

Comparing the emergence of *Echinochloa crus-galli* populations in different locations. Part I: Variations in emergence timing and behaviour of two populations

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Abstract

Echinochloa crus-galli (L.) P. Beauv. is one of the most important weeds. It is distributed worldwide and has adapted to diverse habitats and climatic conditions. This study aimed to compare the emergence patterns of two populations of *E. crus-galli* from different environments at 11 locations across Europe and the Middle East. Seeds of the two populations were collected from maize in Italy and from spring barley in Norway and were then buried in soil in autumn 2015. In the spring of 2016, the soil was disturbed around the usual seedbed preparation date in each location and emergence was recorded. The soil was again disturbed a year later and emergence was recorded for a second season. Total emergence, the times of onset, end and to 50% emergence and the period between 25% and 75% of emergence were analysed by

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two-way ANOVA and principal components analysis. The Italian population showed a higher emergence than the Norwegian population in Southern locations, while the ranking was reversed in Northern locations. In almost all locations, a tendency to emerge earlier was recorded for the Norwegian population, but the periods from 25% to 75% emergence were similar for both populations. Total emergence, and the times of onset and end of emergence seemed to be mainly under genotypic (plus maternal) control, suggesting there were different temperature thresholds for seedling emergence in each population. Conversely, the duration of emergence seemed to be mainly under environmental control. This research confirms the high variability between populations and suggests the need to continue identifying key characteristics for the development of efficient models for seedling emergence in specific climates and/or latitudes.

KEYWORDS

barnyard grass, climate change, temperature thresholds

1 | INTRODUCTION

Echinochloa crus-galli (L.) P. Beauv. is one of the most harmful weed species worldwide (Bajwa et al., 2015). Its seeds germinate over a wide range of temperatures (Masin et al., 2014) and humidity levels (Maun and Barrett, 1986), as long as a soil moisture content above 35% is ensured (Rahn et al., 1968). Because of this ecological plasticity and adaptability, this weed has invaded a large number of contrasting crops and climatic conditions (Holm et al., 1991). Therefore, *E. crus-galli* is one of the most widely distributed naturalised species worldwide (Pysek et al., 2017), including irrigated crops in the Mediterranean area and rainfed crops in temperate climates. Being a C_4 species, its competitiveness is greater than that of C_3 summer crops, like rice or soybean (Bagavathiannan et al., 2011), and it has become one of the most important weeds in rice (Weeraratne et al., 2015). It is also widespread in maize (Golebiowska and Kieloch, 2016), in which its occurrence is increasing in Central Europe (Keller et al., 2014).

Climate change is also modifying weed distributions and some harmful weeds are progressively moving to higher latitudes (Peters et al., 2014). Indeed, as winter periods become shorter, conditions are becoming more favourable for C_4 weeds due to a longer growing season at these latitudes (Peters and Gerowitt, 2015). At the highest latitudes, such as in the Scandinavian and Baltic regions, the spread of *E. crus-galli* will mainly increase due to the rise of temperatures (Peters and Gerowitt, 2015), because the increase in length of the growing season may be constrained by the photoperiod. According to Holm et al. (1991), *E. crus-galli* flowers in 8 to 13-h photoperiods, and at the highest latitudes, such conditions are only found before and shortly after the spring equinox, when temperatures would be too low for its germination. *E. crus-galli* is, however, already infesting lower latitudes, and problems with herbicide-resistant biotypes are now occurring in several crops and countries (Heap, 2019). Thus, the

control of this weed is crucial for optimising crop productivity (Bajwa et al., 2015).

Knowledge about the emergence of weeds is essential for effective weed management (Masin et al., 2014). For this reason, the emergence of *E. crus-galli* has been widely studied. Bagavathiannan et al. (2011) observed a prolonged period of emergence that varied from site to site in Arkansas (USA). These authors developed three models for describing *E. crus-galli* emergence based on time, growing degree days (GDD) and hydrothermal time (HTT), with no significant differences in accuracy among them. In Europe, a model that predicts the emergence of *E. crus-galli* in maize and soybean is also available (Masin et al., 2014; Šoštarčić et al., 2021).

The GDD and HTT concepts assume that emergence patterns change according to the environmental conditions and the 'internal' characteristics of seed populations, such as the base temperature (T_b) and base water potential. In consequence, if a given population is put in different environmental conditions, different emergence patterns will be observed. Likewise, if two populations of the same plant species from different environments and with different thermal time characteristics are put in the same environmental conditions, different emergence patterns should also be observed. This inference has been confirmed in *E. crus-galli* by the different T_b levels of 11.7°C and 9.7°C in Central and Northern Italy (Masin et al., 2014) and Arkansas (Bagavathiannan et al., 2011), respectively. Other estimates of T_b include 5°C (Sadeghloo et al., 2013) and 13.3°C (Loddo et al., 2018) in Iran, 10.8°C in Croatia (Šoštarčić et al., 2021) and 6.2°C in France (Guillemin et al., 2013).

It is expected that different populations of *E. crus-galli* will arise as they adapt to specific agro-ecologies. Therefore, populations from contrasting climatic areas and/or farming systems could show different emergence patterns. If so, emergence models for weed management developed for the species would probably need to be parameterised for each local population. However, this hypothesis

has seldom been tested, *Chenopodium album* and *Stellaria media* being notable exceptions (Grundy et al., 2003). Most studies of weed seed ecology are limited to one or only a few small populations, originating from similar environments.

Based on this background, a common experiment was set up by the working group 'Germination and Early Growth' of the European Weed Research Society. The objective was to increase the knowledge of the emergence timing and behaviour of two populations of *E. crus-galli* originating from Italy and Norway across different climatic conditions in order to understand which emergence traits, such as the onset, duration and end of emergence, and time to 50% emergence, may be driven by population characteristics or by environmental conditions.

2 | MATERIALS AND METHODS

2.1 | Seed collection

Seeds of two populations of *E. crus-galli* were collected between July and September 2015, one in Padova (Italy, 45°20'N, 11°58'E) and the second in Svarstad (Norway, 59°24'N, 9°58'E). The two 'common' populations were distributed to all participants in the study and are hereafter referred to as IT and NO, respectively. In addition, up to three local populations were collected at each location (Table 1). This paper focusses on the results obtained with the two common populations, while the similarities and differences in emergence patterns of the local populations will be reported elsewhere (Royo-Esnal et al., 2022).

