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# 38 Hydrothermal Time as a Tool in Comparative Germination Studies

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**Hydrothermal time (HTT) analysis is an effective method for quantifying germination response to temperature and water potential. Time course curves under any temperature and water potential conditions can be generated from knowledge of the HTT parameters mean base water potential, standard deviation of base water potentials, base temperature, and hydrothermal time requirement. Here we use these parameters as indices for making comparisons among 24 species including shrubs, grasses and perennial herbs from the deserts and semi-deserts of Asia and North America. Halophytes (salt-tolerant species) are characterized by low base water potential values but high hydrothermal time constants. Psammophytes (species that inhabit high-sand soils) are just the opposite, and bodenvags (generalist species with no special soil requirements) display a wide range in all parameter values. Variation in the distribution of base water potentials strongly influences uniformity of germination. The results illustrate that germination rate in water or at reduced water potentials is closely associated with HTT parameters. These findings also have important ecological relevance, in that they help explain differences in germination patterns associated with contrasting habitats.**

## Introduction

Temperature and water potential conditions strongly influence seed germination. Hydrothermal time (equation 38.1) describes progress toward seed germination under various combinations of incubation water potential and temperature:

$$\theta_{\text{HTT}} = (\Psi - \Psi_b(g))(T - T_b)t_g \quad (38.1)$$

where  $\theta_{\text{HTT}}$  is the hydrothermal time required for germination (e.g. MPa-degree-days),  $\Psi$  and  $T$  are the water potential and temperature of the incubation

medium,  $T_b$  is the base temperature at or below which germination will not occur,  $\Psi_b(g)$  is the base water potential at or below which germination of the  $g$  fraction will not occur, and  $t_g$  is the time requirement for germination of fraction  $g$ .

Rearranging equation 1 and using a value of  $g = 50$  illustrates that mean germination rate ( $1/t_{50}$ ) is directly proportional to the differences between  $(\Psi - \Psi_b(50))$  and  $(T - T_b)$ , but is inversely proportional to  $\theta_{HT}$ :

$$\frac{1}{t_{50}} = \frac{(\Psi - \Psi_b(50))(T - T_b)}{\theta_{HT}} \quad (38.2)$$

Increases in  $\theta_{HT}$ ,  $T_b$ , and  $\Psi_b(50)$  all reduce germination rate, while increases in  $\Psi$  (i.e. a less negative number) and  $T$  increase germination rate. Distribution of  $\Psi_b(g)$  is assumed to be approximately normal, with a mean base water potential ( $\Psi_b(50)$ ) below which half of the seeds will not germinate and a standard deviation  $\sigma_{\Psi_b}$ . This normal distribution of  $\Psi_b(g)$  generates the characteristic inverse normal distribution of  $t(g)$ 's observed in germination time courses.

Hydrothermal time was first used to describe the germination rate for sugarbeet (Gummerson, 1986), but has since been applied to tomato (Dahal and Bradford, 1994; Cheng and Bradford, 1999). We have recently used hydrothermal time as a tool in predicting dormancy loss that occurs during dry after-ripening, under both laboratory and field conditions (Christensen *et al.*, 1996; Allen and Meyer, 1998; Bauer *et al.*, 1998). Changes in germination time course curves as seeds afterripen are associated with a progressive decrease in  $\Psi_b(50)$ .

While hydrothermal time and the related hydrotime modelling approaches have received increased attention during the past decade (Bradford 1990, 1995; Finch-Savage *et al.*, 1998), relatively few species have been subjected to HTT analysis. In order to examine the suggestion that hydrothermal time theory has ecological significance as well as an underlying physiological basis, and is not just another way of empirically fitting germination time course data, we here present results of hydrothermal time analysis for 24 different species representing a range of growth habits and ecological habitats. This study had three specific objectives: (i) to evaluate hydrothermal time application across multiple species; (ii) to use hydrothermal time parameters to make comparisons among species; and (iii) to determine whether species with similar germination rates in water have similar hydrothermal time parameters.

## Methods

The 24 species included in this study were grouped into three ecological classifications (Table 38.1). Halophytes are salt-tolerant species and were included because they germinate at low water potential ( $\Psi$ ). Psammophytes are species that inhabit high-sand soils. Such soils are characterized by poor water retention and seeds of psammophytes frequently encounter rapid soil drying (i.e. widely fluctuating  $\Psi$ ). Bodenvags represent generalist species with no specific soil type requirements.

