Vodno-toplinsko modeliranje za prognozu nicanja korova u kukuruzu

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Valentina Šoštarčić

PREDICTING WEED EMERGENCE IN MAIZE WITH HYDROTHERMAL MODELLING

DOCTORAL DISSERTATION

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Sveučilište u Zagrebu

Agronomski fakultet

Valentina Šoštarčić

VODNO-TOPLINSKO MODELIRANJE ZA PROGNOZU NICANJA KOROVA U KUKURUZU

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Mentori:
izv. prof. dr. sc. Maja Šćepanović
izv. prof. dr. sc. Roberta Masin

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With my signature I confirm that:

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Maja Šćepanović is associate professor at University of Zagreb Faculty of Agriculture. In 2011 she obtained a Ph.D. „Effect of repeated low-rate herbicide treatments on weed control and sugar beet yield (*Beta vulgaris* L. var*. altissima* Doll) at University of Zagreb Faculty of Agriculture. In 2018 she became an associate professor and Head of Department of Weed Science at Faculty of Agriculture. She was trained in 2002 in Denmark at two-week training program on "Environmental protection ", and one-week program in 2015 "Training school on Controlling Common Ragweed by Vegetation Management, Germany" and in 2012 "Weed Management and Control, Osijek". She is a professor on several obligatory and elected modules on undergraduate, graduate, and postgraduate level at the University of Zagreb Faculty of Agriculture, in weed science and weed management. Since 2021 she is a coordinator of a Study Programme Phytomedicine. She took part in several national and international scientific, professional, and teaching projects within the topic of integrated plant management such as COST „Sustainable management of *Ambrosia artemisiifolia* in Europe ", Ministry of Agriculture „ Monitoring of resistant pests to plant protection product in Croatia from 2018 to 2020"and Erasmus plus „Harmonization and Innovation in PhD Study Programs for Plant Health in Sustainable Agriculture – HarISA". She is referee of several international journals, among which Plants, Agronomy, Weed Research and Journal of Central European Agriculture and from 2021 she is editor in MDPI journal Plants. She is a member of the Managing Board of Croatian Plant Protection Society and member of European Weed Research Society (EWRS). Her main research topics are weed biology and ecology, allelopathy and sustainable weed management as well as detecting of herbicide resistance weeds. She took part in several national and international congresses and in 2021 she was a guest speaker at 11th Weed Science Congress in Serbia. She is author of 70 peer-reviewed papers within which 18 international peer-reviewed paper (Scopus 2021).

Supervisor information

Roberta Masin is associate professor at the Department of Agronomy Food Natural Resources Animal and Environment (DAFNAE) of Padova University. She was assistant professor in the same department from 2011 to 2017. In 2004, she obtained the PhD in Environmental Agronomy on the use of emergence predictive model for turf weeds. She was visiting scientist at the USDA-ARS North Central Soil Conservation Research Laboratory (Minnesota, USA) and at the Danish Institute of Agricultural Sciences, Slagelse, Denmark. She is engaged in teaching activities in Bachelor and Master degrees at the University of Padova. The main courses are: Weed Science, Environmental Statistics and Weed Control in Organic Agriculture. She is programme coordinator of the Bachelor's Degree in Organic Crop Production and she was vice-coordinator of the PhD course in Crop Science (DAFNAE, University of Padova) from Sept. 2019 to Sept. 2021. She is vice-president of the Italian Weed Research Society (SIRFI).

She took part several national and international projects, in 3 projects of national interest (PRIN), and in 2 European projects (EU FP7 ENDURE - European Network for the Durable Exploitation of Crop Protection Strategies, and EU FP7 PURE - Pesticide Use-and-risk Reduction in European farming systems with Integrated Pest Management). She is referee of several journals, among which Weed Research, Weed Science, and Frontiers in Agronomy. He is member of the Editorial Board of Italian Journal of Agronomy, Plants and Science. She is a member of the Italian Agronomy Society (SIA), Italian Weed Research Society (SIRFI) and European Weed Research Society (EWRS). Her main research topics are weed biology and ecology, with particular emphasis on weed seed germination and early growth, modelling of weed emergence for crop protection, crop-weed competition, development of DSS for weed control, and study of agricultural pesticide mitigation measures. She is author of 52 peer-reviewed international papers (Scopus 2021) and took part in several national and international congresses. Her H-index is equal to 11, for a total of 430 citations (Scopus 2021).

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Summary

Maize is the most widespread crop in Croatia and weeds are the main production limiting factors. In modern maize production herbicide application is performed after weed and crop emergence. Since weed species can differ in the time and duration of emergence to achieve appropriately timed weed control, it is necessary to determine the period in the field when the largest population of weed species is expected. Weed emergence prediction models are being developed to predict peak periods of weeds so that farmers can determine the appropriate time to apply herbicides. Soil temperature and soil moisture are the two main factors affecting weed emergence under field conditions. Therefore, hydrothermal models can be used to predict weed emergence in agricultural crops. Hydrothermal models summarize thermal units subtracted from the value of base temperature (T_b) when the soil water potential is above the value of base water potential of the species (Ψ_b). AlertInf is an Italian hydrothermal model for weed emergence prediction in maize developed in the Veneto region. The possibility of validating this model to Croatian maize crops was tested during this doctoral research. Prior to validate the model, estimation of germination parameters (T_b and Ψ_b of each weed species) is required. In the doctoral research, germination parameters of economically important weeds in maize crop in Croatia (*Amaranthus retroflexus*, *Chenopodium album*, *Echinochloa crus-galli*, *Abutilon theophrasti*, *Setaria pumila*, *Panicum capillare* and *Ambrosia artemisiifolia*) were estimated. Naimely, if the biological parameters of native and foreign populations of the same species differ it is necessary to calibrate the model.

The estimated T_b and Ψ_b of of the studied species are: *Ambrosia artemisiifolia* (1.5°C; -0.89 MPa), *Chenopodium album* (3.4°C; -1.38 MPa), *Abutilon theophrasti* (4.5°C; -0.67 MPa), *Setaria pumila* (6.6°C; -0.71 MPa), *Echinochloa crus-galli* (10.8°C; -0.97 MPa), *Panicum capillare* (11.0°C; -0.87 MPa), *Amaranthus retroflexus* (13.9°C; -0.36 MPa). No statistical difference was found between the Croatian and Italian populations of *Abutilon theophrasti* for both germination parameters. Therefore, the AlertInf model can be validated in the Croatian maize field without calibration. No statistical difference was found between the Croatian and Italian populations of *Echinichloa crus-galli* and *Amaranthus retroflexus* in the base water potential parameters. However, a statistical difference was found in the base temperature parameters for these two species. Therefore, AlertInf should be calibrated for these two species and validated for the base temperature parameters. Statistical differences between Croatian and Italian populations of *Setaria pumila* were found for both parameters studied (T_b and Ψ_b). In order to use the AlertInf model in Croatian maize fields, the model should be calibrated and validated for both studied parameters. Since the AlertInf model does not consider the species *Ambrosia artemisiifolia* and *Panicum capillare*, it should be updated and validated for this two weed species in maize fields in continental Croatia.

The emergence of *Echinochloa crus-galli* in Croatian maize fields was successfully predicted with AlertInf including estimated germination parameters of the native population. The overall performance of the model was evaluated by the root mean square error (RMSE) and modeling efficiency (EF). The RMSE is 1.69 and 1.38 for 2019 and 2020, respectively. In addition, EF is 0.97 and 0.98 in 2019 and 2020, respectively. With the calibrated model AlertInf it is possible to predict the emergence of *Echinochloa crus-galli* in maize fields in continental Croatia.

The results obtained in the PhD thesis also have a practical value for maize growers and fit well with the EU Directive 2009/128/ EC on the sustainable use of pesticides and the European Commission's Green Deal initiatives to reduce pesticide use in agriculture.

Keywords: base temperature, base water potential, intergrated weed management, predictive weed emergence models, weeds

Prošireni sažetak (Extended summary in Croatian):

Naslov doktorske disertacije na hrvatskom jeziku (title of the doctoral thesis in Croatian)**:**

Vodno-toplinsko modeliranje za prognozu nicanja korova u kukuruzu

Kukuruz (*Zea mays* L.) je najraširenija ratarska kultura u Hrvatskoj, a korovi su glavni ograničavajući čimbenici suvremene proizvodnje. Korovna flora kukuruza na području kontinentalne Hrvatske sastavljena je od sljedećih vrsta: koštan (*Echinochloa crus-galli* (L.) P. Beauv.), pelinolisni limundžik (*Ambrosia artemisiifolia* L.), bijela loboda (*Chenopodium album* L.), oštrodlakavi šćir (*Amaranthus retroflexus* L.), sivi muhar (*Setaria pumila (*Poir.) Roem & Schult. (= *Setaria glauca* [L.] P. Beauv.; *Setaria lutescens* [Stuntz] F.T. Hubb.), europski mračnjak (*Abutilon theophrasti* Med.) vlasasto proso (*Panicum capillare* L.). Sukladno Direktivi 2009/128/ EC Europske unije o održivoj uporabi pesticida i novim strategijama europskog Zelenog plana o redukciji unosa pesticida u okoliš, usmjereno suzbijanje korova u suvremenoj proizvodnji kukuruza potrebno je provoditi nakon nicanja korova u tzv. post-emergence periodu. Koncept integrirane zaštite bilja zasniva se na poznavanju ekonomskog praga štetnosti kao ekonomski opravdanoj osnovi za primjenu herbicida. Drugim riječima iznad utvrđenog broja štetne populacije tretiranje treba provesti kako ne bi došlo do redukcije prinosa. Implementacija pragova odluke kod suzbijanja korova, međutim odvija se značajno sporije nego kod ostalih štetnih organizama. Naime, brojnost korova nije dobar pokazatelj potencijalnih šteta koje određena vrsta može nanjeti usjevu. Ukoliko korovna vrsta ponikne ranije u odnosu na usjev štete su veće u odnosu na korovne vrste koje poniknu kasnije u vegetaciji. Stoga se noviji pragovi oduke za suzbijanje temelje na poznavanju dinamike nicanja korovnih vrsta odnosno vremenu i trajanju nicanja korova temeljem čega se prilagođava primjena herbicida i ostale mjere borbe protiv korova. To je posebice važno danas zbog nove strategijje EU Zelenog plana čiji je cilj redukcija unosa pesticida u okoliš.

Temperatura i vlažnost tla dva su osnovna čimbenika koji određuju nicanje korova pa se za prognozu nicanja koriste dva tipa modela: toplinski i vodno-toplinski modeli. Toplinski modeli sumiraju toplinske jedinice iznad biološkog minimuma sjemena (T_b) odnosno utvrđene minimalne temperature za klijanje. Kod toplinskog modela može doći do odstupanja u očekivanom nicanju korova pri nedostatku vlage u tlu pa je predviđanje ovim modelom manje precizno u odnosu na vodno-toplinski model, a pogotovu u ne-navodnjavnim usjevima. Nasuprot tome, vodno-toplinski model zbraja toplinske jedinice od trenutka kad vodni potencijal tla dosegne vrijednost biološkog vodnog potencijala sjemena (Ψ_b), odnosno najnižu vrijednost vodnog potencijala tla pri kojem sjeme pojedine vrste klije.

Vodno-toplinski model AlertInf, jedan je od rijetkih prognoznih modela korištenih i u praktične, a ne samo znanstvene svrhe i dostupan je (https://www.arpa.veneto.it/upload_teolo/agrometeo/infestanti.htm) proizvođačima kukuruza u Italiji za prognozu nicanja korova. AlertInf trenutno prognozira nicanje deset korovnih vrsta: *Abutilon theophrasti*, svračica (*Digitaria sanguinalis* (L.) Scop.), *Echinochloa crus galli*, perzijski dvornik (*Polygonum persicaria* L.), zeleni muhar (*Setaria viridis* (L.) Beauv.), crna pomoćnica (*Solanum nigrum* L.), *Amaranthus retroflexus*, *Chenopodium album*, *Setaria pumila* i divlji sirak (*Sorghum halepense* (L.) Pers.).

Izrada vodno-toplinskog modela nicanja odvija se u četiri faze: (1) laboratorijsko utvrđivanje bioloških parametara klijanja (biološki minimum i biološki vodni potencijal) pojedine korovne vrste; (2) praćenje dinamike nicanja korova i monitoring temperature i vodnog potencijala tla u zoni nicanja korova u poljskim uvjetima; (3) izračun sume vodno-toplinskih jedinica i izrada krivulje dinamike nicanja korova u usjevu – prognoza nicanja te (4) validacija modela u usjevu kukuruza. Primjena vodno-toplinskog modela zahtjeva utvrđivanje vrijednosti bioloških parametara svake vrste uključene u model što dosad nije istraživano u Hrvatskoj. Naime, u različitim geografskim područjima korovne vrste mogu varirati u vrijednostima bioloških parametara, a koje su nužne za primjenu, odnosno korištenje prognoznih modela.

Stoga je preduvjet za implementaciju modela razvijenog na drugom geografskom području preklapanje vrijednosti bioloških parametara domaćih populacija s onim vrijednostima populacija ugrađenim u postojeći model. Ukoliko se vrijednosti bioloških parametara ne razlikuju između dvije populacije, model je moguće validirati u polju praćenjem nicanja istraživanih vrsta. Ukoliko postoji statistička razlika u vrijednostima bioloških parametara model je potrebno kalibrirati za vrijednosti nativnih populacija.

Stoga su postavljene hipoteze istraživanja: (1) bioloških parametri klijanja (biološki minimum i biološki vodni potencijal) nativnih populacija korova razlikovat će se od stranih populacija ugrađenih u AlertInf model; (2) primjenom AlertInf modela s utvrđenim vrijednostima biološkog minimuma i biološkog vodnog potencijala nativnih populacija moguće je prognozirati dinamiku nicanja korova u polju.

Cilj istraživanja u sklopu doktorske disertacije bio je utvrditi: (1) biološke parametre (biološki minimum i biološki vodni potencijal) sedam korovnih vrsta (*Abutilon theophrasti*, *Echinochloa crus-galli*, *Amaranthus retroflexus*, *Chenopodium album*, *Setaria pumila*, *Panicum capillare*, *Ambrosia artemisiifolia*) prisutnih u usjevu kukuruza u Hrvatskoj i usporediti ih s vrijednostima istih vrsta ugrađenih u AlertInf model; (2) dinamiku nicanja korova u polju korištenjem biološkog minimuma i biološkog vodnog potencijala za nativne populacije uključujući dvije vrste koje dosada nisu bile uključene u AlertInf model.

Istraživanje je provedeno u laboratorijskim i poljskim uvjetima. U laboratorijskim uvjetima provedeno je utvrđivanje bioloških parametara. Sjeme ovih vrsta sakupljeno na lokacijama Šašinovec (45°50'59.6"N;16°09'53.9"E), Maksimir (45°49'34.3"N;16°01'49.8"E) i Lipovec Lonjski (45°44'51.9"N;16°23'12.4"E). Očišćeno sjeme je do početka istraživanja hladno uskladišteno (4°C) uz relativnu vlažnost zraka 70 %. Za utvrđivanje biološkog minimuma, u Petrijeve zdjelice na filter papir uz dodatak destilirane vode položeno je 100 sjemenki svake istraživane vrste. Posijano sjeme je postavljeno na klijanje u klima komore pri rasponu od najmanje sedam konstantnih temperatura zraka, ovisno o istraživanoj korovnoj vrsti i fotoperiodu 12h : 12h (dan : noć).

Za određivanje biološkog vodnog potencijala 100 sjemenki je položeno u Petrijeve zdjelice na osam različitih koncentracija polietilen-gilkola (PEG 6000) za simulaciju vodnog potencijala tla. Istraživani vodni potencijali iznosili su: 0,00 (destilirana voda), -0,05, -0,10, -0,25, -0,38, -0,50, -0,80, -1,00 MPa. Pokusi su provedeni pri konstantnoj temperaturi od 22 °C i fotoperiodu 12h : 12h (dan : noć).

U laboratorijskim istraživanjima za utvrđivanje biološkog minimuma i biološkog vodnog potencijala klijavost je provjeravana dva puta dnevno pri visokim temperaturama (> 20 °C) i visokim vodnim potencijalima (> -0,38 MPa), te jednom dnevno na niskim temperaturama (< 20 °C) i niskim vodnim potencijalima (< - 0,38 MPa). Biološki parametri klijanja (biološki minimum i biološki vodni potencijal) utvrđeni su korištenjem podataka o dinamici klijanja pri različitim temperaturama i vodnim potencijalima za svaku vrstu. Krivulja dinamike klijanja izrađena je koristeći logističku funkciju u statističkom programu Bioassay97 s ciljem utvrđivanja srednjeg vremena klijanja (t_{50}). Recipročna vrijednost t_{50} ($1/t_{50}$) korištena je za izradu pravca linearne regresije. Vrijednost biološkog minimuma i biološkog vodnog potencijala predstavljaju točku u kojoj pravac linearne regresije siječe os x. Intervali pouzdanosti za biološki minimum i biološki vodni potencijal utvrđeni su prema bootstrap metodi. Potom su utvrđene vrijednosti bioloških parametara nativnih populacija uspoređene s vrijednostima talijanskih ugrađenih u AlertInf model, prema kriteriju 95 % preklapanja granica pouzdanosti. U slučaju da preklapanja granica pouzdanosti nije bilo, utvrđena je značajna statistička razlika između dvije populacije.

Poljski pokus proveden je tijekom dvije vegetacijske sezone kukuruza na lokaciji Šašinovec praćenjem nicanja korovne vrste *Echinochloa crus-galli* u usjevu. Praćenje nicanja provedeno je postavljanjem 12 kvadrata (0,3 x 0,3 m) između redova kukuruza. Ponikli prvi listovi uklanjani su tri puta tjedno bez dodatnog okretanja tla. Temperatura i vlažnost tla praćeni postavljanjem termometra (HOBO UA-001-08, Onset Computer Corporation, Bourne, MA) i uređaja za mjerenje vlage (ECH2O 10HS Soil Water Content sensor, Meter Group Inc., USA) na dubini do 5 cm unutar istraživanih kvadrata.

Rezultati istraživanja ukazuju da se istraživane korovne vrste razlikuju u vrijednostima bioloških parametra klijanja. Utvrđeni biološki minimumi istraživanih vrsta su: *Ambrosia*

artemisiifolia (1,5°C), *Chenopodium album* (3,4°C), *Abutilon theophrasti* (4,5°C), *Setaria pumila* (6,6°C), *Echinochloa crus-galli* (10,8°C), *Panicum capillare* (11,0°C), *Amaranthus retroflexus* (13,9°C). Utvrđeni biološki vodni potencijalih istraživanih vrsta su: *Chenopodium album* (-1,38 MPa), *Echiniochloa crus-galli* (-0,97 MPa), Ambrosia artemisiifolia (-0,89 MPa), *Panicum capillare* (-0,87 MPa), *Setaria pumila* (-0,71 MPa), *Abutilon theophrasti* (-0,67 MPa) and *Amaranthus retroflexus* (-0,36 MPa). Rezultati istraživanja pokazuju da između hrvatske i talijanske populacije korovne vrste *Abutilon theophrasti* nisu utvrđene statistički značajne razlike u vrijednosti biološkog minimuma i biološkog vodnog potencijala. Ovo je ujedno i jedina vrsta kod koje statistička razlika nije utvrđena između oba parametra u dvije populacije. Nadalje, statistički značajna razlika utvrđena je između hrvatske i talijanske populacije korovnih vrsta *Echinichloa crus-galli* i *Amaranthus retroflexus* u vrijednosti biološkog minimuma dok za iste vrste nije utvrđena statistička razlika u vrijednosti vodnog potencijala. Statistička značajna razlika utvrđena je za oba parametra između hrvatske i talijanske populacije korovnih vrsta *Chenopodium album* i *Setaria pumila*.

Prema navaedenim rezultatima, za korovnu vrstu *Abutilon theophrasti* moguća je diretkna validacija AlertInf modela u polju bez prethodne kalibracije vrijednosti bioloških parametara modela. Za vrste *Echinochloa crus-galli* i *Amaranthus retroflexus* validaciji modela mora prethoditi kalibracija u usjevu s utvrđenim biološkim minimumima, a kalibracija je potrebna za oba istraživana parametra za vrste *Chenopodium album* i *Setaria pumila*. S obzirom da AlertInf ne prognozira nicanje vrsta *Panicum capillare* i *Ambrosia artemisiifolia* za ove vrste potrebno je nadograditi model te ga validirati u polju.

U usjevu kukuruza praćenjem nicanja korovne vrste *Echinochloa crus-galli* validran je model AlertInf u kojeg su ugrađene utvrđene vrijednost biološkog minimuma nativne populacije. Kalibriranim modelom AlertInf uspješno je predviđeno nicanje korovne vrste *Echinochloa crus-galli* u kukuruzu u kontinentalonoj Hrvatskoj. Uspješnost modela modela procijenjena je korijenom srednje kvadratne pogreške (RMSE) i indeksom učinkovitosti modeliranja (EF). RMSE je iznosio 1,69 u 2019., odnosno 1,38 u 2020. a EF je iznosio 0,97 za 2019. i -0,98 za 2020. Stoga rezultati ukazuju da je korištenjem kalibriranog modela AlertInf moguće predvidjeti nicanje korovne vrste *Echinochloa crus-galli* u kukuruzu u kontinentalnoj Hrvatskoj.

Ključne riječi: biološki minimum, biološki vodni potencijal, korovi, prognoza nicanja, integrirano suzbijanje

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Publication No. 1

Šoštarčić, V.; Šćepanović, M.; Masin, R.; Maggoso, D.; Zanin, G. (2018). Estimation of biological parameters for germination of *Abutilon theophrasti* Medik., *Periodicum biologorum*, **120** *(2-3)*, 81-89 doi:10.18054/pb.v120i2-3.5048

Publication No. 2

Šoštarčić, V.; Masin, R.; Turčinov, M.; Carin, N.; Šćepanović, M. (2020). Intrapopulation variability in morphological and functional properties of *Ambrosia artemisiifolia* L. seeds, *Journal of Central European Agriculture*, **21** *(2)*, 366-378, doi:10.5513/JCEA01/21.2.2622

Publication No. 3

Šoštarčić, V.; Masin, R.; Loddo, D.; Brijačak, E.; Šćepanović, M. (2021). Germination parameters of selected summer weeds: transferring of the AlertInf model to other geographical regions, *Agronomy*, **11** *(2)*, 292-307 doi:.org/10.3390/agronomy11020292

Publication No. 4

Šoštarčić, V.; Masin, R.; Loddo, D.; Svečnjak, Z.; Rubinić, V.; Šćepanović, M. (2021). Predicting the emergence of *Echinochloa crus-galli* (L.) P. Beauv.in maize crop in Croatia with hydrothermal model, *Agronomy*

1. GENERAL INTRODUCTION

Maize is one of the world's most important crops, with more than 190 million hectares cultivated annually (FAOSTAT, 2021). The United States accounts for the largest share of global maize production (52.2%), while Europe accounts for 11.2%. Among the 27 countries belonging to the European Union, Romania, France, Hungary, Poland, and Italy are the main leaders in the maize production, while Croatia ranks 11th (EUROSTAT 2021). In Croatia, maize is the most important crop, grown on 285,000 hectares with yield of 7.6 t/ha in 2021. Compared to other cereals, wheat is grown on 144,900 hectares with 6.6 t/ha, barely on 57,000 hectares with 5.40 t/ha, while arable crops such as soybean and sunflower are grown on 85,000 (3.0 t/ha) and 40,000 (3.0 t/ha) hectares, respectively, (EUROSTAT, 2021).

The main production limiting factors in maize are generally weeds, which cause 40.3% (37-44%) potential yield loss of maize without control measures (Oerke, 2005). In Croatia, Goršić (2012) measured 67% of yield losses caused by uncontrolled weeds. In all arable crops weeds are mainly controlled with herbicides. However, herbicide control faces challenges due to the required EU agricultural policy regulated by Directive 2009/128/ EC and the Green Deal strategies presented in December 2019. Directive 2009/128/ EC require sustainable pesticide use and therefore promotes Integrated Pest Management (IPM) as one of the main tools to reduce pesticide use in agriculture. One of the principles of IPM is pest monitoring, i.e., the application of plant protection measures depending on the thresholds for the specific pest. In other words, to avoid yield reductions, pesticides should be only applied when the pest population is above an economic threshold, i.e., above a certain number of pest populations that lead to yield losses. Economic decision thresholds have been used for entomological purposes since the 1970s (Stern, 1973), but the adoption of decision thresholds for weed control has been much slower (Swanton et al., 1999). Although weed density has been successfully used to calculate economic thresholds for decision support models, several problems have arisen in applying the concept of economic thresholds to weeds, including the effects of multiple weed species, environmental conditions varying from site to site or year to year, and weed seed production on future weed problems (Werner et al., 2004).

One of the most important factors affecting yield loss is the timing of weed and plant emergence in the field. For example, Knezevic et al. (1994) found that 0.5 and 8 plants of *Amaranthus retroflexus* L. per maize row reduced maize yield by 5 to 34% when they emerged during the BBCH 14 and BBCH 16 maize growth stages. However, the same number of plants that emerged at BBCH 17 and 19 reduced maize yield by 5 to 10%. This suggests that weeds that emerge early in the growing season have the greatest potential impact on crop productivity, indicating that weed control should be conducted early in the season. In addition, weeds that emerge later may escape early control measures (Sattin et al. 1992). Therefore, for sustainable maize production, it is not only important to calculate economic thresholds but also to know the dynamics of weed emergence in the crop. Although herbicides can be applied as pre-emergence soil herbicides or post-emergence soil/leaf herbicides, the integrated weed management (IWM) approach recommends post-emergence applications. Once the weeds and crop have emerged, it is possible to select an effective herbicide or combination of herbicides based on the composition of the weed flora. However, accurate post-emergence weed control is only possible if the correct timing for herbicide application is known. An early herbicide application may result in a reapplication due to the new weed flush. Conversely, a later application may result in less herbicide efficacy because the weeds are at a phenologically advanced stage that is less sensitive to herbicides. For example, *Chenopodium album* L. develops a thicker wax cuticle (Sanyal et al., 2006; Taylor et al., 1981) and *Abutilon theophrasti* Medik. denser hairs (Sanyal et al., 2006) or *Alopecurus myosuroides* Huds. multiple growing points that may reduce herbicide uptake (Pintar et al., 2021). Therefore, to achieve precise weed control, it is necessary to determine the period in the field when the largest population of weed species can be expected. In other words, the dynamics of weed emergence should be known.

Soil temperature and soil moisture are the two most important factors that determine weed emergence (Forcella et al., 2000). Therefore, two types of models can be used to predict weed emergence: Thermal Time (TT) and Hydrothermal Time (HTT) models (Masin et al., 2010). TT models summarise thermal units subtracted from the base temperature (T_b) value, which is the minimum temperature required for seed germination (Royo-Esnal et al., 2010). However, TT models are generally less accurate than HTT models because they are not able to predict the variation in expected weed emergence in the absence of soil moisture during dry periods (Forcella et al., 2000). The HTT model sums the heat units when the soil water potential reaches the value of the base water potential (Ψ_b), which is the lowest value of soil water potential at which seeds can germinate (Gummerson, 1986). Hence, HTT models used both germination parameters (T_b and Ψ_b) to predict emergence. HTT models are constructed in several phases. In the first phase, the germination parameters (T_b and Ψ_b) must be determined for each weed species in the laboratory. In the second phase, weed emergence in the field is monitored over several years at different locations, recording daily soil microclimatic conditions (T and Ψ in the weed emergence zone). By combining laboratory and field data, emergence curves are generated based on the sum of hydrothermal (HT) units, which represent the prediction of weed emergence. The final stage of model development is to test the validity of the model by using it to predict weed emergence in the field (Masin et al., 2012).

Predictive models have been developed for maize in Italy (Masin et al., 2012) and Spain (Dorado et al., 2009), for soybean in Italy (Masin et al., 2014) and Nebraska (Werle et al., 2014), and for winter cereals in Spain (Royo- Esnal et al., 2015). Most of these predictive emergence models are used for scientific purposes. However, a simplified version of the Italian HTT model AlertInf has been successfully used by maize producers in Italy (Veneto region) to predict weed emergence (Masin et al., 2010) for ten species: *Abutilon theophrasti*, *Digitaria sanguinalis* (L.) Scop., *Echinochloa crus - galli* (L.) P. Beauv., *Polygonum persicaria* L., *Setaria viridis* (L.) Beauv., *Solanum nigrum* L., *Amaranthus retroflexus*, *Chenopodium album*, *Setaria pumila* (Poir.) Roem & Schult. and *Sorghum halepense* (L.) Pers. (Masin et al., 2010).

Currently, there are no models for weed emergence prediction in Croatia and a possible extension of the HTT AlertInf model from Italy to Croatia could be tested. A prerequisite for implementing a model that has already been developed in another geographical area is to check whether the values of the germination parameters of the "native" populations overlap with those of the existing model. Weed species, as non-cultivated species, exhibit variability within and between populations due to adaptation to changing environmental conditions. This is particularly evident in introduced invasive weed species (e.g., *Ambrosia artemisiifolia* L.) (Clematis and Jones, 2021). This feature makes it difficult to apply the same model to larger areas (Fumanal et al. 2007; Loddo et al. 2018) without prior verification of germination parameters and calibration of the model with the estimated parameters. For example, Leiblein-Wild et al. (2014) estimated differences in T_b between European and native North American populations of *Ambrosia artemisiifolia* (2.0°C and 4.2°C, respectively). In addition, Bürger and Colbach (2018) estimated different T_b values for *Chenopodium album* and *Echinochloa crus-galli* between French and German populations (5.8°C vs. 1.5°C and 6.2°C vs. 10.2°C, respectively). Although there are few data on estimated Ψ_b in different populations, Masin et al. (2010) also estimated different values for *Amaranthus retroflexus* in populations of the two regions in Italy (Padua, Pisa). The estimated values Ψ_b for the populations of Padua and Pisa were -0.41 MPa and -0.62 MPa, respectively.

It is evident that weed species in different geographical areas may vary in the values of germination parameters. For five species (*Abutilon theophrasti*, *Echinochloa crus-galli*, *Amaranthus retroflexus*, *Chenopodium album* and *Setaria pumila*), prediction of weed emergence is possible in Croatia based on germination parameters of Italian populations (Masin et al., 2010). If germination parameters of native and foreign populations of the same species differ, the model needs to be updated according to the steps described above. In addition, AlertInf has not yet been used to predict the emergence of *Ambrosia artemisiifolia* and *Panicum capillare* L., which are economically important weed species of maize in Croatia.

1.1. Research hypotheses and objectives

Research hypotheses

1. The germination parameters (T_b and Ψ_b) of native weed populations will differ from the foreign populations built into the AlertInf model.

2. Applying the AlertInf model with determined values of T_b and Ψ_b of native populations, it is possible to predict the weed emergence dynamics in maize field in Croatia.

Objectives of the research

1. To estimate T_b and Ψ_b for seven weed species (*Abutilon theophrasti*, *Echinochloa crus-galli*, *Amaranthus retroflexus*, *Chenopodium album*, *Setaria pumila*, *Panicum capillare*, *Ambrosia artemisiifolia*) in maize in Croatia and compare them with the values of the same species built in the AlertInf model.

2. To determine the weed emergence dynamics in the field using T_b and Ψ_b for native populations including two species that have not been included in the AlertInf model.

2. OVERVIEW OF PREVIOUS RESEARCH

2.1. Weeds of economic importance to maize production

Maize is the most widespread crop in Croatia, with a cultivated area of over 285,000 ha in 2021 (EUROSTAT, 2021). The use of cultivated land and the success of production are reflected in the yield obtained, which varies under the influence of various production factors. Compared to other abiotic and biotic factors that can reduce yield (drought, nutrient deficiency, pests, etc.), the main limiting factors of modern maize production are weeds (Subedu and Ma, 2009). As a botanical pest, weeds share the same trophic level as crop plants, and weed-crop competition for light, water, and nutrients results in substantial crop yield losses (Swanton et al., 2015; Ramesh et al., 2017).

According to Oerke (2005) the potential yield loss due to weeds without control measures is 40.3% (37- 44%), which is equal to the potential damage due to diseases and pests combined. The actual damage, i.e., damage with the implementation of protective measures, caused by weeds in maize is on average 10.5% of the genetically possible yield of maize. The percentage of yield losses depend on the composition of the weed flora, the number and competitiveness of the dominant weeds, and the timing and duration of weed emergence in the crop (Ivanek-Martinčić et al., 2010; Knezevic et al., 1994).

In Croatian maize crops composition of weed flora is made of the following species determined in the 40-yr (1969 - 2009) survey in continental Croatia: *Echinochloa crus-galli* (L.) P. Beauv. (barnyardgrass) > *Ambrosia artemisiifolia* L. (common ragweed) > *Chenopodium album* L. (common lambsquarters) > *Amaranthus retroflexus* L. (redroot pigweed) > *Setaria pumila* (Poir.) Roem & Schult. (= *Setaria glauca* [L.] P. Beauv.; *Setaria lutescens* [Stuntz] F.T. Hubb.) (yellow foxtail) (L.) > *Abutilon theophrasti* Med. (velvetleaf) (Šarić et al., 2011). Moreover, in the last decade *Panicum capillare* L. (witchgrass) has also been regularly detected in the maize crop (Šćepanović et al., 2016; Burul et al., 2020).

Table 1. Impact of weed density on the yield reduction of maize

Species	**Plant/m^2**	**Yield losses (%)**	**References**
Echinochloa crus-galli	18	50	Kropff et al. (1984)
Panicum capillare	5	4-5	Ontario Weed Committee (2021)
Chenopodium album	172	6	Sibuga and Bandeen (1980)
	277	58	Ngouajio et. al. (1999)
Ambrosia artemisiifolia	9	53	Varga et. al. (2000)
	26	71	
Abutilon theophrasti	2	5	Werner et al (2004)
	4	32	Varga et. al. (2000)
	9	53	

Echinochloa crus-galli, *Setaria pumila* and *Chenopodium album* are species native to Europe and Croatia, with a worldwide distribution (Holm et al., 1997). Although some authors reported *Chenopodium album* to be native to western Asia, but the origin of this species is still not fully clear (Anonymus, 2017). *Panicum capillare*, *Ambrosia artemisiifolia* and *Amaranthus retroflexus* are invasive species in Europe (Baskin and Baskin, 1986; Essl et al., 2015; Sauer, 1967), originating from North America, while *Abutilon theophrasti* is an invasive species in Europe, originating from Asia (Spencer, 1984). All species are noxious weeds in Europe and the rest of the world infesting mostly summer crops: corn, soybean, sunflower, sugar beet etc.

All the above species are highly competitive in maize and cause large yield losses. However, these species have been found to compete differently in maize (see Table 1). For example, 50% of maize yield is reduced by 9 individuals *of Ambrosia artemisiifolia*, 18 individuals of *Echinochloa crus-gallli* and 277 individuals of *Chenopodium album* /m^2 (Varga et. al., 2000; Kropff et al., 1984; Ngouajio et. al., 1999).

Apart from competition, allelopathy effect of some weeds on the maize early development have been reported. Several studies have investigated the inhibitory effect of *Amaranthus retroflexus* (Szabó et al., 2018; Konstantinović et al., 2014; Bohowmik and Doll, 1982), *Abutilon theophrasti* (Nádasy et al.,2018; Šćepanović et al., 2007;), *Chenopodium album* (Kakar et al., 2016; Bohowmik and Doll, 1982), *Ambrosia artemisiifolia* (Lehoczky et al., 2011) and *Setaria pumila* (Bohowmik and Doll, 1982) extracts on early germination of maize. Moreover, Bohowmik and Doll (1982) found that the residues of *Ambrosia artemisifolia*, *Abutilon theophrasti*, *Echinochloa crus-galli* inhibited maize growth in the greenhouse.

Except from the negative effects in maize production, *Chenopodium album*, *Amaranthus retroflexus* and *Ambrosia artemisiifolia* are allergenic species causing health issues – allergy to airbone pollen (pollinosis) to humans. The most allergenic species in Croatia and in the rest of the Eastern Europe is *Ambrosia artemisiifolia*. It is estimated that 10-15% of all pollinoses are caused by *Ambrosia artemisiifolia* pollen in Croatia (Galzina et al., 2010). In Hungary even 80% of the population is allergic to pollen of this species (Mezei et al., 1995). It has been found that an atmospheric pollen concentration between 10 and 50 grains/m^3 during 24h causes an allergic reaction (Bergmann et al., 2008; Solomon, 1984). The average daily atmospheric pollen concentration in Zagreb in the period from 2006 to 2009 was 16 - 86 grains/m^3 (Stjepanović et al., 2015).

Echinochloa crus-galli, *Setaria pumila* and *Panicum capillare* are annual monocotyledonous (narrow-leaved) plants belonging to the Poaceae family. The other four species are annual dicotyledonous (broad-leaved) species belonging to different families: *Amaranthus retroflexus* - Amaranthaceae, *Chenopodium album* - Chenopodiaceae, *Ambrosia artemisiifolia* - Asteraceae and *Abutilon theophrasti* - Malvaceae.

According to the carbon fixation pathways, *Abutilon theophrasti*, *Chenopodium album* and *Ambrosia artemisiifolia* are C3 species, while *Amaranthus retroflexus*, *Echinochloa crus-galii*, *Panicum capillare* and *Setaria pumila* are C4 species. With the mechanism avoiding photorespiration C4 species are better adapted to hot, dry environments. However, in today`s rising atmospheric carbon dioxide concentration it is expected that the growth of the C3 species will increase. Ziska and Caulfield (2000) found that the exposure to the 600 μmol mol^{-1} (projected 21st century concentration) carbon dioxide concentration increased *Ambrosia artemisiifolia* growth and pollen production to 320% compared to concentrations estimated during the experimental period (370 μmol mol^{-1}). This highlights the potential treat that can arise from the C3 species, especially *Ambrosia artemisiifolia* in the future.

