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Germination Parameters of Selected Summer Weeds: Transferring of the AlertInf Model to Other Geographical Regions

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Abstract: Effective weed management depends on correct control timing, which depends on seedling emergence dynamics. Since soil temperature and soil moisture are the two main factors that determine weed germination, the hydrothermal time model can be used to predict their emergence. The aim of this study was to estimate the base temperature (T_b) and base water potential (Ψ_b) for the germination of *Chenopodium album*, *Amaranthus retroflexus*, *Setaria pumila*, and *Panicum capillare* collected from fields in continental Croatia and then to compare these values with those of Italian populations embedded in the AlertInf model. Germination tests were performed at seven constant temperatures (ranging from 4 to 27 °C) and eight water potentials (0.00–1.00 MPa). The estimated T_b and Ψ_b were 3.4 °C and −1.38 MPa for *C. album*, 13.9 °C and −0.36 MPa for *A. retroflexus*, 6.6 °C and −0.71 MPa for *S. pumila*, and 11.0 °C and −0.87 MPa for *P. capillare*, respectively. According to the criterion of overlap of the 95% confidence intervals, only the T_b of *C. album* and the Ψ_b of *A. retroflexus* of the Croatian and Italian populations were similar. Further field experiments should be conducted to monitor the weed emergence patterns of *C. album* and calibrate the AlerInf equation parameters.

Keywords: base temperature; base water potential; maize; predictive weed emergence model; weed germination

1. Introduction

Integrated Weed Management (IWM) recommends the use of different weed control methods with the main objective of developing crop production systems that require less herbicide use. To achieve this goal and maximize herbicide efficacy, herbicide mixtures should be closely matched to the specific botanical composition of the weed flora in the field. Achieving an effective control is highly dependent on the timing of the herbicide application, which depends on the weed emergence dynamics [1]. Therefore, knowledge of the timing and duration of weed emergence could facilitate the achievement of effective herbicide application, without subsequent corrective treatments [2]. In addition, compared to standard management practice, it allows for lower herbicide application and lower weed control costs [3]. Weed emergence data constitute a basis for the development of predictive weed emergence models. These models provide the percentage of cumulative weed emergence achieved daily in the field, with the aim of suggesting the best time for farmers to control weeds [1]. Several predictive weed emergence models have been developed and are currently available for growers of maize [4,5], soybeans [2,6], and winter cereals [7,8] in Europe and the United States. These models are often based on the concept of thermal time (TT) or hydrothermal time (HTT) [9], depending on whether they consider only temperature (TT) or temperature and soil moisture (HTT) as triggers for germination.

HTT models start calculating hydrothermal units when the soil temperature and water potential reach the value of the estimated base temperature (T_b) and base water potential (Ψ_b) for the germination of certain weed species. The prediction of HTT models is usually more accurate, as they are able to predict the pauses in seedling emergence caused by a low soil water potential [1,3,10], which could not be predicted by TT models. The calculation of HT requires the estimation of germination parameters, which are the base temperature (T_b) and base water potential (Ψ_b), to simulate the seedling emergence according to the weather trends [11].

In Croatia, maize is the most important annual crop, averaging 260,000 hectares per year, which corresponds to about 32% of the country's arable land [12]. Maize is almost always cultivated without irrigation, and weed control relies mainly on post-emergence herbicides. The prediction of weed emergence is therefore very important for the identication of the correct timing for herbicide application. Currently, there are no developed models; therefore, the transfer of the existing model from geographically close areas [4,11] would greatly benefit maize growers.

The criteria for the transfer of a HTT model developed in another area are the presence of the main weed species in the existing model and overlap between the values of the germination parameters of the domestic and foreign populations built into the model. In case of overlapping values, the existing model can be validated in a maize field, without repeated monitoring of weed emergence dynamics over many years.

The inter-population variability of weed species may limit the transferability of the HTT model, as it has been reported that local populations may develop different germination behavior in adapting to local environmental conditions [11,13]. For example, differences between the T_b values in the Italian and Portuguese populations of *Datura stramonium* L. [13], the American and European populations of *A. artemisiifolia* L. [14], and the German and French populations of *Chenopodium album* L. and *Echinochloa crus-galli* (L.) P. Beauv [15] have been reported. In contrast, similar T_b values were found for *Amaranthus retroflexus* L., *Abutilon theophrasti* Med., and *Chenopodium album* L. between populations from different regions of Italy, Veneto, and Tuscany [1]. Thus, in transfering a model to geographical areas other than that in which it was created, the behavior of the local population should be tested [11].

Of the HTT models developed so far, the one geographically closest to the continental part of Croatia is the AlertInf model, developed in Italy (Veneto) for predicting the emergence of ten weed species: *A. theophrasti*, *Digitaria sanguinalis* (L.) Scop., *E. crus-galli*, *Polygonum persicaria* L., *Setaria pumila* (L.) P. Beauv, *Setaria viridis* (L.) Beauv., *Solanum nigrum* L., *A. retroflexus*, *C. album*, and *Sorghum halepense* (L.) Pers. [1,4].

Previous studies found no statistical difference between the germination parameters adopted for the Italian population of *A. theophrasti* included in the AlertInf model and the values estimated using the same methodology for a Croatian population [16]. The results of these preliminary studies indicate the possibility of transferring AlertInf to other geographical regions (Croatia), but further studies are needed to include other thermophilic weed species that are common in maize fields in Croatia. Hence, the aim of this research was to estimate the T_b and Ψ_b for four weed species: *A. retroflexus*, *C. album*, *S. pumila*, and *Panicum capillare* L. collected from fields in continental Croatia and then to compare these values with the values of the Italian populations embedded in the AlertInf model in order to verify the possibility of using the predicative weed emergence model in geographically and climatologically different areas.

2. Materials and Methods

2.1. Site Description and Comparison

In order to verify the transferability of the AlertInf model from one geographical location to another that is climatologically different, the values of the germination parameters of seeds collected from Croatia (Zagreb) and Italy (Padova) were compared. According to the Köppen-Geiger climate classification [17], Zagreb is classified as Dfb, with a cold

climate, precipitation without a dry season, and a warm summer. Padova is classified as Cfa, with a temperate climate, precipitation without a dry season, and a warm summer. For Padova, the average annual temperatures and precipitation were taken from Masin et al. [6], and for Zagreb, they were taken from the Croatian Meteorological and Hydrological Service. The average annual precipitation in Zagreb is 861.1 mm, with the minimum precipitation in February (44.6 mm) and maximum in September (101.6 mm). The average annual temperature is 11.8 °C, with the minimum temperature in January (−3.2 °C) and maximum in August (25.0 °C). Padova has an average annual precipitation of about 850 mm, which is uniformly distributed throughout the year. The average annual temperature is 12.2 °C, with temperature increases from January (average minimum value: −1.5 °C) to July (average maximum value: 27.2 °C).

