

Differential weed seed removal in dryland cereals

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Abstract Weeds persist in rain-fed cereal fields in NE Spain, despite intense herbicide use and high seed removal rates by granivorous harvester ants. Herbicide resistance is involved, but certain weed species also appear to escape seed removal by granivores. To identify the mechanisms involved, we measured seed removal rates (three fields in 2010) and the timing of seed shed (one field in 2009 and three fields in 2010) and used an existing model, which integrates short-term rates of seed shed, burial and removal, to estimate long-term seed removal rates. Averaged over years, fields and weed species, the long-term seed removal rate was estimated at 72 % (range 46–100 %). Fifteen to 25 % of the seeds of *Bromus diandrus* avoided removal by being less attractive (low removal rates), and another 0–29 % escaped through crop harvest, which made seeds inaccessible to granivores. Similarly, 20–32 % of the *Papaver rhoeas* seeds escaped through crop harvest, while another 13–17 % escaped by burial into the soil (small seed size). Other species, such as *Galium spurium* or *Diploaxis erucoides*, had no means of avoiding seed removal by harvester ants. In

particular, the more troublesome weeds, such as *B. diandrus*, *P. rhoeas* and *L. rigidum*, combined herbicide resistance or tolerance with avoidance mechanisms against granivory.

Keywords Harvester ants · Granivores · Seed removal rate · Seed shed pattern · Seed exposure time · Timing of crop harvest

Introduction

Seed removal (granivory) can contribute to weed control. When enough of the newly produced weed seeds are intercepted by granivores, seed reservoirs in the soil are slowly depleted, resulting in lower weed densities. Estimates of annual removal rates, however, are difficult to obtain experimentally, because both seed shed and seed predation are highly dynamic and erratic over a season, forcing measurements of weed seed shed and weed seed removal rates to be made at a daily or weekly timescale. These episodic point estimates then have to be scaled to long-term removal rates (Davis et al. 2011). It is important to understand the factors that influence the estimates of annual removal rates, in order to predict annual removal rates and long-term effects on weed population dynamics, and to identify management practices that foster high levels of weed seed removal.

In temperate regions, differences in annual removal rates between weed species mainly stem from differences in the degree of overlap between the period of highest activity of granivores and the period that seed are available on the soil surface. For example, in phenologically early crops, such as small-grain cereals, the peak in seed demand by granivores precedes the peak in weed seed shed (Westerman et al. 2003), causing early maturing weed species to sustain higher seed losses than late-maturing weed species.

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Several weed species can cause substantial losses in cereal yields in NE Spain (Escorial et al. 2011; Torra et al. 2011). The presence of these species can partially be explained by resistance or differential susceptibility to herbicides. For example, *Papaver rhoeas* L. and *Lolium rigidum rigidum* (Gaud.) are resistant and *Bromus diandrus* Roth. tolerant to several commonly used herbicides (Escorial et al. 2011; Heap 2012). However, for weed species to persist, they also have to be able to evade seed predation. Seed removal rates are high all season long (Baraibar et al. 2009), and, therefore, weed seeds cannot escape seed predation because of dissimilar periods of seed shed and seed demand. So, how do these seeds avoid predation?

The main seed predator is a harvester ant, *Messor barbarus* L., whose activity is constrained by temperature and internal cues. Foraging activity is high as long as the surface temperatures are between approximately 15 and 35 °C (Azcárate et al. 2007), which is usually from early May until mid-October (Generalitat de Catalunya 2011), with a temporary trough at the end of September or early October caused by colony reproduction (Baraibar et al. 2009). Other seed predators, such as carabid beetles and granivorous rodents, do occur, but their numbers are low (Baraibar et al. 2009), and their foraging activity is negligible compared with that caused by the massive presence of harvester ants (Baraibar and Westerman, pers. obs.). The causes for the low numbers and low activity-densities are probably water and food shortage in the case of rodents (the area is semi-arid) and competition with harvester ants in the case of carabid beetles.

A particular seed species could avoid being collected if other seed species are more attractive, more nutritious or easier to handle. Harvester ants tend to have clear preferences. In general, larger seeds with a soft seed coat are collected more frequently than smaller seeds with a tough seed coat (Willot et al. 2000; Reyes-López and Fernández-Haeger 2002a). Seed selection may further be influenced by the relative abundance of seeds (Willot et al. 2000) and the state of filling of the granaries, which are large subterranean storage rooms for seeds. The fuller the granaries, the smaller the seeds collected (Reyes-López and Fernández-Haeger 2002b). It is unknown how various common species of weed seeds in NE Spain differ in attractiveness to harvester ants.

