Survival Dynamics of *Aphanomyces cochlioides* Oospores Exposed to Heat Stress

A. T. Dyer, C. E. Windels, R. D. Cook, and K. J. Leonard

First author: Montana State University, Department of Plant Sciences and Plant Pathology, Bozeman 59717; second author: University of Minnesota, Northwest Research and Outreach Center, Crookston 56716; third author: University of Minnesota, School of Statistics, St. Paul 55108; and fourth author, University of Minnesota, Department of Plant Pathology, St. Paul.
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ABSTRACT

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To determine how exposure to heat effects their survival, oospores of *Aphanomyces cochlioides* isolate C22 were exposed in water to 35, 40, 45, or 50°C for prescribed times and then examined for viability. The Weibull model was modified to represent the effects of temperature on survival of oospores. The final fitted model gave lethal doses for 50% of the oospores of 251, 49.8, 9.8, and 1.9 h at 35, 40, 45, and 50°C, respectively. To determine if alternating high and low temperatures resulted in (i) recovery from heat damage during low temperature periods, (ii) in-

creased susceptibility to heat damage, or (iii) if effects of heat damage were cumulative, oospores were examined after each of four 24-h cycles at 45°C for 4 h and 21°C for 20 h. Survival of oospores exposed to alternating high and low temperatures fit the cumulative effects model. Significant variability in heat tolerance among five isolates was observed ($P < 0.001$) but model parameters successfully accommodated this variability ($R^2 = 0.96$, $P < 0.001$). This research shows that under wet conditions, there are predictable patterns to mortality for *A. cochlioides* oospores exposed to continuous or fluctuating high temperatures.

Additional keywords: generalized nonlinear model, inoculum, root pathogen, survival model, thermal instability, Weibull model.

The oomycete *Aphanomyces cochlioides* Dresch. causes damping-off and root rot of sugar beet (*Beta vulgaris* L.), resulting in significant losses wherever the crop is grown (11,23,24,30,32,38). This pathogen produces short-lived, motile zoospores and long-lived, dormant oospores. Dormant oospores serve as primary inoculum in any given growing season. Control measures for *Aphanomyces* root rot include planting seed of moderately resistant cultivars treated with the fungicide hymexazol (Tachigaren) and cultural practices (early planting, long rotations between sugar beet crops, cultivation, and drainage to reduce soil moisture). During prolonged warm (>25°C) and wet conditions in heavily infested fields, these control measures fail (3,38). Therefore, avoidance of heavily infested fields is recommended. Solar heating or solarization is an effective control measure for several soilborne plant pathogens (8,17,22,27) and is being explored in the Red River Valley of Minnesota and North Dakota as a method of reducing oospore inoculum in sugar beet fields (6). Additionally, composting and other heat-related disinfestation procedures are being considered at sugar beet factories for the treatment of tare soil (soil and beet refuse collected when sugar beet roots are piled for storage) prior to its return to growers' fields. Bolstering this interest is the recent finding that moderately high temperatures are lethal to oospores of *A. cochlioides* (14).

To define the temperatures and times necessary to reduce plant pathogen populations, plant pathologists have explored thermal death ranges, temperature coefficients, and polynomial survival curves to describe the survival of a variety of organisms and propagules (5,15,22,27,31,39). These studies have provided a basic understanding of the temperatures and times necessary to

inactivate infectious propagules. Advancing this line of inquiry, Pullman et al. (27) described the semi-logarithmic relationship between lethal doses (LD_{90}) and temperatures for four plant pathogens exposed to moderately high temperatures. This first order expression of survival and others like it are particularly attractive because they linearize survival curves allowing simple statistical assessment. While simplifying the statistics involved, these models have consistently underestimated survival in the tails of survival curves (4,25), which limits their utility.

In food microbiology, survival of food contaminants in relation to preparation temperatures and times has been extensively explored. Traditionally, these studies have focused on simple exponential models (25). In more recent studies, this focus has shifted to the Weibull model to describe these response surfaces (25, 26,34). The cumulative form of the Weibull model is defined as

$$S(t) = \exp[-(\Theta_1 \times t)^{\Theta_2}] \quad (1)$$

where S is the proportional survival or probability of survival at time, t ; and where Θ_1 and Θ_2 are rate and shaping parameters, respectively. Weibull distributions have effectively described the success-failure dynamics for a plethora of studies ranging from the survival of cancer patients (36) and other organisms (16,26) to the failure of filament bundles under weight loads (10). The strength of the Weibull model is that the response surface varies from the exponential according to fitted values for Θ_2 (16,33). This slight modification of the exponential model provides flexibility in the tails of the survival curves where the exponential model fails. Recently, the Weibull model was expanded to describe the relationship between temperature-time and survival for the food contaminant *Listeria monocytogenes* (26) as well as the relationship between temperature, relative humidity, time, and survival for *Fusarium oxysporum* and *Sclerotinia rolfii* (29).