Weed species can be monitored from an ecological point of view. Ecological indicator values were proposed by Landolt (1977) and describe a wide range of ecological factors: soil moisture (F), soil reaction (R), soil fertility (N), organic matter (H), texture, light availability (L), temperature (T) and continentality (C). Ecological indicator values are one of the most common tools used to summarize complex knowledge about the ecology of the individual organism (Nimis and Martellos, 2001). These values can help to compare ecological traits between species. In Table 2, ecological indicator values are categorized by Landolt (1977) and described for each species by Knežević (2006). According to the soil moisture factor (F), most species included in the doctoral thesis are indicators of dry sites (2), while *Echiochloa crus-galli* and *Ambrosia artemisiifolia* are described as species growing on moderately dry to moderately moist soils with a wide ecological range (F-3). Soil reaction values (R) indicate that all investigated species grow on moderately acidic soils (4.5 - 7.5). *Echinochloa crus-galli* is also an indicator of over-fertilized soils (N-4). *Ambrosia artemisiifolia* and *Setaria pumila* (H-2) can grow on mineral soils with low humus cover. In addition, *Ambrosia artemisiifolia* (D-2) can grow on rubble, gravel or soils with a high skeleton content (> 2 mm), while *Echinochloa crus-galli*, *Chenopodium album* and *Abutilon theophrasti* grow on soils with a low skeleton content (0.002 - 0.05 mm). *Echinochloa crus-galli* (L-3) is a partial shade plant, rarely growing in full light, but generally in more than 10% relative light. The other species are light pointers but also occur in partial shade. Of the species listed, *Chenopodium album*, as described by Landolt (1977), is a montane zone species that tolerates lower temperatures, while *Panicum capillare*, *Abutilon theophrasti*, and *Ambrosia artemisiifolia* are indicators of a warm environment.

Table 2. Ecological indicator values by Landolt (1977) for studies species described in Knežević (2006)

Weed species	**F**	**R**	**N**	**H**	**D**	**L**	**T**	**C**
Echinochloa crus-galli	3	3	5	3	4	3	4	3
Setaria pumila	2	3	4	2	3	4	4	3
Panicum capillare	2	3	4	3	3	4	5	3
Amaranthus retroflexus	2	3	4	3	3	4	4	3
Chenopodium album	2	3	4	3	4	4	3	3
Ambrosia artemisiifolia	3	3	4	2	2	4	5	3
Abutilon theophrasti	2	3	4	3	4	4	5	3

However, there are certain pitfalls in using ecological indicator values for different populations of the same species due to variation between and within populations of the same species (Nimis and Martellos, 2001). Weed species develop intra- and interpopulation variability as a result of adaption to environmental conditions and gene recombination in the process of hybridization and polyploidy (Fenner, 1991; Clements and Jones, 2021). Spreading of the weed species to wide regions provided gene recombination which is more evident for invasive species crossed with close genus of native. For example, *Amaranthus retoflexus* has approximately 50 variates, form and sub-forms mostly described in Europe (Costea et al, 2004). Similar, for species *Chenopodium album* morphological and physiological different biotypes can be found worldwide and the differences between those is rather hard to determined. Therefore, the studies of weed biology and ecology have to be conducted for the certain geographical area since due to diverse environment, plasticity and the adaptability of the weed species. Different ecological indicators effect development of the species. The focus of the thesis are the germination and emergence process, therefore the factors that influence these stages will be further elaborated.

2.2. Factors influencing seed germination and seedling emergence of weeds

2.2.1. Seed properties and (vertical) distribution in soil

Life cycle of annual plants begins and ends with the seed; therefore, the seed is a crucial phase strongly dependent to the future plant development. According to the life span of the seeds, species can be divided in three groups: microbiotic with seeds whose lifespan does not exceed 3 years; mesobiotic are those whose life-span ranges from 3–15 years; and macrobiotic whose life span ranges from 15 to more than 100 years. Seed (caryopsis) of *Echinochloa crus-galli* and *Setaria pumila* can be classified as mesobiotic seeds. Namely, caryopsis of *Echinochloa crus-galli* and *Setaria pumila* have ability to germinate 10 years after ripening (Campagna i Rapparini, 2008; Dawson and Bruns, 1975). On contrary, *Chenopodium album* can remain in soil ready to germinate 20 years (Wright, 1972), *Ambrosia artemisiifolia* more than 40 years (Cunze et al., 2013) same as *Amaranthus retroflexus* (Kivilaan and Bandurski, 1981) while *Abutilon theophrasti* can remain viabile for more than 50 years (Warwick and Black, 1988). This longevity classifies their seed as macrobiotic. For species *Panicum capillare* there are lack of information about the seed longevity. However, two related species present in the weed flora of Croatia, *Panicum miliaceaum* and *Panicum dichotomiflorum* have seed longevity 4 and 20 year, respectively (James et al, 2011). Longevity of the seed is strongly dependent on the dormancy further elaborated in the next subchapter.

Table 3. Fruit type, number of seeds per plant, seed mass and dimensions of weed species included in thesis

Weed species	**Fruit type**	**Number of seed per plant**	**Seed mass, g (1 000 seed weight)**
Ecinochloa crus-galli	Caryopsis	200 – 10 000 (Păunescu, 1997)	1.7 – 2.1 (Bajwa et. al., 2015)
Setaria pumila	Caryopsis	540 – 8 460 (Peters et al., 1961)	2.0 – 2.7 (Hulina, 1998)
Panicum capillare	Caryopsis	10 000 – 12 000 (Hulina, 1998)	0-1 - 0.3 (Gross et al., 1992; Shipley and Parent, 1991)
Ambrosia artemisiifolia	Cypsela	2 300 – 6 000 (Fumanal et al., 2007)	2.4 – 5.5 (Šoštarčić et al., 2021)
Chenopodium album	Nutlet	3 000 – 20 000 (Korsmo et al., 1981)	0.3 (Lemić et al., 2014)
Amaranthus retroflexus	Nutlet	230 000 – 500 000 (Stevens, 1957)	0.25-0.48 (McWilliams et al., 1968)
Abutilon theophrasti	Capsula	44 200 (Steinmaus et al., 2002)	9.1 (Plodinec et al., 2015)

Except from longevity weed species different in the weight and production of seed per plant (Table 2). Species can be ranked from larger to smaller seed as: *Abutilon theophrasti* >

Ambrosia artemisiifolia > *Setaria pumila* > *Echinochloa crus-galli* > *Panicum capillare* > *Chenopodium album* = *Amaranthus retroflexus*. Production of seed depends on different abiotic and biotic factors such as temperature, light, moisture, nutrient availability, interaction between factors, interaction among species therefore the number of seeds differ between years, production systems or environment conditions (Baskin and Baskin, 2001). Fruit type variates among weed species. Among studies species six of them have seed attached to pericarp of caryopsis, cypsela and nutlet. For this species, seeds remain in the fruit and are usually hard or impossible to split and the experiments are always conducted with the fruit. But for simplified used of the terminiology fruits of *Ecinochloa crus-galli*, *Setaria pumila*, *Panicum capillare, Ambrosia artemisiifolia* and *Amaranthus retroflexus* are usually called seed. *Abutilon theophrasti* posess fruit called capsula. Capsula is cup-shaped, circular cluster of 12-15 carpels (seedpods) and each carpel opens with a vertical slit along outer edge and containing from one to three seeds which during maturation fall off the plant (Warwick and Black, 1988).

2.2.2. Soil temperature and water content

The processes of seed germination and seedling emergence are determined by the influence of various abiotic and biotic factors from the time of seed formation on the plant to the beginning of germination and seedling development. Seed germination is defined as the sum of events beginning with the hydration of the seed and ending with the emergence of the embryonic axis (usually the radicle) from the seed coat (Srivastava, 2002). According to Forcella et al. (2000), emergence represents the point at which a seedling is weaned from dependence on the non-renewable seed reserves originally produced by the parents and photosynthetic autotrophism begins. However, to reach this point, the seed must pass through three stages in the life cycle. Van der Weide (1993) divided the emergence process into three different processes: breaking dormancy, seed germination and pre-emergence growth. Each of these processes can be influenced by various external and internal factors on which the success of plant establishment directly depends.

Of the internal factors, dormancy is the most important and strongly influences the timing of emergence (Forcella et al., 2000). Emergence of seedlings of a particular weed in the field occurs when population dormancy is at its lowest (Probert, 1992). Baskin and Baskin (2004) defined a dormant seed as a seed that is unable to germinate within a specified period under any combination of normal physical environmental factors (temperature, light/darkness, etc.) that are otherwise favourable for its germination, i.e., after the seed is no longer dormant. Similarly, Benech-Arnold et al. (2000) explained dormancy as an internal condition of the seed that impedes its germination under otherwise adequate hydric, thermal, and gaseous conditions. Dormancy can be divided into primary and secondary dormancy according to the time of its occurrence and physiological, morphological, morphophysiological, physical and combined of physiological and physical dormancy according to the mechanism or location (Baskin and Baskin, 2004). Primary dormancy is the innate dormancy possessed by freshly matured seed dispersed from the mother plant. Secondary dormancy is a dormant state induced in non-dormant seed by unfavourable conditions for germination or induced again in seed once dormant, after a sufficiently low dormancy has been achieved. Physiological dormancy, the most common form of dormancy (Baskin and Baskin, 2004), is thought to be caused by a physiological condition of the embryo and possibly by reduced gas permeability of seed coat structures (Baskin and Baskin, 2001). Three levels of physiological are distinguished: non-deep, intermediate, and deep (Baskin and Baskin, 2004). Physical dormancy is due to a water-impermeable seed or fruit coat, morphological dormancy requires that an underdeveloped embryo grow before germination can begin (Baskin and Baskin, 2001). Out of seven species studied, six species have physiological type of dormancy: *Echinochloa crus-galli*, *Setaria pumila*, *Panicum capillare*, *Amaranthus retroflexus*, *Chenopodium album* and *Ambrosia artemisiifolia*. *Abutilon theophrasti* is characteristic for its physical dormancy, which is influenced by the thick, hard seed coat that is impermeable to water (Bello et al., 1995). In laboratory conditions, physiological dormancy can be overcome exposing the seed to the period of stratification in which seeds are sowed in sterilized sand and storage in the refrigerator at 4°C for several weeks, depending on the species (Baskin and Baskin, 2004; Body et al., 2013; Harre et al., 2019). This method has been successfully used for seed of summer annuals as imitation of natural conditions during the winter period (Milberg and Anderson, 1998). Moreover, physiological dormancy in laboratory is also broken with the use of potassium nitrate and thiourea as well as the exposure to light (Tang et al., 2008; Lemić et. al., 2014). Laboratory methods for breaking physical dormancy are use of mechanical or chemical scarification and soaking in hot water (Baskin and Baskin, 2004; Hock et al., 2006; Ghantous and Sandler, 2012). Mechanical scarification is performed with the sandpaper, while chemical scarification includes use of different acid, usually sulfuric acid (H_2SO_4) (Baskin and Baskin, 2020). Breaking physical dormancy in nature occurs through many pathways, including elevated or fluctuating temperatures, which can be spiked by fire or freeze/thaw events, desiccation, passage through the digestive tract of animals or with mechanical tools in agriculture (Kelly et al., 1992; Rolston, 1978). Environmental factors affecting seed population dormancy can be divided into two categories: (1) factors that alter the degree of dormancy (soil temperature and soil moisture) and (2) factors that terminate dormancy or remove the ultimate constraint on germination once the degree of dormancy reaches its minimum (light flux density and quality, fluctuating temperature, nitrate concentration) (Benech-Arnold et al., 2000).

Dormancy begins to develop as the seed matures on the parent plant. Baskin and Baskin (2001) refer to this phenomenon as "preconditioning", referring to all factors that can influence maturation. For example, seeds that matured at lower temperatures during formation may develop a thicker seed coat, resulting in lower emergence and deeper dormancy.

Once seeds enter the soil, the process of secondary dormancy begins, and in some species, such as *Ambrosia artemisiifolia*, secondary dormancy may end and be introduced seasonally, which is defined as cyclic dormancy (Baskin and Baskin, 1980). Temperature is the key factor that triggers the dormancy and germination process. In the context of germination, three cardinal temperatures (minimum, optimum and maximum) describe the temperature range in which seeds of a particular species can germinate (Bewley and Black, 1994). The minimum or base temperature (T_b) is the lowest temperature at which germination can occur, the optimum temperature (T_o) is the temperature at which germination occurs most rapidly, and the maximum or ceiling temperature (T_c) is the highest temperature at which seeds can germinate. It is important to emphasise that the temperature requirements for breaking seed dormancy are different from those that promote germination. For example, in summer annual species, dormancy is broken at low temperatures, while the optimal temperature for germination is found at higher temperatures. For example, the optimal temperature conditions for termination of dormancy of *Panicum miliaceum* L. are at 8°C (Benech-Arnold et al., 2000) while the optimal temperature for germination of this species are between 18 - 25°C (Kalinova and Moudry, 2005; James et al., 2011). Dormancy of *Echinochloa crus-galli* can be overcome with high temperatures of 40 to 50°C and low temperatures of 5°C (Arai and Miyahara, 1960; Vengris et al., 1966) while optimal temperature for germination is 27 and 31°C (Martinkova et al., 2006). Apart from constant temperature, dormancy can be overcome by temperature fluctuations in some species. For example, a temperature variation of 2 to 15°C can terminate dormancy in *Chenopodium album*.

Soil moisture also directly effects the dormancy status of the weed species (Benech-Arnold et al., 2000; Batlla et al., 2014). It has been found that less dormant seeds of *Sinapis arvensis* L. were produced from the mother plants under water stress conditions (Wright et al., 1999). Similar results have been reported regarding either winter annual grass species *Avena fatua* L. or summer perennial *Sorghum halepense* (Peters, 1982; Benech-Arnold et al., 1992).

Once the dormancy had been broken, germination process can begin however as much as the dormancy, the germination is affected by similar abiotic and biotic factors. Seed germination is not possible without the moisture which allows water uptake and the imbibition of seed. Water is essential for cellular metabolism for at least three reasons: enzymatic activity, solubilisation, and transport of reactants, and as a reactant, especially in the hydrolytic digestion of stored reserves of protein, carbohydrate, and fat (Woodstock, 1988).

2.2.3. Soil texture, compaction, and tillage operations

Abiotic factors such as temperature, soil moisture, light, pH, gases are once that can be manipulated indirectly through the management. However, soil fertility, salinity, compaction, tillage, and surface residue which mostly effect emergence can be manipulated more directly through the management by agricultural operations (Forcella et al., 2000). Those factors have great effect on the last phase of emergence cycle – pre-growth emergence seedling elongation and emergence.

Different tillage systems (conventional, reduced, and no-till) affect the distribution of the seed in the soil profiles. Conventional tillage, mostly used in Croatia, buries seed deeper in the soil profile, while reduced and no-till systems, leaves seed on the surface. Burial in soil or surface exposure can have negative and positive effect on emergence. Small-seed species whose germination is not light dependent, like *Echinochloa crus-galli* or *Panicum capillare* when buried in the soil have less chance to emerge due to lack of seed reserves and can be exhausted before reaching the surface (fatal germination). Hence, those species enter in secondary dormancy and present the long-term infestation in soil seed bank with possible germination and emergence with next soil rotation. Or they can be decomposed by influence of abiotic and biotic factors such as soil fauna. On the other hand, large-seed species like *Abutilon theophrasti* can successfully emerge from deeper soil profile. Although optimal depth for germination and emergence of *Abutilon theophrasti* is 1-2 cm (>70%), emergence occurs even when seed is sown at 15 cm (5%) (Nagy et al., 2010). Exposure of the seeds in the surface can help the small-seed species to accomplished emergence but also it can be damaged by the herbivores before the germination.

Another factor that can prevent the seedling growth and emergence is development of crust on the soil surface as a result of combinations of agronomic practices (seedbed prepapration) and weather conditions (rainfall). This factor has less effect on species with smaller cotyledons like *Amaranthus retroflexus* or *Chenopodium album* whose lanceolate cotyledons are thin and in a form of pointed apex at the top of hypocotyl and can easily emerge. Also, weeds from Poaceae family, *Panicum capillare*, *Setaria glauca* and *Echinochloa crus-galli* whose coleptiles are sharp pointed can more successfully elongate through packed soils (Forcella et al., 2000). Seedlings of *Ambrosia artemisiifolia* and *Abutilon theophrasti* are large oval-shaped and kidney shaped and unable to easily growth through compacted soil.

2.2.4. Crop residues, light, and other environmental conditions

In some weed species, germination can be enhancing in presence of light, gaseous (oxygen, carbon dioxide, ethylene, water vapour), nitrates, pH, etc. Residues of crops can have negative effect on the germination and pre-emergence seedling growth inhibiting the growth through exudating of allelopathic substances or creating environment unsuitable for growth (lack of light, moisture, high temperature leading to death). Light is an important ecological aspect for germination and the requirement for light means that seeds will only germinate at or near the soil surface (Chauhan and Johnson, 2011). Effect of light has been study for certain species, and it is knowing that light can terminate the dormancy and promote germination of *Chenopodium album* and *Amaranthus retroflexus* (Bewley and Black, 1982). In some species light may stimulate germination. For example, the germination of *Echinochloa crus-galli* and *Setaria pumila* was stimulated by in the presence of light (Chauhan and Johnson, 2011; Povilaitis, 1956). In study of Baskin and Baskin (1986) *Panicum capillare* germinated better exposed to photoperiod (76 – 100%) compared to germination at complete darkness (1-24%). Farooq et al. (2019) studied the effect of the photoperiod on germination of *Ambrosia artemisiifolia* and found higher germination (> 80%) for all populations at photoperiod (12/12h; light: dark) compared to complete darkness. However, Sang et al. (2011) found good germination of *Ambrosia artemisiifolia* at both light (97%) and complete darkness conditions (83%). Nevertheless, germination of *Abutilon theophrasti* seed has shown good germination regardless of light conditions but only at temperature > 25°C. At lower temperatures germination was higher in the presence of light for 14h at 15 and 25°C, 6 and 7% higher germination (Xiong et al, 2018).

Although soil pH can affect the germination of some species, *Abutilon theoprasti* has shown the consistent germination at different studied pH (5 -10) with > 90% of germination (Xiong et al, 2018; Sadeghloo et al. 2013). On contrary, germination of *Echinochloa crus-galli* has shown to be reduced in the alkaline environment, with the optimum range for germination between pH 4 to 6 (> 61%), and decreased germination at pH 6 to 9, with 11% germination at pH 9 (Sadeghloo et al. 2013). Optimum range for *Ambrosia artemisiifolia* germination is at pH range from 5 to 9 (98%), while germination decreases at pH 10 and 12 (< 70%) and no germination occurs at pH 2 (Sang et al., 2011). For other studied species there is a lack of information about effect of pH on germination.

Oxygen, carbon dioxide and ethylene release by living organism are the three main gaseous in the soil. Gas ration in the soil changes under the influence of environmental conditions. In flooded fields, the ratio of carbon dioxide to oxygen increases and can have detrimental effect on weed seed germination and seedling emergence. However, the studies have shown that grass seeds are more tolerant to flooding enduring the radicle inhibition while coleoptile elongation still may proceed (Kennedy et al., 1987; Kodan and Ashraf, 1990; Wijte and Gallagher, 1996). Namely, shoot elongation into an oxygen-rich aerial environment takes precedence over root growth in oxygen-depleted media (Forcella et al, 2000). This could explain the high occurrence of the grass weed *Echinochloa crus-galli* in the flooded rice fields in the world. Ethylene, a gas with a well- known role as a growth regulator, is also present in the soil environment, with its usual value of the pressure ranging between 0.05 and 1.2 MPa (Corbineau and Côme, 1995). Presence of ethylene in soil has breaking-dormancy effect on seed of *Chenopodium album* and *Amaranthus retroflexus* (Taylorson, 1979). Ethylene was successfully used for breaking seed dormancy of *Ambrosia trifida* L. (Harre et al., 2019) in laboratory conditions.

Although the emergence process is a complex process influences by numerous factor specific for each species most authors (Forcella et al., 2000; Benech-Arnold et al., 2000; Bakin and Baskin, 2001) agree that temperature and moisture are primary and most important ones. Therefore, models predicting weed emergence based on those two factors represent the potential threshold decision making tool for weed emergence management.

2.3. Hydrothermal modelling for the prediction of weed emergence

Consistence of timing and sequence of weed emergence is known to be similar across years within a geographical location and it is called emergence pattern (Werle et. al., 2014). Emergence pattern can be explained by the specific dormancy of the species and environmental conditions therefore they are specific for creation climatic area. Emergence patterns form a basis for creation and practical use of predictive weed emergence models. Obtaining the data of emergence of each species thought several years and multiple locations, predictive weed emergence models provide the information of expected emergence in the next years. Information given by the models is the percentage of weed individuals that will be present in the field at certain time span which helps setting weed control at the peak of emergence. According to the approach predictive weed emergence models can be divided as phenology, empirical and mechanistic models.

Phenology models are based on the study of the phenology of a particular plant species and the study of periodic biological events (Orton 1996; Cardina et al., 2011, Masin et al., 2005). It has been shown that the development of phenophases of some plants in nature precedes or follows the appearance of certain weed species in the crop. For example, research carried out in Northern Italy conducted between 1999 and 2004 showed a correlation between the flowering phase of *Syringa vulgaris* L. and the emergence of weed species *Digitaria sanguinalis*. In addition, the end of flowering period of shrub species *Forsythia viridissima* Lindl.

coincided with the beginning of the emergence of the two weeds *Setaria pumila* and *Setaria viridis* (Masin et al., 2005). By simply observing the flowering of these two woody plants planted near crops each year, the occurrence of these weeds can be predicted.

Mechanistic or reductionist models are physiologically based models since they usually include different biological process that influence seed emergence. As mentioned before the emergence process is divided in three phases: dormancy breaking, seed germination and pre-emergence growth (Van der Weide, 1993). Each phase has own requirements and depends on different factors. Mechanistic models tent to include as much phases as possible to achieve good weed emergence prediction. One of the detailed mechanistic model is AlomySys, which considers the emergence of *Alopecurus myosuroides* Huds. in winter wheat (Colbach et al., 2006). This model is consisted of sub-models: soil environment (climate, structure) resulting from the cropping system and weather, vertical soil seed distribution after tillage, depending on the tool, the characteristics of the tillage implement and the soil structure; and seed mortality, dormancy, germination, and pre-emergent growth depending on soil environment, seed depth, characteristics, and past history. The mechanistic models such as AlomySys require input of large and various amount of data to create reliable prediction of weed emergence.

Empirical models are less complex than mechanistic models and are called monophasic models. These models focus on the emergence excluding the other phases of the weed emergence (dormancy, germination, etc.). There are weather-based models and use meteorological data (temperature, precipitation) and estimated germination parameters (base temperature and base water potential) of certain species to predict the emergence. Currently, there are three types of empirical predictive emergence models: thermal time (TT), hydro-thermal time (HTT) and photohyrothermal time models (PhHTT). TT models are first developed models that included only temperature as factor that triggers the germination. TT models summarize thermal units, growing degree days (GDD) subtracted from the value of base temperature (T_b) which represents the minimum temperature for required for seed germination (Roché et al., 1997). However, TT models are usually less accurate since there are not able to predict deviations in the expected emergence of weeds in the absence of soil moisture during periods of drought (Masin et al, 2010; Forcella et al., 2000; Leguizamon et al., 2005). HTT model sums heat units when the soil water potential reaches the value of the base water potential (Ψ_b), which is the lowest value of the soil water potential at which the seeds can germinate (Gummerson, 1986). The units that HTT model calculates are called hydrothermal units (HT). Recently developed PhHTT models include not only temperature and soil moisture but also light as hourly and solar radiation. This factor is included in germination process in phase of dormancy seed breaking and therefore provides more accurate information of expected emergence in the field (Royo-Esnal et al., 2015). These models are useful for the species whose germination greatly depends on light.

Most predictive weed germination and emergence models are developed using non-linear regression since they are easy to develop and use. This model is sigmoidal (S-shaped) type models which are based on TT or HTT. Weibull, Logistic and Gompertz function are most common function used for predicting weed emergence.

Weibull:

$$Y = k\,(1-\exp(-(a(x-p))^{c}))$$

Logistic:

$$Y = k/(1+\exp\,(a-(x-p)))$$

Gompertz:

$$Y = k\,\exp\,(-\exp(-a-(x-p)))$$

Where Y is cumulative emergence, x is cumulative GDD, a is the slope (emergence rate), p is the inflection point on the axis x, c is the shape factor that determines the skewness and kurtosis of the distribution, and k is the maximum emergence fraction of the model. Although the Logistic and Gompertz functions are similar the main differences are that the Gompertz function approaches the asymptote more gradually, which often matches observation of late-emerging seedlings. On the other hand, the Weibull model is a more flexible function than the Logistic and Gompertz as it can acquire the characteristics of other types of distributions based on the value of the shape parameter c (Gonzalez-Andujar et al., 2016). As it can be seen from the Table 4. Weibull function is more common used in predictive weed emergence modelling.

The development of the predictive emergence model is divided in four steps. The first step is to determine the biological parameters (base temperature and base water potential) required for the germination of a weed species in the laboratory. The second phase refers to monitoring the dynamics of weed emergence in the field over several years and locations while monitoring the daily microclimatic conditions in the soil (temperature and water potential of the soil in the germination zone). Based on the data collected in the field and laboratory and calculating the sum of HT units, weed germination curves in the crop are made, which represent the third phase – prediction of emergence. The final stage of model development refers to the validation of the model using the same to predict emergence in the crop (Masin et al., 2010; Masin et al. 2012; Egea-Cobrero et al., 2020).

Table 4. Predictive models developed for studied weed species adjusted and taken from Chantre and González-Andújar (2020)

Species	Germination	Emergence	TT	HTT	Model	References
Echinochloa crus-galli		x		x	Weibull	Werle et al (2014)
		x	x	x	Weibull	Bagavanthiannan et al. (2011)
	x	x	x	x	Logistic/Gompertz	Masin et al (2010)
		x	x		General logistic	Dorado et al (2009)
Setaria pumila		x		x	Weibull	Werle et al (2014)
	x	x	x	x	Logistic/Gompertz	Masin et al (2010)
Panicum capillare	-	-	-	-	-	-
Amaranthus retroflexus		x		x	Weibull	Werle et al (2014)
	x	x	x	x	Logistic/Gompertz	Masin et al (2010)
		x	x		Logistic/Gompertz/GA	Haj Seyed Hadi and Gonzalez-Andujar (2009)
	x		x	x	Weibull	Oryokot et al. (1997)
Chenopodium album		x		x	Weibull	Werle et al (2014)
	x	x	x	x	Logistic/Gompertz	Masin et al (2010)
		x	x		Weibull	Leblanc et al. (2003, 2004)
		x			Logistic	Grundy et al. (2003)
		x		x	Weibull	Roman et al. (1999)
	x	x	x		Gompertz	Vleeshouwers and Kropff (2000)

Species	**Germination**	**Emergence**	**TT**	**HTT**	**Model**	**References**
Ambrosia artemisiifolia			x	x	Weibull	Barnes et al (2017)
		x		x	Weibull	Werle et al (2014)
	x			x	Weibull	Shrestha et al (1999)
Abutilon theophrasti		x		x	Weibull	Werle et al (2014)
	x	x	x	x	Logistic/Gompertz	Masin et al (2010)
		x	x		Weibull	Dorado et al (2009)

Emergence models have been developed to predict weed emergence in maize (Dorado et al. 2009; Masin et al. 2012), soybean (Masin et al. 2014; Werle et al. 2014) or winter cereals (Royo-Esnal et al. 2010, 2015; García et al. 2013; Izquierdo et al. 2013). Most of the models are not yet used at farm or advisor level. Hydrothermal model AlertInf developed in Italy (Masin et al., 2010), and WeedCast developed United States (Forcella, 1998) are two models whose simplified software version is available and successfully used by the producers. The complexity of development of a completely new model delays the likelihood of their soon availability in Croatia. A faster solution is to introduce an already existing model, validating its use in the other geographical area. However, the attempts to validate the existing model were done but not always successfully (Loddo et. al., 2019; Egea-Cobrero et al., 2020). The validation should be performed with prior calibration of the model with the germination parameters of the certain population. Table 5 present the literature data for germination parameters of weed species included in this thesis. It is evident that the germination parameters of weed germination may vary depending on the geographical area. For example, base temperature for *Abutilon theophrasti* varies from 3.5° C (Loddo et al., 2018) to 8.0° C (Leon et al., 2004), for *Echinochloa crus-galli* of 5.0° C (Sadeghloo et al., 2013) to 13.8° C (Steinamus et al., 2000), and for *Chenopodium album* from 1.5° C (Bürger and Colbach, 2018) to 10.0° C (Gardarin et al., 2010). Species that vary less in base temperature values are: *Amaranthus retroflexus* from 10.0° C (Wiese and Binning, 1987) to 12.3° C (Masin et al., 2010) and *Ambrosia artemisiifolia* from 2.0 to 4.2° C (Leiblein et al., 2014). The authors explain the variation of base temperature for *Ambrosia artemisiifolia* by its adaptation to new agroecological conditions, which is a characteristic of invasive plant species. Namely, European populations of this species have lower base temperature relative to native populations from North America. For *Setaria pumila*, the base temperature found so far is 10.4° C (Masin et al., 2010), while for species *Panicum capillare* there are no data of base temperature in the literature. Furthermore, estimated values of base water potential also vary significantly in the available literature: *Ambrosia artemisiifolia* from -0.80 MPa (Shrestha et al., 1999) to -1.28 MPa (Gullemin et al., 2013); *Chenopodium album* from -0.80 MPa (Gullemin et al., 2013) to -1.04 MPa (Masin et al., 2010); *Abutilon theophrasti* from-0.15 MPa (Archer et al., 2001) to -0.82 MPa (Masin et al., 2010); *Amaranthus retroflexus* from -0.41 MPa (Masin et al., 2010) to - 0.95 MPa (Gullemin et al., 2013); *Echinochloa crus-galli* from −0.10 MPa (Archer et al., 2001) to −1.19 MPa (Gullemin et al., 2013); *Setaria pumila* from −0.75 MPa (Gullemin et al., 2013) to −0.93 MPa (Masin et al., 2010). For the species *Panicum capillare* there are no data of base water potential in the literature.

It is important to highlight that the differences in the values of estimated parameters can occur when using different statistical approaches. Moreover, the statistical approach of the estimation of germination parameters could also lead to the differences in the estimated values between the populations (Steinamus et al, 2000; Loddo et al., 2018). Statistical approach such as linear or nonlinear regression with resampling bootstrap methods (Masin et al. 2010; Onofri et al. 2014), population-based threshold models (Dorado et al. 2009), probit analysis (Zambrano-Navea et al. 2013) or survival analysis (Onofri et al. 2010) can be used for estimation base germination parameters. While transferring the model to another climatic region it is better to use the same approach as it has been used for population included in the model.

The Italian model AlertInf is the geographical and microclimatic closest to the area of continental Croatia. This model is available to farmers at web site of the Regional Agency for Environmental Prevention and Protection of the Veneto (APRAV) as a simple tool that predict the emergence of weed species in maize and soybean. AlertInf allows users to add nearest meteorological station, date of seedbed preparation and select the species whose emergence percentage is required. Masin et al. (2012) successfully validated the AlertInf model and transferred it from Veneto to Tuscany region using the observed emergence data of three maize species *Chenopodium album*, *Sorghum halepense* and *Abutilon theophrasti*. The validation was performed with prior calibrating the model to the germination parameters of the weed species from Veneto population. The successful validation and transfer of the Italian hydrothermal model AlertInf from Veneto to Tuscany region has generated the idea of a possible extension of the model to Croatia. Currently, predicting weed emergence using AlertInf model is possible for ten species: *Abutilon theophrasti*, hairy crabgrass (*Digitaria sanguinalis*), *Echinochloa crus - galli*, spotted ladysthumb (*Polygonum persicaria*), green foxtail (*Setaria viridis*), black nightshade (*Solanum nigrum*), *Amaranthus retroflexus*, *Chenopodium album*, *Setaria pumila* and johnsongrass (*Sorghum halepense*) (Masin et al., 2010). Predicting weed emergence for economically important species in the continental Croatia is possible for five species (*Abutilon theophrasti*, *Echinochloa crus-galli*, *Amaranthus retroflexus*, *Chenopodium album* and *Setaria glauca*) based on the germinationl parameters of Italian populations (Masin et al., 2010) while for species not included in the AlertInf the germination parameters have to be estimated, emergence data collected from the field and validation of the model should be performed.

Table 5. Estimated base temperature and base water potential of the species included in the doctoral thesis

Weed species	Base temperature (°C)	References	Base water potential (MPa)	References
Echinochloa crus-galli	5.0	Sadeghloo et al (2013)	-0.97	Masin et al (2010)
	6.2	Guillemin et al (2013);	-1.19	Guillemin et al (2013)
	10.0	Wise and Binning (1987) Forcella (1998) Sartorato and Pignata (2008)		
	10.2	Bürger and Colbach (2018)		
	10.4	Loddo et al (2018)		
	13.3	Loddo et al (2018)		
	13.8	Steinamus et al (2000)		
Setaria pumila	5.3	Mollaee et al (2020)	-0.52	Mollaee et al (2020)
	8.2	Sartorato and Pignata (2008)	-0.93	Masin et al (2010)
	8.3	Masin i sur (2005)		
Panicum capillare	-		-	
Ambrosia artemisiifolia	2.0	Leiblein et al (2014)	-0.80	Shrestha et al. (1999)
	3.4	Sartorato i Pignata (2008)	-1.28	Guillemin et al (2013);
	3.6	Guillemin et al (2013); Shrestha et al (1999)		
	4.2	Leiblein et al (2014)		
Chenopodium album	2.0	Vleeshauwers and Kropff (2000)	-0.64	Roman et al. (2000)
	4.2	Roman et al (2000)	-0.80	Guillemin et al (2013)
	5.0	Masin et al (2010)	-0.96	Masin et al (2010)
	5.8	Guillemin et al (2013)	-1.04	Masin et al (2010)
	6.0	Wise and Binning (1987)		
Amaranthus retroflexus	10.0	Loddo et al (2018); Wiese and Binning (1987)	-0.41	Masin et al (2010)
	11.6	Benvenuti and Macchia (1993)	-0.62	Masin et al (2010)
	12.0	Loddo et al (2018)	-0.86	Derakhshan et al (2014)
	12.1	Masin et al (2010)	-0.95	Guillemin et al (2013)
	12.3	Loddo et al (2018); Guillemin et al (2013)		

	12.9	Loddo et al (2018)		
Abutilon theophrasti	3.4	Loddo et al (2013)	-0.60	Sadeghloo et al (2013)
	3.5	Loddo et al. (2018)	-0.64	Dorado et al. (2009)
	3.9	Masin et al. (2010)	-0.73	Dorado et al. (2009)
	4.4	Loddo et al. (2013); Masin et al. (2010)	-0.78	Masin et al (2010)
	4.8	Loddo et al.(2013)	-0.82	Masin et al (2010)
	4.9	Loddo et al. (2018)		
	5.0	Loddo et al (2013); Sadeghloo i sur. (2013)		
	6.2	Benvenuti and Macchia (1993)		
	6.5	Sartorato and Pignata (2008)		
	6.8	Dorado et al. (2009)		
	7.2	Dorado et al. (2009)		
	8.0	Leon et al (2004)		

3. GENERAL DISCUSSION

3.1. Estimation of seed germination parameters for weed species

The **first objective** of the doctoral thesis was to estimate base temperature and base water potential for seven maize weed species (*Abutilon theophrasti*, *Echinochloa crus-galli*, *Amaranthus retroflexus*, *Chenopodium album*, *Setaria pumila*, *Panicum capillare*, *Ambrosia artemisiifolia*) in Croatia and compare them with the values of the same species built in the AlertInf model. Laboratory experiments were conducted to estimate the germination parameters (base temperature and base water potential) of the studied species *Abutilon theophrasti* (Publication No. 1), *Chenopodium album*, *Amaranthus retroflexus*, *Setaria pumila* and *Panicum capillare* (Publication No. 3), *Echinochloa crus-galli* (Publication No. 4) and *Ambrosia artemisiifolia* (unpublished data). As expected, weed species differed in the estimated value of the germination parameters. The lowest base temperature was estimated for *Ambrosia artemisiifolia* (1.5°C), while the highest base temperature was estimated for *Amaranthus retroflexus* (13.9°C). According to the estimated base temperature studied weed species can be classified from less to more thermophilic: *Ambrosia artemisiifolia* (1.5°C) *Chenopodium album* (3.4°C) < *Abutilon theophrasti* (4.5°C) < *Setaria pumila* (6.6°C) < *Echinochloa crus-galli* (10.8°C) < *Panicum capillare* (11.0°C) < *Amaranthus retroflexus* (13.9°C). For *Ambrosia artemisifolia* base temperature value of Croatian population was the lowest compared to other studied species. In previouse studies Leiblein et al. (2014) estimated 2.0°C for Euopean populations (France, Hungary, Germany and Czech Republic) and this is the lowest base temperature value found in the literature. The highest value was found for American population of *Ambrosia artemisiifolia*, 4.2°C also by Leiblein et al. (2014). The lowest value of base temperature for *Chenopodium album* found in literature is 2°C (Vleeshauwers and Kropff, 2000) for a population from the Netherlands and 4.2°C in the USA, Ontario (Roman et al., 2000) and is similar to the values in this study. Other authors estimated higher base temperature values of 5.0 in Italy (Masin et al., 2010), 5.8°C in France (Guillemin et al., 2013) and 6.0 in the USA, Wisconsin (Wise and Binning, 1987). In present study, the third lowest value of base temperature was estimated for *Abutilon theophrasti*. Loddo et al (2013) conducted the experiment to estimate the base parameters for germination of *Abutilon theophrasti* seeds collected in Italy, Portugal, and Spain using the same statistical approach. The estimated values were 3.9°C (Italian population), 3.4°C (Portuguese population) and 5.0 (Spanish population). For the Iranian population, the estimated value of the base temperature is 4.9°C (Loddo et al., 2018). In contrast, Dorado et al. (2009) estimated different values for *Abutilon theophrasti* in non-chilled and chilled seed buried in soil in Spain: 6.8 and 7.2°C, respectively. For *Setaria pumila*, there are few references in the literature where the base temperature has been estimated. Mollaee et al. (2020) conducted the experiment with Iranian populations of three *Setaria* species: *Setara verticillata* (L.) P. Beauv., *Setaria viridis*, and *Setaria pumila*. All three species differed in the value of base temperature and *Setaria pumila* had the lowest estimated base temperature (5.3°C), while *Setara verticillata* and *Setaria viridis* had a higher estimated value, 7.8 and 8.6°C, respectively. In Italy, Sartorato and Pignata (2008) and Masin et al. (2010) estimated even higher values for the base temperature of *Setaria pumila*, namely 8.2 and 8.3°C, respectively. Germination parameters for *Echinochloa crus-galli* have been studied by more authors due to its common occurrence and economic importance. The estimated base temperature value for the Croatian population (10.8°C) is similar to the estimated values for the Iranian population (10.4°C) (Loddo et al., 2018) and a German population (10.2°C) (Bürger and Colbach, 2018). A similar value was also reported for a Texas population, 9.7°C (Wiese and Binning, 1987). In contrast, the lowest base temperature of *Echinochloa crus-galli* was estimated for a French population, 6.2°C (Guillemin et al, 2013) while Steinmaus et al. (2000) estimated the highest base temperature in California to be 13.8°C. In the present study the highest base temperature was estimated for *Amaranthus retroflexus* and similarly was found for Italian and Iranian populations around 12.0°C (Masin et al., 2010; Loddo et al., 2018). However, for this weed species lower values were estimated for the German population (10.5°C) (Bürger and Colbach, 2020) and the French population (8.9°C) (Guillemin et al, 2013).

