

Article

# Biological parameters for germination of selected summer weed species: first step to transfer the hydrothermal model AlertInf to Croatia

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**Abstract:** The efficacy of weed management depends on the correct control timing according to the seedling emergence dynamics. Since soil temperature and soil moisture are 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 ( $\Psi_b$ ) for 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 to -1.00 MPa). Estimated  $T_b$  and  $\Psi_b$  were 3.4°C, -1.38 MPa for *C. album*, 13.9°C, -0.36 MPa for *A. retroflexus*, 6.6°C, -0.71 MPa for *S. pumila* and 11.0°C, -0.87 MPa for *P. capillare*, respectively. According to the criterion of overlap of the 95% confidence intervals, only  $T_b$  of *C. album*, and  $\Psi_b$  of *A. retroflexus* were similar between Croatian and Italian populations. Further field experiments should be conducted in the Croatian field to monitor weed emergence patterns of *C. album* and to calibrate the AlertInf equation parameters.

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

## 1. Introduction

Integrated Weed Management recommends the use of post-emergence herbicides when it is possible to select an effective herbicide or combination of herbicides based on the weed flora composition. Although such an application can be tailored to the specific botanical composition of the real weed flora, efficient control is highly dependent on timing according to weed emergence dynamics [1]. Therefore, knowledge of the timing and duration of weed emergence could help to achieve 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 are 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 [6,2] 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 ( $\Psi_b$ ) for 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 low soil water potential [1,3,10], which could not be predicted by TT models. The calculation of HT requires the estimation of biological parameters, which are base temperature ( $T_b$ ) and base water potential ( $\Psi_b$ ) for germination, to simulate the seedlings emergence according to 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. Prediction of weed emergence is therefore very important to identify 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 be of great benefit to 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 the overlap of the values of the biological parameters of the domestic and foreign populations built into the model. In case of overlapping values, the existing model can be validated in maize field without repeated monitoring of weed emergence dynamics lasting 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 as an adaptation to local environmental conditions [11, 13]. For example, differences between  $T_b$  values in Italian and Portuguese populations of *Datura stramonium* L. [13], American and European populations of *A. artemisiifolia* L. [14] and 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, to transfer a model to geographical areas other than that of creation, the behavior of the local population should be tested [11].

Of the HTT models developed so far, the 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 biological 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 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  $T_b$  and  $\Psi_b$  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.

## 2. Materials and Methods

### 2.1. Site Description and Comparison

In order to verify the transferability of the AlertInf model from Italy to Croatia, the values of biological parameters of seeds collected from Croatia (Zagreb) and Italy (Padova) were compared. According to Köppen-Geiger climate classification [17], Zagreb is classified as Dfb as cold climate, precipitation without dry season and warm summer. Padova is classified as Cfa as temperate climate, precipitation without dry season and warm summer. For location Padova average annual temperatures and precipitation were

taken from Masin et al. [6] and for location Zagreb from Croatian Meteorological and Hydrological Service. Average annual precipitation in Zagreb is 861.1 mm with minimum precipitation in February (44.6 mm) and maximum in September (101.6 mm). Average annual temperature is 11.8°C, with minimum temperature in January (-3.2°C) and maximum in August (25.0°C). Padova has an average annual precipitation about 850 mm uniformly distributed throughout the year. 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), while the seeds of *A. retroflexus* were collected at the Experimental Station Maksimir (45°49'34.3"N 16°01'49.8"E) and *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, 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 University of Padova, Department of Agronomy, Food, Natural resources, Animals and Environment and 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 the climate chamber (W87R, KW Aparecchi Scientifici SRL, Italy) at constant temperature (25°C) and photoperiod 12 h:12h (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 four weed species was performed by testing the germination at six or seven constant temperatures with photoperiod 12h:12h (day: night) simultaneously in the different climatic chambers. To prevent the growth of pathogens on the seeds and on filter paper, seeds were sterilized with 1% hydrogen peroxide and washed with distilled water. Three replicates of 100 seeds or five replicates of 50 seeds were placed in the Petri dish on Whatman® filter paper covered with 5 ml distilled water and sealed with parafilm. The initial temperature was defined for each weed species as one degree lower than the base temperature previously established from the literature [11, 18 - 20]. Therefore, *C. album* and *S. pumila* were tested to a constant temperature of 4, 8, 12, 16, 20, 24, 28°C. Furthermore, the germination of *A. retroflexus* was tested at 9, 12, 15, 18, 21, 24, 27°C and *P. capillare* at 6, 9, 12, 15, 18, 21, 24, 27 and 30°C.