Weeds can also avoid predation by retaining their seeds in the seed heads until crop harvest; seed removal rates drop noticeably following crop harvest (Baraibar et al. 2009). Dust, soil and straw produced during harvest cover seeds upon dispersal, making them inaccessible to seed predators (Westerman et al. 2006, 2009). Plant debris hinders the movement and foraging of harvester ants (Atanackovic pers. obs.). Furthermore, the presence of

waste grain, which is an attractive food source, distracts harvester ants from foraging on weed seeds. Once the straw is removed, the weed seeds become accessible again. However, in no-till fields, the straw is usually left on the field for 4–5 weeks, because there is no incentive or need to remove the straw, as fields are left fallow until seeding in late autumn. In conventionally managed fields, the straw is removed more quickly, but followed by tillage, which transports the seeds to deeper soil layers and out of reach of granivores. Either way, seeds shed at crop harvest have a much better chance of avoiding predation than seeds shed during the cropping season.

We hypothesized that certain weed species may suffer lower removal rates than others, if their seeds were (1) less attractive or (2) shed at crop harvest. To test our hypotheses, the timing of seed shed (one field in 2009 and three fields in 2010) and the pattern of seed removal over time (three fields in 2010) were determined in commercial cereal fields in NE Spain. Seed preference was estimated by comparing seed removal rates of four weed species in one of the fields in 2010. Next, an existing model was used to integrate the dynamics of seed shed and seed removal to estimate long-term removal rates (Westerman et al. 2003).

Materials and methods

Locations

Trials were conducted in commercial winter cereal fields in Agramunt (field 1 (41°46'12"N 1°6'2"E); clay soil) in 2009 and 2010 and in Vilanova de Bellpuig (field 2 (41°35'23"N 0°58'23"E) and field 3 (41°35'24"N 0°58'41"E); sandy clay soil) in 2010. Details of crops, date of sowing and harvest, and other management details are provided in Table 1. The region is semi-arid; average annual temperature is 14.7 °C (1971–2000; Agencia Española de Meteorología 2012), and average annual rainfall is 369 mm, concentrated in spring and autumn. Summers are hot (average maximum 33 °C) and winters mild (average minimum 0 °C).

Weed survey

On 8 May 2009, field 1 was surveyed using 30 randomly selected 0.4 m² (0.4 m × 1.0 m) quadrats, which were placed between crop rows in the areas selected for seed shed measurements (see below). Weeds were identified to species level, and densities were expressed as numbers per m² (Table 2). On 17 June 2010, all three fields were surveyed as described for 2009, using 20 random 0.4 m² quadrats in fields 2 and 3, and 25 0.4 m² quadrats in field 1, because weed diversity tended to be higher (Table 2). Occasionally, a rare plant species was encountered, that is,

Table 1 Crop management in the fields 1, 2 and 3 in NE Spain, in 2009 and 2010; location, crop type, dates of sowing, harvest and straw removal, and details on herbicide applications

Field	Size [ha]	Location	Crop	Row dist. (cm)	Date of sowing	Date of			Herbicide application [g ai ha ⁻¹]			
						Harvest	Straw removal	Date	Pre-emergence	Post-emergence	Date	
2009												
1	5.4	Agramunt	Barley	18	25-10-08	22-6-09	25-7-09		Glyphosate (720) + mesosulfuron-methyl (1200)	18-10-08	No herbicides	-
2010												
1	5.4	Agramunt	Wheat, triticale	18	23-10-09	25-6-10	30-7-10		Glyphosate (720) + mesosulfuron-methyl (1200)	16-10-09	No herbicides	-
2	2.1	Vilanova de Bellpuig	Barley	20	29-10-09	25-6-10	25-7-10		Glyphosate (540) + MCPA* (200)	25-10-09	Florasulam (22) + 2,4-D** (1050)	30-1-10
3	6.1	Vilanova de Bellpuig	Barley	20	29-10-09	25-6-10	25-7-10		No herbicides	-	Florasulam (22) + 2,4-D** (1050)	30-1-10

* 2-methyl-4-chlorophenoxyacetic acid; ** 2,4-dichlorophenoxyacetic acid

one or two individuals per field, but these were omitted. The size of the dispersal units, that is, seeds with hulls, fruits or other structures naturally attached to the seed (except awns or hairs), were determined for 18 seeds per species, using an ocular micrometer.