2.2. Seed Material

The seeds of *S. pumila*, *P. capillare*, *A. retroflexus*, and *C. album* were hand-picked from plants in maize fields at physiological maturity. The seeds of *C. album* and *S. pumila* were collected at the Experimental Station of the University of Zagreb Faculty of Agriculture, Sasinovecki Lug (45°50′59.6″ N; 16°09′53.9″ E), the seeds of *A. retroflexus* were collected at the Experimental Station Maksimir (45°49′34.3″ N; 16°01′49.8″ E), and the seeds of *P. capillare* were collected at the site, Lipovec Lonjski (45°44′51.9″ N; 16°23′12.4″ E). The collected seeds were brought to the laboratory and cleaned, sieved, and stored in paper bags in the refrigerator (4 °C) until the beginning of the experiment.

2.3. Germination Experiments

Experiments to estimate base temperature and base water potential for germination were conducted at the University of Padova, Department of Agronomy, Food, Natural Resources, Animals, and Environment, and the University of Zagreb, Faculty of Agriculture, Department of Weed Science, from 2013 to 2020. Prior to the start of the experiments, a preliminary germination test in a climate chamber (W87R, KW Apparecchi Scientifici SRL, via della Resistenza 119, 53035 Monteriggioni, Italy) at a constant temperature (25 °C) and photoperiod of 12 h/12 h (day/night) was conducted to check the germination capacity of the seeds. Seed populations that achieved a germination higher than 60% were included in further studies.

The estimation of the base temperature of the four weed species was performed by simultaneously testing the germination at six or seven constant temperatures, with a photoperiod of 12 h/12 h (day/night) in different climatic chambers. The seeds were calibrated and uniformed before placing them on filter paper. To prevent the growth of pathogens on the seeds and on the filter paper, the seeds were sterilized with 1% hydrogen peroxide and washed with distilled water. According to the amount of uniformed seed, three replicates of 100 seeds of *C. album*, *A. retroflexus*, and *P. capillare* and five replicates of 50 seeds of *S. pumila* were placed in a Petri dish on Whatman® filter paper. The filter paper was covered with 5 mL of distilled water, and the Petri dishes were sealed with parafilm. The initial temperature was defined for each weed species as one degree lower than the base temperature previously established in the literature [11,18–20]. Therefore, *C. album* and *S. pumila* were tested at a constant temperature of 4, 8, 12, 16, 20, 24, and 28 °C. Furthermore, the germination of *A. retroflexus* was tested at 9, 12, 15, 18, 21, 24, and 27 °C, and that of *P. capillare* was tested at 6, 9, 12, 15, 18, 21, 24, 27, and 30 °C.

To estimate the base water potential of each species, a germination test was carried out, exposing the seeds to different levels of water potential, that is, different levels of water availability. As described before, three replicates per 100 seeds or five replicates per 50 seeds were placed in eight different water potential solutions. For this purpose, polyethylene glycol (PEG) 6000 (Sigma-Aldrich Chemie GmbH 25322-68-3, St. Louis, MO, USA) was used to prepare the solutions with eight water stress levels: 0.00 (pure distilled water), −0.05, −0.10, −0.25, −0.38, −0.50, −0.80, and −1.00 MPa, according to Michel and Kaufmann [21]. The seeds were placed in transparent plastic containers with diameter and

height of 10 cm and 7 cm, respectively, as described by Masin et al. [1]. Containers with 50 mL of the prepared solution were placed at a constant temperature of 22 °C, with a photoperiod of 12 h/12 h (day/night).

In both germination experiments, the seeds were defined as germinated when the seed radicle was 1 mm long. Germinated seeds incubated at the temperatures above 20 °C and with all water potentials above −0.38 MPa were counted and removed twice daily, and germinated seeds incubated at temperatures below 20 °C and with water potentials below −0.38 MPa were counted and removed once. The germination test was considered complete when no germination was detected for 10 consecutive days. The germination test lasted between 9 and 95 days, depending on the temperature or water potential and tested weed species.

The temperature in the climate chambers was recorded hourly using temperature data loggers (HOBO UA-001-08, Onset Computer Corporation, Bourne, MA, USA). Temperature deviations ±0.5 °C were considered acceptable.

2.4. Statistical Analysis and Statistical Methods

The effect of temperature and water potential on final germination percentages was evaluated with regression analysis. A parabolic model was used to describe the effect of temperature, as follows:

$$FG = aT^2 + bT + c$$

where, FG is the percentage of final germination, T is the temperature expressed in °C, and a, b, and c are constant numbers with a not equal to zero.

An exponential model was used to describe the effect of water potential, as follows:

$$FG = FG_0 \cdot \exp(-k\Psi)$$

where, FG is the percentage of final germination, FG_0 is the highest percentage when the water potential of the solution is equal to zero, Ψ is the water potential expresses in MPa, and k is rate constant.

The germination dynamics curve was generated using the logistic function in the Bioassay97 statistical program [22] to determine the initial (t_{10}), medium (t_{50}), and final (t_{90}) germination time with the formula:

$$CG = 100/1 + \exp \{a [\ln (t + 0.0000001) - \ln (b)]\}$$

where, CG is the percentage of cumulative germination, t is the time expressed in days, a is the slope of the curve, and b is the inflection point. The initial (t_{10}), medium (t_{50}), and final (t_{90}) germination time, i.e., the time it takes for 10%, 50%, and 90% of the germinating seeds to germinate, are determined by the slope of the curve (b). The effect of the temperature and water potential on germination dynamics, expressed in days (that is on t_{10}, t_{50}, and t_{90}), was analyzed by means of variance analysis (ANOVA). After the significant F-test, the LSD test for $p = 0.05$ was used to compare the mean values.

