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Aggressiveness of *Phytophthora Infestans* Genotypes on Potato Stems and Leaves at Three Temperatures

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Abstract Epidemics of late blight on potato in the semiarid Pacific Northwest since 1991 have been characterized by a prevalence of stem lesions in relation to leaf lesions. This study was conducted in 1997 to test the hypothesis that isolates representing immigrant strains of *Phytophthora infestans* were more adapted at causing stem lesions and more aggressive at higher temperatures than isolates representing the relatively older US-1 strain. A total of 23 *Phytophthora infestans* isolates representing US-1, US-8, and a new A1 compatibility type strain were tested for aggressiveness on leaflets and stems of whole potato plants (cv. Russet Burbank) in 11 trials. Plants in one set of trials were incubated at constant temperatures of 18, 23, and 28 °C for six days with a 16 h photoperiod. Plants in the second set of trials were incubated at the same three day temperatures but the night temperature in all treatments was reduced to 16 °C. Lesion establishment was recorded, and daily severity readings were used to calculate the area under the lesion expansion curve (AULEC). Lesion area, sporulation frequency, sporulation time, and sporulation capacity were also measured. Lesion establishment was higher on stems than on leaves for isolates of all strains. Isolates representing US-8 and new A1 strains often had higher AULEC values but had similar lesion establishment, sporulation frequency, sporulation time, and sporulation capacity values as US-1 isolates. A reduction in components of aggressiveness for all strains was noted at 28 °C, with leaflets being more affected than stems. Sporulation rarely occurred at 28 °C. Few differences in components of aggressiveness were observed between 18 and 23 °C. These findings indicate that isolates from the relatively newer strains (US-8 and new A1) were not better adapted in causing lesions on potato stems than isolates from the old US-1 strain, nor were they better adapted to higher temperatures. The relatively newer strains, however, were generally more aggressive as indicated by higher AULEC on stems and leaflets over the range of temperatures used in this experiment.

Resumen Las epidemias del tizón tardío de la papa en el Pacífico Noroccidental semiárido desde 1991 han sido caracterizadas por una prevalencia de lesiones del tallo sobre las de hoja. Este estudio se efectuó en 1997 para probar la hipótesis de que los aislamientos representando variantes inmigrantes de *Phytophthora infestans* se adaptaron mejor causando lesiones de tallo y fueron más agresivas a temperaturas más altas que los aislamientos representando la variante relativamente más vieja de US-1. Se probó un total de 23 aislamientos de *Phytophthora infestans* representando US-1, US-8 y una nueva cepa de tipo de compatibilidad A1, respecto a agresividad, en pecíolos y tallos de plantas completas de papa (var. Russet Burbank) en 11 ensayos. Se incubaron plantas en un grupo de ensayos a temperaturas de 18, 23, y 28 °C por seis días con un fotoperiodo de 16 hs. Las plantas en el segundo juego de ensayos se incubaron tres días a las mismas temperaturas, pero la temperatura nocturna en todos los tratamientos se redujo a 16 °C. Se registró el establecimiento de la lesión y se usaron lecturas diarias de severidad para calcular el área bajo la curva de expansión de la lesión (AULEC). También se midieron el área de la lesión, la frecuencia, tiempo y capacidad de la esporulación. El establecimiento de la lesión fue más alto en tallos que en hojas para aislamientos de todas las variantes. Los aislamientos US-8 y el nuevo A1 a menudo tuvieron valores de AULEC más altos, pero similar valor para establecimiento de la lesión, frecuencia, tiempo y capacidad de esporulación a los US-1. Se notó una reducción en los componentes de agresividad para

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todas las variantes a 28 °C, con mayor afectación de los pecíolos que de los tallos. La esporulación raramente ocurrió a 28 °C. Se observaron pocas diferencias en los componentes de agresividad entre 18 y 23 °C. Estos hallazgos indican que los aislamientos de las cepas relativamente nuevas (US-8 y nuevo A1) no tuvieron mejor adaptación respecto a causar lesiones en tallos de papa que los aislamientos de la vieja cepa US-1, ni tuvieron mejor adaptación a temperaturas más altas. No obstante, las cepas relativamente más nuevas fueron generalmente más agresivas como se indica por un AULEC más alto en tallos y pecíolos sobre la amplitud de temperaturas usadas en este experimento.

Keywords Late blight

Introduction

Late blight of potato (Solanum tuberosum subsp. tuberosum L.) is caused by the oomycete *Phytophthora infestans* (Mont.) de Bary. *P. infestans* was responsible for the devastating losses which resulted in the Irish potato famine (Large 1940) and re-emerged in the 1990's as one of the most important pathogens of potato worldwide (Fry and Goodwin 1997). Evidence supports that a single clonal lineage of the pathogen, characterized by the A1 compatibility type, metalaxyl sensitivity, 86/100 glucose-6-phospate isomerase (*Gpi*) phenotype, and 92/100 peptidase (*Pep*) phenotype, existed worldwide outside Mexico until the 1980's (Goodwin et al. 1994b). A great deal of interest was generated in characterizing collections of *P. infestans* when the A2 compatibility type was reported from Switzerland in 1984 (Hohl and Iselin 1984). Characterization of *P. infestans* collections from around the world indicated that new strains migrated throughout the world from Mexico (Fry et al. 1993; 1992; Spielman et al. 1991) and replaced the old clonal lineage (Day and Shattock 1997; Dowley 1987; Fry and Goodwin 1997; Fry et al. 1993; Goodwin et al. 1994a; Kadish and Cohen 1989; Miller et al. 1997; Spielman et al. 1991).

Severe late blight epidemics have regularly occurred in the semiarid potato growing region of the Columbia Basin of Washington and Oregon since 1991 (Johnson et al. 1997; 2009; Miller et al. 1997). Epidemics since 1993 have primarily been caused by relatively newer strains of *P. infestans* which are more aggressive than the old US-1 lineage (Miller et al. 1998). Additionally, a high proportion of stem lesions were observed in many fields where newer strains were found (D.A. Johnson, unpublished data). Replacement of the old clonal lineage by new strains could have been influenced by fungicide resistance, overwintering adaptations, increased aggressiveness, temperature adaptations, higher incidence of stem lesions, and increased fitness of the newer strains. It is doubtful that a single factor is solely responsible, and it is more probable that most, if not all, of the above factors have contributed in some manner.

Increased aggressiveness is one factor which may be responsible for this replacement of the old clonal lineage by new strains. Aggressiveness has been defined as "the pathogen's polygenic general pathogenicity" (Browning et al. 1977) and as "the ability of a pathogen to increase to epidemic proportions in a host population" (Knutson and Eide 1961). Races of a pathogen which do not differentially infect host genotypes but differ in the quantitative amount of disease they cause on the same genotype of the host are said to differ in aggressiveness (Vanderplank 1982). Differential aggressiveness has been shown among isolates of different races (Castronovo et al. 1954; Knutson and Eide 1961), among isolates sensitive and insensitive to metalaxyl (Day and Shattock 1997; Kadish and Cohen 1988; 1989; Kadish et al. 1990), among the old A1 clonal lineage and isolates from the sexually reproducing population in Toluca, Mexico (Tooley et al. 1986), and among isolates from the old clonal lineage and newer clonal lineages (Kato et al. 1997; Miller et al. 1998). Components of aggressiveness examined in these studies have included infectivity, tissue colonization, and sporulation capacity. Testing of these aggressiveness components has been done using leaf discs (Dowley 1987), detached leaflets in a laboratory (Day and Shattock 1997; Kadish and Cohen 1988; Kato et al. 1997; Miller et al. 1998), plants in plastic walk-in tunnels (Kadish and Cohen 1988; 1989), and plants in a greenhouse (Bashan et al. 1989; Dowley 1987; Kadish and Cohen 1988; Kadish et al. 1990; Knutson 1962; Tooley et al. 1986).

The increased occurrence of stem lesions may have influenced the replacement of the US-1 strain by the US-8 strain in the Columbia Basin. The US-8 strain dominated in the Columbia Basin from 1994 to 2008 (Johnson et al. 2012; Johnson and Cummings 2009; Porter et al. 2004) and again in 2013 (unpublished data). During periods of hot weather, stem lesions survive better than leaf lesions (Kable and MacKenzie 1980; Rotem and Cohen 1974). Prior to the 1990's, late blight traditionally was not a problem in the semiarid regions of Washington (Johnson et al. 1996). The increased occurrence of late blight epidemics in the 1990's coincided with the appearance of newer strains of the pathogen, prompting speculation that newer strains are better adapted to elevated temperatures than the old US-1 strain. These experiments were conducted to test the hypotheses that new strains cause more severe stem lesions than the old US-1 strain and that new strains are more aggressive on leaves and stems at higher temperatures.

Materials and Methods

Potato Plants Certified Russet Burbank seed tubers were rinsed under warm tap water, surface sterilized in 1 % sodium

hypochlorite for 10 min, and then allowed to dry under ambient laboratory conditions. Seed tubers were then placed in the dark at room temperature for 5 to 7 days to promote sprout formation. Sprouting buds were cut from tubers with a sterile, dish-shaped, melon-ball cutter to produce spherical tuber seed pieces weighing approximately 13 g. Tuber seed pieces were planted at a depth of 10 cm in 15-cm-diameter plastic pots containing a standard potting mix (3:1:1 [vol/vol/vol], peat moss, pumice, and sand). Ten grams of 34–0–0 NPK fertilizer were incorporated into the potting mix prior to planting and plants were watered as needed following emergence.

