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Hydrothermal time models for conidial germination and mycelial growth of the seed pathogen *Pyrenophora semeniperda*

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ABSTRACT

Population-based threshold models using hydrothermal time (HTT) have been widely used to model seed germination. We used HTT to model conidial germination and mycelial growth for the seed pathogen *Pyrenophora semeniperda* in a novel approach to understanding its interactions with host seeds. Germination time courses and mycelial growth rates for *P. semeniperda* were measured on PDA amended to achieve a series of five water potentials (ca. 0 to –6 MPa) at six constant temperatures (5–30 °C). Conidial germination was described with alternative population-based models using constant or variable base and maximum temperature and water potential parameters. Mycelial growth was modeled as a continuous, linear process with constant base temperature and base water potential. Models based on HTT showed reasonable fit to germination and growth rate data sets. The best-fit conidial germination model ($R^2 = 0.859$) was based on variable base and maximum temperature as a function of water potential. The good fit of the linear mycelial growth model ($R^2 = 0.916$) demonstrated the utility of HTT for modeling continuous as well as population-based processes. HTT modeling may be a useful approach to the quantification of germination and growth processes in a wide range of filamentous fungi.

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Introduction

Hydrothermal time (HTT) is a modeling approach that is useful in describing and quantifying the combined effects of temperature and water potential on biological processes (Allen 2003). Time to proceed to completion for a defined fraction of the population is inversely proportional to the amount by which temperature (T) and water potential (Ψ) conditions in the environment exceed given base or threshold values. The

process is inhibited whenever T is below the base temperature (T_b) or Ψ is below (i.e., more negative than) the base water potential (Ψ_b). Variation in time to proceed to completion among individuals within the population is accounted for by variation in T_b and/or Ψ_b . In other words, HTT is a population-based threshold-type model. It was developed to characterize germination and dormancy of seeds (reviewed by Bradford 2002), and has thus far been almost exclusively used for that purpose. However, there is no reason why the fundamental

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concepts should not also be applicable to other biological processes that occur across a range of water potentials, e.g., the growth of organisms that tolerate significant water stress (Alpert 2006). A primary advantage of HTT is that it replaces strictly empirical models with a strong conceptual and mathematical framework, one that allows for prediction of outcomes across a wide range of conditions and also under variable conditions (Bradford 2005; Allen et al. 2007; Meyer & Allen 2009).

In the present study, we apply the principles of HTT to germination and growth of a fungal seed pathogen, *Pyrenophora semeniperda*. This fungus plays an important ecological role in semi-arid ecosystems of the western United States (Beckstead et al. 2014; Meyer et al. 2014). It attacks and kills large numbers of seeds in the seed bank of the invasive winter annual grass *Bromus tectorum*, whose seed germination has been extensively modeled by our group using HTT concepts (Christensen et al. 1996; Bauer et al. 1998; Bair et al. 2006; Meyer & Allen 2009). The goal of this study was to determine how temperature and water potential influence the germination and growth phases of *P. semeniperda*, as part of an overall effort to understand how environmental conditions influence disease outcomes in the *P. semeniperda*–*B. tectorum* pathosystem. We have demonstrated that disease development in this pathosystem can occur at water potentials below those that allow seeds to germinate (Finch et al. 2013). Numerous studies have shown that many fungi can germinate and grow at reduced water potentials (e.g., Marín et al. 1995, 1996; Ramos et al. 1998; Torres et al. 2003; Andersen et al. 2006), and considerable effort has been made to model these processes (reviewed by D'Antigny et al. 2005). The models developed to date are largely empirical and often mathematically complex, making them difficult to use for simulation of processes that take place in variable environments. To our knowledge, this is the first attempt to model germination and growth processes in a fungus using the relatively simple concepts of HTT.

Hydrothermal time models

The HTT concept has been extensively discussed in earlier publications (Bradford 2002; Allen et al. 2007; Meyer & Allen 2009). It was originally proposed by Gummerson (1986), who used the following equation to calculate HTT accumulation in germinating seeds:

$$\theta_{\text{HTT}} = (T - T_b)(\Psi - \Psi_b(g))t_g \quad (1)$$

where θ_{HTT} , the HTT constant, is the amount of HTT (in MPa-degree-time units) that a seed must accumulate to germinate, T_b is the base temperature below which seed germination will not occur, $\Psi_b(g)$ is the base water potential below which germination of the g fraction of the population will not occur, and t_g is the actual time required for germination of the g fraction. T and Ψ represent the temperature and water potential conditions during incubation, respectively. As the difference between incubation temperature or water potential and the corresponding base value increases (i.e., $(T - T_b)$ or $(\Psi - \Psi_b(g))$ becomes larger), HTT accumulates more rapidly, θ_{HTT} is reached more quickly, and germination takes place sooner. θ_{HTT} and T_b are generally assumed to be constants for a given

population, while $\Psi_b(g)$ and t_g are allowed to vary with germination fraction. The distribution of $\Psi_b(g)$ is assumed to be approximately normal (although a Weibull function sometimes yields a better fit; e.g., Watt et al. 2010), with a mean $\Psi_b(50)$ and standard deviation σ_{Ψ_b} . In order to apply Eqn. 1 to a population rather than just an individual seed, Gummerson (1986) developed the following:

$$\text{probit}(g/g_m) = [\Psi - \Psi_b(50) - (\theta_{\text{HTT}} / ((T - T_b)t_g))] / \sigma_{\Psi_b} \quad (2)$$

where g/g_m is the proportional germination fraction (g_m is the maximum possible germination, equivalent to viability). The probit transformation linearizes a cumulative normal distribution, and subsequent probit analysis techniques allow HTT parameters to be determined using repeated linear regression (Bradford 1990, 2005).

In the simplest application of the model, incubation temperature is held constant, and germination time course curves at multiple water potentials are used to determine parameter values for a hydrotime equation (Bradford 1990). Similarly, thermal time modeling has been a common procedure for many biological processes including seed germination, with the assumption that water potential is held constant at 0 MPa (free water; e.g., García-Huidobro et al. 1982). Combining germination time course curves at both multiple temperatures and multiple water potentials results in the more complex model shown in Eqn. 2.

It is also possible to use HTT to explain changes in germination time course curves due to other processes. These processes are proposed to affect the values of HTT parameters assumed to be constant in the original Gummerson model (Eqn. 2). For example, Christensen et al. (1996) found that increased germination speed and percentage in a population of seeds during dormancy loss could be accounted for by a linear decrease in $\Psi_b(50)$ through time. This relationship is inherent in Eqn. 1 because, as the difference between incubation water potential and the corresponding base water potential increases (i.e., $(\Psi - \Psi_b(g))$ becomes larger), HTT accumulates more quickly, and seeds will be able to germinate more quickly. Thus a decrease in the parameter $\Psi_b(50)$ has the same effect on a germination time course as a hypothetical increase in ambient water potential. A similar explanation has been proposed for germination decrease at supraoptimal temperature, i.e., a linear increase in $\Psi_b(50)$ with temperature above the optimum can account for increases in germination time and decreases in percentage as temperature increases (Meyer et al. 2000; Alvarado & Bradford 2002).

Study hypotheses

The study reported here uses *in vitro* experiments on PDA (potato dextrose agar) using glycerol as an osmoticum to address the following hypotheses: 1) Conidial germination as a function of temperature and water potential can be modeled using HTT, 2) Mycelial growth rate as a function of temperature and water potential can be modeled using HTT, but because the process is continuous and not population-based, the time courses will have a linear rather than a cumulative normal distribution, 3) The optimum water potential for mycelial growth will be negative, i.e., mycelial growth will be more rapid under mild water stress than in free water.