The germination parameters were determined using germination dynamics data at different temperatures and water potentials for each species studied. The reciprocal of t_{50} ($1/t_{50}$) was used to establish the linear regression line against the incubation temperature or water potential [1]. The values of T_b and Ψ_b were presented as the point where the linear regression line intersects the abscissa. The 95% confidence intervals for T_b and Ψ_b were determined using the bootstrap method [23]. The values obtained for the germination parameters of the Croatian populations were compared with the values of the Italian built into the AlertInf model, according to the criterion of overlap of the 95% confidence intervals [1]. If there is no overlapping of the confidence intervals between the two populations, a significant difference is determined.

3. Results

3.1. Weeds Germination at Different Temperatures and Water Potentials

Regression analysis showed very different influence of temperature and water potential on germination of the studied species (Figures 1 and 2).
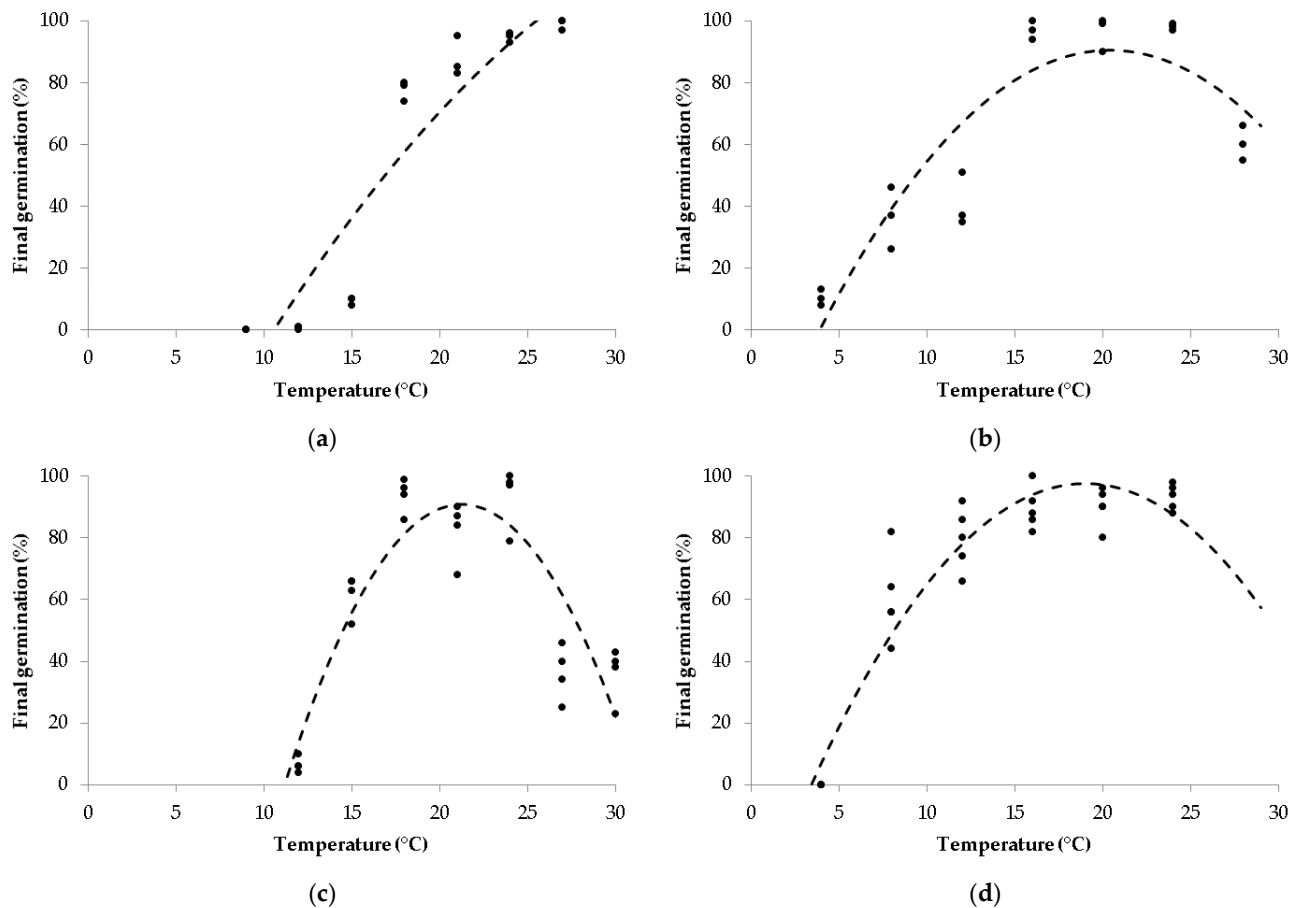

Figure 1. Final germination (%) of *A. retroflexus, C. album, P. capillare,* and *S. pumila* at studied temperatures. The dotted line represents the regression line and the points the observed final germination percentages. Estimated function are: (**a**) *Amaranthus retroflexus* y = $-0.1x^2 + 11.8x - 108.6$, $r^2 = 0.87$; (**b**) *Chenopodium album* y = $-0.3x^2 + 13.6x - 48.0$, $r^2 = 0.82$; (**c**) *Panicum capillare* y = $-0.9x^2 + 38.1 - 314.1$, $r^2 = 0.78$; (**d**) *Setaria pumila* y = $-0.4x^2 + 15.3x - 47.9$, $r^2 = 0.91$.

The germination of the studied species varied between 0.67% and 99%, depending on the incubation temperature or weed species (Figure 1). Among the tested weed species, *C. album* showed the ability to germinate at the lowest temperature (4 °C), while the other species started to germinate at 8 °C (*S. pumila*) or 12 °C (*A. retroflexus* and *P. capillare*). The highest germination percentage of all four species was reached at 24 °C: *C. album* (98%), *A. retroflexus* (95%), *S. pumila* (93%), and *P. capillare* (93%). However, *A. retroflexus* also achieved a similar germination percentage at 27 °C, and *C. album* and *S. pumila* achieved this percentage within a temperature range of 16–24 °C. Germination began to decrease as the temperature dropped, but this process was species-specific. These results indicate that incubation temperatures greatly affect the germination of the tested weed species.

