Influence of burial depth and soil disturbance on the emergence of common weed species in the Iberian Peninsula

Carlos Sousa-Ortega[1] , Ramon G. Leon[2] , Nuria Lopez-Martinez[3] and Pedro Castro-Valdecantos[4]

[1]Postdoctoral Research Scholar, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA; [2]Professor and University Faculty Scholar, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA; [3]Professor, Departamento de Agronomía, ETSIA, Universidad de Sevilla, Spain and [4]Substitute Professor, Departamento de Agronomía, ETSIA, Universidad de Sevilla, Spain

Abstract

North African knapweed (*Centaurea diluta* Aiton), cornflower (*Centaurea cyanus* L.), corn marigold [*Glebionis segetum* (L.) Fourr.], rigid ryegrass (*Lolium rigidum* Gaudin), and corn poppy (*Papaver rhoeas* L.) are weeds of economic importance in the Iberian Peninsula, particularly due to limited herbicide options for effective control. For this reason, information about their seedling emergence has become critical to develop effective integrated management strategies and better time control actions. The aims of this study were to evaluate the effect of seed burial depth and soil disturbance on the emergence of these weed species. Two pot experiments were carried out to (1) quantify seedling emergence at three burial depths (2, 5, and 9 cm) and (2) characterize seedling emergence in response to different frequencies and timings of soil disturbance. Burial depth limited the emergence of *G. segetum* and *P. rhoeas* at 5 and 9 cm, respectively; while seedling emergence of *C. diluta*, *C. cyanus*, and *L. rigidum* were reduced by 92%, 90%, and 67% at 9 cm compared with 2 cm, respectively. Two or more sequential soil disturbance events increased total seedling emergence of *C. diluta*, *P. rhoeas*, and *G. segetum* compared with single events, while *L. rigidum* did not respond to repeated soil disturbance. These results suggest that turning the soil to bury weed seeds down to 5 cm or deeper would be a very effective method to control *G. segetum* and *P. rhoeas* and moderately effective to control *C. cyanus*. Also, the use of a stale seedbed would have some efficacy to reduce *P. rhoeas* and *C. diluta* weed pressure within the crop. This study illustrates how differences among species in seedling emergence in response to soil depth and disturbance can determine distinct emergence patterns ultimately influencing the selection of weed control tools and timing.

Introduction

Weeds interfere with crop access to nutrients, light, water, and space. This interference results in approximately 34% yield loss, which is considerably higher than losses caused by insect pests (18%) or pathogens (16%) (Oerke 2006). Therefore, weeds are one of the greatest challenges in crop protection. In fact, herbicide application comprises 53% of total worldwide chemical phytosanitary consumption (FAOSTAT 2020).

It has become evident that weed management, based solely on chemical control, is ineffective in the long term, due to the evolution of herbicide-resistant biotypes. In fact, more than 500 unique resistance cases involving 267 weed species and 21 modes of action have been recorded (Heap 2022). These cases of resistance, together with the lack of new herbicide active ingredients (Barzman and Dachbrodt-Saaydeh 2011; European Parliament 2009), have limited the number of available herbicides for weed control in many crops. For these reasons, a deeper understanding of weed biology has become critical for the design of weed management strategies. This information can be used to reduce weed growth and survival by implementing control actions that target the times and stages when the weeds are more susceptible. For instance, knowing when and how weeds emerge can help maximize control by implementing actions before weeds become too large and eliminating as many individuals as possible with a single action. Understanding the effects of soil disturbance on weed emergence can help manage the trade-offs between weed seedbank management and soil conservation. Additionally, studying weed seed production and dormancy variability can provide insights into the risk associated with weed escape from control measures due to late or irregular emergence (Van Acker 2009).

The current study is focused on seedling emergence, because this is the most vulnerable stage for most annual weeds, as seed reserves can be quickly depleted before photosynthetic activity

can support seedling growth (Forcella et al. 2000). Therefore, control methods targeting this growth phase tend to be both highly effective and efficient. Because of its importance for weed control and population dynamics, multiple authors have studied weed emergence and developed models to predict it using temperature and humidity; in fact, there are germination and emergence models for almost 100 weed species (Royo-Esnal et al. 2020). However, most of these models do not consider the variation that the emergence pattern may undergo when the soil is disturbed, potentially reducing the model's accuracy under field conditions.

Unlike crops, weed seeds are distributed at different depths, and seed density distribution in the soil profile can be conspicuously influenced by tillage and cultivation practices. The frequency and intensity of soil disturbance affect the physical properties of the soil (e.g., bulk density, porosity, hydraulic conductivity), modifying water and oxygen availability as well as heat transfer capacity (Guérif et al. 2001). The change in the seed's environment caused by soil disturbance can trigger germination events, resulting in changes in seedling emergence patterns (Baskin and Baskin 2014; Finch-Savage and Leubner-Metzger 2006). Frequently, this is the result of several stimuli, such as light exposure, nitrate release, increase in temperature fluctuation, and oxygen availability, that reduce seed dormancy and increase germination rates for various weed species (Dekker 2003; Leon and Owen 2003; Longchamps et al. 2012; Mohler 2001).

Although soil disturbance can induce new emergence, seedbank responses will depend on the particular requirements of each weed species (Nichols et al. 2015). Therefore, knowing the type and timing of soil disturbance would allow not only the anticipation of emergence windows but also the implementation of specific weed control actions to target the emergence pattern expected for each species. For instance, for those species in which tillage induces new emergence, repetitive tillage while preventing new seed production would reduce the seedbank (Gallandt 2006). In addition, this response to tillage is a major determinant of the efficiency of the stale seedbed for weed population reduction before crop sowing (Travlos et al. 2008). Another strategy, albeit less studied, involves the use of mechanical tools to place seeds at depths conducive to fatal germination, which occurs when seeds germinate from depths at which seedlings die before reaching the soil surface. This phenomenon could be caused by seeds having insufficient energy reserves to emerge from those depths or a soil pathogen infecting and killing the seedling during the emergence process (Davis and Renner 2007).

North African knapweed (*Centaurea diluta* Aiton), cornflower (*Centaurea cyanus* L.), corn marigold [*Glebionis segetum* (L.) Fourr.], rigid ryegrass (*Lolium rigidum* Gaudin), and corn poppy (*Papaver rhoeas* L.) are weed species frequently associated with winter cereal crops in the Iberian Peninsula (Saavedra et al. 2016, 2017, 2018; Torra et al. 2022). These weeds were selected because the ability of growers to control them is declining due to limited effective herbicide options; in fact, *L. rigidum* and *P. rhoeas* have already evolved herbicide resistance in the Iberian Peninsula (Torra et al. 2022), as has *C. cyanus* in Europe (Stankiewicz-Kosyl et al. 2021).