Materials and methods

Experiments

Pyrenophora semeniperda strain WRKO (Finch et al. 2013) was first obtained from a killed *Bromus tectorum* seed in the seed bank at Whiterocks, Utah (−112.7780 long, 40.3282 lat, 1446 m), and isolated onto V8 agar. The isolate was sub-cultured onto modified alphacel medium for conidial production (Meyer et al. 2010). Conidia were stored air-dry at room temperature until use.

Experiments were carried out at five water potentials (0, −1.5, −3, −4.5, or −6 MPa) and six constant temperatures (5, 10, 15, 20, 25, or 30 °C). To adjust the water potential of the medium, specific amounts of glycerol were added to full-strength PDA (potato dextrose agar) solution according to established protocols (Dallyn 1978). The amounts were as follows: 0 MPa = no glycerol, −1.5 MPa = 0.6 mol kg^{−1}, −3.0 MPa = 1.2 mol kg^{−1}, −4.5 MPa = 1.8 mol kg^{−1}, −6.0 MPa = 2.4 mol kg^{−1}. The water potential of all media was verified using an AquaLab CX3 unit (Decagon Devices). The water potential of control PDA (no added glycerol) was slightly negative (ca −0.2 MPa), but was assumed to be 0 MPa as a simplification for modeling. All experiments were maintained in continuous darkness, with light exposure only during periodic data collection.

Conidial germination experiment

The conidial germination experiment included six replicates per treatment combination (five water potentials × six temperatures) for a total of 180 experimental units. PDA adjusted to each of the five treatment water potentials was poured onto sets of 36 microscope slides corresponding to each water potential treatment. A conidial suspension was created by adding a small scoop of conidia from a flattened dissection needle to a 5 ml vial containing 4 mL of deionized water with a drop of Tween-80 and shaking vigorously for 30 s, resulting in a very dilute suspension of spores. The suspension was then immediately pipetted onto the individual PDA-coated microscope slides to ensure that none of the conidia were allowed to germinate while resting in the suspension. After 5 min, sufficient time for the conidia to settle onto the agar, the excess water was poured off of each slide.

Conidial germination slides were examined at either 2, 4, 6, 8, 10, and 24 h or 3, 5, 7, 9, 11, and 24 h after inoculation depending on the expected germination rate (as observed from preliminary data). Most conidia were readily distinguishable as individual spores; only clearly individual spores were scored. In order to save time at each examination, video recordings of each slide were created using a microscope equipped with a camera, and germination was tallied at a later time. This effectively divided up the work and allowed for timely scoring even with the large number of slides examined. Germination measurements were carried out by tallying the number germinated out of the first 100 conidia examined on each slide. Conidial germination was defined as clear emergence and growth of the germ tube equal to or longer than the width of the conidium. Germination proportion was

corrected to proportion of total viable conidia for data analysis by dividing by the proportion of viable conidia in the population ($g_m = 0.95$) as determined prior to the experiment (Meyer et al. 2010).

Mycelial growth rate experiment

To create inoculum for mycelial growth experiments, conidia were inoculated onto the center of PDA plates and cultured at 25 °C. After one week of growth, 2-mm agar cores were taken from the outer edge of the mycelial colony and used to inoculate experimental PDA plates (9 cm × 10 mm plastic disposable Petri dishes). The experiment was repeated in time three times, with three replicates and 90 experimental units per repeat, for a total of 270 experimental units.

Mycelial growth plates were examined at 2, 4, 7, 11, and 14 d after inoculation. A maximum of 14 d of growth was chosen in order to stay within the linear growth phase of the mycelium on agar gel, before the nutrients are depleted or the edges of the plate restrict the growth diameter. The mycelial diameter was measured along four 45-degree transects at each examination and an average diameter was determined.

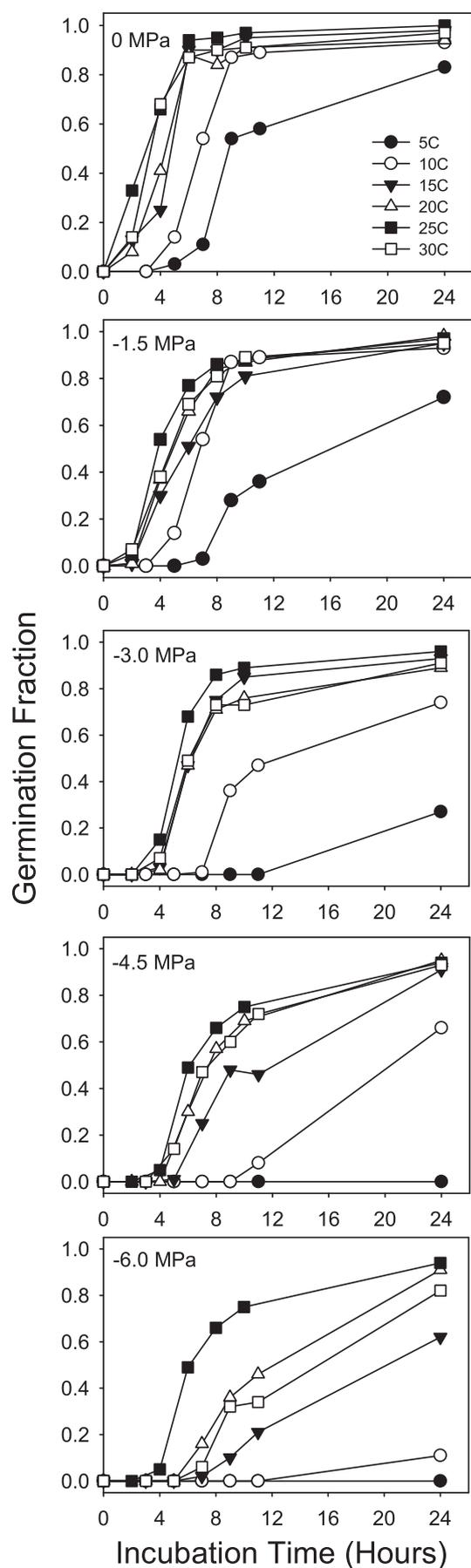
Model development for conidial germination

Provisional model with constant HTT parameters

We first created a provisional HTT model (Model 0) in which all HTT parameters (θ_{HT} , T_b , and $\Psi_b(50)$) were held constant. The modeled data set included conidial germination curves (based on the mean of six replicate values for each treatment combination and scoring time) across all water potentials and all suboptimal incubation temperatures, as previously described for seed germination (Bradford 2002). Based on the observation that there was no increase in conidial germination rate or percentage from 25 to 30 °C (Fig 1), 30 °C was considered to be above the optimal temperature; it was therefore excluded from Model 0. To estimate $\Psi_b(50)$, we used the regression analysis technique based on Eqn. (2) in which we regressed $\text{probit}(g/g_m)$ for $0.05 < g/g_m < 0.95$ versus $\Psi - \theta_{HT} / ((T - T_b)t_g)$, which is equal to $\Psi_b(g)$. The reason for excluding data for germination fractions <0.05 or greater than >0.95 from the regression analysis was to exclude values that deviate widely from predicted values just by chance; the effect of such outliers is exacerbated by the probit transformation. The values of θ_{HT} and T_b were systematically adjusted until the highest R^2 value was obtained. Then, using the regression line of best fit, $\text{probit}(g/g_m) = m(\Psi_b(g)) + b$, the mean base water potential was determined by calculating the x intercept (i.e., $\Psi_b(50) = -b m^{-1}$). The standard deviation of base water potentials (σ_{Ψ_b}) was determined by calculating the reciprocal of the slope of the regression line (i.e., $\sigma_{\Psi_b} = m^{-1}$). As explained below, the HTT model with constant parameters had a relatively poor fit.