Similarly, water potential greatly affected the germination of the weed species. For all tested weed species, germination began to decrease as the water potential dropped (Figure 2). The germination of *S. pumila* and *C. album* showed a slow reduction. *A. retroflexus* presented a fast decrease of germination with a strong inhibition from −0.25 MPa. *P. capillare* germination decreased with an intermediate reduction rate compared to the other species. Almost all weed species presented very low or no germinated seeds at −0.80 and −1.00 MPa, except *C. album*

that showed germination also at −1.00 MPa. Taken together, these findings demonstrate that the germination of all species decreased significantly at lower water potentials, but germination ability at different water potentials was also species-specific.
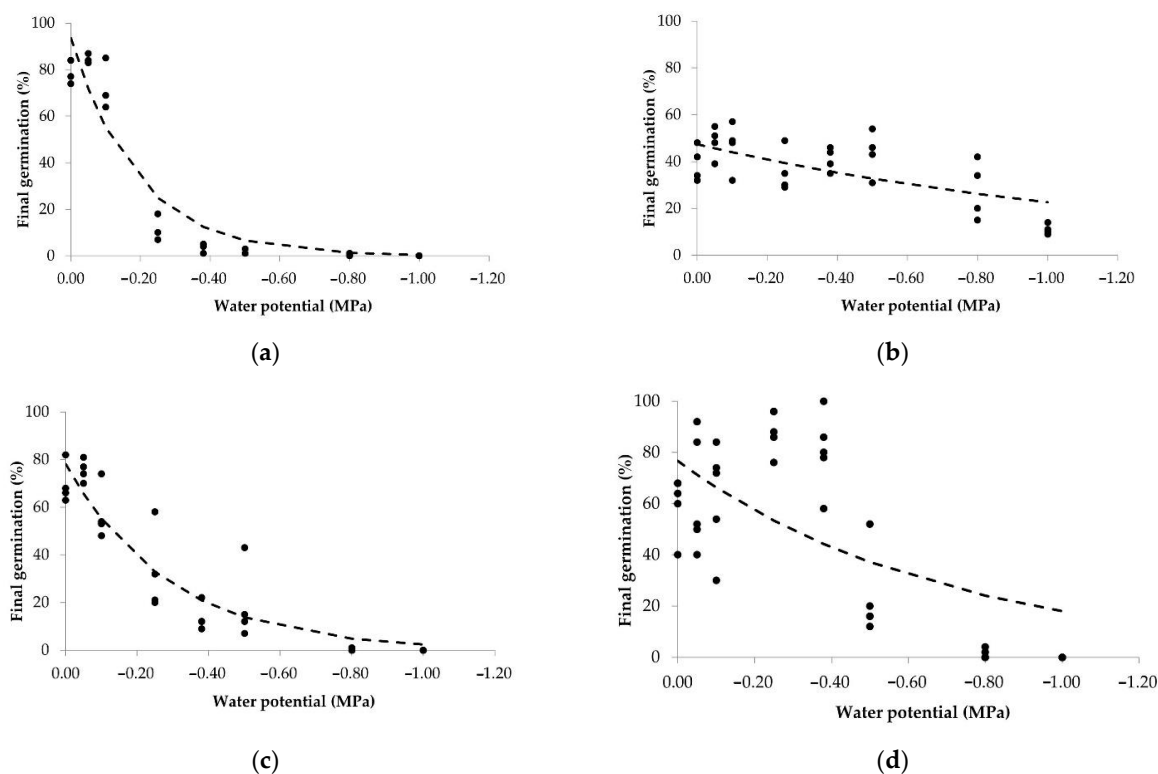

Figure 2. Final germination percentage of the studied weed species at different water potentials (MPa). The dotted line represents the regression line and the points the observed final germination percentages. Estimated functions are: (**a**) *Amaranthus retroflexus* y = 93.5 exp(−5.3x), r^2 = 0.90; (**b**) *Chenopodium album* y = 47.4 exp(−0.7x). r^2 = 0.45; (**c**) *Panicum capillare* y = 78.1 exp(−3.5x), r^2 = 0.87; (**d**) *Setaria pumila* y = 76.7 exp(−1.5x), r^2 = 0.46.

3.2. Germination Dynamics in Response to Different Temperatures and Estimation of Base Temperature

Daily recorded germination data were used to obtain germination dynamic curves at each studied temperature. The germination dynamics were influenced by temperature in all tested species (Table 1). A decrease in temperature led to an increase in the number of days required for the start and end of germination for all species. The duration of germination varied between 0.6 and 76.0 days (d), depending on the incubation temperature and the species studied. At 24 °C, the initial germination (t_{10}) was shortest for *A. retroflexus* (1.0 d) and longest for *C. album* (3.3 d). At the same temperature, *A. retroflexus* needed 1.4 d to achieve medium germination (t_{50}), while *C. album* had a longer trend, and the longest t_{50} value was 4.5 days. In contrast, no statistical difference was found between two monocotyledonous species, *P. capillare* and *S. pumila*, in the medium germination (t_{50}) at a temperature of 24 °C. The end of germination (t_{90}) was reached for *A. retroflexus* in 1.8 d, while *C. album* and *P. capillare* finished germination in 6.2 and 7.0 d.

As expected, lower temperatures prolonged the germination of all investigated species. Due to the low germination capacity of *A. retroflexus* at 12 °C (0.67%), it was not possible to establish a germination curve for this species, unlike at other temperatures. The initial, medium, and final germination of the other three species at 12 °C varied between 5.83 and 24.38 d. *C. album* and *S. pumila* started germination at 5.83 and 8.98 d, while *P. capillare* extended the start of germination to 12.17 d. *S. pumila* was the species that reached, before the others, the end of germination (t_{90} at 12.3 d), while *P. capillare* set the end of germination to 24.38 d.

Table 1. Germination dynamics (t_{10}, t_{50}, and t_{90}) at the different studied temperatures.