To estimate base water potential of each species, germination test was carried out exposing seeds to different level of water potential, that is different level of water availability. Three replicates per 100 seeds or five replicates per 50 seeds were placed at eight different water potential solutions. For this purpose, polyethylene glycol (PEG) 6000 (Sigma-Aldrich Chemie GmbH 25322-68-3) was used to prepare solutions with eight water stress levels: 0.00 (pure distilled water), -0.05, -0.10, -0.25, -0.38, -0.50, -0.80, -1.00 MPa according to Michel and Kaufmann [21]. The seeds were placed in transparent plastic containers 10 cm in diameter and 7 cm high, as described by Masin et al. [1]. Containers with 50 ml prepared solution were placed at a constant temperature of 22°C and a photoperiod of 12h:12h (day: night).

In both germination experiments, seeds were defined as germinated when the seed radicle was 1 mm long. Germinated seeds were counted and removed twice daily for seeds incubated at temperatures above 20°C and all water potentials above -0.38 MPa and once daily for seeds temperatures below 20°C and water potential below -0.38 MPa. The germination test was considered complete when no germination was detected for 10

consecutive days. Germination test lasted from 9 to 95 days depending on the temperature or water potential and tested weed species.

The temperature in the climate chambers was recorded hourly with temperature data loggers (HOBO UA-001-08, Onset Computer Corporation, Bourne, MA). Temperature deviations  $\pm 0.5^{\circ}\text{C}$  were considered acceptable.

#### 2.4. Statistical Analysis and Statistical Methods

Mean percentage of germination was calculated for each treatment. The germination dynamics curve was generated using the logistic function in the Bioassay97 statistical program [22] to determine initial ( $t_{10}$ ), medium ( $t_{50}$ ) and final ( $t_{90}$ ) germination time using the formula:

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

Where CG is the percentage of cumulative germination,  $t$  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 10, 50 and 90 % of the germinating seeds to germinate, are determined by the slope of curve ( $a$ ). The effect of temperature and water potential on germination percentages and germination dynamics ( $t_{10}$ ,  $t_{50}$  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 biological 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  $\Psi_b$  were presented as the point where the linear regression line intersects the abscissa. The 95% confidence intervals for  $T_b$  and  $\Psi_b$  were determined using the bootstrap method [23]. The values obtained for the biological parameters of the Croatian populations were compared with the values of 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

Analysis of variance showed significant influence ( $P < 0.0001$ ) of studied temperatures and water potential to weed germination (Table 1 and 2).

**Table 1.** Final germination (%) of *C. album*, *S. pumila*, *A. retroflexus* and *P. capillare* at studied temperatures.

°C	Final germination (%)														
	4	6	8	9	12	15	16	18	20	21	24	27	28	30	
<i>C. album</i>	10.3 d	-	36.3 c	-	41 c	-	97 a	-	96.3 a	-	98 a	-	60.3 b	-	
<i>S. pumila</i>	0 d	-	60.4 c	-	79.6 b	-	89.6 ab	-	90 ab	-	93.2 a	-	-	-	
<i>A. retroflexus</i>	-	-	0 e	-	0.67 e	9.33 d	-	77.67 c	-	87.67 b	94.67 a	99 a	-	-	
<i>P. capillare</i>		0 e	-	0 e	6.5 e	61.8 c	-	93.8 a	-	82.2 b	93.5 a	36.2 d	-	36.0 d	

Means within a row followed by the same letter are not significantly different ( $P > 0.05$ ) according to the LSD test.

The germination of the species studied varied between 0.67 and 99% depending on incubation temperature or weed species. Among the weed species tested, *C. album* showed the ability to germinate at the lowest temperature (4°C), while 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* achieved similar germination percentage also at 27°C, or *C. album* and *S. pumila* at temperature range 16 - 24°C. Germination began to decrease as the temperature dropped, but this process was species-specific. Germination of *C. album* and *S. pumila* decreased at temperatures  $\leq 8^\circ\text{C}$ . Germination percentage of *P. capillare* decreased at temperatures  $\leq 15^\circ\text{C}$  and then plunged at 12°C, while *A. retroflexus* showed a sharp reduction of germination even at 15°C. These results indicate that incubation temperatures affect greatly the germination of tested weed species.