In the field, a pathogen is exposed to fluctuating temperatures that are highest in the late afternoon and lowest by early morning (17,19,20). In understanding and successfully describing a

Corresponding author: A. T. Dyer; E-mail address: adyer@montana.edu

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propagule's response to these intermittent high temperatures, we need to first identify the character of the response. In the simplest terms, dormant propagules that survive a high temperature period may (i) recover from heat-related damage during low temperature periods, (ii) break dormancy and thereby become more susceptible to successive high-temperature episodes, or (iii) remain inactive or dormant, accumulating heat-related damage. Little research has been conducted on the effects of intermittent high temperatures. Two recent papers (8,29) attempted to model the effects of intermittent high temperatures on survival under field environmental conditions but conclusions as to the type of response the pathogens displayed were not drawn. Understanding the type of response displayed by oospores of *A. cochlioides* exposed to intermittent high temperatures could provide an important insight into the survival biology of this pathogen and allow for better quantification of oospore survival in the field in relation to temperatures experienced.

Equally important, defining the variability in heat tolerance displayed among individuals of a pathogen species could be crucial to assessing feasibility of heat-related disinfection procedures. Such an examination may provide insight into the potential success and durability solarization has as a control measure. It would also enable us to examine permutations that may exist in a population's heat resistance and how to adapt the resulting survival model to the field where population variability may be important. These would be crucial advances in predicting success of solarization and other heat-related control measures. To date, only limited studies have been conducted to examine variability in heat tolerances in relation to population survival (15,27).

Heat-related disinfection procedures such as solarization and composting are complex systems which in the past have resisted holistic approaches to modeling. For that reason, this study focuses solely on the direct effects of heat on oospore survival. By doing so, we hope to provide the tools for isolating the direct effects of heat on survival from the plethora of other factors involved. The objectives of this study were to (i) define the survival relationship for oospores of *A. cochlioides* exposed to constant moderately high temperatures, (ii) identify the type of response oospores have to intermittent high temperatures, and (iii) describe the variability in heat tolerance that exists among isolates of *A. cochlioides*. In addressing these objectives, previously described techniques for in vivo production of oospores and the reliable visual assessment of their viability were employed (14). A preliminary report of this research has been published (13).

MATERIALS AND METHODS

Isolates. Isolates of *A. cochlioides* were obtained from soil samples collected in Cavalier, ND (isolates C10 and C22); Buffalo Lake, MN (isolates B14 and B19); and Bushland, TX (isolate T71) in the summer of 1997 (12). These isolates were selected because they produced abundant oospores in culture (data not shown) and because preliminary analysis of amplified fragment length polymorphisms indicated they were genetically distinct, except for isolates C10 and B14 (12). Isolates were stored in sterile distilled water (SDW) and transferred every 6 months (14). Experiments conducted to define the response of oospores to high temperatures were performed with isolate C22 and the experiments on variability among populations included the other isolates.

Oospore production. Oospores were produced in vivo in sugar beet seedling hypocotyls of cv. American Crystal 205 (14). Seedlings were grown in 10-cm-diameter plastic pots (25 seeds per pot) containing a pasteurized greenhouse soil mix (6:6:5:2, Waukegan silt loam/sand/peat/composted manure by volume, pH 7.2). Greenhouse temperatures were 23°C during the day (14 h) and 16°C at night. Natural daylight was supplemented from October through March with illumination from two 400-W high-

pressure sodium lamps. Hypocotyls were excised (approximately 2 cm length) from 2-week-old seedlings, surface-treated for 30 s in 0.5% NaOCl, rinsed twice with SDW, and placed in 10-cm-diameter petri dishes (two hypocotyls per dish) containing 20 ml of SDW. Dishes were inoculated with a 0.4-cm-diameter core of *A. cochlioides* (one per dish) removed from the margin of an actively growing culture on corn meal agar amended with 50 mg/liter each of rifampicin and penicillin G. Petri dishes containing the hypocotyls then were placed in the dark at 17 to 23°C. All oospores employed in these studies were between 5 and 6 weeks old (beginning at time of hypocotyl inoculation), which was more than adequate for production of mature oospores (14). Because extraction could potentially damage oospores, heat treatments were performed on oospores within hypocotyl tissues. Therefore, statements involving hypocotyls from here on refer to hypocotyls containing oospores.

Heat treatments. Hypocotyls in 20 ml of SDW in 10-cm-diameter petri dishes were exposed to 35, 40, 45, and 50°C ($\pm 1^\circ\text{C}$) in a Thelco Model 4, Low-Temperature Oven (Precision Scientific Co., Winchester, VA). This temperature range represents the temperature at which *A. cochlioides* is reported to stop growing in culture (35°C) (21) to the highest possible temperature (50°C) achievable by solarization in the Red River Valley of Minnesota and North Dakota (*unpublished data*). Water temperatures were monitored with an internal/external thermometer (Model 15-077-8D, Fisher Scientific, Pittsburgh, PA). After oospores had been exposed to prescribed temperature-time treatments, they were stored at 21°C and microscopically examined at $\times 400$ and/or $\times 1,000$ magnification (usually within 24 h and not beyond 7 days after treatment). Prior experience showed no change in oospore appearance over a 7-day interval at 21°C (14). Viable oospores have a densely organized, uniform, granular appearance and dead oospores have a loosely organized, nongranular appearance (14).

Constant temperatures. To determine the response of oospores exposed to constant temperature, hypocotyls containing oospores of isolate C22 were exposed to 35, 40, and 45°C for 0, 6, 12, 24, 48, 72, and 96 h. Based on the results for oospores exposed to 45°C, exposures to 50°C were shortened to 0, 1, 2, 3, 4, and 6 h. Viability was determined for 50 oospores in each of the five hypocotyls in each of the 27 temperature-time treatments for a total of 6,750 oospores examined in each of the two times the experiment was run.