Models with variable HTT parameters

We then investigated two alternative models to better describe conidial germination response. The poor fit of Model 0 appeared to be due to much delayed and reduced germination in low water potential/low temperature incubation treatments that could not be accounted for by simple proximity to



constant base values. We hypothesized two possible explanations for this. First, $\Psi_b(50)$ could be increasing with decreasing temperature below the optimum, in much the same way that $\Psi_b(50)$ increases with increasing temperature above the optimum in some seed germination models (e.g., Meyer et al. 2000). To test this hypothesis, we constructed Model 1, in which $\Psi_b(50)$ was allowed to vary as a function of temperature across the entire suboptimal to supraoptimal range, while other HTT parameters were held constant. This model also accommodated slowed germination rate in the supraoptimal temperature range by adjusting $\Psi_b(50)$ upward as in earlier models. The alternative hypothesis was that T_b might be increasing with decreasing water potential. To test this hypothesis, we designed a model (Model 2) in which T_b was allowed to vary with water potential over the suboptimal temperature range. To accommodate decreased germination at supraoptimal temperature in this model, we incorporated the concept of maximum temperature (T_m), the theoretical temperature above which germination cannot occur. We also allowed this parameter to vary as a function of water potential.

For Model 1, we used essentially the same procedure as Christensen et al. (1996) for accommodating variable $\Psi_b(50)$, namely incorporation of an adjustment term for each condition expected to have a different $\Psi_b(50)$. We used hydrotime models (Bradford 1990) at each temperature to obtain initial estimates for $\Psi_b(50)$ at each temperature (data not shown). We then regressed probit (g/g_m) for $0.05 < g/g_m < 0.95$ versus $[\Psi - \theta_{HT}/((T - T_b)t_g)] - \Psi_b(50)_{adj}$. The adjustment term, $\Psi_b(50)_{adj}$, was different for each temperature and was used to offset the differences between the $\Psi_b(50)$ values at different temperatures. These adjustment terms allowed us to collapse regression lines with different $\Psi_b(50)$ values into a composite regression line that included all the data and could be used to determine the θ_{HT} and T_b with the best fit (highest R^2) overall. Fitting the model required repeated probit regression with systematic adjustment of values for the constants θ_{HT} and T_b , in addition to varying the value of $\Psi_b(50)_{adj}$ for each temperature, until the model with the best fit was found. The σ_{Ψ_b} for the best-fit equation and the best values for $\Psi_b(50)$ at each temperature could then be calculated. This model accounted for reduced germination rate at supraoptimal temperature by an increase in $\Psi_b(50)$ as in earlier models (Alvarado & Bradford 2002). The best-fit values of $\Psi_b(50)$ were then plotted as a function of temperature to determine whether this parameter showed systematic variation.

For HTT analysis using Model 2, the values of T_b for each water potential at suboptimal temperature and T_m for each water potential at supraoptimal temperature were incorporated into the calculation of estimated $\Psi_b(g)$ for probit regression. Initial T_b and T_m estimates were obtained from thermal time models at each water potential (Covell et al. 1986; data not shown). We used $\Psi - \theta_{HT}/((T - T_b)t_g)$ as the estimate for $\Psi_b(g)$ at temperatures at or below the optimum and $\Psi - \theta_{HT}/((T_m - T)t_g)$ as the estimate for $\Psi_b(g)$ at supraoptimal temperature (30 °C). A regression plot combining both sub- and

Fig 1 – *Pyrenophora semeniperda* observed conidial germination time courses at five water potentials and six temperatures.

supraoptimal temperatures could then be created to determine an overall θ_{HT} , $\Psi_b(50)$ and σ_{Ψ_b} . We regressed $\text{probit}(g/g_m)$ for $0.05 < g/g_m < 0.95$ versus the estimates of $\Psi_b(g)$ based on T_b and T_m for sub and supraoptimal temperatures, respectively, at each water potential. The value of θ_{HT} , along with values for T_b and T_m at each water potential, were systematically adjusted until the best fit was obtained. Then, using the regression line of best fit, $\Psi_b(50)$ and σ_{Ψ_b} were determined, as well as the best-fit values of the constant θ_{HT} , and T_b and T_m values at each water potential. The best-fit values of T_m and T_b were then plotted as a function of water potential to determine whether these parameters showed systematic variation.

After determining the values of the HTT parameters for combined HTT models for Model 0, Model 1, and Model 2, conidial germination time course curves could be predicted for each incubation water potential by temperature combination. Using Eqn. 2 and the parameters θ_{HT} , $\Psi_b(50)$, σ_{Ψ_b} , and T_b or T_m , $\text{probit}(g/g_m)$ lines were calculated for each incubation condition, and predicted time course curves were plotted for each combination of T and Ψ . These predicted time course curves were then compared with observed conidial germination time courses for each incubation condition.

Model development for mycelial growth

Modeling mycelial growth required modifications to the basic HTT model. Because fungal radial growth (mycelial diameter increase) in agar was continuous rather than population-based and was linear (as long as the medium provided saturating nutrition and growth was not restricted by the size of the dish), no probit transformation was necessary. We developed the following equation for calculating HTT accumulation for mycelial growth:

$$\theta_{HT}(gr) = [(T - T_b)(\psi - \psi_b)t_{gr}] \quad (3)$$

where $\theta_{HT}(gr)$, the hydrothermal growth time constant, is the amount of HTT (in MPa-degree-time units) that mycelium must accumulate to achieve 1 mm of radial growth, T_b is the base temperature at which mycelial growth will not occur, Ψ_b is the base water potential at which mycelial growth will not occur, and t_{gr} is the time required for a growth increment of one mm. T and Ψ represent the temperature and water potential of incubation, respectively.

Because the optimal water potential for growth could be negative (i.e., <0 MPa; Rosso & Robinson 2001), modeling mycelial growth required an additional parameter, namely, a maximum water potential (Ψ_m), analogous to T_m in a supraoptimal temperature model, that could be positive (>0 MPa). To determine the hydrothermal growth time constant $\theta_{HT}(gr)$ as well as T_b , Ψ_b , and Ψ_m , a regression plot was created using the growth curves from both suboptimal and supraoptimal water potentials. Based on Eqn. 3, we regressed average mycelial diameter at each time on accumulated hydrothermal growth time at optimum Ψ and below, calculated as $(T - T_b)(\Psi - \Psi_b)t_{gr}$. To include hydrothermal growth times at supraoptimal water potential, $(\Psi_m - \Psi)$ was substituted for $(\Psi - \Psi_b)$. After determining that 30°C was supraoptimal for mycelial growth as well as for conidial germination, we incorporated an adjustment term, Ψ_{adj} , in the hydrotime portion of the

regression (i.e., $(\Psi - \Psi_b + \Psi_{adj})$ or $(\Psi_m - \Psi - \Psi_{adj})$) for data points from supraoptimal temperature and sub- and supraoptimal water potential incubation, respectively. Based on inspection of growth curves, we determined that -1.5 MPa was the optimal water potential for growth at suboptimal temperature and -3.0 MPa was the optimal water potential at the supraoptimal temperature of 30°C . Values of T_b , Ψ_{adj} , and Ψ_b or Ψ_m were adjusted until the best fit was obtained for the combined regression. The value of $\theta_{HT}(gr)$ (the HTT required for 1 mm of radial growth) was determined using the regression line of best fit, $\text{growth diameter} = m(\text{hydrothermal time}) + b$, by calculating the reciprocal of the slope (i.e., $\theta_{HT}(gr) = m^{-1}$).