To ensure the natural variability within each population was sampled, seeds were collected at maturity from at least 20 plants in single fields of maize in Italy and spring barley in Norway. Maturity was defined as occurring when the seeds shed easily upon shaking

the spikelets. Immediately after harvest, seed samples were dried at room temperature (20–25°C) for one week by laying the seeds in a thin layer on trays. Exposure to direct sunlight and high humidity during this process was avoided. Seeds were then cleaned by sieving; unwanted chaff being removed by rubbing the seeds followed by cleaning using a seed blower. The two populations were sent to HerbiSeed in the UK which prepared phytosanitary certificates and then immediately despatched sub-samples to all participants. Sub-samples were also sent to the Seed Science Laboratory at the University of Reading, where seed characterisation was performed. Seeds were stored in airtight containers and placed in a refrigerator at 3 to 5°C until ready for use.

2.2 | Germination test

For the seed characterisation, the weight of 1000 seeds was obtained. Because the field experiment started with an autumn sowing, so that seeds over-wintered in the soil before spring emergence, the characterisation experiment focussed on the effect of chilling on subsequent germination. Three periods of chilling (0, 4 and 14 days at 4°C) were tested with two replicates for each seed lot. Each replication consisted of 50 seeds which were placed in 9-cm diameter polystyrene Petri dishes containing two 9-cm diameter circles of Whatman 181 Seed Testing Paper moistened by 4.5 ml deionised water. Petri dishes were placed in a polythene bag and the 4- and 14-days treatments were placed in the cold room for the required chilling period. After chilling, all Petri dishes were moved into an incubator for germination in an alternating temperature regime of 10°/20°C (12 h/12 h). After 14 days, an intermediate germination count was carried out considering 2 mm root growth as the germination criterion. The germinated seeds were removed and their number was recorded. The final count took place 14 days later using the same germination criterion.

TABLE 1 Locations of the experiments, coordinates, altitude and climatic classification from Köppen. Soil characteristics of the locations are also provided, including structure (percentage of sand, silt and clay), organic matter content (OM), and pH

Country	Location	Latitude	Longitude	Altitude (m)	Climate ^a	Sand (%)	Silt (%)	Clay (%)	OM (%)	pH
Sweden	Uppsala	59°49'N	17°39'E	11	Dfb	62.0	31.0	8.0	7.0	7.3
Norway	Ås	59°40'N	10°46'E	90	Dfb	48.5	31.5	19.5	4.2	6.2
Latvia	Carnikava	57°05'N	24°12'E	2	Dfb	52.0	35.0	13.0	2.4	5.8
Denmark	Aarhus	55°19'N	11°24'E	20	Cfb	73.7	12.8	12.4	0.6	7.2
Poland	Kraków	50°05'N	19°52'E	230	Dfb	39.0	52.0	9.0	5.6	6.8
Italy	Padova	45°20'N	11°58'E	15	Cfa	16.0	64.9	19.1	1.8	8.0
Spain	Lleida	41°37'N	0°35'E	180	Csa	34.3	46.3	19.4	2.1	7.4
Turkey (North)	Düzce	40°50'N	31°09'E	196	Cfb	60.6	7.0	32.4	–	–
Portugal	Oeiras	38°42'N	9°18'W	33	Csa	55.7	18.9	25.4	0.8	8.0
Turkey (South)	Antakya	36°16'N	36°14'E	85	Csa	38.3	20.4	41.2	0.6	7.4
Iran	Ilam	33°39'N	46°23'E	1319	Csa	45.0	20.0	35.0	2.0	7.3

^aClimate classification according to the updated Köppen-Geiger classification (Kottek et al., 2006): Cfa, Temperate with no dry season, hot summer; Cfb, Temperate with no dry season, warm summer; Csa, Temperate with dry and hot summer (warm Mediterranean); Dfb, Cold with no dry season and warm summer (continental).

2.3 | Field experiment

Field experiments were set up in 10 countries (Denmark, Iran, Italy, Latvia, Norway, Poland, Portugal, Spain, Sweden and Turkey) with two locations in Turkey (North and South), encompassing a wide variation in latitude and climate (Kottek et al., 2006) (Table 1). Emergence assays were performed in pots with five replicate pots per population. Pots had an upper diameter of 25 cm and an extra hole through 10% of its bottom area in order to ensure good contact with the soil outside the pots. The typical soil of each location was used to fill the pots (Table 1) after sieving to remove stones. Pots were filled up to 1 cm below the rim and were buried in the ground to the appropriate depth, so that this 1 cm of the pot extended above the ground.

Seeds were incorporated into the upper 5 cm of the soil in each pot in autumn 2015, the actual dates ranging from early October to November, depending on the location. To incorporate the seeds, the upper 5-cm layer of soil was removed from each pot and carefully mixed by hand with 200 seeds. The soil was then replaced in the pot. Six control pots were included in which the soil was removed and replaced as above, but without adding the seeds. A temperature logger (HOBO Pendant®, Onset Computer Corporation) was buried horizontally 2.5 cm below the soil surface in one of the control pots, while the other five were used to check for possible contamination of *E. crus-galli* seeds in the local soils.

In spring 2016, the soil was disturbed to a depth of 5 cm at the time of the typical sowing date for the crops where *E. crus-galli* is found in each location (Table 2). The method of disturbance was similar to the autumn sowing (removal of the upper 5 cm, mixing in a tray and replacing in the pots) except that an NPK fertilizer, at a rate of 50 kg N ha⁻¹, was incorporated during mixing.

Daily maximum and minimum air temperatures and precipitation data were obtained from the nearest weather station in each location. The pots were rainfed throughout the experiment except in Iran, Italy, Spain and Turkey South, where supplementary irrigation was applied at the same times as in adjacent fields at those locations. All irrigation treatments were recorded.

Emergence was recorded non-destructively at weekly intervals until the soil was disturbed in the spring or the first seedlings emerged, after which the main emergence flush started and seedlings were counted every 2 to 3 days, until this flush finished, depending on the location, between late spring and early summer (June–July). Thereafter weekly counts continued throughout the summer. Newly emerged seedlings were marked with metallic wires to avoid double counting. After the main emergence flush, seedlings (and wires) were carefully removed, except for three individuals in each pot that were allowed to grow to maturity. The soil was disturbed again in autumn 2016 when tillage was being carried out in the locality. Any seedlings emerging from September onward were counted, but were not considered for analysis because they are supposed to be killed in the autumn disturbance.