Weed seed shed

In each field, an area of 50 m × 50 m was selected at least 5 m from the nearest field margin. Thirty-six seed traps were placed in each area, organized in nine transects with four traps per transect. In the case of field 3, a 25 m × 100 m area was selected, because not the entire area had been treated with herbicides in October 2009, causing weed densities to be uncharacteristically high in 2010. The selected area was such that it fit inside the herbicide-treated area. Here, traps were placed along two transects with 18 traps per transect. The distance between transects was always 5 m and between traps within transects 10 m. Traps were emptied once per week, and collected seeds were stored in plastic bags until further processing. In the case of excessive rainfall, the contents of the traps were poured through a sieve and the seeds dried in laboratory for 4 h at 40 °C. Measurements started on 29 April in 2009 (field 1) and on 13 April in 2010 (all fields), prior to the onset of weed seed shed. The experiment was terminated shortly after harvest on 22 June 2009 (field 1) and 27 June 2010 (all fields). Crop harvest stopped weed seed production altogether; weed plants were either cut during harvest or smothered by the straw left on the field. Soil moisture after harvest is usually insufficient to induce another flush of weed seedlings.

Seed traps were modified after Westerman et al. (2003) and consisted of two aluminium trays (25 cm × 13 cm × 3 cm; 1 × w × h; total trap surface 0.0645 m²) placed next to each other between two crop rows on the soil surface and fixed in position by nails. Each trap was covered by small-mesh (6 mm) metal netting to keep out vertebrates. The external walls of the trays were coated with fluon (Polytetrafluoroethylene, BioQuip Products Inc. Rancho Dominguez, CA, USA) to keep out insects. To measure seed shed during harvest, traps were dug into the soil a few days before harvest, such that the top of each trap was flush with the soil surface. Traps and their contents were retrieved immediately after harvest. Seeds of most species entered the traps without difficulty. However, bigger seeds, such as those of *Avena sterilis* L. and *B. diandrus*, accumulated on the mesh on top of the trays and these seeds were carefully collected and included in the seed samples. It is possible that some seeds had already been gathered and consumed by seed predators.

Seeds collected were identified and counted. The average number of seeds caught per m² per week, *Y*, and the total number of seeds per m² for the entire season were

Table 2 Densities for the main weed species found in fields 1, 2 and 3, in NE Spain, in 2009 and 2010 (plants m⁻²; mean ± SE). Data for 2009 are based on 30, 0.4 m² quadrats (0.4 m × 1.0 m), and for 2010 on 25, 20 and 20, 0.4 m² quadrats in fields 1, 2 and 3, respectively

Years	Density (mean ± SE) [m ⁻²]			
	2009		2010	
Weed species field	1	1	2	3
<i>Galium spurium</i>	7.2 ± 1.7	1.6 ± 0.4	–	–
<i>Bromus diandrus</i>	99.1 ± 8.8	9.2 ± 3.3	–	–
<i>Lolium rigidum</i>	0.2 ± 0.1	–	1.6 ± 1.0	9.4 ± 1.1
<i>Avena sterilis</i>	–	–	3.6 ± 0.6	20.3 ± 2.7
<i>Diptotaxis erucoides</i>	–	–	–	27.9 ± 4.4
<i>Papaver rhoeas</i>	35.5 ± 5.5	24.6 ± 2.7	–	–
<i>Chenopodium album</i>	2.8 ± 1.0	11.3 ± 2.0	2.3 ± 1.4	–
<i>Anacyclus clavatus</i>	17.2 ± 2.6	–	4.1 ± 1.0	–
<i>Kochia scoparia</i>	–	4.8 ± 1.4	–	2.4 ± 2.4

estimated for each weed species. To match seed shed with the seed removal rate, seed shed per two days, Y_i , was calculated as $Y \times 2/7$ (m⁻²) for each 2 days period from mid-April until and including harvest, assuming that the daily seed shed was constant within each collection period.