Using Eqn. 3, the parameters generated by the regression, and the values of T and Ψ for each incubation condition, predicted growth time courses were calculated for each incubation condition. These predicted growth curves could then be compared with observed mycelial growth curves.

Results

Conidial germination modeling

When conidial germination time courses were plotted as a function of incubation water potential and temperature, the overall pattern was similar to patterns observed in earlier HTT models for seed germination, with decreasing germination rate and percentage occurring in concert as conditions departed further from the optimum (Fig 1). Several differences were immediately apparent, however. Conidia could germinate much faster under optimal conditions than even fast-germinating *Bromus tectorum* seeds (>24 h to 50 % seed germination; Christensen et al. 1996) and there was no strong germination suppression even at water potentials as low as -6 MPa, as long as temperatures were near optimum. When both temperature and water potential were low, conidial germination was strongly inhibited.

Model 0 (Constant HTT parameters)

The classical HTT model fit to conidial germination curves across all water potentials and suboptimum temperatures had a relatively poor fit, suggesting that further modifications to the basic model might cause improvement (Table 1, Fig 2A; $R^2 = 0.721$). This model had a small θ_{HT} (512 MPa degree hours), indicating a rapid germination rate, even given the relatively high constant values of T_b (-1.5C) and $\Psi_b(50)$ (-7.414 MPa). These high values would tend to reduce germination rate over the experimental range of temperatures and water potentials, but this was compensated by the small θ_{HT} .

Model 1 (Mean base water potential varying with temperature)

When the HTT model with $\Psi_b(50)$ varying as a function of temperature was constructed, it had a somewhat better fit ($R^2 = 0.778$; Table 1) than the constant-parameter model, but in fact was surprisingly similar to that model in terms of the distribution of $\Psi_b(g)$ (Fig 2A, B). As predicted, $\Psi_b(50)$ varied systematically with temperature, with the highest value at the lowest temperature and a significant decrease over the suboptimal range (5 – 25°C), as well as the predicted increase above

Table 1 – Hydrothermal time parameter values for conidial germination Model 0, Model 1, and Model 2. See Fig 2 for probit regressions and Electronic Supplement 1 for observed versus predicted germination time courses in each incubation condition.

	θ_{HT}	σ_{Ψ_b}	R^2	$\Psi_b(50)$ (MPa)	10 °C	15 °C	20 °C	25 °C	30 °C	T_b/T_m (°C)	0 MPa	-1.5 MPa	-3.0 MPa	-4.5 MPa	-6.0 MPa
Model 0	512	3.306	0.7212	-7.414	Constant for all temperatures	Not included	-1.5/-	Constant for all water potentials							
Model 1	1130	3.007	0.7781	-3.996	-5.696	-7.796	-7.496	-8.296	-6.996	-22.6/-	-1.7/53	1.5/45	4.1/45	4.1/45	7.3/40
Model 2	1100	6.519	0.8588	-13.263	Constant for all temperatures	-6.996	-3.6/56	-	-	-	-	-			

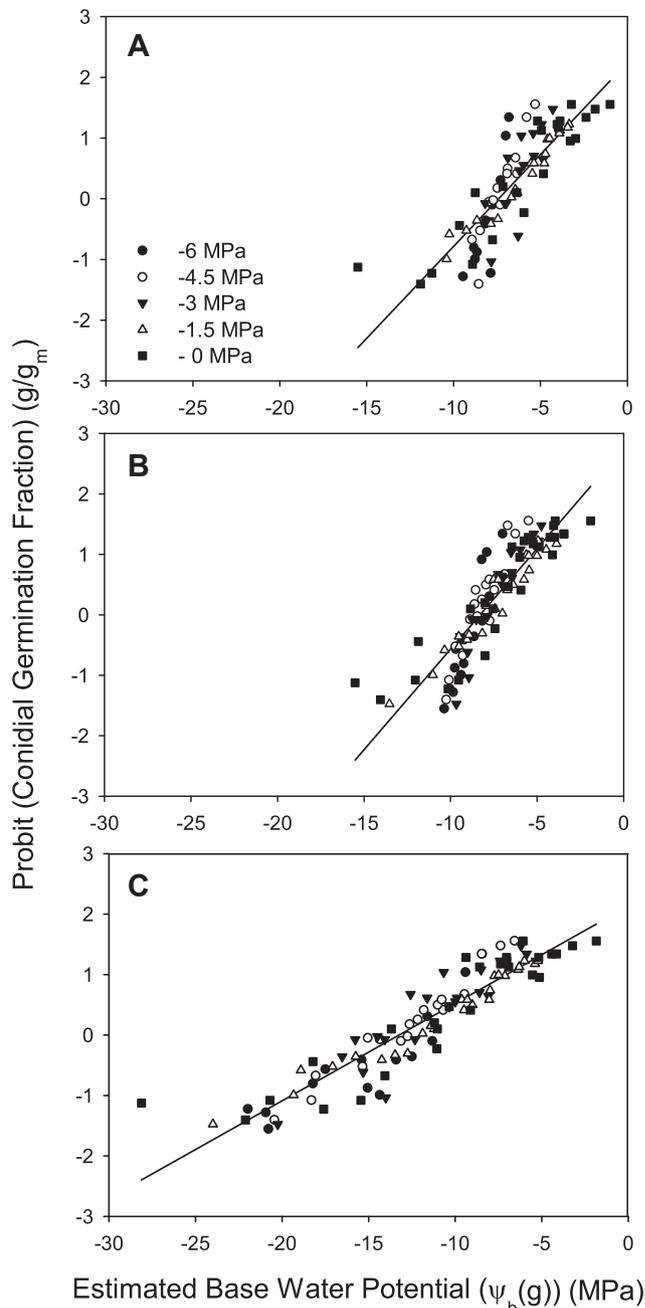


Fig 2 – Fitted hydrothermal time models for conidial germination of *Pyrenophora semeniperda*: (A) Model 0, with constant hydrothermal time parameters across all water potentials and all suboptimal temperatures, (B) Model 1, with variable mean base water potential as a function of temperature, (C) Model 2, with variable base and maximum temperature as a function of water potential. See Table 1 for parameter values.

the optimum (Fig 3A). Estimated T_b in this model was much lower than in the constant $\Psi_b(50)$ model, with a value of -22.6 °C (Table 1). The effect of temperature was minimized in this model because the decrease in germination at lower temperature was mainly accounted for by higher $\Psi_b(50)$, making the slope of the direct relationship with temperature

rather flat. θ_{HT} was about twice as large in the variable $\Psi_b(50)$ model (1130 MPa-degree days) as in the constant parameter model (Model 0). The two models had similar values for σ_{Ψ_b} .