Isolate Collection, Maintenance, and Characterization Isolates of *P. infestans* were obtained from naturally infected potato foliage in the Columbia Basin of Washington and Oregon from 1994 to 1996, and recovered from experimental plots in Pullman, WA in 1997 (Table 1). Cultures were obtained by transferring sporangia from the margin of a single sporulating lesion onto rye extract agar (Ribeiro 1978) amended with 100 μg/ml each of penicillin, pimaricin, and polymyxin or by removing a small area of infected leaf tissue near the lesion margin and plating it onto rye extract agar containing 10 μl/ml of pimaricin, 250 U/ml of ampicillin, and 10 μg/ml of rifampicin. After initial growth on media was obtained, isolates were derived by transferring a hyphal tip or single sporangium to unamended rye extract agar. Isolates were maintained on rye extract agar at 18 °C in darkness and were transferred every 4 to 5 weeks. Isolates were inoculated on detached leaves and reisolated at the initiation of the study to select against isolates which had lost pathogenicity in culture. Isolates were characterized for compatibility type (Miller et al. 1998), glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*) allozyme phenotype (Goodwin et al. 1995), and restriction fragment length polymorphism (RFLP) genotype as determined by the RG57 nuclear DNA probe (Goodwin et al. 1992). Isolates were selected from three multilocus genotypes (Goodwin et al. 1994a), or strain classifications: US-1, US-8, or new A1 (Table 1). RFLP characterization was not obtained for the new A1 strain.

Inoculum Production Sporangia were washed from fresh rye extract agar cultures (1 to 3 weeks old) with distilled water. A 50 μl drop of this unquantified suspension was placed on a 1 cm^2 piece of Whatman no. 1 filter paper. The filter paper square was then placed face down on the abaxial leaflet surface of several detached leaves. Detached leaves were placed in 16×150 mm test tubes containing 22 ml of a 6.5 g/L sucrose solution with 0.435 g/L 20–20–20 NPK fertilizer and incubated for 5–6 days in a plastic box lined with moistened paper towels in a growth chamber at 18 °C with a 16 h photoperiod (57 to 59 μmol m^{-2} s^{-1}). Sporangia from sporulating lesions were washed from leaves with distilled water and the resulting suspension was adjusted to 1× 10^4 sporangia/ml. The sporangial suspension was chilled at 4 °C for two hours to induce zoospore liberation prior to use in inoculation.

Inoculation and Data Collection Plants were inoculated as described above on the adaxial surface of 5 leaflets and on three different locations of the main plant stem. Plants were then placed in a mist chamber for 20 h. Plants were removed from the mist chamber, the inoculation sites were tagged, filter paper squares were removed, and plants were placed in one of three constant temperature growth chambers set at 18 °C, 23 °C, or 28 °C with a 16 h photoperiod (208–254 μmol m^{-2} s^{-1}). Leaflet and stem lesions were evaluated daily for 6 days.

Leaflets were scored for the percentage of the leaflet affected and stem lesions or infections were rated on a 0 to 4 scale:

0 = No visible lesion.
0.5 = Up to 5 small necrotic flecks.
1 = Lesion < 2.5 cm long.
2 = Lesion 2.5 to < 5 cm long.
3 = Lesion 5 to 10 cm long.
4 = Lesion over 10 cm long, or 5 to 10 cm long with stem girdled.

Lesion establishment was calculated as the number of inoculation sites developing lesions. The area under the lesion expansion curve (AULEC) was determined by plotting daily lesion ratings over time and calculating the resultant curve (Campbell and Madden 1990).

Leaflets and stem sections with lesions were then cut from the plants and placed in glass Petri dishes (15×100 mm) containing moistened filter paper. Lesions were incubated in growth chambers at the corresponding temperatures with a 16 h photoperiod (57–59 μmol m^{-2} s^{-1}) and examined every other hour and the time required for sporulation (sporulation time) was noted. After lesions had been incubated for 24 h, leaflet lesion areas were recorded by placing overhead transparencies over the Petri dishes and tracing around the edges of the lesions. The lesion area tracings on the overhead transparencies were measured using the Agvision image analysis system (Decagon Devices, Pullman, WA). Stem lesion areas were determined by estimating the length and width of each lesion with a ruler. Sporangia were then collected by placing leaflets and stem pieces in 50 ml polystyrene bottles containing approximately 10 ml of sporangial fixative (90:5:5 [vol/vol/vol], 90 % ethanol, glacial acetic acid, and formalin) and shaken vigorously. The total number of sporangia dislodged from each lesion was determined with a hemacytometer (8 counts per lesion). The sporulation capacity of each lesion was determined by dividing the total number of sporangia by the lesion area. The sporulation frequency, or percentage of

Table 1 Characteristics of *Phytophthora infestans* isolates used in constant temperature and reduced night temperature trials[a]

Strain[b] Isolate	Date Collected	Compatibility Type	*Gpi* Genotype	*Pep* Genotype	RFLP Genotype[b]
US-1					
1–1 Pl 63	July 1992	A1	86/100	92/100	10111010110011010000110011
1–2 Fi 2	July 1994	A1	86/100	92/100	10111010110011010000110011
1–3 412–1	July 1994	A1	86/100	92/100	10111010110011010000110011
1–4 412–2	July 1994	A1	86/100	92/100	10111010110011010000110011
1–5 FS2–3	August 1997	A1	86/100	92/100	10111010110011010000110011
1–6 FS2–9	August 1997	A1	86/100	92/100	10111010110011010000110011
1–7 FS4B	August 1997	A1	86/100	92/100	10111010110011010000110011
US-8					
8–1 584–1	August 1994	A2	100/111/122	100/100	10011000010011010000110111
8–2 584–6	August 1994	A2	100/111/122	100/100	10011000010011010000110111
8–3 5–149	July 1995	A2	100/111/122	100/100	10011000010011010000110111
8–4 B9–1	June 1996	A2	100/111/122	100/100	10011000010011010000110111
8–5 EM1	August 1996	A2	100/111/122	100/100	10011000010011010000110111
8–6 CF1	August 1996	A2	100/111/122	100/100	10011000010011010000110111
8–7 Vol 2	November 1996	A2	100/111/122	100/100	10011000010011010000110111
8–8 FS1–5	August 1997	A2	100/111/122	100/100	10011000010011010000110111
8–9 FS4A	August 1997	A2	100/111/122	100/100	10011000010011010000110111
New A1					
N-1 366–1	July 1996	A1	100/111/122	100/100	Untested
N-2 366–2	July 1996	A1	100/111/122	100/100	Untested
N-3 366–3	July 1996	A1	100/111/122	100/100	Untested
N-4 366–4	July 1996	A1	100/111/122	100/100	Untested
N-5 366–6	July 1996	A1	100/111/122	100/100	Untested
N-6 366–9	July 1996	A1	100/111/122	100/100	Untested
N-7 366–10	July 1996	A1	100/111/122	100/100	Untested

[a] Isolates were collected from naturally infected potato foliage. Six constant temperature trials (designated trials A through F) and five reduced night temperature trials (designated trials G through K) were carried out over a 10 month period

[b] Strain determined by compatibility type, glucose-6-phosphate isomerase and peptidase allozyme genotype, and restriction fragment length polymorphism (RFLP) genotype

[c] RFLP genotype for the new A1 strain was not tested

inoculation sites which developed into lesions and sporulated under high humidity within 24 h, was also determined.

Experimental Design and Statistical Analysis Only three or four isolates could be tested simultaneously in growth chambers so isolates were tested in a series of trials. Isolate 1–2 (US-1, Table 1) was included in every trial as a standard because it reliably produced adequate amounts of inoculum and was subjectively judged to be moderately aggressive for US-1 isolates in previous tests (Miller et al. 1998). The experiment was performed twice. The first set of trials was performed at constant temperature and is referred to as constant temperature trials (trials A through F). In the second set of trials, the growth chamber temperatures during the 6 day incubation period were modified so that each chamber would drop to 16 °C during the 8 h "night" period. This set of trials was referred to as the reduced night temperature trials (trials G through K). A total of 23 isolates was tested over 11 trials (Table 1). All trials were designed as randomized complete blocks with three replications. Replications were individual plants and different leaflet or stem inoculation sites on each plant were treated as subsamples.

Data were analyzed with the SAS GLM and CORR procedures (SAS Institute, Cary, NC). Testing for significant differences among model effects was done using analysis of variance (ANOVA) according to trial and lesion type (stem and leaf) with the following model statement: $Y = \mu + \text{Temperature} + \text{Block (Temperature)} + \text{Isolate} + \text{Isolate*Temperature} + \text{Error}$. The mean squared error term for the Block (Temperature) effect was used to test the Temperature effect. Mean separation of model effects was

performed using Fisher's LSD with a critical value of $P \leq 0.05$. In the absence of significant interactions among isolates and temperatures, mean separation was performed on marginal means. When significant interactions were observed, mean separation was performed on isolates at each level of temperature and on temperatures at each level of isolate. Transformation of raw data was occasionally needed when residuals of the raw data failed to conform to the assumptions of normality or equal variances. Linear contrasts were done to evaluate the effect of isolates grouped according to strain. The model statement was then modified to include the effect of lesion site (stem or leaflet) and ANOVA was done to test for differences between stem and leaflet aggressiveness components. Additionally, tests for correlations among aggressiveness components were performed using Pearson's correlation coefficients.