Intermittent high temperatures. To determine the effect intermittent high temperatures has on oospore survival, five hypocotyls containing oospores of isolate C22 were exposed to 45°C for 4 h and then removed and held at 21°C for 20 h. This high/low temperature cycle was repeated an additional three times. Prior to the initial 45°C treatment, a 4-mm segment was removed from each hypocotyl and 50 oospores per segment were observed for viability. Additional 4-mm hypocotyl segments were removed and oospores were examined after each successive 24-h treatment cycle. This experiment was repeated once.

During this experiment, there was a drop in water temperature caused by the return of hypocotyls to the 45°C petri dish. Consequently, water temperatures were recorded at 5-min intervals until water temperatures returned to 45°C. The average water temperature drop was 1.25°C with an average recovery time of 36.3 min (variation ranged from no temperature decrease to a 2°C drop in water temperature with a 70-min recovery time). To correct for water temperature disturbance, average temperatures were calculated employing a trapezoid function for each 5-min time interval. The time required for water to return to 45°C was accounted for as a portion of the 4 h/45°C treatment.

Isolate variability. For the analysis of heterogeneity, isolates C10, C22, B14, B19, and T71 were evaluated based on results of trials with isolate C22. For each isolate, five hypocotyls were exposed for 0, 48, 96, 144, and 192 h at 35°C; 0, 24, 48, 72, and

96 h at 40°C; 0, 6, 12, 24, and 48 h at 45°C; and 0, 1.5, 3.0, 4.5, and 6.0 h at 50°C. For the first experiment, only one oospore remained viable after exposure to 45°C for 48 h so when the experiment was repeated, exposure periods at 45°C were changed to 0, 6, 12, 18, and 24 h. At 45 and 50°C, treatments were processed individually to minimize changes in water temperature. For each of the five isolates, 50 oospores were examined for viability for each of five hypocotyls for each of the 20 temperature–time treatments for a total of 25,000 oospores examined in each of the two times the experiment was run.

Statistical analyses. For all trials, the 50 oospores examined per hypocotyl were treated as a subsample. Results of repeated trials were essentially the same. So with the exception of the intermittent high-temperature experiment, where we present the results of one trial, results were combined as replicates for purposes of linear regression analysis. The total number of surviving oospores was considered a binomial random variable with probability $S(t)$ and total trials equal to the total oospores exposed to temperature treatments. Because $\log\{S(t)/[1 - S(t)]\}$ is a nonlinear function of the Θ 's, the regression analysis required generalized nonlinear modeling techniques (9). Our data analyses employed the regression program ARC version 1.03 (University of Minnesota, Applied Statistics, St. Paul, MN). A new generalized nonlinear model update (gnm.lsp) of the program enabled the fitting of the Weibull survival model with a binomial distribution function. Output for fitted models include deviance values (G^2) to compare nested models and provide a statistic of model fit (9). G^2 values are a summation of the statistical distances between observed and fitted values. Through reiterative calculations, the computer program searches for a convergence of fitted parameter values that minimize total deviance. Where deviance values were large, deviance-residuals were plotted against predicted means and examined for trends employing locally weighted scatter plot smoother (*lowess*, $f = 0.6$) for a best-fit line (7). A locally weighted scatter plot smoother uses weighted local response values to estimate local means within a scatter plot. For the fluctuating temperature experiments, where estimates of variance were needed, the model predictions were assumed fixed and a binomial variance was calculated for the observed sample. To provide measures of fit for comparisons between trials, predicted values were compared with mean observed values employing

linear regression (1). The resulting R^2 values are summary values and not a true test statistic.

RESULTS

Constant temperatures. Oospore viability of isolate C22 averaged 98.5% prior to exposure to high temperatures. As temperature increased, viability declined rapidly such that exposure to 35°C for 96 h reduced viability to 85.2% while exposure at 50°C for 4 h reduced viability to 2.6% (Fig. 1). Exposure to 45°C for 48 or 72 h reduced viability to 0.4 and 0.0%, respectively. Oospores exposed to 50°C for 6 h did not survive.

To determine the relationship between temperature and survival, a slightly modified Weibull model was fitted for the data at each temperature with the following formula:

$$S(t) = \exp[\Theta_0 - (\Theta_1 \times t)^{\Theta_2}] \quad (2)$$

where Θ_0 is an offset parameter that adjusts the model for differences in initial levels of oospore viability. Values for Θ_0 ranged from a low of -0.099 to a high of -0.004 (mean = -0.023). Values for Θ_1 varied from a low of 0.00424 at 35°C to a high of 0.434 at 50°C. Values of Θ_2 varied from a low of 1.64 at 35°C to a high of 4.86 at 45°C. Regression between $\ln\Theta_1$ and temperature showed a strong linear relationship (Fig. 2A, $R^2 = 0.98$, $P < 0.001$). No significant relationship was discerned between Θ_2 and temperature (Fig. 2B, $R^2 = 0.08$, $P = 0.48$). Therefore, in expanding to a continuous temperature model, a constant value was fitted for Θ_2 and $\exp(\Theta_{1A} + \Theta_{1B} \times T)$ replaced Θ_1 . The expanded model for all temperatures was

$$S(t) = \exp\{\Theta_0 - [\exp(\Theta_{1A} + \Theta_{1B} \times T) \times t]^{\Theta_2}\} \quad (3)$$

where T = temperature (°C).