Model 2 (Base and maximum temperature varying with water potential)

When the HTT model with varying T_b and T_m was fit to the conidial germination data set, it had a substantially better fit than the model with constant HTT parameters ($R^2 = 0.859$; Table 1, Fig 2C). There was a strong pattern of change in both T_b and T_m as a function of water potential (Fig 3B, C). As water potential decreased from 0 to -6 MPa, T_b showed a significant linear increase (Fig 3B) and T_m showed a significant linear decrease (Fig 3C). This pattern of change would have the effect of slowing germination at lower water potentials more than would be predicted by increasing proximity to $\Psi_b(50)$, and this slowing would be particularly evident at lower and at supraoptimal temperatures.

The constant $\Psi_b(50)$ for Model 2 was quite low, -13.85 MPa, but because of the pattern of change in T_b and T_m at lower water potential, germination at this low water potential would theoretically be possible only at optimum temperature. In this model, reduced germination at low water potential is largely accounted for by high T_b or T_m , reducing the direct effect of water potential over the experimental range and therefore lowering the estimate of $\Psi_b(50)$, similar to the effect on T_b in Model 1. Model 1 and Model 2 had similar θ_{HT} values, but Model 2 had a much larger σ_{Ψ_b} than either Model 0 or Model 1 (Table 1). This is because $\Psi_b(g)$ was allowed to reach lower values in Model 2, so that the spread of estimated values for $\Psi_b(g)$ was much greater (Fig 2). This was not strongly reflected in the actual germination time courses because of the very strong constraining effects of higher T_b and lower T_m on germination rate at low water potentials.

Model 2, the HTT model in which T_b and T_m were allowed to vary systematically with water potential but $\Psi_b(50)$ was kept constant, had the strongest empirical support (highest R^2) of the three models tested. It therefore represents the current best approximation for the HTT parameters underlying the variation in conidial germination that was observed (Fig 1). The fit of predicted curves from the three HTT models to observed conidial germination time courses under each experimental condition is shown in Electronic Supplement 1.

Mycelial growth modeling

Examination of mycelial growth rates as a function of temperature and water potential led to the discovery that these environmental factors showed a strong interactive effect on growth (Fig 4). Growth generally increased with increasing water potential over the range -6 to -1.5 MPa for temperatures at or below the 25 °C optimum, but did not increase over the range -1.5 to 0 MPa. At the supraoptimal temperature of 30 °C, growth was reduced relative to the 25 °C growth rate across all water potentials, but also showed a very strong reduction with water potential above -3 MPa. This suggested that the optimum water potential for growth was <0 MPa at temperatures at or below optimum, and that it was even lower at supraoptimal temperature.

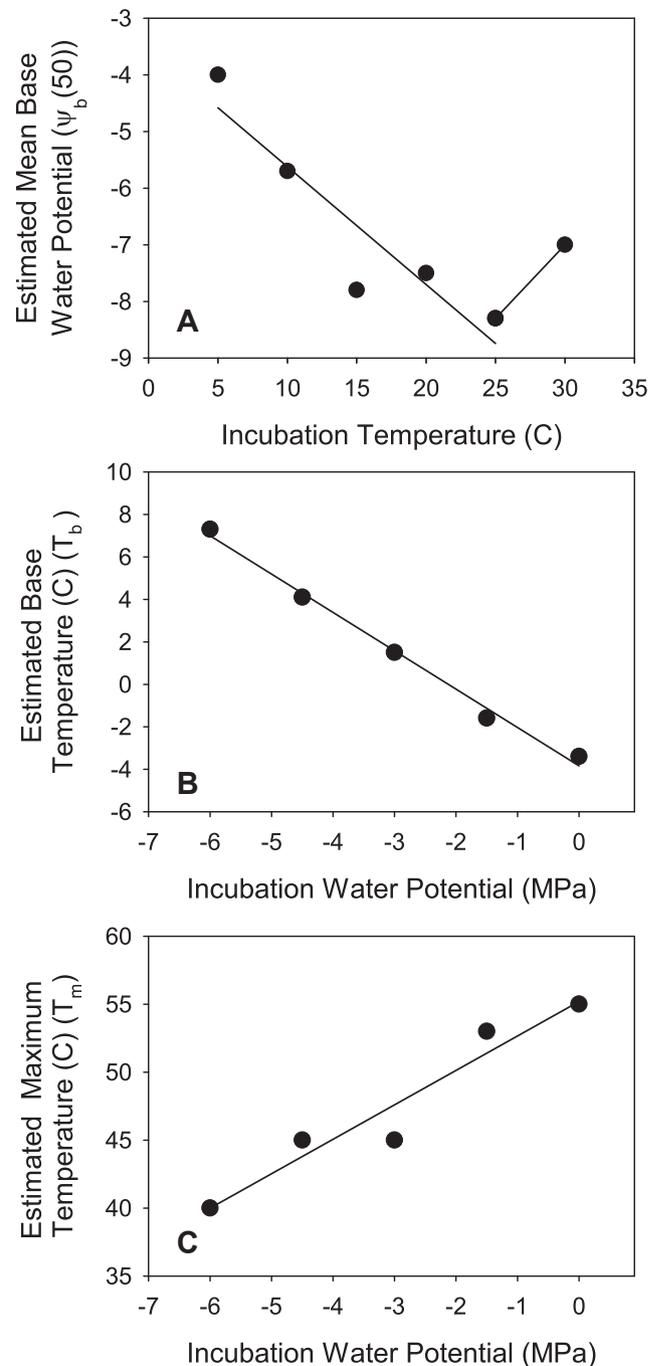


Fig 3 – (A) Estimated mean base water potentials from *Pyrenophora semeniperda* conidial germination Model 1 plotted as a function of incubation temperature, (B) Estimated base temperatures from *Pyrenophora semeniperda* conidial germination Model 2 plotted as a function of incubation water potential (C) Estimated maximum temperatures from *Pyrenophora semeniperda* conidial germination Model 2 plotted as a function of incubation water potential. Regressions were significant at: $P = 0.025$ ($r = -0.923$, d. f. = 3) for mean base water potential as a function of suboptimal temperature (A); $P = 0.0003$ (d. f. = 3, $r = -0.996$), for base temperature as a function of water potential (B); and $P = 0.0078$ (d. f. = 3, $r = +0.965$) for maximum temperature as a function of water potential (C).

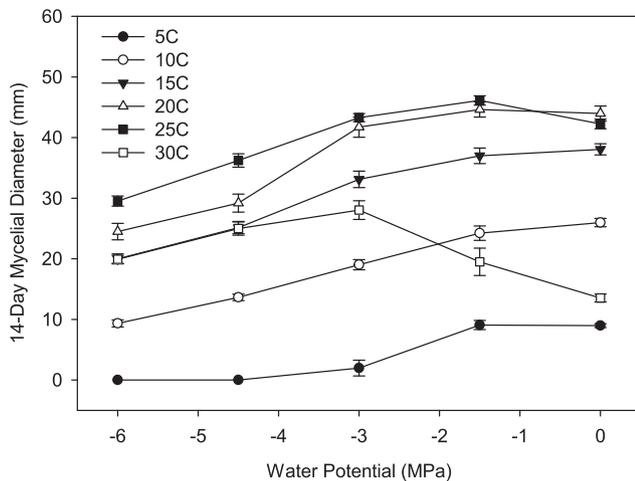


Fig 4 – Fourteen-day *Pyrenophora semeniperda* mycelial diameter plotted as a function of incubation temperature and water potential for six temperatures and five water potentials. Error bars represent standard error of the mean ($n = 9$).