Weed seed removal rate

Seed removal was measured once per month from April to June 2010, as the percentage of seed removal per two days averaged over 25 feeding stations per weed species per field. Farmers would not allow trials to interfere with management, and, therefore, seed removal rates could not be determined after crop harvest. As with seed shed, seed removal was measured in 50 m × 50 m areas. All areas were at least 2 m away from the selected areas in which seed shed was measured and 5 m away from the nearest field edge. Because of its unusual shape and the fact that it had to accommodate four times as many feeding stations as the other fields, two areas were selected in field 1: 1a (50 m × 90 m) and 1b (50 m × 30 m). Forty feeding stations were placed in area 1a (40), organized in four rows and ten columns, and 60 stations were placed in area 1b, organized in six rows and ten columns. In fields 2 and 3, 25 feeding stations were organized along a regular grid of five rows and five columns. The distance between feeding stations was always 10 m. The experimental unit is the feeding station.

In field 1, 25 feeding stations each were filled with 2 g of *Lolium rigidum* (Gaud.) (2.59 ± 0.043 mg seed⁻¹, Herbiseed, Reading, UK), *Papaver rhoeas* L. (0.0118 ± 0.002 mg seed⁻¹, Herbiseed, Reading, UK), *Galium spurium* L. (0.61 ± 0.007 mg seed⁻¹, Herbiseed, Reading, UK) or *Bromus diandrus* Roth (13.183 ± 1.1 mg seed⁻¹, collected in Agramunt in 2009) to allow testing for seed preference. *Lolium rigidum* is readily taken by harvester ants and

other seed predators (Baraibar et al. 2009) and is naturally present in the area. The other species were among the most abundant weed species in field 1 in 2009. The distribution of seed species was chosen randomly from the available positions in areas 1a and 1b. In fields 2 and 3, feeding stations were filled with 2 g of *L. rigidum*. All seeds were dried for 4 h at 40 °C prior to weighing, to standardize seed weight.

Each feeding station consisted of one half of a plastic, 9-cm-diameter Petri dish with two 15-mm-wide openings in the sides to facilitate entry to harvester ants and other invertebrates. Previous studies had indicated that two openings were more than sufficient to allow full utilization of the available seeds (Díaz 1992; Baraibar et al. 2009). Dishes were covered by 1-cm mesh metal cages (10 cm × 11 cm × 3 cm) and served to keep out vertebrates, if any. Feeding stations were installed on 13 April, 26 May and 25 June 2010. Two days later, the remaining seeds were retrieved, dried and weighed. Seed removal rates were calculated as the weight of seeds removed per two days, relative to the initial amount provided. Seed removal rates for each two-day interval from April to June were obtained via linear interpolation.

Statistical analysis

The weight of seeds recovered per feeding station was analysed to test for the effect of weed species and sampling date on seed removal rate in field 1 in 2010. A regression model (GLM) was used with a logit link and a binomial variance function allowing for overdispersion (Genstat 11; Genstat 5 Committee 1993). Significance was evaluated in terms of mean deviance ratios, which were, in turn, evaluated by comparison with *F*-distributions ($\alpha = 0.05$). For significant effects, the *t* test was used to rank means. Similarly, the weight of *L. rigidum* recovered per feeding station in all three fields in 2010 was analysed to test for differences between fields.

Long-term seed removal rates

Many weeds have extended periods of seed shed. As a result, seeds that are produced on weed plants become available to epigeaic seed predators gradually. The longer the seeds are exposed on the soil surface, the higher the probability that they will be consumed by granivores. Once covered by soil or debris, they have a very low probability of being found and consumed. The speed with which seeds are incorporated into the soil matrix is influenced by, for example, size, shape and soil conditions (Westerman et al. 2009). Most weed seeds tend to be quickly buried due to their small size. Because of the differential timing of seed shed and seed burial, each seed cohort will be exposed to granivores for a different period of time during the season. At the same time, seed removal rates vary over the season, resulting in cohort-specific seasonal predation rates. Ergo, granivory over an entire season cannot be measured directly as the proportion of seeds removed at the end of the season, but has to be calculated as the mean of predation rates sustained by each of the seed cohorts.

A model that combines information on the timing of seed shed, differences between weed species in the duration of seed exposure, and seasonal variability in seed removal by granivores is available to estimate seasonal predation rates (Westerman et al. 2003). It follows the fate of seeds in each cohort (consumed, buried or on the soil surface) over time, and seed losses per cohort are combined into a single estimate, the long-term removal rate (\bar{M}), via a weighted mean, with cohort size as the weighting factor.