Fitting the expanded model to data from 35, 40, 45, and 50°C resulted in a large deviance value ($G^2 = 1,366$, $P < 0.001$). From examination of residuals, one outlier was found at a temperature and time (45°C for 48 h) in which sample size was inadequate to effectively measure survival. Removal of this outlier gave -17.06 for Θ_{1A} , 0.324 for Θ_{1B} , and 2.09 for Θ_2 (Fig. 1). Deviance was still large ($G^2 = 1,311$, $P < 0.001$). However, deviance-residual plots were flat and centered on 0 indicating that the model effectively described observed means and the large deviance value

Fig. 1. Plot of observed and predicted survival of oospores of *Aphanomyces cochlioides* isolate C22. Predictions were based on the expanded temperature (T) time (t), survival model, $S(t) = \exp\{\Theta_0 - [\exp(\Theta_{1A} + \Theta_{1B} \times T) \times t]^{\Theta_2}\}$, ($G^2 = 1,311$, degrees of freedom = 266; $R^2 = 0.97$, $P < 0.001$, regression between observed mean survival and predicted survival). Each observed data point represents 500 oospores.

was the result of overdispersion. This conclusion was reconfirmed by the strong relationship between mean observed values and fitted values ($R^2 = 0.972$, $P < 0.001$). The final fitted model gave lethal dose values for 50% of the oospores (LD_{50}) of 251 h at 35°C, 49.8 h at 40°C, 9.8 h at 45°C, and 1.9 h at 50°C. Extrapolating to lower temperatures, the fitted model predicts LD_{50} values at 30°C of 53 days, at 25°C of 268 days, and at 20°C of 3.7 years. Extrapolating to higher temperatures, LD_{50} values are 23 min at 55°C and 4.6 min at 60°C.

Intermittent high temperatures. At a continuous high temperature (45°C), the percent survival of oospores declines over time in an S-shaped curve described by the modified Weibull model. The loss of viability starts slowly and then the rate of mortality increases over time until finally the rate of mortality begins to decrease as the percent survival nears 0. In the partial recovery model for intermittent high temperature episodes, survivors are assumed to recover from heat-related damage during the lower temperature periods. Thus, the S-shaped curve is assumed to start anew for survivors of the previous high temperature episode (equation 4, Fig. 3A). That is, the rate of loss of viability will be relatively slow at first and then speed up as time at high temperature progresses for each new high temperature

episode. We defined the partial recovery model as

$$S(t) = \exp\left(\Theta_0 - \sum_i \{[\exp(\Theta_{1A} + \Theta_{1B} \times T_i) \times t_i]^{\Theta_2}\}\right) \quad (4)$$

where t_i is the duration of the i th high temperature episode.

For the cumulative effects model, oospores are assumed to not recover during the lower temperature periods. Thus, if the first high temperature episode resulted in the survival curve reaching the down turn in the S-shaped curve toward more rapid loss of viability, the next high temperature episode causes a continuation down the S-shaped curve giving even more rapid loss of viability (equation 5, Fig. 3B) rather than starting again down the top of a new S-shaped curve. We defined the cumulative effects model as

$$S(t) = \exp\left(\Theta_0 - \left\{\sum_i [\exp(\Theta_{1A} + \Theta_{1B} \times T_i) \times t_i]\right\}^{\Theta_2}\right) \quad (5)$$

Fig. 2. Regression of temperature on **A**, natural log (Θ_1), $R^2 = 0.98$, $P < 0.001$, and **B**, Θ_2 , $R^2 = 0.08$, $P = 0.48$ for the survival model, $S(t) = \exp[\Theta_0 - (\Theta_1 \times t)^{\Theta_2}]$, for oospores of *Aphanomyces cochlioides* isolate C22 (t = time in hours).

Fig. 3. Potential survival patterns for oospores of *Aphanomyces cochlioides* when exposed to four 24-h cycles of 4 h at 45°C and 20 h at 21°C. Predictions from two models are **A**, partial recovery during low temperature periods, $S(t) = \exp\{\Theta_0 - \sum_i [\exp(\Theta_{1A} + \Theta_{1B} \times T_i) \times t_i]^{\Theta_2}\}$ and **B**, cumulative effects, $S(t) = \exp\{\Theta_0 - \left\{\sum_i [\exp(\Theta_{1A} + \Theta_{1B} \times T_i) \times t_i]\right\}^{\Theta_2}\}$. Survival (S) is given as a function of temperature (T , °C) and time (t , hours).

where t_i is the duration for the i th temperature experienced, that is, all temperatures experienced cumulatively affect oospore health. As seen in the comparison of Figure 3A and B, repeated episodes of high temperature cause a more rapid decline in oospore viability in the cumulative model than in the partial recovery model. If oospores became more susceptible to successive high temperature episodes, observed viability was expected to be significantly lower than predictions of the cumulative effects model. These distinctions become very important in predicting the effects of solarization treatments in which daily episodes of high temperature are interrupted by lower temperatures each night.