Similarly, water potential greatly affected the germination of weed species. For all tested weed species germination began to decrease as the water potential dropped (Table 2). Germination of *S. pumila* and *C. album* showed a reduction from water potential of -0.50 and -0.38 MPa, respectively. *A. retroflexus* presented a first decrease of germination at -0.10 MPa and then a strong inhibition from -0.25 MPa. *P. capillare* ceased germination at -1.00 MPa. All weed species presented almost no germinated seeds at -0.80 and -1.00 MPa. Taking together, germination of all species decreased significantly at lower water potentials, but the ability for germination at different water potential was also species specific.

**Table 2.** Final germination of studied weed species at different water potentials (MPa).

Species	Water potential (MPa)							
	0.00	-0.05	-0.10	-0.25	-0.38	-0.50	-0.80	-1.00
<i>A. retroflexus</i>	78.3 ab	84.7 a	72.7 b	11.7c	3.3 cd	1.7 d	0.7 d	0.0 d
<i>P. capillare</i>	69.8 ab	75.5 a	57.2 b	32.8 c	13.8 de	19.2 d	0.5 e	0.0 e
<i>C. album</i>	66.3 a	67.0 a	67.7 a	63.0 a	20.7 b	7.3 c	2.3 c	0.0 c
<i>S. pumila</i>	88.4 a	80.4 ab	63.6 bc	62.8 bc	60.0 c	23.0 d	1.2 e	0.0 e

Means within a row followed by the same letter are not significantly different ( $P > 0.05$ ) according to the LSD test.

### 3.2. Germination dynamic in response to different temperatures and estimation of base temperature

Daily recorded germination data was used to obtain germination dynamic curves at each studied temperature. Estimated time required for  $t_{10}$ ,  $t_{50}$  and  $t_{90}$  is expressed in days (d) as decimal number (Table 3). The germination dynamics were influenced by temperature in all tested species. 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 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* continued with a prolonged trend with the longest  $t_{50}$  value of 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 as was the case at other temperatures. The initial, medium and final germination of the other three species at 12°C varied between 5.83 and 24.38 days. *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 3.** Germination dynamic ( $t_{10}$ ,  $t_{50}$ ,  $t_{90}$ ) at the different studied temperatures.

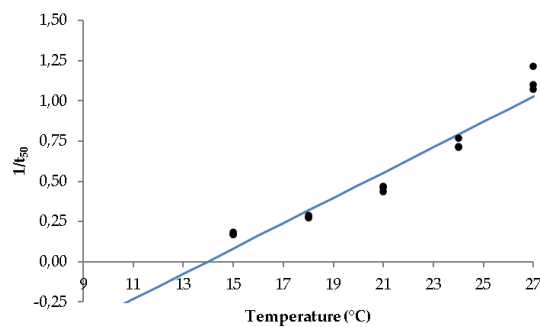
°C	<i>P. capillare</i>			<i>C. album</i>			<i>S. pumila</i>			<i>A. retroflexus</i>		
	$t_{10}$	$t_{50}$	$t_{90}$	$t_{10}$	$t_{50}$	$t_{90}$	$t_{10}$	$t_{50}$	$t_{90}$	$t_{10}$	$t_{50}$	$t_{90}$
4	-	-	-	31.2 c	48.3 d	76.0 d	-	-	-	-	-	-
8	-	-	-	18.0 b	33.9 c	63.9 c	24.3 d	28.0 d	32.4 d	-	-	-
12	12.17 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 statistical difference in each parameter separately for one species by Fisher's Least Significant Difference (LSD) test at  $p < 0.05$ .

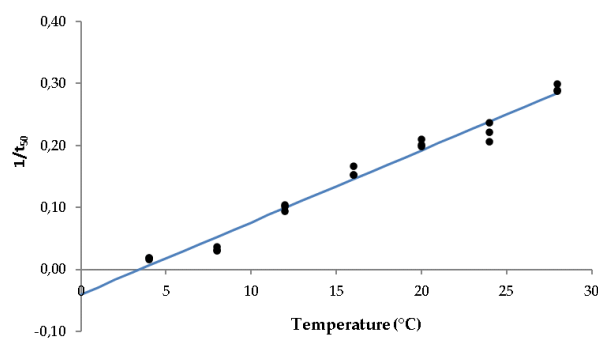
Different big letters (A -D) indicate statistical difference between species in each parameter separately ( $t_{10}$ ,  $t_{50}$ ,  $t_{90}$ ) by Fisher's Least Significant Difference (LSD) test at  $p < 0.05$ .