The values of base temperature for germination estimated in the present study are consistent with the occurrence of this species in the field. Werle et al. (2014) categorized the species according to the accumulated growing degree days (GDD) and their emergence in the field as early emerging (< 70 GDD), middle emerging (70-140 GDD) and late emerging (> 140 GDD). From the description, *Chenopodium album* (19 GDD) and *Ambrosia artemisiifolia* (33 GDD) are an early emerging species, *Abutilon theophrasti* (77 GDD), *Setaria pumila* (121 GDD), *Echinochloa crus-galli* (103 GDD) are middle emerging species, while *Amaranthus retroflexus* (220 GDD) belongs to a late emerging species. This is the reason why a later emergence of *Amaranthus retroflexus* is observed in maize fields, compared to the other studied weeds (Myers et al., 2004).

In addition, the base water potential was estimated for each species in the present study. The highest values of base temperature were estimated for *Chenopodium album* (-1.38 MPa), indicating that this species can tolerate water stress and germinate in soils with low water content. This contrasts with the less tolerant species to water stress *Amaranthus retroflexus* with a base water potential of -0.36 MPa. Depending on tolerance to water stress, weed species of mainland Croatia can be classified from more to less tolerant: *Chenopodium album* (-1.38 MPa), *Echiniochloa crus-galli* (-0.97 MPa), *Ambrosia artemisiifolia* (-0.89 MPa), *Panicum capillare* (-0.87 MPa), *Setaria pumila* (-0.71 MPa), *Abutilon theophrasti* (-0.67 MPa) and *Amaranthus retroflexus* (-0.36 MPa). There is less information in the literature on the base water potential of the species studied. For example, the base water potential for *Chenopodium album* in the USA, Ontario (Roman et al., 2000) is -0.64 MPa and the French population is -0.80 MPa (Guillemin et al, 2013). Masin et al. (2010) estimated a higher base water potential for the population in Pisa (-1.04 MPa) than in Padova (-0.96 MPa). Shrestha et al (1999) estimated -0.80 MPa for Ontario population of *Ambrosia artemisiifolia* which is similar to the values estimated in this research. However, estimated value for French population is much lower, -1.28 MPa (Guillemin et al, 2013). The value of the base water potential of *Echinochloa crus-galli* is the same for the Croatian and Italian populations (-0.97 MPa) (Masin et al., 2010), while the French population has higher values (-1.19 MPa) (Guillemin et al, 2013). The response to osmotic stress and base values of *Panicum capillare* have been less studied. However, in Wu et al (2021), limited germination occurred at a water potential of -0.96 MPa under optimal alternate temperatures of 30/25°C. This was not the case in the doctoral study, where germination ceased at lower water potentials. But in doctoral thesis experiment, germination at different water potential levels was observed at lower constant temperature (22°C). Values for the base water potential of *Setaria pumila* differ from those in the literature, with -0.52 MPa estimated for the Iranian population (Mollaee et al., 2020) and -0.93 MPa for the Italian population (Masin et al., 2010). As noted for the Croatian population, *Abutilon theophrasti* also has lower base water potential in other populations. Sadeghloo et al. (2013) estimated -0.60 MPa, while Dorado et al. (2009) estimated -0.64 MPa for non-chilled seeds and -0.73 MPa for naturally chilled seeds. Even lower than the Croatian values, Masin et al. (2010) estimated -0.78 MPa for the base water potential of the Padova population and -0.82 MPa for the Pisa population. The base water potential value for the Croatian population of *Amaranthus retroflexus* is also the highest estimated to date. Similar to the Croatian population, the population in Padova has a value of -0.41 MPa (Masin et al., 2010). In contrast, the population in Pisa has an estimated base water potential of -0.62 MPa (Masin et al., 2010). Derakhshan et al. (2014) estimated -0.86 MPa for the Iranian population, while Guillemin et al. (2013) estimated -0.95 MPa for the French population. Taken together it can be concluded that germination parameters estimated in present study differed greatly compared to different populations of the same species.

The difference between populations can be explained by variability between populations of weed species as a result of environment and genetic (Baskin and Baskin, 2001). However, in order to strongly state that this variation is a consequence of interpopulation variability, more information on genetic origin and environment conditions should be available to compare these values from this point of view. Apart from variation among populations, differences among estimated values of germination parameters may also be connected to the use of different statistical approaches. Steinmaus et al. (2000) compared different approaches to estimating temperature thresholds for nine species collected in California. They used the x-axis intercept of conventional germination rate indices regressed on temperature (percent germination per day, reciprocal time to mean germination, germination rate index), repeated probit analysis, and a mathematical approach. Differences were observed between approaches for the same species at 1-2°C higher or lower base temperature. For example, the base temperature of *Echinochloa crus-galli* was estimated to be 13.8°C using reciprocal time to median germination, whereas it was 12.5°C using probit analysis. An even greater difference was observed for *Setaria pumila*, whose base temperature was estimated to be 9.8°C using the reciprocal time to median germination, while the probit analysis yielded a value of 14.0°C. Based on statistical criteria and biological relevance, the author determined that reciprocal time to median germination was the best choice because this method distinguished summer annuals with relatively high base temperature values from winter annuals with lower values. A similar study was conducted by Loddo et al. (2018), who used the same two approaches to estimate the base temperature of five weed species. Higher base temperature values were estimated using probit analysis compared to reciprocal time to median germination for the species *Amaranthus albus* L. (13.1 vs 10.4°C), *Amaranthus hybridus* L. (7.6 vs 9.6°C), *Amaranthus retroflexus* two seed lots (10.0 vs 12.3 and 12.0 vs 12.9°C), *Echinochloa crus-galli* (10.4 vs 13.3°C), *Sorghum halepense* (10.1 vs 12.4°C). Therefore, in building the global weed emergence prediction model proposed by Grundy (2003), it is important to harmonize the methodology and approach for testing the germination parameters of the species.

Reciprocal time to median germination was the method used in this study and it was the same method used by Masin et al. (2010) for estimating germination parameters incorporated in the AlertInf model. The estimated germination parameters of the Croatian populations were compared with those of the Italian populations fitted to the AlertInf model. The **first hypothesis** of the doctoral thesis was that the germination parameters (base temperature and base water potential) of the native weed populations will differ from those of the foreign populations fitted to the AlertInf model. This hypothesis was partially confirmed. Of the species tested, both germination parameters of *Abutilon theophrasti* overlapped between the Croatian and Italian populations (*Publication No. 1*). The Croatian population of *Setaria pumila* did not overlap with the Italian population in any of the parameters. *Echinichloa crus-galli* and *Amaranthus retroflexus* overlapped only in the parameter of base water potential, while *Chenopodium album* overlapped in the parameter of base temperature (*Publication No. 3*). The germination parameters of *Panicum capillare* and *Ambrosia artemisiifolia* were not previously tested for the Italian population, so it was not possible to compare them. The laboratory experiments to estimate the germination parameters for *Ambrosia artermisiifolia* were not published until the end of the doctoral thesis due to additional experiments that were performed during the study period. Due to the uneven germination in the laboratory experiments, the seeds of twenty plants of *Ambrosia artemisiifolia* were tested separately to estimate the germination dynamics and possible differences in germination of the seeds collected from the different plants as a precursor to testing the base temperature (*Publication No. 2*). The presence of intrapopulation variation in seed germination is well known for weeds and other wild species (Baloch et al., 2001; Elison, 2001; Genton et al., 2005) and the laboratory experiments of testing germination parameters tend to be reliable and repeatable because this data is implemented in predictive weed emergence models used in the field. Intrapopulation variation has been defined as variation in morphology (seed weight, size, texture, colour) or function (degree of dormancy, mortality, germinability). Morphological and functional polymorphism can be found for seeds originated from the same inflorescence, within the same plant, within different plants from same population and between different population of the same species (Fenner, 1991). The possible occurrence of the intrapopulation variation of *Ambrosia artemisiifolia* seed was tested. The aims of this research were to determine existence and degree of morphological polymorphism (variation in seed weight within the population), and to determine existence and degree of functional polymorphism (percentage of seed germination, mortality, dormancy and dynamics of germination) within the two populations in continental Croatia: Jastrebarsko (J) and Popovača (P). As expected intrapopulation variation within seed population was found to be statistically significant. However, determined intrapopulation variation, degrees of variability between important researched parameters (final germination, dormancy and medium germination – T_{50}) were weak or moderate and therefore acceptable for further seed experiments. This study (*Publication No. 2*) has shown that the germination dynamic differed between the plants, but the coefficient of variation was low. This mean that in the further experiments the seeds could be mixed from the large number of plants (> 20) to obtain representative mixture and to provide the sufficient germination dynamic for estimation of base parameters.

The comparison of germination parameters of Croatian and Italian population was crucial to determine the direction of the experiments. 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 (Masin et al., 2010) estimated by the bootstrap method (Efron, 1979). If there is no overlapping of the confidence intervals between the two populations, a significant difference is determined. The assumption was made that the Croatian population will have the lower base temperature values than the Italian due to climatic characteristics of the two locations. Namely, 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 minimum (Kottek et al., 2006). The average annual temperature in Zagreb is

11.8 °C, while the average annual temperature in Padova is 12.2°C. Although these climatic conditions could affect the preference of the species to the temperature has not been the pattern for all studied species. When comparing base temperature Croatian populations of dicotyledonous species had higher estimated base temperature than Italian populations. Croatian population of *Abutilon theophrasti* is estimated to have 0.6°C higher base temperature than Italian but no statistical differences were found between this population. Similar, *Chenopodium album* base temperature did not differ between two populations but was 0.8°C higher for Croatian population. Populations of *Amaranthus retroflexus* differ in the base temperature parameter and were 1.6°C higher for Croatian population. Monocotyledonous species has lower base temperature estimated for Croatian populations compared to Italian. Both species have the base temperature statistically different from the Italian populations. Croatian population of *Echinochloa crus-galli* has 0.97°C lower base temperature than the Italian, while *Setaria pumila* has 3.8°C estimated lower base temperature than the Italian population of this species. Similar, when observing and comparing base water potential between two populations no specific rule could be found. Although, *Abutilon theophrasti* and *Amaranthus retroflexus* have higher base water potential estimated in Croatia there is no statistical differences between Croatian and Italian populations for this weed species. On contrary, *Chenopodium album* and *Setaria pumila* have lower base water potential than the Italian population and the statistical differences have been found. This means that the Croatian population of two species have better tolerance to water stress than the Italian one. 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 (Fenner, 1991; Baskin and Baskin, 2001). Bürger and Colbach (2018) also found the difference in base temperature for different species, and it was not possible to find a pattern connected to climate conditions. They found a 4.3°C lower base temperature for *Chenopodium album*, and a 4.0°C higher base temperature for *Echinochloa crus-galli* in Germany, compared to France.

Since the germination parameters of *Abutilon theophrasti* overlapped between two populations, it can be assumed that the AlertInf model can be validated in the field with the same parameters already fitted in the model without further calibration. *Chenopodium album* emergence could also be predicted using AlertInf, but as a thermal time (TT) model that excludes base water potential and uses only base temperature. This means that the occurrence of *Chenopodium album* can be predicted with AlertInf in irrigated maize fields or in seasons with sufficient rainfall during sowing and early growth of maize. Therefore, in further studies, the validation of Alertinf in irrigated maize crops must be tested using the parameters already fitted to the model.

For other species (*Echinochloa crus-galli*, *Amaranthus retroflexus*, *Setaria pumila*), the model should be calibrated and for *Panicum capillare* and *Ambrosia artemisiifolia* upgraded since these species are are not built in AlertInf. From species included in the thesis *Echinochloa crus-galli* was first species whose parameters (base temperature) was used to calibrate AlertInf model and validate the emergence using the AlertInf in the field. The importance and high occurrence in the maize field was already elaborated in the previous chapters, therefore, this species was used as a model species to test the possibility of transferring the calibrated model AlertInf.

3.2. Validation of hydrothermal model AlertInf for predicting weed emergence in maize

The **second objective** of the dissertation was to determine the dynamics of weed emergence in the field using base temperature and base water potential for native populations (*Publication No. 4*). Experiments were performed by calibrating the AlertInf model with germination parameters (base temperature) of *Echinochloa crus-galli*. The occurrence of this species in the experimental field was highest with a density of 933 plants/m^2 in 2019 and 834 plants/m^2 in 2020. This high density suppressed the occurrence of other weed species and model validation for other weed species could not be performed. Therefore, monitoring of the occurrence of the other species is still ongoing. The emergence of *Echinochloa crus-galli* was monitored during a two-year field experiment on the Croatian mainland. The emergence data were used to validate the AlertInf model, which was calibrated with the values of the base temperature (10.8°C) of the Croatian population. The successful validation of the model with the calibrated values gave the possibility to use the model for this species. In this work (*Publication No. 4*), the second hypothesis of the doctoral thesis was confirmed, which stated that the application of the AlertInf model with the determined values of base temperature and base water potential of the native populations can predict the dynamics of weed emergence in maize fields in Croatia. The hypothesis was confirmed for one species and monitoring of the occurrence of other studied species whose germination parameters were estimated, is still ongoing and will be publish later. Data on the emergence of *Echinochloa crus-galli* were collected over 2 years at the field in Sasinovecki Lug and fitted to the prediction of the AlertInf model calibrated to the value of the base temperature. Monitoring of emergence dynamics started after maize sowing on 8 May 2019 and 5 May 2020 until at least two weeks after maize canopy closure (BBCH 18-19) when no new emergence was observed, which was mid to late

June in both years. The cessation of emergence with the closure of the leaf canopy has been previously documented (Forcella et al., 2000) and explained by the change in soil climate. However, studies based on the determination of emergence pattern are often conducted in a crop-free field (Zambrano-Navea et al., 2013; Vasileiadis et al., 2016). Nevertheless, when *Echinochloa crus-galli* emergence was observed at 12 sites in the United States without a crop, emergence extended into September (Bagavathiannan et al., 2011), much longer than in our study. However, Werle et al. (2014) also observed similar emergence dynamics for *Echinochloa crus-galli* in the crop-free experiment as in our experiment. The emergence dynamics were expressed by the GDD units. In this experiment, GDD units were accumulated from 1 January, using uniform values for the base temperature of 9°C for all 23 species in the study. Emergence of *Echinochloa crus-galli* began at 103 accumulated GDD, reached in late April and early May. Based on the timing of emergence, Werle et al. (2014) classified this species as middle-emerging species. According to Myers et al.'s (2004) classification of duration of emergence, *Echinochloa crus-galli* is an intermediate species, ending its emergence at 439 GDD (Werle et al., 2014). In the study by Werle et al. (2014), emergence ended in mid-June. In our experiments, soil moisture was also used to predict the emergence of *Echinochloa crus-galli* and the estimated hydrothermal units (HT) were slightly lower than the GDD values calculated by Werle et al. (2014), which is expected. Considering calendar days, field emergence followed a similar trend to the emergence dynamics in the Werle et al. (2014) study. The initial emergence (10%) of *Echinichloa crus-galli* in our study in 2019 was observed on 26 May, 18 days after sowing (DAS), middle emergence (40-50%) was observed from 4-8 June, while the end of emergence (99-100%) occurred on 28 June. In 2020, initial emergence (10 %) was observed in the field on 18-22 May, i.e. between 13-17 DAS. Middle emergence observed from 22 to 25 May (32 - 63%), i.e. between 17 and 20 DAS. The end of emergence (99 -100%) was observed in the field from 12 June.

To validate the AlertInf model, the estimated emergence dynamics of *Echinochloa crus-galli* in Sasinovecki lug were adjusted to the predicted emergence of the model. In 2019, the AlertInf model predicted initial emergence (11%) at 29.1 cumulative HT units on 31 May, mid-emergence (52%) on 6 June at 96.2 cumulative HT units, and end of emergence (99-100%) on 26 June at 312 HT units, whereas in the field this was on 28 June. In 2020, the onset of emergence (10%) is predicted for May 16 at 27.3 HT units. The middle emergence predicted by the model was reached on 24 May with a cumulative 93.1 HT units. The end of emergence was predicted for 17 June with 342.5 HT units, while it was observed in the field from 12 June (*Publication No. 4*). The objective of the study was to determine the fit of the model to the observed emergence and to evaluate the overall performance of the model. Using the root mean square error (RMSE) index and the modeling efficiency (EF), the performance of the model can be considered satisfactory with EF indices of 0.97 and 0.98 in 2019 and 2020, respectively. The RMSE is 1.69 and 1.38 in 2019 and 2020, respectively. Therefore, the use of AlertInf model for predicting the occurrence of *Echinochloa crus-galli* in Sasinovecki Lug (Croatia) is quite feasible considering the threshold EF of 0.5 for acceptable model prediction (Ramanarayanan et al., 1997). Similar validation of the models has been proposed by other authors and the effectiveness of the model indicates the goodness of the prediction and the possibility of transferring it to another region. For example, when transferring the AlertInf from Veneto to Tuscany, Masin et al. (2012) estimated EF values for *Abutilon theophrasti* (0.98), *Chenopodium album* (0.99) and *Sorghum halepense* (0.98) emergence. In contrast, Egea-Cobrero et al. (2020) used the Myers et al. (2004) data set from the United States to validate the emergence of *Abutilon theophrasti* in Golega (Portugal) and Minnesota (United States) and obtained EFs of 0.30 and 0.97, respectively. Due to the low EF in Portugal, the authors concluded that the same model could not be used in Portugal.

The satisfactory performance index of the model in our case is consistent with the **second hypothesis** of the study and represents the possible use of the model with calibrated values in our region. This is valuable information for further experiments and possible extension of the use of the model in another geographical area. Nevertheless, the possible use of this model in Croatia for *Echinichloa crus-galli* encourages testing other weed species whose germination parameters differ from the Italian ones included in the model *(Setaria pumila, Chenopodium album* and *Amaranthus* retroflexus). However, the result of the study must be taken with caution. At the moment, it is difficult to generalize the application of the model to other environmental conditions and agronomic practices. For example, in this study, tillage (conventional tillage) and seedbed preparation did not differ in the two years. In both years, experimental field was mouldboard ploughed in autumn of each year. Shallow spring-tooth harrowing in early spring (mid-March) for soil loosening was followed by field cultivator for seedbed preparation just before sowing. The effect of tillage on the vertical distribution of seed in the seed bank is well known, and different tillage practices can significantly affect field emergence (Clements et al., 1996). However, Vasileiadis et al. (2006) concluded that emergence of *Echinochloa crus-galli* was stable over the years under different simulated tillage systems (conventional, reduced and no-till), so AlertInf could be adapted for maize grown under different tillage conditions taking this into account. Another factor that could influence the emergence and effectiveness of the model is soil type, which was not considered in this experiment and should be further investigated. A good example is the study by Leblanc et al. (2004), who calibrated a predictive mathematical model to different soil types by adjusting the base temperature of *Chenopodium album* seedlings to the soil texture.

Echinochloa crus-galli in maize fields in Croatia is almost always controlled with pre-emergence or post-emergence herbicides, usually in combination with inter-row cultivation. According to Oriade and Forcella (1999), the efficacy of inter-row cultivation is highest when

60% of *Setaria viridis*, another important monocot maize species, has emerged. Based on experiment data, inter-row cultivation should be applied from 96 to 113 cumulative HT units. The best efficacy of post-emergence foliar herbicides is achieved when 70–80% of weeds have emerged in the field (Masin et al., 2010; Otto et al., 2009). According to AlertInf, foliar application should be made at a cumulative 140–144 HT units. Finally, the AlertInf model can be used to support the adoption of integrated weed control tactics and post-emergence band application with inter-row cultivation, which can significantly reduce herbicide use in maize (Loddo et al., 2020).

Taken all results together, further field trials are needed and should be conducted in two directions depending on the weed species. First, AlertInf needs to be further validated for *Chenopodium album* or *Abutilon therophrasti* based on their estimated base temperature overlap with Italian populations by comparing species emergence in maize fields with those predicted by AlertInf. Second, the model should be calibrated for *Setaria pumila* and *Amaranthus retroflexus* species, as germination parameters differ significantly. Third, AlertInf should be extended *to Panicum capillare* and *Ambrosia artemisiifolia*, whose germination parameters have now been estimated.

4. CONCLUSIONS

Based on the conducted research, the following conclusions can be made:

1. The weed species differed in the estimated value of the base temperature. The estimated base temperatures of the studied species are: *Ambrosia artemisiifolia* (1.5°C), *Chenopodium album* (3.4°C), *Abutilon theophrasti* (4.5°C), *Setaria pumila* (6.6°C), *Echinochloa crus-galli* (10.8°C), *Panicum capillare* (11.0°C), *Amaranthus retroflexus* (13.9°C). In addition, the studied species differed in the estimated values of the base water potential. The estimated base water potentials for the studied species are: *Chenopodium album* (-1.38 MPa), *Echiniochloa crus-galli* (-0.97 MPa), *Ambrosia artemisiifolia* (-0.89 MPa), *Panicum capillare* (-0.87 MPa), *Setaria pumila* (-0.71 MPa), *Abutilon theophrasti* (-0.67 MPa) and *Amaranthus retroflexus* (-0.36 MPa).

2. No statistical difference was found between the Croatian and Italian populations of *Abutilon theophrasti* in both germination parameters (base temperature and base water potential). Therefore, the AlertInf model can be validated in the Croatian maize field without calibration.

3. No statistical difference was found between the Croatian and Italian populations of *Echinichloa crus-galli* and *Amaranthus retroflexus* in the base water potential parameters. However, a statistical difference was found for these two species in the base temperature parameters. Therefore, AlertInf should be calibrated for these two species and validated for the base temperature parameters. Moreover, between the Croatian and Italian populations of *Chenopodium album* no statistical difference was found in the base temperature parameter.

4. Statistical differences were found between Croatian and Italian populations of *Setaria pumila* for both parameters studied (base temperature and base water potential). To use the AlertInf model in Croatian maize fields, the model should be calibrated and validated for both studied parameters.

5. Since the AlertInf model does not consider the species *Panicum capillare* and *Ambrosia artemisiifolia*, it should be updated and validated for this weed species in maize fields in continental Croatia.

6. The emergence of *Echinochloa crus-galli* in Croatian maize field was successfully predicted. The overall performance of the model was evaluated by the root mean square error (RMSE) and modeling efficiency (EF). The RMSE is 1.69 and 1.38 in 2019 and 2020, respectively. Moreover, EF is 0.97 and 0.98 in 2019 and 2020, respectively. Using calibrated model AlertInf, it is possible to predict the emergence of *Echinochloa crus-galli* in conventionaly produced maize fields in continental Croatia.

5. REFERENCES:

2. Archer DW, Forcella F, Eklund JJ, Gunsolus J (2001) WeedCast Version 4.0. http://www.ars.usda.gov/services/software/software. htm. Accessed September 15, 2021
3. Anonymous (2017) Invasive species compendium database, CAB International. available at: www.cabi.org/isc., accessed 20 September 2021.
4. Arai, M., Miyahara, M. (1960). Physiological and ecological studies on barnyardgrass (*Echinochloa crus-galli* Beauv. var. *oryzicola ohwi*). I. On the primary dormancy of the seed. (1) Relation of the seed covering to dormancy, and effects of temperature and oxygen on breaking dormancy. Crop Science Society Japan Proceedings 29:130-132.
5. Bagavathiannan, M. V., Norsworthy, J. K., Smith, K. L., Burgos, N. (2011). Seedbank Size and Emergence Pattern of Barnyardgrass (*Echinochloa crus-galli*) in Arkansas. Weed Science, 59(3), 359–365.
6. Bajwa, A., Jabran, K., Shahid, M., Ali, Hafiz H., Chauhan, B. Ehsanullah. (2015). Eco-biology and management of *Echinochloa crus-galli*. Crop Protection. 75. 151-162.
7. Baloch, H., Tommaso, A., Watson, A. (2001) Intrapopulation variation in *Abutilon theophrasti* seed mass and its relationship to seed germinability. Seed Science Research, 11 (4), 335-343.
8. Barnes E. R., Werle, R., Sandell, L.D., Lindquist, J.L., Knezevic, S.Z., Sikkema, P.H., Jhala A.J. (2017). Influence of tillage on common ragweed (*Ambrosia artemisiifolia*) emergence pattern in Nebraska. Weed Technol 31:623–631.
9. Baskin, C. C., and Baskin, J. M. (2020). Breaking seed dormancy during dry storage: A useful tool or major problem for successful restoration via direct seeding? Plants (Basel, Switzerland), 9(5), 636.
10. Baskin, J.M., Baskin, C.C. (2004). A classification system for seed dormancy. Seed Science Research, 14(1), 1-16.
11. Baskin, C. C., Baskin, J. M. (2001). Seed ecology, biogeography, and evolution of dormancy and germination. School of Biological Sciences, University of Kentucky, Academic press, 181 – 219.
12. Baskin, J. M., Baskin, C. C. (1986). Seasonal changes in the germination responses of buried witchgrass (*Panicum capillare*) seeds. Weed Science, 34, 22–24
13. Baskin, J. M., and Baskin, C. C. (1980). Ecophysiology of secondary dormancy in seeds of *Ambrosia artemisiifolia*. Ecology, 61(3), 475–480.

14. Batlla, D., Benech-Arnold, R. (2014). Weed seed germination and the light environment: Implications for weed management. Weed Biology and Management, 14, 77–87.
15. Bello, I., A., Micheal D. K. Owen, and Harlene M. Hatterman-Valenti. (1995). Effect of shade on velvetleaf (*Abutilon theophrasti*) growth, seed production, and dormancy. Weed Technology 9, (3): 452–55.
16. Benech-Arnold, R. L., Sanchez, R. A., Forcella, F., Kruk, B. C., Ghersa, C. M. (2000). Environmental control of dormancy in weed seed banks in soil. Field Crops Research 67:105-122.
17. Benech Arnold, R.L., Fenner, M., & Edwards, P. J. (1992). Changes in dormancy level in *Sorghum halepense* seeds induced by water stress during seed development. Functional Ecology, 6(5), 596–605.
18. Benvenuti, S., M. Macchia. (1993). Calculation of threshold temperature for the development of various weeds. Agricoltura Mediterranea, 123:252-256
19. Bergmann, C. K., Werchan, D., Maurer, M., Zuberbier, T. (2008). The threshold value for number of Ambrosia pollen including acute nasal reactions is very low. Allergo Journal, 17, 375-376
20. Bewley, J. D., Black, M. (1994). Seeds: Physiology of development and germination, second edition. Plenum Press. New York and London. 445 pp.
21. Bewley, J. D. and Black, M. (1982). Physiology and biochemistry of seeds in relation togermination: Volume 2: Viability, dormancy, and environmental control. Springer Science & Business Media
22. Bhowmik, P. C. and Doll, J. D. (1982). Corn and soybean response to allelopathic effects of weed and crop residues. Agronomy Journal, 74(4), 601.
23. Boddy, L. G., Bradford, K. J., Fischer, A. J. (2013). Stratification requirements for seed dormancy alleviation in a wetland weed. Plos one, 8 (9), e71457. doi: 10.1371/journal.pone.0071457.
24. Burul, F.; Šoštarčić, V.; Šćepanović, M. (2020). Biologija i ekologija korovnih vrsta roda Panicum L., Fragmenta phytomedica, 34, 5; 40-62.
25. Bürger J., Malyshev, A.V., Colbach, N. (2020). Populations of arable weed species show intraspecific variability in germination base temperature but not in early growth rate. PLoS ONE,15(10): e0240538. https://doi.org/10.1371/journal.pone.0240538
26. Bürger, J. and Colbach, N. (2018). Germination base temperature and relative growth rate of 13 weed species – comparing populations from two geographical origins, 28th German Conference on Weed Biology and Weed Control, Julius-Kuhn-Archiv 458: 419-426.

27. Campagna, G. and Rapparini, G. (2008). Erbe infestanti delle colture agrarie Riconoscimento, biologia e lotta. Edizion L`Informatore Agrario, 17 – 547
28. Cardina, J., Herms, C., and Herms, D. (2011). Phenological Indicators for Emergence of Large and Smooth Crabgrass (*Digitaria sanguinalis* and *D. ischaemum*). Weed Technology, 25(1), 141-150.
29. Chantre, G., R. and González-Andújar, J.L. (2020). Decision Support Systems for Weed Springer Nature Switzerland AG Management, 85-116.
30. Chauhan, S., Johnson, D.E. (2011). Ecological studies on *Echinochloa crus-galli* and the implications for weed management in direct-seeded rice, Crop Protection, 30 (11) 1385-1391.
31. Clements, D.R. and Jones, V.L. (2021). Rapid Evolution of Invasive Weeds Under Climate Change: Present Evidence and Future Research Needs. Frontiers in Agronomy, 3:664034.
32. Clements, D. R., Benoit, D. L., Murphy, S. D, Swanton, C. J. (1996). Tillage effects on weed seed return and seedbank composition. Weed Science 44: 314-322.
33. Colbach, N.; Dürr, C.; Roger-Estrade, J.; Chauvel, B.; Caneill, J. (2006). AlomySys: Modelling black-grass (*Alopecurus myosuroides* Huds.) germination and emergence, in interaction with seed characteristics, tillage and soil climate: I. Construction. European Journal of Agronomy, 24(2), 95–112. https://doi.org/10.1016/j.eja.2005.07.001
34. Corbineau, F., and Côme, D. (1995). “Control of seed germination and dormancy by the gaseous environment,” in Seed Development and Germination, eds J. Kigel, and A. Galili (New York, NY: Marcel Dekker), 397–424.
35. Costea, M., Weaver, S., Tardif, F. (2004). The biology of Canadian weeds. 130. *Amaranthus retroflexus* L., *A. powellii* S. Watson and *A. hybridus* L., Canadian Journal of Plant Science. 84. 631-668.
36. Cunze, S., Leiblein, M. C., Tackenberg, O. (2013). Range expansion of *Ambrosia artemisiifolia* in Europe is promoted by climate change. ISRN Ecology, 1-9.
37. Dawson, J.H., Bruns, V.F. (1975). Longevity of barnyardgrass, green foxtail, and yellow foxtail seeds in soil. Weed Science, 23, 437–440.
38. Derakhshan, A., Akbari, H., Gherekhloo, J. (2014) Hydrotime modeling of *Phalaris minor*, *Amaranthus retroflexus* and *A. blitoides* seed germination. Iranian Journal of Seed Sciences and Research, 1(1): 83-97.
39. Dorado J., Sousa E., Cahla I.M., González-Andújar J.M., Fernández-Quintanilla C. (2009). Predicting weed emergence in maize crops under two contrasting climatic conditions. Weed Research, 49: 251–260.

40. Duary, B. (2008). Recent advances in herbicide resistance in weeds and its management. Indian J. Weed Science, 24, 124–135
41. Efron, B. (1979). Bootstrap methods: another look at the jackknife, The Annals of Statistics, 7(1): 1-26.
42. Egea-Cobrero, V.; Bradley, K.; Calha, I. M.; Davis, A. S.; Dorado, J.; Forcella, F.; Lindquist, J. L.; Sprague, C. L.; Gonzalez-Andujar, J. L. (2020). Validation of predictive empirical weed emergence models of *Abutilon theophrasti* Medik based on intercontinental data. Weed Research, 60(4), 297–302.
43. Ellison, A. M. (2001). Interspecific and intraspecific variation in seed size and germination requirements of Sarracenia (Sarraceniaceae), American Journal of Botany 88, 429-437.
44. Essl, F., Biró, K., Brandes, D., Broennimann, O., Bullock, J., Chapman, D., Chauvel, B., Dullinger, S., Fumanal, B., Guisan, A., Karrer, G., Kazinczi, G., Kueffer, C., Laitung, B., Lavoie, C., Leitner, M., Mang, T., Moser, D., Müller-Schärer, H., and Follak, S. (2015). Biological Flora of the British Isles: Ambrosia artemisiifolia. Journal of Ecology. 103. 1069-1098.
45. EUROSTAT (2021). https://ec.europa.eu/eurostat/data/statistics-a-z, accessed 15 September 2021.
46. FAOSTAT (2021). Food and Agriculture Organization of the United Nations: http://www.fao.org/faostat/, access 15 September 2021.
47. Farooq, S., Onen, H., Özaslan, C., Baskin, C.C., Gunal, H. (2019). Seed germination niche for common ragweed (*Ambrosia artemisiifolia* L.) populations naturalized in Turkey. South African Journal of Botany. 123. 361-371.
48. Fenner M. (1991). The effects of the parent environment on seed germinability. Seed Science Research, 1, 75–84.
49. Forcella, F., R. L. Benech Arnold, R. Sanchez, C. M. Ghersa (2000). Modelling seedling emergence. Field Crop Research 67:123-139
50. Forcella, F. (1998). Real-time assessment of seed dormancy and seedling growth for weed management. Seed Science Research, 8(2), 201-210.
51. Fumanal, B., Chauvel, B., Sabatier, A., Bretagnolle, F. (2007). Variability and cryptic heteromorphism of *Ambrosia artemisiifolia* seeds: what consequences for its invasion in France? Annals of Botany, 100, 305-313.
52. Galzina, N., Barić, K., Šćepanović, M., Goršić, M., Ostojić, Z. (2010). Distribution of the invasive weed *Ambrosia artemisiifolia* L. in Croatia. Agriculture Conspectus Scientificus, 75(2), 75-81.
53. García, A. L., Recasens, J., Forcella, F., Torra, J., Royo-Esnal, A. (2013). Hydrothermal emergence model for ripgut brome (*Bromus diandrus*). Weed Science, 61(1), 146–153.

54. Gardarin, A., Guillemin, J.P., Munier-Jolain, N., Colbach, N. (2010). Estimation of key parameters for weed population dynamics models: Base temperature and base water potential for germination. European Journal of Agronomy, 32. 162-168.
55. Genton, B.J., Shykoff, J.A., Giraud, T. (2005) High genetic diversity in French invasive populations of common ragweed, *Ambrosia artemisiifolia*, as a result of multiple sources of introduction. Molecular Ecology, 14 (14), 4275-85
56. Ghantous, K.M., Sandler, H.A. (2012). Mechanical scarification of dodder seeds with a handheld rotary tool. Weed Technology 26: 485-489
57. Gonzalez-Andujar, J. Chantre, G., Morvillo, C., Blanco, A., Forcella, F. (2016). Predicting field weed emergence with empirical models and soft computing techniques. Weed Research, 56, 415-423.
58. Goršić, M. (2012). Učinak kombinacija topramezona i adjuvanata na vrste *Abutilon theophrasti* Med., *Ambrosia artemisiifolia* L. i *Amaranthus retroflexus* L. u kukuruzu, PhD thesis, University of Zagreb Faculty of Agriculture.
59. Gross, K. L., Maruca, D. and Pregitzer, K. S. (1992). Seedling growth and root morphology of plants with different life-histories. The New Phytologist, 120: 535–542
60. Grundy, A. C. (2003) Predicting weed emergence: A review of approaches and future challenges. Weed Research, 43(1), 1–11
61. Guillemin, J.P., Gardarin, A., Granger, S., Reibel, C., Munier-Jolain, N. & Colbach, N. (2013) Assessing potential germination period of weeds with base temperatures and base water potentials. Weed Research, 53, 76–87.
62. Gummerson R. J. (1986). The effect of constant temperatures and osmotic potential on germination of sugar beet. Journal of Experimental Botany, 41:1431-1439.
63. Haj Seyed Hadi, M.R., Gonzalez-Andujar, J.L. (2009) Comparison of fitting weed seedling emergence models with nonlinear regression and genetic algorithm, Computers and Electronics in Agriculture, 65:19–25.
64. Harre, N. T., Weller, S. C., Young, B. G. (2019). An improved method to shorten physiological dormancy of giant ragweed (*Ambrosia trifida*) seed. Weed Science, 67, 205-213.
65. Hock, S. M., Knezevic, S. Z., Petersen, C. L., Eastin, J., Martin, A. R. (2006). Germination techniques for Common lambsquarters (*Chenopodium album*) and Pennsylvania smartweed (*Polygonum pensylvanicum*). Weed technology, 20: 530-534.
66. Holm, L.; Doll, J.; Holm, E.; Pancho, J.; Herberger, J. (1997). World Weeds: Natural Histories and Distribution, John Wiley & Sons, New York.
67. Hulina, N. (1998). Korovi. Školska knjiga. Zagreb, 127-133.