Results

Constant Temperature Studies Lesion establishment for some US-1 isolates was statistically similar to that of some US-8 or what we termed new A1 isolates (N designation) on stems (trials A, C, D, E, and F; Table 2). Some US-8 isolates had lower lesion establishment values on stems than the standard US-1 isolate (1–2 in trials B, D, and F). On leaflets, US-1 isolates had lesion establishment values similar to (trials C and E) or significantly higher than (trials D and F) US-8 or new A1 isolates. Two older US-1 isolates (1–1 and 1–3, trial C) did not establish on leaflets. The older of these, 1–1, was isolated from the field in 1992 (Table 1). On stems, results were similar (trials A, B, C, E, and F). Clear trends for AULEC on leaflets and stems were not observed for isolates. On leaflets, significant differences among isolates were only observed in three of the six trials, and in these trials US-1 and US-8 isolates were similar. Significant differences were observed in all tests for stem AULEC. Isolate 1–2 had the lowest stem AULEC on stems in trial A and the highest in trial D. Differences in sporulation frequency on leaflets were observed in three of the six trials with the standard US-1 isolate (1–2) similar to US-8 (8–4, trial C), or higher (8–2 and 8–2, trial D). Isolate 1–2 had a lower sporulation frequency than isolate N-1 (trial F), but was similar to all other new A1 isolates tested (trials A, B, and E). Sporulation frequency on stems was variable with isolate 1–2 being similar to new A1 isolates (trial B) or higher than US-8 (8–1 in trial B and 8–3 in trial F) or other new A1 isolates (N-1 in trial F. In trial E isolate 1–2 did not sporulate. Isolate 1–3 was similar to N-6 for all aggressiveness components measured (trial A), but isolates 1–1 and 1–3 were generally less aggressive than isolate 8–4 (trial C). Final lesion area, sporulation time, and sporulation capacity values exhibited high variability and fewer significant differences were observed.

Table 2 Lesion establishment, area under the lesion expansion curve (AULEC) and sporulation frequency when *Phytophthora infestans* isolates representing three multilocus genotypes were inoculated on Russet Burbank potato leaflets and stems and incubated at three constant temperatures for 6 days[a]

Test	Lesion		AULEC[d]		Sporulation	
Isolate[b]	Establishment[c]				Frequency[e]	
	Leaflet	Stem	Leaflet	Stem	Leaflet	Stem
A						
N-6	14	97 a	51	11.2 a	17	92
1–3	25	92 a	39	9.7 a	17	75
1–2	8	78 b	12	6.0 b	4	50
B						
N-5	42	94 a	84	8.1 a	39	100 a
N-7	39	94 a	20	5.6 b	27	50 bc
8–1	16	43 b	14	2.0 c	13	14 c
1–2	40	94 a	37	5.6 b	33	67 ab
C						
8–4	33 a	67 ab	29 a	3.9 a	40 a	33 a
1–4	0 b	50 b	0 b	1.4 b	0 b	0 b
1–2	36 a	89 a	60 a	4.2 a	40 a	37 a
1–1	0 b	9 c	0 b	0.9 b	0 b	0 b
D						
8–2	11 b	**	25 b	2.9 b	17 b	7 b
8–1	13 b	**	50 a	3.5 b	7 b	0 b
1–2	38 a	**	48 a	7.7 a	50 a	33 a
E						
N-2	44 a	52 b	**	2.5 b	31	0 b
8–6	11 b	30 b	**	1.4 b	7	0 b
8–5	36 a	96 a	**	12.8 a	40	70 a
1–2	44 a	54 b	**	1.8 b	10	0 b
F						
N-1	7 b	72 a	25	**	41 a	31 b
8–3	10 b	44 b	13	**	7 b	17 b
1–2	32 a	89 a	53	**	10 b	70 a

[a] Isolates were tested in 6 trials, designated A through F. Testing for significant differences among model effects within each trial was done using ANOVA. Mean separation of model effects was determined using Fisher's LSD. Isolate means within each trial followed by the different lowercase letters are statistically different ($P \leq 0.05$). "**" used to denote significant isolate × temperature interaction. Interaction values are given in Table 4. "–" indicates no data

[b] First character refers to multilocus genotype (1 = US-1, 8 = US-8, and N = "New" A1; see Table 1)

[c] Percentage of inoculation sites developing into lesions. Values in trial B were transformed with the ln (x+1) transformation prior to mean separation to satisfy the assumptions of normality and equal variances. Untransformed values are listed in the table

[d] Area under the lesion expansion curve. Values in trials A, C, E, and F were transformed with the ln (x+1) transformation and values in trial B were transformed with the reciprocal transformation (1/x) prior to mean separation to satisfy the assumptions of normality and equal variances. Untransformed values are listed in the table

[e] Number of sporulating lesions divided by the number of inoculation sites. Lesions from the 28 °C temperature treatment did not sporulate. Due to the number of missing values, valid ANOVA cannot be performed for trial E

Table 3 Lesion establishment, area under the lesion expansion curve and sporulation frequency when isolates of *Phytophthora infestans* were inoculated on Russet Burbank potato leaflets and stems and incubated at 16 °C during an 8 h night period and at three different temperatures during an artificially lighted day period for 6 days[a]

Test	Lesion		AULEC[c]		Sporulation	
Temp (C)	Establishment[b]				Frequency[d]	
	Leaflet	Stem	Leaflet	Stem	Leaflet	Stem
A						
18	22	86 a	39	9.5 a	17	70
23	11	100 b	55	11.5 a	8	75
28	14	86 a	8	5.9 b	–	–
B						
18	43	88	64	6.7 a	33	54
23	37	82	50	6.6 a	23	61
28	23	75	2	2.6 b	–	–
C						
18	20	42	91 a	1.4	20	8
23	25	65	82 a	3.8	20	27
28	7	54	13 b	2.6	–	–
D						
18	36	**	60	4.7	31	13
23	22	**	27	6.3	18	15
28	4	**	1	3.3	–	–
E						
18	43	51	**	3.0 a	28	4
23	43	51	**	8.1 b	15	25
28	15	71	**	2.8 a	–	–
F						
18	28	61	42	**	20	33
23	21	81	50	**	18	44
28	0	63	0	**	–	–

[a] *P. infestans* isolates were tested in 6 trials, designated A through F. Testing for significant differences among model effects within each trial was done using ANOVA. Mean separation of model effects was determined using Fisher's LSD. Temperature means within each trial followed by the different lowercase letters are statistically different ($P \leq 0.05$). "**" used to denote significant isolate × temperature interaction. Interaction values are given in Table 4. "–" indicates no data

[b] Percentage of inoculation sites developing into lesions. Values in trial B were transformed with the ln (x+1) transformation prior to mean separation to satisfy the assumptions of normality and equal variances. Untransformed values are listed in the table

[c] Area under the lesion expansion curve. Values in trials A, C, E, and F were transformed with the ln (x+1) transformation and values in trial B were transformed with the reciprocal transformation (1/x) prior to mean separation to satisfy the assumptions of normality and equal variances. Untransformed values are listed in the table

[d] Number of lesions which sporulated divided by the total number of inoculation sites. Lesions from the 28 °C temperature treatment did not sporulate. Due to the number of missing values, valid ANOVA cannot be performed for trial E

[e] Time required in hours for lesions to sporulate when placed at high humidity. Lesions from the 28 °C temperature treatment did not sporulate. Due to the number of missing values, valid ANOVA cannot be performed for leaflet lesions in trial A and stem lesions in trials D and E

[f] Number of sporangia (×1,000) per cm^2 lesion area. A sporulation capacity of 0 indicates sporangia were observed with the aid of a dissecting microscope, but not detected in the spore fixative used to rinse the sporulating lesion. Lesions from 28 °C did not sporulate. Values in trials A and E were transformed with the ln (x+1) transformation and values in trial D were transformed with the reciprocal transformation (1/x) prior to mean separation to satisfy the assumptions of normality and equal variances. Untransformed values are listed in the table. Due to missing values, valid ANOVA cannot be performed for stem lesions in trial E and leaflet lesions in trial F

Significant differences among temperatures were observed for lesion establishment on stems in trial A and significant interactions were found between the main effects for stem lesions in trial D (Table 3). Lesion establishment was highest at 23 °C in trial A and in trial D for isolate 8–1. Values for AULEC on stems and leaflets were sometimes lower at 28 °C than at the

other temperatures (stems in trials A and B, leaflets in C) and were similar at 18 and 23 °C in all trials except for stems in trial E. Lesion area values exhibited high variability and fewer significant differences were observed than with AULEC (data not shown). None of the isolates tested sporulated at 28 °C, and significant differences were not observed between 18 and 23 °C for sporulation frequency and sporulation time. Sporulation capacity was higher at 18 °C for leaflets in trial A and stems in trial F.