Among the species mentioned, *L. rigidum* is the one whose germination and seedling emergence requirements have been studied in the most detail. Several studies reported higher seedling emergence after photo-induction than under darkness (Steadman 2004; Steadman et al. 2003; Vila-Aiub et al. 2005). This behavior has been attributed to light sensitivity triggering germination of a large proportion of *L. rigidum* seeds (50% to 95%) (Steadman et al.

2003). Furthermore, the seeds of this species germinate more easily when close to the surface, and below 2 cm, seedling emergence is reduced considerably (more than 61% reduction at 8-cm depth) (Vila-Aiub et al. 2005). In the case of *P. rhoeas*, there are studies indicating that the seeds of this species exhibit morphophysiological dormancy that is not easily broken. Burial under moist conditions with alternating temperature can break the physiological component of the dormancy, while light favors reduction of the morphological component (Baskin et al. 2002; McNaughton and Harper 1964). Additionally, Cirujeda et al. (2006) reported that seed dormancy was not affected by increases in burial depth. Both *Centaurea* species are problematic weeds in winter cereals fields, mainly because of their innate tolerance to acetolactate synthase (ALS)-inhibiting herbicides (Palma-Bautista et al. 2023). Although limited research has been published about the biology of *C. diluta* and *C. cyanus*, results from species within the same genus suggest that these weeds exhibit low dormancy (Joley et al. 1997, 2003) and high seed production (Gutierrez et al. 2005). Additionally, it is known that the seedbank of *C. diluta* can be rapidly depleted after two growing seasons if seed rain is prevented (Sousa-Ortega et al. 2020b). As is the case for *Centaurea* species, little published information on methods for controlling *G. segetum* exists, except for a technical report mentioning the challenge of controlling this weed species in winter cereal crops due to its tolerance to herbicides from the ALS and synthetic auxin families (Saavedra et al. 2016).

Despite the knowledge that has been generated about the effect of tillage on weed emergence and germination requirements of multiple species, it is still not clear how the combined effects of soil disturbance (e.g., light exposure, rearrangement of seeds in the soil, oxygenation, change in bulk density, temperature changes, soil moisture) affect weed seedling emergence, especially without the confounding effects that are usually part of field studies (e.g., soil heterogeneity, soil pathogen–dependent mortality, spatial and temporal variability of temperature and moisture). Those confounding effects have limited our ability to describe the distinct responses of weed species to soil disturbance, which may affect emergence patterns and weed community assembly during the growing season.

We hypothesized that the five aforementioned weed species differ in germination and emergence patterns in response to changes in the soil environment, which could generate unique behaviors in response to soil management practices. Therefore, the aim of this research was to assess the impact of soil disturbance and seed burial depth on seedling emergence of these weed species in order to inform the design of soil management strategies to reduce weed seedbanks.

Materials and Methods

Plant Material

Species selection was based on difficulty to control in winter cereal crops in Spain (Table 1). Seeds were collected from more than 20 individuals grown in a common garden with no herbicide history during the previous growing season at the Higher Technical School of Agricultural Engineering of the University of Seville (ETSIA; 37.35°N, 5.93°W). This was done to minimize any maternal effects that could influence seedling emergence. Seed collection was conducted between April and June 2018; seeds were cleaned manually to remove plant residues and nonviable seeds (i.e., small, not fully developed). Following cleaning, seeds were stored in a refrigerator at 4 C under dark and dry conditions using silica gel.

Table 1. Species, family, and sowing density for each species used in each experiment.

Species	Family	Seed size	Seeds per pot	
			Burial depth experiment	Soil disturbance experiment
		mm		
Centaurea cyanus	Asteraceae	3.1 × 1.5	100	—[a]
Centaurea diluta	Asteraceae	2.7 × 1.3	100	300
Glebionis segetum	Asteraceae	2.0 × 1.0	250	750
Lolium rigidum	Poaceae	5.0 × 2.0	100	300
Papaver rhoeas	Papaveraceae	0.6 × 0.4	500	1,500

[a]*Centaurea cyanus* was not included in the soil disturbance experiment due to limited seed availability.

Experimental Design

Two individual experiments were performed at ETSIA with the aim of evaluating burial depth and soil disturbance, respectively. Seeds were sown in pots (10 cm by 10 cm and 10.5-cm height, 1,050 cm³), with holes at the base to allow drainage. Pots were filled with a 50% mixture of a sterilized peat moss (Gramoflor, Gramoflor GmbH& Co. Kg, Neuenkirchen-Vörden) and silica sand (Sisanflor, Sisanflor S.L, Sisante) instead of soil to enhance workability (bulk density = 36 g 100 cm⁻³) and achieve better seed distribution. This mix provided optimal conditions for seed germination. Pots were placed in a growth chamber with 24/18 ± 1 C cycle, 60% relative humidity, and supplementary lighting providing a photosynthetic photon flux density of 1,000–1,200 μmol m⁻² s⁻¹ with a 12-h photoperiod. Temperature range was determined based on average temperatures in southern Spain during October and November, which is when these weeds typically emerge. All pots were irrigated twice per week with tap water to field capacity. The experiments were carried out in a randomized complete block design with three replications and were conducted twice. The first experiment was conducted in May 2019, followed immediately by a second repetition.

Burial Depth Experiment

Weed seeds were sown at 2-, 5-, and 9-cm depths. Pots were filled to the depth selected, the seeds were gently placed, covered with more substrate until 1,000 cm³ (or 0.5 cm below the top of the pot), and then moistened. The sowing density varied according to the species and seed size in order to recruit a high number of emerged seedlings (Table 1). Seed number was higher for *G. segetum* and *P. rhoeas*, because their dormancy takes more time to decline than that of *L. rigidum* and both *Centaurea* species. The seed densities chosen ensured enough seedling emergence to properly characterize emergence patterns with individual seedlings representing a small fraction of the cumulative emergence (~ 0.2% to 7%). Seedling recruitment in the present study was similar to or higher than other experiments conducted under similar conditions (Nosratti et al. 2017; Thompson et al. 2021).

Soil Disturbance Experiment

For this experiment, there were six treatments based on timing and number of disturbance events: sowing (D0); at sowing and 3 wk after sowing (WAS) (D3); at 0 and 6 WAS (D6); at 0 and 9 WAS (D9); at 0, 3, and 6 WAS (D3-D6), and at 0, 3, 6, and 9 WAS (D3-D9). The 3-wk interval was selected because this would be the maximum interval to ensure proper weed control with cultivation in the Iberian Peninsula. Furthermore, in our experience, that is enough time for cumulative emergence to level off for the studied species.

