater ($P < 0.05$) for moisture level 1 (wettest) than for moisture level 4 (driest) for shaded conditions but did not significantly differ among moisture levels under nonshaded conditions.

In the second trial, there were significant interactions between solar irradiance and moisture level for the AUSSC ($P = 0.0503$) and MDSS ($P = 0.001$). The AUSSC was not significantly different ($P > 0.05$) between the nonshaded/moisture level 1 and the shaded/moisture level 3, and the MDSS was not significantly different between nonshaded/moisture level 1 and the shaded/moisture level 4. In addition, the AUSSC and MDSS were significantly greater ($P < 0.05$) for shaded treatments at moisture levels 1 and 2 than for all other moisture levels and solar irradiances (Table 5). Mean solar irradiance, soil temperature, and water potential are recorded for both trials in Table 5.

Effect of solar irradiance on survival. In both trials, the AUSSC significantly decreased ($P < 0.001$) as solar irradiance increased (Table 6). Coefficients of determination were 0.69 and 0.80 for the first and second trials, respectively. The MDSS also significantly decreased ($P < 0.001$ and < 0.01 for the first and second trials, respectively) as solar irradiance increased (Table 6). Coefficients of determination were 0.68 and 0.59 for the first and second trials, respectively. Mean solar irradiance, soil temperature, and water potential are recorded for both trials in Table 6.

DISCUSSION

Quantifying length of sporangia survival of *P. infestans* in soil is a key component to understanding and modeling the potential spread of the late blight pathogen within and between potato fields and in predicting tuber infection. Survival of sporangia of *P. infestans* in soil has never been assessed in the Columbia Basin, and particularly, the survival of metalaxyl-resistant genotypes of the pathogen. The semi-arid environment present in the Columbia Basin is not conducive for long-term survival of sporangia directly exposed to the environment, and although the A1 and A2 mating types (9) of *P. infestans* have coexisted in the Columbia Basin for several years (23), production of sexual oospores

Table 3. Mean area under the spore survival curve (AUSSC) and mean maximum days of sporangia survival (MDSS) of three isolates of *Phytophthora infestans* exposed to two solar irradiances^a

Isolate/irradiance ^v	Trial 1		Trial 2		Trial 3	
	AUSSC ^w	MDSS ^x	AUSSC	MDSS	AUSSC	MDSS
110b						
Shaded	782	9.3	... ^y	...	532	9.7
Nonshaded	712	4.7	...	...	439	9.7
02						
Shaded	782	10.1	1,039 a	14.0	679	9.7
Nonshaded	...	...	467 b	11.7	593	8.3
GE-7						
Shaded	1,131	11.7	1,173 a	14.0	...	...
Nonshaded	700	4.7	537 b	9.3	...	...
Combined mean						
Shaded	898 ^z	10.4	1,106*	14.0*	606	9.7
Nonshaded	706	4.7	502	10.5	516	9.0

^a Trial 1 was conducted from April to May of 2004. Trials 2 and 3 were conducted during May of 2004. Values followed by the same letter are not significantly different at $P < 0.05$ based on Fisher's least significant difference. If no letters are present, values are not statistically different at $P < 0.05$. Isolate 110b, 02, and GE-7 are US-11, US-8, and US-8 isolates of *P. infestans*, respectively. See Table 4 for solar irradiance, soil temperature, and water potential information.

^v Shaded = sporangia completely shaded from direct sunlight by black nontransparent polyethylene or fiberglass and nonshaded = sporangia not shaded. Combined mean = since there were no significant differences between isolates for AUSSC and MDSS, a mean for all isolates combined was determined for each of these dependent variables. An asterisk next to a combined mean signifies a significant difference ($P < 0.5$) between shaded and nonshaded AUSSC or MDSS combined mean values, respectively, within a trial.

^w AUSSC determined using the equation given in Porter and Johnson (29).

^x Values in parentheses are the maximum range of detected survival measured in days.

^y ... = Not assessed or missing data.