68. Ivanek-Martinčić, M., Ostojić, Z., Barić, K. i Goršić, M. (2010). Važnost poznavanja kritičnoga razdoblja zakorovljenosti poljoprivrednih kultura. Poljoprivreda, 16 (1), 57-61.
69. Izquierdo J.; Bastida F.; Lezaún J.M.; Sánchez del Arco M.J.; González-Andújar J.L. (2013). Development and evaluation of a model for predicting *Lolium rigidum* emergence in winter cereal crops in the Mediterranean area. Weed Research, 53, 269–278.
70. James, T.K.; Rahman, A.; Mcgill, C.R.; Trivedi, P.D. (2011). Biology and survival of broom corn millet (*Panicum miliaceum*) seed. New Zealand Plant Protection, 64, 142-148.
71. Kalinová, J. and Moudrý, J. (2005). Frost resistance evaluation of the common millet (*Panicum miliaceum* L.) varieties. Agricultura, 3:10–12.
72. Kakar, G., Laghari, G., Kakar, A., & Memon, M., Kakar, H., Jatoi, G. (2016). Allelopathic effect of common lambsquarter (*Chenopodium album*) on seedling growth of different maize varieties. Sci.Int.(Lahore). 28, 4595-4600.
73. Kelly, K.M., Van Staden, J. and Bell, W.E. (1992). Seed coat structure and dormancy. Plant Growth Regulation,11, 201–209.
74. Kennedy, R.A., Rumpho, M.E., Fox, T.C., (1987). Germination physiology of rice and rice weeds: metabolic adaptations to anoxia. In: Special Publication of the Brittish Ecological Society. Blackwell Scientic Publications, Oxford,193-203.
75. Kivilaan, A., Bandurski R.S. (1981) The one hundred year period for Dr Beal's seed viability experiment. American Journal of Botany 68, 1290-1292.
76. Knežević, M. (2006). Atlas korovne, ruderalne i travnjačke flore. Poljoprivredni fakultet Osijek. Udžbenici Sveučilišta Josipa Jurja Strossmayera u Osijeku. 258-260
77. Knezevic, S, Weise, S. F., Swanton, C.J. (1994) Interference of Redroot Pigweed (*Amaranthus retroflexus*) in Corn (*Zea mays*), Weed Science, 42:568-573
78. Konstantinovic, B., Blagojević, Konstantinović, B., M., Samardzic, N. (2014). Allelopathic effect of weed species *Amaranthus retroflexus* L. on maize seed germination. Romanian Agricultural Research, 31, 315-321.
79. Kordan, H.A., Ashraf, M.1(1990). Environmental anoxia is unnecessary for inhibiting chloroplast photomorphogenesis in rice coleoptiles. Journal of Experimental Botany. 41, 435-440.
80. Korsmo, E, Torstein, V, Fykse, H. (1981). Korsmos' Ugras Plansjer. Oslo, Norway: Norsk Landbruk/Landbruks Forlaget
81. Kottek, M.; Grieser, J.; Beck, C.; Rudolf, B.; Rubel, F. (2006). World map of the Koppen-Geiger climate classification updated. Meteorologische Zeitschrift, 15, 259–263

82. Kropff, M. J., Vossen, F. J. H, Spitters, C. J. T, Groot, De W. (1984). Competition between a maize crop and a natural population of *Echinochloa crus-galli* (L.). Netherlands Journal of Agricultural Science, 35, 324–327
83. Landolt, E. (1977). Ökologischer Zeigerwerte zur Schweizer Flora. In: Veroffentlichungen des Geobotanischen Institutes der Eidg. Techn. Hochschule. Stiftung Rubel, 208.
84. Leblanc, M. L., Cloutier, D. C., Stewart, K. A., Hamel, C. (2004) Calibration and validation of a common lambsquarters (*Chenopodium album*) seedling emergence model. Weed Science, 52(1), 61–66.
85. Leblanc, M.L., Cloutier, D.C., Stewart, K.A., Hamel C. (2003) The use of thermal time to model common lambsquarters (*Chenopodium album*) seedling em emergence in corn. Weed Science, 51:718–724
86. Leguizamon, E.S., Fernandez-Quintanilla, C., Barroso, J., Gonzalez-Andujar, J.L. (2005) Using thermal and hydrothermal time to model seedling emergence of *Avena sterilis* spp. *ludoviciana* in Spain. Weed Research, 45:149-156.
87. Lehoczky E, Gólya G, Szabó R, Szalai A. (2011). Allelopathic effects of ragweed (*Ambrosia artemisiifolia* L.) on cultivated plants. Communications in Agricultural and Applied Biological Sciences, 76(3):545-9.
88. Leiblein-Wild, M.C., Kaviani, R., Tackenberg, O. (2014). Germination and seedling frost tolerance differ between the native and invasive range in common ragweed. Oecologia; 174: 739–750
89. Lemić, M., Šćepanović, M., Barić, K., Svečnjak, Z., Jukić, Ž. (2014.). Metode prekidanja dormantnosti sjemena bijele lobode (*Chenopodium album* L.). Agronomski glasnik, 1-2: 45-60.
90. Leon R. G., Knapp A. D., Owen, M. D. K. (2004). Effect of temperature on the germination of common waterhemp (*Amaranthus tuberculatus*), giant foxtail (*Setaria faberi*), and velvetleaf (*Abutilon theophrasti*), Weed Science 52, 67-73.
91. Loddo, D., Scarabel, L., Sattin, M., Pederzoli, A., Morsiani, C., Canestrale, R., Tommasini, M.G. (2020). Combination of herbicide band application and inter-row cultivation provides sustainable weed control in maize, Agronomy, 10, 20
92. Loddo, D.; Bozic, D.; Calha, I. M.; Dorado, J.; Izquierdo, J.; Šćepanović, M.; Barić, K.; Carlesi, S.; Leskovsek, R.; Peterson, D.; Vasileiadis, V. P.; Veres, A.; Vrbničanin, S.; Masin, R. (2019). Variability in seedling emergence for European and North American populations of *Abutilon theophrasti*. Weed Research, 59(1), 15–27.
93. Loddo D., Ghaderi-Far F., Rastegar Z., Masin R. (2018). Base temperatures for germination of selected weed species in Iran. Plant Protection Science, 54: 60–66.
94. Loddo, D.; Sousa E.; Masin R.; Calha, I.; Zanin, G.; Fernández-Quintanilla, C.; Dorado, J. (2013). Estimation and Comparison of Base Temperatures for Germination of

European Populations of Velvetleaf (*Abutilon theophrasti*) and Jimsonweed (*Datura stramonium*). Weed Science, 61, 3, 443-451

95. Martinkova, Z., Honek, A., and Lukas, J. (2006). Seed age and storage conditions influence germination of barnyardgrass (*Echinochloa crus-galli*). Weed Science, 54(2), 298-304.
96. Masin R., Loddo D., Gasparini V., Otto S., Zanin G. (2014). Evaluation of weed emergence model AlertInf for maize in soybean. Weed Science, 62: 360–369.
97. Masin R., Loddo D., Benvenuti S., Otto S., Zanin G. (2012). Modeling weed emergence in Italian maize fields. Weed Science, 60: 254–259.
98. Masin R., Loddo D., Benvenuti S., Zuin M.C., Macchia M. i Zanin G. (2010). Temperature and water potential as parameters for modeling weed emergence in central-northern Italy. Weed Science, 58:216-222.
99. Masin, R., Zuin, M. C., Zanin, G., Tridello, G. (2005). Weed Turf: software for improving summer annual weed control in turf. Italian Journal of Agrometeorology, 50(3), 46–50
100. McWilliams, E. L., Landers, R. Q., & Mahlstede, J. P. (1968). Variation in Seed Weight and Germination in Populations of *Amaranthus Retroflexus* L. Ecology, 49(2), 290–296.
101. Mezei, G., Jarai-Komoldi, M., Medzihradsky, Z., Cserhati, E. (1995). Seasonal allergic rhinitis and pollen count (a 5-year survey in Budapest). Orvosi hetilap, s136, 1721–1724.
102. Milberg, P., Andersson, L. (1998). Does cold stratification level out differences in seed germinability between populations? Plant Ecology 134, 225–234.
103. Mollaee, M., Darbandi, E.I., Aval, M.B., Chauhan, B.S. (2020). Germination response of three Setaria species (*S. viridis*, *S. verticillata*, and *S. glauca*) to water potential and temperature using non-linear regression and hydrothermal time models. Acta Physiologiae Plantarum, 42, 149.
104. Myers, M.W.; Curran, W.S.; VanGessel, M.J.; Calvin, D.D.; Mortensen, D.A.; Majek, B.A. (2004). Predicting weed emergence for eight annual species in the northeastern United States. Weed Science, 52, 913–919
105. Nádasy E., Pásztor G., Béres I., Szilágyi G. (2018). Allelopathic effects of *Abutilon theophrasti*, *Asclepias syriaca* and *Panicum ruderale* on maize, Julius-Kühn-Archiv, 458, 453-457.
106. Ngouajio, M, Lemieux, C, Leroux, GD. (1999). Prediction of corn (*Zea mays*) yield loss from early observations of the relative leaf area and the relative leaf cover of weeds. Weed Science, 47(3):297-304.

107. Nimis, P.L., Martellos, S. (2001). Testing the predictivity of ecological indicator values. A comparison of real and `virtual' relevés of lichen vegetation. Plant Ecology 157, 165–172.
108. Oerke E.C. (2005). Crop losses to pests. Journal of Agricultural Science 44: 31-43
109. Onofri, A., Mesgaran, M., Neve, P., Cousens, R. (2014). Experimental design and parameter estimation for threshold models in seed germination. Weed Research. 54. 10.1111/wre.12095.
110. Onofri A, Gresta F, Tei F (2010) A new method for the analysis of germination and emergence data of weed species. Weed Research 50:187–198
111. Oriade, C., Forcella, F. (1999). Maximizing efficacy and economics of mechanical weed control in row crops through forecasts of weed emergence. Journal of Crop Production, 2, 189–205.
112. Orton, D. A. (1996). Using plants to time pest control. Grounds Maint. 1:14–19
113. Oryokot JOE, Murphy SD, Thomas AG, Swanton CJ (1997) Temperature- and moisture-dependent models of seed germination and shoot elongation in green and redroot pigweed (*Amaranthus powellii*, *A. retroflexus*). Weed Sci 45:488–496.
114. Otto, S.; Masin, R.; Casari, G.; Zanin, G. (2009). Weed–Corn Competition Parameters in Late-Winter Sowing in Northern Italy. Weed Science, 57, 194–201
115. Păunescu, G. (1997). Researches regarding the depth influence and durability upon caryopsis germination of *Echinocloa crus-galli*. Proplant, 97, 155-164.
116. Peters, N. C. B. (1982). The dormancy ofwild oat seed (*Avena fatua* L.) from plants grown under various temperature and soil moisture conditions. Weed Res. 22, 205–212.
117. Peters, R. A., Yokum, H. C. (1961). Progress report on a study of the germination and growth of yellow foxtail (*Setaria glauca* L. Beauv.) NEWCC Proc., 15, 350-355.
118. Pintar, A., Svečnjak Z., Šoštarčić V., Lakić J., Barić K., Brzoja D., Šćepanović M. (2021) Growth stage of *Alopecurus myosuroides* huds. determines the efficacy of pinoxaden. Plants, 10(4):732.
119. Plodinec, M., Šćepanović, M., Barić, K., Jareš, D. (2015). Inter-populacijska varijabilnost u nicanju korovne vrste *Abutilon theophrasti* Med., Agronomski glasnik, 77(1-2), 23-40.
120. Probert, R.J. (1992). The role of temperature in germination ecophysiology. In: Fenner, M. (Ed.), Seeds: The Ecology of Regeneration in Plant Communities. CAB International, Wallingford, UK, 285-325.
121. Povilaitis, B. (1956). Dormancy studies with seeds of various weed species. Proceedings of the International Seed Testing Association, 21, 87–111.

122. Ramanarayanan, T.S.; Williams, J.R.; Dugas, W.A.; Hauck, L.M.; McFarland, A.M.S. (1997). Using APEX to Identify Alternative Practices for Animal Waste Management. Part I: Model Description and Validation. St. Joseph, MI: American Society of Agricultural Engineers, 1997, ASAE Paper No. 972209
123. Ramesh, K., Matloob, A., Aslam, F., Florentine, S., and Chauhan, B. S. (2017). Weeds in a changing climate: vulnerabilities, consequences, and implications for future weed management. Frontiers in Plant Science, 8:95.
124. Roché, C., Thill, D., Shafii, B. (1997). Estimation of base and optimum temperatures for seed germination in common crupina (Crupina vulgaris). Weed Science, 45(4), 529-533.
125. Rolston, M.P. (1978). Water impermeable seed dormancy, Botanical Review, 44, (3), 365-396.
126. Roman, E.S., Murphy, S.D., Swanton, C.J. (2000). Simulation of seedling emergence. Weed Science 48:217–224.
127. Roman, E.S., Thomas, A.G., Murphy, S.D., Swanton, C.J. (1999). Modelling germination and seedling elongation of common lambsquarters (*Chenopodium album*). Weed Sci 47:149–155.
128. Royo-Esnal A., García A.L., Torra J., Forcella F., Recasens J. (2015). Describing *Polygonum aviculare* emergence in different tillage systems. Weed Research, 55: 387–395.
129. Royo-Esnal, A., J. Torra, J.A., Conesa, F. Forcella, J. Recasens (2010). Modeling the emergence of three arable bedstraw (Galium) species. Weed Science 58: 10 -15.
130. Sadeghloo A., Ashgari J., Ghaderi-Far F. (2013). Seed germination and seedling emergence of velvetleaf (*Abutilon theophrasti*) and barnyardgrass (*Echinochloa crus-galli*). Planta Daninha, 31: 259–266.
131. Sang, W., Xinyan, L., Axmacher, J. (2011). Germination and emergence of *Ambrosia artemisiifolia* L. under changing environmental conditions in China. Plant Species Biology. 26, 125-133.
132. Sanyal, D., Bhowmik, P. and Reddy, K. (2006). Leaf characteristics and surfactants affect primisulfuron droplet spread in three broadleaf weeds. Weed Science, 54(1), 16-22.
133. Sartorato, I., G. Pignata. (2008). Base temperature estimation of 21 weed and crop species. Pages 112–113 in Proceedings of the 5th International Weed Science Congress. Vancouver, Canada: International Weed Science Society.
134. Sattin, M., Zanin, G., Berti, A. (1992). Case history for weed competition/population ecology: velvetleaf (*Abutilon theophrasti*) in corn (Zea mays). Weed technology 6:213-219.

135. Sauer J.D. (1967). The grain amaranths and their relatives: A revised taxonomic and geographic survey. Annals of the Missouri Botanic Garden, 54:103-137.
136. Shipley, B. and Parent, M. (1991). Germination responses of 64 wetland species in relation to seed size, minimum time to reproduction and seedling relative growth rate. Functional Ecology, 5: 111–11
137. Shrestha, A., Erivelton, S.R., Thomas, A.G. & Swanton, C.J. (1999) Modeling germination and shoot-radicle elongation of *Ambrosia artemisiifolia*. Weed Science, 47, 557–562
138. Sibuga, K. P., Bandeen, J. D. (1980.): Effects of green foxtail and lamb'squarters interference in field corn. Canadian Journal of Plant Science, 60(4):1419-1425.
139. Solomon, W. R. (1984). Aerobiology of pollinosis. The Journal of Allergy and Clinical Immunology, 74(4), 449-61.
140. Spencer, N. R. (1984). Velvetleaf, *Abutilon theophrasti* (Malvaceae), history and economic impact in the United States. Economic Botany, 38: 407–416.
141. Srivastava (2002). CHAPTER 19 - Seed Germination, Mobilization of Food Reserves, and Seed Dormancy Plant Growth and Development Hormones and Environment, 447-471
142. Steinmaus, S.J., Norris, R. F. (2002). Growth analysis and canopy architecture of velvetleaf grown under light conditions representative of irrigated Mediterranean type agroecosystems. Weed Science, 50 (1), 42-5.
143. Steinmaus, S. J., Prather, T. S., Holt, J. S. (2000). Estimation of base temperatures for nine weed species. Journal of Experimental Botany. 51, 275–286
144. Stern, V.M. (1973). Economic thresholds. Annual Review of Entomology, 18:259-28
145. Stevens, O. (1957). Weights of seeds and numbers per plant. Weeds, 5:46-55.
146. Stjepanovic, B., Svecnjak, Z., Hrga, I., Večenaj, A., Šćepanović, M., Barić, K. (2015). Seasonal variation of airborne ragweed (*Ambrosia artemisiifolia* L.) pollen in Zagreb, Croatia. Aerobiologia, 31(4), 525-535.
147. Subedi, K.D, B.L. Ma (2009). Assessment of some major yield-limiting factors on maize production in a humid temperate environment, Field Crops Research 110: 21-26.
148. Swanton, C. J., Nkoa, R., and Blackshaw, R. E. (2015). Experimental methods for crop–weed competition studies. Weed Science, 63, 2–11.
149. Swanton, C. J., Weaver, S., Cowan, P., Van Acker, R., Deen, W. and Shreshta, A. (1999). Weed Thresholds: Theory and Applicability in Expanding the Context of Weed Management, Taylor and Francis, Douglas D. Buhler, 9-31.

150. Szabó, R., Nádasy, E., Pásztor, G. (2018). Study on the allelopathic effect of *Amaranthus retroflexus* L., *Datura stramonium* L. and *Panicum miliaceum* L. on the germination of maize. Julius-Kühn-Archiv, 458, 459-468.
151. Šarić T., Ostojić Z., Stefanović L., Deneva Milanova S., Kazinczi G., Tyšer L. (2011). The changes of the composition of weed flora in southeastern and central europe as affected by cropping practices. Herbologia 12: 8-12
152. Šćepanović, M.; Barić, K.; Ostojić, Z.; Pintar, A. (2016). Važnost poznavanja bioloških i ekoloških značajki divljih prosa u integriranom sustavu suzbijanja, Glasilo biljne zaštite, 60. seminar biljne zaštite, Opatija, Hrvatska, 9-12.02.2016.
153. Šćepanović, M., Novak, N., Barić, K., Ostojić, Z., Galzina, N., Goršić, M. (2007). Alelopatski utjecaj korovnih vrsta *Abutilon theophrasti* Med. i *Datura stramonium* L. na početni razvoj kukuruza. Agronomski glasnik, 69 (6), 459-472.
154. Šoštarčić, V., Višić, D., Šćepanović, M. (2021). Inter-populacijska varijabilnost sjemena ambrozije - mehanizam uspješne prilagodbe na različite okolišne uvjete, Glasilo future, 4; 65-78.
155. Tang, D. S., Hamayun, M., Ko, Y. M., Zhang, Y. P., Kang, S. M., Lee, I. J. (2008). Role of red light, temperature, stratification and nitrogen in breaking seed dormancy of Chenopodium album. Journal of Crop Science and Biotechnology, 11 (3): 199-204.
156. Taylor, F., Davies, L. Cobb, A. (1981). An analysis of the epicuticular wax of *Chenopodium album* leaves in relation to environmental change, leaf wettability and the penetration of the herbicide bentazone. Annals of Applied Biology. 98. 471 - 478.
157. Taylorson, R. B. (1979). Response of weed seeds to ethylene and related hydrocarbons. Weed Science, 27, 7–10.
158. Van der Weide, R. Y. (1993.) Population dynamics and population control of Galium aparine L. PhD Thesis, Agricultural University, Wageningen, The Netherlands.
159. Varga, P., Béres, I., Reisinger, P. (2000). Yield decreasing effect of weed species on the maize. (In Hungarian) Hung. Weed Research, Technol. 1, 45-51.
160. Vasileiadis, V. P., Froud-Williams, R. J., Loddo, D., Eleftherohorinos, I. G. (2016). Emergence dynamics of barnyardgrass and jimsonweed from two depths when switching from conventional to reduced and no-till conditions, Spanish Journal of Agricultural Research, 14(1), 1–7.
161. Vengris, J., A. E. Kacperska-Palacz, Livingston, R. E. (1966). Growth and development of barnyardgrass in Massachusetts. Weeds 14:299-301.
162. Vleeshouwers, L. M., Kropff, M. J. (2000) Modelling field emergence patterns in arable weeds. New Phytologist, 148(3), 445–457.
163. Warwick, S. I. and L. D. Black. (1988). The biology of Canadian Weeds. 90. Abutilon theophrasti, Canadian Journal of Plant Science. 68:1069-1085

164. Werle R., Sandell L.D., Buhler D.D., Hartzler R.G., Lindquist J.L. (2014). Predicting emergence of 23 summer annual weed species. Weed Science, 62: 267–279.
165. Werner, E. L., Curran, W.S., Harper, J. K., Gregory W. Roth, & Knievel, D. P. (2004). Velvetleaf (*Abutilon theophrasti*) Interference and Seed Production in Corn Silage and Grain. Weed Technology, 18(3), 779–783.
166. Wiese, A.M., Binning, L. K. (1987). Calculating the threshold temperature of development for weeds. Weed Science 35, 177-179.
167. Wijte, A.H., Gallagher, J.L. (1996). Effects of oxygen availability and salinity on early life history stages of marsh plants. I. Different germination strategies of *Spartina alterniflora* and *Phragmites australis* (Poaceae). American Journal of Botany, 83, 1337-1342.
168. Wright, K., Seavers, G., Peters, N., Marshall, M. A. (1999). Influence of soil moisture on the competitive ability and seed dormancy of Sinapis arvensis in spring wheat. Weed Research. 39. 309 - 317.
169. Wright, R. H. (1972). What good is a weed? Ecology in Action. Lothrop, Lee & Shepard Co., New York
170. Woodstock, L. W. (1988). Seed imbibition: a critical period for successful germination, Journal of Seed Technology, 12 (1), 1-15.
171. Wu H., Asaduzzaman M., Shephard A. and Ma X. (2021) Seed Biology of Witchgrass (*Panicum capillare* L.) Ensures Its Success Under Different Environmental Conditions. Frontiers in Agronomy, 3:657785.
172. Xiong, R. C., Ma, Y., Wu, H.W., Jiang, W.l. and Ma, X.Y. (2018). Effects of Environmental Factors on Seed Germination and Emergence of Velvetleaf (*Abutilon theophrasti*). Planta Daninha. 36.
173. Zambrano-Navea, C., Bastida, F., Gonzalez-Andujar, J. L. (2013). A hydrothermal seedling emergence model for Conyza bonariensis. Weed Research, 53(3), 213–220.
174. Ziska, L. and Caulfield, F. (2000). Rising CO_2 and pollen production of common ragweed (*Ambrosia artemisiifolia* L.), a known allergy-inducing species: implications for public health. Functional Plant Biology. 27. 893-898.

AUTOBIOGRAPHY

Valentina Šoštarčić was born in Zagreb on 14 February 1993. She completed her primary and secondary education in Ivanić Grad. Since April 2016 she has been working as an assistant in the Department of Weed Sciences at the Faculty of Agriculture. Since 2017, she is a PhD student in agricultural sciences at the University of Zagreb, Faculty of Agriculture. In October 2020, she defended the topic of her PhD thesis: "Prediction of weed emergence in maize with hydrothermal modelling". In her previous work, she has published 19 professional, 22 national citated scientific papers (CROSBI), including eight CC papers (Web of Science). She actively participates in national and international scientific and professional conferences. Shee is a collaborator in the implementation of the international teaching project HARISA "Harmonization and innovation in doctoral study programs in plant health for sustainable agriculture" and in the project funded by Ministry of Agriculture "Monitoring of resistance of harmful organisms to pesticides." Under the Erasmus program, she spent a total of eight months at the University of Padua. Moreover, she spent one month at the University of Belgrade Faculty of Agriculture as part of HARISA project mobility. She participates in performing exercises in a total of nine subjects of undergraduate, graduate, and postgraduate studies. She actively participates in the planning and implementation of extracurricular activities "Intriguing World of Weeds", as well as in activities related to the Faculty Promotion Days and popularization of science. She is a member of the Croatian Society for Plant Protection (HDBZ) and the European Society for Weed Research (EWRS).

APPENDICES

Scientific publication 1.

Šoštarčić, V.; Šćepanović, M.; Masin, R.; Maggoso, D.; Zanin, G. (2018). Estimation of biological parameters for germination of *Abutilon theophrasti* Medik., Periodicum biologorum, 120 (2-3), 81-89.

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Estimation of biological parameters for germination of *Abutilon theophrasti* Medik.

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Background and Purpose: *Velvetleaf seed longevity and prolonged emergence complicate assessing appropriate time for its control. Estimating biological parameters (base temperature and base water potential) is a first step towards development of emergence predictive model for this weed species in summer crops. Since, development of new model is time consuming, the aim of the research was to provide the data set of biological thresholds for Croatian ecotype and then to compare it with Italian velvetleaf thresholds to assess the implementation of AlertInf predictive weed emergence model from Italy to Croatia.*

Materials and methods: *Laboratory experiments were conducted with 100 seeds per three replicates at seven constant temperatures (4, 8, 12, 16, 20, 24, 28°C) to estimate base temperature (T_b) and at seven water stress levels (0.00, –0.05, –0.10, –0.25, –0.38, –0.50, –0.80, –1.00 MPa) to estimate base water potential (Ψ_b).*

Results: *Germination was fastest at temperature > 20°C with 3.6 days and at water stress level > –0.25 MPa with 4.9 days to complete 50% of germination (t_{50}). The slowest t_{50} occurred at 4°C (41.9 days) and < –0.38 MPa (10.1 days). Estimated Croatian velvetleaf biological parameters are: 4.5°C (T_b) and –0.67 MPa (Ψ_b) with no significant difference compared to Italian ecotype, according to 95% confidence intervals overlapping.*

Conclusion: *The results indicate that it could be possible to implement AlertInf model from Italy to Croatia for this weed species without additional adjustment. Nevertheless, next step of this implementation should include validation of estimated results in the field conditions.*

INTRODUCTION

Velvetleaf (*Abutilon theophrasti* Medik.) is an annual weed species common in corn, soybean and sugar beet fields. Its success to establish in row summer crops is mostly attributed to poor weed control by pre-emergence (PRE) herbicides *(1)*. Escaped and then not controlled individuals usually produce high number of seed per plant, up to 44 200 *(2)* with high physical seed dormancy *(3)* which could have long-term consequences for weed management. Along with this, herbicide absorption could be enabled due to velvetleaf burial depth in the soil. Typically, PRE herbicides have a good efficacy on the seeds with low seed weight, capable to emerge from the shallow soil depths *(4)* in the herbicide application zone, but provide scarce control of seeds with higher seed weight such as velvetleaf with 1000 seed weight of 9.1g *(5)*. Although, most of velvetleaf seeds emerge in the early period of vegetation season, its emergence can be prolonged during the midgrowing season as well *(6)* when applied herbicides are not efficient anymore.

Such insufficient control of PRE herbicide required a switch to a post-emergence (POST) application, today recognized as a part of Integrated Weed Management. This approach permits the selection of the most effective herbicide, or combination of herbicides, based on emerged weeds in the field *(7)*. Despite all unquestionable benefits, efficacy of POST herbicides depends mostly on time and duration of weed emergence in the field *(8)*. Thus the knowledge of weed emergence is crucial to assess the right time for weed control. It is usually suggested that farmers apply POST herbicides when approximately 70 to 80% of weeds had emerged *(9)*. For example, with too early applications is very likely to have new flush of weed emergence. As consequence, herbicide application should be repeated, which is not environmentally safe and economically profitable. Otherwise, too late application can be less effective especially on weeds with morphological barriers such as waxes and hairs on leaf surface *(10)*. Velvetleaf develops hairs on the leaf surface that slower herbicide absporbion. Therefore is recommended to apply herbicides at earliest growth stage (cotyledons) were is found out that absorption of triflusurfuron (common used herbicide in sugar beet) is about 28% better than applied at first true leave *(11)*.

In order to provide information to farmers about right time for weed control, predictive weed emergence models are developed *(12)*. The main goal of these models is to assess the time span in which most individuals in populations had emerge in the field. Currently, three types of predictive weed emergence models are developed: phenological, empirical and mechanistic (reductionist) models. Most used are empirical models based on monitoring weed emergence at precise field for several years with the intention to estimate their time of emergence in the future years. Often, empirical models combine calendar days of seedling appearance in the field with temperature requirements of each weed species. First generation of weed emergence models were based primary on thermal time (TT) concept *(13, 14, 15)* where daily mean soil (eventually air) temperature is accumulated above a specific threshold (base temperature – T_b), during the cropping season until weed emergence is completed *(16)*. Since temperature is not the only factor that influences germination and these models are not able to accurately predict seedling emergence in condition of water stress, second generation of predictive weed models are developed by integrating soil water potential and soil temperature into hydrothermal time (HTT) models. These models can be better at predicting emergence than TT models *(17)* as they include soil water availability, necessary for seed imbibition and germination (water potential – Ψ_b). Biological parameters (T_b and Ψ_b) are specific for each weed species, but also for different ecotype of species due to different growing and environmental area of development *(12, 18, 19, 20)*.

AletInf is a weed emergence model developed for predicting emergence of some common summer weed species in maize and soybean fields in Veneto region. The model is based on HTT concept and it is available as an interactive web service on the web site of meteorological station ARPAV for farmers in the Veneto Region (http://www.arpa.veneto.it/upload_teolo/agrometeo/infestanti.htm). The information provided to farmers by AlertInf is the percentage of weeds that have already emerged in the field out of the total number of weeds that may potentially emerge until the end of season *(12)*.

Currently in Croatia there are no developed predictive models. The idea of this study is to transfer AlertInf model from Italy to Croatia. Hypothesis is that for some weed species the same biological parameters could be used in different geographical areas, without conducting specific experiments that are time and sources consuming. Therefore the first step, before implementation of Alertinf in Croatia is estimation of biological parameters (T_b and Ψ_b) for Croatian velvetleaf ecotype in order to compare them with Italian ecotypes for the same species. This comparison is necessary to verify the difference or similitude in germination-emergence behaviour of ecotypes of species simulated by AlertInf and therefore to evaluate the transferability of the model.

Velvetleaf was chosen to be the first weed species for estimation and comparison of biological thresholds. According to farmer reports, velvetleaf is often unsuccessfully controlled in sugar beet and soybean crops. Because of its seed longevity, high seed weight and prolonged emergence in the field, producers usually have problem with estimating appropriate time for a weed control. Therefore, development of predicting weed emergence model for this species would have practical and positive effect on its successful control in important summer crops. Therefore the objectives of this study were i) to provide data set of base temperatures and base water potential for weed species *A. theophrasti* and ii) to compare Italian and Croatian ecotype of velvetleaf with the intension to verify the possibility of introduction AlertInf predictive emergence model from Italy to Croatia using same biological parameters.

MATERIALS AND METHODS

Experiments to determinate base temperature were conducted at the University of Zagreb, Faculty of Agriculture, Department of Weed Science in 2012. Experiments to determinate base water potential were conducted at the University of Padova, Department of Agronomy, Food, Natural resources, Animals and Environment in 2014. Both experiments were performed at constant temperature in germination chamber equipped with UV lamps.

Seed collection

Seeds were collected from spontaneous population of velvetleaf in maize field in summer of 2011 at experimental station of Faculty of Agriculture, University of Zagreb, Sasinovecki Lug (45°52'0''N, 16°10'0.01''E). Velvetleaf mature capsules were collected and then seeds were gently hand cleaned and stored in dry conditions until experiment initiation. Before using the seeds in the experiment, seeds were mechanical scarificated with sandpaper *(12)* in order to break physical dormancy and enable seed imbibition.

Base temperature for germination

Base temperature experiment was composed of three replicates with 100 seeds per Petri dish. Seeds were placed on filter paper imbibed with 10 ml of deionized water into 19-cm-diam Petri dishes sealed with parafilm. Petri dishes were then incubated in germination chambers at seven different constant temperatures (4, 8, 12, 16, 20, 24, 28°C) and photoperiods of 12:12 h (light: dark).

Base water potential for germination

Eight treatments were conducted to determine the effect of osmotic stress on germination. Polyethylene glycol (PEG) 6000 (Sigma-Aldrich Chemie GmbH 25322-68-3) was used to prepare solutions with eight water stress levels: 0.00, –0.05, –0.10, –0.25, –0.38, –0.50, –0.80, –1.00 MPa. Treatments with polyethylene glycol solution consisted of three replicates of 100 seeds per each transparent plastic containers 10-cm-diam, 7-cm-high as described by Masin et al. *(12)* Containers with 50 ml of prepared solution were placed at a constant temperature of 22°C and photoperiod of 12:12h (light: dark).

In both experiment germination was recorded twice daily at higher temperatures (> 20 °C) and lower PEG solutions (> –0.38 MPa) and daily at lower temperatures (< 20°C) and higher PEG solutions (< –0.38 MPa). Germinated seeds were removed from each Petri dish. The seeds were defined as germinated if the radicle was > 1 mm, in both experiments.

Statistical procedures

Mean values of germination rate at different temperatures and water potentials data from experiment were subjected to ANOVA in SAS 8.0 (21). Fisher's protected LSD ($\alpha \le 0.05$) was used to separate means.

Data set of final germination at different constant temperatures and different concentrations of PEG solutions was used to create germination time course for estimation of time necessary for 50% seeds to germinate (t_{50}). In order to observe difference in the speed of cumulative germination between studied temperatures statistical difference was estimated using analysis of variance. The germination time course to determinate t_{50} was analysed using a logistic function in the Bioassay97 program (22) and then transferred into germination rate or reciprocal of time to 50% of germination. Germination linear regression line was created according to bootstrap method *(23)*. The base temperature and base water potential were estimated as the intercept of the regression line with the temperature or water potential axis *(12, 24)*. The bootstrap distribution of the estimated base temperature and base water potential was used to determine 95% confidence interval. Values of base temperature and water potential of the Croatian ecotypes of velvetleaf were then compared to the Italian ecotype, according to the criterion of 95% confidence intervals overlapping. If did not overlap, they were considered statistically different (P = 0.05), as already adopted in similar studies *(25)*.

RESULTS AND DISCUSSION

Base temperature

Final germination rate of *A. theophrasti* was above 50% at each studied temperature and the highest germination was observed at 16 and 24 °C with an average of 79.0 and 77.0%, respectively (data not shown). Although viability tests were not performed, final percentage of germination suggests that the dormancy was not fully broken. Several authors reported optimum temperature for germination of velvetleaf around 24°C *(26)* or between 24 to 30°C *(27)*. However, all temperatures above 35°C *(27)* or 45°C *(26)* could lead to declined germination.

Cumulative germination was recorded for all studied temperatures described at germination graphs (Figure 1). The relationship between temperature and seed germination is described as a two-parameter logistic function for all temperatures regimes. Observed germination at different temperatures for all three replicates are shown as the black dots while solid line represents the predicted value determinate from the logistic function.

The graphs shows typical S-shaped behaviour with initial lag phase (onset) decreased with increasing temperature. As expected, shortest onset (ED 10) occurred at the temperature of 28, 20 and 16°C with no significant difference between. The longest onset was observed at lowest temperature (4°C) where even 31 days were needed for seeds to germinate (Table 1).

Using cumulative germination curve, the information about the time needed for 50% seed to germinated (t_{50}) was obtained as a crucial step in the determination of base temperature (Table 1). As expected, velvetleaf germination speed varied at the different temperatures increasing the days required for 50% germination significantly at temperatures < 20°C. Fastest germination was achieved at temperatures > 20°C with about 4 days needed to complete 50% of germination. The highest temperatures (28°C, 24°C and 20°C) were not significantly different between t_{50}. Lowering the temperatures germination began to slow. At the lowest temperature (4°C) seeds needed almost 42 days to achieve 50% germination which is more than 11-fold lower compared to temperatures > 20°C.

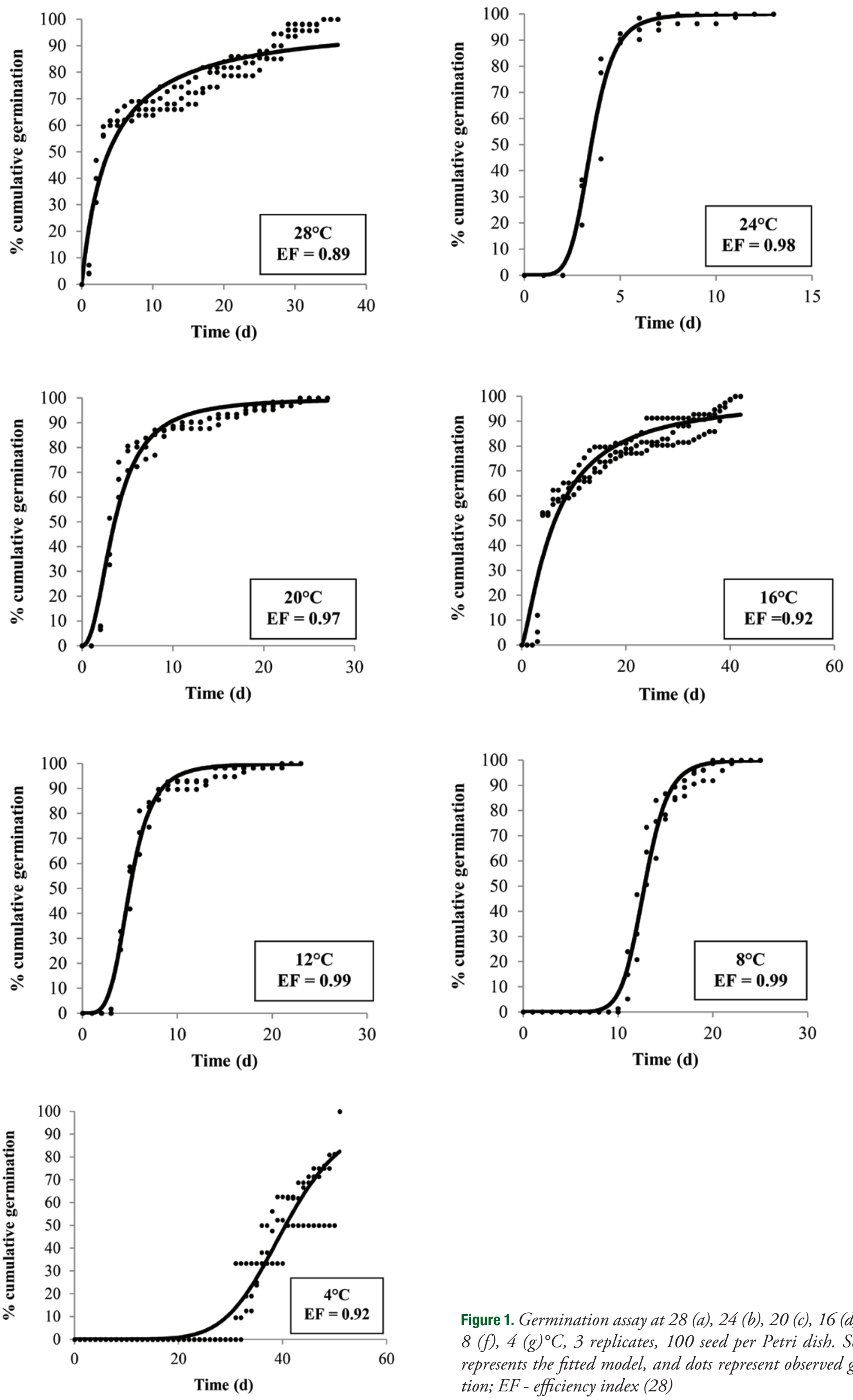

Figure 1. *Germination assay at 28 (a), 24 (b), 20 (c), 16 (d), 12 (e), 8 (f), 4 (g)°C, 3 replicates, 100 seed per Petri dish. Solid line represents the fitted model, and dots represent observed germination; EF - efficiency index (28)*

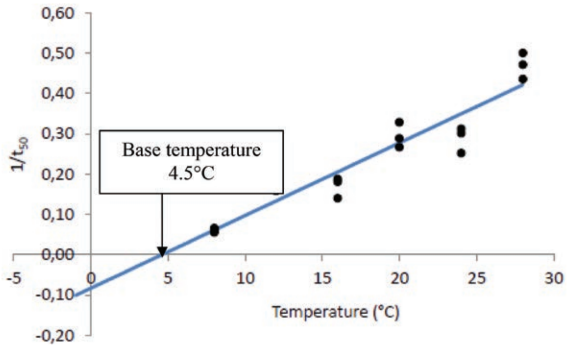

Figure 2. *The solid line represents the linear regression line (y = 0,018x - 0,0826, R^2 = 0,90) and the points are the calculated germination rate ($1/t_{50}$)*

Linear regression across all temperatures showed increasing germination rate by increasing the incubation temperature following a linear trend (Figure 2). The accuracy of the linear estimation is high with an R^2 of 0.90.