In this experiment, sowing was done after manually mixing the seeds with the substrate to ensure that seeds were evenly distributed throughout the soil column within the pot. Soil disturbance was accomplished by emptying the pot's substrate into a transparent plastic bag, followed by intense manual agitation, before returning the mixture to its original pot. Seed density was tripled compared with the burial depth experiment to ensure the presence of enough seeds throughout the soil profile. Due to limited *C. cyanus* seed, this species was not included in this experiment.

Seedling Measurements

In both experiments, seedling emergence was recorded daily, and emerged seedlings were removed from the pot after being counted. The interval between samplings increased as the emergence rate decreased. The experiment was terminated after a month with no further emergence.

Statistical Analysis

Results were analyzed separately per species. Because no statistical differences were found between experimental repetitions (Supplementary Table S1), the analysis was conducted by pooling them. One-way ANOVA determined the effects soil disturbance or burial depth, with treatment as the fixed effect and block and experimental repetition as random effects. Means separation was done with the Tukey's honestly significant difference test ($\alpha = 0.05$), while Levene and Shapiro-Wilk tests were performed to evaluate homoscedasticity and normality of the data. When necessary, data underwent a square-root transformation to meet ANOVA requirements (Supplementary Table S2). These analyses were performed with R software (v. 4.2.1) (R Development Core Team 2017).

Seedling emergence was expressed as a percent of the total seeds sown, while seedling emergence pattern was defined as the cumulative fraction of emergence based on total emerged seedlings, as described by Sousa-Ortega et al. (2021). The emergence pattern analysis focused on the time needed to reach the 10, 50, and 90 percentiles (i.e., p10, p50, and p90, respectively). The estimation of p10, p50, and p90 was performed using linear interpolation.

Results and Discussion

The Effect of Burial Depth on Weed Emergence

When data were averaged across depths, *L. rigidum* exhibited the highest seedling emergence (77%), while *P. rhoeas* showed the lowest (2%). Among the species studied, both *C. cyanus* and *C. diluta* had an average seedling emergence of 28% and 9%,

Table 2. Total emergence and days since sowing elapsed until reaching 10%, 50%, and 90% of the accumulated emergence for each species at different sowing depths.[a]

| Species | Burial depth | Seedling emergence (%)[b] | Speed of emergence[c] days | | |
			10	50	90
Centaurea cyanus	2 cm	44.20 (2.31) a	0.66 (0.13) b	3.18 (0.26) a	24.00 (4.82)
	5 cm	36.80 (0.92) b	0.98 (0.34) b	2.88 (0.57) a	18.06 (7.29)
	9 cm	4.40 (2.25) c	28.05 (9.3) a	34.25 (10.49) b	45.20 (13.92)
	P-value	**< 0.001**	**< 0.001**	**0.003**	0.126
Centaurea diluta	2 cm	14.50 (3.50) a	1.40 (0.25) b	14.12 (5.87)	31.77 (11.44)
	5 cm	10.80 (1.85) a	2.14 (0.40) b	10.02 (5.13)	19.78 (7.57)
	9 cm	1.20 (0.49) b	21.43 (9.84) a	21.83 (9.84)	22.23 (9.84)
	P-value	**< 0.001**	**< 0.001**	0.489	0.6443
Glebionis segetum	2 cm	3.60 (1.05)	6.70 (1.23)	18.70 (6.15)	39.84 (12.08)
	5 cm	n.d.	n.d.	n.d.	n.d.
	9 cm	n.d.	n.d.	n.d.	n.d.
	P-value	n.d.	n.d.	n.d.	n.d.
Lolium rigidum	2 cm	98.6 (5.54) a	0.94 (0.18) b	2.58 (0.12) b	5.26 (0.57) b
	5 cm	99.40 (3.78) a	1.58 (0.14) b	2.96 (0.23) b	5.34 (0.37) b
	9 cm	32.8 (4.91) b	11.42 (1.74) a	16.16 (1.83) a	20.76 (2.18) a
	P-value	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Papaver rhoeas	2 cm	3.92 (0.65)	2.16 (0.42)	4.00 (0.79)	8.44 (1.44)
	5 cm	0.96 (0.41)	4.40 (0.53)	5.08 (0.39)	18.70 (11.33)
	9 cm	n.d.	n.d.	n.d.	n.d.
	P-value	**0.003**	**0.011**	0.257	0.541

[a]Different letters indicate significant differences between rows for the same species according to Tukey's test for a confidence level of 95%. In those cases where there were only two levels of treatment, Student's *t*-test was performed. Significant P-values (P < 0.05) are shown in bold.
[b]Seedling emergence indicates the percentage of emerged seedlings from the total of seeds sown. n.d., no seedling emergence detected.
[c]Days needed to reach up the 10%, 50%, and 90% of the accumulated seedlings emergence from total emerged seedlings.

respectively. Finally, *G. segetum* was only able to emerge from the 2-cm deep (4%). The average seedling emergence in the burial depth experiment, as in the soil disturbance experiment, fell within the range reported in previous studies under similar controlled conditions. For instance, similar seedling emergence was documented for *L. rigidum* by Thompson et al. (2021) and Steadman et al. (2003). Additionally, the seedling emergence of *C. diluta* and *C. cyanus* was comparable to that of other species of the same genus, such as Iberian starthistle (*Centaurea iberica* Trevir. ex Spreng.) (Nosratti et al. 2017) and yellow starthistle (*Centaurea solstitialis* L.) (Joley et al. 2003). In the case of *G. segetum* and *P. rhoeas*, low emergence percentage was expected due to the deep seed dormancy typical of these species (McNaughton and Harper 1964; Roberts and Neilson 1981). Therefore, to compensate for low germination rates, we increased seed number per experimental unit, resulting in a similar number of emerged seedlings for *G. segetum* and *P. rhoeas* compared with the other species studied. This enabled us to properly characterize the emergence patterns of *G. segetum* and *P. rhoeas*.

Seedling emergence was affected by burial depth for all species (Table 2). *Papaver rhoeas* and *G. segetum* did not emerge from 9- and 5-cm depths, respectively. Additionally, *P. rhoeas* emergence decreased by 76% when burial depth increased from 2 to 5 cm. Similarly, when burial depth increased from 2 to 9 cm, seedling emergence was reduced by 67%, 90%, and 92% for *L. rigidum*, *C. cyanus*, and *C. diluta*, respectively. Seedling emergence of *C. diluta* and *L. rigidum* was only reduced at 9 cm compared with 2 and 5 cm. In contrast, the three burial depths caused clear variation in *C. cyanus* emergence, with a marked decline as depth increased (Table 2).