Interactions among temperatures and isolates were observed for lesion establishment on stems in trial D, and for AULEC on leaflets in trial E and stems in trial F (Table 4). In trial D, lesion establishment was similar for all isolates at 23 °C, but differences were observed at 18 and 28 °C. Isolate 1–2 had similar values at all temperatures while lesion establishment for the two US-8 isolates varied. In trial E, AULEC was similar for all isolates at 28 °C. Isolate 8–6 had significantly lower AULEC values than 8–5 and N-2 at 18 and 23 °C. AULEC on stems in trial F was similar for all isolates at 28 °C, but isolate N-1 and 8–3 had significantly lower values than isolate 1–2 at 18 and 23 °C.

Table 4 Interactions among *Phytophthora infestans* isolates and temperatures for lesion establishment and area under the lesion expansion curve (AULEC) when three multilocus genotypes of *Phytophthora infestans* were inoculated on Russet Burbank potato leaflets and stems and incubated at three constant temperatures for 6 days[a]

Parameter (Test, tissue type)	Temperature (°C)		
Isolate[b]	18	23	28
Lesion establishment[c] (D, stems)			
1–2	100 a A	100 a A	89 a A
8–2	100 a A	100 a A	50 b B
8–1	34 b B	100 a A	17 b B
AULEC[d] (E, leaflets)			
8–5	52 a A	91 a A	1 a B
N–2	33 a A	39 a A	1 a B
1–2	23 ab A	23 a A	11 a A
8–6	9 b A	1 b A	13 a A
AULEC[d] (F, stems)			
1–2	11.5 a A	12.6 a A	3.2 a B
N–1	5.3 b AB	5.9 b A	3.0 a B
8–3	2.7 b AB	3.7 b A	0.8 a B

[a] *P. infestans* isolates were tested in 6 trials, designated A through F. Testing for significant differences among model effects within each trial was done using ANOVA. Due to significant isolate × temperature interaction, mean separation of isolates was performed by temperature and mean separation of temperature was performed by isolate using Fisher's LSD. Isolate means with different lowercase letters within each temperature (column) are significantly different and temperature means with different uppercase letters within each isolate (row) are statistically different ($P \leq 0.05$)

[b] First character refers to multilocus genotype (1 = US-1, 8 = US-8, and N = "New" A1; see Table 1)

[c] Percentage of inoculation sites developing into lesions

[d] Area under the lesion expansion curve. Values in trials E and F were transformed with the ln (x+1) transformation prior to mean separation to satisfy the assumptions of normality and equal variances. Untransformed values are listed in the table

Reduced Night Temperature Trials Significant differences among isolates for lesion establishment were found in trials I and K for leaflet lesions and in trials J and K for stem lesions (Table 5). In trial J, isolate 8–7 had lower values than 1–2 or two of the new A1 isolates. Significant isolate × temperature interactions were observed in trials G and H. On leaflets, the standard US-1 isolate was statistically similar to US-8 and new A1 isolates with the highest values in all trials. Significant interactions among temperatures and isolates for AULEC were observed in trials G, H, J, and K. When significant differences among isolates were observed, US-8 or new A1 isolates had AULEC values significantly higher than US-1 isolates (trials G, I, J, and K) at 18 and 23 °C, and trials I, J, and K). Clear trends were not observed among isolates for sporulation frequency. Isolates 8–9 and 1–5 had higher leaflet values than two other US-1 isolates in trial G. In other trials, US-1 and US-8 isolates were similar. In trial J, new A1 isolates had higher values than both the US-1 and US-8 isolates. Additionally, the new A1 isolates in trial J has shorter sporulation times on leaflets. Sporulation time of 8–9 and 1–5 on stems were also shorter than the standard 1–2 in trial G. Differences among isolates for sporulation capacity were only observed in trial K where isolate N-7 produced more sporangia per lesion area than isolates 8–1, N-5, and 1–2).

In three of the five trials significant differences were observed among temperatures for lesion establishment (Table 6). Lesion establishment was lower at 28 °C than at 18 or 23 °C for leaflets in trials G and K and for stems in trial H. AULEC values were lower at 28 °C than at the other temperatures for both leaflet (trials G and I) and stem (trial I). Sporulation of lesions from the 28 °C treatment was observed in the reduced night temperature trials in trials I, J, and K for stems and in trial I for leaflets. Sporulation frequency for stem lesions at 28 °C was lower than at 18 or 23 °C in trial I, but significant differences were not observed in trials J and K. Sporulation frequency for leaflets was significantly higher at 18 than at 23 °C in trials G and K. When sporulation occurred at 28 °C, sporulation time was significantly longer than at the other temperatures on both stems and leaflets in trial I. Significant differences were not observed between 18 and 23 °C on either stems or leaflets. Sporulation capacity for stem lesions was similar at all temperatures, but was higher at 18 °C than at 23 (trials G, H, and I) or 28 °C (trial I) for leaflet lesions.

Significant interactions in the reduced night experiments were observed for lesion establishment (leaflets in trial G, stems in trial H), AULEC (leaflets in trial H, J, and K, stems

Table 5 Lesion establishment, area under the lesion establishment curve (AULEC), sporulation frequency, sporulation time, and sporulation capacity of *Phytophthora infestans* isolates representing three multilocus genotypes when inoculated on Russet Burbank potato leaflets and stems and incubated at 16 °C during an 8 h night period and at three different temperatures during an artificially lighted day period for 6 days[a]

Test	Lesion		AULEC[d]		Sporulation		Sporulation		Sporulation	
Isolate[b]	Establishment[c]				Frequency[e]		Time[f]		Capacity[g]	
	Leaflet	Stem	Leaflet	Stem	Leaflet	Stem	Leaflet	Stem	Leaflet	Stem
G										
8–9	82	**	82 a	**	60 a	75 a	13.7	5.2 a	2.9	19.4
1–5	70	**	46 b	**	56 a	83 a	14.4	7.2 a	2.7	13.2
1–7	56	**	29 b	**	20 b	47 ab	17.4	10.0 ab	1.2	1.4
1–2	62	**	29 b	**	23 b	20 b	19.1	17.6 b	0.7	3.7
H										
8–8	**	50	**	3.8	75 a	33	15.1	15.1	4.4	6.4
1–6	**	48	**	3.8	57 a	25	16.9	13.2	3.8	5.9
8–4	**	39	**	2.1	57 a	42	15.7	11.5	6.5	1.8
1–2	**	37	**	1.9	10 b	0	20.0	–	3.0	–
I										
8–5	84 a	96	65 a	8.4 a	69 a	70	4.3	8.2	14.0	5.7
1–2	64 a	94	34 b	6.8 b	53 ab	57	3.8	10.2	21.1	3.7
8–6	66 a	91	40 b	6.6 b	46 bc	48	4.7	11.1	17.2	3.7
N-1	29 b	91	28 b	6.3 b	27 c	56	8.8	13.2	13.6	1.2
J										
N-4	60 a	100 a	**	5.4 a	**	46 a	7.8 a	10.7	16.5	8.9
N-3	61 a	94 a	**	4.7 a	**	30 ab	7.8 a	13.8	26.2	7.7
1–2	38 a	96 a	**	2.7 b	**	6 b	16.7 b	24.0	10.7	5.9
8–7	9 b	41 b	**	1.6 b	**	0 b	21.7 c	–	1.6	–
K										
N-7	61 a	74 a	**	3.1 a	44 a	15 a	10.0	19.9	20.9 a	4.2
8–1	11 b	24 b	**	0.8 b	18 b	0 b	16.1	–	2.3 b	–
N-5	38 ab	19 b	**	1.1 b	14 b	0 b	19.0	–	6.2 b	–
1–2	35 ab	17 b	**	0.8 b	8 b	0 b	20.9	–	1.8 b	–

[a] Isolates were tested in 5 trials, designated G through K. Testing for significant differences among model effects within each trial was done using ANOVA. Mean separation of model effects was determined using Fisher's LSD. Isolate means within each trial followed by the different lowercase letters are statistically different ($P \leq 0.05$). "**" used to denote significant isolate × temperature interaction. Interaction values are given in Table 7. "–" indicates no data

[b] First character refers to multilocus genotype (1 = US-1, 8 = US-8, and N = "New" A1; see Table 1)

[c] Percentage of inoculation sites developing into lesions. Values in trial I were transformed with the arcsin transformation prior to mean separation to satisfy the assumptions of normality and equal variances. Untransformed values are listed in the table

[d] Area under the lesion expansion curve

[e] Number of lesions which sporulated divided by the total number of inoculation sites. Values in trial H (stems) and J (leaflets) were transformed with the reciprocal (1/x) transformation prior to mean separation to satisfy the assumptions of normality and equal variances. Untransformed values are listed in the table

[f] Time required in hours for lesions to sporulate when placed at high humidity. Lesions from the 28 °C temperature treatment did not sporulate. Due to the number of missing values, valid ANOVA cannot be performed for stem values of trial K

[g] Number of sporangia (×1,000) per cm^2 lesion area. A sporulation capacity of 0 means that sporangia were observed with the aid of a dissecting microscope, but were not detected in spore fixative used to rinse the sporulating lesion. Values in trials H and I were transformed with the ln (x+1) transformation prior to mean separation to satisfy the assumptions of normality and equal variances. Untransformed values are listed in the table. Due to missing values, valid ANOVA cannot be performed for stem lesions in trial K

in trial G), and sporulation frequency (leaflets in H). In trial G isolates did not differ for lesion establishment at 18 and 23 °C, but did at 28 °C with isolate 8–9 having a higher value than the three US-1 isolates (Table 7). Stem lesion establishment was lower at 28 than at the other two temperatures for the three US-1 isolates. Lesion establishment was variable for leaflets