\bar{M} was calculated via \bar{S} , the proportion of newly produced seeds that survive removal; $\bar{M} = 1 - \bar{S}$, with

$$\bar{S} = \frac{\sum_{i=1}^n \left(Y_i \prod_{j=i}^k S_j \right)}{\sum_{i=1}^n Y_i} \quad (1)$$

The denominator in (1) is the total number of seeds produced over the entire period, and the numerator is the total number of seeds not removed by predators. The latter is calculated by subdividing seed production into n 2-day cohorts of size Y_i and following their fate over time. Survival of each seed cohort was calculated as the product of survival chances per two days, $S_i = 1 - M_i$, during the k , 2-day periods that seeds are exposed to seed predators on the soil surface, with M_i the seed removal rate for the i th time period. For further details on the model, refer to Westerman et al. (2003). The implicit assumption to be able to use the data gathered in this study in model calculations is that proportions based on weight are

equivalent to proportions based on numbers. It was furthermore assumed that both seed shed and seed removal ended immediately after crop harvest. The model was therefore adapted to $\bar{M}_h = 1 - (\bar{S}_h + H)$, where \bar{M}_h is the long-term removal rate from the onset of seed shed until and including crop harvest, \bar{S}_h is the proportion of seeds not removed by granivores between the onset of seed shed and crop harvest, and H is the proportion of seeds shed during harvest. Thus, $\bar{S}_h + H$ serves as an estimate of the long-term seed survival rate, \bar{S} . Species with a peak in seed shed that coincides with crop harvest will have a large H ; species with a low preference by harvester ants (low removal rate) will have higher estimates of \bar{S}_h . Species-specific, long-term removal rates \bar{M}_h were calculated for those species, for which both species-specific seed shed and seed removal data were available, namely for *P. rhoeas*, *G. spurium* and *B. diandrus* in field 1 in 2009 and all four weed species in 2010, and *L. rigidum* in fields 2 and 3 in 2010.

Unfortunately, one of the variables in Eq. 1, namely the mean duration of exposure of seeds on the soil surface ($k \times 2$ -days) was unknown. The exposure time is directly related to seed size; the bigger the seed, the slower the rate of incorporation into the soil matrix. Using a rough extrapolation of the data by Westerman et al. (2009), the mean exposure time for *P. rhoeas* seeds (seed size 1.0 mm) would be 8 days, for *G. spurium* 10 days (1.1 mm), and for *B. diandrus* (length 21.2 mm; width 1.8 mm) and *L. rigidum* (length 6.6 mm; width 1.0 mm) 4 w. Probably, true exposure times were longer, because soils in NE Spain tend to form hard, dry crusts in summer, with little or no cracks. To test the sensitivity of \bar{M}_h to the exposure time, \bar{M}_h was calculated using the removal rates for three of the weed species in field 1 in 2010, while varying the duration of exposure from 2 days after seed shed ($k = i$) to continuous exposure ($k = n$); that is, as if seeds could shrink and expand. These preliminary calculations showed that \bar{M}_h increased with exposure period until it levelled off after 6 (*G. spurium*, *P. rhoeas*) and 10 days (*B. diandrus*) (Fig. 1). Seed removal was so fast that maximal removal ($1 - (\bar{S}_h + H)$) was achieved within 10 days, which corresponds to a seed size of approximately 1 mm. For all seeds larger than 1 mm, the estimate of \bar{M}_h will change little or nothing whether the exposure time was 10 days or longer, because seed burial rate (0.04 2-days^{-1}) could not compete with seed removal rate (between 0.2 and $\approx 1.0 \text{ 2-days}^{-1}$). Only in the case of very small seeds ($< 1 \text{ mm}$, e.g. *P. rhoeas*) could seeds escape into the subsoil (0.17 2-days^{-1}) before they could be removed by ants. Calculations of \bar{M}_h were, therefore, conducted with the appropriate exposure times as estimated above.