Employing parameter estimates for isolate C22 (Table 1), the partial recovery model predicted that oospore survival would decrease to a low of 0.293 after the fourth temperature cycle in run 1 (Fig. 4) and to 0.281 for run 2 (data not shown). Survival predictions for the cumulative effect after the fourth temperature cycle were 0.003 for the first run (Fig. 4) and 0.0023 for the second run (data not shown). Observed survival for the intermittent high temperature runs (four cycles of 45°C, 4 h/21°C, 20 h) started at 0.996 and 0.992 for runs 1 and 2, respectively, and decreased to 0.023 at the end of the fourth temperature cycle for run 1 (Fig. 4) and 0.004 for run 2 (data not shown). Predictions for the cumulative effects model were within the 95% confidence interval for six of the eight mean survival observations from the

TABLE 1. Mean and fitted Θ 's for expanded temperature survival models^a for five isolates of *Aphanomyces cochlioides* and a composite population of the five isolates

Isolate	Θ_0^b	Θ_{1A}	Θ_{1B}	Θ_2
B14	-0.219	-16.280	0.307	1.94
B19	-0.270	-16.361	0.310	2.11
C10	-0.012	-18.150 ^c	0.334	2.10
C22	-0.015	-13.419 ^c	0.255	2.13
T71	-0.039	-15.513	0.291	1.90
Mean values	-0.111	-15.944	0.300	2.02
Composite population	-0.104	-16.102	0.302	1.44

^a $S(t) = \exp\{\Theta_0 - [\exp(\Theta_{1A} + \Theta_{1B} \times T) \times t]^{\Theta_2}\}$, T = temperature (°C) and t = time (hours).

^b Significantly different (χ^2 , $P < 0.001$).

^c Significantly different (t test, $P < 0.001$).

Fig. 4. Oospore survival of *Aphanomyces cochlioides* exposed to four cycles of 4 h at 45°C and 20 h at 21°C. Predictions from two models (cumulative effect of temperature and partial recovery during low temperature periods) are compared with the observed mean survival (bars indicate 95% confidence intervals).

two runs, while partial recovery predictions were within the 95% confidence interval after the first temperature cycle in the second run.

Isolate variability and effects of heterogeneity. Significant variation was identified among isolates. The most dramatic differences in survival were seen between isolates C22 and C10 both from North Dakota. For instance, the 35°C/144-h treatment resulted in only 5% of the oospores surviving for isolate C22, while for isolate C10, 89.6% of oospores survived (data not shown). At 40°C, the LD₅₀ values as predicted by the fitted models (Table 1) for isolates C22 and C10 were 21 and 101 h, respectively. Additionally, isolates B14 and B19 had significantly lower initial viability (80.3 and 76.3%, respectively) when compared with the other isolates that ranged from 96.1 to 98.8% (χ^2 , $P < 0.001$). In terms of model parameters, linear regression of $\ln\Theta_1$ values of the five isolates against temperature ($R^2 = 96$, $P < 0.001$) indicated that Θ_{1A} values for isolates C22 and C10 were significantly different from the other isolates (t test, both at $P < 0.001$). Variation in Θ_{1B} was not significant ($P = 0.493$) and little variation was observed in Θ_2 , which ranged from 1.90 to 2.11 (Table 1). When constant population mean values of 0.30 and 2.0 replaced Θ_{1B} and Θ_2 , respectively, fitted values for Θ_{1A} were -15.311, -15.786, -15.892, -15.926, and -16.712 for isolates C22, B19, B14, T71, and C10, respectively.

Field populations typically consist of heterogeneous mixtures of clones. While it is not possible to test every clone in a population for sensitivity to heat treatment, the appropriate values for parameters in the modified Weibull model for a heterogeneous population may be approximated based on responses of representative isolates from the population. To this end, the response of a hypothetical population was simulated by combining the survival data from the five isolates to generate a single curve for oospores in the mixed population. Parameter values for the modified Weibull model were calculated and the model performed well in describing the hypothetical population (Fig. 5; $R^2 = 0.96$, $P < 0.001$). The LD₅₀ values for the hypothetical population were 180 h for 35°C, 39.8 h for 40°C, 8.8 h for 45°C, and 1.9 h for 50°C. The parameter values for Θ_0 , Θ_{1A} , and Θ_{1B} for the hypothetical population closely matched means for the individual isolate models. The value for Θ_2 of 1.44, however, was noticeably lower than the mean of 2.02 for the five isolates (Table 1). The behavior of Θ_2 relative to the variance in individual isolate Θ_{1A} values for the population was determined for mixtures of all combinations of three, four, or all five isolates tested to see how this parameter related to the degree of heterogeneity in the population (Fig. 6). With this knowledge, it should be possible to estimate a value for Θ_2 for a heterogeneous population based on tests of selected isolates in the population.

DISCUSSION

We demonstrated that oospores of *A. cochlioides* are susceptible to moderately high temperatures and susceptibility dramatically increases as temperatures increase. As such, the soil temperatures reportedly achievable either through solarization of fields or through composting of soil and plant refuses (17,19,20,27) may have a significant effect on infestations of this pathogen. In comparisons with other studies, oospores of *A. cochlioides* appear more heat tolerant than mycelium and monilioid cells of *Rhizoctonia solani*, oospores of *Pythium ultimum*, microsclerotia of *Verticillium dahliae*, and chlamydospores of *Phytophthora nicotianae* (8,27). The greater heat tolerance of *A. cochlioides* is not surprising given its reputation as a warm weather pathogen.

