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Article

A Modified Thermal Time Model Quantifying Germination Response to Temperature for C_3 and C_4 Species in Temperate Grassland

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Abstract: Thermal-based germination models are widely used to predict germination rate and germination timing of plants. However, comparison of model parameters between large numbers of species is rare. In this study, seeds of 27 species including 12 C_4 and 15 C_3 species were germinated at a range of constant temperatures from 5 °C to 40 °C. We used a modified thermal time model to calculate germination parameters at suboptimal temperatures. Generally, the optimal germination temperature was higher for C_4 species than for C_3 species. The thermal time constant for the 50% germination percentile was significantly higher for C_3 than C_4 species. The thermal time constant of perennials was significantly higher than that of annuals. However, differences in base temperatures were not significant between C_3 and C_4, or annuals and perennial species. The relationship between germination rate and seed mass depended on plant functional type and temperature, while the base temperature and thermal time constant of C_3 and C_4 species exhibited no significant relationship with seed mass. The results illustrate differences in germination characteristics between C_3 and C_4 species. Seed mass does not affect germination parameters, plant life cycle matters, however.

Keywords: germination rate; base temperature; thermal time constant; seed size

1. Introduction

Temperature not only affects seed formation and development, but also influences seed germination and seedling establishment [1,2]. Fastest germination usually occurs at optimal temperatures [3] or over an optimal temperature range [4]. Seeds germinate at lower percentages and rates at temperatures lower or higher than the optimum [5]. Extreme high temperature will kill seeds [6], while extreme low temperature impedes the start of germination-physiological processes [7].

The rate of germination (defined as the reciprocal of the time taken for 50% seeds to germinate) usually increases linearly with temperature in the suboptimal range and then decreases linearly [8–10]. Garcia-Huidobro *et al.* [11] developed a linear thermal time model (TT model) to calculate the cardinal temperatures and the thermal time constant at suboptimal ($\theta_1(g)$) and supraoptimal temperatures ($\theta_2(g)$) of different subpopulations (germination fractions/percentiles) g in a seed lot. The two equations are:

$$GR_g = 1/t_g = (T - T_b(g)) / \theta_1(g) \quad T < T_o \tag{1}$$

$$GR_g = 1/t_g = (T_c(g) - T) / \theta_2(g) \quad T > T_o \tag{2}$$

For any given subpopulation, germination rate can be described by two straight lines. The slopes of the two lines are $\theta_1(g)$ and $\theta_2(g)$ with the intersection of the two lines defined as T_o. The two points where germination percentages equal zero were defined as the base, $T_b(g)$, and maximal temperature, $T_c(g)$, respectively [11].

Recently, we showed that for ryegrass and tall fescue species, germination rate was not significantly different over an optimal temperature range, thus we proposed a modified thermal time model (MTT model), with equations as follows [4]:

$$GR_g = 1/t_g = (T - T_b(g)) / \theta_1(g) \quad T < T_{ol}(g) \tag{3}$$

$$GR_g = 1/t_g = \mathrm{K} \quad T_{ol}(g) \le T \le T_{ou}(g) \tag{4}$$

$$GR_g = 1/t_g = (T_c(g) - T) / \theta_2(g) \quad T > T_{ou}(g) \tag{5}$$

Where T_{ol} is the lower limit of the optimum temperature range and T_{ou} is the upper limit of the optimum temperature range. Different subpopulations in a seed population may have different T_{ol} and T_{ou} values. K is the average value of T_{ol} to T_{ou} for a given subpopulation.

The base temperature and thermal time constant in the model have great significance, and can be used to compare germination timing between different species or for the same species in different habitats or climatic conditions [12,13]. However, most studies use the thermal time model to investigate intraspecific variation of germination or differences between several species [3,14–16]. However, comparison of thermal time model parameters between large numbers of species and between different functional groups is lacking [17,18]. Knowing and comparing the base temperature and thermal time constant at the species level can increase our ability to predict species distribution shift under climate change. It may also provide useful information for plant breeding purposes.

It is widely accepted that high temperature favours C_4 species while low temperature favours C_3 species. Physiological models predict that the C_3 *vs.* C_4 crossover temperature of net assimilation rates (*i.e.*, the temperature above which C_4 plants have higher net assimilation rates than C_3 plants) is approximately 22 °C [19]. However, it remains unclear whether there are significant differences in germination base temperature and thermal time constant between the two groups.

Seed mass is one of the most important functional traits, which affects many aspects of species' regeneration processes [20], including germination. Compared with small seeded species, large seeded species generally germinate better under drought [21], shade [22] and salt conditions [23]. The relationship between seed mass and thermal time parameters has not been tested.

In this study, we used the modified thermal time model to calculate the base temperatures and thermal time constants of different C_3 and C_4 species in the Songnen grassland. We had two main objectives: (1) to compare the difference of germination response and model parameters between C_3 and C_4 species; (2) to test the relationship between model parameters and seed size within the two group species.