^z * = Significant difference between the shaded and nonshaded combined mean.

Table 4. Mean solar irradiance (SI), soil temperature (ST), and water potential (WP) when three isolates of *Phytophthora infestans* were exposed to two solar irradiances during three trials^a

	Trial 1		Trial 2		Trial 3	
	Shaded ^y	Nonshaded	Shaded	Nonshaded	Shaded	Nonshaded
SI	31.3 ± 6.48	233.4 ± 28.23	8.1 ± 1.93	204.9 ± 22.22	8.9 ± 2.21	20.2 ± 23.20
ST ^z	11.4 ± 0.72 (4,22)	15.0 ± 1.09 (3,28)	12.2 ± 0.85 (6,24)	12.8 ± 0.94 (6,25)	11.7 ± 0.73 (5,17)	13.9 ± 1.57 (7,24)
WP	13 ± 0.9	11 ± 0.9	10 ± 2.1	8 ± 1.2	11 ± 0.8	5.0 ± 1.2

^a Values of environmental data are the daily means over the duration of the trial plus or minus the standard error. Solar irradiance was measured at the soil surface in Watts/m² using a pyranometer. Water potential was measured in centibars using Watermark water potential sensors (Spectrum Technologies Inc., Plainsfield, IL), located in the center of a microplot and buried 1 cm deep at a 45° angle. Temperature was measured in degrees Celsius and each sensor was placed in the center of a microplot at a 1 cm depth.

^y Shaded = sporangia completely shaded from direct sunlight by black nontransparent polyethylene or fiberglass. Nonshaded = sporangia not shaded.

^z Numbers in parentheses represent the minimum and maximum temperature for each treatment.

(survival spores) has not been observed (24). In addition, the spore viability of metalaxyl-resistant genotypes may be negatively impacted since the development of fungicide resistance by a pathogen can often reduce the fitness of that pathogen (7). Therefore, determining the viability of sporangia of *P. infestans* in soil in the Columbia Basin would advance the development of models used to understand late blight epidemics and tuber infection.

Sporangia of *P. infestans* of metalaxyl-resistant US-11 and US-8 clonal lineages were capable of surviving between 23 and 30 days in two soil types common to po-

tato production in the Columbia Basin (Table 1). This range of survival is similar to previous reports in which the maximum length of survival of *P. infestans* sporangia of the US-1 genotype under natural environmental conditions in artificially infested soil in pots (26) and naturally infested soil (18) was 21 and 32 days, respectively. Since the length of sporangia survival of the US-8 and US-11 isolates from this study were similar to the durations previously reported for the US-1 genotype, preliminary findings indicated that the development of phenylamide resistance by the pathogen has not significantly im-

pacted the ability of the sporangia to survive and germinate, although a larger population size would need to be tested to substantiate this finding.

Solar radiation can deleteriously affect the survival of spores (32). Reduced solar irradiance is likely the greatest factor influencing the viability of sporangia of *P. infestans* in soil since the AUSSC were significantly greater for shaded versus nonshaded treatments in eight of nine trials (Tables 1, 3, 5, 6). Spores directly exposed to sunlight are exposed to higher levels of UV-radiation than those that are shaded, and UV-radiation has been shown to nega-

Table 5. Solar irradiance, soil temperature, water potential, area under the spore survival curve (AUSSC), and mean maximum days of sporangia survival (MDSS) of *Phytophthora infestans* at four soil moistures when the soil was shaded or nonshaded in two trials^s