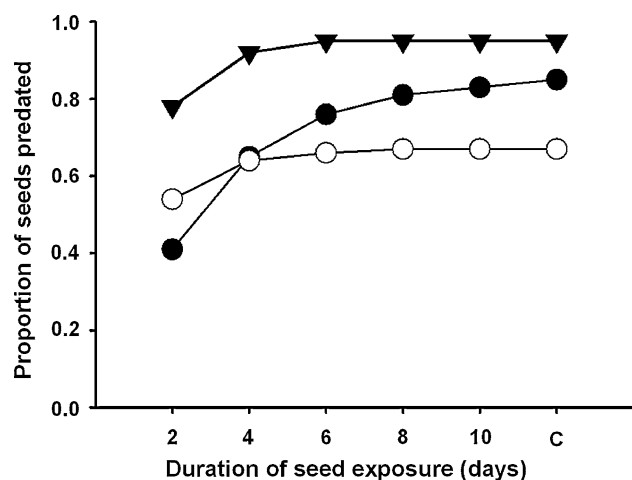


Fig. 1 Proportion of annual seed loss due to removal, \bar{M}_h , as a function of the exposure time in field 1 in 2010 for *Bromus diandrus* (filled circle), *Papaver rhoeas* (open circle) and *Galium spurium* (filled down pointing triangle). 'C' refers to continuous seed exposure

Results

Weed seed shed

The timing of seed shed and the number of seeds produced differed between weed species and fields. Seed shed of *Diplo-taxis erucoides* (L.) DC. started and peaked early, while that of *P. rhoeas*, *B. diandrus* and *A. sterilis* started and peaked late (Fig. 2). No seeds of *Chenopodium album* L. *Anacyclus clavatus* (Desf.) Pers. or *Kochia scoparia* (L.) Roth were collected, despite the fact that these species had been present (Table 2). Seeds of *A. clavatus* were unripe and retained in the seed heads; most plants of *K. scoparia* were seedlings or small, vegetative plants; and plants of *C. album* remained vegetative.

Seed removal by granivores

Regression analysis showed that in field 1, the rate of seed removal increased significantly over time ($p = 0.04$) and did so differently for the four weed species (weed species, $p < 0.001$; interaction weed species \times sampling date, $p < 0.001$). In May 2010, a significantly higher proportion of *G. spurium* (0.7 ± 0.08) and *L. rigidum* (0.9 ± 0.05) and in June a significantly higher proportion of *L. rigidum* (1.0 ± 0.00) and *P. rhoeas* (0.9 ± 0.06) were removed, compared with the other two species (Fig. 3a). In June, the proportion seed removal of *B. diandrus* (0.2 ± 0.07) was significantly lower than that of *G. spurium* (0.6 ± 0.09). Overall, *B. diandrus* was the least and *L. rigidum* the most preferred seed species (Fig. 3a).

Seed removal rates of *L. rigidum* increased significantly over time ($p < 0.001$) (Fig. 3b), but did not differ between

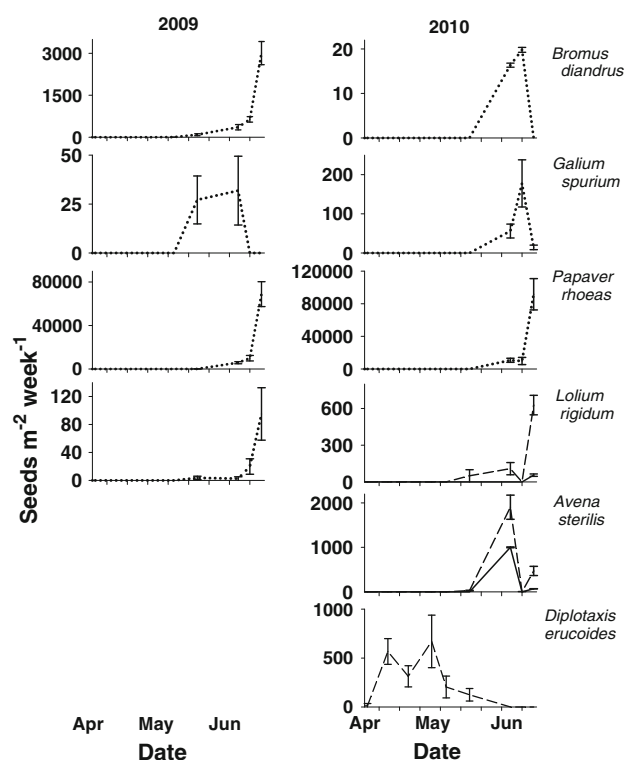


Fig. 2 Weed seed production (seeds $m^{-2} week^{-1}$) as measured in 36 seed traps per selected area, each consisting of two aluminium trays (25 cm \times 13 cm \times 3 cm; 1 \times w \times h; total trap surface 0.0645 m^2), in field 1 (dotted line), field 2 (black line) and field 3 (dotted line) in 2009 (left side) and 2010 (right side), for *Bromus diandrus*, *Galium spurium*, *Papaver rhoeas*, *Lolium rigidum*, *Avena sterilis* and *Diplo-taxis erucoides*. Bars represent standard errors

the three fields ($p = 0.94$), indicating that the estimates were consistent over space. Furthermore, seed removal rates in this study corresponded reasonably well with those obtained for *L. multiflorum* by Baraibar et al. (2009, 2011), suggesting that seed removal patterns are consistent over time as well.