2. Materials and Methods

2.1. Plant Materials and Habitats

Twenty seven species were used in this study, among which *Plantago asiatica*, *Saussurea glomerata*, *Lactuca indica*, *Cynanchum sibiricum*, *Dracocephalum moldavica*, *Cynanchum chinense*, *Allium odorum*, *Convolvulus arvensis*, *Pharbitis purpurea*, *Bidens parviflora*, *Achillea mongolica*, *Potentilla chinensis*, *Stipa baicalensis*, *Lappula echinata*, *Incarvillea sinensis* were C_3 species, *Kochia prostrate*, *Artemisia anethifolia*, *Salsola collina*, *Portulaca oleracea*, *Setaria viridis*, *Chenopodium album*, *Amaranthus retroflexus*, *Amaranthus blitoides*, *Chloris virgata*, *Eriochloa villosa*, *Euphorbia humifusa*, *Echinochloa crusgalli* were C_4 species [24,25]. Species information was given in Table 1. Mature seeds were collected in autumn from wild populations in Changling, Jilin Province of China. The seeds were stored in cloth bags in a fridge at 4 °C until used. Mean seed mass was calculated by weighing 30 seeds of each species on a microbalance, with five replicates.

2.2. Germination Tests

The experiments were conducted in programmed chambers (HPG-400HX; Harbin Donglian Electronic and Technology Co. Ltd., Harbin, China) under a 12-h light/12-h dark photoperiod, with light at approximately 200 $\mu mol \cdot m^{-2} s^{-1}$ supplied by cool white fluorescent lamps (Sylvania). Eight constant temperature treatments from 5 °C to 40 °C at 5 °C intervals were set in different chambers. There were four replicates at each temperature. For each replicate, 100 seeds were germinated on two layers of filter paper in Petri dishes (10 cm in diameter). The filter paper was kept moistened with distilled water. Seeds were considered to have germinated when the radicle emerged, and germinated seeds were removed. Germination was recorded every 8 h in the first week, every 12 h in the second week and then once a day as germination rates decreased. Germination tests were terminated when no seeds had germinated for 3 consecutive days.

Table 1. Photosynthetic-type (P), family, life cycle, single seed weight (calculated from 30 seeds, $n = 5$) of 27 wild species in this study.

P	Species	Family	Life Cycle	Seed Weight (mg)
C_4	*Kochia prostrata*	Amaranthaceae	Annual	0.762 ± 0.013
	Chenopodium album	Amaranthaceae	Annual	0.579 ± 0.006
	Salsola collina	Amaranthaceae	Annual	1.632 ± 0.064
	Amaranthus blitoides	Amaranthaceae	Annual	0.965 ± 0.019
	Amaranthus retroflexus	Amaranthaceae	Annual	0.502 ± 0.006
	Setaria viridis	Poaceae	Annual	0.815 ± 0.007
	Chloris virgata	Poaceae	Annual	0.629 ± 0.025
	Echinochloa crusgalli	Poaceae	Annual	1.836 ± 0.028
	Eriochloa villosa	Poaceae	Annual	3.549 ± 0.353
	Portulaca oleracea	Portulacaceae	Annual	0.134 ± 0.003
	Euphorbia humifusa	Euphorbiaceae	Annual	0.434 ± 0.007
	Artemisia anethifolia	Compositae	Biennial	1.019 ± 0.012
C_3	*Lappula echinata*	Boraginaceae	Annual	2.170 ± 0.052
	Incarvillea sinensis	Bignoniaceae	Annual	0.660 ± 0.010
	Dracocephalum moldavica	Labiatae	Annual	1.892 ± 0.031
	Bidens parviflora	Compositae	Annual	5.530 ± 0.139
	Saussurea glomerata	Compositae	Perennial	2.843 ± 0.077
	Lactuca indica	Compositae	Perennial	1.031 ± 0.028
	Achillea mongolica	Compositae	Perennial	0.030 ± 0.001
	Allium odorum	Liliaceae	Perennial	2.187 ± 0.017
	Convolvulus arvensis	Convolvulaceae	Perennial	31.82 ± 0.131
	Pharbitis purpurea	Convolvulaceae	Perennial	28.55 ± 0.442
	Cynanchum sibiricum	Asclepiadaceae	Perennial	5.973 ± 0.124
	Cynanchum chinense	Asclepiadaceae	Perennial	4.217 ± 0.070
	Potentilla chinensis	Rosaceae	Perennial	0.411 ± 0.010
	Stipa baicalensis	Poaceae	Perennial	7.980 ± 0.194
	Plantago asiatica	Plantaginaceae	Perennial	0.229 ± 0.002

2.3. Data Analysis

Germination data were arcsine transformed before being subjected to statistical analysis. For modeling purposes, a seed population was considered to be composed of subpopulations defined by differences in their relative germination rates (Garcia-Huidobro *et al.*, 1982 [11]). In this study, the 1st and 50th germination percentiles were used to calculate thermal time model parameters, as they represent first germination and half of the seeds germination. Germination rates were defined as the reciprocal of 1% and 50% germination times. The differences between germination rates at different constant temperatures were tested by One-Way ANOVA ($p < 0.05$). The base temperature (T_b) and thermal time constant (θ_1) at suboptimal temperatures of each species were predicted by the modified thermal time model (Equation (3) in the introduction). Differences in T_b and θ_1 of C_3 and C_4 species were examined using Independent-Samples T test ($p < 0.05$). Linear regression was used to test the relationship between T_b, θ_1 and seed mass of C_3 and C_4 species. Data transformation and analysis of variance were carried out in SPSS (version 13.0, SPSS Inc., Chicago, IL, USA). Regression and calculation of model parameters were carried out in SigmaPlot (version 10.0, Systat Software Inc., Richmond, CA, USA).