Intercept between linear regression line and temperature axis points value, estimated velvetleaf base temperature of 4.5°C. Velvetleaf base temperature determinate in this study is consistent to those found in literature, closest to those estimated in Italy, Portugal and Spain. Using the same "x-intercept" method *(29)* for Italian velvetleaf ecotype base temperature is 3.9°C *(12)*, for Portugal ecotype 3.4 to 4.8°C, while Spain ecotype of velvetleaf had slightly higher temperature threshold from 4.4 to 5.0°C *(19)*. Although the lots deriving from the Spanish population were characterised by higher base temperatures at all cultivation sites, these differences were not significant according to the criteria of the overlap of confidence intervals *(19)*. Similar value was also determinate for Iran ecotype of 5.0°C *(20)*. Moreover, within the same country, base temperature was tested for different ecotypes. For example, in Italy, for Padua and Pisa velvetleaf ecotypes similar base temperature was estimated, 3.9 and 4.4°C, respectively *(12)*. On the contrary, in central Spain different value of 6.8 °C in non-chilled seeds and 7.2 °C in chilled seeds were reported *(18)*. The highest base temperature of 8.0°C value was estimated in Iowa *(26)*.

Base water potential

Final germination of velvetleaf seed significantly varied with different osmotic potential of the solution. With the increasing of water stress final germination was slightly decreasing to the point where no germination occurs. The greatest germination rate (77.3 %) was achieved at 0.00 MPa (deionized water) and was not significantly different to –0.05 MPa solutions (72.0%). Germination ceased at 0.80 and –1.00 MPa (data not shown). Similar data are reported in the literature by different authors. For example, Illinois population of velvetleaf achieved to germinate in the range of –0.10 to –0.90 MPa, although the germination was decreased rapidly with decreasing potential from 0.00 MPa *(30)* In the same research, velvetleaf was described as species more tolerant to water stress when compared to another researched weed species such as *Ambrosia artemisiifolia* L. which completely stopped germination at –0.60 MPa.

The cumulative germination curves for all studied water potential were fitted to a two-parameter logistic function with EF always higher than 0.88.

The lower water potential i.e. higher osmotic solution lengthened the time taken for seeds to germinate (Figure 3). The highest germination lag (ED 10) occurs from 0.00 MPa and –0.25 MPa (Table 1), while decreasing of onset is followed by increasing of the water potential level. As

Table 1. *Speed of germination presented through the days required for 10% and 50% of germination rate at different incubated temperatures and water potential levels*

Temperature (°C)	ED 10[a]	SE[b]	ED 50[a]	SE[b]	Water potential levels (MPa)	ED 10[a]	SE[b]	ED50[a]	SE[b]
28	0.43 a	0.12	3.83 a	0.41	0.00	0.86 a	0.08	2.56a	0.12
24	2.41 b c	0.08	3.52 a	0.05	–0.05	1.63 a	0.10	3.12a	0.08
20	1.37 ab	0.14	3.60 a	0.16	–0.10	1.19 a	0.16	4.77a	0.27
16	1.25 a	0.20	6.39 b	0.40	–0.25	2.19 a	0.13	4.49a	0.11
12	3.23 c	0.13	5.01 b	0.09	–0.38	4.81 b	0.24	10.62b	0.26
8	10.42 d	0.17	12.90 c	0.14	–0.50	6.26 b	0.28	9.66b	0.23
4	30.38 e	0.75	41.95 d	0.45	–	–	–	–	–
Fisher's LSD	1.31	–	1.81	–	–	2.26	–	3.48	–

[a] values within a column followed by the same letter are not significantly different at P ≥ 0.05
[b] SE; standard error

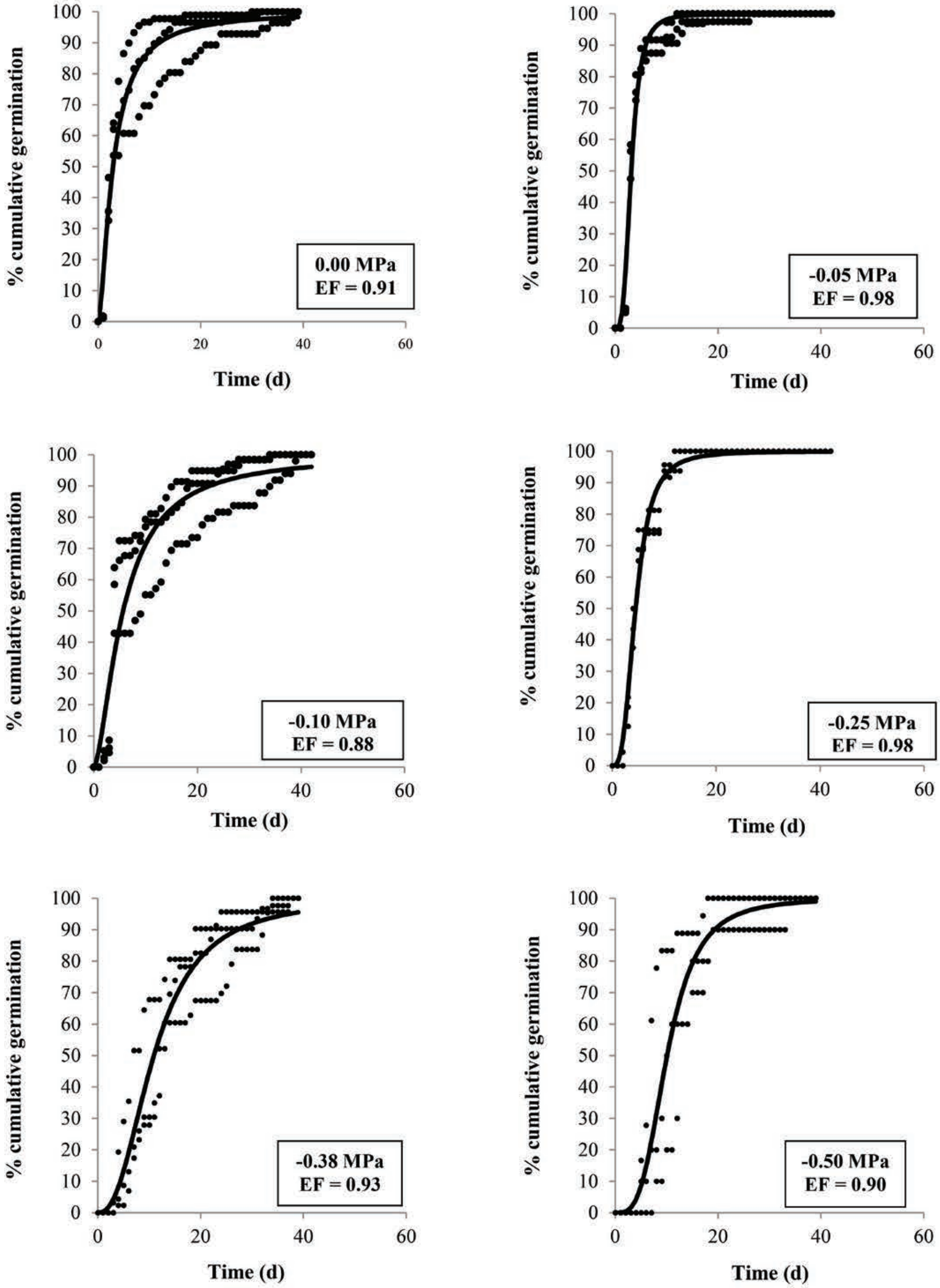

Figure 3. *Germination assay at 0.00 (a), –0.05 (b), –0.10 (c), –0.25 (d), –0.38 (e), –0.50 (f) MPa, 3 replicates, 100 seed per Petri dish. Solid line represents the fitted model, and dots represent observed germination; EF – efficiency index (28)*

confirmed in the literature in field conditions, moisture stress decreases initial germination of velvetleaf which is consequently followed by slower root and shoots elongation *(31)*.

There was no significant difference in time necessary for 50% seeds to germinate at water potential from 0.00 to –0.25 MPa, with an average of 4.9 days (Table 1). Statistical difference occurred at water potential < –0.38 MPa, where it took approximately 10 days for seeds to achieve 50% germination.

Estimated base water potential of velvetleaf is –0.67 MPa (Figure 4) which shows high sensitivity of this weed species to water stress. Although, there are small amount of literature data of base water potential for velvetleaf,

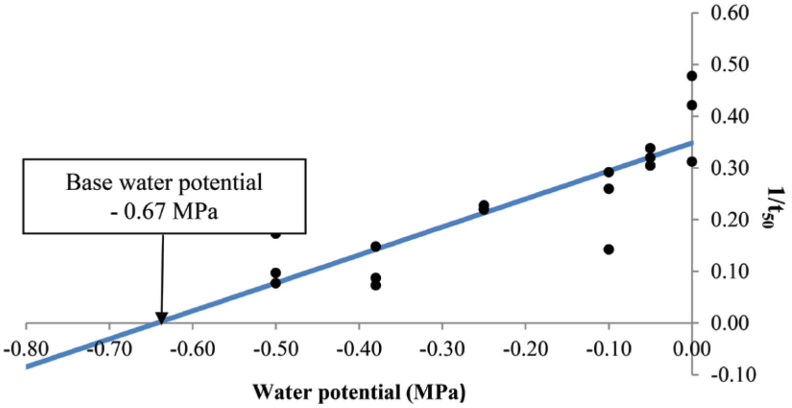

Figure 4. *The solid line represents the linear regression line ($y = 0.3485x + 0.5421$, $R^2 = 0.76$) and the points are the calculated germination rate ($1/t_{50}$)*

available values are not quite different among different population. The recorded base water potential for Spain population is at the range from –0.64 to –0.73 MPa *(18)*, similar with Italian ecotype of –0.78 MPa *(12)* and Iran ecotype of –0.60 MPa *(20)*. It is interesting to observe that the WeedCast predictive emergence model applied within the north-central region of the United States and neighbouring Canada *(32)* uses the highest base water potential of –0.15 MPa.

Comparison of biological parameters among Croatian and Italian ecotypes

The main aim of the research was to determine if there is a difference in biological parameters between Croatian and Italian ecotype of velvetleaf in order to transfer Italian predictive weed emergence model to Croatia. According to the criteria of the overlap of 95% of confidence level, Croatian (4.5°C) and Italian (3.9°C) ecotype showed statistical similar values (Figure 5). Similarly, base water potential between Croatian (–0.67 MPa) and Italian (–0.78 MPa) ecotypes were also not significantly different (Figure 5).

For this weed species the literature does not report a great variability in biological parameters as is for example for weed species *Echinochloa crus-galli* L. (P. Beauv.) with base temperature estimated in the wide range from 5.0 to 13.8°C *(2, 20)* which indicates existence of the high diversities in this population worldwide. However, the differences among velvetleaf ecotypes of different geographic origins still exist. Variation in temperature threshold are explained as the inter-population variability among different geographic origin affected by various climatic conditions under which mother plant is maturating. In the literature this phenomenon is described with the term „preconditioning“ *(33)*. Often, for weed species germination requirements are conditioned with differences in dormancy. For instance, in velvetleaf case physical dormancy is caused by hardness of seed *(34)* which can be less or more developed depending on climatic area *(35)*. Another factor which defines variations within the same species is caused due to genetic characteristic of the seeds. Sexual reproduction and exchanging of genetic material gives the opportunity for a wide spread weed to adjust current environment and therefore to allocate species with most suitable features in the certain environment *(33)*. Hence, importance of the estimation of biological parameters for local ecotype is crucial.

It is considerable to point out that this biological parameter has never been estimated before for any of Croatian weed species. Since results of our study showed no difference in velvetleaf biological parameters compared with ecotype used is AlertInf model, here is open ability

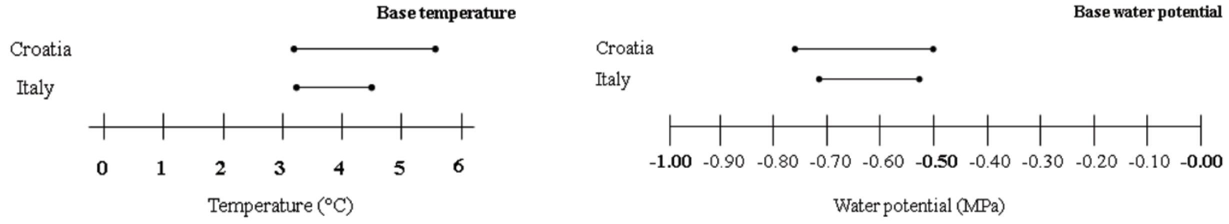

Figure 5. *Comparison of base temperature (left) and base water potential (right) between estimated Croatian and Italian ecotype of velvetleaf determined by Masin et al (22)*

to implement this predictive weed emergence model in Croatia for this species without additional adjustment.

Before implementation it is necessary to valid this estimated parameters in the field conditions where germination is influenced by other factors not included in this research. It is well know that temperature and water potential are not the only factors that influence germination in the field. The benefit of velvetleaf characteristic is the fact that its germination is not influenced by light, like some other weed species, for example *Veronica hederifolia* L. *(29)*. In addition to that, germination is not dependent on different pH of soil like it is for *Echinochloa crus-galli* L. (P. Beauv) which shows sensitivity to acid soils decreasing significantly germination *(20)*. On the contrary, the oxygen levels in different soil type can affect the germination rate of velvetleaf, and with decreasing of the oxygen level in the soil, germination of velvetleaf is inhibited *(36)*. Furthermore, velvetleaf germination could be decreased in high-salinity soil at concentration of 225 mM NaCl. Also, uptake of water by seeds at the different stress levels is increased by the temperature *(20)*, which was not indeed included in our study, where only one temperature was used at different water stress levels.

From all above mentioned it is clear that velvetleaf can germinate under various environmental conditions, although in some cases germination could be reduced. Thus, emergence studies at the field conditions and at different soil type conditions should be performed for this weed species in order to re-evaluate laboratory estimated biological parameters.The first obtained results are quiet positive which encourages further development of the model AlertInf and future studies to test its transferability to Croatia not only for velvetleaf but for other problematic weeds in Croatia.

CONCLUSIONS

Following the aim of the experiment, i) estimation of biological parameters of velvetleaf and ii) comparison of Croatian and Italian biological parameters, was accomplished by determining base temperature of Croatian ecotype at 4.5°C and base water potential at –0.67 MPa with no significant difference compared to Italian ecotype, according to the criteria of the overlap of 95% of confidence level. Therefore, the possibility of implementation of predictive weed emergence model AlertInf for velvetleaf is for now possible. However, to make this statement for sure, it is important to conduct field experiments throughout the several years to see if the laboratory estimated values are valid in the field conditions.

Since, lacking of water can result in absence of germination, although there are optimal temperatures for germination in the field. Combination of two parameters (T_b i Ψ_b) in the future experiments will provide more accurate information about the time of emergence in the field.

Further experiments should be also focused on estimation of biological parameters for other problematic weed species in row crops. Moreover, by including more weed species in predictive model, weed control would be more efficient and useful in summer crops were mixed weed populations usually occur.

REFERENCES

1. ZANIN, G., SATTIN, M 1988 Threshold level and seed production of velvetleaf (*Abutilón theophrasti* Medicus) in maize. Weed Research 28: 347-352. https://doi.org/10.1111/j.1365-3180.1988.tb00813.x
2. STEINMAUS, S J, NORRIS, R F 2002 Growth analysis and canopy architecture of velvetleaf grown under light conditions representative of irrigated Mediterranean-type agroecosystems. Weed Science 50:42-53. https://doi.org/10.1614/0043-1745(2002)050[0042:GAACAO]2.0.CO;2
3. BASKIN, J M, BASKIN, C C 1989 Physiology of dormancy and germination in relation to seed bank ecology. In M. A. Leck, V. T. Parker and R. L. Simpson, eds. Ecology of Soil Seed Banks. New York: Academic Press, pp. 53-66. https://doi.org/10.1016/B978-0-12-440405-2.50009-9
4. BENVENUTI, S, MACCHIA, M, MIELE, S 2001 Quantitative analysis of emergence of seedlings from buried weed seeds with increasing soil depth, Weed Science, 49(4), pp. 528–535. https://doi.org/10.1614/0043-1745(2001)049[0528:QAOEOS]2.0.CO;2
5. PLODINEC, M, ŠĆEPANOVIĆ, M, BARIĆ, K, JAREŠ, D 2015 Inter-populacijska varijabilnost u nicanju korovne vrste *Abutilon theophrasti* Med., Agronomski glasnik. 63, 1-2; 23-40.
6. EGLYE, G H, WILLIAMS, R D 1991 Emergence periodicity of six summer annual weed species. Weed Science 4.595-600.
7. JURSÍK M, SOUKUP J, VENCLOVÁ V, HOLEC J 2011 POST Herbicide combinations for velvetleaf (*Abutilon theophrasti*) control in sugarbeet. Weed Technology, 25:14–18. https://doi.org/10.1614/WT-D-10-00059.1
8. GRUNDY, A C 2003 Predicting weed emergence: a review of approaches and future challenges. Weed Research, 43: 1 – 11. https://doi.org/10.1046/j.1365-3180.2003.00317.x
9. WEEDCAST VERSION 4.0. Technical documentation. Available: https://www.ars.usda.gov/research/software/, accessed 08 March 2017.
10. BATTLA, D, BENECH-ARNOLD, R L 2007 Predicting changes in dormancy level in weed seed soil banks: implications for weed management. Crop Prot. 26:189–197. https://doi.org/10.1016/j.cropro.2005.07.014
11. STARKE, R J, K A RENNER 1996. Velvetleaf (*Abutilon theophrasti*) and sugarbeet (*Beta vulgaris*) response to triflusulfuron and desmedipham plus phenmedipham. Weed Technology. 10:121 -126. https://doi.org/10.1017/S0890037X00045814
12. MASIN R, LODDO D, BENVENUTI S, ZUIN M C, MACCHIA, M, ZANIN G 2010 Temperature and water potential as parameters for modeling weed emergence in central-northern Italy. Weed Science, 58:216-222. https://doi.org/10.1614/WS-D-09-00066.1
13. ROMAN, E S, MURPHY, S D, SWANTON, C, J, 2000 Simulation of *Chenopodium album* seedling emergence. Weed Science, 48:217–224. https://doi.org/10.1614/0043-1745(2000)048[0217:SOCASE]2.0.CO;2

14. MARTINSON, K, DURGAN, B, FORCELLA, F, WIERSMA, J, SPOKAS, K ARCHER, D 2007 An emergence model for wild oat (*Avena fatua*). Weed Science, 55: 584-591. https://doi.org/10.1614/WS-07-059.1

15. IZQUIERDO, J, GONZÁLEZ-ANDÚJAR J, BASTIDA F, LEZAÚN J A, SÁNCHEZ-DEL M J 2009 A thermal time to predict corn poppy (*Papaver rhoeas*) emergence in cereal fields. Weed Sci. 57: 660 – 664. https://doi.org/10.1614/WS-09-043.1

16. GONZALEZ-ANDUJAR J L, CHANTRE G R, MORVILLO C, BLANCO A M FORCELLA F 2016 Predicting field weed emergence with empirical models and soft computing techniques. Weed Research 56, 415–423. https://doi.org/10.1111/wre.12223

17. MCGIFFEN M, SPOKAS K, FORCELLA F, ARCHER D, POPPE, S, FIGUEROA R 2008 Emergence prediction of common groundsel (*Senecio vulgaris*). Weed Science 56, 58–65. https://doi.org/10.1614/WS-07-060.1

18. DORADO, J, FERNANDEZ-QUINTANILLA C, GRUNDY A C 2009 Germination patterns in naturally chilled and non-chilled seeds of fierce thornapple (*Datura ferox*) and velvetleaf (*Abutilon theophrasti*). Weed Science, 57:155–162. https://doi.org/10.1614/WS-08-122.1

19. LODDO D, SOUSA E, MASIN R, CALHA I, ZANIN G, FERNANDEZ-QUINTANILLA C, DORADO J 2013 Estimation and Comparison of Base Temperatures for Germination of European Populations of Velvetleaf (*Abutilon theophrasti*) and Jimsonweed (*Datura stramonium*). Weed Science 61:443–451. https://doi.org/10.1614/WS-D-12-00162.1

20. SADEGHLOO, A, ASGHATI, J, GHADERI-FAR, F 2013 Seed germination and seedling emergence of velvetleaf (*Abutilon theophrasti*) and barnyardgrass (*Echinochloa crus-galli*). Planta Daninha, Viçosa-MG, v. 31, n. 2, p. 259-266. https://doi.org/10.1590/S0100-83582013000200003

21. SAS INSTITUTE 1997 SAS/STAT Software: Changes and enhancements through Rel. 6.12. SAS Inst., Cary, NC.

22. ONOFRI, A 2001 BIOASSAY97: A New EXCEL tVBA Macro to Perform Statistical Analyses on Pesticide Dose-Response Data http://www.agr.unipg.it/disaprov/bioassay97/bioassay97.htm; last accessed 10 January 2017.

23. EFRON, B 1979 Bootstrap methods: another look at the jackknife. Ann. Stat. 7:1–26. https://doi.org/10.1214/aos/1176344552

24. MASIN R, ZUIN M C, ARCHER, D W, ZANIN, G 2005 WeedTurf: a predictive model to aid control of annual summer weeds in turf. Weed Science, 53:193-201. https://doi.org/10.1614/WS-04-066R1

25. LODDO, D, MASIN R, OTTO S, ZANIN G 2012 Estimation of base temperature for *Sorghum halepense* rhizome sprouting. Weed Research 52:42–49. https://doi.org/10.1111/j.1365-3180.2011.00886.x

26. LEON R G, KNAPP A D, OWEN, M D K 2004 Effect of temperature on the germination of common waterhemp (*Amaranthus tuberculatus*), giant foxtail (*Setaria faberi*), and velvetleaf (*Abutilon theophrasti*). Weed Science 52, 67-73. https://doi.org/10.1614/P2002-172

27. HOROWITZ, M, TAYLORSON, R B 1984 Hardseededness and germinability of velvetleaf (*Abutilon theophrasti*) as affected by temperature and moisture. Weed Science 32: 111 – 115.

28. LOAGUE, K, GREEN, R E 1991 Statistical and graphical methods for evaluating solute transport models: overview and application. J. Cont. Hydrol. 7:51-73. https://doi.org/10.1016/0169-7722(91)90038-3

29. GUILLEMIN, J P, GARDARIN, A, GRANGER, S , REIBEL, C, MUNIER-JOLAIN, N, COLBACH, N 2013 Assessing potential germination period of weeds with base temperatures and base water potential, Weed Research 53: 76 – 87. https://doi.org/10.1111/wre.12000

30. RAYNAL, D, J, BAZZAZ, F, A 1973 Establishment of early successional plant populations on forest and prairie soil. Ecol. 54: 1335 – 1341. https://doi.org/10.2307/1934196

31. BECKER, R L 1979 Weed seedling emergence under osmotic stress. Abstract. Proc. North Cent., Weed Control Conf. 34: 45.

32. ARCHER, D W, FORCELLA F, EKLUND J J, AND J GUNSOLUS 2001 WeedCast Version 2.0. www.morris.ars.usda.gov

33. BASKIN, C C, BASKIN, J M 2001 Seed ecology, biogeography, and evolution of dormancy and germination. School of Biological Sciences, University of Kentucky, Academic press: 181 – 219.

34. WARWICK, S I, BLACK, L D 1988. The biology of Canadian weeds. 90. *Abutilon theophrasti*. Canadian Journal of Plant Science 68 : 1069-1085. https://doi.org/10.4141/cjps88-127

35. CARDINA, J, SPARROW, DH 1997 Temporal Changes in Velvetleaf (*Abutilon theophrasti*) Seed Dormancy, Weed Science, 45: 61-66.

36. HOLM, R E 1972 Volatile metabolites controlling germination in buried weed seeds. Plant Physiol. 50: 293 – 297. https://doi.org/10.1104/pp.50.2.293

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Intrapopulation variability in morphological and functional properties of *Ambrosia artemisiifolia* L. seeds

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Morfološka i funkcionalna intrapopulacijska varijabilnost sjemena korovne vrste *Ambrosia artemisiifolia* L.

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ABSTRACT

The aim of the research was to determinate the degree of variation in morphological (seed weight) and functional polymorphism (final germination, dormancy, seed mortality and germination dynamic) within two seed populations of *Ambrosia artemisiifolia* L. Seeds were collected separately from 20 plants/population in Jastebarsko (45°40'18˝N; 15°39'03˝E), population-J and Popovača (45°34´1˝N; 16°40´43˝E), population-P in 2014. Seed weight of J and P populations had weak variability (CV-J = 18.2%, CV-P= 29.8%) with an average of 4.05 g (J) and 4.54 g (P). Estimated variability was strong for amount of death seeds in the J (CV = 67.6%), but weak for geminated (CV = 4.9%) and moderate for dormant seeds (CV = 51.0%). Variability within P population was moderate for both germinated and dead seeds (CV = 34.5%; 34.1%), while variability within amount of dormant seeds was weak (CV = 14.2%). Variability in medium germination (T_{50}) was relatively low for both experimental populations (CV – J = 12.0%; CV – P = 22.4%). Despite determined intrapopulation variation, degrees of variability between researched parameters (final germination, dormancy and medium germination – T_{50}) were weak or moderate and therefore acceptable for further experiment base on seed testing.

Keywords: common ragweed, dormancy, germination dynamic, germination, seed weight

SAŽETAK

Cilj istraživanja bio je utvrditi stupanj morfološke (masa sjemena) i funkcionalne (klijavost, dormantnost, smrtnost sjemena i dinamika klijanja) varijabilnosti unutar dvije populacije sjemena korovne vrste *Ambrosia artemisiifolia* L. (ambrozija) sakupljene u sjeverozapadnom dijelu Hrvatske: Jastrebarsko (45 ° 40'18˝N; 15 ° 39'03˝E), populacija-J i Popovača (45 ° 34´1˝N; 16 ° 40´43˝E), populacija-P. Prikupljeno je sjeme s 20 biljaka/populaciji u listopadu 2014. Rezultati istraživanja ukazuju na slabu varijabilnost (CV-J = 18,2%, CV-P = 29,8%) mase 1 000 sjemenki s prosječnom masom od 4,05 g (J) i 4,54 g (P). Intrapopulacijska varijabilnost bila je relativno visoka za udio mrtvog sjemena u populaciji-J (CV = 67,6%), ali vrlo slaba za udio klijavog (CV = 4,9%) te umjerena za udio dormantnog sjemena (CV = 51,0%). Varijabilnost unutar populacije-P bila je umjerena za udio klijavog i mrtvog sjemena (CV = 34,5%; 34,1%), a relativno slaba za udio dormantnog sjemena (CV = 14,2%). Dinamika klijanja (T_{10}, T_{50} i T_{90}) obje populacije bila je slabo ili umjereno varijabilna. Sredina klijanja (T_{50}) bila je relativno slabo varijabilna za obje istraživane populacije (CV - J = 12,0%; CV - P = 22,4%).

Ključne riječi: ambrozija, dinamika klijanja, dormantnost, klijavost, masa sjemena

DETAILED ABSTRACT

Predictive weed emergence models are one of the advanced tools in Integrated Weed Management (IWM). These models are based on two parameters specific for each species: base temperature (minimum temperature for seed germination) and base water potential (minimum amount of water seed germination). These biological parameters are used in the equation for calculation time of seed germination and later emergence in the field. Procedure for estimation of biological parameters begins with setting germination tests at different constant temperature for base temperature, and different water potential for base water potential estimation. These laboratory experiments tend to be reliable and repeatable because this data is implemented in predictive weed emergence models used in the field. While conducting germination test on *Ambrosia artemisiifolia* L. seed, difference in germination rate and dynamic were observed on the seed with the same origin previously subjected to same storage conditions. Presence of intrapopulation variation in seed germination is well known for weeds and other wild species. Intrapopulation variation has been defined as variation in morphology (seed weight, size, texture, colour) or function (degree of dormancy, mortality, germinability). Morphological and functional polymorphism can be found for seeds originated from the same inflorescence, within the same plant, within different plants from same population and between different population of the same species. Before testing the seed germination in laboratory and estimating biological parameters for *Ambrosia artemisiifolia* it is interesting to investigate possible intrapopulation variation of this invasive weed species. The aims of this research were: (1) to determine existence and degree of morphological polymorphism (variation in seed weight within the population), and (2) to determine existence and degree of functional polymorphism (percentage of seed germination, mortality, dormancy and dynamics of germination) within the population. Seeds were collected separately from 40 plants (20/population) in October 2014. Chosen plants were two to three meters away from each other at 100 m^2 of experimental fields located in the northwest part of Croatia: Jastebarsko (45°40'18˝N ; 15°39'03˝E), population-J and Popovača (45°34´1˝N; 16°40´43˝E), population-P. Weight of 1 000 seeds was measured and the number of seed per each plant was calculated. Seeds were cleaned and storage at dry and dark conditions until the beginning of the experiment. During 2017 (three years after harvest), germination test was conducted in germination chamber with photoperiods of 12h: 12h (light: dark) at alternating 25/15 °C (day : night) temperatures. Non- germinated seeds were subjected to tetrazolium test to distinguish dormant from dead seed. Results indicate weak variability of seed weight of population-J and -P (CV-J = 18.2%, CV-P= 29.8%) with an average weight of 4.05 g (J) and 4.54 g (P). Results of germination and tetrazolium test indicate significant ($P<0.05$) difference in final germination, dormancy, seed mortality and germination dynamic among all individuals at each location. Mean germination of population-J was 87.2% and population-P was 23.4%. Amount of dormant seed within population-J was 8.8%, and within population-P was 18.3%. Within population-P higher amount of dead seed was found (58.3%) than within population-P (3.9%). Estimated variability was strong for amount of death seed in the population-J (CV = 67.6%), but weak for geminated (CV = 4.9%) and moderate for dormant seed (CV = 51.0%) in the same population. Variability within population-P was moderate for both germinated and dead seed (CV = 34.5%; 34.1%) while variability within amount of dormant seed was weak (CV = 14.2%). Germination dynamic (T_{10}, T_{50} and T_{90}) had low or moderate variability for both populations. Variability in medium germination (T_{50}) was relatively weak for both experimental populations (CV – J = 12.0%; CV – P = 22.4%). Furthermore, based on overlapping of medium germination values within population-J plants are divided into two groups. First group reached medium germination within 4.98 to 7.68 days, and second within 9.34 to 16.48 days. Specific pattern of overlapping of medium germination values within population-P was not found. In conclusion, as expected intrapopulation variation within seed population was found to be statistically significant. Despite determined intrapopulation variation, degrees of variability between important researched parameters (final germination, dormancy and medium germination – T_{50}) were weak or moderate and therefore acceptable for further experiment based on seed testing.

UVOD

Intrapopulacijska varijabilnost predstavlja genetsku i fenotipsku različitost (veličina, masa, boja) sjemena formiranog unutar različitih biljaka iz iste populacije (Harper i Obeid, 1967; Moles i sur., 2000). Prema Fenneru (1991) dva su faktora koja uvjetuju intrapopulacijsku varijabilnost: genetska raznolikost i majčinski efekt – okolišni uvjeti kojima je podložna majčinska biljka u vrijeme formiranja i dozrijevanja sjemena. Homogenost u prirodnim uvjetima okoliša narušena je djelovanjem mikroklimatskih uvjeta kao i intraspecijskim odnosima između susjednih biljaka. Osim dostupnosti prirodnih resursa i genetskih obilježja, rekombinacija gena kao posljedica seksualne reprodukcije također definira sposobnost usvajanja resursa u datom okolišu. Sjeme koje se pritom razvija očekivano je u manjoj ili većoj mjeri varijabilno u različitim karakteristikama pa se pojava tipološki varijabilnog sjemena unutar iste populacije ili unutar iste biljke naziva sjemenskim polimorfizmom. Ova pojava razvija se na razini cvata, pojedinačne biljke, klona, pojedine vrste i populacije, a moguće ga je podijeliti na: morfološki i funkcionalni polimorfizam (Fenner, 1991). Morfološki polimorfizam podrazumijeva razvoj sjemena različite boje, veličine, teksture i mase na jednoj pojedinačnoj biljci, između biljaka iz iste populacije ili različitih populacija iste vrste (Milberg i sur., 1996, Baloch i sur, 2001; Ellison, 2001). Funkcionalni polimorfizam podrazumijeva razvoj sjemena različite sposobnosti klijanja odnosno različitog stupnja dormantnosti. Kod pojedinih vrsta funkcionalni polimorfizam povezan je s morfološkim polimorfizmom. Tako sjeme koje sadrži tvrđu sjemenu ovojnicu posjeduje veći stupanj dormantnosti od sjemena mekše sjemene ovojnice. Različiti stupanj dormantnog sjemena unutar populacije osigurava opstanak vrste u varijabilnim uvjetima okoliša što je posebice izraženo kod invazivnih korovnih vrsta kao što je ambrozija. Uspješno širenje invazivnih vrsta na određenom lokalitetu najviše i ovisi o prilagodbi na okolišne čimbenike. Populacije s izraženijom genetičkom raznolikošću u pravilu imaju veću stopu invazivnosti koja kod ambrozije rezultira rasprostranjivanjem i do 20 km godišnje (Jovanović-Radovanov i Božić, 2015).

Molekularnim tehnikama potvrđen je visok nivo heterozigotnosti kod autohtonih i alohtonih populacija ambrozije pa se pretpostavlja da je iz Amerike u Europu unijet veći broj manjih populacija ove vrste (Genton i sur., 2005).

Ambrosia artemisiifolia je na području Republike Hrvatske ekonomski štetan korov u poljoprivrednoj proizvodnji, ali i značajan medicinski problem (alergije). Prema učestalosti pojave, kao jedna od napasnih širokolisnih korovnih vrsta, ambrozija se nalazi na prvom mjestu u okopavinskim usjevima u kontinentalnoj Hrvatskoj (Ostojić, 2011). Velika produkcija sjemena, dugovječnost sjemena u tlu, adaptabilnosti kao i obilna produkcija polena čine ovu vrstu jednom od problematičnih korova ruderalnih i segetalnih površina. Istraživanjem provedenim na 39 lokacija diljem Europe utvrđena je visoka varijabilnost između lokacija u produkciji sjemena i polena ambrozije na području cijele Europe. Najveća produkcija sjemena i polena utvrđena je na području Rumunjske, Hrvatske i Mađarske (Lommen i sur., 2018).

S ciljem uspješnijeg suzbijanja, ambrozija je jedna od vrsta čije se biološke karakteristike sjemena trenutno nastoje ugraditi u model prognoze nicanja koji je jedan od naprednijih metoda detekcije i suzbijanja korova u integriranoj zaštiti bilja. Razvoj i implementacija prognoznih modela temelji se na pouzdanoj i ispravnoj nadogradnji prognoznog software-a preciznim laboratorijskim i vjerodostojnim poljskim podacima. Temeljni podaci koji se ugrađuju u svaki prognozni model odnose se na biologiju pojedine korovne vrste, a potom i na okolišne uvjete koji moraju biti zadovoljeni da bi pojedina vrsta ostvarila svoj ekološki uspjeh (razvoj klijanca, odrasle biljke i plodonošenje). U tom smislu, potrebno je utvrditi biološke parametre potrebne za početan rast i razvoj pojedine vrste biološki minimum(minimalna temperatura potrebna za nicanja pojedine vrste) i biološki vodni potencijal (minimalna količina vlage u tlu koja je potrebna za početak nicanja). Ovi biološki parametri utvrđuju se provođenjem testova klijavosti metodološki posebno prilagođenih za svaku istraživanu vrstu (raspon temperatura i vodnih potencijala).

U preliminarnim istraživanjima bioloških parametara za ambroziju često je primijećen intrapopulacijski nesklad u postotku klijavog sjemena. Poznato je da ambrozija posjeduje intrapopulacijsku sjemensku varijabilnost, ali nije poznata jačina te intrapopulacijske varijabilnosti. Poznavanje jačine sjemenske varijabilnosti odredit će daljnju pouzdanost korištenih podataka i po potrebi modificiranje metodologije utvrđivanja bioloških parametara klijanja za ovu korovnu vrstu. Stoga je cilj istraživanja bio utvrditi: (1) postojanje i jačinu morfološkog polimorfizma, u masi sjemena unutar populacije te (2) postojanje i jačinu funkcionalnog polimorfizma, u postotku klijavosti, smrtnosti, dormantnosti i dinamici klijanja unutar različitih biljaka iz iste populacije.

MATERIJALI I METODE

Sjeme korovne vrste *Ambrosia artemisiifolia* sakupljeno je tijekom listopada 2014 na dvije lokacije u sjeverozapadnom dijelu Hrvatske: Jastrebarsko - (populacija-J) i Popovača (populacija-P). Podaci o geografskom smještaju, tipu tla i zakorovljenosti ambrozijom (m^2) na lokacijama Jastrebarsko i Popovača prikazani su u Tablici 1.

U godini sakupljanja sjemena, zakorovljene površine na obje lokacije bile su zapušteni travnjaci, iako se prethodnih godina uzgajao krumpir (Jastrebarsko) i održavao vinograd (Popovača). Veličina parcela na kojima je skupljano sjeme iznosila je oko 100 m^2. Na obje lokacije sjeme je odvojeno sakupljeno s dvadeset biljaka, a biljke su međusobno bile dva do tri metra udaljene jedna od druge. Nakon sakupljanja sjeme je očišćeno od nečistoća i upakirano u papirnate vrećice. Prije skladištenja izmjerena je pojedinačna masa sjemena sakupljenog sa svake biljke (g). Do provođenja pokusa sjeme je skladišteno na suhom i mračnom mjestu pri sobnoj temperaturi.