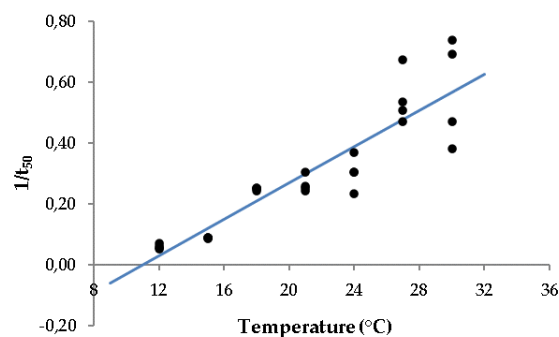
A linear regression line was used to estimate the  $T_b$  of the weed species studied (Figure 1). The highest  $T_b$  value was estimated for *A. retroflexus* ( $13.9 \pm 0.36^\circ\text{C}$ ) and the lowest for *C. album* ( $3.4 \pm 0.36^\circ\text{C}$ ), while *P. capillare* and *S. pumila* presented intermediate values ( $11.0 \pm 1.99^\circ\text{C}$ ) and ( $6.6 \pm 0.09^\circ\text{C}$ ), respectively.



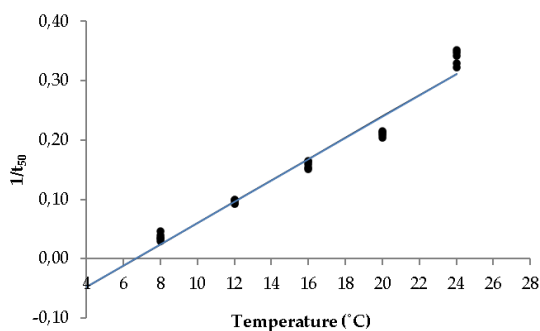
a) *Amaranthus retroflexus* base temperature ( $13.9 \pm 0.36^\circ\text{C}$ )



b) *Chenopodium album* base temperature ( $3.4 \pm 0.36^\circ\text{C}$ )



c) *Panicum capillare* base temperature ( $11.0 \pm 1.99^\circ\text{C}$ )



d) *Setaria pumila* base temperature ( $6.6 \pm 0.09^\circ\text{C}$ )

**Figure 1.** Estimated base temperatures. The solid line represents the linear regression line and the points are the calculated germination rate ( $1/t_{50}$ ). a) *Amaranthus retroflexus* ( $T_b = 13.9^\circ\text{C}$ ;  $y = 0.079x - 1.010$ ;  $r^2 = 0.96$ ) b) *Chenopodium album* ( $T_b = 3.4^\circ\text{C}$ ;  $y = 0.012x - 0.04$ ,  $r^2 = 0.98$ ) c) *Panicum capillare* ( $T_b = 11.0^\circ\text{C}$ ;  $y = 0.030x - 0.328$ ;  $r^2 = 0.82$ ) d) *Setaria pumila* ( $T_b = 6.6^\circ\text{C}$ ;  $y = 0.018x - 0.119$ ;  $r^2 =$



0.96).

3.3. Germination dynamic in response to different water potential and estimation of base water potential for tested weed species

The duration of 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 germination increased with the decrease in water potential. The germination was extended in the range depending on the species (Table 4).

*A. retroflexus* showed the highest sensitivity to water stress. After a very low germination at a water potential  $< -0.25$  MPa, it was even not 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 ( $t_{90}$ ). Other species required longer time to reach 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.

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Table 4. Germination dynamic at different water potential (MPa) at 22°C.