3. Results and Discussion

3.1. Germination Responses of C_3 and C_4 Species to Temperature

The twelve C_4 species exhibited a variety of responses to constant temperatures (Figure 1). Seeds of *Kochia prostrata*, *Salsola collina*, *Chloris virgata*, *Echinochloa crusgalli* and *Artemisia anethifolia* germinated well (>80%) at a wide range of temperatures from 5–35 °C or 10–35 °C. The germination percentages of *Amaranthus retroflexus*, *Eriochloa villosa* and *Portulaca oleracea* increased with temperature until 30 °C, and then kept constant or decreased slightly. The germination percentages of *Chenopodium album* and *Euphorbia humifusa* increased with temperature, then decreased greatly above 30 °C. For *Amaranthus blitoides* and *Setaria viridis*, more than half of the seeds did not germinate at most temperatures.

For most C_3 species, the relationship between germination percentage and temperature resembled an upside-down "U" or "V" (Figure 2). Only three species *Dracocephalum moldavica*, *Bidens parviflora* and *Lactuca indica* had more than 90% seed germination at all temperatures from 5 °C until 30 °C or 35 °C.

The germination rate either increased with temperature until 40 °C, or increased until an optimal temperature, then decreased, irrespective of whether they were C_3 or C_4 species (Figures 3 and 4). The trends of germination rate change with temperature were similar for 1% and 50% germination percentiles of each species. The germination rates of C_4 species were generally higher than those of C_3 species, with *S. collina* most rapid, and *C. virgata* next.

C_3 and C_4 species are classified by their photosynthetic pathway. C_3 species are mainly distributed to high latitude regions with cooler climate, while C_4 species are generally found at low latitudes with warmer climate and strong light [26]. From our study, the two types of species also had different germination responses to temperature [27]. The twelve C_4 species used were all annual or biennial and distributed widely in the research region; More than half these species had a wide optimal temperature range. At 5 °C, seven species exhibited no seed germination or lower than 10 percent of seeds germinated; however, all the C_4 species could germinate at 40 °C (Figure 1). By contrast, twelve of fifteen C_3 species could germinate at 5 °C and ten of the fifteen species could not germinate at 40 °C (Figure 2). Seeds of C_4 species germinated faster than those of C_3 species at the optimal 30 °C ($p < 0.05$). Plant responses to temperature reflect the environments in which those species live, thus the differences in germination optima between species may have ecological significance [12].