During the 2016–17 season, emergence was followed again at some locations to see whether seeds that remained dormant during the first season emerged in the second season. Disturbance and

fertilisation were repeated in spring 2017 and the emergence counts continued until autumn in the same way as in 2016.

The number of viable seeds remaining in the soil in autumn 2017 was estimated by removing the upper 5 cm layer of soil, and spreading it out in trays in a greenhouse. The emergence of seedlings over a 2 or 3-month period was assessed.

2.4 | Statistical analyses

In the seed characterisation test, because one population did not germinate, the final percentage germination of the remaining population was analysed by one-way ANOVA, considering the stratification period as a factor. For buried seeds, the total percentage of emerged seedlings from disturbance until the end of July (% of sown seeds, E_{tot}) in each pot was subject to two-way ANOVA with location (11 levels), population (2 levels) and their interaction as the experimental factors. In both cases, data were square root transformed prior to analyses in order to satisfy the basic assumptions of normality and homoscedasticity of residuals. Back-transformed means and standard deviations are reported in tables. Means on the back-transformed scale were compared using a generalised multiple comparison procedure, with multiplicity adjustment (Bretz et al., 2011).

As a second step, the emergence from the time of soil disturbance in spring 2016 until the corresponding end of the main emergence flush in each location, and no longer than end of July, was considered as a single cohort. The median time to emergence and the times to emergence for the 25th and 75th percentiles were calculated on a GDD, as follows:

$$GDD = \sum (T_i - T_b)$$

where T_i is the mean soil temperature at day i after disturbance, and T_b is the base temperature for emergence. If T_i is lower than T_b , then $T_i - T_b = 0$. Due to the variation of the T_b observed in literature (Bagavathiannan et al., 2011; Guillemin et al., 2013; Loddo et al., 2018; Masin et al., 2014; Sadeghloo et al., 2013), a typical value of 10°C was considered as a first approximation for this work.

Times (t) to emergence in GDD of a selected percentile, g (i.e., 25, 50 or 75) were calculated as follows:

$$t_g = t_1 + \left(\frac{Ng}{100} - N_1 \right) \frac{t_2 - t_1}{N_2 - N_1}$$

where N is the total number of emerged seedlings in the pot, t_1 is the last inspection time in GDD when the cumulative count of emerged seedlings was lower than $Ng/100$, t_2 is the first inspection time in GDD when the cumulative count of emerged seedlings is higher than $Ng/100$, N_1 is the cumulative count at t_1 and N_2 is the cumulative count at t_2 .

The median emergence time (T_{50}) was taken as a measure of emergence 'speed', while T_{25} and T_{75} were used to calculate the interquartile range (IR) and used as a measure of the duration of

TABLE 2 Dates of sowing (autumn 2015) and soil disturbance (spring 2016) and the emergence periods from first to final (100%) emergence for the Italian (IT) and Norwegian (NO) seed populations in each experimental location

Country	Location	Sowing (2015)	Disturbance (2016)	Emergence period					
				IT			NO		
Sweden	Uppsala	28/10	11/05	18/05	–	13/07	18/05	–	13/07
Norway	Ås	23/10	26/04	26/05	–	07/08	16/05	–	20/07
Latvia	Carnikava	27/10	13/04	05/05	–	29/08	12/05	–	29/08
Denmark	Aarhus	11/11	03/05	12/05	–	07/07	11/05	–	07/07
Poland	Krakow	26/10	05/04	25/04	–	27/06	15/04	–	13/06
Italy	Padova	27/10	29/03	29/03	–	22/06	27/03	–	17/05
Spain	Lleida	28/10	24/03	24/03	–	07/06	24/03	–	06/05
Turkey (North)	Düzce	23/10	12/04	^a 23/11/2015	–	02/09	^a 02/11/2015	–	02/09
Portugal	Oeiras	11/11	08/04	^a 16/12/2015	–	06/06	^a 16/12/2015	–	20/05
Turkey (South)	Antakya	23/11	05/03	18/03	–	18/07	28/03	–	04/07
Iran	Ilam	15/11	20/04	01/05	–	17/06	20/04	–	09/05

^aEmergence started prior to the disturbance of the soil in spring.

emergence, that is, the narrower the interquartile range, the more synchronous the seedling emergence.

E_{tot} , T_{50} and IR were used to distinguish populations and locations using principal component analysis. Data were standardised to zero mean and unit variance prior to principal components analysis and the first two principal components were displayed on a distance biplot (row-metrics preserving; Legendre and Legendre, 2012).

3 | RESULTS

3.1 | Seed characterisation

The weight of 1000 seeds of the IT population was lower (1.44 g) than that of the NO population (2.30 g). Seeds of the IT population did not germinate in any stratification treatment. By contrast, germination of the NO population increased with the period of stratification at 4°C (60.7%, 61.0% and 71.6%, respectively for 0, 4 and 14 days), the difference between 4 and 14 days being significant.

3.2 | Climatic conditions

Climatic conditions differed among locations mainly with respect to monthly temperature. Norway had the coldest winter month (−5.9°C in January 2016) and the coolest summer month (17.3°C), while Lleida (Spain) and Antakya (South Turkey) experienced the highest summer month temperatures, with 35.6°C (July 2016) and 33.3°C (July 2017), respectively (Figure 1). The difference between the coldest and hottest months of the year was also greatest in these two locations, with a range of 28.0°C in Lleida and 28.5°C in Antakya compared with a range of only 8.9°C in Oeiras (Portugal).

3.3 | Emergence season

In most locations, emergence began in spring 2016, an average of 11.5 days after soil disturbance, but with very wide variability across locations (ranging between 1 and 30 days). On average, IT seedlings started to emerge 12.4 days after soil disturbance, while NO seedlings started in 10.6 days from disturbance, although the difference was not statistically significant ($p = 0.71$). The emergence behaviour was different in Portugal and Turkey (North), because both populations started emerging in autumn 2015, immediately after sowing (Table 2). Emergence continued until late spring/early summer in Iran, Italy, Poland, Portugal and Spain, which showed the highest summer temperatures. In other locations (Latvia, Norway, Sweden and Turkey) emergence continued until late summer/early autumn (Table 2).