°C	*P. capillare*			*C. album*			*S. pumila*			*A. retroflexus*		
	t_{10}	t_{50}	t_{90}	t_{10}	t_{50}	t_{90}	t_{10}	t_{50}	t_{90}	t_{10}	t_{50}	t_{90}
4	-	-	-	31.2 c	48.3 d	76.0 d	-	-	-	-	-	-
8	-	-	-	18.0 b	33.9 c	63.9 c	24.3 d	28.0 d	32.4 d	-	-	-
12	12.17 e B	17.03 d A	24.38 d C	5.8 a A	9.7 b A	16.4 b B	8.8 c AB	10.4 c B	12.3 c A	-	-	-
15	9.87 d	11.34 c	13.04 c	-	-	-	-	-	-	3.5 c	5.7 e	9.5 d
16	-	-	-	4.1 a	6.6 ab	10.8 ab	5.0 b	6.3 b	8.1 b	-	-	-
18	3.46 bc	4.03 b	4.70 ab	-	-	-	-	-	-	3.0 c	3.6 d	4.2 c
20	-	-	-	3.6 a	4.9 a	6.8 a	3.6 ab	4.8 ab	6.2 ab	-	-	-
21	2.87 bc	3.80 b	5.11 ab	-	-	-	-	-	-	1.7 b	2.2 c	2.8 b
24	1.64 a–c B	3.39 b B	7.06 b C	3.3 a D	4.5 a C	6.2 a C	2.3 a C	3.0 a B	3.8 a B	1.0 ab A	1.4 b A	1.8 ab A
27	1.48 ab	1.87 a	2.37 a	-	-	-	-	-	-	0.6 a	0.9 a	1.5 a
28	-	-	-	1.9 a	3.4 a	6.3 a	-	-	-	-	-	-
30	0.75 a	1.89 a	6.28 b	-	-	-	-	-	-	-	-	-

Different small letters (a–e) within a column indicate a statistical difference in each parameter separately for one species according to Fisher's Least Significant Difference (LSD) test at $p < 0.05$. Different capital letters (A–D) indicate a statistical difference between species in each parameter separately (t_{10}, t_{50}, and t_{90}) according to Fisher's Least Significant Difference (LSD) test at $p < 0.05$.

A linear regression line was used to estimate the T_b of the studied weed species (Figure 3). The highest T_b value was estimated for *A. retroflexus* (13.9 ± 0.36 °C) and the lowest was estimated for *C. album* (3.4 ± 0.36 °C), while *P. capillare* and *S. pumila* presented intermediate values (11.0 ± 1.99 °C and 6.6 ± 0.09 °C, respectively).
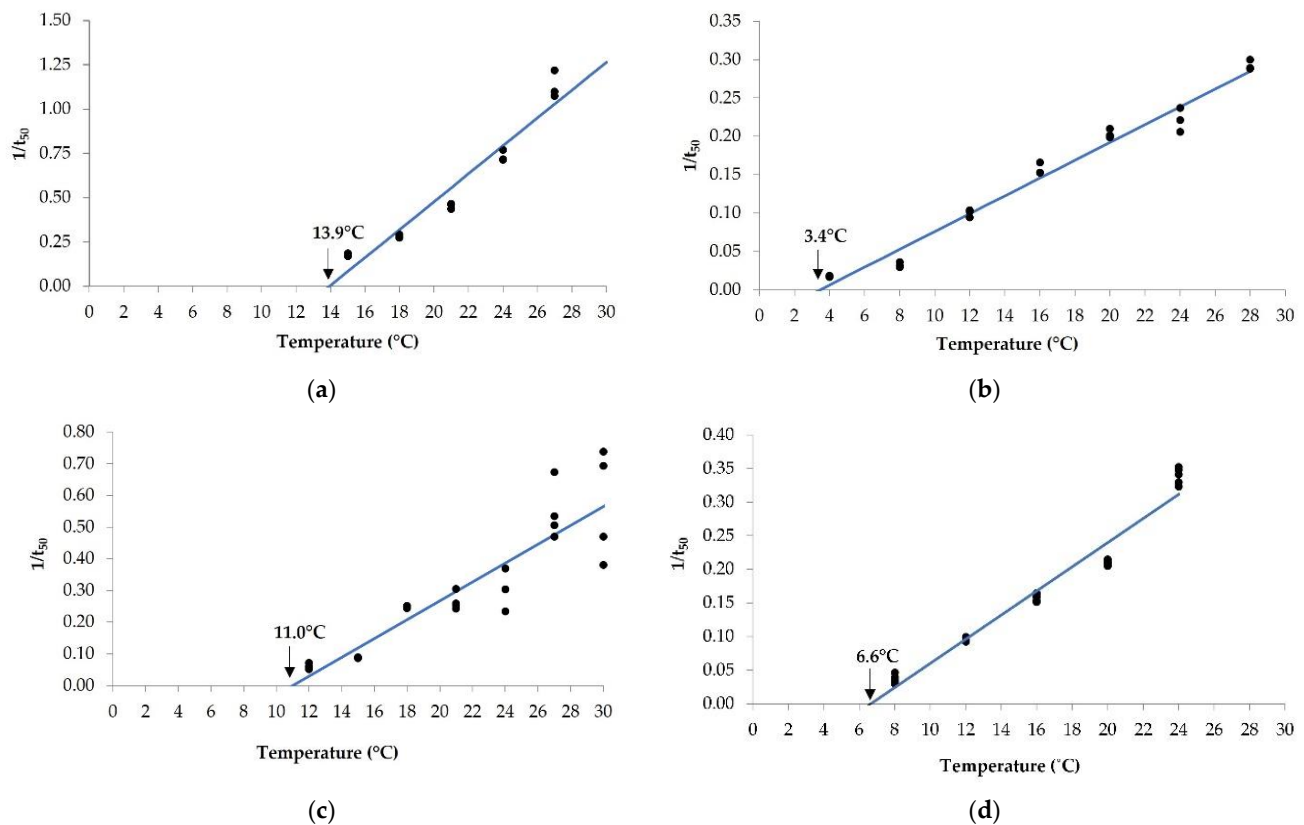

Figure 3. Estimated base temperatures. The solid line represents the linear regression line and the points represents the inverse value of the time necessary to reach 50% of germination ($1/t_{50}$). The estimated value of the base temperature is the intersection of the regression line with the *x*-axis: (**a**) *Amaranthus retroflexus* ($T_b = 13.9 \pm 0.36$ °C; $y = 0.079x - 1.010$; $r^2 = 0.96$); (**b**) *Chenopodium album* ($T_b = 3.4 \pm 0.36$ °C; $y = 0.012x - 0.04$, $r^2 = 0.98$); (**c**) *Panicum capillare* ($T_b = 11.0 \pm 1.99$ °C; $y = 0.030x - 0.328$; $r^2 = 0.82$); (**d**) *Setaria pumila* ($T_b = 6.6 \pm 0.09$ °C; $y = 0.018x - 0.119$; $r^2 = 0.96$).