Table 6 Effect of temperature on lesion establishment, area under the lesion establishment curve (AULEC), sporulation frequency, sporulation time, and sporulation capacity of *Phytophthora infestans* isolates when inoculated on Russet Burbank potato leaflets and stems and incubated at 16 °C during an 8 h night period and at three different temperatures during an artificially lighted day period for 6 days[a]

Test Temp (C)	Lesion establishment[b]		AULEC[c]		Sporulation frequency[d]		Sporulation time[e]		Sporulation capacity[f]	
	Leaflet	Stem	Leaflet	Stem	Leaflet	Stem	Leaflet	Stem	Leaflet	Stem
G										
18	91 a	**	72 a	**	58 a	61	15.7	9.8	3.1 a	16.1
23	77 a	**	61 a	**	22 b	52	15.3	9.4	0.4 b	2.7
28	34 b	**	7 b	**	0	0	–	–	–	–
H										
18	**	58 ab	**	3.2	57	25	15.3	15.6	6.9 a	5.6
23	**	60 a	**	4.4	43	25	17.7	12.1	2.4 b	3.5
28	**	21 b	**	1.2	0	0	–	–	–	–
I										
18	70	93	47 a	8.2 a	70 a	83 a	4.4 a	10.9 a	31.8 a	6.1
23	68	90	64 a	8.0 a	63 a	76 a	4.3 a	9.1 a	5.2 b	1.6
28	45	96	14 b	5.0 b	13 b	14 b	15.9 b	18.0 b	0.8 b	1.0
J										
18	61	85	**	4.6 a	**	31	13.2	16.7	18.1	5.8
23	54	85	**	3.6 ab	**	22	8.1	9.3	15.8	10.7
28	11	79	**	2.5 b	**	8		12.0	–	12.9
K										
18	66 a	42	**	1.8	49 a	6	15.8	21.5	12.2	10.6
23	38 b	29	**	1.2	14 b	3	11.5	19.0	8.0	0.0
28	4 c	29	**	1.4	0 b	3	–	15.0	–	2.0

[a] *P. infestans* isolates were tested in 5 trials, designated G through K. Testing for significant differences among model effects within each trial was done using ANOVA. Mean separation of model effects was determined using Fisher's LSD. Temperature means within each trial followed by the different lowercase letters are statistically different ($P \leq 0.05$). "**" used to denote significant isolate × temperature interaction. Interaction values are given in Table 7. "–" indicates no data

[b] Percentage of inoculation sites developing into lesions. Values in trial I were transformed with the arcsin transformation prior to mean separation to satisfy the assumptions of normality and equal variances. Untransformed values are listed in the table

[c] Area under the lesion expansion curve

[d] Number of lesions which sporulated divided by the total number of inoculation sites. Values in trial H (stems) and J (leaflets) were transformed with the reciprocal (1/x) transformation prior to mean separation to satisfy the assumptions of normality and equal variances. Untransformed values are listed in the table

[e] Time required in hours for lesions to sporulate when placed at high humidity. Lesions from the 28 °C temperature treatment did not sporulate. Due to the number of missing values, valid ANOVA cannot be performed for stem values of trial K

[f] Number of sporangia (×1,000) per cm^2 lesion area. A sporulation capacity of 0 means that sporangia were observed with the aid of a dissecting microscope, but were not detected in spore fixative used to rinse the sporulating lesion. Values in trials H and I were transformed with the ln (x+1) transformation prior to mean separation to satisfy the assumptions of normality and equal variances. Untransformed values are listed in the table. Due to missing values, valid ANOVA cannot be performed for stem lesions in trial K

in trial H with no clear trends for isolates or temperatures. Stem AULEC in trial G was higher at 18 and 23 than at 28 °C with the exception of isolate 1–2. Leaflet AULEC in trial H, J, and K varied for isolates at 18 and 23, but not at 28°C and was generally higher at 18 and 23 than at 28 for all isolates. Sporulation frequency was similar at 18 and 23 °C for all isolates but 1–2 in trial J, where sporulation was lower at 23 than at 18 °C.

A significant negative correlation was observed between lesion establishment and AULEC for constant temperature trials (Table 8). This was not observed in the reduced night temperature trials, however. Lesion establishment and AULEC were both positively correlated with sporulation frequency on both sets of trials. Lesion area and sporulation frequency were negatively correlated with sporulation time in both trials and positively correlated with sporulation capacity in the reduced night trials. Sporulation time and sporulation capacity were negatively correlated in both sets of trials.

Table 7 Interactions among isolates of *Phytophthora infestans* and temperatures for lesion establishment, area under the lesion expansion curve (AULEC), and sporulation frequency when three multilocus genotypes of *Phytophthora infestans* were inoculated on Russet Burbank potato leaflets and stems and incubated at three constant temperatures for 6 days[a]

Parameter (Test, tissue type)	Temperature (°C)		
Isolate[b]	18	23	28
Lesion establishment[c] (G, stems)			
8–9	100 a A	100 a A	100 a A
1–5	100 a A	100 a A	67 b B
1–7	100 a A	100 a A	67 b B
1–2	100 a A	89 a A	40 c B
Lesion establishment[c] (H, leaflets)			
8–8	93 a A	72 b B	20 a C
1–6	80 a A	93 a A	0 b B
8–4	53 b B	73 b A	20 a C
1–2	13 c B	67 b A	27 a AB
AULEC[d] (G, stems)			
8–8	8.1 a A	9.5 a A	3.3 a B
1–6	6.0 b A	5.9 b A	3.7 a B
8–4	2.9 c A	3.2 c A	0.8 b B
1–2	2.8 c AB	4.1 c A	1.8 b B
AULEC[d] (H, leaflets)			
8–8	151 a A	141 a A	4 a B
1–6	95 b A	72 b A	24 a B
8–4	79 b A	68 bc A	0 a B
1–2	25 c A	29 c A	3 a A
AULEC[d] (J, leaflets)			
N-4	84 a A	92 a A	3 a B
N-3	56 b A	78 a A	6 a B
1–2	13 c A	5 b A	0 a A
8–7	8 c A	0 b A	0 a A
AULEC[d] (K, leaflets)			
N-7	87 a A	37 a B	5 C
8–1	34 b A	13 b AB	0 B
N-5	14 b A	0 b A	0 A
1–2	18 b A	6 b A	0 A
Sporulation frequency[e] (J, leaflets)			
N-4	87 a A	87 a A	0
N-3	52 a A	87 a A	0
1–2	52 a A	7 b B	0
8–7	20 b A	0 b A	0

[a] *P. infestans* isolates were tested in 5 trials, designated G through K. Testing for significant differences among model effects within each trial was done using ANOVA. Due to significant isolate × temperature interaction, mean separation of isolates was performed by temperature and mean separation of temperature was performed by isolate using Fisher's LSD. Isolate means with different lowercase letters within each temperature (column) are significantly different and temperature means with different uppercase letters within each isolate (row) are statistically different ($P \leq 0.05$)

[b] First character refers to multilocus genotype (1 = US-1, 8 = US-8, and N = "New" A1; see Table 1)

[c] Percentage of inoculation sites developing into lesions

[d] Area under the lesion expansion curve

[e] Number of lesions which sporulated divided by the total number of inoculation sites. Values in trial J were transformed with the reciprocal (1/x) transformation prior to mean separation to satisfy the assumptions of normality and equal variances. Untransformed values are listed in the table. Due to "0" values for all isolates, data from 28 °C not included in ANOVA

Table 8 Pearson's correlation coefficients for the relationships among lesion establishment, the area under the lesion expansion curve (AULEC), lesion area, sporulation frequency, sporulation time, and sporulation capacity when stems and leaflets of potato were infected with various isolates of *Phytophthora infestans* and maintained at three constant temperatures or at three temperatures with a reduced night temperature[a]

	AULEC	Lesion area	Sporulation frequency	Sporulation time	Sporulation capacity
Constant Temperature Trials					
Lesion Establishment[b]	−0.31**	−0.38	0.52**	−0.08	−0.17
AULEC[c]		0.49**	0.22*	−0.08	0.09
Lesion Area[d]			0.64**	−0.48**	0.07
Sporulation Frequency[e]				−0.39**	0.09
Sporulation Time[f]					−0.46**
Sporulation Capacity[g]					
Reduced Night Temperature Trials					
Lesion Establishment	0.10	0.15*	0.59**	−0.04	−0.11
AULEC		0.55**	0.45**	−0.07	0.06
Lesion Area			0.70**	−0.62**	0.30**
Sporulation Frequency				−0.41**	0.21*
Sporulation Time					−0.47**
Sporulation Capacity					

[a] Potato plants were incubated at 18, 23, and 28 °C in constant temperature trials and at the same day temperatures with an 8 h 16 °C night temperature in reduced night temperature trials. * significant at $P \leq 0.05$ and ** significant at $P \leq 0.0001$

[b] Percentage of inoculation sites developing into lesions

[c] Area under the lesion expansion curve

[d] Lesion area (cm^2) after 6 days incubation

[e] Percentage of inoculation sites developing sporulating lesions when placed at high humidity

[f] Time required for lesions to sporulate when placed at high humidity

[g] Number of sporangia (×1,000) produced per cm^2 lesion area

Stem and Leaf, and Strain Comparisons Lesion establishment on stems was significantly higher than leaflets in 5 of 6 trials showing no interactions among lesion type and temperature (Table 9). When significant interactions among lesion type and temperatures were present, leaflet lesions showed a reduced lesion establishment at 28 °C compared to 18 or 23 °C (Table 10). However, differences among temperatures were observed only in trial D for stem lesions. Consistent trends were not observed with sporulation frequency, sporulation time, or sporulation capacity (Table 9). Sporulation frequency was higher on stems than leaflets in trials A and B. Sporulation time was shorter on stems than leaflets in trials A and G, and longer on stems in trial I. Stem and leaflet sporulation capacity was quite variable, but was higher on leaflets than stems in trials D, I, J, and K. Stem and leaflet AULEC values could not be compared because different rating scales were used.