Long-term seed removal rates

Averaged over years, fields and weed species, the long-term seed removal rate, \bar{M}_h , was estimated at 72 % (range 46–100 %) (Table 3). \bar{M}_h was highest for *G. spurium* in both years and lowest for either *P. rhoeas* or *B. diandrus*. Estimates of \bar{M}_h varied between years, causing the ranking of \bar{M}_h for weed species to differ between years as well.

Long-term seed removal rate for *D. erucoides* and *A. sterilis* could not be properly estimated, because no species-specific removal rates had been determined. However, using average removal rates over all four weed species in field 1 and an exposure time of 14 days for *D. erucoides* (seed size 1.3 mm) and 4 w for *A. sterilis* (seed length 15.6 mm; width 2.0 mm), \bar{M}_h would have been 100 % in either case.

Fig. 3 Seed removal rate (proportion seeds 2-days⁻¹) as measured in 25 feeding stations per weed species per field for *Bromus diandrus* (filled circle), *Papaver rhoeas* (open circle), *L. rigidum* (open triangle) and *G. spurium* (filled down pointing triangle) in field C (a) for *Lolium rigidum* in field A (dashed line), field B (black line) and field C (dotted line) (b), and in April, May and June 2010. Bars represent standard errors

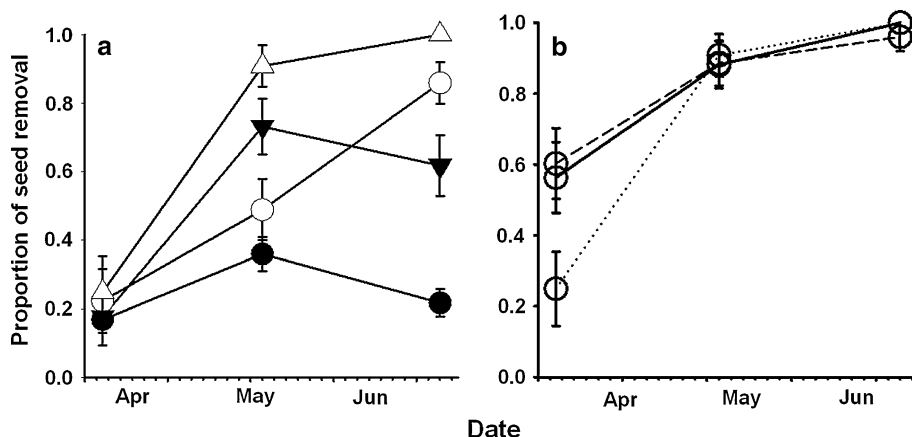


Table 3 Seed production ($\sum_{i=1}^n Y_i$) and the estimated fate of seeds of *Papaver rhoeas*, *Galium spurium*, *Lolium rigidum* and *Bromus diandrus* in field 1 in 2009 and 2010; with \bar{M}_h , the proportion of seeds removed up to the moment of crop harvest; \bar{S}_h , the proportion of seeds not removed up to the moment of harvest; H , the proportion of seeds shed at harvest; and $\bar{S} \approx \bar{S}_h + H$, the long-term seed survival rate

Weed species	$\sum_{i=1}^n Y_i$ [m ⁻²]	\bar{M}_h	\bar{S}_h	H	$\bar{S} = \bar{S}_h + H$
Field 1; 2009					
<i>Papaver rhoeas</i>	84,604	0.51	0.17	0.32	0.49
<i>Galium spurium</i>	59	1.00	0.00	0.00	0.00
<i>Lolium rigidum</i>	114	0.67	0.02	0.31	0.33
<i>Bromus diandrus</i>	4,089	0.46	0.25	0.29	0.54
Field 1; 2010					
<i>Papaver rhoeas</i>	112,130	0.67	0.13	0.20	0.33
<i>Galium spurium</i>	248	0.95	0.02	0.03	0.05
<i>Bromus diandrus</i>	36	0.85	0.15	0.00	0.15
<i>Lolium rigidum</i> ; 2010					
Field 2	56	0.60	0.00	0.40	0.40
Field 3	598	0.75	0.00	0.25	0.25