When growth curves from all sub- and supraoptimal temperatures and all sub- and supraoptimal water potentials were combined into a single HTT regression, the continuous linear model showed a generally good fit (Fig 5; $R^2 = 0.915$). The estimated $\theta_{HT}(gr)$ was 69.93 MPa $^\circ$ -days; this represents the HTT increment necessary for a 1-mm increment in mycelial radial growth for this strain on full strength PDA. The model was successfully fit with a constant T_b of 1.3 $^\circ$ C, a constant suboptimal-temperature Ψ_{base} of -11.4 MPa, and a constant suboptimal-temperature Ψ_{max} of 9.9 MPa. For temperatures at or below the optimum (25 $^\circ$ C), the optimum water potential was -1.5 MPa. At supraoptimal temperature (30 $^\circ$ C), the estimated Ψ_{base} increased to -7.8 MPa and the estimated Ψ_{max} decreased to 1.3 MPa, which accounted for slower growth at supraoptimal temperature below and especially above the optimum water potential of -3 MPa. A comparison of predicted versus observed growth curves at each incubation can be found in [Electronic Supplement 2](#). The poorest fit of the model was seen at low temperature, suggesting that the relationship of mycelial growth rate to temperature might not be linear near the lower temperature limit for growth.

Discussion

It was possible to successfully model both conidial germination and mycelial growth for *Pyrenophora semeniperda* using HTT. For the conidial germination model, major modifications to the basic HTT framework were required, and some apparently counter-intuitive parameter values were obtained (Table 1). This was because temperature and water potential interacted strongly in their effect on conidial germination. For host *Bromus tectorum* seeds, constant T_b across water potentials and constant $\Psi_b(50)$ across suboptimal temperatures produced models that could adequately predict dormancy loss and germination (Christensen et al. 1996; Bauer et al.

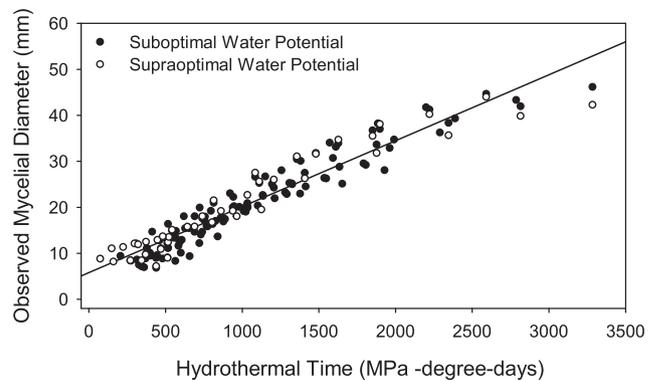


Fig 5 – A fitted continuous linear hydrothermal time model for mycelial growth rate of *Pyrenophora semeniperda* based on a constant base temperature (T_{base}), a constant base water potential (Ψ_{base}) for optimal water potentials and below, and a constant maximum water potential (Ψ_{max}) for supraoptimal water potentials. These Ψ_{base} and Ψ_{max} values were used at and below the optimum temperature of 25 $^\circ$ C. To account for slowed growth at supraoptimal temperature (30 $^\circ$ C), Ψ_{base} for suboptimal water potentials was adjusted upward, and Ψ_{max} for supraoptimal water potentials was adjusted downward. Final parameter values were: $\theta_{HT}(gr) = 69.93$ MPa $^\circ$ days, $T_{base} = 1.3$ $^\circ$ C, $\Psi_b = -11.4$ MPa, Ψ_b at supraoptimum $T = -7.8$ MPa, $\Psi_m = 9.9$ MPa, and Ψ_m at supraoptimum $T = 1.3$ MPa. The R^2 for the overall regression was 0.916.

1998; Meyer & Allen 2009). In seed germination HTT modeling, it is generally not necessary to allow either of these parameters to vary as a function of incubation conditions below the optimum (Bradford 2005). For *P. semeniperda*, it was necessary to include either variable $\Psi_b(50)$ as a function of temperature or variable T_b and T_m as a function of water potential to account for their combined effects under conditions far from the optimum. These parameters varied systematically and predictably as a function of environmental conditions in their respective models (Fig 3), suggesting that there is an underlying physiological process in *P. semeniperda* conidia that accounts for these shifts. This systematic variation in base and maximum values will also make it relatively simple to incorporate their effects in a simulation modeling context.

In Model 1, the best-fit T_b was very low, well below freezing (Table 1). It is important to realize that in threshold models, the best-fit base value only applies over the range of experimental conditions included, i.e., T_b is the base temperature that best explains the slope of the linear relationship with temperature over the range 5–25 $^\circ$ C, given that $\Psi_b(50)$ is also increasing with decreasing temperature. Similarly, a T_m of 56 $^\circ$ C at optimum water potential in Model 2 only applies over the supraoptimal temperature range examined, namely 25–30 $^\circ$ C. It quantitatively incorporates the fact that the decrease in germination rate from 25 to 30 $^\circ$ C was not very great (Fig 1). Threshold values should not be interpreted to have biological meaning over a wider range, but this does not limit their usefulness in modeling these processes in variable

environments over the biologically relevant range included in this experiment.

The parameter values in each of the three conidial germination models acted in complementary fashion to approximate as closely as possible the actual germination time courses observed (Table 1, Electronic Supplement 1). Model 0 had a small θ_{HT} that compensated for relatively high constant T_b and $\Psi_b(50)$ values to give rapid germination predictions at the optimum, but this combination did a poor job of predicting germination time courses under conditions far from the optimum (Electronic Supplement 1). Models 1 and 2 had much larger θ_{HT} values that were necessary to yield realistic time courses under near-optimum conditions, given the low T_b in Model 1 and the low $\Psi_b(50)$ in Model 2, both of which would tend to generate extremely rapid germination at the optimum. This large θ_{HT} value combined with increasing $\Psi_b(50)$ as temperature decreased or increased relative to the optimum in Model 1 did a fair job of approximating germination time courses across the range of incubation conditions. Model 2, with a large θ_{HT} combined with increasing T_b and decreasing T_m as water potential decreased, provided the best fit of the three models. The σ_{Ψ_b} in Model 2 was twice as large as in the other two models, probably because constraining germination rate with higher T_b and T_m at low water potentials allowed $\Psi_b(g)$ to reach lower values than in the other two models and therefore caused it to vary over a wider range. As this is the first effort to model conidial germination using HTT, it is difficult to say which model provides the best description of actual environmental controls on conidial germination. It will require additional studies to validate these HTT models with multiple strains of *P. semeniperda* and also in other filamentous fungi.

The HTT model developed for *P. semeniperda* mycelial growth rate represents a straightforward application of HTT, as constant T_b and Ψ_b adequately accounted for growth rate variation as a function of temperature and water potential in the suboptimum range. This model represents a simple extension of continuous thermal time models used to model many physiological processes in plants, and is also not substantially different from models for fungi that use mean growth rate as the response parameter. In these models, linear regression (or a more complex model, e.g., Baranyi & Roberts 1994) is used to fit the essentially linear time courses of radial growth exhibited by filamentous fungi, in order to derive a simple parameter, growth rate, that can then be used in predictive models. Because fungi often have a Ψ_{opt} for growth that is < 0 MPa, the concept of a Ψ_m for mycelial growth used here has also been included in earlier fungal growth models (Rosso & Robinson 2001; Sautour et al. 2001a).