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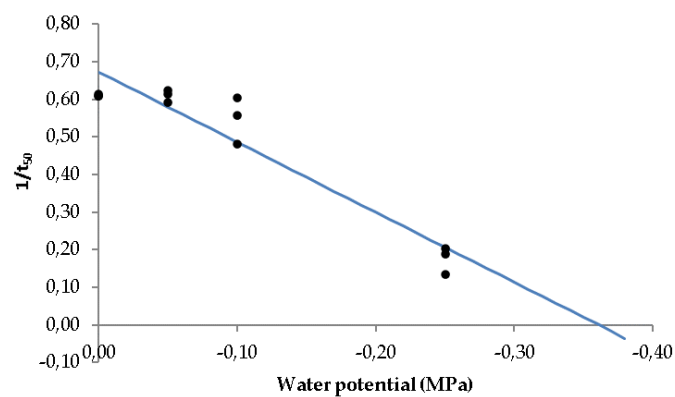
MPa	<i>P. capillare</i>			<i>C. album</i>			<i>S. pumila</i>			<i>A. retroflexus</i>		
	t <sub>10</sub>	t <sub>50</sub>	t <sub>90</sub>	t <sub>10</sub>	t <sub>50</sub>	t <sub>90</sub>	t <sub>10</sub>	t <sub>50</sub>	t <sub>90</sub>	t <sub>10</sub>	t <sub>50</sub>	t <sub>90</sub>
0.00	4.6 bc	5.0 a	5.5 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	9.1a	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 initial (t<sub>10</sub>), medium (t<sub>50</sub>) and final (t<sub>90</sub>) germination under different water potential by one-way analysis of variance (ANOVA). Different small letters (a-e) within a column indicate statistical difference by Fisher's Least Significant Difference (LSD) test at p < 0.05.

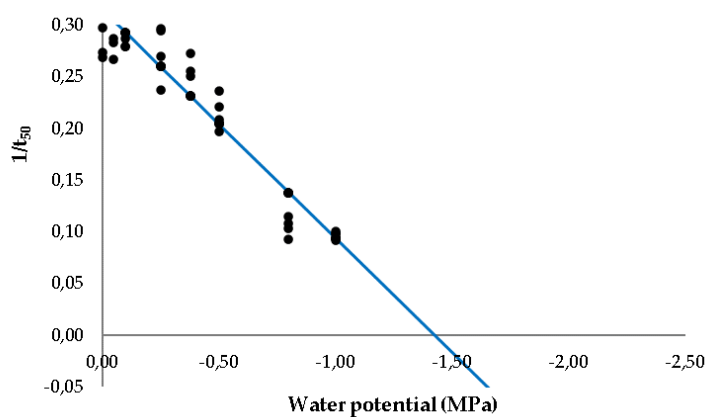
82

83

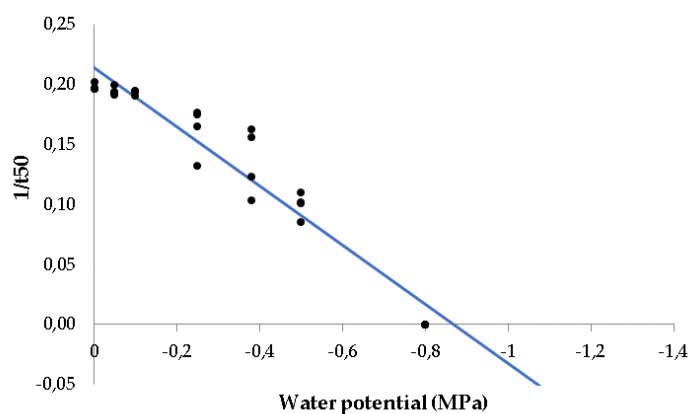
Consequently,  $\Psi_b$  was estimated with the germination dynamics shown in Table 3. 84  
 and presented in Figure 2. The lowest value was estimated for *C. album* ( $-1.38 \pm 0.14$  85  
 MPa) and the highest for *A. retroflexus* ( $-0.36 \pm 0.03$  MPa) while *S. pumila* ( $-0.71 \pm 0.07$  86  
 MPa) and *P. capillare* ( $-0.86 \pm 0.07$  MPa) had an intermediate value. 87