On average, the duration of emergence was slightly and non-significantly longer in the IT population than with the NO population (respectively, 111 and 101 days from early to late emergence; $p = 0.77$). For the IT population, the emergence season ranged from 40 to 50 days (Denmark, Sweden, Iran) to more than 200 days (Portugal and Turkey). The NO population showed lower values, being lowest in Iran, where the emergence season only lasted for 19 days (Table 2). In general, the duration of emergence showed a higher variability across locations, than between the populations at each location.

3.4 | Total emergence

The total percentage of emerged plants (E_{tot}) was significantly affected by the 'population × location' interaction, meaning that the two populations had different emergence behaviours in the different locations. This was confirmed by the variance component for

FIGURE 1 Mean monthly soil temperatures at sites used for emergence study of *Echinochloa crus-galli* seeds collected from two populations

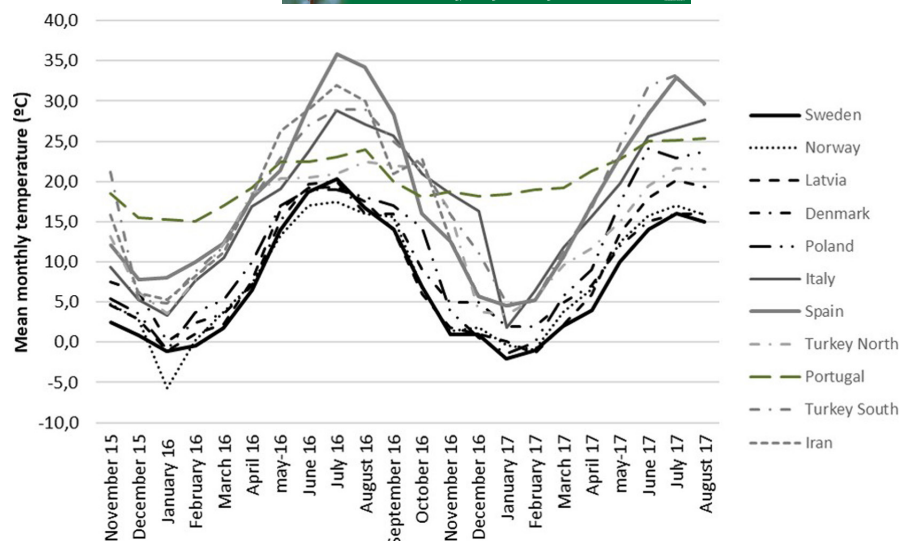


TABLE 3 Average total percentage emergence (E_{tot}) of the Italian (IT) and Norwegian (NO) seed populations in each location during the first season (2015/2016) from the first observed seedling in the spring until the end of July. The standard deviation (SD) is provided in brackets. Different letters denote significant differences at $p < 0.05$; capital letters are for the comparisons across locations within each population, small letters are for the comparisons across populations within each location. The 'population $\times$ location' interaction was significant ($p < 0.0001$)

Location	IT		NO	
Sweden	28.3 (4.07)	DE-b	56.1 (5.73)	BC-a
Norway	24.8 (3.81)	EF-b	52.9 (5.57)	BC-a
Latvia	25.0 (3.83)	EF-b	47.2 (5.26)	CD-a
Denmark	18.0 (3.25)	FG-b	36.5 (4.63)	D-a
Poland	21.4 (3.54)	EF-b	62.5 (6.05)	B-a
Italy	23.2 (3.69)	EF-a	6.8 (2.00)	E-b
Spain	36.3 (4.61)	CD-a	10.3 (2.46)	E-b
Turkey North	64.3 (6.14) ^a	A-a	87.3 (7.15) ^a	A-a
Portugal	12.6 (2.72) ^a	G-b	48.9 (5.35) ^a	BCD-a
Turkey South	53.8 (5.62)	AB-a	52.5 (5.55)	BC-a
Iran	42.7 (5.00)	BC-a	1.2 (0.84)	F-b
Mean	31.1 (15.40)		39.3 (26.80)	

^aEmergence started in autumn 2015, prior to the spring 2016 soil disturbance.

'population $\times$ location' interaction, which was much higher than the variance component for the 'location' effect (3.47 and 0.44, respectively).

On average, IT had lower but less variable emergence over locations (31.1%, SD = 15.4) with respect to NO (39.3%, SD = 26.80) (Table 3). More IT seedlings emerged in southern latitudes (e.g. Iran, Italy, Spain; 34%, on average) compared with northern latitudes (e.g. Denmark, Latvia, Norway, Poland, Sweden; 23.5%, on average). Conversely, NO showed much higher emergence in northern (51.1%, on average) than in southern latitudes (6.1% on average). The differences between populations were not, however, significant in

TABLE 4 Average total percentage emergence (E_{tot}) of the Italian (IT) and Norwegian (NO) seed populations in each location in the 2016/2017 season, from disturbance of the soil in spring (2017) to the end of July; emergence after the upper 5 cm layer of soil in each pot was put in a greenhouse at each location in 2018; and the average percentage of seeds for each population that were unaccounted for by emergence in the field or greenhouse after 2.5 years

Location	2016/2017 (E_{tot})		Greenhouse 2018		Not emerged ^a	
	IT	NO	IT	NO	IT	NO
Norway	1.9	3.2	1.6	1.8	71.7	42.1
Latvia	11.6	5.1	1.5	1.4	61.9	46.3
Poland	2.1	0.8	0.0	0.0	76.5	36.7
Spain	0.2	0.1	0.1	0.6	63.0	88.3
Turkey South	37.4	39.8	0.0	0.0	7.8	6.6
Iran	0.4	0.4	0.0	0.0	68.5	60.3

^aIt is unknown if the seeds which failed to emerge had lost viability, suffered fatal germination or predation or were still dormant.

Denmark, Latvia and Turkey (North and South). The highest emergence was reached by NO in Turkey North (87.3%), Poland (62.5%), Sweden (56.1%), Norway (52.9%) and Latvia (47.2%); IT reached its highest emergence in Turkey North (64.3%), Turkey South (53.8%) and Iran (42.7%).

Although in most locations, emergence occurred after the soil was disturbed in spring (Table 2), in Portugal, most emergence of NO (48%) occurred in the autumn, prior to disturbance, with only 0.9% emergence in the spring. By contrast for IT, autumn emergence in Portugal was 0.4% compared with 12.2% in the spring. In Turkey North, emergence prior to disturbance was also considerable, with 64.5% and 30% respectively for NO and IT, but spring emergence was also relatively high, with 23.3% (NO) and 35.2% (IT). In some locations (Spain, Turkey North and South), some seedlings emerged in autumn 2016 1 year after sowing, although numbers were small ranging between 0.5 and 2.8% of sown seeds.