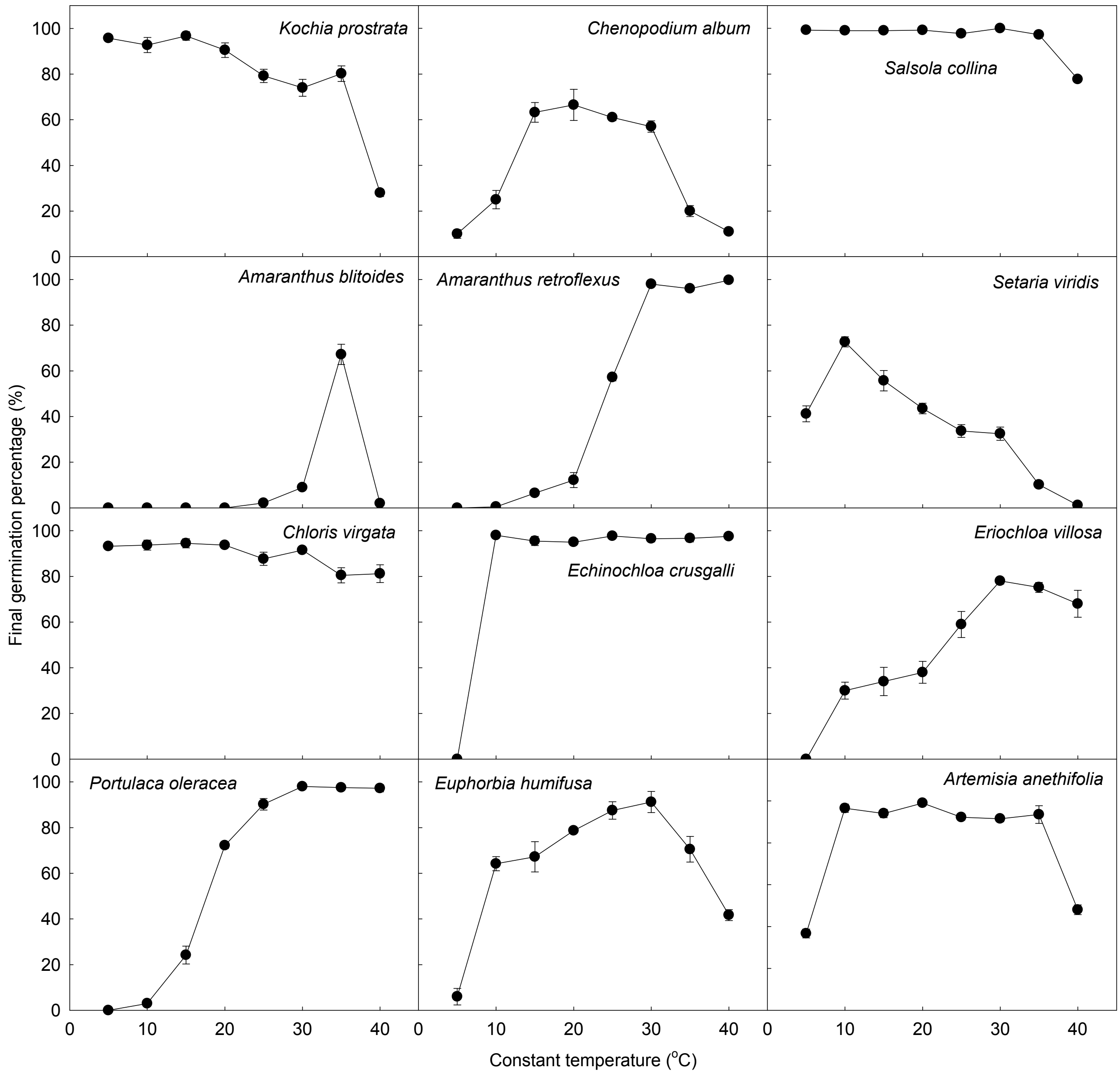

Figure 1. Final germination percentages of C_4 species at a range of constant temperatures from 5 °C to 40 °C. Bars represent ±SE (n = 4).

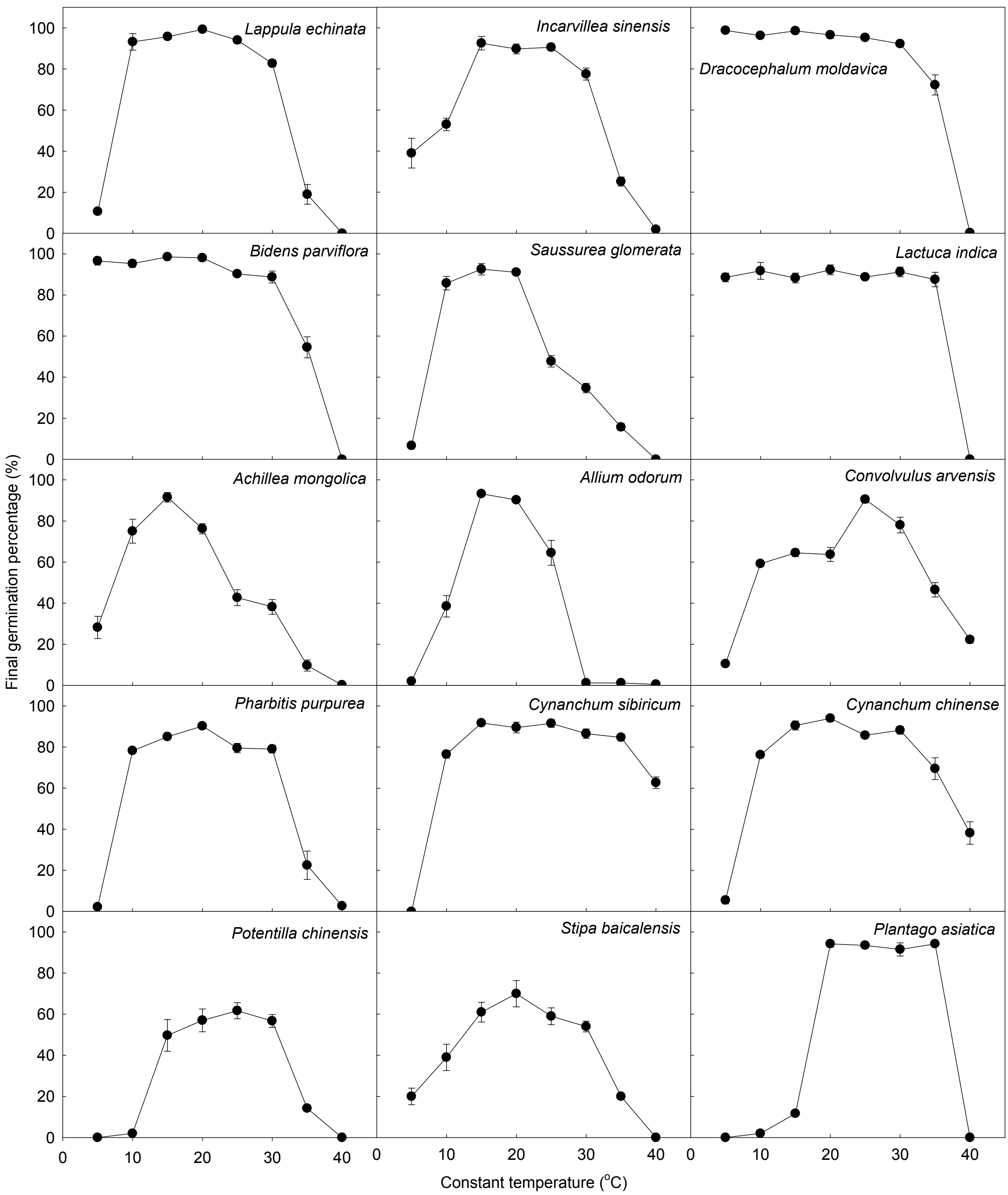

Figure 2. Final germination percentages of C_3 species at a range of constant temperatures from 5 °C to 40 °C Bars represent ±SE (n = 4).