In contrast to HTT modeling, the general approach to modeling germination and growth in fungi has commonly involved the development of primary and secondary models (D'Antigny et al. 2005). Primary models are used to fit individual time courses using empirically derived equations (Zwietering et al. 1990; D'Antigny et al. 2007, 2011). Fitting these models generates parameter values, e.g., maximum rates for conidial germination time courses. Secondary models use these parameter values as response variables in regression equations that aim to predict the effect of one or more environmental variables (Ratkowsky et al. 1983; Davey 1989;

Zwietering et al. 1991; Rosso et al. 1993). These approaches were first used to model temperature responses in bacteria and later extended to fungi and also to water potential response (Rosso & Robinson 2001; Sautour et al. 2001b; Gougouli & Koutsoumanis 2010, 2012; Yue et al. 2011). An exception is Andersen et al. (2006), where a population-based approach was used to examine the effect of water potential on conidial germination of fungal insect pathogens. Only a handful of papers model the simultaneous effects of temperature and water potential on fungal growth or conidial germination, and most of these use a polynomial regression approach (Sautour et al. 2001b; Lahlali et al. 2005; Samapundo et al. 2005; Dagno et al. 2011; Leggieria et al. 2014).

The HTT modeling approach used in this study contrasts with these alternative modeling approaches in several important ways. First, the HTT regression analysis uses all the time course data for germination or mycelial growth directly in the final model rather than using parameter values derived from primary models. This eliminates the need for primary and secondary models, and should also lead to more accurate characterizations of the studied relationships, even though it incorporates more variance, which can lead to lower R^2 values. Second, rather than using nonlinear regression, HTT for conidial germination utilizes the probit transformation of the normal distribution, generating simple linear relationships and easily understood model parameters that potentially have physiological and ecological relevance. Third, HTT models combine water potential and temperature as independent variables without the necessity for high-order polynomial regression. Fourth, the method provides a means of calculating predicted time courses over the full range of experimental conditions, not just plotting the value of a derived dependent variable as a function of independent variables. And last, the method is easily applied in a simulation modeling framework under field-realistic fluctuating temperature and water potential conditions, as has been demonstrated for host seed germination (Meyer & Allen 2009).

This study examined the germination and growth response to water potential generated using an osmoticum (glycerol) dissolved in a highly water-conducting medium (PDA). Most of the HTT modeling for seeds is based on data sets generated using polyethylene glycol (PEG8000) water suspensions on germination blotter paper. PEG8000 can be considered to generate matric water stress rather than osmotic water stress (Ramirez et al. 2004), but the hydraulic conductivity of the medium is still high. Matric water stress in soil is much more severe than osmotic water stress at a given low water potential, probably due to reduced hydraulic conductivity (Adebayo & Harris 1971). This indicates that results from germination and growth experiments that manipulate osmotic potential may not be directly transferable to conditions in field seed beds.

Previous work has demonstrated that inoculated *B. tectorum* seeds held in PEG8000 solutions at -2 MPa became infected by *P. semeniperda*, indicating that both conidial germination and penetration are possible at matric water potentials of -2 MPa (Finch et al. 2013). Inoculated seeds held at a matric water potential of -4 MPa over a saturated salt solution following a 24-h imbibition period were quickly killed upon transfer to free water. This indicates that *P. semeniperda* can

grow inside seeds at matric water potentials as low as -4 MPa (Finch 2013). The present study supports the hypothesis that this pathogen can germinate, infect, and grow at water potentials well below those that permit seed germination. The ability of *P. semeniperda* to remain active at low water potentials explains how it can cause mortality even of potentially rapidly germinating host seeds under the fluctuating moisture conditions in field seed beds following small and intermittent autumn storms.

Conclusions

This study has demonstrated that the HTT model framework developed to describe the effects of temperature and water potential on physiological processes in seeds can also be successfully applied to germination and growth processes in an ascomycete seed pathogen, *Pyrenophora semeniperda*. It was necessary to adapt the HTT model framework in order to apply these models to germination and growth processes in a fungus. For conidial germination, the best-fit population-based threshold model utilized base and maximum temperature parameters that varied systematically as a function of incubation water potential. For mycelial growth, a continuous linear threshold model that incorporated both base and maximum water potential parameters produced a satisfactory fit. The good fit of these models will make it possible to model germination and growth processes simultaneously in host and pathogen under fluctuating field seed bed conditions. This modeling approach should lead to a clearer understanding of how environmental conditions influence disease outcomes in the *Bromus tectorum*–*Pyrenophora semeniperda* pathosystem. In addition, the successful application of HTT modeling to *P. semeniperda* suggests that this methodology could be usefully added to the extensive suite of modeling methods already available to study germination and growth processes in filamentous fungi.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.funbio.2015.04.004>.

REFERENCES

- Adebayo AA, Harris RF, 1971. Fungal growth responses to osmotic as compared to matric water potential. *Soil Science Society of America Journal* 35: 465–469.
- Allen PS, 2003. When and how many? Hydrothermal models and the prediction of seed germination. *New Phytologist* 158: 1–3.
- Allen PS, Benech-Arnold RL, Batlla D, Bradford KJ, 2007. Modeling of seed dormancy. In: Bradford KJ, Nonogaki H (eds), *Annual Plant Reviews Volume 27: Seed Development, Dormancy and Germination*. Blackwell Publishing, Oxford, UK, pp. 72–112.
- Alpert P, 2006. Constraints of tolerance: why are desiccation-tolerant organisms so small or so rare. *Journal of Experimental Biology* 209: 1575–1584.
- Alvarado V, Bradford KJ, 2002. A hydrothermal time model explains the cardinal temperatures for seed germination. *Plant, Cell and Environment* 25: 1061–1069.
- Andersen M, Magan N, Mead A, Chandler D, 2006. Development of a population-based threshold model of conidial germination for analyzing the effects of physiological manipulation on the stress tolerance and infectivity of insect pathogenic fungi. *Environmental Microbiology* 8: 1625–1634.
- Bauer MC, Meyer SE, Allen PS, 1998. A simulation model to predict seed dormancy loss in the field for *Bromus tectorum* L. *Journal of Experimental Botany* 49: 1235–1244.
- Bair NB, Meyer SE, Allen PS, 2006. A hydrothermal after-ripening time model for seed dormancy loss in *Bromus tectorum* L. *Seed Science Research* 16: 17–28.
- Baranyi J, Roberts TA, 1994. A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology* 23: 277–294.
- Beckstead J, Meyer SE, Reinhart KO, Bergen KM, Holden SR, Boekweg HF, 2014. Factors affecting host range in a generalist seed pathogen of semi-arid shrublands. *Plant Ecology* 215: 427–440.
- Bradford KJ, 1990. A water relations analysis of seed germination rates. *Plant Physiology* 94: 840–849.
- Bradford KJ, 2002. Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Science* 50: 248–260.
- Bradford KJ, 2005. Threshold models applied to seed germination ecology. *New Phytologist* 165: 338–341.
- Christensen M, Meyer SE, Allen PS, 1996. A hydrothermal time model of seed after-ripening in *Bromus tectorum* L. *Seed Science Research* 6: 155–163.
- Covell S, Ellis RH, Roberts EH, Summerfield RJ, 1986. The influence of temperature on seed germination rate in grain legumes I. A comparison of chickpea, lentil, soyabean and cowpea at constant temperatures. *Journal of Experimental Botany* 37: 705–715.
- Dagno K, Lahlali R, Diourte M, Jijakli MH, 2011. Effect of temperature and water activity on spore germination and mycelial growth of three fungal biocontrol agents against water hyacinth (*Eichhornia crassipes*). *Journal of Applied Microbiology* 110: 521–528.
- Dallyn H, 1978. *The Effect of the Substrate Water Activity on the Growth of Certain Xerophilic Fungi* PhD dissertation. Polytechnic of the South Bank, London, United Kingdom.
- D'Antigny P, Guilmar A, Bensoussan M, 2005. Basis of predictive mycology. *International Journal of Food Microbiology* 100: 187–196.
- D'Antigny P, Marín S, Beyer M, Magan N, 2007. Mould germination: data treatment and modelling. *International Journal of Food Microbiology* 114: 17–24.
- D'Antigny P, Nanguy SPM, Judet-Correia D, Bensoussan M, 2011. A new model for germination of fungi. *International Journal of Food Microbiology* 146: 176–181.