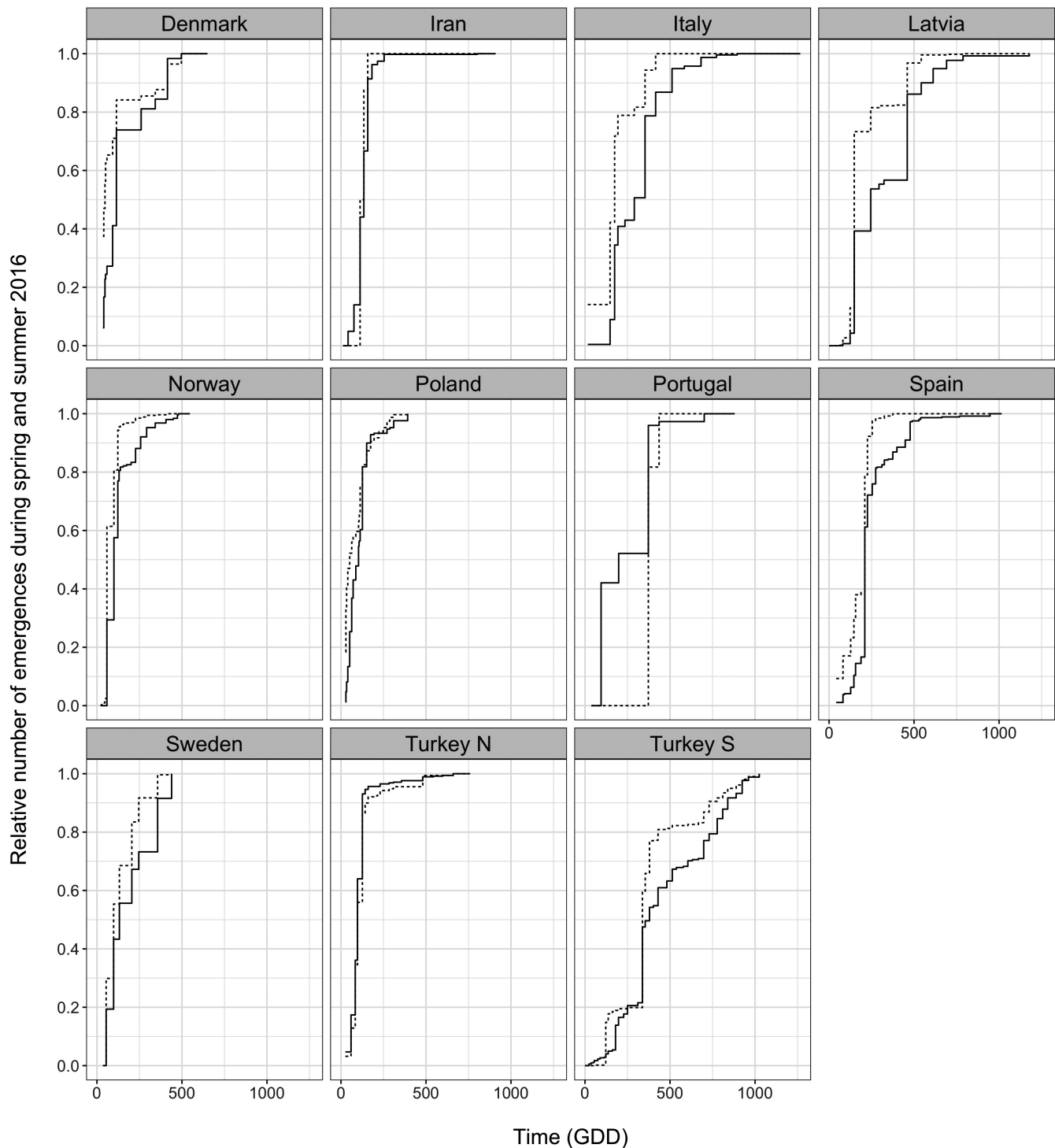


FIGURE 2 Time-course for the main flush of seedling emergence of the two populations of *Echinochloa crus-galli*. Seeds were collected in Italy (solid lines) and Norway (dashed lines) during summer 2015, and sown in pots in autumn 2015. The soil in the pots was disturbed in spring 2016, and zero on the time scale is the date of disturbance. The main emergence flush occurred thereafter. The length of the lines corresponds to the observation period in each location, until the end of the main emergence flush and no more than end of July

The E_{tot} during the second season was lower than in the first season in all locations, with values varying between 0.1 and 11.1% for Latvia, Poland and Spain, while it was higher in Turkey South (28.8–43.7%; Table 4). When soil samples were tested in the greenhouse after the 2017 growing season, less than 2% emergence was

observed in Latvia, Norway and Spain, while no seedlings emerged in Poland, Iran and Turkey South (Table 4). In these locations, the proportion of non-emerged seeds was higher for IT than NO, except in Spain (Table 4). The portion of non-emerged seeds varied between 61.9% (Latvia) and 76.5% (Poland) for IT and from 36.7%

TABLE 5 Growing degree days (GDD, °C d) for the median emergence time (T_{50}) and the duration of emergence (the interquartile period $T_{75}-T_{25}$) from disturbance of the soil in spring until the end of July, assuming $T_b = 10^{\circ}\text{C}$, for the Italian (IT) and Norwegian (NO) populations at each location. Different letters show significant differences ($p < 0.05$); capital letters are for the comparisons across locations within each population, small letters are for the comparisons across populations within each location. The 'population $\times$ location' interaction was significant ($p < 0.0001$)

Location	IT		NO	
GDD to the median emergence time (T_{50})				
Sweden	127.4	AC-a	91.7	ABD-a
Norway	84.8	A-a	55.3	AB-a
Latvia	249.8	B-a	138.7	ACD-b
Denmark	95.3	A-a	44.7	B-a
Poland	81.4	A-a	40.3	B-a
Italy	252.3	B-a	153.4	CD-b
Spain	204.5	BC-a	183.0	C-a
Turkey North	90.5	A-a	94.7	ABCD-a
Portugal	152.2	AC-a	155.3	CD-a
Turkey South	353.3	D-a	299.9	E-a
Iran	117.8	AC-a	60.5	AB-b
Mean	165.1		116.9	
SD	88.5		76.9	
GDD for duration of emergence ($T_{75}-T_{25}$)				
Sweden	184.9	CD-a	120.4	A-a
Norway	81.6	ABC-a	40.1	A-a
Latvia	259.0	D-a	53.8	A-b
Denmark	110.0	ABC-a	94.3	A-a
Poland	71.6	ABC-a	86.4	A-a
Italy	179.6	BCD-a	120.4	A-a
Spain	60.4	AB-a	86.0	A-a
Turkey North	39.3	A-a	42.9	A-a
Portugal	191.7	CD-a	155.3	AB-a
Turkey South	434.9	E-a	256.2	B-b
Iran	52.4	A-a	47.0	A-a
Mean	147.7		97.4	
SD	120.9		67.5	

(Poland) to 88.3% (Spain) for NO, except in Turkey South, where the proportions were much lower (7.8 and 6.6% for IT and NO, respectively).