Istraživanje s ciljem utvrđivanja varijabilnosti u klijanju, dinamici (brzini) klijanja te masi između biljaka iz iste populacije započeto je u siječnju 2017 godine. Masa 1 000 sjemena i brojnost sjemena po biljci utvrđena je za dvadeset biljaka u svakoj populaciji. Klijavost i brzina klijanja utvrđivana je na ukupno deset biljaka unutar svake populacije. Biljke uključene u istraživanje klijavosti i dinamike klijanja nasumično su odabrane tu su označene kao: B1 – B10, gdje slovo B podrazumijeva biljku, dok brojevi od 1 do 10 označuju broj biljke.

Prije postavljanja testa klijavosti sjeme nije podvrgnuto niti jednom tretmanu prekidanja dormantnosti. Test klijavost proveden je na standardni način postavljanjem sjemena u Petrijeve zdjelice na filter papir prekriven s 5 ml destilirane vode s ukupno 1 000 sjemena po populaciji (25 sjemena x 4 repeticije x 10 biljka). Petrijeve zdjelice zatvorene su parafilmom kako bi se spriječio gubitak vlage te su postavljene u klima komoru na optimalnu temperaturu za klijanje ambrozije od 25 °C/15 °C (Willemsen, 1975) s fotoperiodom od 12 sati dana i 12 sati noći te relativnom vlagom zraka od 60%. Klijavost je provjeravana tri tjedna svakoga dana, zatim svaka tri dana sljedeća dva tjedna i na kraju jednom tjedno, ukupno 43 dana. Proklijale sjemenke uklanjane su sterilnom pincetom, a proklijalim sjemenom je smatrano svako sjeme s radikulom > 1 mm. Test klijavosti prekinut je u trenutku kad deset dana za redom nije utvrđena nova klijavost u pojedinoj Petrijevoj posudi.

Temeljem podatka o dnevnoj klijavosti utvrđena je dinamika klijanja (brzina klijanja) unutar istraživanog perioda. Brzina klijanja označena je kao T_{10}, T_{50}, T_{90},

Table 1. Geographical location, soil type and common ragweed infestation (m^2) at Jastrebarsko and Popovača

Tablica 1. Geografski smještaj, tip tla i zakorovljenost ambrozijom (m^2) na lokacijama Jastrebarsko i Popovača

Location	Latitude	Longitude	Altitude	Density (m^2)	Soil type
Lokacija	Zemljopisna širina	Zemljopisna dužina	Nadmorska visina (m)	Zakorovljenost (m^2)	Tip tla
Jastrebarsko	45°42´73˝	15°39´47˝	230	41	Muljevita glinenasta ilovača
Popovača	45°34´1˝	16°40´43˝	196	10	Muljevita ilovača

gdje T_{10} označava početnu (inicijalnu) klijavost, odnosno vrijeme, izraženo u broju dana, potrebno da 10% klijavog sjemena proklije. Oznaka T_{50}, označava sredinu klijavosti, i broj dana potrebno za klijanje 50% sjemena, a T_{90} završnu klijavost i broj dana da proklije 90% sjemena. Nakon provedenog testa klijavosti neproklijalo sjeme podvrgnuto je standardnom tetrazolium testu (ISTA, 1985) kako bi se odvojilo dormantno od mrtvog sjemena te utvrdio postotak viabilnog sjemena (dormantno + klijavo sjeme) za svaku pojedinu biljku ambrozije unutar obje populacije.

Dobiveni podaci (% klijavog, dormantnog i mrtvog sjemena, te dinamika klijanja između biljaka) obrađeni su analizom varijance pri čemu je korišten kompjuterski program SAS 8.0 (SAS Institute, 1997). Nakon signifikantnog F-testa, za usporedbu srednjih vrijednosti korišten je LSD test uz P ≤ 0,05. Podaci o dnevnom utvrđivanju klijavosti za svih deset biljaka unutar dvije populacije korišteni su za prikaz dinamike klijanja koristeći logističku funkciju u statističkom programu Bioassay97 (Onofri, 2001). Za utvrđivanje varijabilnosti u masi, broju sjemena, % klijavog, mrtvog i dormantnog sjemena i dinamika klijanja između biljaka unutar jedne populacije korišten je koeficijent varijabilnosti (CV) izražen u postotcima.

REZULTATI I RASPRAVA

Masa i brojnost sjemenki unutar populacije

Prosječna masa 1 000 sjemenki 20 biljaka na lokaciji Jastrebarsko iznosi 4,05 g, dok je medijana nešto viša i iznosi 4,08 g. Utvrđena prosječna masa 1 000 sjemenki sakupljenih s 20 biljaka na lokaciji Popovača nije se uvelike razlikovala te iznosi 4,54 g, s medijanom od 4,18 g (Tablica 2). Prema dosad poznatim podacima, raspon težine 1 000 sjemenki ambrozije kreće se od 1,72 do 10,0 g. Za šest različitih populacija ambrozije na području Francuske, Fumanal i sur. (2007) utvrđuju masu 1 000 sjemena od: 1,72 g (Concoeur-Coroin), 3,07 g (Labergement), 3,08 g (Lux), 3,48 g (Chaponnay), 3,60 g (Alex), 3,28 g (St. Pierre de Chandieu). Nitzsche (2010) utvrđuje prosječnu masu 1 000 sjemena za njemačku i mađarsku populaciju od oko 5,00 g, s odstupanjem jedne njemačke populacije

Table 2. Weight of the 1 000 seeds (g) per plants collected from twenty different plants at location Jastrebarsko – population J and Popovača – population P

Tablica 2. Utvrđena masa 1 000 sjemena (g) po pojedinoj biljci ambrozije lokacije Jastrebarsko – populacija J i Popovača – populacija P

Plant No. Br. biljke	JASTREBARSKO (J)	POPOVAČA (P)
B1	4,81	5,44
B2	2,98	3,93
B3	3,20	4,98
B4	3,39	5,70
B5	3,71	8,13
B6	3,47	4,08
B7	4,10	4,89
B8	3,20	4,78
B9	4,41	4,44
B10	3,98	3,86
B11	3,42	2,80
B12	4,70	4,13
B13	4,23	3,56
B14	4,43	3,18
B15	4,59	3,48
B16	4,27	4,14
B17	6,11	3,06
B18	3,19	7,50
B19	4,06	4,57
B20	4,66	4,21
median medijana	4,08	4,18
mean prosjek	4,05	4,54
CV (%)[a]	18,2	29,8

[a] CV - coefficient of variation

[a] CV – koeficijent varijabilnosti

čija masa 1 000 sjemena iznosi 10,0 g. Prosječna masa tri američke populacije ambrozije prema Leiblein-Wild i sur. (2014) iznosi 5,7 g. Nadalje, Kazinczi i sur. (2008) navode masu 1 000 sjemenki za mađarsku populaciju od 2 - 2,7 g.

Posljednjih 40-tak godina, istraživanjima je utvrđeno da masa sjemena korovnih vrsta može varirati između različitih populacija ili uvjeta rasta (obradivo tlo, neobradivo tlo), između biljaka iz iste populacije, pa čak i sjemena razvijenog u istom plodu. Mjerenjem mase 300 individualnih sjemena unutar jedne populacije Guillemin i Chauvel (2011) utvrđuju značajno variranje mase sjemena od 1,2 pa do 7,7 mg s medijanom od 3,98 i prosječnom masom od 4,08 mg. Ovisno o težini autori kategoriziraju sjemenke u pet kategorija: lagano sjeme do 3,1 mg, srednje lagano > 3,1 do 3,7 mg; srednje >3,7 do 4,3 mg; srednje teško > 4,3 do 5 mg te teško > 5mg. Shodno navedenoj kategorizaciji podjednak postotak mase sjemena biljaka ambrozije iz populacije-J pripada skupini srednje lakšeg (35%), srednje (20%) i srednje teškog sjemena (35%). Manji postotak, odnosno samo sjeme s jedne biljke pripada u kategoriju lakšeg (5%) i teškog sjemena (5%). Masa sjemena populacije-P također većim djelom pripada kategoriji srednje (30%), srednje teškog (30%) i teškog (20%) sjemena. Manji postotak sjemena iz populacije-P ubraja se u skupinu laganog (10%) i srednje laganog sjemena (15%) (Tablica 2). I u ovom istraživanju, prosječna masa 1 000 sjemenki ambrozije razlikuje se između biljaka iz iste populacije. Najmanja masa 1 000 sjemenki populacije-J (2,98 g) utvrđena je za B2 (Tablica 2). Slična vrijednost (2,80 g) utvrđena je i za B11 unutar populacije-P. Najveća utvrđena masa 1 000 sjemenki biljaka ambrozije populacije-J utvrđena je za B17 (6,11 g), dok je najveća utvrđena masa unutar populacije-P utvrđena za B5 (8,13 g). Iako, je kod svake od istraživanih biljaka utvrđena različita masa sjemena, varijabilnost izražena koeficijentom varijabilnosti (CV) za masu sjemena iz populacije-J iznosi tek 18,2% što podrazumijeva relativno slabu varijabilnost. Promatrano kroz isti raspon, varijabilnost koja se javlja kod populacije-P također je definirana kao relativno slaba (29,8%). Fumanal i sur. (2007) također utvrđuju relativnu slabu varijabilnost u masi sjemena korovne vrste *Ambrosia artemisiifolia* unutar biljaka iz iste populacije (22,2%) što je Obeso (1993) potvrdio i za vrstu *Asphodelus albus* Mill.

Klijavost ambrozije unutar istraživanih sjemenskih populacija

Rezultati analize varijance za udio klijavog, dormantnog i mrtvog sjemena između deset biljaka unutar dvije populacije ukazuju na postojanje značajne statističke razlike u navedenim parametrima, izuzev % dormantnog sjemena između biljaka iz populacije Popovača (P) gdje nije utvrđena statistički značajna razlika (Tablica 3). Najveći postotak biljaka ambrozije unutar populacije Jastrebarsko (J) imao je visoki udio vijabilnog sjemena (klijavo + dormantno sjeme), 96,0%. Čak devet od deset biljaka imale su više od 80,0% klijavog sjemena. Prosječni postotak klijavog sjemena u populaciji-J iznosio je 87,2%, dormantnog sjemena 8,8% te mrtvog sjemena tek 3,9%. Najveća klijavost (99,0%) utvrđena je kod B2, čija se klijavost statistički nije razlikovala od klijavosti B4 (95,0%), B7 (96,0%) te B9 (97,0%). Najmanja prosječna klijavost utvrđena je kod B8 (50,5%). Od ukupnog udjela dormantnog sjemena u populaciji veći broj biljaka posjeduje nedormantno do slabo dormantno sjeme. Uz najmanju utvrđenu klijavost, B8 ističe se po najvećom postotku dormantnog sjemena (29,4%). Najmanji postotak dormantnog sjemena ima B2 (0,0%) te se statistički ne razlikuje od B7, B9, B4, B6, B5 i B1 s postotkom dormantnog sjemena od 1,0 do 9,0%. Najveći postotak mrtvog sjemena imala je B8 (20,2%), dok su najmanji postotak mrtvog sjemena imale B1, B2, B4, B9 i B10 s 1% mrtvog sjemena. Statistički značajna razlika nije utvrđena ni između B3, B7 i B6 čiji se % mrtvog sjemena kretao od 2,0 do 6,0% (Tablica 3).

Ukupni udio vijabilnog sjemena populacije-P između svih deset biljaka korištenih u pokusu bio je 2,3 puta niži u odnosu na populaciju-J, te je iznosio 41,7%.Utvrđena klijavost između deset biljaka unutar ove populacije iznosila je 23,4%.Najveću prosječnu klijavost ostvarile su biljke B1 (50,8%), B4 (47,0%) i B10 (58,7%) te između istih nije utvrđena značajna statistička razlika. Najmanju prosječnu klijavost ostvarile su B2 (1,0%), B8 (1,0%), B9 (10,0%) i B7 (13,0%) između kojih nije utvrđena značajna statistička

Table 3. Total percentage of germinated, dormant and dead seed of ten plants within the population

Tablica 3. Ukupni postotak klijavosti, dormantnosti i mrtvog sjemena za 10 biljaka unutar populacije

	JASTREBARSKO (%)			POPOVAČA (%)		
Plant No.	germination	mortality	dormancy	germination	mortality	dormancy
Br. biljke	klijavost	smrtnost	dormantnost	klijavost	smrtnost	dormantnost
B1	90,0 bcd*	1,0 b	9,0 bc	50,8 a	32,9 de	16,3 a
B2	99,0 a	1,0 b	0,0 c	1,0 c	83,6 a	15,4 a
B3	84,0 d	2,0 b	14,0 b	16,2 bc	62,7 bc	21,1 a
B4	95,0 abc	1,0 b	4,0 c	47,0 a	35,0 de	18,0 a
B5	89,0 bcd	3,0 b	8,0 bc	30,3 b	49,5 cd	20,0 a
B6	88,0 cd	6,0 b	6,0 bc	6,0 bc	81,0 a	13,0 a
B7	96,0 abc	3,0 b	1,0 c	13,0 c	76,0 ab	11,0 b
B8	50,5 e	20,2 a	29,4 a	1,0 c	74,0 ab	25,0 a
B9	97,0 ab	1,0 b	2,0 c	10,0 c	69,0 ab	21,0 a
B10	84,0 d	1,0 b	15,0 b	58,7 a	19,2 e	22,1 a
CV^{a} (%)	4,9	67,6	51,0	34,5	34,1	14,2

* values followed by the same letter are not significantly different
LSD values:
Jastrebarsko:% germination = 8,86; % dormancy = 9,21; % mortality = 5,44
Popovača: % germination = 16,58; % dormancy = 13,37; % mortality = 17,0
^{a} CV - coefficient of variation

* vrijednosti označene istim slovima međusobno se ne razlikuju
LSD vrijednosti:
Jastrebarsko: % klijavog sjemena = 8,86; % dormantnog sjemena = 9,21; % mrtvog sjemena = 5,44
Popovača: % klijavog sjemena = 16,58; % dormantnog sjemena = 13,37; % mrtvog sjemena = 17,0
^{a} CV – koeficijent varijabilnosti

razlika. Suprotno populaciji-J, gdje je veći udio sjemena iskazao visoku klijavost (>80%), unutar populacije-P sjeme je većinom definirano kao mrtvo sjeme. Ukupni postotak mrtvog sjemena u populaciji-J iznosio je 58,3%. Ukupni postotak dormantnog sjemena u populaciji-P iznosio je 18,3%, s rasponom od 11,0 do 25,0% između različitih biljaka (Tablica 3). Statistička razlika u klijavosti između sjemena iz iste populacije utvrđena je i kod drugih vrsta. Martinez-Fernandez i sur. (2014) utvrđuju razliku u klijavosti sjemena vrste *Astragalus gines-lopezii* Talavera, Podlech, Devesa, F.M.Vazquez za sjeme sakupljeno unutar iste populacije i iste godine. Mnogi autori razliku u viabilnosti sjemena objašnjavaju različitom masom sjemena. U ovom istraživanju regresijskom analizom nije utvrđena povezanost mase i viabilnosti sjemena (R^2 = 0,12). U istraživanju Guillemin i Chauvel (2011) također nije utvrđena povezanost između mase i klijavost sjemena ambrozije. U sličnim istraživanjima nije utvrđena ni povezanost u klijavosti i masi sjemena za vrstu *Centaurea eriophora* L. kao ni *Dactylis glomerata* L. te *Andropogon tectorum* Schumach. Suprotno tome, Milberg i sur. (1996) utvrđuju povezanost težine sjemena (veća težina veća klijavost) i klijavosti vrste *Lithospermum arvense* L., isto vrijedi i za vrstu *Abutilon theophrasti* Medik. (Baloch i sur., 2001), dok sjeme *Erodium brachycarpum* (Godron) Thell. (Stamp, 1990) pokazuje veću klijavost pri manjoj težini sjemena. Jačina varijabilnost u sastavu klijavog, dormantnog i mrtvog sjemena unutar populacije razlikuje se za populaciju J i P. Unutar populacije-J između sjemena sposobnog ostvariti klijanje utvrđena je vrlo slaba varijabilnost (4,9%). Umjerena varijabilnost u sposobnosti klijanja utvrđena je unutar sjemena populacije-P (34,5%). To podrazumijeva relativno ujednačenu klijavost sjemena sakupljenog s različitih biljaka unutar dvije populacije. Varijabilnost udjela mrtvog sjemena između različitih biljaka unutar iste populacije razlikovao se za dvije populacije. Unutar populacije-J utvrđena je relativno jaka varijabilnost u postotku mrtvog sjemena (67,6%) iako je za sve biljke utvrđen nizak postotak mrtvog sjemena (izuzev B8) dok je unutar populacije-P varijabilnost u postotku mrtvog sjemena bila umjerena (34,1%). Varijabilnost utvrđena u udjelu dormantnog sjemena izraženija je kod populacije-J (51,0%) te je definirana kao vrlo jaka. Nasuprot tome, varijabilnost u udjelu dormantnog sjemena populacije-P definirana je kao relativno slaba (14,2%).

Dinamika klijanja ambrozije za istraživane populacije

Utvrđena slaba (P-J) i umjerena (P-P) varijabilnost u viabilnosti sjemena nije toliko značajna za laboratorijske pokuse utvrđivanja bioloških parametara klijanja korovnih vrsta. U tom pogledu značajnije je utvrditi postoji li razlika u dinamici klijanja između deset biljaka iz iste populacije s obzirom da se vrijednost T_{50} koristi kod određivanja biološkog minimuma i biološkog vodnog potencijala.

Sjeme iz obje populacije ambrozije statistički se značajno razlikovalo u dinamici klijanja (Tablica 4) odnosno vremenu potrebnom da 10%, 50% i 90% sjemena pojedine biljke proklije. Utvrđena jačina varijabilnosti (CV) između deset istraživanih biljaka za obje populacije u sva tri parametra (T_{10}, T_{50} i T_{90}) je relativno do umjereno slaba (12,0 – 32,7). Unutar istraživanog razdoblja za populaciju-J

Table 4. Estimated germination dynamics – T (10%, 50%, 90%) in Biostat97 model and ANOVA for 10 plants expressed in days

Tablica 4. Procijenjena dinamika klijanja – T(10%, 50%, 90%) prema Biostat97 modelu te ANOVA za 10 biljaka izražena u danima

Plant No.	JASTREBARSKO (%)			POPOVAČA (%)		
Br. biljke	T_{10}	T_{50}	T_{90}	T_{10}	T_{50}	T_{90}
B1	6,46 ab*	15,67 a	39,0 ab	5,76 b	11,23 c	22,16 b
B2	3,57 d	7,68 cd	16,7 def	0,00 c	0,00 d	0,00 c
B3	7,28 a	14,50 a	29,3 bc	12,18 a	21,82 a	39,10 a
B4	3,61 d	7,29 cde	17,0 def	6,85 b	14,64 bc	32,40 ab
B5	3,23 d	6,65 de	13,9 ef	7,37 b	16,78 ab	39,80 a
B6	4,91 bcd	10,58 b	23,1 cde	0,00 c	0,00 d	0,00 c
B7	3,29 d	4,98 e	7,8 f	12,10 a	20,95 a	38,30 a
B8	6,38 ab	16,48 a	44,5 a	0,00c	0,00 d	0,00 c
B9	5,42 bc	11,28 b	24,7 cd	11,16 a	14,50 bc	18,90 b
B10	4,35 cd	9,34 bc	20,6 cde	6,48 b	13,13 bc	27,40 ab
CV (%)^a	17,7	12,0	22,4	22,2	22,4	32,7

*values followed by the same letter are not significantly different
Jastrebarsko: $LSD_{0.05}$ T_{10} = 1.76; $LSD_{0.05}$ T_{50} = 2.57; $LSD_{0.05}$ T_{90} = 10.89
Popovaca: $LSD_{0.05}$ T_{10} = 2.81; $LSD_{0.05}$ T_{50} = 5.18; $LSD_{0.05}$ T_{90} = 14.69
^{a} CV - coefficient of variation

*vrijednosti označene istim slovima međusobno se ne razlikuju
Jastrebarsko: $LSD_{0.05}$ T_{10} = 1,76; $LSD_{0.05}$ T_{50} = 2,57; $LSD_{0.05}$ T_{90} = 10,89
Popovača: $LSD_{0.05}$ T_{10} = 2,81; $LSD_{0.05}$ T_{50} = 5,18; $LSD_{0.05}$ T_{90} = 14,69
^{a} CV – koeficijent varijabilnosti

za ostvarenje početne klijavosti bilo je potrebno prosječno 4,8 dana (d), za sredinu klijavosti prosječno 10,4 (d), a za završetak klijavosti bilo je potrebno u prosjeku 23,6 (d) (Grafikon 1).

Početak klijavosti izražen u danima između deset različitih biljaka iz populacije-J kretao se u rasponu od 3,23 do 7,28 dana. Najbrže početno klijanje unutar populacije-J ostvarile su B5 (3,23 d), B7 (3,29 d), B2 (3,57 d) i B4 (3,61 d) sa statistički neopravdanom razlikom između tih biljaka. Najsporije klijanje ostvareno je kod sjemena B3 (7,28 d), B1 (6,46 d) i B8 (6,38 d) bez utvrđene statistički značajne razlike između istih. Sredina klijavosti (50%) između deset biljaka unutar populacije-J ostvarena je u rasponu od 4,98 do 16,48 dana. Sjemenu B7, B5 i B4 bilo je potrebno 4,98 do 7,29 dana kako bi ostvarile 50% klijavost od ukupnog broja posijanog sjemena, te između njih nije utvrđena statistička razlika. Statistička razlika nije utvrđena ni između B3 (14,50 d), B1(15,67 d) i B8 (16,48 d) koje su najsporije dosegle 50% klijavosti. Kraj klijavosti za deset biljaka unutar populacije-J kretao se u rasponu od 7,8 do 44,5 dana. S klijanjem je najbrže završila B7 (7,8 d), dok je najsporiji završetak klijavosti ostvarila B8 (44,5 d).

Sjemenska populacija-P ostvarila je dvostruko razvučenije klijanje u istom istraživanom razdoblju. Raspon početne klijavosti između deset istraživanih biljaka kretao se od 5,76 do 12,18 dana, prosječno 8,84 d. Za ostvarenje sredine klijavosti bilo je potrebno 16,15 d, kraj klijavosti prosječno je ostvaren unutar 31,15 d (Grafikon 2).

Najbrže početno klijanje ostvarile su B1 (5,76 d), B10 (6,48 d), B4 (6,85 d) te B5 (7,37 d). Početna klijavost sjemena populacije-P odvijala se sporo za sjeme biljaka B3, B7 i B9 s rasponom od 12,18 do 11,16 dana (Grafikon 2). Dinamika ostvarivanja sredine klijavosti unutar populacije-P razvučena je kao posljedica sporog ostvarenja inicijalne klijavosti. Raspon unutar kojeg su biljke unutar populacije-P ostvarile sredinu klijavosti kretao se od 11,23 do 21,82 dana. Najbrža srednja klijavost ostvarena je kod sjemena biljaka B1 (11,23 d), B10 (13,13 d), B9 (14,5 d) te B4 (14,64 d) bez utvrđene statistički značajne razlike između istih. Najsporije ostvarena srednja klijavost populacije-P utvrđena je za biljke B3 (21,8 d) i B7 (20,9 d) pri čemu se iste nisu statistički značajno razlikovale. Raspon završne klijavosti kretao se od 18,9 do 39,8 dana. Pri tom su biljke B9 (18,9 d) i B1 (22,2 d) ostvarile najbržu završnu klijavost dok su B5 (39,8 d), B3 (39,1 d) i B7 (38,3 d) najsporije završile klijavost (Grafikon 2).

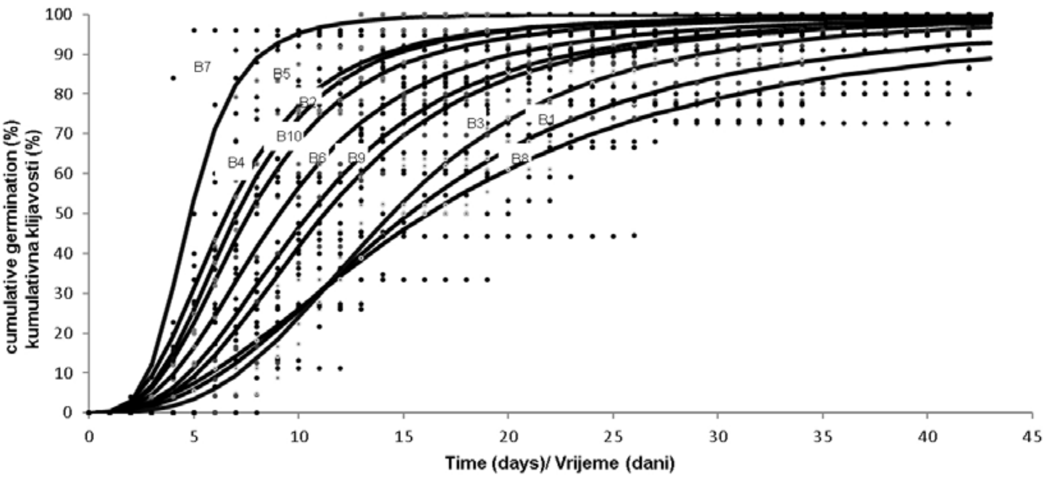

Figure 1. Cumulative germination and the time required for initial (T_{10}), middle (T_{50}) and final germination (T_{90}) of the seed collected from ten different plants from the same population (Jastrebarsko)

Grafikon 1. Kumulativna klijavost i procijenjeno vrijeme potrebno za početak (T_{10}), sredinu (T_{50}) i kraj klijavosti (T_{90}) sjemena sakupljenog s deset različitih biljaka iz iste populacije (Jastrebarsko)

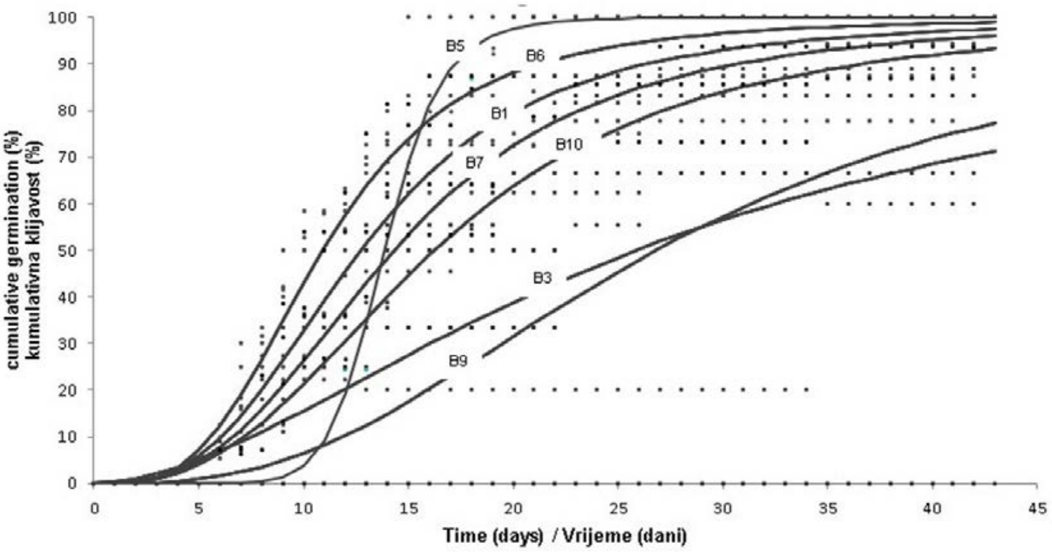

Figure 2. Cumulative germination and the time (days) required for initial (T_{10}), middle(T_{50}) and final germination (T_{90}) of the seed collected from ten different plants from the same population (Popovaca)

Grafikon 2. Kumulativna klijavost i procijenjeno vrijeme (dani) potrebno za početak (T_{10}), sredinu (T_{50}) i kraj klijavosti (T_{90}) sjemena sakupljenog s deset različitih biljaka iz iste populacije (Popovača)

Sposobnost sjemena da ostvari brzu inicijalnu klijavost u kratkom vremenu prednost je u uvjetima ograničenih resursa okoliša. Takva sposobnost definira kompetitivnost određene vrste odnosno jedinke (Ross i Harper, 1972). U tom pogledu, sjeme populacije-J kompetitivnije je u kraćem vremenu ostvariti klijavost. Utvrđivanjem stupnja varijabilnosti između biljka iz ove populacije (Tablica 4), vidljivo je da iako statistička razlika između sjemena postoji, stupanj varijabilnosti u početnoj klijavosti je relativno slab (17,7%). Početna klijavost sjemena populacije-P razvučenija je, ali trend usporene klijavosti prisutan je ravnomjerno kod svog sjemena u populaciji. Iz tog razloga, kao i kod populacije-J i kod populacije-P varijabilnost između sjemena je relativno slaba (22,2%). Iako je utvrđena statistička razlika između istraživanih biljaka unutar dvije populacije u vremenskom razdoblju ostvarivanja srednje klijavosti varijabilnost koja je pritom utvrđena relativno je slaba pa za populaciju-P iznosi 22,4%, dok za populaciju-J iznosi svega12,0% (Tablica 4). Fumanal i sur. (2007) utvrđuju vrijednosti srednje klijavosti sjemena ambrozije šest populacija u rasponu od 3,6 do 16,5 d što je sličnije rezultatima dobivenim za populaciju-J. Leiblein-Wild i sur. (2014) utvrđuju prosječnu vrijednost srednje klijavosti (T_{50}) za sjeme američke i europske populacije ambrozije od 13,4 d pri konstantnih 25 °C. Pri tome, utvrđuju razliku između dvije navedene populacije, pa tako vrijednost srednje klijavosti autohtone (američke) i invazivnih (europskih) populacija ambrozije, iznosi 22,2 i 4,5 d što ukazuje na značajno brže dostizanje sredine klijavosti europskih populacija i time na njen jak invazivni potencijal. Kao što je vidljivo prosječne vrijednosti sredine klijavosti utvrđene u ovom istraživanju nalaze se unutar navedenog raspona stranih autora.

Utvrđena varijabilnost u završnoj klijavosti unutar biljaka iz populacije-J pri tome je relativno slaba (22,4%), dok je kod populacije-P umjerena (32,7%) (Tablica 4).

Kako se za utvrđivanje biološkog minimuma korovnih vrsta koristi podatak o T_{50} važno je utvrditi postoji li određena povezanost, odnosno preklapanje u vremenu potrebnom da deset biljaka unutar iste populacije ostvari 50% klijavosti. Za svaku biljku stoga je utvrđena gornja i donja vrijednost T_{50} kao vrijednost dobivena analizom četiri repeticije za svaku biljku.

S obzirom na vrijednost T_{50} i njihovo međusobno preklapanje biljke iz populacije-J podijeljene su u dvije različite grupe (Grafikon 3). Prva grupa obuhvaća skupinu biljaka čija je vrijednost T_{50} ostvarena u rasponu od 4,98 do 7,68 dana te je ujedno i najbrža grupa po ostvarenom T_{50}. Unutar ove grupe nalaze se biljke B7, B5, B4, B2.

Drugu skupinu obuhvaćaju biljke B10, B9, B8, B6, B3 i B1 čija je vrijednost T_{50} ostvarena u rasponu od 9,34 do 16,48 dana. Nasuprot tome na populaciji-P utvrđena su preklapanja u vremenskom razdoblju ostvarenja srednje klijavosti ostvarena za svih sedam biljaka koje su unutar ove populacije ostvarile klijavost (Grafikon 4).

Dobiveni rezultati istraživanja ukazuju na slabo izraženu varijabilnost u dinamici srednje klijavosti za obje populacije. Vrijednost srednje klijavosti osnova je utvrđivanja bioloških parametra klijavosti stoga slabo izražena varijabilnost unutar ovog parametra ukazuje na mogućnost dobivanja pouzdanih podataka o biološkim parametrima čitave populacije temeljem korištenja miješanog sjemena s različitih biljaka. Ipak, između sjemena dvije populacije vidljiva je razlika u srednjoj klijavosti sjemena zbog čega bi buduća istraživanja trebala biti usmjerena na istraživanje većeg broja populacija na području RH.

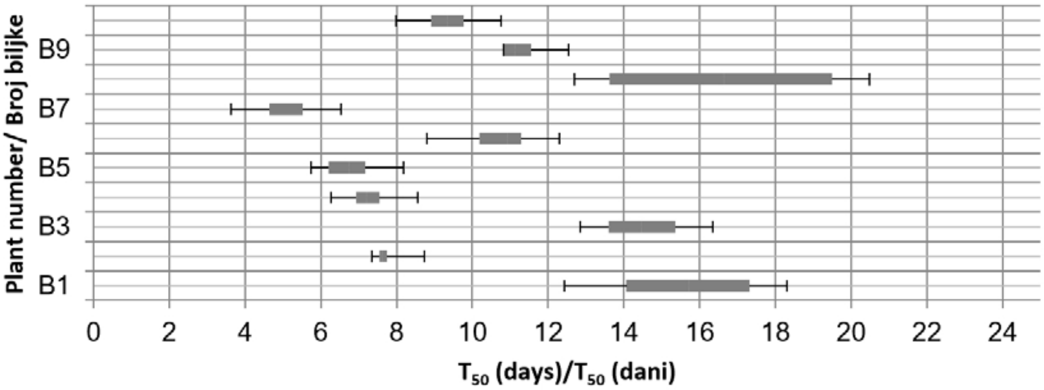

Figure 3. The time required for seed of the ten plants from the same population to achieve T_{50} and the estimated overlapping between each (Jastrebarsko)

Grafikon 3. Vremenski raspon unutar kojeg je ostvaren T_{50} za deset različitih biljaka u populaciji i utvrđena preklapanja istih (Jastrebarsko)

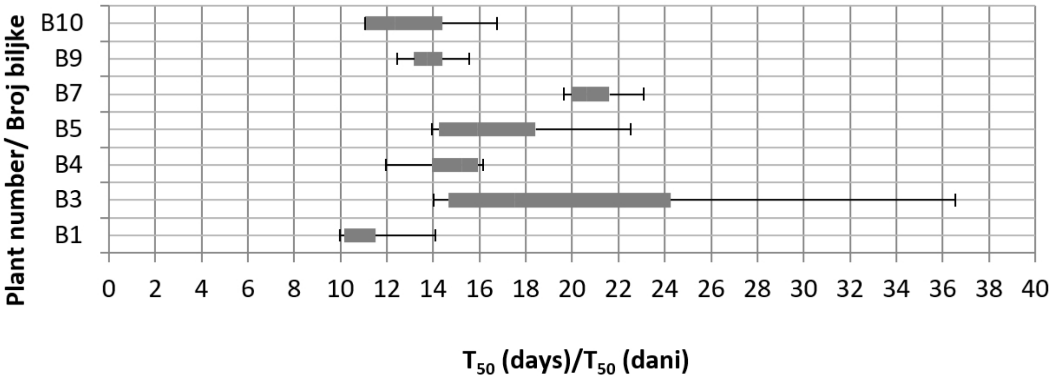

Figure 4. The time required for seed of the ten plants from the same population to achieve T_{50} and the estimated overlapping between each (Popovaca)

Grafikon 4. Vremenski raspon unutar kojeg je ostvaren T_{50} za deset različitih biljaka u populaciji i utvrđena preklapanja istih (Popovača)

ZAKLJUČAK

Očekivana prisutnost morfološkog polimorfizma u masi sjemena utvrđena je kod obje istraživane populacije. Ipak, utvrđena varijabilnost slabo je izražena za sjeme unutar obje istraživane populacije. Funkcionalna varijabilnost utvrđena provođenjem testa klijavosti i tetrazolium testa razlikuje se između dvije istraživane populacije te je unutar obje utvrđena statistički značajna razlika između biljaka iz iste populacije. Dokazana je intrapopulacijska varijabilnosti između različitih biljaka u postotku klijavosti, dormantnosti, smrtnosti i dinamici klijanja. Iako je između navedene tri funkcionalne karakteristike sjemena utvrđena varijabilnost, stupanj varijabilnosti za iste nalazi se u rasponu od slabo do umjereno varijabilnog. Takav stupanj varijabilnosti prihvatljiv je prilikom korištenja u daljnjim istraživanjima bioloških parametara. Ipak, dobiveni podaci daju samo mali uvid u sjemenske karakteristike izražene unutar sjemena iz iste populacije. Za bolje razumijevanje intrapopulacijskih sjemenskih karakteristika slična istraživanja trebala bi biti usmjerena na proučavanje varijabilnosti svojstava sjemena i na razini pojedinačne biljke (položaju cvatova na biljci). Također, uključivanju i različitih okolišnih faktora koji utječu na razvoj varijabilnosti unutar populacija proučavanjem posrednih i neposrednih okolišnih mikroklimatskih uvjeta.

LITERATURA

Baloch, H., Tommaso, A., Watson, A. (2001) Intrapopulation variation in *Abutilon theophrasti* seed mass and its relationship to seed germinability. Seed Science Research, 11 (4), 335-343. DOI: https://doi.org/10.1079/SSR200190

Ellison, A. M. (2001) Interspecific and intraspecific variation in seed size and germination requirements of Sarracenia (Sarraceniaceae), American Journal of Botany 88, 429-437. DOI: https://doi.org/10.2307/2657107

Fenner, M. (1991) The effects of the parent environment on seed germinability. Seed Science Research 1, 75–84. DOI: https://doi.org/10.1017/S0960258500000696

Fumanal, B., Chauvel, B., Sabatier, A., Bretagnolle, F. (2007) Variability and cryptic heteromorphism of *Ambrosia artemisiifolia* seeds: what consequences for its invasion in France? Annals of Botany, 100 (2), 305-13 DOI: https://doi.org/10.1093/aob/mcm108

Genton, B.J., Shykoff, J.A., Giraud, T. (2005) High genetic diversity in French invasive populations of common ragweed, *Ambrosia artemisiifolia*, as a result of multiple sources of introduction. Molecular Ecology, 14 (14), 4275-85. DOI: https://doi.org/10.1111/j.1365-294X.2005.02750.x

Guillemin, J.P., Chauvel, B. (2011) Effects of the seed weight and burial depth on the seed behavior of common ragweed (*Ambrosia artemisiifolia*). Weed Biology and Management. 11 (4), 217-223. DOI: https://doi.org/10.1111/j.1445-6664.2011.00423.x

Harper, J.L., Obeid, M. (1967) Influence of seed size and depth of sowing on the establishment and growth of varieties of fiber and oil seed flax. Crop Science 7, 527–532. DOI: https://doi.org/10.2135/cropsci1967.0011183X000700050036x

ISTA (1985) International rules for seed testing. Seed Science Technology,13, 300–520.