Overall genotype comparisons showed significant variation within clonal lineages (Table 11). The US-8 genotype had both significantly lower lesion establishment (trials B, D, and F) and significantly higher lesion establishment (trials C and G) than the standard US-1 isolate. The US-8 genotype had the highest stem AULEC in trials C and G and the new A1 genotype had the highest stem AULEC in trials A, F, J, and K. For leaflet AULEC, the US-8 or new A1 genotype had significantly higher values than the US-1 in trials A, B, C, G, H, I, J, and K. The US-1 genotype had a significantly higher AULEC than the US-8 genotype in trials D and F for stem lesions and in trial D for leaflet lesions. New A1 isolates sporulated more frequently than US-1 isolates in trials J and K. The US-1 genotype had a higher sporulation frequency than the US-8 genotype in trials C and D, whereas the converse was true in trials E and G. The US-8 and new A1 genotype had shorter sporulation times than the US-1 genotype in trial G and in trials A and F, respectively. Significant differences among genotypes for sporulation capacity were not found.

Discussion

The findings from this experiment indicate that the relatively new genotypes (US-8 or new A1) were not better adapted at causing stem lesions. Rather, new strains seem to cause more severe stem lesions. Isolates representing newer genotypes were more aggressive in terms of lesion expansion on both

Table 9 Lesion establishment, sporulation frequency, sporulation time, and sporulation capacity of *Phytophthora infestans* isolates inoculated on stems and leaves of potato plants and incubated in growth chambers at three constant temperatures and at three constant temperatures with a reduced night temperature

Component	Constant temperature trials						Reduced night temperature trials				
Lesion type	A	B	C	D	E	F	G	H	I	J	K
Lesion Establishment[b]											
Stem	89 a	82 a	54 a	**	**	69 a	89 a	44	**	**	**
Leaflet	16 b	34 b	17 b	**	**	16 b	67 b	51	**	**	**
Sporulation Frequency[c]											
Stem	72 a	58 a	18	14	15	**	38	17	58	20	**
Leaflet	12 b	28 b	20	24	22	**	27	33	49	33	**
Sporulation Time[d]											
Stem	11.2 a	8.3	12.4	13.6	9.4	9.6	9.6 a	13.5	11.0 b	13.5	19.3
Leaflet	14.1 b	12.3	10.5	11.3	15.9	10.8	15.5 b	16.5	5.7 a	11.1	14.6
Sporulation Capacity[e]											
Stem	5.0	7.5	10.9	1.7 b	6.1	7.1	10.3	4.3	3.5 b	8.2 b	5.8 b
Leaflet	3.2	3.4	16.6	19.4 a	6.0	7.7	2.1	4.7	16.0 a	17.1 a	10.9 a

[a] Plants in trials A through G were held at constant temperatures of 18, 23, and 28 °C. In trials G through K the temperature was reduced to 16 °C for an 8 h night period. ** Lesion type means cannot be listed due to significant interactions with another model effect (See Table 14) for lesion establishment. Values with different lowercase letters are statistically different ($P \geq 0.05$) within each column and within each aggressiveness component

[b] Percentage of inoculation sites developing into lesions

[c] Percentage of inoculation sites which develop into lesions and sporulate when placed at high humidity. Analysis in trials A through H was done excluding values for 28 °C since sporulation did not occur at that temperature. The reciprocal transformation was performed on data in trial H so that the data set conformed to the assumptions of normality and equal variance

[d] Time in hours required for lesions to sporulate when placed at high humidity. The reciprocal transformation was performed on data in trials C and D so that the data set conformed to the assumptions of normality and equal variance

[e] Number of sporangia (×1,000) per cm^2 lesion area. The reciprocal transformation was performed on data in trial D and the logarithm (x+1) transformation was performed on trials A, G, and I so that the data sets conformed to the assumptions of normality and equal variance

stems and leaflets at 18 and 23 °C. The most aggressive US-8 and new A1 isolates had significantly higher AULEC values than US-1 isolates in a majority of the trials. These results on whole potato plants support work done using detached leaflets (Kato et al. 1997; Miller et al. 1998) and support the idea that newer strains are more aggressive than the old clonal lineage (Day and Shattock 1997). Additionally, isolates from the newer strains do not appear to be adapted to higher temperatures. When significant interactions among isolates and temperatures were observed, significant differences among isolates were usually present at 18 and 23 °C, but not at 28 °C. All isolates grew poorly at 28 °C, compared to the two lower temperatures. But isolates of *P. infestans*, presumably of the old clonal lineage, have been observed to grow in potato plants to some degree at temperatures above 28 °C (Kable and MacKenzie 1980; Rotem and Cohen 1974).

Table 10 Interactions among lesion type and temperature for lesion establishment of isolates of *Phytophthora infestans* inoculated on stems and leaflets of potato plants and incubated in growth chambers for 6 days

Lesion type	Trial[a]				
Temperature	D	E	I	J	K
Stem					
18	80 a	51	93	85	42
23	100 a	51	90	85	29
28	52 b	71	96	79	29
Leaflet					
18	36 a	43 a	70 a	61 a	65 a
23	22 ab	43 a	68 a	53 a	38 b
28	4 b	15 b	45 b	11 b	4 c

[a] Plants in trials D and E were held at constant temperatures of 18, 23, and 28 °C. In trials I, J, and K the temperature was reduced to 16 °C for an 8 h night period. Values with the same lowercase letter are statistically similar ($P \geq 0.05$) within each column and within each lesion type category

Using whole plants to test aggressiveness components of isolates is more cumbersome and time consuming than using detached leaflets. Due to space constraints in the mist and growth chambers, a maximum of four isolates could be tested at one time. Several trials had to be performed in order to test a large number of isolates. However, in a previous study (Dorrance and Inglis 1997), results of whole plant tests dealing with host resistance were found to correlate more closely with field results than detached leaflet tests. Additionally, other studies have shown that greenhouse tests for host

Table 11 Lesion establishment, area under the lesion expansion curve (AULEC), sporulation frequency, sporulation time, and sporulation capacity of *Phytophthora infestans* genotypes inoculated on stems and leaves of potato plants and incubated in growth chambers at three constant temperatures and at three constant temperatures with a reduced night temperature

Component[b]	Constant temperature trials						Reduce night temperature trials				
Strain[c]	A	B	C	D	E	F	G	H	I	J	K
Lesion Establishment											
US-8	–	29	50	40	45	39	85	50	84	25	17
New A1	55	68	–	–	48	27	–	–	60	79	48
US-1	51	67[y]	31[y]	67[y]	45	61[xy]	71[y]	45	79	67[y]	26[z]
AULEC-Stems											
US-8	–	2.0	4.2	3.3	7.1	2.4	6.1	3.8	7.5	1.6	1.1
New A1	11.2	6.8	–	–	2.5	9.1	–	–	6.3	5.0	2.0
US-1	7.9[z]	5.6[y]	2.1[y]	7.7[y]	1.8	4.7[zy]	2.6[y]	2.0	6.8	2.7[xz]	0.8[z]
AULEC-Leaflets											
US-8	–	14	60	21	28	13	64	81	53	3	5
New A1	51	60	–	–	28	53	–	–	28	53	29
US-1	26[z]	20[z]	10[y]	48[y]	16	25	29[y]	34[y]	34[y]	6[xz]	8[xz]
Sporulation Frequency											
US-8	–	14	13	8	29	12	46	34	58	3	4
New A1	54	60	–	–	16	40	–	–	41	45	19
US-1	36	38	37[y]	42[y]	7[xy]	36	18[y]	15	55	13[xz]	7[z]
Sporulation Time											
US-8	–	14.0	8.3	14.0	10.6	14.0	10.1	14.7	7.1	**	19.0
New A1	10.4	8.7	–	–	16.7	5.8	–	–	7.3	**	14.4
US-1	13.0[z]	12.1	13.7	10.8	20.0	12.4[z]	16.8[y]	16.7	11.0	**	20.9
Sporulation Capacity											
US-8	–	1.2	17.3	12.7	9.2	6.2	8.7	4.8	10.4	1.6	6.2
New A1	5.7	6.7	–	–	2.9	8.0	–	–	11.6	15.4	11.4
US-1	3.8	5.1	11.6	14.1	2.5	7.2	1.5	4.1	7.8	9.5	1.8