- Davey KR, 1989. A predictive model for combined temperature and water activity on microbial growth during the growth phase. *Journal of Applied Bacteriology* **67**: 483–488.
- Finch H, 2013. *The Bromus Tectorum-Pyrenophora Semeniperda Pathosystem* M.S. thesis. Brigham Young University, Provo, Utah, USA.
- Finch H, Allen PS, Meyer SE, 2013. Environmental factors influencing *Pyrenophora semeniperda*-caused seed mortality in *Bromus tectorum*. *Seed Science Research* **23**: 57–66.
- García-Huidobro J, Monteith JL, Squire GR, 1982. Time, temperature and germination of pearl millet (*Pennisetum typhoides* S. & H.) I. Constant temperature. *Journal of Experimental Botany* **33**: 288–296.
- Gougouli M, Koutsoumanis KP, 2010. Modelling growth of *Penicillium expansum* and *Aspergillus niger* at constant and fluctuating temperature conditions. *International Journal of Food Microbiology* **140**: 254–262.
- Gougouli M, Koutsoumanis KP, 2012. Modeling germination of fungal spores at constant and fluctuating temperature conditions. *International Journal of Food Microbiology* **152**: 153–161.
- Gummerson RJ, 1986. The effect of constant temperatures and osmotic potentials on the germination of sugar beet. *Journal of Experimental Botany* **37**: 729–741.
- Lahlali R, Serrhini MN, Jijakli MH, 2005. Studying and modelling the combined effect of temperature and water activity on the growth rate of *P. expansum*. *International Journal of Food Microbiology* **103**: 315–322.
- Leggieria MC, Mitchell D, Aldred D, Battilana P, Magan N, 2014. Hydro- and thermotimes for conidial germination kinetics of the ochratoxigenic species *Aspergillus carbonarius* in vitro, on grape skin and grape flesh. *Fungal Biology* **118**: 996–1003. <http://dx.doi.org/10.1016/j.funbio.2014.09.005>.
- Marín S, Sanchis V, Magan N, 1995. Water activity, temperature, and pH effects on growth of *Fusarium moniliforme* and *Fusarium proliferatum* isolates from maize. *Canadian Journal of Microbiology* **41**: 1063–1070.
- Marín S, Sanchis V, Teixidó A, Saenz R, Ramos AJ, Vinas I, Magan N, 1996. Water and temperature relations and microconidial germination of *Fusarium moniliforme* and *Fusarium proliferatum* from maize. *Canadian Journal of Microbiology* **42**: 1045–1050.
- Meyer SE, Allen PS, 2009. Predicting seed dormancy loss and germination timing for *Bromus tectorum* in a semi-arid environment using hydrothermal time models. *Seed Science Research* **19**: 225–239.
- Meyer SE, Debaene-Gill SB, Allen PS, 2000. Using hydrothermal time concepts to model seed germination response to temperature, dormancy loss, and priming effects in *Elymus elymoides*. *Seed Science Research* **10**: 213–223.
- Meyer SE, Merrill KT, Allen PS, Beckstead J, Norte AS, 2014. Indirect effects of an invasive annual grass on seed fates of two native perennial grass species. *Oecologia* **174**: 1401–1413.
- Meyer SE, Stewart T, Clement S, 2010. The quick and the deadly: growth vs virulence in a seed bank pathogen. *New Phytologist* **187**: 209–216.
- Ramos A, Labernia N, Mann S, Sanchis V, Magan N, 1998. Effect of water activity and temperature on growth and ochratoxin production by three strains of *Aspergillus ochraceus* on a barley extract medium and on barley grains. *International Journal of Food Microbiology* **44**: 133–140.
- Ramirez ML, Chulze SN, Magan N, 2004. Impact of osmotic and matric water stress on germination, growth, mycelial water potentials and endogenous accumulation of sugars and sugar alcohols in *Fusarium graminearum*. *Mycologia* **96**: 470–478.
- Ratkowsky DA, Lowry RK, McMeekin TA, Stokes AN, Chandler RE, 1983. Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *Journal of Bacteriology* **154**: 1222–1226.
- Rosso L, Lobry JR, Flandrois JP, 1993. An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. *Journal of Theoretical Biology* **162**: 447–463.
- Rosso L, Robinson TP, 2001. A cardinal model to describe the effect of water activity on the growth of moulds. *International Journal of Food Microbiology* **63**: 265–273.
- Samapundo S, Devlieghere F, De Meulenaer B, Geeraerd AH, Van Impe JF, Debevere JM, 2005. Predictive modelling of the individual and combined effect of water activity and temperature on the radial growth of *Fusarium verticillioides* and *F. proliferatum* on corn. *International Journal of Food Microbiology* **105**: 35–52.
- Sautour M, D'Antigny P, Divies C, Bensoussan M, 2001a. A temperature-type model for describing the relationship between fungal growth and water activity. *International Journal of Food Microbiology* **67**: 63–69.
- Sautour M, Rouget A, D'Antigny P, Divies C, Bensoussan M, 2001b. Prediction of conidial germination of *Penicillium chrysogenum* as influenced by temperature, water activity and pH. *Letters in Applied Microbiology* **32**: 131–134.
- Torres MR, Ramos AJ, Soler J, Sanchis V, Marín S, 2003. SEM study of water activity and temperature effects on the initial growth of *Aspergillus ochraceus*, *Alternaria alternata* and *Fusarium verticillioides* on maize grain. *International Journal of Food Microbiology* **81**: 185–193.
- Watt MS, Xu V, Bloomberg M, 2010. Development of a hydrothermal time seed germination model which uses the Weibull distribution to describe base water potential. *Ecological Modelling* **221**: 1267–1272.
- Yue X, Sui J, Niu T, Liu Y, Liu X, 2011. Modeling the effect of temperature and water activity on the growth rate and lag phase of *Aspergillus flavus* during rice drying. *Drying Technology* **29**: 1306–1312.
- Zwietering MH, Jongenburger I, Rombouts FM, Van't Riet K, 1990. Modeling of the bacterial growth curve. *Applied and Environmental Microbiology* **56**: 1875–1881.
- Zwietering MH, De Koos JT, Hasenack BE, De Witt JC, Van't Riet K, 1991. Modeling of bacterial growth as a function of temperature. *Applied and Environmental Microbiology* **57**: 1094–1101.