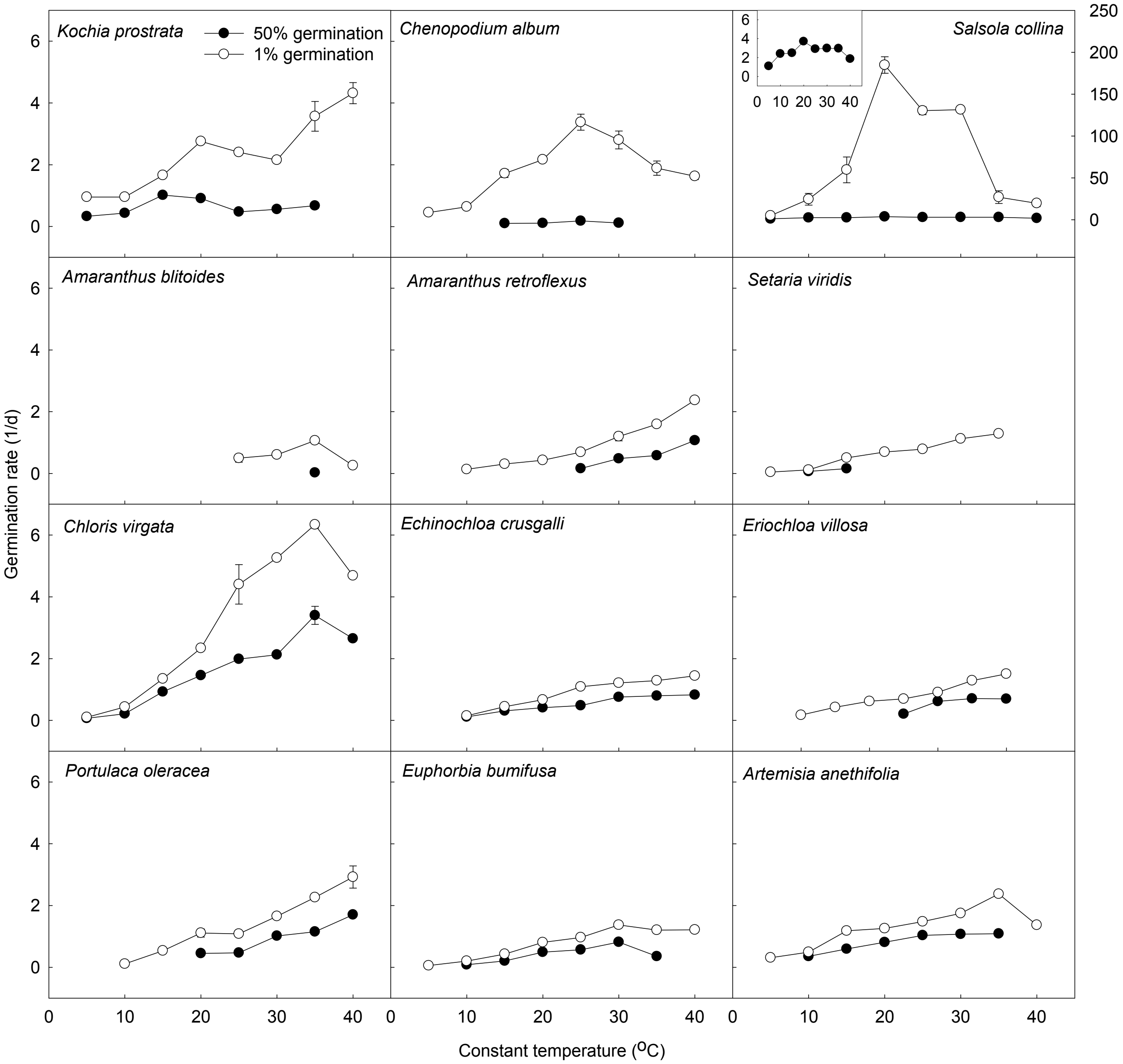

Figure 3. Germination rates of C_4 species for 1% (○) and 50% (●) germination percentiles at a range of constant temperatures from 5 °C to 40 °C. Bars represent ±SE (n = 4). For *Salsola collina*, scaling of y axis was given on the right-hand side; the enlarged figure was for 50% germination percentile.