[a] Plants in trials A through G were held at constant temperatures of 18, 23, and 28 °C. In trials G through K the temperature was reduced to 16 °C for an 8 h night period. – Isolates representing this strain were not used in this trial. ** Strain means cannot be listed due to significant interactions with another model effect

[b] Lesion establishment is the percentage of inoculation sites developing into lesions, sporulation frequency in the percentage of inoculation sites which develop into lesions and sporulate when placed at high humidity, sporulation time is the time in hours required for lesions to sporulate when placed at high humidity, and sporulation capacity is the number of sporangia (×1,000) per cm^2 lesion area. In trials A through H data from 28 °C was not analyzed for sporulation frequency, sporulation time, and sporulation capacity

[c] Strain refers to the multilocus genotype determined by compatibility type, *Gpi* and *Pep* genotype, and restriction fragment length polymorphism (RFLP) genotype

[x] Linear contrast of US-1 vs. US-8 and New A1 significant ($P \leq 0.05$)

[y] Linear contrast of US-1 vs. US-8 significant ($P \leq 0.05$)

[z] Linear contrast of US-1 vs. New A1 significant ($P \leq 0.05$)

resistance using whole plants were more similar to field results than laboratory tests (Guzmán-N 1964; Knutson 1962). The use of individual lesions on both stems and leaflets in this test made it possible to evaluate lesion establishment and growth on a daily basis. Sporulation data in this study were obtained from detached plant parts since constant observations were needed to record the time required for lesions to sporulate at high humidity.

Conditions during the inoculation period of these trials (18–22° C with free water present on plant surfaces for 20 h) were such that all inoculation sites should have developed lesions. However, lesion establishment was occasionally reduced at higher temperatures. This was true more for lesions on leaflets than on stems. Sporangial dispersal has been observed to be greater on hot, dry days compared to cool, wet days (Rotem and Cohen 1974). If sporangia dispersal in early

or late morning were followed by high temperatures later in the day, establishment of lesions on leaves may be reduced compared to stems. The effect of temperature on reducing leaflet lesion establishment was similar for all three genotypes used in this experiment. US-1 isolates established lesions on stems and leaflets just as effectively as isolates of the US-8 or new A1 genotype (trials G, H, I, J, and K). Thus, newer genotypes, such as the US-8 or new A1 genotype, do not appear to be better adapted at causing stem lesions than the old US-1 strain. Rather, *P. infestans* seems more likely to establish stem lesions than leaflet lesions at higher temperatures (28 °C).

Analyzing lesion expansion on a stem or leaf is different than analyzing the progression of an epidemic in a field. However, the growth of a lesion can be modeled in a manner similar to modeling the growth of an epidemic by using the area under the lesion expansion curve (AULEC). Lesion expansion may be the most important component of aggressiveness because it can account for more than 95 % of diseased area in a late blight epidemic (Berger et al. 1997). The AULEC on a single plant basis is analogous to the area under the disease progress curve (AUDPC) on a whole field basis. The AUDPC and AULEC incorporate time of disease onset, growth rate, and final disease severity into a single value and these values are useful when the rate of change for disease severity is irregular over time (Campbell and Madden 1990).

The effect of elevated temperature was more evident for AULEC values than for lesion establishment. Many of the stem and leaflet AULEC values at 28 °C were significantly lower than at 18 or 23 °C. This was true more so for the reduced night temperature trials than for the constant temperature trials. The highest AULEC values were not always found at the same temperature, and values from 18 and 23 °C rarely differed from each other. Isolates representing newer genotypes (US-8 and new A1) generally had higher AULEC values, but the differences were not always significant. Previous tests on detached leaflets resulted in significant differences between US-8 and US-1 isolates (Kato et al. 1997; Miller et al. 1998). When US-1 isolates in this test were tested against US-8 or new A1 isolates originating from the same year (1997), the US-1 isolates generally had significantly lower AULEC values on both stems and leaves (trials G and H). The US-1 standard isolate collected from an earlier year (1994) had similar or even higher AULEC values than some US-8 isolates collected from the same year (trials B and D). The number of isolates used in these trials was small. Thus, caution must be used in interpreting these results. It is possible that other isolates may have responded differently in these tests. However, the results of these tests support previously published reports where greater numbers of isolates were used (Kato et al. 1997; Miller et al. 1998).

All isolates used in these tests were grown on potato tubers and leaves periodically throughout the study to maintain pathogenicity. Zoosporangia used for inoculation were always taken from infected potato tissue. This was done to ensure that isolates retained their pathogenicity to potato. Isolates did retain pathogenicity as evidenced by isolate 1–2. Isolate 1–2 was highly aggressive when first isolated in 1994 and was still aggressive in these tests conducted in 1997.

Leaflet and stem lesions sporulated with similar frequency. Lesions also sporulated with similar frequency and time at either 18 or 23 °C. Sporulation did not occur at 28 °C in constant temperature trials and was very rare at 28 °C for the reduced night temperature trials. Four isolates on stems and two on leaflets sporulated at 28 °C and all of these were from trials where the night temperature was reduced to 16 °C. One of these isolates was the standard isolate and it did not sporulate at 28 °C in any of the other trials. Rotem and Cohen (1974) observed that leaf lesions exposed to a constant temperature of 30 °C in a growth chamber for 5–8 days did not sporulate while 30 of 75 stem lesions sporulated after 20 days at the same temperature. Kable and MacKenzie (1980) additionally noted that sporangial production was not reduced by subjecting potato stem lesions to 30 °C for 7 days but was reduced when lesions were exposed to temperatures above 32.5 °C. Sporulation was induced at 18–20 °C in these two tests (Kable and MacKenzie 1980; Rotem and Cohen 1974). In our test, however, sporulation was induced at 18, 23, and 28 °C. Sporulation is reported to be optimal near 21 °C (Hooker 1981). It is unlikely that any lesion would be subjected to constant high temperatures in the field for 6 or 7 days in the Columbia Basin of Washington and Oregon. The cyclic pattern of daily temperature would provide periods of time where the temperature was favorable for growth or sporulation. Cool night temperatures may provide periods of growth for the pathogen between hot, dry daytime conditions. In our constant temperature trials, no lesions sporulated at 28 °C. In some trials, after the 24 h incubation period at high humidity, lesions from the 28 °C treatment were placed at 18 °C. After another 24 h incubation period, these lesions still did not sporulate. This is in contrast to the reduced night temperature trials where lesions from the 28 °C treatment sporulated in three of five trials.

The time required for lesions to sporulate was similar for stems and leaves, and similar for the different strains, indicating that newer genotypes do not sporulate sooner than the old US-1 strain. Some significant differences were observed with isolates from the newer strains sporulating in less time than US-1 isolates, but the trend was not present in all trials. The number of sporangia produced per unit of lesion area was highly variable. Even though means of isolates grouped according to strain were often quite divergent, significant differences were not observed. Previous tests examining sporulation capacity on detached leaflets indicate that the US-8 genotype has a higher sporulation capacity than the US-1 strain. Kato et al. (1997) found the sporulation capacity of US-8

isolates to be significantly higher than that of US-1 isolates. Miller et al. (1998) observed significant differences between the US-8 and US-1 isolates on the moderately resistant cultivar Legend. But lesions were allowed to progress on detached leaflets for the above cited tests, not on whole plants. Experimental design may also play a role in detecting differences among isolates. More replications are possible with detached leaflet assays than with whole plant studies, increasing the power in the statistical procedures for detached leaflet assays and increasing the probability of detecting significant differences.

Some aggressiveness components were independent of each other while others were highly correlated. AULEC was negatively correlated with lesion establishment in constant temperature trials, but this may have resulted from chance since there does not seem to be a biological reason for isolates which establish lesions less often to have more aggressive lesion growth. Rather, it seems more logical that isolates which can successfully infect more often do so because the sporangia germinate to a greater extent or else the pathogen penetrates more quickly. These isolates may also grow more quickly in tissue and thus have a higher AULEC value than isolates which establish themselves to a lesser degree. Additionally, a significant correlation between AULEC and lesion establishment was not found in the reduced night trials. Naturally, lesion area was significantly correlated with AULEC since the final lesion size is incorporated into the AULEC value. Since the inoculation sites which did sporulate (sporulation frequency) were a subset of the inoculations sites which developed into lesions (lesion establishment), it is not surprising that sporulation frequency and lesion establishment were positively correlated with each other. Sporulation capacity was negatively correlated with sporulation time in both constant temperature and reduced night temperature trials. This is intuitive since the sooner a lesion begins to sporulate, the more sporangia will be produced. A larger lesion area was not always associated with a higher sporulation capacity. Sporulation capacity standardizes the total number of sporangia to the lesion area. The presence of a significant correlation would indicate that larger lesions produce more sporangia per unit area than smaller lesions. This observation was not consistent over both sets of trials in this experiment.