Centaurea cyanus exhibited the earliest emergence pattern, reaching p10 within the first day at 2 and 5 cm. Subsequently, *L. rigidum*, *C. diluta*, and *P. rhoeas* achieved p10 within 4 d at 2- and 5-cm depths (Table 2; Figure 1), while *G. segetum* took a week to reach the same emergence (Figure 2). Regarding emergence duration (p90 − p10), *L. rigidum* had the fastest emergence rate, reaching maximum germination in 5 to 6 d at 2- and 5-cm treatments. On the other hand, the slowest emergence pattern was observed in *G. segetum*, which took more than a month to reach p10 at 2-cm depth (Figure 2). The *P. rhoeas*, *C. cyanus*, and *C. diluta* emergence patterns showed intermediate duration averaging 14, 21, and 21 d when averaging 2- and 5-cm depths, respectively (Table 2; Figures 1 and 2).

Burial depth not only influenced the total number of seedlings emerging but also the emergence pattern. In *P. rhoeas*, significant differences were only found for the time to reach p10, doubling from 2 to 5 cm, and no significant differences among depths were observed for p50 and p90 (Table 2). *Centaurea diluta* p10 was delayed 20 d at 9-cm depth compared with the 2- and 5-cm depths, but no significant differences among depths were observed for p50 and p90, which took on average 15 and 24 d after sowing (DAS), respectively (Table 2; Figure 1). Conversely, *L. rigidum* was the only species in which burial depth affected the three percentiles. Thus, compared with 2 and 5 cm, burial at 9 cm increased the time to reach p10, p50, and p90, by 1,114%, 526%, and 294%, respectively (Table 2; Figure 1). *Centaurea cyanus* followed a similar trend for p10 and p50, with 1,430% and 55% increases at 9 cm compared with 2- and 5-cm burial depths, respectively (Table 2; Figure 1). Finally, the *G. segetum* emergence time to p10, p50, and p90 at 2-cm depth was 6.7, 18.7, and 39.8 DAS, respectively (Figure 2).

The current work was carried out under conditions conducive to maximize seedling emergence, because the sand–peat mixture is a light substrate that favors emergence compared with heavier soils such as clay soils (Vila-Aiub et al. 2005). In addition, because the substrate was sterilized, differences in seedling emergence are more likely to result from seed germination differences than seedling mortality due to pathogens. Also, burying the seeds delayed the start of seedling emergence for all species more than 50% and 1,000% at 5 and 9 cm compared with 2 cm, which may provide a
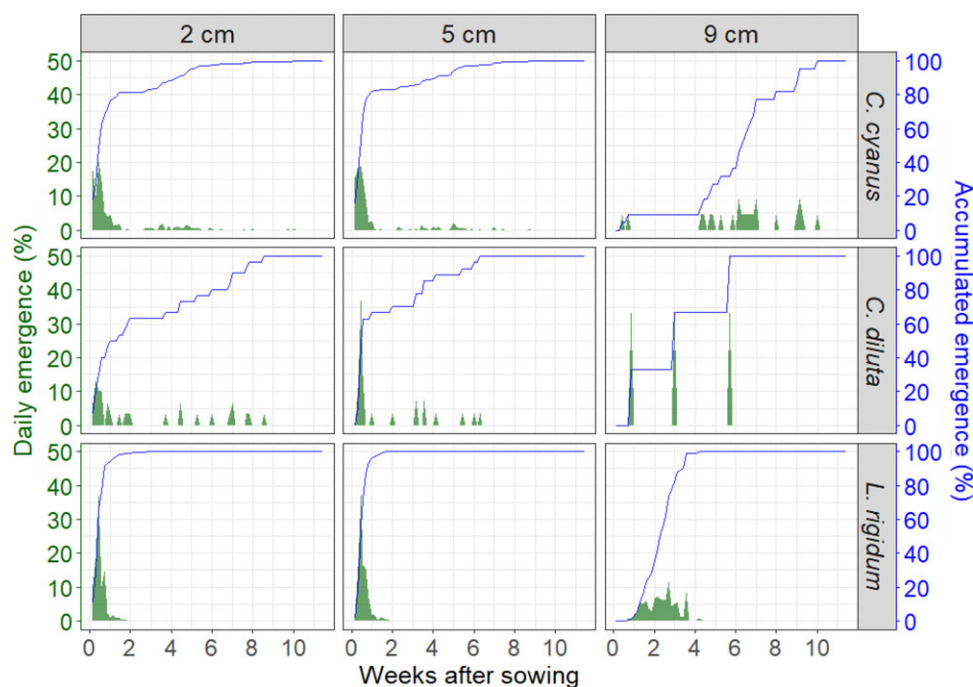
Figure 1. Daily and accumulated emergence in percentage for *Centaurea cyanus*, *Centaurea diluta*, and *Lolium rigidum* in weeks after sowing. The blue line indicates the accumulated emergence, and the green area indicates the daily emergence.
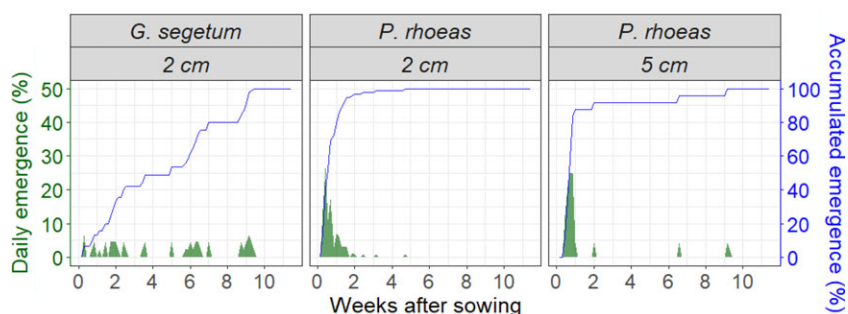

Figure 2. Daily and accumulated emergence in percentage for *Glebionis segetum* and *Papaver rhoeas* in weeks after sowing. The blue line indicates the cumulated emergence, and the green area indicates the daily emergence.

competitive advantage to the crop against these weeds. This advantage may be of particular value for small-seeded crops such as oilseed rape (*Brassica napus* L.) that are poor competitors during early phases of the growing season (Knezevic and Datta 2015).

Effect of Soil Disturbance on Weed Emergence

Average seedling emergence for *C. diluta* was 17%, varying between 8% and 24% depending on the treatment. The number of soil disturbance events increased seedling emergence by more than 165% for both D3-6 and D3-9, compared with D0 (P-value = 0.02). On the other hand, seedling emergence did not differ among treatments with two disturbance events regardless of the duration of the interval between them (D3, D6, or D9). The *C. diluta* emergence pattern was defined by soil disturbance to the point that there was a consistent and sharp increase in emergence rate immediately after each disturbance (Figure 3).