Jovanović-Radovanov, K., Božić, D. (2015) Invazivni procesi introdukovanih alohtonih korova i štete u novokolonizovanoj sredini. U: Vrbničanin, S., ur. Invazivni korovi – invazivni procesi, ekološko-genetički potencijal, unošenje, predviđanje, rizici, širenje, štete i kartiranje. Beograd: Herbološko društvo Srbije, pp. 167- 233.

Kazinczi, G., Béres, I., Novák, R., Bíró, K., Pathy, Z. (2008) Common ragweed (*Ambrosia artemisiifolia*): A review with special regards to the results in Hungary. I. Taxonomy, origin and distribution, morphology, life cycle and reproduction strategy. Herbologia, 9 (1), 55-91.

Leiblein-Wild, M.C., Kaviani, R., Tackenberg, O. (2014) Germination and seedling frost tolerance differ between the native and invasive range in common ragweed. Oecologia, 174, 739–750. DOI: https://doi.org/10.1007/s00442-013-2813-6

Lommen, S, Hallmann, C, Jongejans, E, Chauvel, B, Leitsch Vitalos, M, Aleksanyan, A, Toth, P, Preda, C, Šćepanović, M, Onen, H, Tokarska-Guzik, B, Anastasiu, P, Dorner, Z, Annamária, F, Karrer, G, Nagy, K, Pinke, G, Tiborcz, V, Zagyvai, G, Müller-Schärer, H. (2018) Explaining variability in the production of seed and allergenic pollen by invasive *Ambrosia artemisiifolia* across Europe. Biological Invasions, 20 (6), 1475 - 1491. DOI: https://doi.org/10.1007/s10530-017-1640-9.

Martínez-Fernández, V., Martínez-García, F., Pérez-García, F. (2014) Census, reproductive biology, and germination of *Astragalus gines-lopezii* (Fabaceae), a narrow and endangered endemic species of SW Spain. Turkish Journal of Botany, 38 (4), 686-695.

Milberg, P., Andersson, L., Elfverson, C. Regnér, S. (1996) Germination characteristics of seeds differing in mass. Seed Science Research 6 (4),191 – 198. DOI: https://doi.org/10.1017/S0960258500003251

Moles, A.T., Hodson, D.W., Webb, C.J. (2000) Seed size and shape and persistence in the soil in the New Zealand flora. Oikos 89, 541–545. DOI: https://doi.org/10.1034/j.1600-0706.2000.890313.x

Nitzsche, J. (2010) *Ambrosia artemisiifolia* L. (Beifuß-Ambrosie) in Deutschland. Biologie der Art, Konkurrenzverhalten und Monitoring. PhD thesis. Braunschweig: University of Braunschweig.

Obeso, J.R. (1993) Seed mass variation in the perennial herb *Asphodelus albus*: sources of variation and position effect. Oecologia, 93, 571–575. DOI: https://doi.org/10.1007/BF00328967

Onofri, A. (2001) BIOASSAY97: A New EXCELt VBA Macro to Perform Statistical Analyses on Pesticide Dose-Response Data.

Ostojić, Z. (2011) The changes of the composition of weed flora in Southeastern and Central Europe as affected by cropping practices – Croatia. U Šarić T., Ostojić Z., Stefanović L., Deneva Milanova S., Kazinczi G., Tyšer L., ur. The changes of the composition of weed flora in Southeastern and Central Europe as affected by cropping practices. Herbologia, 12 (1), 8-12.

Ross, M.A., Harper, J.L. (1972) Occupation of biological space during seedling establishment. Journal of Ecology, 60 (1), 77–88. [Online] Available at: https://www.jstor.org/stable/2258041 [Accessed 8 May 2020].

SAS Institute (1997) SAS/STAT Software: Changes and Enhancements through Release 6.12. Cary, NC: SAS Institute Inc.

Stamp, N.E. (1990) Production and effect of seed size in a grassland annual (*Erodium brachycarpum* Geraniaceae). American Journal of Botany, 77 (7), 874 - 882.
DOI: https://doi.org/10.1002/j.1537-2197.1990.tb15182.x

Willemsen, R. W. (1975) Effect of stratification temperature and germination temperature on germination and the induction of secondary dormancy in common ragweed seeds. American Journal of Botany, 62 (1), 1-5.
DOI: https://doi.org/10.1002/j.1537-2197.1975.tb12333.x

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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

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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:

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

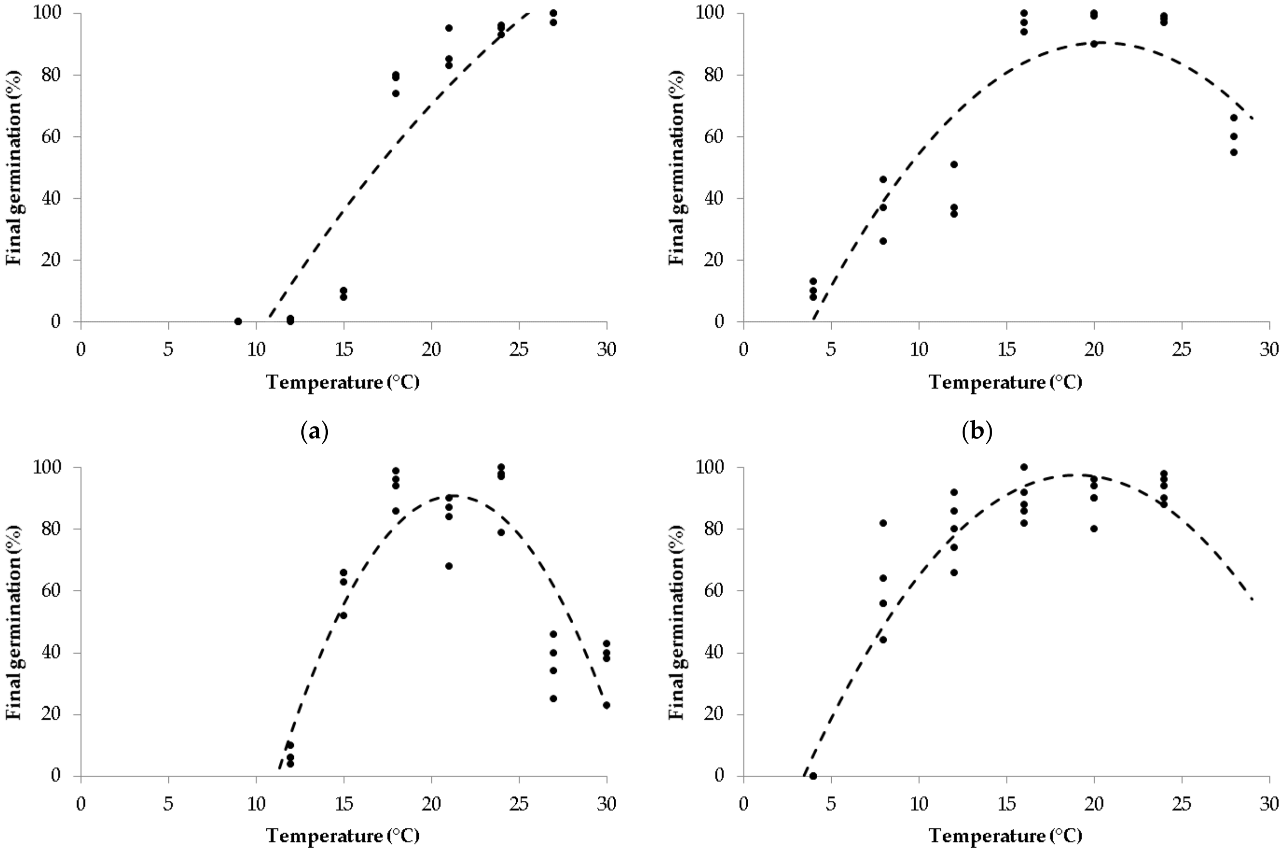
where, CG is the percentage of cumulative germination, t is the time expressed in days, a is the slope of the curve, and b is the inflection point. The initial (t_{10}), medium (t_{50}), and final (t_{90}) germination time, i.e., the time it takes for 10%, 50%, and 90% of the germinating seeds to germinate, 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).



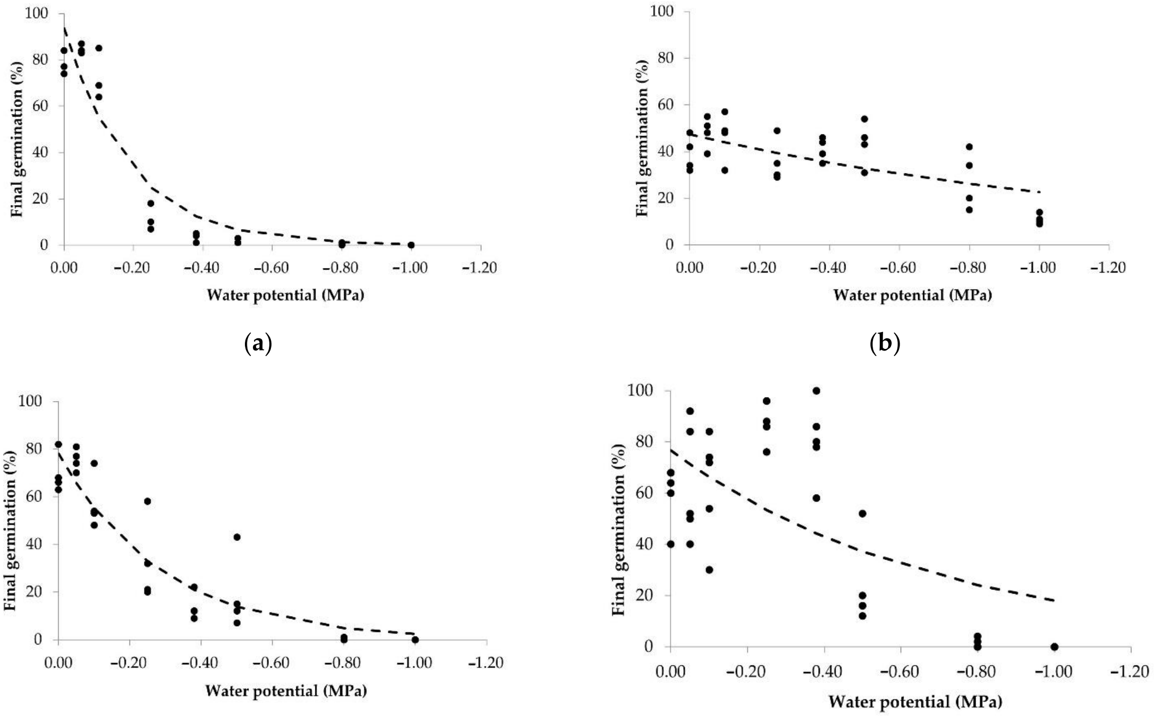
(**a**) (**b**)

(**c**) (**d**)

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.



(**a**) (**b**)

(**c**) (**d**)

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.

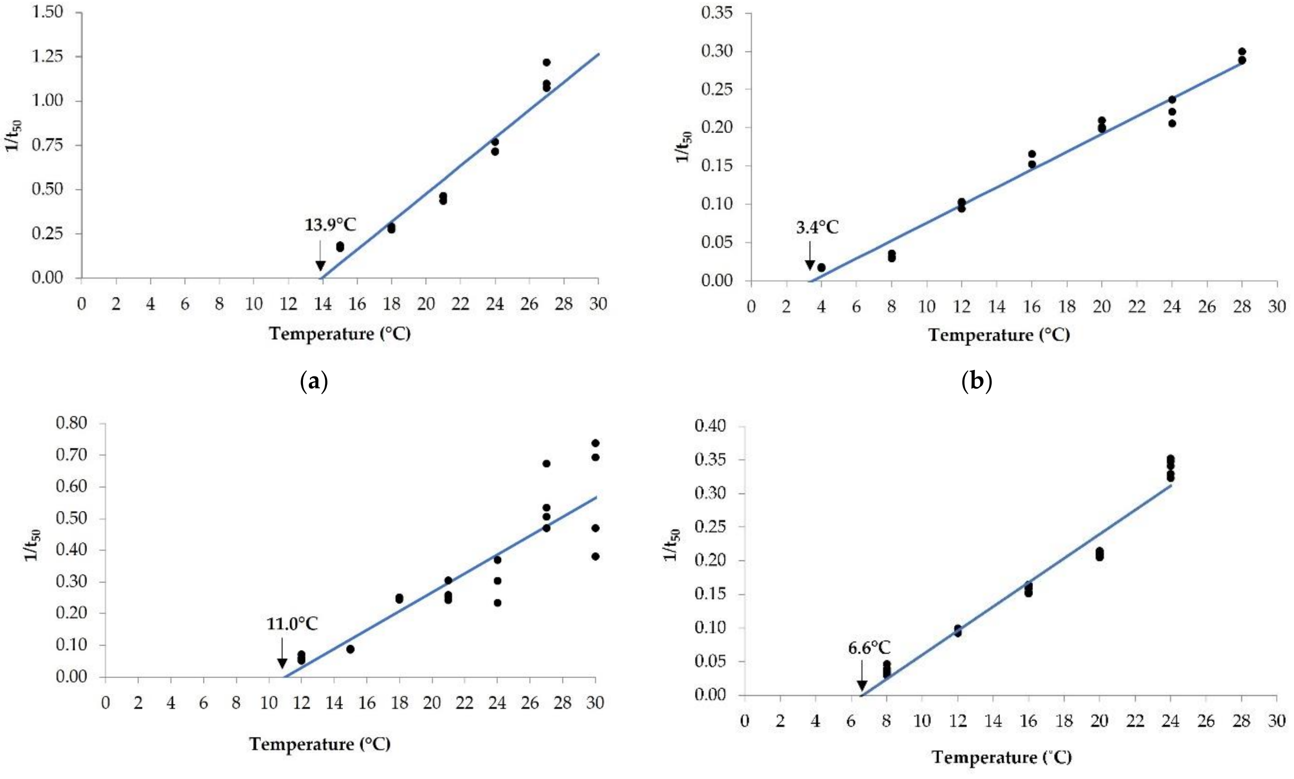
As expected, lower temperatures prolonged the germination of all investigated species. Due to the low germination capacity of *A. retroflexus* at 12 °C (0.67%), it was not possible to establish a germination curve for this species, unlike at other temperatures. The initial, medium, and final germination 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).



(**a**) (**b**)

(**c**) (**d**)

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 ± 0.36 °C; y = 0.079x − 1.010; r^2 = 0.96); (**b**) *Chenopodium album* (T_b = 3.4 ± 0.36 °C; y = 0.012x − 0.04, r^2 = 0.98); (**c**) *Panicum capillare* (T_b = 11.0 ± 1.99 °C; y = 0.030x − 0.328; r^2 = 0.82); (**d**) *Setaria pumila* (T_b = 6.6 ± 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.

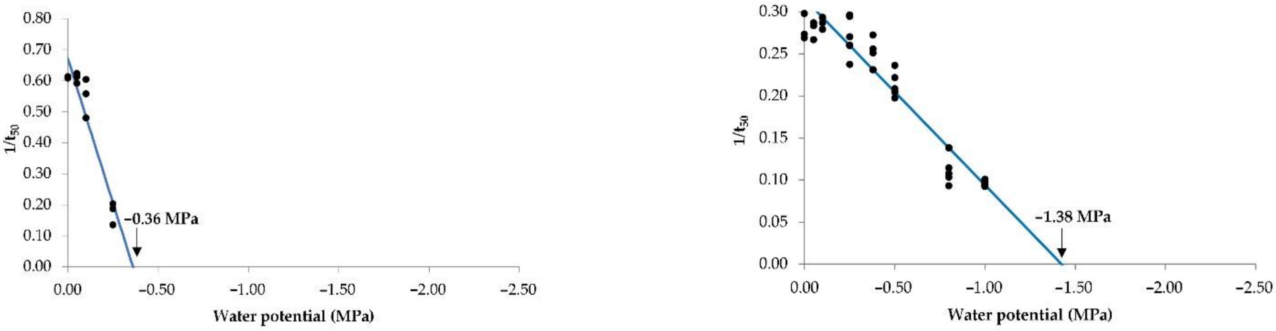
MPa	*P. capillare*			*C. album*			*S. pumila*			*A. retroflexus*		
	t_{10}	t_{50}	t_{90}	t_{10}	t_{50}	t_{90}	t_{10}	t_{50}	t_{90}	t_{10}	t_{50}	t_{90}
0.00	4.6 bc	5.0 a	5.5 a	2.1 a	3.4 a	5.7 a	2.5 a	3.5 a	4.8 a	1.4 a	1.6 a	1.9 a
−0.05	4.7 bc	5.2 a	5.7 a	2.2 a	3.4 a	5.5 a	3.1 a	3.9 a	5.0 a	1.2 b	1.6 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.

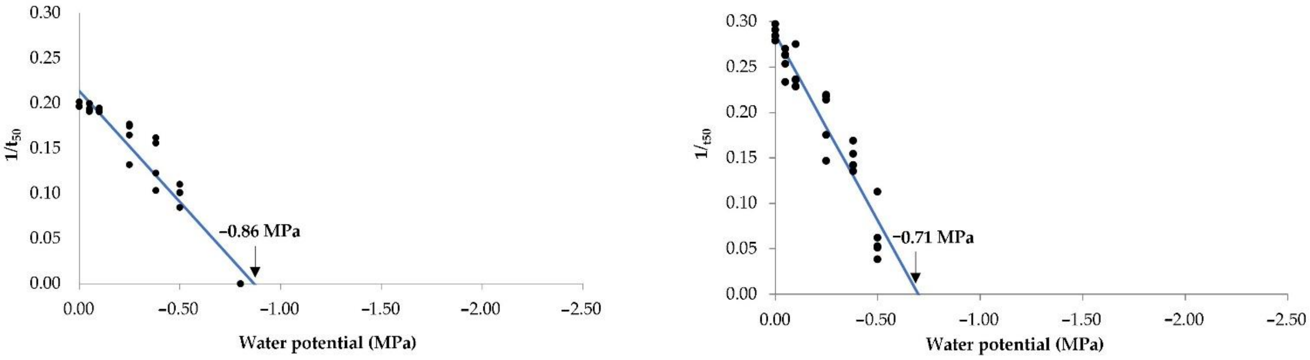
Consequently, Ψ_b was estimated with the germination dynamics shown in Table 2 and is presented in Figure 4. The lowest value was estimated for *C. album* (−1.38 ± 0.14 MPa), and the highest was estimated for *A. retroflexus* (−0.36 ± 0.03 MPa), while *S. pumila* (−0.71 ± 0.07 MPa) and *P. capillare*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

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References

1. Masin, R.; Loddo, D.; Benvenuti, S.; Zuin, M.C.; Macchia, M.; Zanin, G. Temperature and water potential as parameters for modeling weed emergence in central-northern Italy. *Weed Sci.* **2010**, *58*, 216–222. [CrossRef]
2. Werle, R.; Sandell, L.D.; Buhler, D.D.; Hartzler, R.G.; Lindquist, J.L. Predicting emergence of 23 summer annual weed species. *Weed Sci.* **2014**, *62*, 267–279. [CrossRef]
3. Forcella, F.; Benech Arnold, R.L.; Sanchez, R.; Ghersa, C.M. Modelling seedling emergence. *Field Crops Res.* **2000**, *67*, 123–139. [CrossRef]
4. Masin, R.; Loddo, D.; Benvenuti, S.; Otto, S.; Zanin, G. Modeling weed emergence in Italian maize fields. *Weed Sci.* **2012**, *60*, 254–259. [CrossRef]
5. Dorado, J.; Sousa, E.; Cahla, I.M.; González-Andújar, J.M.; Fernández-Quintanilla, C. Predicting weed emergence in maize crops under two contrasting climatic conditions. *Weed Res.* **2009**, *49*, 251–260. [CrossRef]
6. Masin, R.; Loddo, D.; Gasparini, V.; Otto, S.; Zanin, G. Evaluation of weed emergence model AlertInf for maize in soybean. *Weed Sci.* **2014**, *62*, 360–369. [CrossRef]
7. Royo-Esnal, A.; García, A.L.; Torra, J.; Forcella, F.; Recasens, J. Describing *Polygonum aviculare* emergence in different tillage systems. *Weed Res.* **2015**, *55*, 387–395. [CrossRef]
8. Izquierdo, J.; Bastida, F.; Lezaún, J.M.; Sánchez del Arco, M.J.; González-Andújar, J.L. Development and evaluation of a model for predicting *Lolium rigidum* emergence in winter cereal crops in the Mediterranean area. *Weed Res.* **2013**, *53*, 269–278. [CrossRef]
9. Bradford, K.J. Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Sci.* **2002**, *50*, 248–260. [CrossRef]
10. Leguizamon, E.S.; Fernandez-Quintanilla, C.; Barroso, J.; Gonzalez-Andujiar, J.L. Using thermal and hydrothermal time to model seedling emergence of *Avena sterilis* ssp. *ludoviciana in Spain. Weed Res.* **2005**, *45*, 149–156. [CrossRef]
11. Loddo, D.; Ghaderi-Far, F.; Rastegar, Z.; Masin, R. Base temperatures for germination of selected weed species in Iran. *Plant Prot. Sci.* **2018**, *54*, 60–66.
12. CBS—Croatian Bureau of Statistics. Available online: https://www.dzs.hr/default_e.htm (accessed on 15 November 2020).
13. Loddo, D.; Sousa, E.; Masin, R.; Calha, I.; Zanin, G.; Fernández-Quintanilla, C.; Dorado, J. Estimation and Comparison of Base Temperatures for Germination of European Populations of Velvetleaf (*Abutilon theophrasti*) and Jimsonweed (*Datura stramonium*). *Weed Sci.* **2013**, *61*, 443–451. [CrossRef]
14. Leiblein-Wild, M.C.; Kaviani, R.; Tackenberg, O. Germination and seedling frost tolerance differ between the native and invasive range in common ragweed. *Oecologia* **2014**, *174*, 739–750. [CrossRef] [PubMed]
15. Bürger, J.; Colbach, N. Germination base temperature and relative growth rate of 13 weed species—Comparing populations from two geographical origins. In Proceedings of the 28th German Conference on Weed Biology and Weed Control, Braunschweig, Germany, 27 February–1 March 2018; Volume 458, pp. 419–426.

16. Šoštarčić, V.; Šćepanović, M.; Masin, R.; Magosso, D.; Zanin, G. Estimation of biological parameters for germination of *Abutilon theophrasti* Medik. *Period. Biol.* **2018**, *120*, 81–89. [CrossRef]
17. Kottek, M.; Grieser, J.; Beck, C.; Rudolf, B.; Rubel, F. World map of the Koppen-Geiger climate classificationupdated. *Meteorol. Z.* **2006**, *15*, 259–263. [CrossRef]
18. Steinmaus, S.J.; Prather, T.S.; Holt, J.S. Estimation of base temperatures for nine weed species. *J. Exp. Bot.* **2005**, *1*, 275–286. [CrossRef]
19. Guillemin, J.P.; Gardarin, A.; Granger, S.; Reibel, C.; Munier-Jolain, N.; Colbach, N. Assessing potential germination period of weeds with base temperatures and base water potentials. *Weed Res.* **2013**, *53*, 76–87. [CrossRef]
20. Masin, R.; Zuin, M.C.; Archer, D.W.; Zanin, G. WeedTurf: A predictive model to aid control of annual summer weeds in turf. *Weed Sci.* **2005**, *53*, 193–201. [CrossRef]
21. Michel, B.E.; Kaufmann, M.R. The Osmotic Potential of Polyethylene Glycol 6000. *Plant Physiol.* **1973**, *51*, 914–916. [CrossRef]
22. Onofri, A. Bioassay97: A new Excel VBA macro to perform statistical analyses on herbicide dose-response data. *Ital. J. Agrometeorol.* **2001**, *3*, 40–45.
23. Efron, B. Bootstrap methods: Another look at the jackknife. *Ann. Stat.* **1979**, *7*, 1–26. [CrossRef]
24. Steckel, E.L.; Sprague, C.L.; Stoller, E.W.; Wax, L.M. Temperature Effects on Germination of Nine Amaranthus Species. *Weed Sci.* **2004**, *52*, 217–221. [CrossRef]
25. Mollaee, M.; Darbandi, E.I.; Aval, M.B.; Chauhan, B.S. Germination response of three *Setaria species* (*S. viridis*, *S. verticillata*, and *S. glauca*) to water potential and temperature using non-linear regression and hydrothermal time models. *Acta Physiol. Plant* **2020**, *42*, 149. [CrossRef]
26. Rivera, C.M.; Peters, R.A. A preliminary report on formation of seed and germination of fall panicum (*Panicum dichotomiflorum* Michx.) and witchgrass (*P. capillare* L.). *Proc. NEWCC* **1971**, *25*, 30.
27. Vengris, J.; Damon, R.A. Field growth of fall panicum and witchgrass. *Weed Sci.* **1976**, *24*, 205–208. [CrossRef]
28. Baskin, J.M.; Baskin, C.C. Seasonal changes in the germination responses of buried witchgrass (*Panicum capillare*) seeds. *Weed Sci.* **1986**, *34*, 22–24. [CrossRef]
29. Smith, A.A. The Effects of Seed Mass and Emergence Order on Growth and Reproduction of Fall Panicum (*Panicum dichotomiflorum* Michx.). Master's Thesis, The Ohio State University, Columbus, OH, USA, 1986.
30. Kalinová, J.; Moudrý, J. Frost resistance evaluation of the common millet (*Panicum miliaceum* L.) varieties. *Agricultura* **2005**, *3*, 10–12.
31. James, T.K.; Rahman, A.; Mcgill, C.R.; Trivedi, P.D. Biology and survival of broom corn millet (*Panicum miliaceum*) seed. *N. Z. Plant Prot.* **2011**, *64*, 142–148. [CrossRef]
32. Altenhofen, L.M. The Effects of Light, Temperature, After-Ripening, Nitrate and Water on *Chenopodium album* Seed Germination. Master's Thesis, Iowa State University, Ames, IA, USA, 2009.
33. Myers, M.W.; Curran, W.S.; VanGessel, M.J.; Calvin, D.D.; Mortensen, D.A.; Majek, B.A.; Karsten, H.D.; Roth, G.W. Predicting weed emergence for eight annual species in the northeastern United States. *Weed Sci.* **2004**, *52*, 913–919. [CrossRef]
34. Bürger, J.; Malyshev, A.V.; Colbach, N. Populations of arable weed species show intraspecific variability in germination base temperature but not in early growth rate. *PLoS ONE* **2020**, *15*, e0240538. [CrossRef] [PubMed]
35. Vleeshouwers, L.M.; Kropff, M.J. Modelling field emergence patterns in arable weeds. *New Phytol.* **2000**, *148*, 445–457. [CrossRef]
36. Loddo, D.; Bozic, D.; Calha, I.M.; Dorado, J.; Izquierdo, J.; Šćepanović, M.; Barić, K.; Carlesi, S.; Leskovsek, R.; Peterson, D.; et al. Variability in seedling emergence for European and North American populations of *Abutilon theophrasti*. *Weed Res.* **2019**, *59*, 15–27. [CrossRef]
37. Fenner, M. The effects of the parent environment on seed germinability. *Seed Sci. Res.* **1991**, *1*, 75–84. [CrossRef]
38. Baskin, C.C.; Baskin, J.M. *Seed Ecology, Biogeography, and Evolution of Dormancy and Germination*; School of Biological Sciences, University of Kentucky: Lexington, KY, USA; Academic Press: Cambridge, MA, USA, 2001; pp. 181–219.
39. Holm, L.; Doll, J.; Holm, E.; Pancho, J.; Herberger, J. *World Weeds: Natural Histories and Distribution*; Wiley: New York, NY, USA, 1997.
40. Šarić, T.; Ostojić, Z.; Stefanović, L.; Deneva Milanova, S.; Kazinczi, G.; Tyšer, L. The changes of the composition of weed flora in southeastern and central europe as affected by cropping practices. *Herbologia* **2011**, *12*, 8–12.

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Predicting the Emergence of *Echinochloa crus-galli* (L.) P. Beauv. in Maize Crop in Croatia with Hydrothermal Model

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Abstract: *Echinochloa crus-galli* (L.) P. Beauv. is the most common monocotyledonous weed in maize crops in Croatia. Crop–weed interference is influenced by weed emergence patterns, and knowledge of the timing of weed emergence is crucial for the development of an efficient integrated weed-management program. Therefore, two-year field experiments were conducted in a maize crop sown in early May in continental Croatia to determine the emergence pattern of *E. crus-galli* from natural seedbank. In laboratory studies, the estimated base temperature and base water potential for the Croatian ecotype of *E. crus-galli* were 10.8 °C and −0.97 MPa, respectively. Then, the estimated germination parameters were compared with the values embedded in the AlertInf model from Italy (Veneto) to calibrate this hydrothermal model. The estimated hydrothermal units were around 28 for the onset (10%) and 93 for the middle (50%) emergence of *E. crus-galli*. Our findings showed that the AlertInf model satisfactorily simulated the emergence of *E. crus-galli* in maize crop in Croatia (EF = 0.97 in 2019 and 0.98 in 2020), indicating its potential use in other geographical areas

Keywords: AlertInf; base temperature; base water potential; barnyardgrass; integrated weed management; validation

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1. Introduction

Crop-weed interference is affected by the timing of weed emergence, and knowledge of the peak weed emergence period is critical to developing an effective integrated weed-management program [1]. According to Vleeshouwers and Kropff [2], emergence is the result of two different processes: germination and pre-emergence growth. Each process is influenced by different abiotic and biotic environmental factors, which consequently determine the timing of weed emergence. Seed germination is influenced by soil temperature, soil moisture, light exposure, temperature fluctuations, nitrate concentration, soil pH, and gaseous environment in the soil [3]. After successful germination, the developed seedlings or coleoptile emerge from the soil, and the factors that can influence the pre-emergence growth and emergence are soil type, soil texture, timing of tillage, depth of tillage, type of tillage, crop residues, etc. [4]. The timing of weed emergence can be predicted using weed emergence models.

Adequate soil temperature and moisture are the main factors triggering emergence [5]; both mechanistic and empirical models include these factors. Mechanistic models are developed in growing chambers using a different range of temperatures and water potentials, while empirical models rely on the observations of the emergence in the field and the periodicity of occurrence of certain species as a function of weather data [6–8]. Both models describe weed emergence with hydrothermal time (HTT), which is based on two germination parameters - base temperature (T_b) and base water potential (Ψ_b). T_b is the lowest temperature at which germination can occur, while Ψ_b is the minimum value of soil water potential at optimal temperature under which the germination rate is zero [9,10]. Based on these parameters, hydrothermal units (HT) are accumulated during the growing season when the average daily soil or air temperature and soil water potential were above Ψ_b, respectively, until the end of weed emergence. HT units are growing degree day-like measurements that accumulate the heat units above T_b only on days when soil moisture is above Ψ_b.

These models have been shown to be sufficiently accurate in predicting weed emergence in specific areas where they have been developed. For example, in maize, two HTT models are available as software applications for growers: AlertInf in Italy [11] and WeedCast in the United States [12].

The idea of extending the models to other climatic zones was proposed by Grundy [1]. However, for reliable application of the predictive emergence model, the germination parameters of the local populations should be estimated. This is because the conditions specific for seed development as well as numerous other abiotic and biotic factors can influence the adaptation and behavior of the plant in a given climatic region [13]. In addition, climate change may affect weed germination behavior. Therefore, it is important to study the biology of weed germination for each population. Laboratory experiments have shown the differences in the values of the germination parameters of different populations of the same species. For example, Leiblein-Wild et al. [14] estimated the differences in T_b between European and North American populations of *Ambrosia artemisiifolia* L. (2.0 °C and 4.2 °C, respectively) and explained this variation as an adaptation to new agroecological conditions, which is a characteristic of invasive plant species. In addition, Bürger and Colbach [15] estimated different T_b values for *Chenopodium album* L. and *Echinochloa crus-galli* (L.) P. Beauv. between the French and German populations, with 5.8 °C vs. 1.5 °C and 6.2 °C vs. 10.2 °C, respectively. Although the data of estimated Ψ_b between populations of the same weed species are limited, Masin et al. [7] estimated different values of Ψ_b for two populations of *Amaranthus retroflexus* L. in Italy (Padova, Pisa). The estimated values of Ψ_b for the populations in Padova and Pisa were 0.41 MPa and −0.62 MPa, respectively. Excluding specific population germination parameters, Loddo et al. [13] attempted to validate the AlertInf model for *Abutilon theophrasti* Med. with populations collected at eight sites in Europe and the USA, and validation was successful at only two sites. However, model validation is more successful when calibrated for the specific geographic location. For example, Leblanc et al. [16] successfully predicted *C. album* emergence by calibrating the model for different soils at three sites in Canada. Additionally, Masin et al. [11] in Italy successfully transferred the AlertInf model from the Veneto to Tuscany regions for three species (*C. album*, *Sorghum halepense* (L.) Pers., and *A. theophrasti*) in which the estimated germination parameters were not statistically different between the two populations.

Currently, weed emergence prediction using the AlertInf model is possible for ten summer species: *A. theophrasti*, *Digitaria sanguinalis* (L.) Scop., *E. crus-galli*, *Polygonum persicaria* L., *Setaria viridis* (L.) Beauv., *Solanum nigrum* L., *A. retroflexus*, *C. album*, *Setaria pumila* (Poir.) Roem & Schultz, and *S. halepense* [7]. Among the species included in the model, *E. crus-galli* is by far the most interesting to test the ability to extend the prediction of its emergence in Croatia. Being one of the most problematic weeds in the world [17], it is also the important weed in maize crop in Croatia occurring in 91% of the fields on the Croatian mainland monitored over a 40-year period (1969–2009) [18]. In untreated plots in Croatian soybean field trials, this weed species was present with an average of 48 plants per square meter [19]. The population density of *E. crus-galli* is similar in other row crops such as maize, where the competition caused by this species reduces grain yield by up to 50%, depending on the density of *E. crus-galli* and the crop growth stage [20].

The successful validation and transfer of the model AlertInf from Veneto to the Tuscany region has generated the idea of possibly using the model in maize crop in Croatia. Namely, maize is the most important arable crop in Croatia, and the HTT model could be a useful tool for farmers to adjust herbicide application based on predicted field emergence. Predicting weed emergence helps determine the appropriate time to apply herbicides when the largest population of weed species is present in the field. This approach contributes to low pesticide use in agriculture, as required by new EU agricultural strategies (The EU Green Deal).

Therefore, the first objective of the study was to estimate the germination parameters (T_b and Ψ_b) of a Croatian population of *E. crus-galli* and then to compare them with the Italian population modeled by AlertInf. The second objective was to validate the AlertInf HTT model with the emergence data of *E. crus-galli* observed in a maize field in continental Croatia.

2. Materials and Methods

2.1. Laboratory Experiments—Estimation of Base Temperature and Base Water Potential

Freshly matured seeds of *E. crus-galli* were collected in October 2013 from 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 were cleaned, placed in paper bags, and stored in a refrigerator (4 °C) until the start of the experiment.

The laboratory germination experiments were conducted in 2014 and performed at different temperatures and water potentials in the germination chambers. To estimate T_b, 100 seeds per three replicates were sown in Petri dishes containing distilled water and sealed with parafilm to prevent evaporation. Petri dishes with sown seeds were placed in germination chambers (W87R, KW Apparecchi Scientifici SRL, Monteriggioni, Italy) at different temperatures (8, 12, 16, 20, 24, and 28 °C) and a photoperiod of 12 h:12 h (day–night). To estimate the Ψ_b, the same number of seeds was sown in plastic containers (10 cm diameter and 7 cm high) [7]. The seeds were sown in these containers to obtain a sufficient amount of solution at each water potential throughout the experimental period. Polyethylene glycol (PEG) 6000 (Sigma-Aldrich Chemie GmbH 25322-68-3, St. Louis, MO, USA) was used to achieve different water potentials, and the solutions were prepared 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 [21]. The PEG 6000 solutions were prepared according to the methodology described by Michel and Kaufman [21] using the formula:

$$OP = (-1.18 \cdot 10^{-2}) \cdot C - (1.18 \cdot 10^{-4}) \cdot C + (2.67 \cdot 10^{-4}) \cdot C{\cdot}T + (8.39 \cdot 10^{-7}) \cdot C^2T \quad (1)$$

where OP is the osmotic pressure, C is the PEG concentration expressed as different weights to reach different stress levels, and T is the temperature (24 °C).

Plastic containers with 50 mL of PEG solution were placed at 24 °C with a photoperiod of 12 h:12 h (day–night) in the germination chamber. Temperature was chosen according to the preliminary experiments where the germination rate of *E. crus-galli* was highest at 24 °C. The photoperiod used in the experiment was the same as used in the experiment of Masin et al. [7]. The light intensity in the chamber was 40–50 µmol m^{-2}.

The monitoring of germination was performed as described in Šoštarčić et al. [22] and Masin et al. [7]. Germination was recorded daily to analyze the germination dynamics at different temperatures and water potentials. Germination lasted between 2 and 64 days, depending on the temperature and water potential.

2.2. Field Experiments and Laboratory Analyses

2.2.1. Monitoring of *E. crus-galli* Emergence in Maize

During the two growing seasons of maize, the emergence of *E. crus-galli* was monitored at the experimental station of Sasinovecki Lug to verify the transferability of the AlertInf model. The field experiment was set in a maize crop highly infested by *E. crus-galli* observed in a previous year. Maize was grown under recommended agronomic practices and operations. Previous crops in rotation were winter wheat (*Triticum aestivum* L.) and winter barley (*Hordeum vulgare* L.) for maize crops grown in 2019 and 2020, respectively. After harvesting winter cereals, an experimental field was moldboard ploughed in the autumn of each year. Shallow spring-tooth harrowing in early spring (mid-March) for soil loosening was followed by seedbed preparation using a field cultivator just before sowing. Hybrid Bc 418 was sown on 8 May 2019 and 5 May 2020 at the recommended rate (75.188 seeds per ha) in rows 70 cm apart.

Monitoring of *E. crus-galli* emergence was carried out three times a week by placing 12 metal formed squares (0.3 × 0.3 m) between maize rows. The first seedlings to emerge (with visible true leaves) of *E. crus-galli* were counted and then removed by gently plucking three times a week, without additional soil rotation.