While solarization may provide control initially, the significant variability in heat tolerance displayed in the Θ_{1A} parameter among the five isolates suggests repeated use of this control option for the field will quickly result in less satisfactory control. Surpris-

ingly, the isolates that display the greatest heat tolerance (isolate C10) and sensitivity (isolate C22) come from the same field in northern North Dakota. In other studies, isolates from this location have displayed significant variation in other phenotypic characters (*unpublished data*). Still, we expected that isolate T71 from Texas would display the greatest heat tolerance and the two isolates from North Dakota would display the least given their relative climates. At this time, we are unable to give a logical explanation why the most heat tolerant isolate comes from the northernmost location.

Previously, it was uncertain whether oospores of *A. cochlioides* would display a repair mechanism or simply accumulate heat-related damage when exposed to intermittent high temperature experiences. Survival of bacteria in fluctuating high temperature (>100°C) experiments involved simple kinetics, but bacterial endospores are stimulated to germinate by short exposures to high temperatures when followed by moderate temperatures (28). Being considerably larger, oospores have potentially more resources than bacteria to repair environmentally related damage. Results from studies of other plant pathogens appear inconclusive on this subject (8,29). The strong performance of the cumulative effects model and the poor performance of the partial recovery model were conclusive, in this situation, that oospores of *A. cochlioides* follow simple kinetics. While of limited scope, this experiment represents the first successful demonstration of predictive power of a temperature survival model for an organism experiencing high-low temperature fluctuations. If oospore survival in the intermittent high temperature trials had indicated either increased susceptibility or recovery during low temperature periods, additional studies would have been necessary to define the phenomena. Results of the intermittent high temperature trials indicated that calculations for practical survival predictions are feasible and simple.

The continuous relationship described by this study was significantly different from that defined for *Listeria monocytogenes* (26). Both our study and the *Listeria* study employed Weibull base models and resulted in comparable temperature functions for Θ_1 . For the *Aphanomyces* model, a constant value was fitted for Θ_2 while a temperature function was fitted for the *Listeria* model. Authors of the *Listeria* study were skeptical of the temperature function as they described the plot of Θ_2 against temperature as

one of “considerable scatter”. For dozens of other food contaminants relationships between Θ_2 and temperatures have been inconsistent (34). In our study, no relationship was distinguished between Θ_2 and temperature. These differences in handling Θ_2 may be the result of the different base formulas employed. Weibull (35) defined a distribution function with the formula $S(t) = \exp(\Theta_1 \times t^{\Theta_2})$. In performing calculations, however, Weibull employed an alternative formula, $S(t) = \exp(\Theta_1 \times t)^{\Theta_2}$. While both formulas gave the same fitted mean values for iso-stress data, we observed that the values for Θ_1 were heavily

Fig. 6. Regression between the standard deviation of Θ_{1A} values among member isolates of 16 hypothetical composite populations and values of Θ_2 from fitted models for the composite populations ($R^2 = 0.90$, $P < 0.001$). The 16 hypothetical populations were made up of isolates B14, B19, C10, C22, and T71, or any of the 15 possible three- or four-isolate combinations drawn from these isolates.

Fig. 5. Observed and predicted survival for *Aphanomyces cochlioides* oospores of a hypothetical population of the five isolates exposed to various temperatures and times ($R^2 = 0.96$). Each observed data point is based on 2,500 oospores.

influenced by the values for Θ_2 in the first formula but were insulated from Θ_2 in the second formula. Peleg et al. (26) employed the first formula. Because Θ_2 was relatively unstable in our initial trials with isolate C22, we used the second Weibull formula to stabilize the Θ_1 values. By using the second formula, we did not need to force a fit on the Θ_2 parameter that may not exist. Choosing the second formula had additional benefits in providing for simpler summation of intermittent high temperature experiences, an opportunity to normalize data within Θ_2 model parameter, and an easier comparison of heat tolerances among study subjects.

From the examination of five isolates and hypothetical populations based on them, we have concluded that it would be ill-conceived to apply model parameters fitted for individual isolates and expect them to provide measures of temperature experiences relevant to the field. Based on results from the hypothetical populations, mean fitted values for Θ_{1A} and Θ_{1B} would adequately serve field observations but mean values for Θ_2 obtained from individual isolates would confound model relevancy. In order to provide a measure of thermal treatments as suggested by MacDonald (18) and attempted by Mihail and Alcorn (22), we propose, instead, that one first calculate prior experience values (we define prior experience as $\sum_i \exp(\Theta_{1A} + \Theta_{1B} \times T_i) \times t_i$) for the temperature data. These prior experience values can then be normalized against the natural log of the biological response (i.e., viable oospores, measure of disease, etc.) through a Box-Cox power transformation (9). This Box-Cox transformation value is an estimate of Θ_2 that may be applied along with cumulative effects model (equation 5) to provide a measure of thermal experience potentially applicable to field studies.

We demonstrated significant variability in heat tolerance among isolates. Variation in heat tolerance may be influenced by other factors in addition to genetics. For example, observed heat tolerance for isolate C22 dropped considerably between the first trials and the population trials conducted 6 months later. Nutrition as well as temperature at time of oospore formation may play a role in these differences. We have noted in past research that hypocotyl quality plays a significant role in oospore production (14) and this may also affect oospore durability. Variability in prior temperature experiences among an oospore population is another source of variation that will exist in the field which we have not addressed at this time.