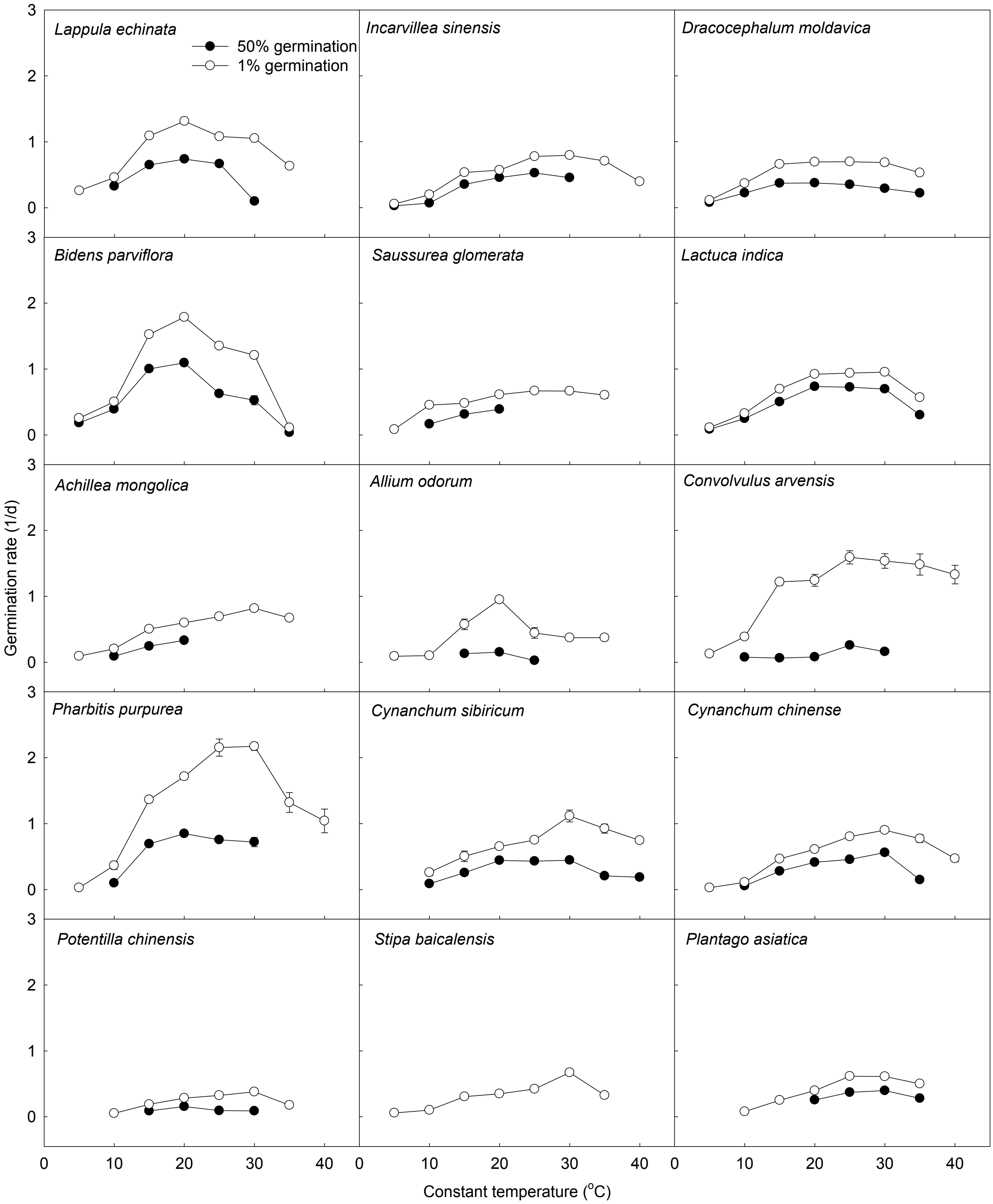

Figure 4. Germination rates of C_3 species for 1% (○) and 50% (●) germination percentiles at a range of constant temperatures from 5 °C to 40 °C. Bars represent ±SE (n = 4).

3.2. Comparison of Model Parameters between C_3 and C_4 Species

We used the modified thermal time model to predict base temperature and thermal time constant of a range of C_4 and C_3 species (Tables 2 and 3). As a whole, the estimation was accurate ($p < 0.05$), except the 50% germination percentile for several species (e.g., *K. prostrata*, *Lappula echinata*) and 1% germination percentile for *S. collina*, *A. blitoides*, and *Saussurea glomerata*. The poor fit for these species was due to the lower number of regression points (three or four). The average base temperature of C_3 species for the 1% germination percentile (T_b = 3.8 ± 0.4 °C, n = 14) was lower than that for C_4 species (T_b = 4.9 ± 1.4 °C, n = 10), with the difference approaching significance (p = 0.074). The average thermal time constant of C_3 species for 1% germination percentile (θ_1 = 24.7 ± 4.0 °C·d, n = 14) was higher than that of C_4 species (θ_1 = 15.2 ± 2.2 °C·d, n = 10) (p = 0.45). The differences between model parameters of C_3 and C_4 species for 50% germination percentile (n = 5 for C_3, n = 7 for C_4) were similar, with a significant difference in θ_1 ($p < 0.05$). Among the 27 species in this study, the differences in base temperature were not significant between annuals and perennials, but the differences in thermal time constant were significant between annuals and perennials (16.0 °C·d and 26.2 °C·d, respectively; $p < 0.01$). This result was consistent with previous research [17], which indicated that germination responses to temperature was related to plant life cycle.

Table 2. Estimated parameters of thermal time model for 1% and 50% germination percentiles (G) of C_4 species at suboptimal temperatures.