The *G. segetum* emergence pattern was staggered in response to disturbance events (Figure 3). The time to p10 for D0 was considerably lower (P-value = 0.001) from the four-disturbance

treatment (D3-9). This difference was due to soil disturbance producing a peak of subsequent emergence, which delayed the emergence time to p10 by 18.9 d. A similar response was observed for p50 (P-value = 0.0258); however, in this case, the later the soil disturbance, the longer the time to reach p50. On the other hand, no significant differences were found at p90 (Table 3; Figure 3).

The seedling emergence of *L. rigidum* averaged 89% for all treatments, and the emergence pattern was not affected by soil disturbance, reaching p10, p50, and p90 at 5, 7, and 16 DAS, respectively (Table 3; Figure 3).

Papaver rhoeas seedling emergence was very sensitive to disturbance (P-value < 0.001), thus three and four disturbance events increased emergence 225% and 278% compared with D0. Interestingly, the magnitude of the emergence event was larger when the interval between disturbances was longer (Table 3). Similar to *C. diluta* and *G. segetum*, *P. rhoeas* had a seedling emergence rate that increased sharply in response to soil disturbance, and this occurred even when the emergence pattern had leveled off for 2 to 8 wk after the previous disturbance event (Figure 3). The response of *G. segetum* to soil disturbance was
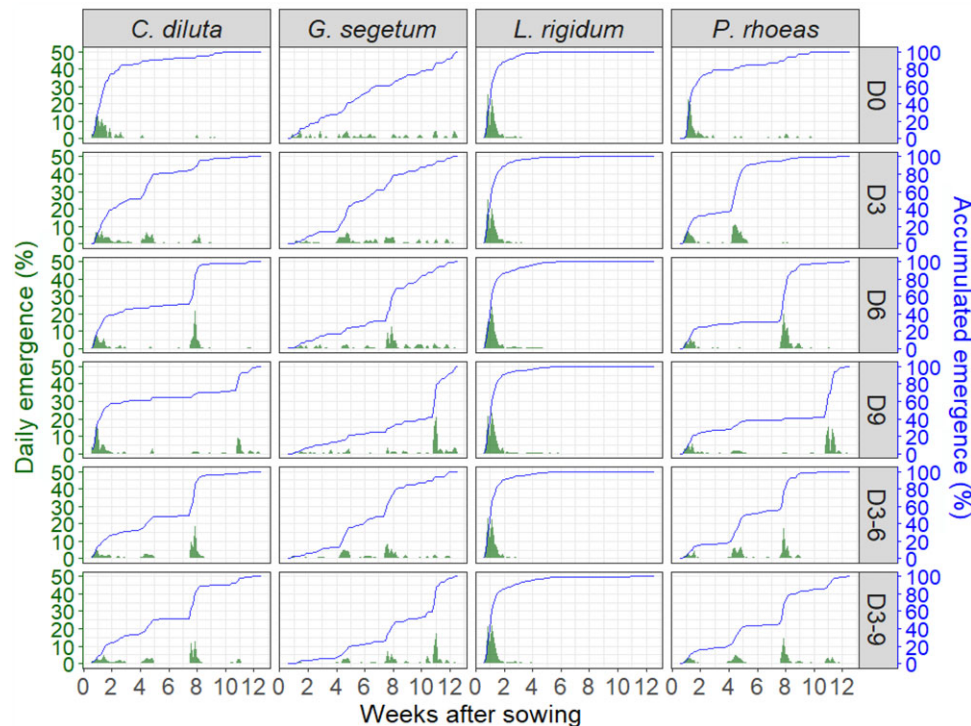
Figure 3. Soil disturbance effects on the emergence pattern. The blue line indicates the accumulated emergence, and the green area indicates the daily emergence. The treatment involves soil disturbance at specific time points: D0 at the time of sowing; D3 at both sowing and 3 wk after sowing (WAS); D6 at 0 and 6 WAS; D9 at 0 and 9 WAS; D3-6 at 0, 3, and 6 WAS; and D3-9 at 0, 3, 6, and 9 WAS.

similar to that exhibited by *C. diluta*, in which seedling emergence increased with disturbance number (P-value < 0.001) (Table 3).

Multiple disturbance events modified the magnitude and pattern of emergence of three out of the four studied species, with *L. rigidum* being the exception, whose total seedling emergence and emergence pattern remained unaltered regardless of the disturbance regime after D0. This behavior might be the result of its rapid and defined emergence pattern, in which most seedlings emerged before the soil disturbance treatment. Such rapid emergence gives this weed an advantage to colonize and acquire resources early in the season, increasing its chances to outcompete the other weed species studied here as well as crops (Knezevic and Datta 2015). The negative aspect of this emergence strategy is that if effective control tools are available, most of the population can be controlled with a single or very few actions. This process could have played a significant role in the evolution of herbicide-resistant biotypes in this species, as it promotes high selective pressure. Conversely, by generating multiple cohorts, other species such as *P. rhoeas* and *C. diluta* might have a better chance of successfully establishing a population in systems that have mortality events early in the growing season, especially if those mortality events are determined by cultivation. The presence of multiple cohorts is likely attributable to the emergence of seeds located in upper soil layers, as a result of their recirculation via soil disturbance. These results illustrate how different weed species might be able to optimize the trade-off between access and competition for resources and the need to spread the risk of mortality over time (Tørresen et al. 2017).

We propose that the response to disturbance described in the present study can be explained by soil temperature variation acting as the main environmental signal for the germination of the studied weed species. This is because seed dormancy release and germination tend to be proportional to the magnitude of the diurnal temperature fluctuations (Leon et al. 2004; Thompson and Grime 1983; Thompson et al. 1977). Two key factors support this idea. First, in all species, seeds buried closer to the surface germinated at higher rates, and soil temperature fluctuations decrease with depth (Kegode et al. 1998). Although the literature frequently cites light exposure as the main trigger for germination in response to soil disturbance (Milberg and Andersson 1997; Penny and Neal 2003; Sester et al. 2006), it is unlikely that light exposure alone can explain the differences in seedling emergence observed here, because all the seeds were buried, and before sowing, they all were exposed to light in the same way. Second, every disturbance event that generated another seedling cohort did so because soil mixing placed ungerminated seeds at depths where environmental signals such as temperature fluctuation can reduce seed dormancy and trigger their germination. We cannot rule out that evaporation rates close to the surface might create soil moisture oxygen concentration variations that could also be signals for seed germination and seedling emergence, but if that were the case, the emergence patterns would be more constant, because pots were watered to maintain soil moisture close to field capacity.