3.3. Germination Dynamic in Response to Different Water Potentials and Estimation of the Base Water Potential for the Tested Weed Species

The duration of the germination of all weeds varied between 0.4 and 32.3 d, depending on the incubation water potentials and the species tested. In general, the duration of the germination increased with the decrease in water potential. The germination was extended within the abovementioned range, depending on the species (Table 2).

Table 2. Germination dynamics at different water potentials (MPa) at 22 °C.

MPa	P. capillare			C. album			S. pumila			A. retroflexus		
	t_{10}	t_{50}	t_{90}	t_{10}	t_{50}	t_{90}	t_{10}	t_{50}	t_{90}	t_{10}	t_{50}	t_{90}
0.00	4.6 bc	5.0 a	5.5 a	2.1 a	3.4 a	5.7 a	2.5 a	3.5 a	4.8 a	1.4 a	1.6 a	1.9 a
−0.05	4.7 bc	5.2 a	5.7 a	2.2 a	3.4 a	5.5 a	3.1 a	3.9 a	5.0 a	1.2 b	1.6 a	2.3 a
−0.10	4.6 bc	5.2 a	5.8 a	2.2 a	3.5 a	5.5 a	3.4 a	4.2 a	5.2 a	0.5 bc	1.3 a	3.6 a
−0.25	4.0 ab	6.2 ab	10.0 ab	2.3 a	3.7 a	5.8 a	3.3 a	5.3 a	7.4 a	1.9 c	5.9 b	18.6 b
−0.38	3.5 a	7.5 b	17.9 b	2.0 a	3.7 a	6.8 a	3.9 a	6.8 a	12.1 b	-	-	-
−0.50	4.9 c	7.5 b	12.8 ab	2.4 a	4.7 b	9.1 a	10.1 b	18.0 b	32.3 c	-	-	-
−0.80	-	-	-	4.5 c	9.6 d	21.4 b	-	-	-	-	-	-
−1.00	-	-	-	3.7 b	8.2 c	18.8 b	-	-	-	-	-	-

Differences between the initial (t_{10}), medium (t_{50}), and final (t_{90}) germination under different water potentials according to one-way analysis of variance (ANOVA). Different small letters (a–d) within a column indicate a statistical difference according to Fisher's Least Significant Difference (LSD) test at $p < 0.05$.

A. retroflexus showed the highest sensitivity to water stress. After a very low germination at a water potential $<−0.25$ MPa, it was not even possible to estimate the germination dynamic curve. At a water potential $>−0.25$ MPa, germination lasted from 0.5 to 3.6 d (t_{10}–t_{90}), and at −0.25 MPa, germination lasted 18.6 d (t_{90}). The other species required a longer time to reach the initial germination phase (t_{10}) at a water potential $<−0.25$ MPa, but then they were able to maintain similar germination dynamics until −0.38 and −0.50 MPa for *S. pumila*, *C. album*, and *P. capillare*, respectively. *C. album* was the only species with the ability to germinate at all investigated water potentials.

Consequently, Ψ_b was estimated with the germination dynamics shown in Table 2 and is presented in Figure 4. The lowest value was estimated for *C. album* ($−1.38 \pm 0.14$ MPa), and the highest was estimated for *A. retroflexus* ($−0.36 \pm 0.03$ MPa), while *S. pumila* ($−0.71 \pm 0.07$ MPa) and *P. capillare* ($−0.86 \pm 0.07$ MPa) had intermediate values.

It is important to underline that, for *P. capillare*, it was not possible to use the logistic regression model to identify the t_{50} at −0.80 MPa due to the low germination (Figure 2). However, a value of $1/t_{50}$ close to zero was used at −0.80 MPa to estimate the base water potential. It was necessary to avoid underestimation of the base parameter.
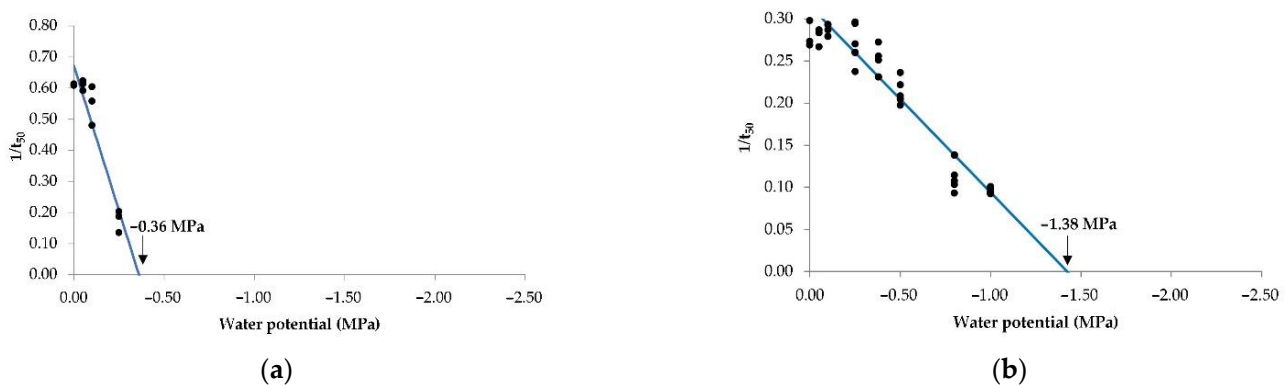

(a)

(b)