Treatment ^t	Trial 1					Trial 2				
	Solar irradiance ^u	Soil temperature ^v	Mean ψ_w ^w	AUSSC ^x	MDSS ^y	Solar irradiance	Soil temperature	Mean ψ_w	AUSSC	MDSS
Shaded										
1	0.2 ± 0.08	20.4 ± 0.83 (16,26)	11 ± 2.8	1267 a	16.0 (16) a	1.1 ± 6.64	19.0 ± 0.95 (14,27)	8 ± 0.8	712 a	14.0 (17) a
2	0.2 ± 0.08	20.7 ± 1.16 (16,25)	22 ± 4.5	1030 ab	14.7 (16) ab	1.1 ± 6.64	19.8 ± 0.98 (14,26)	2 ± 0.9	775 a	15.0 (17) a
3	0.2 ± 0.08	22.4 ± 1.94 (16,24)	14 ± 2.2	916 b	13.3 (16) ab	1.1 ± 6.64	21.6 ± 0.84 (15,26)	11 ± 1.9	485 bc	8.7 (11) b
4	0.2 ± 0.08	22.1 ± 2.03 (16,25)	20 ± 3.1	807 b	10.7 (12) b	1.1 ± 6.64	23.3 ± 1.14 (16,30)	12 ± 2.5	510 b	6.7 (8) bc
Nonshaded										
1	313 ± 38.9	20.1 ± 1.13 (17,31)	17 ± 4.8	267 c	1.7 (5) c	294 ± 38.4	19.3 ± 2.04 (13,35)	2 ± 0	330 cd	4.0 (4) cd
2	313 ± 38.9	21.0 ± 1.01 (17,27)	18 ± 1.5	325 c	3.3 (5) c	294 ± 38.4	21.6 ± 2.04 (14,36)	17 ± 1.7	203 d	1.3 (4) d
3	... ^z	...	...	...	...	294 ± 38.4	22.0 ± 1.54 (16,35)	14 ± 1.9	203 d	1.3 (4) d
4	313 ± 38.9	22.0 ± 2.03 (16,26)	17 ± 1.6	279 c	3.3 (5) c	294 ± 38.4	22.5 ± 1.57 (16,33)	33 ± 2.2	210 d	1.3 (4) d

^s Trials were conducted from June to July of 2004. Values followed by the same letter are not significantly different at $P < 0.05$ based on Fisher's least significant difference. Values of environmental data are the daily means over the duration of the trial plus or minus the standard error. *P. infestans* isolate used was US-8 isolate 02.

^t Treatments are: shaded = sporangia completely shaded from direct sunlight by black nontransparent polyethylene or fiberglass; and nonshaded = sporangia not shaded. Soil moisture levels: level 1 = watered to maintain a moist soil surface; level 2 = watered every other time that "1" was watered; level 3 = watered every third time "1" was watered; and level 4 = not watered after the initial water was applied when the sporangia were applied to the soil.

^u Solar irradiance was measured at the soil surface in Watts/m² using a pyranometer.

^v Temperature was measured in degrees Celsius and sensors were placed in the center of a microplot at a 1 cm depth. Numbers in parentheses represent the minimum and maximum temperature for each treatment.

^w Water potential was measured in centibars using Watermark water potential sensors (Spectrum Technologies Inc., Plainsfield, IL), located in the center of a microplot and buried 1 cm deep at a 45° angle.

^x AUSSC determined using the equation given in Porter and Johnson (29).

^y Values in parentheses are the maximum range of detected survival measured in days.

^z Since there was no detected survival at day 5, AUSSC and MDSS could not be determined.

Table 6. Mean solar irradiance, soil temperature, water potential, area under the spore survival curve (AUSSC), and mean maximum days of sporangia survival (MDSS) when sporangia of *Phytophthora infestans* were exposed to four solar irradiance levels^t

Irradiance level ^u	Trial 1					Trial 2				
	Solar irradiance ^v	Soil temperature ^w	Mean ψ_w ^x	AUSSC ^y	MDSS ^z	Solar irradiance	Soil temperature	Mean ψ_w	AUSSC	MDSS
1	1 ± 0.2	21.8 ± 1.10 (15,25)	13 ± 0.6	638	15.0 (15)	2 ± 0.3	18.2 ± 1.40 (12,24)	8 ± 1.6	920	15.0 (15)
2	16 ± 2.8	22.4 ± 1.04 (16,26)	14 ± 1.3	573	12.3 (15)	15 ± 2.3	17.5 ± 1.40 (12,24)	8 ± 2.0	981	15.0 (15)
3	49 ± 6.2	23 ± 1.30 (15,27)	14 ± 0.6	524	11.0 (11)	38 ± 5.2	18.1 ± 1.39 (12,24)	9 ± 2.0	817	15.0 (15)
4	253 ± 26.6	25.7 ± 1.74 (15,31)	12 ± 0.7	413	5.3 (8)	211 ± 23.9	20.1 ± 1.84 (12,28)	11 ± 1.7	529	10.3 (15)