**Table 38.1.** Descriptive information for 24 species included in this study.

Species	Family	Habit	Habitat	Distribution
Halophytes				
<i>Arthrocnemum indicum</i>	Chenopod	Succulent	Salt marsh	Old World tropics
<i>Suaeda fruticosa</i>	Chenopod	Succulent	Salt desert	Old World
<i>Salicornia utahensis</i>	Chenopod	Succulent	Salt desert	Temperate western US
<i>Triglochin maritima</i>	Arrowgrass	Perennial	Salt marsh	Temperate western US
<i>Atriplex triangularis</i>	Chenopod	Perennial	Salt marsh	North America
<i>Polygonum aviculare</i>	Buckwheat	Annual	Wide range	Cosmopolitan
Psammophytes				
<i>Asclepias tuberosa</i>	Milkweed	Perennial	Sandhills	Temperate western US
<i>Artemisia cana</i>	Aster	Shrub	Sandhills	Temperate western US
<i>Eriogonum alatum</i>	Buckwheat	Perennial	Sand desert	Temperate western US
<i>Heterotheca villosa</i>	Aster	Perennial	Sand desert	Temperate western US
<i>Arabis pulchra</i>	Crucifer	Perennial	Sand desert	Southwestern US
<i>Stipa arida</i>	Grass	Perennial	Sand desert	Southwestern US
<i>Hymenoxys scaposus</i>	Aster	Perennial	Sand desert	Western US
Bodenvags				
<i>Kochia prostrata</i>	Chenopod	Shrub	Cold desert	Central Asia
<i>Poa secunda</i>	Grass	Perennial	Cold desert	Western US
<i>Carrichtera annua</i>	Crucifer	Annual	Warm desert	Mediterranean
<i>Elymus elymoides</i>	Grass	Perennial	Cold desert	Western US
<i>Asclepias asperula</i>	Milkweed	Perennial	Cold desert	Western US
<i>Ceratoides lanata</i>	Chenopod	Shrub	Cold desert	Western US
<i>Bromus tectorum</i>	Grass	Annual	Cold desert	Cosmopolitan
<i>Brachypodium distachyon</i>	Grass	Annual	Warm desert	Mediterranean
<i>Bromus fasciculatus</i>	Grass	Annual	Warm desert	Mediterranean
<i>Stipa capensis</i>	Grass	Perennial	Warm desert	Mediterranean
<i>Ephedra nevadensis</i>	Ephedra	Shrub	Cold desert	Western US

All seeds included were non-dormant at the time studies were conducted. Seeds of several species required dry afterripening to relieve primary dormancy. Germination data for halophytes are from previously published work (Khan and Ungar, 1984, 1997, 2000a,b; Khan and Weber, 1986; Khan and Gul, 1998). For these species, germination at reduced water potentials was achieved by imbibing seeds in liquid contact with water or solutions of sodium chloride as described in the original publications. Germination experiments for all other species involved imbibing seeds on germination blotters saturated with water or polyethylene glycol solutions at a range of  $\Psi$  values (Christensen *et al.*, 1996; Bauer *et al.*, 1998).

Germination time course data for each species were analysed by repeated probit regression to calculate values for  $\theta_{HT}$ ,  $\Psi_b(50)$ ,  $T_b$  and  $\sigma_{\Psi_b}(50)$  (the standard deviation of mean base water potentials, which is important in applying hydrothermal time to seed populations). This approach is described in detail by Christensen *et al.* (1996) and Bauer *et al.* (1998), based on earlier work by Ellis *et al.* (1986), Gummerson (1986) and Bradford (1990, 1995). The only