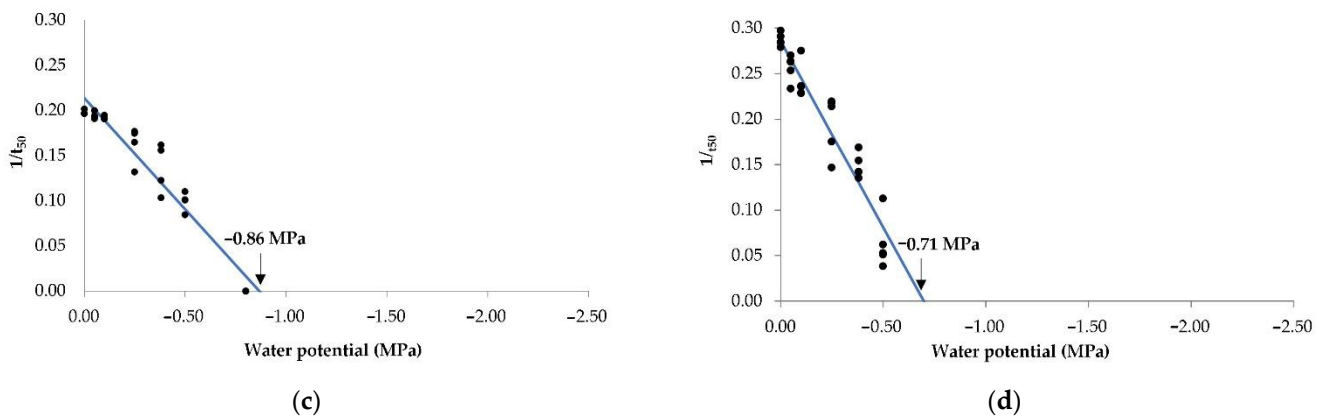**Figure 4.** *Cont.*

(c)

(d)

Figure 4. Estimated base water potentials (Ψ_b). The solid line represents the linear regression line, and the points represent the inverse value of the time necessary to reach 50% of germination ($1/t_{50}$). The estimated value of the base water potential is the intersection of the regression line with the *x*-axis: (**a**) *Amaranthus retroflexus* ($\Psi_b = -0.36 \pm 0.03$ MPa; y = 0.672x + 1.862; $r^2 = 0.91$); (**b**) *Chenopodium album* ($\Psi_b = -1.38 \pm 0.14$ MPa; y = 0.221x + 0.315; $r^2 = 0.87$); (**c**) *Panicum capillare* ($\Psi_b = -0.86 \pm 0.07$ MPa; y = 0.2466x + 0.2138; $r^2 = 0.94$); and (**d**) *Setaria pumila* ($\Psi_b = -0.71 \pm 0.07$ MPa; y = 0.409x + 0.286; $r^2 = 0.92$).

3.4. Comparison of the Germination Parameters of Two Populations from Different Geografical Areas

According to the criterion of overlap of the 95% confidence interval [1] between the Italian and Croatian populations, two out of the three species tested have similar values in an estimated parameter (Table 3). For *P. capillare*, it was not possible to make a comparison because the germination parameters of the Italian population of this species have not yet been estimated.

Table 3. Base temperature (T_b) and base water potential (Ψ_b) of weed species for the Italian and Croatian populations, with the confidence interval (95% CI) and coefficient of determination (r^2). The Italian germination parameters for the three weed species (*A. retroflexus*, *C. album*, and *S. pumila*) were estimated by Masin et al. [1].

Species	Italy			Croatia			Italy			Croatia		
	T_b (°C)	±95% CI	r^2	T_b (°C)	±95% CI	r^2	Ψ_b (MPa)	±95% CI	r^2	Ψ_b (MPa)	±95% CI	r^2
A. retroflexus	12.3	1.12	0.92	13.9	0.36	0.96	−0.41	0.07	0.92	−0.36	0.03	0.95
C. album	2.6	0.77	0.84	3.4	0.36	0.98	−0.96	0.10	0.84	−1.38	0.14	0.87
S. pumila	10.4	0.95	0.97	6.59	0.09	0.96	−0.93	0.11	0.85	−0.71	0.07	0.92
P. capillare	-	-	-	11.0	1.99	0.82	-	-	-	−0.87	0.07	0.94

The Croatian population of *A. retroflexus* had a 1.6 °C higher base temperature compared to the Italian populations, and the overlap was not even found if the extreme of the two confidence intervals were close. Thus, these two values of T_b can be considered as statistically different. In the Croatian population of *C. album*, the T_b was 0.8 °C higher than in the Italian population, but the confidence intervals overlapped. These two values are therefore not statistically different. In contrast, the T_b value estimated for the Croatian population of *S. pumila* is 3.81 °C lower, compared to the Italian population, and it was found that they differed significantly.

The base water potential of *A. retroflexus* was 0.05 MPa higher for the Croatian population, compared to the Italian, but no significant difference was found. A lower base water potential was determined for the Croatian population of *C. album*, and a higher base water potential was determined for *S. pumila*, although significant differences from the Italian population were found in both cases. Taken together, these findings indicate that similar values between Italian and Croatian populations were found only for *C. album* regarding T_b and for *A. retroflexus* regarding Ψ_b.

4. Discussion

In the present study, germination parameters (T_b and Ψ_b) of four summer weed species collected in Croatia were estimated. Germination tests at different temperatures showed a species-specific preference for higher or lower temperatures. The species ranged from less to more thermophilic as follows: *C. album* > *S. pumila* > *P. capillare* > *A. retroflexus*. This is consistent with previous studies, in which *A. retroflexus* was also found to germinate best at temperatures >25 °C [24] and *S. pumila* at temperatures of 24.5 to 34.9 °C [25]. In the present study, *P. capillare* had the highest germination at temperatures of 18–24 °C, which is again consistent with a previous study, where *P. dichotomiflorum* achieved the highest germination capacity at temperatures of 25 °C [26–29] or *P. miliaceaeum* at temperatures between 18 and 25 °C [30,31]. The optimal temperature for the germination of *C. album* has been reported between 15 and 25 °C [32], which is again in line with the germination data of the present study (Figure 1).

If we compare the temperature preferences that define germination with the time of emergence in the field, as suggested in a previous study [2], the species from the present study could be divided into three categories: early (*C. album*), middle (*S. pumila* and *P. capillare*), and late emerging species (*A. retroflexus*). The germination dynamic data shown in this study (Table 1) also reflected the species-specific sensitivity to different temperatures. In particular, *A. retroflexus* had the shortest germination at all temperatures investigated. For example, the mean germination of this species stopped completely after 5.7 days, which is slightly faster, compared to the other studied species (Table 1). No germination was observed at and below 12 °C, confirming the thermophilic behavior of the species [2]. The T_b values for *A. retroflexus* was estimated to be 13.9 °C, which is the highest T_b among the species tested in the present study. This is the reason why a later emergence is observed for *A. retroflexus* in maize fields, compared to the other studied weeds [33]. This T_b is slightly higher than the values around 12 °C reported for the Italian and Iranian populations [1,11], and even lower values, i.e., 10.5 and 8.9 °C, have been reported for other populations from Germany and France, respectively [19,34]. In contrast, our study estimated that the T_b for *C. album* is 3.4 °C, making it the species with the lowest temperature requirement, thus confirming its early germination behavior [2]. This value is similar to the T_b reported for Italian and Dutch populations [1,35], while a significantly higher value was reported for a French population [19]. The value of T_b (6.6 °C) estimated for the Croatian population of *S. pumila* is lower than the range of values (8.6–10.4 °C) reported in previous studies on populations from Italy, France, and California [1,19,30]. In our study, we also estimated the T_b for *P. capillare*, and as far as we know, this is the first report globally on the base temperature for this weed species. We found that 11 °C is the base temperature for *P. capillare*. Our results are consistent with another study, in which the minimum temperature for the germination of *P. miliaceaum* was also estimated to be 11 °C [31].