^t Trial 1 was conducted during August of 2004. Trial 2 was conducted August to September of 2004. AUSSC and MDSS significantly decreased as solar irradiance increased. $P < 0.001$ for each of the AUSSC for trial 1 and 2 and $P < 0.001$ and $P < 0.01$ for the MDSS for trials 1 and 2, respectively, on the basis of regression analysis. Values of environmental data are the daily means over the duration of the trial plus or minus the standard error. Sporangia viability was assessed 10, 16, 23, and 30 days in trial 1 and 7, 17, 22, and 27 days in trial 2, after sporangia were applied to the soil.

^u Level 1 = sporangia completely shaded from direct sunlight by black nontransparent polyethylene or fiberglass, level 2 = sporangia shaded by six layers of nylon screen, level 3 = sporangia shaded by four layers of light brown nylon screen, and level 4 = sporangia not shaded.

^v Mean solar irradiance was measured at the soil surface in Watts/m² using a pyranometer.

^w Mean soil temperature in degrees Celsius at a 1 cm depth. Each temperature sensor was placed in the center of a microplot. Numbers in parentheses represent the minimum and maximum temperature for each treatment.

^x Water potential was measured in centibars using Watermark water potential sensors (Spectrum Technologies Inc., Plainsfield, IL), located in the center of a microplot and buried 1 cm deep at a 45° angle.

^y AUSSC was determined using the method published by Porter and Johnson (29).

^z Values in parentheses are the maximum range of detected survival measured in days.

tively impact the survival of sporangia of *P. infestans* (25,33,34). In addition, sporangia directly exposed to solar radiation most likely dehydrate at a faster rate than those that are shaded (11,35), and in every trial, maximum temperatures were greater for nonshaded versus shaded treatments raising the possibility that elevated soil temperature contributed to sporangia death.

A common cultural practice in the Columbia Basin is to apply a desiccant to potato vines or allow vines to naturally senesce 2 weeks prior to harvest. Desiccating potato vines so that vines are completely dead for 2 weeks before harvest offers the advantage of exposing sporangia to sunlight for an extended time period. This should allow many sporangia to die before harvest. However, many growers in the Columbia Basin harvest tubers when vines are green. Green vines would provide better shade conditions than vines that have been killed and more sporangia would be expected to survive under these shaded conditions on this basis of this research. However, previous research in the Columbia Basin demonstrated that there were no significant differences in tuber blight between tubers harvested from blighted fields when vines were green or after vines were desiccated (14). Important to note is that these fields were treated with fungicide until harvest and tubers were harvested during dry weather conditions (14).

Potato harvest in the Columbia Basin usually begins during the first part of August with most of the harvest completed by the end of October. Environmental conditions conducive for sporangia survival based on this and previous research (29) become more favorable from August to October. During this time period in the Columbia Basin, the photoperiod becomes shorter, solar irradiance values are reduced, the temperatures become cooler, and there tends to be more cloud cover, which also lowers the solar irradiance values. All of these factors reduce the likelihood of dehydration and exposure of sporangia to UV-radiation, which would increase sporangia survival and longevity (11,25,29). Therefore, it would be expected that natural tuber infection would be more likely for tubers growing or harvested later in the fall than for tubers growing or harvested at the first of the harvest period in August.