3.5 | Time course of emergence (main flush)

The time course of emergence shows emergence relative to the total number of emerged seedlings during this period (Figure 2). The

median emergence periods (T_{50}) and the differences between T_{75} and T_{25} (IR) were calculated (Table 5).

Very high and significant differences in T_{50} were observed between the locations (Table 5), while the differences between populations within each location were generally not significant except in Latvia, Italy and Iran. A similar situation can be seen for the IR, which differed between but not within locations except in Latvia and Turkey South. Although NO appeared to show faster emergence ($T_{50} = 116.9$ GDD) and had a shorter IR (97.4 GDD) than IT (165.1 GDD and 147.7 GDD), respectively, these differences were not significant, due to high SD values.

Principal component analysis of total emergence, T_{50} and IR showed that with a few exceptions, the populations were well discriminated, mainly based on differences in the total numbers of emerged seedlings (Figure 3). Within each population, locations show an association such that T_{50} and IR were inversely correlated so that a low T_{50} was associated with a long IR.

4 | DISCUSSION

The *E. crus-galli* populations behaved differently under the various climatic conditions to which they were subjected. IT had higher emergence in southern latitudes, where the soil was disturbed between March and April, whereas NO emerged more abundantly in northern latitudes, where disturbance generally occurred in May (Tables 1 and 3). However, some results deviated from this general pattern. Thus, the NO population showed higher emergence in Portugal and Turkey North (48% and 70%, respectively) where it mostly emerged in the autumn rather than after the soil was disturbed in the spring (0.9% and 17%). The autumn emergence may be accounted for by the warmer autumn–winter temperatures, which were similar to spring temperatures in northern locations. For both Portugal and Turkey North, the emergence of IT in the spring was higher than NO (10% > 0.9% and 50% > 17%, respectively), but it was, nonetheless, lower than that observed for NO during the whole autumn–winter and spring periods. This result implies that *E. crus-galli* seeds were in conditional dormancy when dispersed in autumn (Baskin and Baskin, 2014) and that NO had less primary dormancy, as was confirmed by the seed characterisation; its seeds were, therefore, able to germinate (and hence emerge) already in autumn after sowing, provided they were exposed to favourable temperatures and adequate soil moisture. Conversely, the IT seeds, which did not even germinate in incubators, had much deeper primary dormancy that appeared to require a long period of after-ripening or stratification in the soil over winter. Therefore, its emergence was negligible in the autumn after sowing, even with favourable environmental conditions. The presence of a high level of primary dormancy at the time of seed dispersal for weed populations originating from locations with relatively mild winters has already been described for other species, such as *Datura stramonium* and *Chenopodium album* (Loddo et al., 2014; Murdoch et al., 2010). This trait can be regarded as an

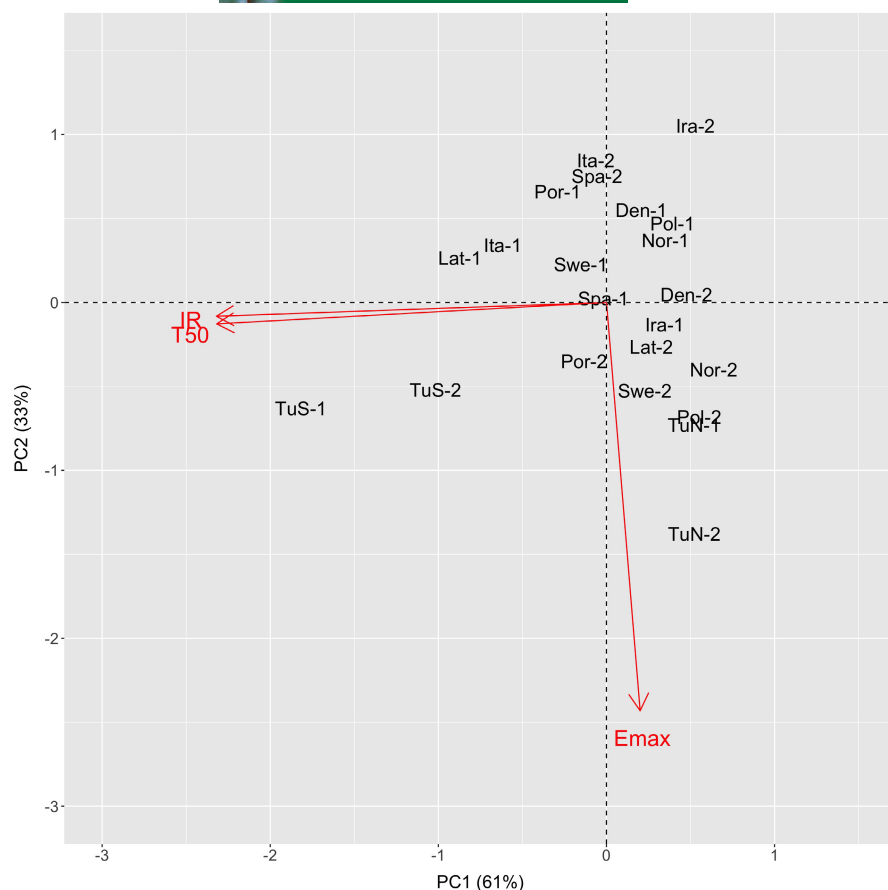


FIGURE 3 Biplot from principal components analysis (PCA) where the main emergence features for each population of *Echinochloa crus-galli* and site are analysed. Emax, total emergence; T50, time to 50% emergence, IR, interquartile range or time from T25 to T75. Den, Denmark; Ira, Iran; Ita, Italy; Lat, Latvia; Nor, Norway; Pol, Poland; Por, Portugal; Spa, Spain; Swe, Sweden; TuN, Turkey North; TuS, Turkey South. The numbers 1 and 2 denote the common populations from Italy and Norway, respectively. The percentage variance accounted for by each axis is shown

ecological adaptation by which germination in unfavourable periods for plant establishment and growth is prevented.