Since temperature was not the only factor found to trigger germination in the present study, we also observed the germination capacity of the same weed species under different water potentials. Depending on their water requirements, the species in this study ranged from less to more tolerant as follows: *A. retroflexus* (Ψ_b −0.36 MPa) < *S. pumila* (Ψ_b −0.71 MPa) < *P. capillare* (Ψ_b −0.86 MPa) < *C. album* (Ψ_b −1.38 MPa) (Figure 4). In addition to the study performed to calibrate the AlertInf model [1], only a single study conducted in France has determined the Ψ_b for these species [19]. The value of Ψ_b estimated for the Croatian population of *A. retroflexus* is similar to the Ψ_b used in AlertInf for a population from Northern Italy, while lower values were reported for other populations from Central Italy (−0.62 MPa) and France (−0.95 MPa) [1,19]. Regarding the Ψ_b for *S. pumila*, an almost identical value was described for a French population (−0.75 vs. −0.71 MPa), while a lower value was determined for the Italian population included in AlertInf [1,19]. Finally, the Croatian population of *C. album* had a lower Ψ_b in comparison with the values previously reported for both Italian (−0.96 and −1.04 MPa) and French (−0.80 MPa) populations [1,19]. As far as we know, there are no data of Ψ_b for the species *P. capillare*, in the literature. The germination behavior of the investigated species at different

temperature and water potential regimes shows that the species with a better tolerance to lower temperatures also had a better tolerance to a lower water potential.

The main objective of the present study was to compare the estimated values of germination parameters for *C. album*, *S. pumila,* and *A. retroflexus* seeds collected at different geographical locations in order to verify the possibility of using the hydrothermal model, AlertInf, in different geographical locations. We wanted to examine if the T_b and Ψ_b values estimated in this study would be comparable with those estimated in Alertinf [4], as a first step of transferring the weed predictive model, AlertInf, out of its original geographical region. Two out of the three Croatian species had a parameter overlapping with the Italian population: *C. album* had a similar T_b but a different Ψ_b, *A. retroflexus* had a different T_b but a similar Ψ_b, and *S. pumila* differed in both germination parameters (Table 3).

The present study therefore showed that a similar germination of Croatian and Italian populations may only be expected for *C. album,* since the T_b value was similar. However, this is valid only in conditions where soil water is not limited, since the Ψ_b differed significantly between the two populations. The next step will therefore be to evaluate the weed emergence patterns of *C. album* in irrigated maize fields and then try to calibrate the AlerInf equation parameters. The prediction of *A. retroflexus* and *S. pumila* using the AltertInf model with its original parameters is unfortunately impossible, even in irrigated maize crops, since the T_b values of Croatian populations differ statistically from those of the Italian populations present in the hydrothermal model. This variability in germination parameters has also been documented in previous studies and is usually explained as an adaptation process of weed species to local climate conditions [13,36]. The annual air temperature in Zagreb is, on average, lower than that in Padova. Moreover, comparing the temperatures in spring (March-June) and summer (July-October) in Zagreb (6.4–19.4 °C; 21.1–11.0 °C) and Padova (9.0–22.0 °C; 23.1–14.0 °C) during a thirty-year period, it is evident that Zagreb has lower air temperatures. Therefore, we expected that the populations in Zagreb and the surrounding area would have lower T_b values, compared to those in Padova, as suggested earlier [1,12]. This was found to be case for *S. pumila* and *C. album*; however, this phenomenon was not found for *A. retroflexus*, where the T_b was found to be higher for the Croatian (colder climate) than for the Italian population (warmer climate). Unfortunately, the complexity of weed seed biology, especially in the period of seed ripening, can influence the germination behavior of seeds. The involvement of various factors that determine the characteristics of the seed (position on the mother plant, micro-environmental conditions, availability of nutrients, etc.) can cause differences in dormancy and germination requirements [37,38]. An attempt to implement the model in another agro-ecological area was also made by Bürger and Colbach [15] using the FlorSys model. The difference in base temperature for different species was also species-specific, and it was not possible to find a pattern connected to climate conditions. They found a 4.3 lower T_b for *C. album*, and a 4.0 °C higher T_b for *E. crus-galli* in Germany, compared to France.

5. Conclusions

C. album, *A. retroflexus*, *S. pumila*, and *P. capillare* are highly distributed weed species globally [39] and in Croatian maize fields [40]. The present study offers a way to implement the predictive emergence model, AlertInf, for *C. album* in a non-irrigated field.

However, further field trials are necessary and should be carried out in two directions depending on the weed species. First, for *C. album* or *A. therophrasti*, due to their estimated T_b overlap with the Italian populations [16], AlertInf needs to be further validated by comparing the emergence of the species in maize fields with those predicted by AlertInf. Second, the model should be adjusted for the species *S. pumila* and *A. retroflexus*, as the germination parameters differ significantly. Third, AlertInf should be extended to *P. capillare*, whose germination parameters have now been estimated for the first time. Furthermore, the results of this study highlight the importance of the need to develop and adapt a predictive weed emergence model for a specific agro-ecological area. With a

few exceptions [11], the comparison made in this study andin some other studies [1,11,15] showed that emergence models cannot be applied globally, and it is important to establish a dataset with local weed germination parameters to introduce the emergence model into a new area. However, the results are valuable as they provide estimated biological germination parameters for four species that have never been estimated before in Croatia. This is the first and obligatory step towards the development/transfer of a model to predict their emergence

Conflicts of Interest: The authors declare no conflict of interest.

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