The impact of solar irradiance on sporangia survival can be greatly influenced by the natural medium (air, water, or soil) protecting the spores. Prior research under semiarid conditions determined that the survival of sporangia of metalaxyl-resistant genotypes of *P. infestans* exposed to direct sunlight was 4 h or less (33), while sporangia in water exposed to direct sunlight survived as much as 8 days (29). Soil, however, may provide the best conditions for spore survival since sporangia in

soil exposed to direct sunlight survived as much as 23 days in this study.

Soil types can differ in chemical properties (1,2), physical properties (29), and microorganisms (17,26,36), which all may impact survival of sporangia of *P. infestans*. The survival of sporangia in two soil types common to potato production in the Columbia Basin were assessed for their effect on sporangia survival in this study. The Quincy loamy fine sand and the Shano silt loam soils selected represent the two most common soil types used in potato production in Grant and Adams counties (10,22), which rank first and third, respectively, in number of potato acres harvested in the state of Washington (5). Although sporangia survival has differed among soil types from previous research (18,36), there were no significant differences between soil types in the current study in the mean AUSSC for both trials (Table 1). However, there was a trend in both trials toward greater AUSSC values in the Quincy loamy fine sand, and the MDSS was also significantly greater for the Quincy loamy fine sand in one of two trials (Table 1).

One potential reason for numerically higher AUSSC values for the Quincy loamy fine sand and a significantly greater MDSS in one of two trials may be the pore size difference between the two soil types. The pore size of the Shano silt loam and the Quincy loamy fine sand likely range from 2 to 50 and 100 to 250 μm , respectively, based on previous research (21). Sporangia of *P. infestans* are 29 to 36 μm long by 19 to 22 μm wide (8) and zoospores are 11 to 15 μm long and 7.5 to 8.5 μm wide (3). Approximately 70% of the pore sizes in a silt soil were 20 μm in diameter and 30% were 10 μm in diameter (21). The Quincy loamy fine sand has a pore size that would allow the movement of sporangia and zoospores through the soil, but the pore size of the Shano silt loam could prevent the movement of both spore types through the soil. Therefore, it would be expected that spores applied to the surface of a Shano silt loam would be less likely to penetrate the soil surface than spores applied to the Quincy loamy fine sand. This would increase the likelihood of spore exposure to UV-radiation and dehydration at the soil surface, which may account for the tendency of AUSSC values and MDSS values to be greater or significantly longer in the Quincy loamy fine sand.

Presence of sufficient soil moisture can reduce the likelihood of spore dehydration and facilitate the movement of spores to greater depths in the soil profile (18), where protection from UV-radiation would be greater than at the soil surface. However, increased soil moisture did not significantly increase the AUSSC or MDSS of spores in nonshaded conditions (Table 5). Soil moisture is important in preventing the dehydration of spores, but sporangia of

P. infestans may not be moving deep enough into the soil profile to reduce the negative effects of dehydration and excessive exposure to UV-radiation that can limit survival. Sporangia of *P. infestans* were only washed through soil columns down to 5 cm deep (13) and only the top 5 cm of field soils were infective in previous studies (27). Also in a previous study, assessing soil types common to potato production in the Columbia Basin, most infected tubers were found at the soil surface and infection was rare on tubers at 5 cm or deeper in the soil (28). The physical properties of the soils in our study may be limiting the movement of sporangia deep into the soil profile, and regardless of the amount of water applied to the soil surface, the majority of the sporangia may be confined to the surface where under nonshaded conditions, the surface soil is directly exposed to UV-radiation and can quickly dry making it so there are no significant differences between moisture levels under nonshaded conditions.

However, moisture levels did significantly impact AUSSC and MDSS under shaded conditions (Table 5). Sporangia in soils receiving more water applications survived longer than those with reduced applications. Therefore, when UV-radiation is reduced as a factor through shading, moisture level may increase in importance for sporangia survival. In addition, in shaded conditions, the soil surface does not dry as quickly and the spore exposure to UV-radiation is decreased.