Potential Applications to Inform Management Decisions

Currently, there are mathematical models to predict the emergence for three of the five weed species studied in this work: *L. rigidum* (Sousa-Ortega et al. 2020a), *C. diluta* (Sousa-Ortega et al. 2020b), and *P. rhoeas* (Izquierdo et al. 2009). One of the main challenges for these and other emergence models is how to determine the appropriate moment to initiate thermal or hydrothermal time (TT and HTT, respectively) accumulation, although models normally use arbitrary criteria such as the first relevant rainfall (>10 mm) for

Table 3. Soil disturbance effects on total seedling emergence and on the emergence profile.[a]

Species	Treatment[b]	NOS[c]	Seedling emergence (%)[d]	Speed of emergence[e] —days— p10	p50	p90
Centaurea diluta	D0	1	8.13 (0.68) b	5.62 (0.62)	9.20 (1.04) b	36.32 (10.90)
	D3	2	13.00 (1.94) ab	6.92 (0.6)	23.22 (3.05) ab	44.70 (4.85)
	D6	2	16.73 (1.88) ab	7.46 (2.09)	31.20 (9.23) ab	56.72 (1.12)
	D9	2	16.22 (4.8) ab	5.37 (0.21)	21.70 (5.31) ab	77.88 (0.83)
	D3-6	3	21.60 (2.8) a	6.32 (0.5)	41.00 (4.93) a	59.58 (3.29)
	D3-9	4	24.87 (3.09) a	7.80 (0.91)	30.22 (7.36) ab	54.14 (11.99)
	P-value		**0.002**	0.2089	**0.0185**	0.0651
Glebionis segetum	D0	1	2.80 (0.52) b	11.48 (2.43) b	41.94 (4.5) c	75.72 (4.53)
	D3	2	3.79 (0.21) b	20.16 (3.92) ab	37.2 (1.99) c	71.76 (4.73)
	D6	2	4.99 (0.98) b	14.45 (2.22) ab	46.17 (9.72) bc	75.05 (2.47)
	D9	2	6.00 (1.11) ab	25.6 (8.35) ab	67.95 (4.75) a	82.33 (1.54)
	D3-6	3	6.53 (1.57) ab	23.04 (3.72) ab	49.72 (1.81) bc	69.26 (3.58)
	D3-9	4	9.12 (0.47) a	30.36 (0.97) a	66.14 (4.64) ab	78.38 (1.26)
	P-value		**0.001**	**0.0258**	**< 0.001**	0.1701
Lolium rigidum	D0	1	88.33 (4.02)	5.16 (0.02)	7.46 (0.10)	15.14 (1.62)
	D3	2	90.27 (6.10)	5.08 (0.05)	7.40 (0.07)	16.56 (2.62)
	D6	2	85.33 (8.71)	5.18 (0.16)	7.44 (0.05)	15.68 (1.8)
	D9	2	88.53 (3.58)	5.20 (0.03)	7.56 (0.1)	15.40 (2.95)
	D3-6	3	92.67 (3.73)	5.12 (0.14)	7.18 (0.21)	15.52 (2.67)
	D3-9	4	88.40 (8.15)	5.24 (0.06)	7.46 (0.12)	20.52 (3.52)
	P-value		0.9751	0.8615	0.3737	0.7165
Papaver rhoeas	D0	1	1.44 (0.17) b	7.22 (0.19)	9.76 (0.51) c	54.32 (1.92) b
	D3	2	3.31 (0.51) ab	6.96 (0.34)	25.32 (4.11) b	37.3 (1.66) c
	D6	2	3.32 (0.56) ab	17.04 (9.15)	48.02 (6.46) ab	60.34 (0.96) b
	D9	2	5.13 (0.43) a	8.96 (1.07)	59.14 (10.53) a	79.56 (0.12) a
	D3-6	3	4.68 (0.98) a	13.32 (4.04)	45.40 (3.97) ab	57.98 (0.65) b
	D3-9	4	5.45 (0.45) a	12.74 (4.09)	49.72 (4.38) a	72.96 (4.4) a
	P-value		**< 0.001**	0.349	**< 0.001**	**< 0.001**

[a]Mean and standard error (in brackets) are shown. Different letters indicate significant differences between rows for the same species according to Tukey's test for a confidence level of 95%. In those cases where there were only two levels of treatment, Student's *t*-test was performed. Significant P-values (P < 0.05) are shown in bold.
[b]The treatment involves soil disturbance at specific time points: D0 at the time of sowing; D3 at both sowing and 3 wk after sowing (WAS); D6 at 0 and 6 WAS; D9 at 0 and 9 WAS; D3-6 at 0, 3, and 6 WAS; and D3-9 at 0, 3, 6, and 9 WAS.
[c]Number of soil disturbances.
[d]Seedling emergence indicates the percentage of emerged seedlings from the total of seeds sown.
[e]Days needed to reach 10%, 50%, and 90% of the accumulated seedling emergence from total emerged seedlings.

this point. The present study suggests that as observed for *C. diluta* and *P. rhoeas*, soil disturbance might be a valuable starting point for TT and HTT accumulation for several weed species. It is important to highlight that sequential soil disturbance events trigger multiple cohorts with similar emergence patterns, and therefore, seedling emergence models might be used for multiple cohorts by resetting the TT and HTT accumulation after each soil disturbance (e.g., cultivation).

The results obtained in this work allow estimation of the usefulness of various control methods such as a stale seedbed or tillage for seed burial. The use of a stale seedbed is probably effective on *C. diluta*, *L. rigidum*, and *P. rhoeas*, as they exhibit early emergence (Table 3; Figures 1 and 2). On the other hand, this control method would not be effective in *G. segetum* because of its continuous emergence. Although a stale seedbed would have some efficacy on *P. rhoeas* and *C. diluta*, the soil should not be disturbed during the removal of emerged weeds; otherwise, seedling emergence might be induced again. In the case of *L. rigidum*, there is more flexibility regarding the control tools that can be used after the stale seedbed, but weed community composition and the presence of soil disturbance–sensitive weeds might reduce the number of options for control. The use of the stale seedbed approach would be particularly appropriate in the southern Iberian Peninsula, where a crop tillage system with a rotation between winter wheat (*Triticum durum* Desf.) and sunflower (*Helianthus annuus* L.) is extensively practiced. In such situations, the stale seedbed could easily be applied before the winter cereal sowing. Additionally, during the fallow period before sunflower sowing, multiple cultivations could be implemented to reduce the seedbank by promoting emergence and preventing seed production. This could be useful for controlling *C. diluta*, *G. segetum*, and *P. rhoeas*, as their emergence was increased between 3 and 5 times when soil disturbance was done multiple times in a sequential manner. Finally, given that the emergence of these species is facilitated by soil tillage, the optimal timing for preemergence herbicide application would be after the secondary tillage before winter cereal sowing.