In six locations, NO started emerging before IT in the spring, in two, IT preceded NO while in the remaining three, they coincided. Similarly, emergence ceased earlier in NO than in IT in seven locations, and in the other four locations, the last seedlings emerged at the same date. Thus, there was a tendency for the onset and the end of the emergence period to differ between these two populations (Table 3 and Figure 2), but more evidence is needed to confirm this behaviour. Such differences could be under both genotypic and environmental control; the adaptation to the contrasting climates of Italy and Norway may have induced differences in the temperature thresholds for dormancy breaking, germination, emergence and dormancy induction. Genetic variation is known to be an important factor influencing seed dormancy of *E. crus-galli*, even within a geographically restricted area (Honek and Martinkova, 1996). Thus, the different populations may have adapted to their respective maturation environments so that differences in thermal-time parameters including the base, optimum and ceiling temperatures and in the GDD required for the germination and emergence of non-dormant seeds, would not be unexpected. Similar effects were previously hypothesised by Cochrane et al. (2014), who reported differences in the upper but not in the lower temperature thresholds for germination for four *Banksia* species, as being a factor determining their adaptation to climate change. On the other hand, the growing conditions in the

year of collection and especially during the period of maturation of the NO and IT populations will have influenced seed development, primary dormancy (Menegat et al., 2018) and germination requirements. In the present study, it is not possible to distinguish genotypic and maternal effects, and thus, genotype and maternal factors should be considered together in interpreting the behaviour of the two populations.

Despite the differences between the populations, once emergence had started, the flushes seemed to be mainly under environmental control, as the periods between the onset and the end of emergence and its duration (IR) were similar for both populations in the same conditions. In addition, the median times to emergence (T_{50}) of the two populations differed only in Latvia, Italy and Iran (Table 5), while the durations of emergence (IR) were generally similar except in Latvia and Turkey South. The generally lower duration of emergence of NO might be due to lower ceiling temperatures, which is supported by an earlier date for the last emergence (Table 2). This would have allowed more time for IT to emerge after the spring disturbance than NO, mainly in southern countries. Because the emergence flushes were recorded after soil disturbance in the spring, the date of disturbance may also have played a role in the variability of T_{50} and IR. Indeed, the disturbance date was different across locations, as it was selected according to the usual date of seedbed preparation in each location.

These results show that a common emergence model for all populations of *E. crus-galli* may be difficult to develop: the two populations reacted differently to the same environmental conditions. The need

to determine model parameters for different populations and environments is supported by differences in T_b which have been found for different locations/populations (Bagavathiannan et al., 2011; Guillemin et al., 2013; Loddo et al., 2018; Masin et al., 2014; Sadeghloo et al., 2013; Šoštarčić et al., 2021). Among these values, the estimated T_b for *E. crus-galli* in Central and Northern Italy (11.7°C) may be appropriate for our IT population, although this needs to be confirmed. On the other hand, an appropriate estimate of T_b is not available in the literature for Norway and should be estimated by future research. Differences in threshold temperatures, such as lower T_b values for populations coming from colder climates, have been also reported for other species (Loddo et al., 2013; Valencia-Gredilla et al., 2020).

The adaptability of *E. crus-galli* to different climates and habitats is well known (Roy et al., 2000). The weed has always been associated with summer crops, implying a certain thermal time requirement for its development. However, considering climate change scenarios, it is moving northward, as Peters et al. (2014) has argued for other C_4 species, so that it is not surprising that *E. crus-galli* is starting to become a serious problem in countries like Norway and Sweden (VKM, 2016; personal observation by K.S. Tørresen). These observations are important for weed management because of the high frequency of freezing temperatures, which means that these countries have not traditionally been regarded as providing ideal environments for this species. Clements and Ditomasso (2011) were already aware that this species had the potential to expand beyond the areas in which it was then occurring. This research suggests that such adaptability may result from changes in threshold temperatures for dormancy breaking, germination and emergence. Indeed, a reduction in T_b would allow earlier emergence of populations in northern latitudes, compared with those in southern latitudes. A lower T_b would have allowed higher germination and emergence in this experiment, but it may also have led to the NO emerging in the autumn and winter. Likewise, a lower ceiling temperature for emergence in northern populations would reduce emergence in the middle of summer, when there may be insufficient time for completion of its life cycle in northern latitudes, where temperatures fall rapidly in the autumn. The combination of climate change (warmer temperatures) and high adaptability therefore makes *E. crus-galli* an important weed to be considered also in areas where it is not yet a harmful weed.

5 | CONCLUSIONS

In *E. crus-galli*, the onset and the end of emergence during the year seem to be under population control, and are consequently influenced by genotypic factors, while the duration of emergence would be mainly under environmental control. These differences may relate to variation in the values of thermal time parameters between populations and may partly explain why *E. crus-galli* adapts to different climatic conditions. Due to these differences, parameterisation of emergence models for populations from different latitudes is necessary before they can be recommended for use in devising weed management strategies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All the raw data about germination, emergence or temperature, in any of the experimental location, is available for anybody who might be interested in consulting them.

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REFERENCES

- Bagavathiannan, M.V., Norstworthy, J.K., Smith, K.L. & Burgos, N. (2011) Seedbank size and emergence pattern of Barnyardgrass (*Echinochloa crus-galli*) in Arkansas. *Weed Science*, 59, 359–365.
- Bajwa, A.A., Jabran, K., Shahid, M., Ali, H.H., Chauhan, B.S. & Ehsanullah (2015) Eco-biology and management of *Echinochloa crus-galli*. *Crop Protection*, 75, 151–162.
- Baskin, C.C. & Baskin, J.M. (2014) *Seeds: ecology, biogeography, and evolution of dormancy and germination*. San Diego, CA: Academic Press, p. 1600.
- Bretz, F., Hotorn, T. & Westfall, P. (2011) *Multiple comparisons using R*. Boca Raton, FL: CRC Press.
- Clements, D.R. & Ditomasso, A. (2011) Climate change and weed adaptation: can evolution of invasive plants lead to greater range expansion than forecasted? *Weed Research*, 51, 227–240. <https://doi.org/10.1111/j.1365-3180.2011.00850.x>
- Cochrane, A., Hoyle, G.L., Yates, C.J., Wood, J. & Nicotra, A. (2014) Predicting the impact of increasing temperatures on seed germination among populations of Western Australian *Banksia* (Proteaceae). *Seed Science Research*, 24, 195–205.
- Golebiowska, H. & Kieloch, R. (2016) The competitive ability of *Chenopodium album* and *Echinochloa crus-galli* in maize crops depending on the time of their occurrence or removal. *Acta Agrobotanica*, 69, 1688.