There were no significant differences among the metalaxyl-resistant isolates in AUSSC and MDSS under shaded and nonshaded conditions, respectively, indicating that genotypes of *P. infestans* may not significantly differ in the quantity of sporangia surviving over time or the duration of sporangia survival. The US-8 genotype has replaced the US-1 and US-11 genotypes in the Columbia Basin, but there is no early indication based on this research that it would be due to the ability of the sporangia of the US-8 genotype to survive better than the US-11 and US-1 genotypes since the US-11 and US-8 genotypes in our study did not significantly differ in AUSSC and MDSS. A study using a large number of US-8 and US-11 isolates from the Columbia Basin would be desirable, but we are currently limited since US-11 and US-1 isolates from the Columbia Basin have not been detected for several years and isolates in long-term storage have been lost or have lost their pathogenicity.

ACKNOWLEDGMENTS

Funding for this study was provided by the Washington State Potato Commission. We thank Tom Cummings and Frank Jones for technical assistance. PPNS No. 0438, Department of Plant Pathology, College of Agricultural, Human, and Natural Resource Sciences Agricultural Research Center Project No. 0678, Washington State University, Pullman, WA 99164-6430.

LITERATURE CITED

1. Andrivon, D. 1994. Fate of *Phytophthora infestans* in a suppressive soil in relation to pH. Soil Biol. Biochem. 26:954-956.
2. Andrivon, D. 1995. Inhibition by aluminium of mycelial growth and of sporangial production and germination in *Phytophthora infestans*. Eur. J. Plant Pathol. 101:527-533.
3. Barr, D. J. S., and Desaulniers, N. L. 1990. The flagellar apparatus in the *Phytophthora infestans* zoospore. Can. J. Bot. 68:2112-2118.
4. Bentley, E. M. and Johnson, D. A. 1992. Late blight in central Washington. Proceedings of the 31st annual Washington state potato conference and trade fair. Pp. 1-5.
5. Census of Agriculture. 2002. National Agricultural Statistics Service, Washington DC.
6. Deahl, K. L., DeMuth, S. P., Pelter, G., and Ormrod, D. J. 1993. First report of resistance of *Phytophthora infestans* to metalaxyl in eastern Washington and southwestern British Columbia. Plant Dis. 77:429.
7. Dekker, J. 1982. Can we estimate the fungicide-resistance hazard in the field from laboratory and greenhouse tests? Pages 128-138 in: Fungicide Resistance in Crop Protection. J. Dekker and S. G. Georgopoulos, eds. Wageningen, The Netherlands. Centre for Agricultural Publishing and Documentation.
8. Erwin, D. C., and Ribeiro, O. K. 1996. *Phytophthora* Diseases Worldwide. American Phytopathological Society, St. Paul, MN.
9. Gallegly, M. E., and Galindo, J. 1958. Mating types and oospores of *Phytophthora infestans* in nature in Mexico. Phytopathology 48:274-277.
10. Gentry, H. R. 1984. Soil survey of Grant County, Washington. United States Department of Agriculture, Soil Conservation Service, Washington, DC.
11. Glendinning, D., MacDonald, J. A., and Grainger, J. 1963. Factors affecting the germination of sporangia in *Phytophthora infestans*. Trans. Brit. Mycol. Soc. 46:595-603.
12. Goodwin, S. B., Smart, C. D., Sandrock, R. W., Deahl, K. L., Punja, Z. K., and Fry, W. E. 1998. Genetic change within populations of *Phytophthora infestans* in the United States and Canada 1994 to 1996: Role of migration and recombination. Phytopathology 88:939-949.
13. Jensen, J. L. 1887. Moyens de combattre et de détruire le *Peronospora* de la pomme de terre. Mem. Soc. Nat. Agric. Fr. 131:31-156.