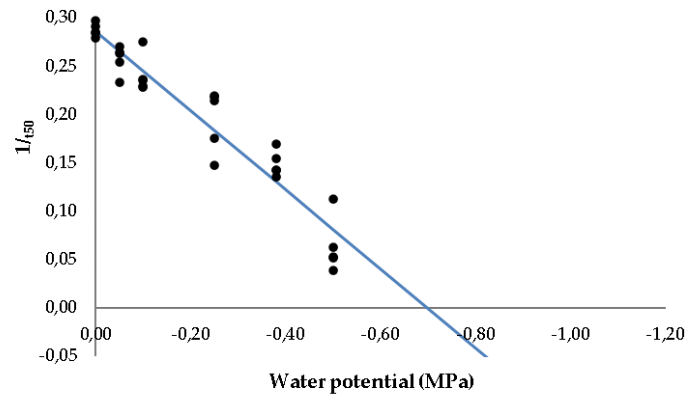
a) *Amaranthus retroflexus* ( $-0.36 \pm 0.03$  MPa)



b) *Chenopodium album* ( $-1.38 \pm 0.14$  MPa)



c) *Panicum capillare* ( $-0.86 \pm 0.07$  MPa)



d) *Setaria pumila* ( $-0.71 \pm 0.07$  MPa)

**Figure 2.** Estimated base water potentials ( $\Psi_b$ ). The solid line represents the linear regression line and the points are the calculated germination rate ( $1/t_{50}$ ): a) *Amaranthus retroflexus* ( $\Psi_b = -0.36$  MPa;  $y = 0.672x + 1.862$ ;  $r^2 = 0.91$ ) b), *Chenopodium album* ( $\Psi_b = -1.42$  MPa;  $y = 0.221x + 0.315$ ;  $r^2 = 0.87$ ) c), *Panicum capillare* ( $\Psi_b = -0.86$  MPa;  $y = 0.2466x + 0.2138$ ;  $r^2 = 0.94$ ) and d) *Setaria pumila* ( $\Psi_b = -0.70$  MPa;  $y = 0.409x + 0.286$ ;  $r^2 = 0.92$ ).

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 (Table 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.

### 3.4. Comparison of biological parameters of Italian and Croatian populations

According to the criterion of overlap of the 95% confidence interval [1] between Italian and Croatian populations, two out of three species tested have similar values in an estimated parameter (Table 5). For *P. capillare* it was not possible to make a comparison because the biological parameters of the Italian population of this species have not yet been estimated.

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

Base water potential of *A. retroflexus* was  $0.05$  MPa higher for the Croatian population compared to Italy but no significant difference was found. Lower base water potential was determined for the Croatian population of *C. album*, and higher for *S. pumila*, anyhow significant differences from the Italian population were found in both cases. Taken together, similar values between Italian and Croatian populations were found only for *C. album* regarding  $T_b$  and for *A. retroflexus* regarding  $\Psi_b$ .

**Table 5.** Base temperature ( $T_b$ ) and base water potential ( $\Psi_b$ ) of weed species for Italian and Croatian population, confidence interval (95 % CI) and coefficient of determination ( $r^2$ ). Italian biological parameters for three weed species (*A. retroflexus*, *C. album*, *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	$r^2$	$\Psi_b$ (MPa)	± 95% CI	$r^2$	$\Psi_b$ (MPa)	± 95% CI	$r^2$
<i>A. retroflexus</i>	12.3	1.12	0.92	13.9	0.36	0.96	-0.41	0.07	0.92	-0.36	0.03	0.95
<i>C. album</i>	2.6	0.77	0.84	3.4	0.36	0.98	-0.96	0.10	0.84	-1.42	0.14	0.87
<i>S. pumila</i>	10.4	0.95	0.97	6.59	0.09	0.96	-0.93	0.11	0.85	-0.70	0.07	0.92
<i>P. capillare</i>	-	-	-	11.0	1.99	0.82	-	-	-	-0.86	0.07	0.94

#### 4. Discussion

In the present study biological parameters for germination ( $T_b$  and  $\Psi_b$ ) 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. Species are ranged from less to more thermophilic as follow: *C. album* > *S. pumila* > *P. capillare* > *A. retroflexus*. This is consistent with previous studies in which *A. retroflexus* also germinated 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 again consistent with previous study where *P. dichotomiflorum* achieved the highest germination capacity at temperatures of 25°C [26-29] or *P. miliaceum* at temperatures between 18 and 25°C [30,31]. Optimal temperature for 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 (Table 1).