Burying seeds at a depth of 5 cm using soil-turning tools would be a very effective method to control *G. segetum* and *P. rhoeas* and moderately effective to control *C. cyanus*. Furthermore, because burial would not increase the dormancy of *P. rhoeas* (Cirujeda et al. 2006), it is likely that fatal germination would increase, reducing seedbank persistence. On the other hand, *L. rigidum* and *C. diluta* will require soil-turning tools to at least 10 cm. However, if the seedbank density of *L. rigidum* is high, the use of this tool at 10 cm might not be effective, because seedling emergence could still be more than 30% (Table 3). Implementing this control method would be particularly useful in the northern Iberian Peninsula, where no-till systems with monoculture of winter cereal crops is typical, as most of the seeds are distributed in the upper soil layer (Feldman et al. 1996). Therefore, this technique would not involve digging up seeds. This control method could be repeated in the

medium term for *G. segetum*, because its seedbank would be drastically reduced after 7 yr, based on the 36% annual seedbank decline described by Chancellor (1986). However, for controlling the *P. rhoeas* population, this technique could only be used once and not repeated in the long term, because the annual seedbank decline for this species is the only 6% (Chancellor 1986). Therefore, the previously buried seed would be placed within adequate germination conditions close to the surface.

Soil disturbance affected the emergence of *C. diluta*, *G. segetum*, and *P. rhoeas*. In general, increasing the number of soil disturbance events and the duration of the interval between events results in higher seedling emergence. *Glebionis segetum*, *P. rhoeas*, and *C. cyanus* emergence was suppressed when buried at a depth of 5 cm or greater, while *C. diluta* and *L. rigidum* emergence was only suppressed when buried at 9 cm. The present study illustrates how the sensitivity of seeds of different weed species to soil disturbance can dramatically determine weed emergence patterns and potentially shape weed emergence sequences and weed community composition. One of the limitations of the present study is that it did not have shallower burial depths. Therefore, it would be important for future experiments to include a treatment with unburied seeds and one in which seeds are buried in the top 0.5 cm of soil, which would provide further insights about responses under conditions similar to no-till systems. It is also critical that further studies be conducted under field conditions, as the present study was carried out under optimal germination conditions. We recognize that emergence patterns might differ with more environmental variation, and field data will be critical for fine-tuning predictive emergence models and weed control timing. However, it is unlikely that the differences among species observed here will not be expressed to some extent in the field.

References

Barzman M, Dachbrodt-Saaydeh S (2011) Comparative analysis of pesticide action plans in five European countries. Pest Manag Sci 67:1481–1485

Baskin CC, Baskin JM (2014) Variation in seed dormancy and germination within and between individuals and populations of a species. *In* Seeds. 2nd ed. San Diego: Academic Press. Pp 277–373

Baskin CC, Milberg P, Andersson L, Baskin JM (2002) Non-deep simple morphophysiological dormancy in seeds of the weedy facultative winter annual *Papaver rhoeas*. Weed Res 42:194–202

Chancellor RJ (1986) Decline of arable weed seeds during 20 years in soil under grass and the periodicity of seedling emergence after cultivation. J Appl Ecol 23:631

Cirujeda A, Recasens J, Taberner A (2006) Dormancy cycle and viability of buried seeds of *Papaver rhoeas*. Weed Res 46:327–334

Davis AS, Renner KA (2007) Influence of seed depth and pathogens on fatal germination of velvetleaf (*Abutilon theophrasti*) and giant foxtail (*Setaria faberi*). Weed Sci 55:30–35

Dekker J (2003) The foxtail (*Setaria*) species-group. Weed Sci 51:641–656

European Parliament (2009) Regulation (EC) No 1107/2009 of the European Parliament and of the Council Of 21 October 2009 Concerning the Placing of Plant Protection Products on the Market and Repealing Council Directives 79/117/EEC and 91/414/EEC. Pp 1–50. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0001:0050:en:PDF. Accessed: January 15, 2023

FAOSTAT (2020) Pesticides Use. https://www.fao.org/faostat/en/#data/RP. Accessed: November 25, 2022

Feldman SR, Alzugaray C, Torres PS, Lewis P (1996) The effect of different tillage systems on the composition of the seedbank. Weed Res 37:71–76

Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. New Phytol 171:501–523

Forcella F, Benech Arnold RL, Sanchez R, Ghersa CM (2000) Modeling seedling emergence. Field Crops Res 67:123–139

Gallandt ER (2006) How can we target the weed seedbank? Weed Sci 54:588–596

Guérif J, Richard G, Dürr C, Machet JM, Recous S, Roger-Estrade J (2001) A review of tillage effects on crop residue management, seedbed conditions and seedling establishment. Soil Tillage Res 61:13–32

Gutierrez AP, Pitcairn MJ, Ellis CK, Carruthers N, Ghezelbash R (2005) Evaluating biological control of yellow starthistle (*Centaurea solstitialis*) in California: a GIS based supply–demand demographic model. Biol Control 34:115–131

Heap I (2022) The International Herbicide-Resistant Weed Database. http://www.weedscience.org. Accessed: January 15, 2023

Izquierdo J, Gonzalez-Andujar J, Bastida F, Lezaún J, Arco M (2009) A thermal time model to predict corn poppy (*Papaver rhoeas*) emergence in cereal fields. Weed Sci 57:660–664

Joley DB, Maddox DM, Schoenig SE, Mackey BE (2003) Parameters affecting germinability and seed bank dynamics in dimorphic achenes of *Centaurea solstitialis* in California. Can J Bot 81:993–1007

Joley DB, Schoenig SE, Casanave KA, Maddox DM, Mackey BE (1997) Effect of light and temperature on germination of dimorphic achenes of *Centaurea solstitialis* in California. Can J Bot 75:2131–2139

Kegode GO, Pearce RB, Bailey TB (1998) Influence of fluctuating temperatures on emergence of shattercane (*Sorghum bicolor*) and giant foxtail (*Setaria faberi*). Weed Sci 46:330–335

Knezevic SZ, Datta A (2015) The critical period for weed control: revisiting data analysis. Weed Sci 63:188–202

Leon RG, Knapp AD, Owen MDK (2004) Effect of temperature on the germination of common waterhemp (*Amaranthus tuberculatus*), giant foxtail (*Setaria faberi*), and velvetleaf (*Abutilon theophrasti*). Weed Sci 52:67–73