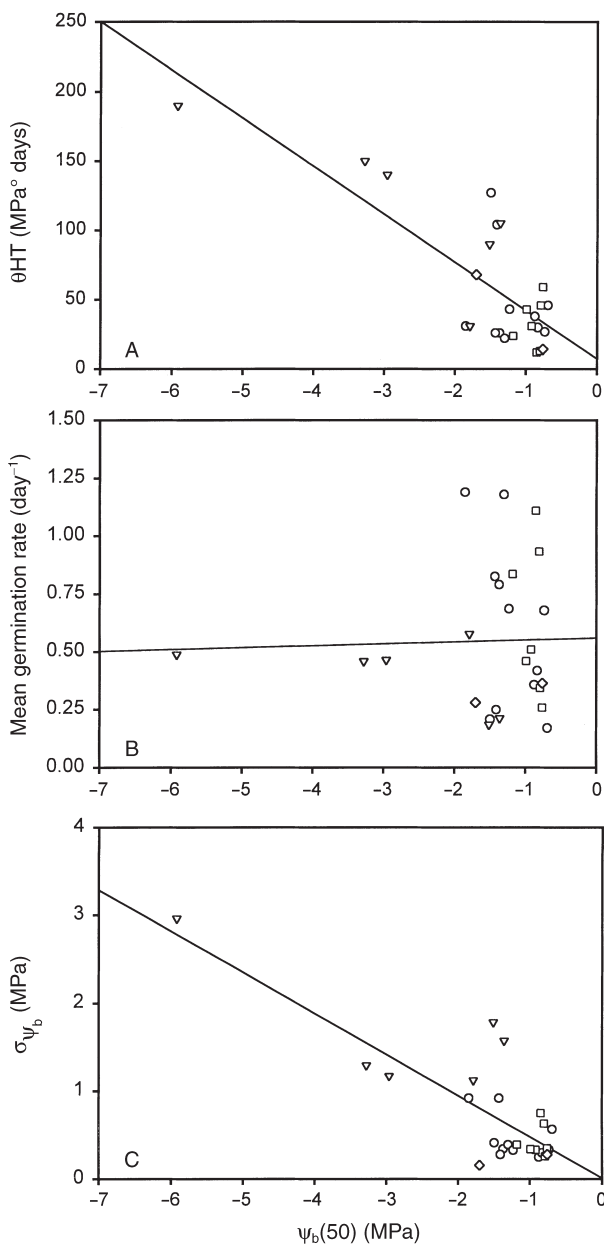
modification from our earlier procedure was that  $T_b$  was also allowed to vary until the best model fit (highest  $R^2$ ) was obtained, as outlined in Dahal and Bradford (1994).

## Results and Discussion

Species varied considerably in  $T_b$  (Table 38.2), reflecting the range of ecological habitats represented in this study (Table 38.1). As expected, tropical and sub-tropical species had high  $T_b$  values; most temperate species had  $T_b$  values near 0°C. Values for  $\theta_{HT}$ ,  $\Psi_b(50)$ ,  $\sigma_{\Psi_b}$  and mean germination rate also showed wide variation, sometimes with greater than a 10-fold increase from the lowest to the highest value obtained. In spite of these widely differing parameter values, the hydrothermal time model generally fit germination time course data quite well. When all probit-transformed germination data for a given species were regressed on  $\Psi_b(g)$ , only four species had  $R^2$  values less than 0.7. Otherwise,  $R^2$  values were similar to those reported for tomato (e.g. Cheng and Bradford, 1999) and sugarbeet (Gummerson, 1986). For those species with low  $R^2$  values, the model fit was improved if a separate hydrothermal time equation was fitted for each incubation temperature (data not shown), as was observed with tomato (Dahal *et al.*, 1993).

Several generalizations regarding hydrothermal time parameters can be made for each ecological class (Table 38.2, Fig. 38.1). Values for  $\Psi_b(50)$  and  $\theta_{HT}$  were negatively correlated, largely due to the extreme values for halophytes (Fig. 38.1A). Halophytes were characterized by low  $\Psi_b(50)$  values, with the extreme observation of -5.9 MPa for *Arthrocnemum indicum*. Halophytes also tended to have very large  $\theta_{HT}$  and high  $\sigma_{\Psi_b}$  values, which resulted in a greater spread of germination times in water or at reduced water potentials (Bradford, 1990). Psammophytes were characterized by high  $\Psi_b(50)$  and low  $\theta_{HT}$  values and intermediate to low  $\sigma_{\Psi_b}$  values. This is of considerable ecological significance, as seeds of these species have a rapid germination rate in water but are strongly inhibited from germinating at reduced water potentials. This reduces risk of germinating into the rapidly drying seedzone environments characteristic of sandy soils. Bodenvags displayed a range of  $\Psi_b(50)$  values, generally intermediate between the halophytes and psammophytes. The lowest  $\Psi_b(50)$  for any bodenvag was -1.85 MPa (*Kochia prostrata*), as compared to -1.18 MPa, the minimum value for a psammophyte (*Asclepias tuberosa*). Values of  $\theta_{HT}$  for bodenvags were distributed nearly across the entire range of values for all species. Data for two crop species, sugarbeet (Gummerson, 1986) and tomato (Cheng and Bradford, 1999), are included in Fig. 38.1 for comparison. Sugarbeet (diamond on regression line in Fig. 38.1A) had a  $\Psi_b(50)$  of nearly -2 MPa, reflecting the halophytic origin of this species. In contrast, tomato (diamond near origin) has both a low  $\Psi_b(50)$  and a low  $\theta_{HT}$ . Note that both species have similar mean germination rates (Fig. 38.1B).