While displaying resilience with hypothetical populations, not all variability in heat tolerance is going to be accommodated by our model. For instance, we observed that desiccated oospores display a condition known as anhydrobiosis. Anhydrobiosis is a condition resulting from desiccation in which an organism's metabolism dramatically decreases and the organism becomes resistant to the damaging effects of heat, UV light, and other deleterious stresses (2). Upon rehydration, cells regain their metabolic capabilities without any apparent consequences. This phenomenon has also been witnessed recently with *Fusarium oxysporum* and *Sclerotinia rolfii* (29). Preliminary observations for *A. cochlidioides* indicated that desiccated oospores are highly resistant to heat (*unpublished data*). For a field population containing both desiccated oospores on or near the soil surface and moist oospores deeper in the soil profile, adjustments in the Θ_2 parameter are unlikely to accommodate their major differences in heat tolerance. In those instances, the desiccated and moist populations will need to be treated separately. While our model may be appropriate for oospores lower in the soil profile, the model proposed by Shelvin et al. (29) may be appropriate for those oospores near the soil surface. This problem will not affect solarization trials where the soil is moistened and then covered with plastic.

Because of the sensitivity of Θ_2 to observations falling within the tails of survival curves, we felt it was important to fit our model parameters using a binomial distribution function and

generalized nonlinear modeling techniques. While this approach is rarely used in plant pathology, we believe we obtained better estimates of model parameters by using generalized modeling techniques than would be obtained with linear regression. Unfortunately, generalized modeling techniques have their weaknesses. In particular, they provide weaker assessments of model adequacy due to the high occurrence of overdispersion, as was our situation. Overdispersion is characterized by large deviance values (G^2) and flat deviance-residual plots centered on 0. Overdispersion is common where binomial distribution functions are employed to describe biological systems (1). Overdispersion indicates that sources of variation within the experiment either are not accounted for by the model or are not controlled within the experimental design. This causes the observed variance to be underestimated by the model's binomial variance function. In our experiments, numerous sources of variation may account for overdispersion including oospore position within the hypocotyls (affecting both the nutrition and temperature experience of the oospores), variability in the microenvironment of the petri dishes in which oospores were treated and error in the assessment of viability. Models exist for describing this extra-binomial variance (37) but we have found them ineffective for describing variance behavior in our experiments. While the binomial distribution function underestimated observed variance in our system, it is important to note that the model's ability to describe the mean observed survival was very good as measured by correlations between observed and fitted model values.

This is the first heat-related study to define how to appropriately quantify a variable temperature experience for a variable biological population. This result may be broadly applicable not just to plant pathogen systems but also to food microbiology and anywhere heat sterilization is applied. Because fitted constants are not as important as the mathematical dynamics described by the formulas, our results may be applied through linear regression analysis to isolate and quantify the direct effects of high temperatures on survival within heat-related trials. Currently, we have field trials examining oospore survival under solarization and will be applying our model. Under field conditions, the direct effect of heat on oospore survival is only one source of mortality among many that need to be addressed. As such, predictions based on our model may be taken as conservative estimates of mortality in field trials. Addressing these other sources of mortality, however, is beyond the scope of the work described. Our survival model is fitted to observations made at 35 to 50°C but extrapolations to lower and higher temperatures provide plausible predictions worth exploring.

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LITERATURE CITED

- Agresti, A. 1996. An Introduction to Categorical Data Analysis. Wiley Interscience, New York.
- Aguilera, J. M., and Karel, M. 1997. Preservation of biological material under desiccation. Crit. Rev. Food Sci. Nutr. 37:287-309.
- Beale, J. W., Windels, C. E., and Kinkel, L. L. 2002. Spatial distribution of *Aphanomyces cochlidioides* and root rot in sugar beet fields. Plant Dis. 86:547-551.
- Benson, D. M. 1994. Inoculum. Pages 1-33 in: Epidemiology and Management of Root Diseases. C. L. Campbell and D. M. Benson, eds. Springer-Verlag, New York.
- Benson, D. M., and Baker, R. 1974. Epidemiology of *Rhizoctonia solani* preemergence damping-off of radish survival. Phytopathology 64:1163-1168.