Monitoring of the emergence ended when no emergent seedlings were observed for at least two weeks after closing the maize canopy (BBCH 18-19). In both years, monitoring started after maize sowing and lasted until 30 June and 29 June in 2019 and 2020, respectively.

Average daily soil temperature and soil moisture were monitored in the field by installing a temperature data logger (HOBO UA-001-08, Onset Computer Corporation, Bourne, MA, USA) and a moisture measuring devices (ECH2O 10HS Soil Water Content sensor, Meter Group Inc., Pullman, WA, USA) at the soil depth of up 5 cm. The data on air temperature and precipitation were recorded from the university meteorological station located at the experimental field, in order to compare the meteorological conditions between the two experimental years. The air temperature, soil temperature, and precipitation during the experimental period are shown in Table 1.

Table 1. Average air and soil temperature and precipitation for the Sasinovecki Lug field site, recorded during the two experimental periods. Air temperature and precipitation were provided by the university meteorological station located at the experimental field, while soil temperature was recorded with the temperature data logger (HOBO UA-001-08, Onset Computer Corporation, Bourne, MA, USA).

Experimental Period	Average Air Temperature (°C)	Average Soil Temperature (°C)	Precipitation (mm)
2019			
8–31 May	13.0	15.2	54.8
1–30 June	22.6	24.9	85.6
1–5 July	22.0	22.6	12.4
2020			
5–31 May	15.3	17.8	58.6
1–29 June	19.3	22.1	85.6

2.2.2. Soil Analysis

Before the start of the experiment, a 1 m deep soil pit was dug in order to describe, sample, and classify the soil at the site. The soil profile is a Calcaric Endogleyic Fluvisol (Aric, Siltic) with the following horizons according to IUSS Working Group WRB [23]: Ap-C-Cl [24]. From each soil horizon, along with the disturbed samples, the undisturbed samples were taken using 100 cm^3 cores. In this paper, only the selected properties of the 30 cm deep topsoil (ploughed layer, i.e., Ap horizon) are presented (Table 2).

The disturbed soil samples were air-dried and sieved through the 2 mm sieve. The soil particle size distribution was determined by the pipette method. The soil organic matter (SOM) was analyzed as humus content by wet oxidation and back titration (Tyurin method). Soil carbonates were determined volumetrically as the content of $CaCO_3$. Soil pH was measured electrometrically using a glass electrode. Soil bulk density was obtained gravimetrically from triplicate undisturbed samples, with the mean value reported in the paper (Table 2).

Table 2. Selected properties of the analyzed topsoil (Ap horizon).

Soil Physicochemical Properties [a]					Soil Water Retention Properties (Vol %) [b]		
Texture	Organic matter %	$CaCO_3$ %	pH_{KCl}	Bulk density g cm^{-3}	Field capacity	Plant wilting point	Plant-available water
Silt loam [25]	2.7 [25]	7.0	7.28 [25]	1.16	44.0	20.6	23.4

[a] The results for soil texture, organic matter, and pH_{KCl} were adapted from Pintar et al. [25]. [b] The soil field capacity (FC) was measured at 0.033 MPa, and plant wilting point (PWP) was measured at 1.5 MPa; the plant-available water (PAW) volume was calculated as PAW = FC − PWP.

Soil water retention was analyzed from the disturbed samples using the Soilmoisture Equipment Corp. extractors by applying pressures of 0.01, 0.033, 0.625, and 1.5 MPa and then by determining the corresponding soil water contents gravimetrically. The volumetric soil water contents were calculated by multiplying the mass soil water contents with the soil bulk density (Table 2). The RETC computer program (U.S. Salinity Laboratory, USDA, ARS: Riverside, CA, USA) [26] was used to build the water retention curve of the studied soil, which was then used to obtain the water potentials that correspond to the soil water contents measured during the experiment.

2.3. Statistical Analysis

A parabolic model was used to describe the effect of temperature, while a logistic model was used to describe the effect of water potential. T_b and Ψ_b were estimated using cumulative germination data, which were presented as germination dynamics. The effect of the temperature and water potential on germination dynamics, expressed in days (t_{10}, t_{50}, and t_{90}), was analyzed using means of variance analysis (ANOVA). After a significant F-test, the LSD test for $p = 0.05$ was used to compare the mean values. The germination dynamics curve was generated using the logistic function in the Bioassay97 statistical program [27] to determine the initial (t_{10}), medium (t_{50}), and final (t_{90}) germination times. A linear regression, estimated using the bootstrap method [28], provided the best fit of germination rate (reciprocal of time to 50% germination) against incubation temperature or water potential. The values of T_b and Ψ_b were estimated as the intercept of the regression line with the temperature or water potential axis [7,22,29].

Then, the germination parameters of *E. crus-galli* were compared with the germination parameters of the Italian population. The aim of the comparison was to verify whether inserting the value of the Croatian population into the AlertInf model is necessary, which would be a model recalibration, whether statistical difference is found between the values of the Italian and Croatian populations, and whether the model can be used without recalibration. The criterion of overlap of the 95% confidence intervals estimated with the bootstrap method was used to compare the values of the germination parameters of Croatian and Italian populations of *E. crus-galli* as described in Šoštarčić et al. [22].

The soil temperature and soil moisture data during the experimental period in the field were used to calculate hydrothermal units (HT) according to Masin et al. [30]:

$$\begin{gathered} HTi = n \cdot \max(T_{si} - T_b, 0) + HTi\text{-}1 \\ T_{si} < T_o\text{: } n = 0 \text{ if } \Psi_{si} \leq \Psi_b;\ n = 1 \text{ if } \Psi_{si} > \Psi_b \\ T_{si} > T_o\text{: } n = 0 \text{ if } \Psi_{si} \leq \Psi_b + K_t(T_{si} - T_o);\ n = 1 \text{ if } \Psi_{si} > \Psi_b + K_t(T_{si} - T_o) \end{gathered} \quad (2)$$

where T_{si} and Ψ_{si} are the average daily soil temperature and water potential at a depth of 5 cm, T_b is the base temperature, Ψ_b is the base water potential, T_o is the optimal temperature for seed germination, and K_t is the slope of the relationship between Ψ_b and Tsi in the supra-optimal temperature range.

Model Validation

In order to verify the applicability of the model to maize in Croatia, the weed emergence dynamics of *E. crus-galli* were simulated by the Italian AlertInf model [30]. Emergence dynamics are expressed by the Gompertz function according to the following equation:

$$\mathrm{CE} = 100 \cdot \exp\left(-\mathrm{a} \cdot \exp\left[-\mathrm{b} \cdot \mathrm{HT}\right]\right) \tag{3}$$

where CE represents cumulative emergence, a is related to an HT lag before emergence starts, and b is related to the slope of the curve.

The simulation used the daily average values of soil temperature and soil water potential recorded in the field. The germination parameters used were the estimated T_b and Ψ_b of the Croatian population, the optimal temperature (T_o), the slope (K_t), and the Gompertz coefficients (a and b) estimated for Italian populations. The cumulated emergence percentage of both years was simulated, and the predictions were compared with the observations. The overall model performance was evaluated using root mean square error (RMSE) and modeling efficiency (EF), calculated as follows:

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(P_i - O_i)^2} \tag{4}$$

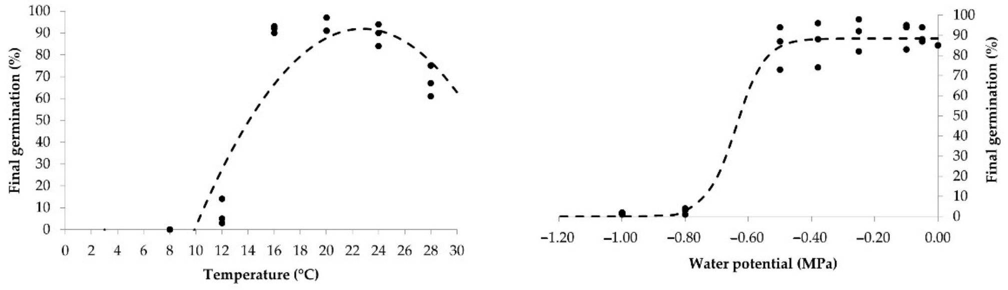
$$\mathrm{EF} = \frac{\sum_{i=1}^{n}(O_i - \overline{O})^2 - \sum_{i=1}^{n}(P_i - O_i)^2}{\sum_{i=1}^{n}(O_i - \overline{O})^2} \tag{5}$$

where P_i is the simulated value, O_i is the measured value, $\bar{O}$ is the mean of the measured value, and N is the number of observations.

3. Results and Discussion

3.1. Estimation of Base Temperature and Base Water Potential

The final germination of *E. crus-galli* at different temperatures ranged from 0 to 93% (Figure 1a). The highest germination was recorded at temperatures 16, 20, and 24 °C (92, 93, and 89%, respectively). Germination decreased at 12 °C (7%), while no germination was recorded at 8 °C. In addition, at the highest studied temperature, 28 °C, germination decreased (68%). High germination was observed across all water potentials from 0.00 to −0.50 MPa (85–86%), and germination decreased at −0.80 MPa (3%) and −1.00 MPa (2%) (Figure 1b).



(**a**) (**b**)

Figure 1. Estimated models of the final germination of *Echinochloa crus-galli*. A parabolic model was used to describe the effect of temperature (**a**), while a logistic model was used to describe the effect of water potential (**b**). The black dots represent the observed germination, while the dashed line represents the model.

The daily recorded germination was analyzed as germination dynamics over the studied period, and the results are presented in Table 3. The germination dynamics at the different studied temperatures lasted from 3.4 to 18.7 days. As expected, the onset of germination was fastest at 28 °C, starting after 1.6 days, whereas it was the slowest at 12 °C, starting after 13.9 days. The same trend was observed for mean germination (t_{50}) and final germination (t_{90}). Germination decreased with the decrease in water potential, with statistical differences observed in t_{10} and t_{50}. The germination dynamics were the fastest at 0.00 MPa (water-saturated environment), while taking 50 days at −0.80 MPa (dry environment).

Table 3. Germination dynamics (t_{10}, t_{50}, and t_{90}) at the different studied temperatures and water potentials at 24 °C.

°C	t_{10}	t_{50}	t_{90}	MPa	t_{10}	t_{50}	t_{90}
28	1.6 a	2.4 a	3.4 a	**0.00**	1.3 a	1.6 a	2.1 a
24	3.2 b	3.8 ab	4.6 ab	**−0.05**	1.6 b	2.2 ab	3.0 a
20	3.1 b	4.6 b	6.7 b	**−0.10**	1.8 b	2.6 ab	3.6 a
16	5.2 e	8.1 c	12.6 c	**−0.25**	2.3 c	3.3 bc	4.7 a
12	13.9 d	16.2 d	18.7 d	**−0.38**	2.6 e	3.6 bc	4.3 a
				−0.50	2.9 d	3.9 c	5.8 a
				−0.80	1.6 b	9.0 d	50.0 b

Differences between the initial (t_{10}), medium (t_{50}), and final (t_{90}) germination under different temperatures and 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 $p < 0.05$.

Reciprocal time to t_{50} was used to create a linear regression line and estimate T_b (10.8 °C, Figure 2) and Ψ_b (−0.97 MPa, Figure 3) for the Croatian population of *E. crus-galli*. The estimated T_b value for the Croatian population is similar to the estimated values for the Iranian population (10.4 °C) [31] and a German origin (10.2 °C) [15]. A similar value was also reported for a Texas population, 9.7 °C [32]. In contrary, the lowest T_b of *E. crus-galli* was estimated for a French population: 6.2 °C [33]. Meanwhile, Steinmaus [34] estimated the highest T_b, 13.8 °C, in California. To our knowledge, little information is found in the literature on the Ψ_b of *E. crus-galli*. However, Guillemin et al. [33] reported the Ψ_b value of −1.19 MPa for a French population.

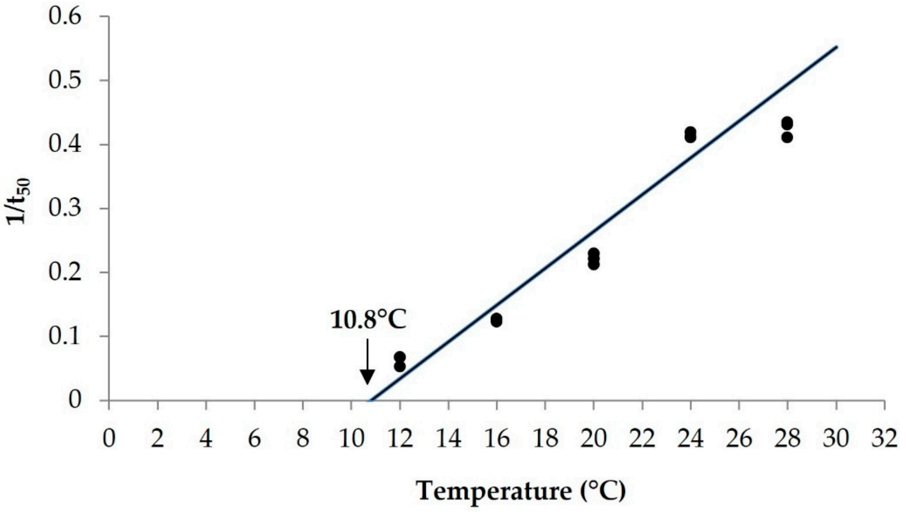


Figure 2. Estimated base temperatures (T_b) for the germination of *Echinochloa crus-galli*. The solid line represents the linear regression line, and the points represent the inverse value of the time necessary to reach 50% of germination ($1/t_{50}$) estimated for the single replicates. The estimated value of the base temperature is the intersection of the regression line with the X-axis, $T_b = 10.8 \pm 0.27$; $y = 0.0288x - 0.3119$, and $R^2 = 0.94$.

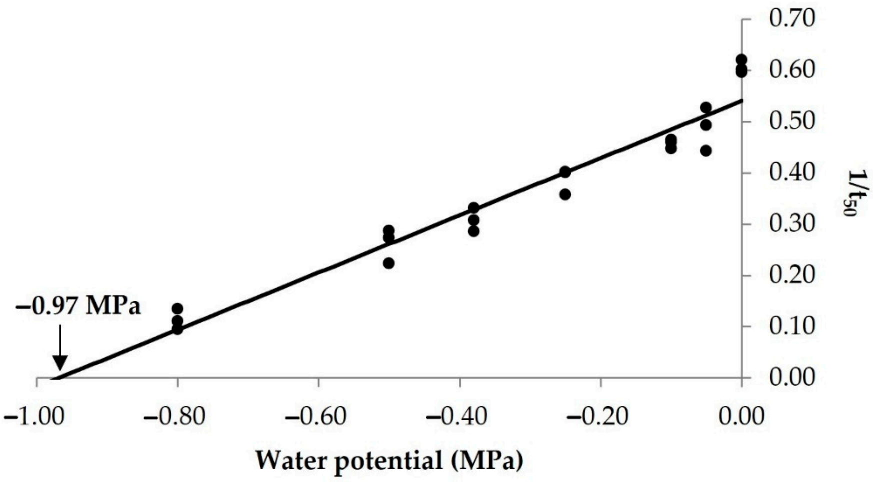


Figure 3. Estimated base water potentials (Ψ_b) for the germination of *Echinochloa crus-galli*. The solid line represents the linear regression line, and the points represent the inverse value of the time necessary to reach 50% of germination ($1/t_{50}$) estimated for the single replicates. The estimated value of the base water potential is the intersection of the regression line with the X-axis, $\Psi_b = -0.97 \pm 0.06$; $y = 0.5578x + 0.5404$, and $R^2 = 0.94$.

The T_b value of the Croatian population of *E. crus-galli* is 0.9 °C lower than the T_b value of the Italian population (Table 4). No overlap in the T_b values between the two populations is found; therefore, the populations differ in this parameter. However, the Ψ_b values of the two populations overlap, indicating that the Croatian and Italian populations of *E. crus-galli* do not differ in this parameter. Among other factors that might influence the difference in the estimated value, the climatic conditions at the two sites might have affected the T_b value. Namely, 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 minimum [35]. The average annual temperature in Zagreb is 11.8 °C, while the average annual temperature in Padova is 12.2 °C. A similar trend was observed for another grass weed species, *S. pumila*, of which the estimated T_b of the Croatian population is 6.6 °C, while the Italian population has a T_b of 10.4 °C [22].

Table 4. Comparison of base temperature (T_b) and base water potential (Ψ_b) of *Echinochloa crus-galli* Croatian and Italian population estimated with the bootstrap method [28], 95% confidence interval (95% CI), and coefficient of determination (r^2). Estimated values of the Italian population were adapted from Masin et al. [7].

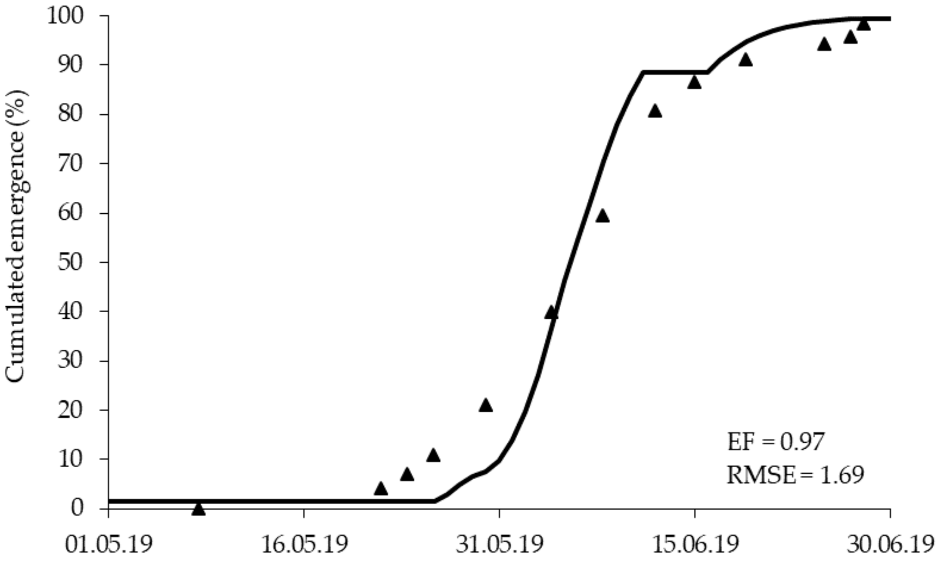
Population	T_b (°C)	±95 CI	r^2	Ψ_b (MPa)	±95 CI	r^2
Croatia	10.8	0.27	0.94	−0.97	0.06	0.94
Italy [7]	11.7	0.28	0.89	−0.97	0.04	0.95

3.2. Field Experiments

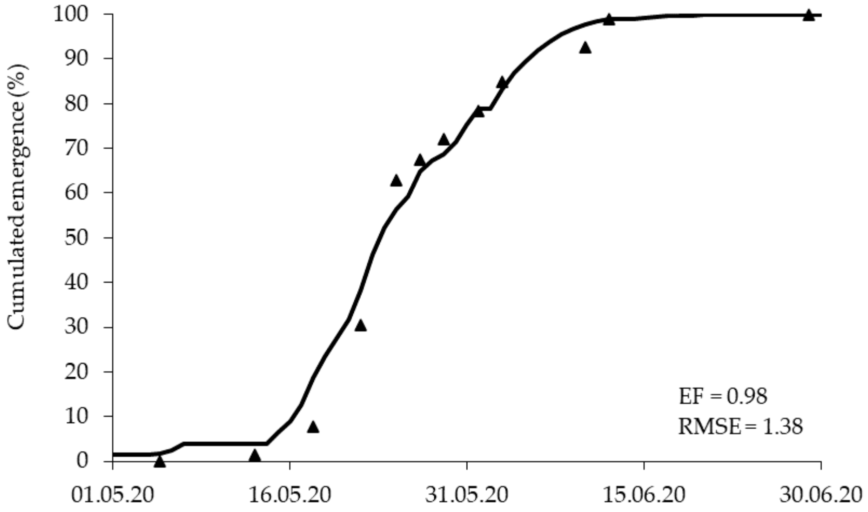
Data on the emergence of *E. crus-galli* in maize crops at Sasinovecki Lug were used to validate the AlertInf model developed with germination parameters (T_b = 10.8 °C and $\Psi_b = -0.97$ MPa) of the Croatian population. The optimal temperature (T_o = 26 °C), the slope of the relationship (K_t = 0.1) between Ψ_b and T_{si}, and the Gompertz coefficient (a = 4.17, and b = 0.02) were adopted from Masin et al. [30].

As dominant weed species in a natural seedbank in a Croatian field [18], *E. crus-galli* was present in high density in Sasinovecki Lug. The average densities were 933 and 834 plants/m^2 in 2019 and 2020, respectively, which highlight the reliability of the model stimulation. Indeed, a prediction of *E. crus-galli* emergence by AlertInf in Veneto was performed by a much lower weed density (56.6 plants/m^2) [30]. Model simulation of

E. cus-galli emergence in maize at Sasinovecki Lug is shown in Figure 4a,b for 2019 and 2020, respectively. The AlertInf model fit the observed data satisfactorily, with EF indices of 0.97 and 0.98 in 2019 and 2020, respectively. However, the emergence data show that the model underestimated the onset of emergence in 2019 and overestimated it in 2020. In addition, a slight overestimation was observed in 2019 from the middle to the end of emergence. The RMSE is 1.69 and 1.38 in 2019 and 2020 respectively, which means that the average deviation predicted from measured values is small.



(**a**)



(**b**)

Figure 4. Cumulative emergence of *Echinochloa crus-galli* observed in the field (triangle) during 2019 (**a**) and 2020 (**b**) and the AlertInf prediction of emergence (line). Hydrothermal units are expressed as calendar days. The seed bed preparation was performed on 8 May 2019 and 5 May 2020.

In 2019, the AlertInf model predicted an initial emergence (11%) of *E. crus-galli* at 29.1 cumulative HT units on 31 May (Figure 4a). However, in the field, this emergence was observed 5 days earlier, on 26 May, 18 days after sowing (DAS). The model predicted the middle emergence (52%) on 6 June at 96.2 cumulative HT units. Field observations at this time were similar, with emergence monitored from 4 to 8 June (40–50%). In addition, according to the model, an 81% emergence at 157.9 HT units should have occurred on 10 June. However, in the field, this was achieved on 12 June. The model predicted 91% of emergence only one day earlier than the value observed in the field (18 June vs. 19 June). Similarly, the model predicted the end of emergence (99–100%) on 26 June at 312 HT units, whereas in the field; this was on 28 June.

In contrast to 2019, in 2020, the model overestimated the onset of emergence (0–30%), while the middle and the end of emergence data were consistent with predicted values. In 2020, the onset of emergence (10%) was predicted for 16 May at 27.3 HT units (Figure 4b). In the field, this emergence was observed on 18 and 22 May, i.e., between 13 and 17 DAS. The middle emergence predicted by the model was reached at a cumulative 93.1 HT units on 24 May. The field situation was also similar, with middle emergence observed from 22 to 25 May (32–63%), i.e., 17 to 20 DAS. Moreover, the prediction of 80% of emergence was achieved at 156.3 HT units, which should have been achieved on 3 June according to the model. In the field, the 85% emergence of *E. crus-galli* was observed two days earlier (85%) on 1 June. The end of emergence was predicted on 17 June at 342.5 HT units, while it was observed in the field from 12 June.

As previously mentioned, the observed field emergence of *E. crus-galli* varied only slightly between 2019 and 2020 when calendar days were considered. In both years, emergence began after sowing (early May) and continued through the end of June, coinciding with the closing of the maize canopy and the concomitant decline in *E. crus-galli* emergence. The cessation of emergence with the closing of the leaf canopy has already been documented [5] and explained by the change in soil climate. However, studies based on determination of emergence pattern often are performed in a crop-free field [36–38]. Nevertheless, when *E. crus-galli* emergence was observed without the crop at 12 sites in the United States, the emergence extended into September [39]—much longer than in our study. Therefore, conducting these experiments and observing emergence within a crop and between different crops are necessary due to the differences in crop canopy architecture. [30].

Our findings suggest that the use of the AlertInf model for predicting the emergence of *E. crus-galli* in Sasinovecki Lug (Croatia) is fully feasible considering the threshold EF value of 0.5 for an acceptable model prediction [40]. When transferring the AlertInf from Veneto to Tuscany, Masin et al. [11] estimated the EF values for the emergence of *A. theophrasti*, *C. album*, and *S. halepense* to be 0.98, 0.99, and 0.98, respectively. On the contrary, Egea-Cobrero et al. [41] used the Myers et al. [42] dataset from the United Sates to validate the emergence of *A. theophrasti* in Golega (Portugal) and Minnesota (United States), obtaining EFs of 0.30 and 0.97, respectively. Due to the low EF in Portugal, the authors concluded that the same model could not be used in Portugal. However, the results of the current study do not allow us to generalize the application of the model to different environmental conditions and agronomic practices. For example, in this study, tillage and seedbed preparation did not differ in both years. The effect of tillage on the vertical distribution of seed in the seed bank is well known, and different tillage practices can significantly affect field emergence. However, Vasileiadis et al. [37] concluded that the emergence *of E. crus-galli* was stable over the years under different simulated tillage systems (conventional, reduced, and, no-till), so AlertInf could be adopted for maize grown under different tillage conditions considering this fact. Another factor that could influence the emergence and effectiveness of the model is soil type, which was not considered in this experiment and should be further investigated. A good example is the study by Leblanc et al. [16], who calibrated a predictive mathematical model to different soil types by adjusting the base temperature of *C. album* seedlings to the soil texture.

In order to set an appropriate time for weed control, determining the time of weed emergence in the field is important, which according to our study can be predicted for *E. crus-galli* by AlertInf. In Croatian maize fields, *E. crus-galli* is almost always controlled with pre-emergence or post-emergence herbicides, usually in combination with inter-row cultivation. According to Oriade and Forcella [43], the efficacy of inter-row cultivation is highest when 60% of *S. viridis*, another important monocot maize species, has emerged. Based on our experiment, inter-row cultivation should be applied from 96 to 113 cumulative HT units. The best efficacy of post-emergence foliar herbicides is achieved when 70–80% of weeds have emerged in the field [7,44]. According to AlertInf, foliar application should be made at a cumulative 140–144 HT units. Finally, the AlertInf model can be used to support the adoption of integrated weed control tactics and post-emergence band application with inter-row cultivation, which can significantly reduce herbicide use in maize [45].

4. Conclusions

E. crus-galli is the most important monocotyledonous weed in maize in Croatia and other geographical areas. The possible use of the existing weed emergence model for this species could be useful for weed control programs. In this study, the Italian AlertInf model had to be calibrated with the T_b values of the Croatian population of *E. crus-galli*, as there were statistical differences with the Italian population embedded in the model. The calibrated AlertInf model showed good prediction of *E. crus-galli* emergence when validated with field data from continental Croatia (Šašinovečki Lug). Therefore, the use of AlertInf for predicting *E. crus-galli* at this site is successful.

Future experiments should focus on increasing the complexity of the AlertInf model by including previously mentioned factors such as environmental conditions (soil type) and agronomic practices (type of tillage) that could influence the emergence of *E. crus-galli*. For practical purposes, the use of AlertInf by Croatian farmers could be a good way to predict the emergence of *E. crus-galli* without the need to measure and monitor all of the parameters required as inputs by complex mechanistic models [1,17]. The results of this study encourage us to extend AlertInf to other important maize weed species for which the germination parameters have already been estimated [22]. The possible extension of AlertInf to other geographical areas could be the focus of further experiments.

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References

1. Grundy, A.C. Predicting weed emergence: A review of approaches and future challenges. *Weed Res.* **2003**, *43*, 1–11. [CrossRef]
2. Vleeshouwers, L.M.; Kropff, M.J. Modelling field emergence patterns in arable weeds. *New Phytologist.* **2000**, *148*, 445–457. [CrossRef]
3. Merfield, C.N. *False and Stale Seedbeds: The Most Effective Non-Chemical WeedManagement Tools for Cropping and Pasture Establishment*; The BHU Future Farming Centre: Lincoln, New Zealand, 2013; p. 23.
4. Mohler, C.L. Weed life history: Identifying vulnerabilities. In *Ecological Management of Agricultural Weeds*; Liebman, M., Mohler, C.L., Staver, C.P., Eds.; Cambridge University Press: Cambridge, UK, 2001; pp. 40–98. [CrossRef]
5. Forcella, F.; Benech-Arnold, R.L.; Sanchez, R.; Ghersa, C.M. Modeling seedling emergence. *Field Crop Res.* **2000**, *67*, 123–139. [CrossRef]
6. García, A.L.; Recasens, J.; Forcella, F.; Torra, J.; Royo-Esnal, A. Hydrothermal Emergence Model for Ripgut Brome (*Bromus diandrus*). *Weed Sci.* **2013**, *61*, 146–153. [CrossRef]
7. Masin, R.; Loddo, D.; Benvenuti, S.; Zuin, M.C.; Macchia, M.; Zanin, G. Temperature and Water Potential as Parameters for Modeling Weed Emergence in Central-Northern Italy. *Weed Sci.* **2010**, *58*, 216–222. [CrossRef]

8. Leguizamon, E.S.; Fernandez-Quintanilla, C.; Barroso, J.; Gonzalez-Andujar, J.L. Using thermal and hydrothermal time to model seedling emergence of *Avena sterilis* spp. *ludoviciana in Spain. Weed Res.* **2005**, *45*, 149–156. [CrossRef]
9. Gummerson, R.J. The effect of constant temperatures and osmotic potentials on the germination of sugar beet. *J. Exp. Bot.* **1986**, *37*, 729–741. [CrossRef]
10. Bradford, K.J. Water relations in seed germination6. In *Seed Development and Germination*; Kigel, J., Galili, G., Eds.; Marcel Dekker, Inc.: New York, NY, USA, 1995; pp. 351–396.
11. Masin, R.; Loddo, D.; Benvenuti, S.; Otto, S.; Zanin, G. Modeling Weed Emergence in Italian Maize Fields. *Weed Sci.* **2012**, *60*, 254–259. [CrossRef]
12. Archer, D.W.; Forcella, F.; Eklund, J.J.; Gunsolus, J. WeedCast Version 2.0. 2001. Available online: http://www.morris.ars.usda.gov (accessed on 15 July 2021).
13. Loddo, D.; Bozic, D.; Calha, I.M.; Dorado, J.; Izquierdo, J.; Šćepanović, M.; Barić, K.; Carlesi, S.; Leskovsek, R.; Peterson, D.; et al. Variability in seedling emergence for European and North American populations of *Abutilon theophrasti*. *Weed Res.* **2019**, *59*, 15–27. [CrossRef]
14. Leiblein-Wild, M.C.; Kaviani, R.; Tackenberg, O. Germination and seedling frost tolerance differ between the native and invasive range in common ragweed. *Oecologia* **2014**, *174*, 739–750. [CrossRef]
15. Bürger, J.; Colbach, N. Germination base temperature and relative growth rate of 13 weed species—Comparing populations from two geographical origins. In Proceedings of the 28th German Conference on Weed Biology and Weed Control, Braunschweig, Germany, 27 February–1 March 2018; Volume 458, pp. 419–426. [CrossRef]
16. Leblanc, M.L.; Cloutier, D.C.; Stewart, K.A.; Hamel, C. Calibration and validation of a common lambsquarters (Chenopodium album) seedling emergence model. *Weed Sci.* **2004**, *52*, 61–66. [CrossRef]
17. Holm, L.; Doll, J.; Holm, E.; Pancho, J.; Herberger, J. *World Weeds: Natural Histories and Distribution*; John Wiley & Sons: New York, NY, USA, 1997.
18. Šarić, T.; Ostojić, Z.; Stefanović, L.; Deneva Milanova, S.; Kazinczi, G.; Tyšer, L. The changes of the composition of weed flora in southeastern and central europe as affected by cropping practices. *Herbologia* **2011**, *12*, 8–12.
19. Šćepanović, M.; Ostojić, Z.; Barić, K. Ograničenja mogućnosti suzbijanja korova u soji nakon nicanja. In *Zbornik Sažetaka 56*; seminara biljne zaštite. Cvjetković, B. (ur.); Hrvatsko društvo biljne zaštite: Opatija, Croatia, 2012; pp. 27–28.
20. Bosnic, A.C.; Swanton, C.J. Influence of barnyardgrass (*Echinochloa crus-galli*) time of emergence and density on corn (*Zea mays*). *Weed Sci.* **1997**, *45*, 276–282. [CrossRef]
21. Michel, B.E.; Kaufmann, M.R. The Osmotic Potential of Polyethylene Glycol 6000. *Plant Physiol.* **1973**, *51*, 914–916. [CrossRef] [PubMed]
22. Šoštarčić, V.; Masin, R.; Loddo, D.; Brijačak, E.; Šćepanović, M. Germination parameters of selected summer weeds: Transferring of the Alertinf model to other geographical regions. *Agronomy* **2021**, *11*, 292. [CrossRef]
23. IUSS Working Group WRB. *World Reference Base for Soil Resources. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*; World Soil Resources Reports; No. 106; FAO: Rome, Italy, 2014.
24. FAO. *Guidelines for Soil Description*, 4th ed.; Food and Agriculture Organization of the United Nations: Rome, Italy, 2006; p. 97.
25. Pintar, A.; Stipičević, S.; Lakić, J.; Barić, K. Phytotoxicity of mesotrione residues on sugar beet (*Beta vulgaris* L.) in agricultural soils differing in adsorption affinity. *Sugar Tech.* **2020**, *22*, 137–142. [CrossRef]
26. van Genuchten, M.T.; Leij, F.J.; Yates, S.R. *The RETC Code for Quantifying the Hydraulic Functions of Unsaturated Soils, Version 1.0*; EPA Report 600/2-91/065; U.S. Salinity Laboratory, USDA, ARS: Riverside, CA, USA, 1991.
27. Onofri, A. Bioassay97: A new Excel VBA macro to perform statistical analyses on herbicide dose-response data. *Ital. J. Agrometeorol.* **2001**, *3*, 40–45.
28. Efron, B. Bootstrap methods: Another look at the jackknife. *Ann. Stat.* **1979**, *7*, 1–26. [CrossRef]
29. Masin, R.; Zuin, M.C.; Zanin, G.; Tridello, G. Weed Turf: Software for improving summer annual weed control in turf. *Ital. J. Agrometeorol.* **2005**, *50*, 46–50.
30. Masin, R.; Loddo, D.; Gasparini, V.; Otto, S.; Zanin, G. Evaluation of Weed Emergence Model AlertInf for Maize in Soybean. *Weed Sci.* **2014**, *62*, 360–369. [CrossRef]
31. Loddo, D.; Ghaderi-Far, F.; Rastegar, Z.; Masin, R. Base temperatures for germination of selected weed species in Iran. *Plant Prot. Sci.* **2018**, *54*, 60–66. [CrossRef]
32. Wiese, A.M.; Binning, L.K. Calculating the Threshold Temperature of Development for Weeds. *Weed Sci.* **1987**, *35*, 177–179. [CrossRef]
33. Guillemin, J.P.; Gardarin, A.; Granger, S.; Reibel, C.; Munier-Jolain, N.; Colbach, N. Assessing potential germination period of weeds with base temperatures and base water potentials. *Weed Res.* **2013**, *53*, 76–87. [CrossRef]
34. Steinmaus, S.J.; Prather, T.S.; Holt, J.S. Estimation of base temperatures for nine weed species. *J. Exp. Bot.* **2000**, *51*, 275–286. [CrossRef]
35. Kottek, M.; Grieser, J.; Beck, C.; Rudolf, B.; Rubel, F. World map of the Koppen-Geiger climate classification updated. *Meteorol. Z.* **2006**, *15*, 259–263. [CrossRef]
36. Zambrano-Navea, C.; Bastida, F.; Gonzalez-Andujar, J.L. A hydrothermal seedling emergence model for *Conyza bonariensis*. *Weed Res.* **2013**, *53*, 213–220. [CrossRef]

37. Vasileiadis, V.P.; Froud-Williams, R.J.; Loddo, D.; Eleftherohorinos, I.G. Emergence dynamics of barnyardgrass and jimsonweed from two depths when switching from conventional to reduced and no-till conditions. *Span. J. Agric.* **2016**, *14*, e1002. [CrossRef]
38. Werle, R.; Sandell, L.D.; Buhler, D.D.; Hartzler, R.G.; Lindquist, J.L. Predicting emergence of 23 summer annual weed species. *Weed Sci.* **2014**, *62*, 267–279. [CrossRef]
39. Bagavathiannan, M.V.; Norsworthy, J.K.; Smith, K.L.; Burgos, N. Seedbank Size and Emergence Pattern of Barnyardgrass (*Echinochloa crusgalli*) in Arkansas. *Weed Sci.* **2011**, *59*, 359–365. [CrossRef]
40. Ramanarayanan, T.S.; Williams, J.R.; Dugas, W.A.; Hauck, L.M.; McFarland, A.M.S. *Using APEX to Identify Alternative Practices for Animal Waste Management. Part I: Model Description and Validation*; ASAE Paper No. 972209; American Society of Agricultural Engineers: St. Joseph, MI, USA, 1997.
41. Egea-Cobrero, V.; Bradley, K.; Calha, I.M.; Davis, A.S.; Dorado, J.; Forcella, F.; Lindquist, J.L.; Sprague, C.L.; Gonzalez-Andujar, J.L. Validation of predictive empirical weed emergence models of *Abutilon theophrasti* Medik based on intercontinental data. *Weed Res.* **2020**, *60*, 297–302. [CrossRef]
42. Myers, M.W.; Curran, W.S.; VanGessel, M.J.; Calvin, D.D.; Mortensen, D.A.; Majek, B.A. Predicting weed emergence for eight annual species in the northeastern United States. *Weed Sci.* **2004**, *52*, 913–919. [CrossRef]
43. Oriade, C.; Forcella, F. Maximizing efficacy and economics of mechanical weed control in row crops through forecasts of weed emergence. *J. Crop Prod.* **1999**, *2*, 189–205. [CrossRef]
44. Otto, S.; Masin, R.; Casari, G.; Zanin, G. Weed–Corn Competition Parameters in Late-Winter Sowing in Northern Italy. *Weed Sci.* **2009**, *57*, 194–201. [CrossRef]
45. Loddo, D.; Scarabel, L.; Sattin, M.; Pederzoli, A.; Morsiani, C.; Canestrale, R.; Tommasini, M.G. Combination of herbicide band application and inter-row cultivation provides sustainable weed control in maize. *Agronomy* **2020**, *10*, 20. [CrossRef]