There was essentially no relationship between  $\Psi_b(50)$  and mean germination rate (Fig. 38.1B). A low  $\Psi_b(50)$ , which would result in a greater



**Fig. 38.1.** Relationships between hydrothermal time parameters. (A) Relationship between mean base water potential ( $\Psi_b(50)$ ) and hydrothermal time requirement ( $\theta_{HT}$ ). (B) Relationship between mean base water potential ( $\Psi_b(50)$ ) and mean germination rate ( $1/t_{50}$ ). (C) Relationship between mean base water potential ( $\Psi_b(50)$ ) and standard deviation of base water potentials ( $\sigma_{\psi_b}$ ). Data for crop species include previously published values for sugarbeet (Gummerson, 1986) and tomato (Cheng and Bradford, 1999).  $\circ$ , Bodenvags;  $\nabla$ , halophytes;  $\square$ , psammophytes;  $\diamond$ , crops.

**Table 38.2.** Hydrothermal time parameters and mean germination rates for 24 species representing halophytes (salt tolerant), psammophytes (high-sand soils), and bodenvags (no special soil requirements).

	$T_b$	$\theta_{HT}$	$\sigma_{vb}$	$R^2$	$\Psi_b(50)$						Mean germination rate													
					10	15	20	25	30	10	15	20	25	30										
<b>Halophytes</b>																								
<i>A. indicum</i>	9	190	2.96	0.850	–	–5.92	–5.12	–	–	–4.42	–	0.19	0.30	–	0.49									
<i>S. fruticosa</i>	9	150	1.29	0.774	–	–3.28	–3.28	–	–	–3.28	–	0.13	0.24	–	0.46									
<i>S. utahensis</i>	8	140	1.17	0.756	–	–2.96	–2.96	–	–	–2.96	–	0.14	0.25	–	0.46									
<i>T. maritima</i>	9	90	1.78	0.770	–	–1.51	–1.51	–	–	–	–	0.10	0.18	–	–									
<i>A. triangularis</i>	3	105	1.57	0.688	–1.36	–1.21	–	–1.01	–	–	0.09	0.14	–	0.21	–									
<i>P. aviculare</i>	0	31	1.12	0.745	–1.79	–0.94	–0.24	–	–	–	0.58	0.45	0.16	–	–									
<b>Psammophytes</b>																								
<i>A. tuberosa</i>	8	24	0.39	0.544	–	–0.75	–	–1.18	–	–	–	0.22	–	0.84	–									
<i>A. cana</i>	0	43	0.34	0.708	–0.86	–	–0.99	–	–	–	0.20	–	0.46	–	–									
<i>E. alatum</i>	3	31	0.33	0.764	–0.62	–0.92	–0.82	–0.72	–	–	0.14	0.36	0.45	0.51	–									
<i>H. villosa</i>	1	12	0.75	0.746	–0.75	–0.85	–0.70	–0.50	–	–	0.56	0.99	1.11	1.00	–									
<i>A. pulchra</i>	0	13	0.63	0.432	–	–0.80	–	–0.48	–	–	–	0.93	–	0.93	–									
<i>S. arida</i>	0	46	0.26	0.883	–0.79	–	–0.79	–	–	–	0.17	–	0.34	–	–									
<i>H. scaposus</i>	0	59	0.35	0.823	–0.51	–	–0.76	–	–	–	0.09	–	0.26	–	–									

Bodenvags													
<i>K. prostrata</i>	0	31	0.92	0.856	-1.50	-	-1.85	-	-	0.48	-	1.19	-
<i>P. secunda</i>	0	127	0.41	0.773	-1.49	-	-1.31	-	-	0.12	-	0.21	-
<i>C. annua</i>	0	26	0.92	0.975	-	-1.43	-	+0.97	-	-	0.83	-	nil
<i>E. elymoides</i>	0	104	0.28	0.952	-	-	-1.41	-	-0.88	-	-	0.25	-
<i>A. asperula</i>	10	26	0.34	0.480	nil	-0.95	-1.17	-1.37	-	nil	0.18	0.45	0.79
<i>C. lanata</i>	0	22	0.39	0.900	-1.30	-	-1.30	-	-	0.59	-	1.18	-
<i>B. tectorum</i>	0	43	0.33	0.829	-	-1.23	-	-1.18	-	-	0.43	-	0.69
<i>B. distachyon</i>	0	38	0.25	0.897	-	-0.87	-	-0.54	-	-	0.34	-	0.36
<i>B. fasciculatus</i>	0	30	0.30	0.815	-	-0.83	-	-0.50	-	-	0.42	-	0.42
<i>S. capensis</i>	0	27	0.33	0.831	-	-0.73	-	-0.73	-	-	0.41	-	0.68
<i>E. nevadensis</i>	0	46	0.56	0.773	-0.69	-	-0.39	-	-	0.15	-	0.17	-