6. Brantner, J. R., Windels, C. E., and Dyer, A. T. 2002. Green manure crops and soil solarization effects on *Aphanomyces cochlioides*. (Abstr.) Phytopathology 92(suppl.):S9.
7. Cleveland, W. 1979. Robust locally weighted regression and smoothing scatterplots. J. Am. Stat. Assoc. 74:829-836.
8. Coelho, L., Mitchell, D. J., and Chellemi, D. O. 2000. Thermal inactivation of *Phytophthora nicotianae*. Phytopathology 90:1089-1097.
9. Cook, R. D., and Weisberg, S. 1999. Applied Regression Including Computing and Graphics. John Wiley & Sons, New York.
10. Creasy, T. S. 2000. A method of extracting Weibull survival model parameters from filament bundle load/strain data. Composites Sci. Technol. 60:825-832.
11. Didelot, D., Muchembled, C., Delannoy, V., and Delafosse, P. 1995. Fatigue of soils in sugarbeet crop. The importance of root necrosis and identification of casual agents. Phytoma 477:29-33.
12. Dyer, A. T., Szabo, L. J., and Windels, C. E. 2004. Characterization and spatial distribution of *Aphanomyces* in sugarbeet fields. J. Sugar Beet Res. 41:1-16.
13. Dyer, A. T., and Windels, C. E. 2002. Modeling the survival of oospores of *Aphanomyces cochlioides*. (Abstr.) Phytopathology 92(suppl.):S22.
14. Dyer, A. T., and Windels, C. E. 2003. Viability and maturation of oospores of *Aphanomyces cochlioides*. Mycologia 95:321-326.
15. Fay, J. C., and Fry, W. E. 1997. Effects of hot and cold temperatures on the survival of oospores produced by United States strains of *Phytophthora infestans*. Am. Potato J. 74:315-323.
16. Kachman, S. D. 1999. Applications in survival analysis. J. Anim. Sci. 77(suppl. 2)/J. Dairy Sci. 82(suppl. 2):147-153.
17. Katan, J. 1996. Soil solarization: Integrated control aspects. Pages 250-278 in: Principles and Practices of Managing Soilborne Plant Pathogens. R. Hall, ed. The American Phytopathological Society, St. Paul, MN.
18. MacDonald, J. D. 1994. The soil environment. Pages 82-116 in: Epidemiology and Management of Root Diseases. C. L. Campbell and D. M. Benson, eds. Springer-Verlag, New York.
19. Mahrer, Y. 1979. Predictions of soil temperatures of a soil mulched under transparent polyethylene. J. Appl. Meteorol. 18:1263-1267.
20. Mahrer, Y. 1991. Physical principles of solar heating of soils by plastic mulching in the field and in glasshouses and simulation models. Pages 75-86 in: Soil Solarization. J. K. Katan and J. E. DeVay, eds. CRC Press, Boca Raton, FL.
21. Mckeen, W. E. 1949. A study of sugar beet root rot in southern Ontario. Can. J. Res. Sect. C Bot. Sci. 27:284-311.
22. Mihail, J. D., and Alcorn, S. M. 1984. Effects of soil solarization on *Macrophomina phaseolina* and *Sclerotium rolfsii*. Plant Dis. 68:156-159.
23. Papavizas, G. C., and Ayers, W. A. 1974. *Aphanomyces* species and their root diseases in pea and sugarbeet. U.S. Dep. Agric. Agric. Res. Serv. Tech. Bull. 1485. Washington, D.C.
24. Payne, P. A., Asher, M. C. C., and Kershaw, C. D. 1994. The incidence of *Pythium* spp. and *Aphanomyces* associated with sugar beet growing soils in Britain. Plant Pathol. 43:300-308.
25. Peleg, M., and Cole, M. B. 1998. Reinterpretation of microbial survival curves. Crit. Rev. Food Sci. 38:353-380.
26. Peleg, M., Penchina, C. M., and Cole, M. B. 2001. Estimation of the survival curve of *Listeria monocytogenes* during non-isothermal heat treatments. Food Res. Int. 34:383-388.
27. Pullman, G. S., DeVay, J. E., and Garber, R. H. 1981. Soil solarization and thermal death: A logarithmic relationship between time and temperature for four soilborne plant pathogens. Phytopathology 71:959-964.
28. Sapru, V., Smerage, A., Teixeira, A., and Lindsay, J. A. 1993. Comparison of predictive models for bacterial spore population resources to sterilization temperatures. J. Food Sci. 58:223-228.
29. Shelvin, E., Saguy, I. S., Mahrer, Y., and Katan, J. 2003. Modeling the survival of two soilborne pathogens under dry structural solarization. Phytopathology 93:1247-1257.
30. Smith, H. 1997. California sugar beet: Its pests and diseases. Brit. Sugar Beet Rev. 65:43-45.
31. Smith, J. H. 1923. The killing of *Botrytis cinerea* by heat, with a note on the determination of temperature coefficients. Ann. Appl. Biol. 10:335-347.
32. Szymczak, N. J., and Banaszak, H. 1994. Importance of seedling damping-off in the integrated sugar beet production. Pages 170-174 in: 34th Res. Session, Inst. Plant Protection. S. Pruszyński and J. J. Lipam, eds. Inst. Ochrony Roslin, Poznań.
33. Thal, W. M., Campbell, C. L., and Madden, L. V. 1984. Sensitivity of Weibull model parameter estimates to variation in simulated disease progression data. Phytopathology 74:1425-1430.
34. Van Boekel, M. J. A. S. 2002. On use of the Weibull model to describe thermal inactivation of microbial vegetative cells. Int. J. Food Microbiol. 74:139-159.
35. Weibull, W. 1951. A statistical distribution function of wide applicability. J. Appl. Mech. 18:293-297.
36. Weller, E. A., Feuer, E. J., Frey, C. M., and Wesley, M. N. 1999. Parametric relative survival regression using generalized linear models with application to Hodgkin's lymphoma. Appl. Statist. 48:79-89.
37. Williams, D. A. 1982. Extra-binomial variation in logistic linear models. Appl. Statist. 31:144-148.
38. Windels, C. E., and Lamey, H. A. 1998. Identification and control of seedling diseases, root rot, and rhizomania on sugar beet. N.D. State Univ. Ext. Serv./Univ. Minn. Ext. Serv. PP-1142-BU-7192-S.
39. Yarwood, C. E. 1975. Temperature coefficients in plant pathology. Phytopathology 65:1198-1201.