Species	G	N	T_b (°C)	θ_1 (°C·d)	R^2	p
Kochia prostrata	1%	8	−3.6	11.1	0.85	0.001
	50%	3	1.4	14.6	0.86	0.24
Chenopodium album	1%	5	3.7	6.8	0.95	0.0044
	50%	3	1.7	126.6	0.81	0.28
Salsola collina	1%	4	6.5	0.09	0.84	0.08
	50%	4	−2.8	6.3	0.92	0.04
Amaranthus blitoides	1%	3	17.3	17.6	0.89	0.21
	50%					
Amaranthus retroflexus	1%	7	11.6	13.9	0.92	0.0006
	50%	4	22.3	17.7	0.94	0.031
Setaria viridis	1%	7	4.8	23.3	0.98	< 0.0001
	50%	2				
Chloris virgata	1%	6	7.0	4.5	0.95	0.0008
	50%	7	6.3	9.4	0.96	0.0001
Echinochloa crusgalli	1%	7	4.4	22.9	0.95	0.0002
	50%	5	5.8	34.4	0.95	0.0042
Eriochloa villosa	1%	7	6.4	23.3	0.97	< 0.0001
	50%	2				
Portulaca oleracea	1%	7	9.4	11.3	0.96	< 0.0001
	50%	5	14.9	15.7	0.93	0.008
Euphorbia humifusa	1%	6	5.4	18.9	0.98	0.0001
	50%	5	8.1	27.3	0.98	0.0016
Artemisia anethifolia	1%	7	0.3	15.6	0.96	0.0001
	50%	4	2.0	22.2	0.99	0.0002

N, number of values; T_b, base temperature; θ_1, thermal time constant; R^2 and p represent the coefficient of determination and probability for the fitting.

Table 3. Estimated parameters of thermal time model for 1% and 50% germination percentiles (G) of C_3 species at suboptimal temperatures.

Species	G	N	T_b (°C)	θ_1 (°C·d)	R^2	p
Lappula echinata	1%	4	2.2	13.2	0.95	0.0241
	50%	3	1.0	25	0.90	0.20
Incarvillea sinensis	1%	5	3.2	27.5	0.95	0.0041
	50%	5	4.6	36.1	0.94	0.0072
Dracocephalum moldavica	1%	3	3.1	18.2	0.99	0.0231
	50%	3	2.3	34.4	0.99	0.0045
Bidens parviflora	1%	4	3.4	8.9	0.93	0.0352
	50%	3	3.6	12.2	0.93	0.17
Saussurea glomerata	1%	4	−0.2	31.1	0.84	0.08
	50%	3	2.0	44.6	0.96	0.11
Lactuca indica	1%	4	3.3	17.9	0.99	0.0057
	50%	4	3.6	22.7	0.99	0.0037
Achillea mongolica	1%	6	1.1	33.8	0.96	0.0006
	50%	3	5.7	41.8	0.98	0.09
Allium odorum	1%	4	5.5	16.4	0.90	0.0494
	50%	2				
Convolvulus arvensis	1%	5	2.9	13.2	0.92	0.0099
	50%	4	6.7	89.3	0.61	0.21
Pharbitis purpurea	1%	5	5.0	8.9	0.97	0.0026
	50%	3	7.7	13.3	0.90	0.20
Cynanchum sibiricum	1%	5	3.2	25.5	0.96	0.0037
	50%	3	7.6	28.2	0.99	0.0196
Cynanchum chinense	1%	6	4.6	26.6	0.97	0.0004
	50%	5	5.1	42.0	0.93	0.0076
Potentilla chinensis	1%	5	4.3	63.3	0.94	0.0058
	50%	2				
Stipa baicalensis	1%	6	3.8	43.1	0.94	0.0013
	50%					
Plantago asiatica	1%	4	7.9	28.6	0.96	0.0001
	50%	3	0.8	70.9	0.88	0.22

N, number of values; T_b, base temperature; θ_1, thermal time constant; R^2 and p represent the coefficient of determination and probability for the fitting.

Compared to tropical and subtropical legumes [18], the temperate grassland species in our study had lower base temperature and thermal time constants. This is not completely coincident with other studies. Trudgill [28] found the base temperature of tropical plants was higher than that of temperate plants, but they also demonstrated that the thermal time constant of tropical plants was lower than that for temperate plants [12]. Therefore, tropical plants germinate faster than temperate plants. They suggested that temperate plants will suffer frost injury if they germinate too early, while tropical plants might suffer high temperature or drought if they germinate too late. The germination response to temperature is also related to phylogeny. Plants in the Poaceae and Cyperaceae have been noted to have lower and higher base temperature, respectively [29].

3.3. Relationship between Seed Mass and Germination Parameters

The relationship between germination rate and seed mass depended on plant functional type and temperature. For C_4 species, big seeds had higher germination rates only at 5 °C ($p < 0.05$, Figure 5a). For C_3 species however, germination rate increased with seed mass significantly over the temperature range 15–40 °C ($p < 0.05$, Figure 5b). Neither the base temperature nor thermal time constant of either C_3 or C_4 species had a significant relationship with seed mass (Figure 5c,d). The T_b of C_3 species were more clustered around 5 °C, while C_4 species were scattered from −3.6 °C to 11.6 °C. The opposite was noted for the thermal time constant. θ_1 of C_4 species was confined to 5–23 °C·d, but that of C_3 species distributed from 9 °C·d to 63 °C·d.