The increased occurrence of stem lesions concomitant with the occurrence of new strains may be a result of fungicide residue patterns, rather than an inherent preference in the pathogen for stem tissue or an inherent difference in host tissue susceptibility. The presence of newer, more aggressive genotypes led to an increase in fungicide use in the Columbia Basin of Washington and Oregon (Johnson et al. 1997; Kato et al. 1997). Results of tests done in the Columbia Basin indicate that as the volume of water used in a fungicide application by aircraft decreases, the concentration of fungicide reaching the stems decreases, compared to leaves (Hamm et al. 2006; 2008). Thus, the protection against *P. infestans* on a fungicide-treated potato crop will be greater for leaves than stems, making invasion by the pathogen more likely on stem tissue. Because the US-8 genotype is more aggressive, these stem lesions grow faster and consume more tissue compared to the old US-1 genotype. This may be why a perceived increase in the number of stem lesions has occurred with the appearance of the US-8 strain. Further residue testing of potato stems subjected to various fungicide treatment methods and water rates is needed to validate this hypothesis.

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References

Bashan, B., D. Kadish, U. Levy, and Y. Cohen. 1989. Infectivity to potato, sporangial germination, and respiration of isolates of *Phytophthora infestans* from metalaxyl-sensitive and metalaxyl-resistant populations. *Phytopathology* 79: 832–836.

Berger, R.D., A. Bergamin Filho, and L. Amorim. 1997. Lesion expansion as an epidemic component. *Phytopathology* 87: 1005–1013.

Browning, J.A., M.D. Simons, and E. Torres. 1977. Managing host genes: Epidemiologic and genetic concepts. Pages 191–212 in. Plant Disease, Volume I. J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York. 465 pp.

Campbell, C.L., and L.V. Madden. 1990. *Introduction to Plant Disease Epidemiology*. New York: John Wiley & Sons. 532 pp.

Castronovo, A.H., D. Thurston, and C.J. Eide. 1954. Parasitic aggressiveness and its relation to late blight tolerant potato varieties and the survival of the pathogen. (Abstr.) Am. *Potato J* 31: 366–367.

Day, J.P., and R.C. Shattock. 1997. Aggressiveness and other factors relating to displacement of populations of *Phytophthora infestans* in England and Wales. *European Journal of Plant Pathology* 103: 379–391.

Dorrance, A.E., and D.A. Inglis. 1997. Assessment of greenhouse and laboratory screening methods for evaluating potato foliage for resistance to late blight. *Plant Disease* 81: 1206–1213.

Dowley, L.J. 1987. Factors affecting the survival of metalaxyl resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. *Potato Research* 30: 473–475.

Fry, W.E., and S.B. Goodwin. 1997. Resurgence of the Irish potato famine fungus. *Bioscience* 47: 363–371.

Fry, W.E., S.B. Goodwin, J.M. Matuszak, L.J. Spielman, and M.G. Milgroom. 1992. Population genetics and intercontinental migrations of *Phytophthora infestans*. *Annual Review of Phytopathology* 30: 107–129.

Fry, W.E., S.B. Goodwin, A.T. Dyer, J.M. Matuszak, A. Drenth, P.W. Tooley, L.S. Sujkowski, Y.J. Koh, B.A. Cohen, L.J. Spielman, K.L. Deahl, D.A. Inglis, and K.P. Sandlan. 1993. Historical and recent migrations of *Phytophthora infestans*: Chronology, pathways, and implications. *Plant Disease* 77: 653–551.

Goodwin, S.B., A. Drenth, and W.E. Fry. 1992. Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. *Current Genetics* 22: 107–115.

Goodwin, S.B., B.A. Cohen, K.L. Deahl, and W.E. Fry. 1994a. Migration from northern Mexico as the probable cause of recent genetic changes in populations of *Phytophthora infestans* in the United States and Canada. *Phytopathology* 84: 553–558.

Goodwin, S.B., B.A. Cohen, and W.E. Fry. 1994b. Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proceedings of the National Academy of Science* 91: 11591–11595.

Goodwin, S.B., R.E. Schneider, and W.E. Fry. 1995. Use of cellulose acetate electrophoresis for rapid identification of allozyme genotypes of *Phytophthora infestans*. *Plant Disease* 79: 1181–1185.

Guzmán-N, J. 1964. Nature of partial resistance of certain clones of three Solanum species to *Phytophthora infestans*. *Phytopathology* 54: 1398–1404.

Hamm, P.B., T.F. Cummings, and D.A. Johnson. 2006. Comparison of deposition patterns in two programs for applying protectant fungicides to potato stems and leaves for the control of late blight (*Phytophthora infestans*). *American Journal of Potato Res* 83: 473–484.

Hamm, P.B., R.A. Boydston, C.W. Hoy, W.R. Stevenson, and P.J.S. Hutchinson. 2008. Applying pesticides. Pages 113–121 in. Potato Health Management, D.A. Johnson, Ed. American Phytopathological Society Press, St. Paul.

Hohl, H.R., and K. Iselin. 1984. Strains of *Phytophthora infestans* with the A2 mating type behaviour. *Transactions of the British Mycological Society* 83: 529–530.

Hooker, W.I. (ed.). 1981. *Compendium of Potato Diseases*. St. Paul, MN: American Phytopathological Society.

Johnson, D.A., and T.F. Cummings. 2009. Latent infection of potato seed tubers by *Phytophthora infestans* during long-term cold storage. *Plan Dis.* 93: 940–946.

Johnson, D.A., J.R. Alldredge, and D.L. Vakoch. 1996. Potato late blight forecasting models for the semiarid environment of south-central Washington. *Phytopathology* 86: 480–484.

Johnson, D.A., T.F. Cummings, P.B. Hamm, R.C. Rowe, J.S. Miller, R.E. Thornton, G.Q. Pelter, and E.J. Sorensen. 1997. Potato late blight in the Columbia Basin: An economic analysis of the 1995 epidemic. *Plant Disease* 81: 103–106.

Johnson, D.A., T.F. Cummings, R. Abi Ghanem, and J.R. Alldredge. 2009. Association of solar irradiance and days of precipitation with incidence of potato late blight in the semiarid environment of the Columbia Basin. *Plant Disease* 93: 272–280.

Johnson, D.A., P.B. Hamm, J.S. Miller, and L.D. Porter. 2012. Late blight epidemics in the Columbia Basin. In. Sustainable Potato Production: Global Case Studies. Eds Z. He, R. Larkin, W. Honeycutt. Pgs 141–162. Springer.

Kable, P.F., and D.R. MacKenzie. 1980. Survival of *Phytophthora infestans* in potato stem lesions at high temperatures and implications for disease forecasting. *Plant Disease* 64: 165–167.

Kadish, D., and Y. Cohen. 1988. Fitness of *Phytophthora infestans* isolates from metalaxyl-sensitive and-resistant populations. *Phytopathology* 78: 912–915.

Kadish, D., and Y. Cohen. 1989. Population dynamics of metalaxyl-sensitive and metalaxyl-resistant isolates of *Phytophthora infestans* in untreated crops of potato. *Plant Pathology* 38: 271–276.

Kadish, D., M. Grinberger, and Y. Cohen. 1990. Fitness of metalaxyl-sensitive and metalaxyl-resistant isolates of *Phytophthora infestans* on susceptible and resistant potato cultivars. *Phytopathology* 80: 200–205.

Kato, M., E.S. Mizubuti, S.B. Goodwin, and W.E. Fry. 1997. Sensitivity to protectant fungicides and pathogenic fitness of clonal lineages of *Phytophthora infestans* in the United States. *Phytopathology* 87: 973–978.

Knutson, D.W. 1962. Studies on the nature of field resistance of the potato to late blight. *Am. Potato J.* 39: 152–161.

Knutson, D.W., and C.J. Eide. 1961. Parasitic aggressiveness in *Phytophthora infestans*. *Phytopathology* 51: 286–290.

Large, E.C. 1940. *The Advance of the Fungi*. London: Jonathan Cape. 488 pp.

Miller, J.S., P.B. Hamm, and D.A. Johnson. 1997. Characterization of the *Phytophthora infestans* population in the Columbia Basin of Oregon and Washington from 1992 to 1995. *Phytopathology* 87: 656–660.

Miller, J.S., D.A. Johnson, and P.B. Hamm. 1998. Aggressiveness of isolates of *Phytophthora infestans* for the Columbia Basin of Washington and Oregon. *Phytopathology* 88: 190–197.

Porter, L.D., D.A. Inglis, and D.A. Johnson. 2004. Identification and characterization of resistance to *Phytophthora infestans* in leaves, stems, flowers, and tubers of potato clones in the Pacific Northwest. *Plant Disease* 88: 965–972.

Ribeiro, O.K. 1978. *A Source Book of the Genus Phytophthora*. Vaduz: J. Cramer.

Rotem, J., and Y. Cohen. 1974. Epidemiological patterns of *Phytophthora infestans* under semi-arid conditions. *Phytopathology* 64: 711–714.

Spielman, L.J., A. Drenth, L.C. Davidse, L.J. Sujkowski, W. Gu, P.W. Tooley, and W.E. Fry. 1991. A second world wide migration and population displacement of *Phytophthora infestans*? *Plant Pathology* 40: 422–430.

Tooley, P.W., J.A. Sweigard, and W.E. Fry. 1986. Fitness and virulence of *Phytophthora infestans* isolates from sexual and asexual populations. *Phytopathology* 76: 209–1212.

Vanderplank, J.E. 1982. *Host-Pathogen Interaction in Plant Disease*. New York: Academic. 207 pp.