If we related the temperature preferences that define germination to 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 3) 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 other species in the study (Table 3). Germination stopped completely at 12°C confirming its thermophilic behavior [2].  $T_b$  values for *A. retroflexus* is 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 later emergence is observed for *A. retroflexus* in maize fields compared to other weeds studied [33]. This  $T_b$  is slightly higher than the values around 12°C reported for 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  $T_b$  for *C. album* is 3.4°C making it the species with the lowest temperature requirement 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 described 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, French and California [1, 19, 30]. In our study we also estimated  $T_b$  for *P. capillare*, and as far as we know, this is the first report globally on base temperature for this weed species. We found that 11°C is the base temperature for *P. capillare*. Our results are consistent with the study where the minimum temperature for germination of *P. miliaceum* was estimated to be also 11°C [31].

Since temperature is not the only factor triggering germination in the present study, we also observed the germination capacity of the same weed species under different water potentials. Depending on water requirements, the species in this study are ranged from less to more tolerant as follow: *A. retroflexus* ( $\Psi_b$  -0.36 MPa) < *S. pumila* ( $\Psi_b$  -0.71 MPa) < *P. capillare* ( $\Psi_b$  -0.86 MPa) < *C. album* ( $\Psi_b$  -1.38 MPa) (Figure 2). In addition to the study performed to calibrate AlertInf model [1], only a single study conducted in France has already determined  $\Psi_b$  for these species [19]. The value of  $\Psi_b$  estimated for the Croatian population of *A. retroflexus* is similar to the  $\Psi_b$  used in AlertInf for a population from Northern Italy, while lower values were reported for other populations from Central Italy (-0.62 MPa) or France (-0.95 MPa) [1,19]. Regarding  $\Psi_b$  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  $\Psi_b$  in comparison with the values previously reported for both Italian (-0.96 and -1.04 MPa) and French (-0.80 MPa) populations [1,19]. For the species *P. capillare* as far as we know there are no data of  $\Psi_b$  in the literature. Germination behavior of the investigated species at different temperature and water po-

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

The main objective of the present study was to compare the estimated values of the Croatian population with Italian populations of *C. album*, *S. pumila* and *A. retroflexus* included in the hydrothermal model AlertInf. We wanted to examine if the  $T_b$  and  $\Psi_b$  values estimated in this study would be comparable with those in AlertInf [4] as a first step of transferring weed predictive model AlertInf in Croatian maize fields. Two out of three Croatian species had a parameter overlapping with the Italian population: *C. album* had similar  $T_b$ , but different  $\Psi_b$ , *A. retroflexus* different  $T_b$  but similar  $\Psi_b$ , and *S. pumila* differed in both germination parameters (Table 5).

Present study therefore showed that similar germination of Croatian and Italian population 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  $\Psi_b$  differed significantly between two populations. Next step therefore will be to evaluate the weed emergence patterns of *C. album* in irrigated maize fields in Croatia and then try to calibrate the AlertInf equation parameters. Prediction of *A. retroflexus* and *S. pumila* by AlertInf model with its original parameters is unfortunately impossible, even in irrigated maize crops, since the  $T_b$  values differ statistically with those Italian presents in the hydrothermal model. This variability in germination parameters has also been documented in previous studies and usually explained as adaptation process of weed species to local climate conditions [13, 36]. The annual air temperature in Zagreb is on average lower than those in Padova (Table 1). 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 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 accomplished for *S. pumila* and *C. album*, however this phenomenon was not found for *A. retroflexus*, where  $T_b$  is 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 the difference in dormancy and germination requirements [37, 38]. An attempt to implement the model in another agro-ecological area was also made by these Bürger and Colbach [15] for the FlorSys model. The difference in base temperature for different species was also species-specific and unable to find the pattern connected to climate conditions. They have found 4.3 lower  $T_b$  for *C. album* and 4.0 °C higher  $T_b$  *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]. Present study offers the ability to implement the predictive emergence model AlertInf only for *C. album* in non-irrigated field. However, the results are valuable since it provides estimated biological parameters of four species, which have never been estimated before in Croatia. This is the first and obligatory step towards developing/transferring a model to predict their emergence.

However, further field experiment is necessary and should be performed in two directions depending on the weed species. First, for *C. album* or *A. theophrasti* since their estimated  $T_b$  are overlapping with Italian populations [16], the AlertInf has to be further validated by comparing the emergence of species in maize fields with those predicted with AlertInf. Secondly, the model should be adapted for species *S. pumila* and *A. retroflexus* since biological parameters differed significantly. And the third, AlertInf should be upgraded for *P. capillare* whose biological parameters have now been estimated for the first time.



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