To our knowledge, this is the first study to test the relationship between germination parameters and seed mass. It is interesting that larger seeds germinated faster for C_4 species at low temperature, while seed mass was positively related to germination rate for C_3 species at high temperatures. We speculate that larger seeds have an advantage under unfavorable conditions, although this hypothesis needs further study.

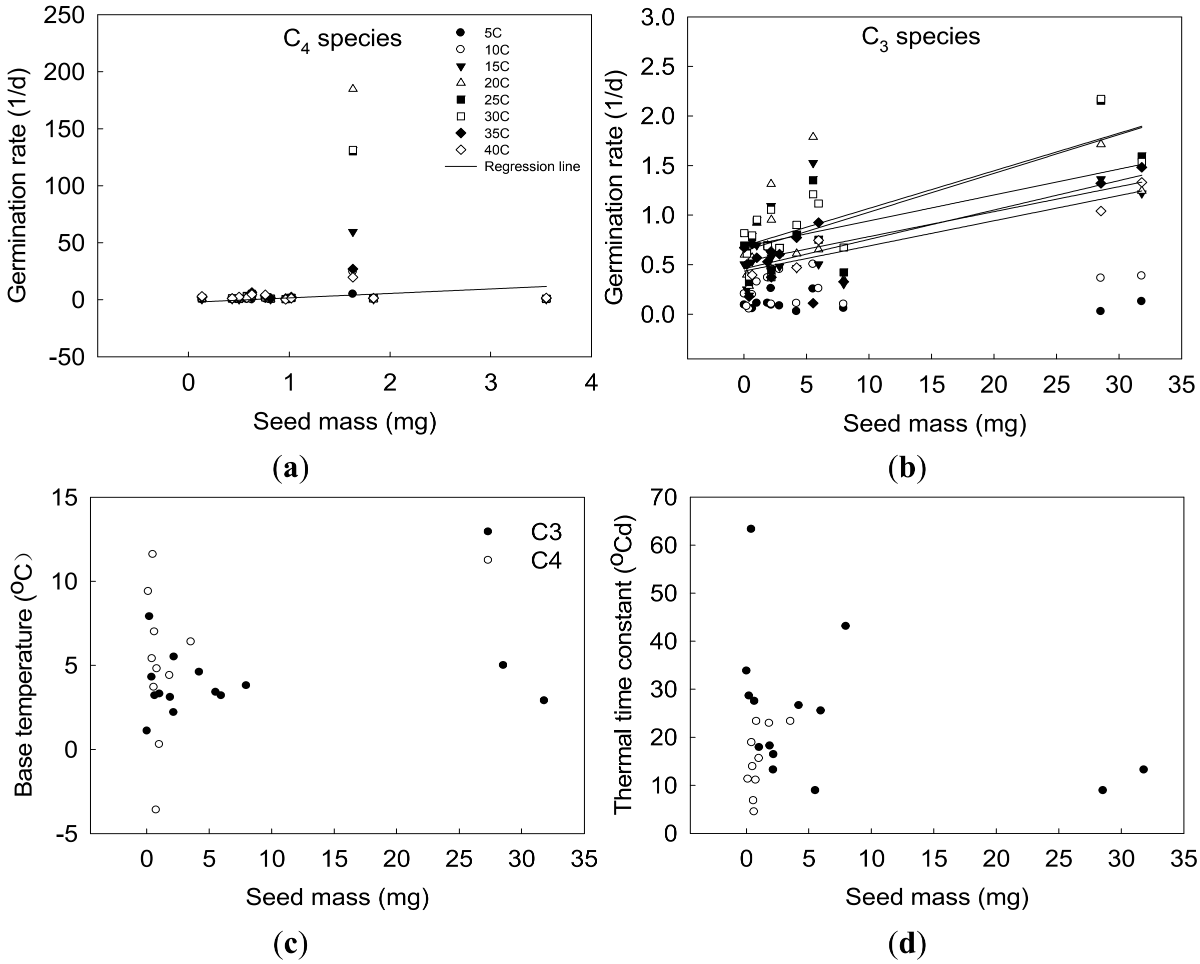

Figure 5. The relationship between seed mass and germination parameters of C_3 and C_4 species (germination rate, (**a**), (**b**); base temperature, (**c**); thermal time constant, (**d**); significant linear regressions were given in figures (**a**), 5 °C; (**b**), 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C).

4. Conclusions

The germination response to temperature was species-dependent. Significant differences in the thermal time constants were noted between C_3 and C_4, and between annual and perennial species. Although seed mass significantly influenced germination rate at certain temperatures for C_3 and C_4, base temperature and thermal time constant were not related to seed mass.

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Author Contributions

Conceived and designed the experiments: Hongxiang Zhang and Daowei Zhou. Performed the experiments: Hongxiang Zhang and Yu Tian. Analyzed the data and manuscript writing: Hongxiang Zhang.

Conflicts of Interest

The authors declare no conflict of interest.

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