14. Johnson, D. A., Martin, M., and Cummings, T. F. 2003. Effect of chemical defoliation, irrigation water, and distance from the pivot on late blight tuber rot in center-pivot irrigated potatoes in the Columbia Basin. Plant Dis. 87:977-982.
15. Jones, L. R., Giddings, N. J., and Lutman, B. F. 1912. Investigations of the potato fungus *Phytophthora infestans*. Pages 9-94 in: Vermont Agric. Exp. Stn. Bull. No. 168. Burlington Free Press Printing Co., Vermont.
16. King, J. E., Colhoun, J., and Butler, R. D. 1968. Changes in the ultrastructure of sporangia of *Phytophthora infestans* associated with indirect germination and ageing. Trans. Br. Mycol. Soc. 51:269-281.
17. Kostrowicka, M. 1959. Interaction between *Phytophthora infestans* and *Rhizoctonia solani*. Proc. 9th Int. Bot. Congr. 2:201.
18. Lacey, J. 1965. The infectivity of soils containing *Phytophthora infestans*. Ann. Appl. Biol. 56:363-380.
19. Lacey, J. 1967. The role of water in the spread of *Phytophthora infestans* in the potato crop. Ann. Appl. Biol. 59:245-255.
20. Larance, R. S., and Martin, W. J. 1954. Comparison of isolates of *Phytophthora infestans* at different temperatures. Phytopathology 44:495.
21. Leamer, R. W., and Lutz, J. F. 1940. Determination of pore-size distribution in soils. Soil Sci. 49:347-360.
22. Lenfesty, C. D. 1967. Soil survey of Adams County, Washington. United States Department of Agriculture, Soil Conservation Service, Washington, DC.
23. Miller, J. S., Hamm, P. B., and Johnson, D. A. 1997. Characterization of the *Phytophthora infestans* population in the Columbia Basin of Oregon and Washington from 1992-1995. Phytopathology 87:656-660.
24. Miller, J. S., and Johnson, D. A. 2000. Competitive fitness of *Phytophthora infestans* isolates under semiarid field conditions. Phytopathology 90:220-227.
25. Mizubuti, E. S. G., Aylor, D. E., and Fry, W. E. 2000. Survival of *Phytophthora infestans* sporangia exposed to solar radiation. Phytopathology 90:78-84.
26. Murphy, P. A. 1922. The bionomics of the conidia of *Phytophthora infestans*. Sci. Proc. R. Dublin Soc. 16:442-466.
27. Murphy, P. A., and McKay, R. 1925. Further experiments on the sources and development of blight infection in potato tubers. J. Dep. Lds. Agric., Dublin 25:10-21.
28. Porter, L. D., Dasgupta, N., and Johnson, D. A. 2005. Effects of tuber depth and soil moisture on infection of potato tubers in soil by *Phytophthora infestans*. Plant Dis. 89:146-152.
29. Porter, L. D., and Johnson, D. A. 2004. Survival of *Phytophthora infestans* in surface water. Phytopathology 94:380-387.
30. Rasmussen, J. J. 1971. Soil survey of Benton County area, Washington. United States Department of Agriculture, Oil Conservation Service, Washington, DC.
31. Rotem, J., Cohen, Y., and Putler, J. 1971. Relativity of limiting and optimum inoculum loads, wetting durations, and temperatures for infection by *Phytophthora infestans*. Phytopathology 61:275-278.
32. Rotem, J., Wooding, B., and Aylor, D. E. 1985. The role of solar radiation, especially ultraviolet, in the mortality of fungal spores. Phytopathology 75:510-514.
33. Sunseri, M. A., Johnson, D. A., and Dasgupta, N. 2002. Survival of detached sporangia of *Phytophthora infestans* exposed to ambient, relatively dry atmospheric conditions. Am. J. Potato Res. 79:443-450.
34. Vishtynetskaya, T. A., D'yakov, Y. T., and Zav'yalova, L. A. 1973. Effect of UV radiation on *Phytophthora infestans* (Mont.) de Bary. Sov. Genet. 9:36-42.
35. Warren, R. C., and Colhoun, J. 1975. Viability of sporangia of *Phytophthora infestans* in relation to drying. Trans. Br. Mycol. Soc. 64:73-78.
36. Zan, K. 1962. Activity of *Phytophthora infestans* in soil in relation to tuber infection. Trans. Br. Mycol. Soc. 45:205-221.