Leon RG, Owen MDK (2003) Regulation of weed seed dormancy through light and temperature interactions. Weed Sci 51:752–758

Longchamps L, Panneton B, Simard M-J, Leroux GD (2012) Could weed sensing in corn interrows result in efficient weed control? Weed Technol 26:649–656

McNaughton IH, Harper JL (1964) *Papaver* L. J Ecol 52:767–793

Milberg P, Andersson L (1997) Seasonal variation in dormancy and light sensitivity in buried seeds of eight annual weed species. Can J Bot 75:1998–2004

Mohler CL (2001) Weed life history: identifying vulnerabilities. Pages 40–98 *in* Mohler CL, Staver CP, Liebman M, eds. Ecological Management of Agricultural Weeds. Cambridge: Cambridge University Press

Nichols V, Verhulst N, Cox R, Govaerts B (2015) Weed dynamics and conservation agriculture principles: a review. Field Crops Res 183:56–68

Nosratti I, Abbasi R, Bagheri A, Bromandan P (2017) Seed germination and seedling emergence of Iberian starthistle (*Centaurea iberica*). Weed Biol Manag 17:144–149

Oerke E-C (2006) Crop losses to pests. J Agric Sci 144:31–43

Palma-Bautista C, Vázquez-García JG, De Portugal J, Bastida F, Alcántara-de La Cruz R, Osuna-Ruiz MD, Torra J, De Prado R (2023) Enhanced detoxification via Cyt-P450 governs cross-tolerance to ALS-inhibiting herbicides in weed species of *Centaurea*. Environ Pollut 322:121140

Penny GM, Neal JC (2003) Light, temperature, seed burial, and mulch effects on mulberry weed (*Fatoua villosa*) seed germination. Weed Technol 17:213–218

R Development Core Team (2017) R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing

Roberts HA, Neilson JE (1981) Seed survival and periodicity of seedling emergence in twelve weedy species of Compositae. Ann Appl Biol 97:325–334

Royo-Esnal A, Torra J, Chantre GR (2020) Weed emergence models. Pages 85–116 *in* Chantre GR, González-Andújar JL, eds. Decision support systems for weed management. Cham: Springer International Publishing

Saavedra M, Fuentres M, Carranza R, Sanchez-Gamonoso M, Álcantaea C (2017) Ensayo de herbicidas de preemergencia en condiciones de sequía contra *Centaurea diluta* en Tigo duro. Sevilla: CAPDER Instituto de Investigación y Formación Agraria y Pesquera. 15 p

Saavedra M, Pérez D, Hidalgo JC, Hidalgo J, Vega V, Leyva A (2016) Ensayo de herbicidas contra *Chrysanthemum segetum*. Sevilla: CAPDER Instituto de Investigación y Formación Agraria y Pesquera. 23 p

Saavedra M, Sánchez-Gamonoso M, Carranza R, Fuentes M, Alcántara C (2018) Ensayo de Herbicidas de Postemergencia contra *Centaurea diluta* en Trigo Duro. Sevilla: CAPDER Instituto de Investigación y Formación Agraria y Pesquera. 14 p

Sester M, Dürr C, Darmency H, Colbach N (2006) Evolution of weed beet (*Beta vulgaris* L.) seed bank: quantification of seed survival, dormancy, germination and pre-emergence growth. Eur J Agron 24:19–25

Sousa-Ortega C, Chamber E, Urbano JM, Izquierdo J, Loureiro I, Marí AI, Cordero F, Vargas M, Saavedra M, Lezaun JA, Paramio JA, Fernández JL, Torra J, Royo-Esnal A (2020a) Should emergence models for *Lolium rigidum* be changed throughout climatic conditions? The case of Spain. Crop Prot 128:105012

Sousa-Ortega C, Royo-Esnal A, DiTommaso A, Izquierdo J, Loureiro I, Marí AI, Cordero F, Vargas M, Saavedra M, Paramio JA, Fernández JL, Torra J, Urbano JM (2020b) Modeling the emergence of North African knapweed (*Centaurea diluta*), an increasingly troublesome weed in Spain. Weed Sci 68:268–277

Sousa-Ortega C, Royo-Esnal A, Loureiro I, Marí AI, Lezáun JA, Cordero F, Saavedra M, Paramio JA, Fernández JL, Torra J, Urbano JM (2021) Modeling emergence of sterile oat (*Avena sterilis* ssp. *ludoviciana*) under semiarid conditions. Weed Sci 69:341–352

Stankiewicz-Kosyl M, Haliniarz M, Wrochna M, Synowiec A, Wenda-Piesik A, Tendziagolska E, Sobolewska M, Domaradzki K, Skrzypczak G, Łykowski W,

Krysiak M, Bednarczyk M, Marcinkowska K (2021) Herbicide resistance of *Centaurea cyanus* L. in Poland in the context of its management. Agronomy 11:1954

Steadman KJ (2004) Dormancy release during hydrated storage in *Lolium rigidum* seeds is dependent on temperature, light quality, and hydration status. J Exp Bot 55:929–937

Steadman KJ, Crawford AD, Gallagher RS (2003) Dormancy release in *Lolium rigidum* seeds is a function of thermal after-ripening time and seed water content. Funct Plant Biol 30:345–352

Thompson K, Grime JP (1983) A comparative study of germination responses to diurnally-fluctuating temperatures. J Appl Ecol 20:141

Thompson K, Grime JP, Mason G (1977) Seed germination in response to diurnal fluctuations of temperature. Nature 267:147–149

Thompson M, Mahajan G, Chauhan BS (2021) Seed germination ecology of southeastern Australian rigid ryegrass (*Lolium rigidum*) populations. Weed Sci 69:454–460

Torra J, Montull JM, Calha IM, Osuna MD, Portugal J, De Prado R (2022) Current status of herbicide resistance in the Iberian Peninsula: future trends and challenges. Agronomy 12:929

Tørresen KS, Karlsson LM, Gonzalez-Andujar JL (2017) Seed biology and population dynamics. Pages 85–113 *in* Hatcher PE, Froud-Williams RJ, eds. Weed Research: Expanding Horizons. Chichester, UK: Wiley

Travlos I, Giannopolitis CN, Paspatis EA (2008) Wild oat variability in wheat fields of Viotia in central Greece. Hell Plant Prot J 1:107–112

Van Acker RC (2009) Weed biology serves practical weed management. Weed Res 49:1–5

Vila-Aiub MM, Neve P, Steadman KJ, Powles SB (2005) Ecological fitness of a multiple herbicide-resistant *Lolium rigidum* population: dynamics of seed germination and seedling emergence of resistant and susceptible phenotypes. J Appl Ecol 42:288–298