- Grundy, A.C., Peters, N.C.B., Rasmussen, I.A., Hartmann, K.M., Sattin, M. & Andersson, L. (2003) Emergence of *Chenopodium album* and *Stellaria media* of different origins under different climatic conditions. *Weed Research*, 43, 163–176.
- 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, 74–87.
- Heap, I. (2019) The International survey of herbicide resistant weeds. Available at: <http://www.weedscience.org> (last accessed 22 August 2019).
- Holm, L., Doll, J., Holm, E., Pancho, J.V. & Herberger, J.P. (1991) *The world's worst weeds: distribution and biology*. Malabar, FL: Krieger Publishing Company.
- Honek, A. & Martinkova, Z. (1996) Geographic variation in seed dormancy among populations of *Echinochloa crus-galli*. *Oecologia*, 108, 419–423.
- Keller, M., Böhringer, N., Möhring, J., Rueda-Ayala, V., Gutjahr, C. & Gerhards, R. (2014) Long-term changes in weed occurrence, yield and use of herbicides in maize in south-western Germany, with implications for the determination of economic thresholds. *Weed Research*, 54, 457–466.
- Kottek, M., Grieser, J., Beck, C., Rudolf, B. & Rubel, F. (2006) World map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift*, 15, 259–263.
- Legendre, P. & Legendre, L. (2012) *Numerical ecology*. Amsterdam, The Netherlands: Elsevier.
- Loddo, D., Ghaderi-Far, F., Rastegar, Z. & Masin, R. (2018) Base temperature for germination of selected weed species in Iran. *Plant Protection Science*, 54, 60–66.
- Loddo, D., Sousa, E., Masin, R., Calha, I.M., 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, 443–451.
- Loddo, D., Sousa, E., Masin, R., Calha, I.M., Zanin, G., Fernández-Quintanilla, C. & Dorado, J. (2014) Germination response of local Southern European populations of *Datura stramonium* at a range of constant temperatures. *Weed Research*, 54, 356–365.
- 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.
- Maun, M.A. & Barrett, S.C.H. (1986) The biology of Canadian weeds. 77. *Echinochloa crus-galli* (L.) Beauv. *Canadian Journal of Plant Science*, 66, 739–759.
- Menegat, A., Milberg, P., Nilsson, A.T.S., Andersson, L. & Vico, G. (2018) Soil water potential and temperature sum during reproductive growth control seed dormancy in *Alopecurus myosuroides*. *Ecology and Evolution*, 8, 7186–7194.
- Murdoch, A.J., Isik, D., Nicholls, R.A., González-Andújar, J.L., Beloit, D. & Davis, A. (2010) Dormancy and germination of *Chenopodium album* seeds from different latitudes in Europe and North America. In: *Proceedings 15th EWRS Symposium* (ed Bastiaans, L. et al) (12–15 July, Kaposvar, Hungary), 74. Pannonia- Print Ltd., Budapest, Hungary.
- Peters, K., Breitsameter, L. & Gerowitt, B. (2014) Impact of climate change on weeds in agriculture: a review. *Agronomy for Sustainable Development*, 34, 707–721.
- Peters, K. & Gerowitt, B. (2015) Weed growth properties of *Amaranthus retroflexus*, *Echinochloa crus-galli* and *Setaria viridis* as influenced by shifts in the maize cropping season. *Journal of Plant Diseases and Protection*, 122, 49–55.
- Pyšek, P., Pergl, J., Essl, F., Lenzner, B., Dawson, W., Kreft, H. et al. (2017) Naturalized alien flora of the world: species diversity, taxonomic and phylogenetic patterns, geographic distribution and global hotspots of plant invasion. *Preslia*, 89, 203–274.
- Rahn, E.M., Sweet, R.D., Vengris, J. & Dunn, S. (1968) Life history study as related to weed control in the Northeast. 5.-Barnyardgrass. *Agric. Exp. Sta. Univ. Delaware Bull.*, 368, 1–46.
- Roy, S., Simon, J.P. & Lapointe, F.J. (2000) Determination of the origin of the cold-adapted populations of barnyard grass (*Echinochloa crus-galli*) in eastern North America: a total-evidence approach using RAPD DNA and DNA sequences. *Canadian Journal of Botany*, 78, 1505–1513.
- Royo-Esnal, A., Onofri, A., Taab, A., Loddo, D., Necajeva, J. & Uludag, A. (2022) Comparing the emergence of *Echinochloa crus-galli* populations in different locations II: similarities and threshold parameters. *Weed Research*, 62. <https://doi.org/10.1111/WRE.12529>
- Sadeghloo, A., Ashgari, J. & Ghaderi-Far, R. (2013) Seed germination and seedling emergence of velvetleaf (*Abutilon theophrasti*) and barnyardgrass (*Echinochloa crus-galli*). *Planta Daninha*, 31, 259–266.
- 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*, 11, 2072.
- Valencia-Gredilla, F., Supiciche, M.L., Chantre, G.R., Recasens, J. & Royo-Esnal, A. (2020) Germination behaviour of *Conyza bonariensis* to constant and alternate temperatures across different populations. *Annals of Applied Biology*, 176, 36–46.
- VKM. (2016) Risk assessment of cockspur grass (*Echinochloa crus-galli*). Scientific Opinion of the Panel on Plant Health of the Norwegian Scientific Committee for Food Safety, ISBN: 978-82-8259-213-0, Oslo, Norway.
- Weerathne, L.V.Y., Suriyagoda, L.D.B. & Marambe, B. (2015) Barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv.) is less competitive in rice (*Oryza sativa* L.) when phosphorous (P) is applied to deeper layers in P-deficient and moisture-limited soils. *Plant and Soil*, 391, 1–17.

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