Model parameters were derived from repeated regression analyses using estimated base water potential as the independent variable and the probit-transformed germination fraction as the dependent variable.

accumulation rate for hydrothermal time, was typically offset by a high  $\theta_{HT}$ . For example, the four halophytes with the lowest base water potentials had similar mean germination rates (approximately 0.5). Psammophytes tended to have closely similar base water potentials (Table 38.2); the variation in mean germination rates among these species was largely a function of variation in  $\theta_{HT}$ . Bodenvags with rapid germination rates typically had a low  $\Psi_b(50)$  or a low  $\theta_{HT}$ , but usually not both.

A weak relationship existed between  $\Psi_b(50)$  and  $\sigma_{\Psi_b}$ , primarily due to the large  $\sigma_{\Psi_b}$  values of halophytes (Fig. 38.1C). Psammophytes and bodenvags had low to intermediate  $\sigma_{\Psi_b}$ . While  $\sigma_{\Psi_b}$  does not affect mean germination rate, an increase in  $\sigma_{\Psi_b}$  leads to an increased germination rate for all fractions faster than the mean and a decreased germination rate for all fractions slower than the mean.

A distinct advantage of hydrothermal time analysis for seeds incubated across a range of  $T$  and  $\Psi$  conditions is that variation or similarity in germination rate can be ascribed to specific underlying factors. For example, a fast germination rate in water may be due either to a low hydrothermal time requirement or a low base water potential. By incubating seeds at various water potentials, the relative importance of  $\Psi_b(50)$  and  $\theta_{HT}$  can be evaluated.

The number of incubation temperatures included in this study ranged from two to four for a particular species. Variation in  $\Psi_b(50)$  associated with incubation temperature produced distinct patterns (Table 38.2). First, for some species there was no difference in  $\Psi_b(50)$  across the range of incubation temperatures tested (e.g. *Suaeda fruticosa* and *Salicornia pacifica*). For seeds of most species,  $\Psi_b(50)$  increased with higher incubation temperature (e.g. *Arthrocnemum indicum* and *Bromus* spp.). In still other cases a higher incubation temperature corresponded to a lower  $\Psi_b(50)$  (e.g. *Hymenoxys scaposus* and *Kochia prostrata*). We believe there is an underlying explanation for these patterns that would be more evident if species had been incubated across the entire range of germination-permissive temperatures. Our evidence suggests that there is a range of temperatures over which  $\Psi_b(50)$  remains constant. Above that range, it increases linearly with temperature until the maximum (no germination). As incubation temperature approaches  $T_b$ ,  $\Psi_b(50)$  also appears to increase. This is most evident for species that were incubated at four temperatures (e.g. *Eriogonum alatum* and *Heterotheca villosa*), but is also supported by other studies in which application of hydrothermal time resulted in a poor fit at incubation temperatures near  $T_b$  unless  $\Psi_b(50)$  was allowed to increase (S.E. Meyer and P.S. Allen, unpublished).

Temperature sensitivity varied considerably among species. Strongly temperature-dependent  $\Psi_b(50)$  values may or may not result in a similar degree of variation in germination rate. For example, *Elymus elymoides* had a  $\Psi_b(50)$  that progressively increased with incubation temperature (Table 38.2 and S.E. Meyer and P.S. Allen, unpublished). This offsets the increased hydrothermal time accumulation at increased incubation temperature and results in a germination rate that is nearly constant at incubation temperatures from 10 to 30°C. In contrast, the increasing  $\Psi_b(50)$  with increasing temperature in *Arthrocnemum indicum* seeds is not great enough to offset the increased



germination rate at higher incubation temperatures. With seeds of *Polygonum aviculare*, the increase in  $\Psi_b(50)$  associated with increased incubation temperature is so large that mean germination rate decreases with increasing temperature.

Many simplifying assumptions associated with hydrothermal time theory were applied herein (e.g. constant  $\theta_{HT}$ ,  $T_b$ , and  $\sigma_{\Psi_b}$  across combinations of incubation  $\Psi$  and  $T$ ), assumptions which occasionally have been shown to reduce reliability of model predictions (see Bradford, 1995). In spite of these limitations, hydrothermal time appears to provide a modelling approach that can be used to explain germination data across numerous experimental conditions and species.

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