The proportion of seeds shed at crop harvest

In field 1, the proportion of seeds shed during harvest, H , varied from 0 to 0.32 in 2009 and from 0 to 0.40 in 2010 (Table 3). Weed species that dispersed a large proportion of their seeds during harvest were *L. rigidum* ($H = 0.25-0.40$) and *P. rhoeas* ($H = 0.20-0.32$). In contrast, *G. spurium* in 2009 ($H = 0$), and *G. spurium* ($H = 0.03$), *B. driandrus* ($H = 0$), *D. erucoides* ($H = 0$) and *A. sterilis* ($H = 0.09$ and 0.05) in 2010 shed all or almost all of their seeds prior to crop harvest.

The effect of seed preference

The lower seed removal rates obtained for *B. diandrus* (Fig. 3a) translated into higher estimates of \bar{S}_h (0.25 for 2009 and 0.15 for 2010) (Table 3) than for any of the other species. *P. rhoeas* also had relatively high estimates of \bar{S}_h (0.17 for 2009 and 0.13 for 2010) (Table 3). However, these did not originate from lower seed removal rates, but

from a higher seed burial rate, which is equivalent to a shorter exposure period.

Discussion

Crop harvest created good opportunities for weed seeds to escape removal by harvester ants. The proportion of seeds shed at harvest, H , depended solely on the shape of seed dispersal curve relative to the timing of crop harvest. Differences in the estimates of H between years were, therefore, caused by differences in the phenology of the weeds and in harvest date. Advancing or delaying harvest by a few days could have a major impact on the value of H . For example, had crop harvest in 2010 occurred a week earlier, a large proportion of the seeds of *B. diandrus*, *G. spurium* and *A. sterilis* would have been shed during instead of prior to harvest (Fig. 2). However, the timing of crop harvest will have little influence on H of, for example, *D. erucoides*, because the peak in seed shed of this weed

species occurs in May, which is too far off from the date of crop harvest. Only weed species whose phenology is more or less synchronized with that of the crop, such that the timing of weed seed shed is close to crop harvest, may escape predation via crop harvest. Similarly, the timing of crop harvest will not influence H of *P. rhoeas* either, but for entirely different reasons. The capsules that contain the ripe *P. rhoeas* seeds are constructed in such a way that they will retain (most) seeds, as long as they are kept in an upright position. Seeds will be shed when the capsules are upturned, such as during harvest.

Part of the newly shed seeds may still fall victim to granivores, because removal rates, although reduced, were not zero after crop harvest (Baraibar et al. 2009); consequently, H was probably overestimated. Furthermore, the straw that covered the seeds was removed after 4–5 w. Seeds that had not disappeared into the subsoil by then could fall victim to harvester ants again. The estimates of H will be more accurate if crop harvest is immediately followed by tillage. Tillage moves seeds to deeper soil layers and out of reach of harvester ants. Depending on the implement used, only about 0.03–40 % of the seeds will remain on the soil surface (e.g. Mohler et al. 2006; Spokas et al. 2007).

Low preference also provided opportunities to weed species to avoid removal by harvester ants. Seeds of *B. diandrus* were not preferred by harvester ants, as evidenced by much lower 2-day removal rates compared with the three other weed species (Fig 3a). The low removal rates bought the seeds enough time to disappear in the subsoil or to remain on the soil surface untouched by harvester ants until crop harvest. This resulted in relatively high estimates of \bar{S}_h for *B. diandrus*. It is unknown why seeds of *B. diandrus* were not preferred; they should be attractive because they are large seeds with soft seed coats. Maybe they had a low nutritional value or contained toxic secondary metabolites; maybe they were difficult to transport because of chaff and awns (Hulme and Benkman 2002). Harvester ants were frequently observed removing the awns before transportation of the seeds. Larger workers with stronger jaws were required for this job (B. Baraibar and P. R. Westerman, pers. obs.), thus limiting foraging efficiency.

The mechanism that is usually responsible for preventing seed removal by granivores in temperate regions, namely seed burial into the soil matrix (Westerman et al. 2006, 2009), was largely ineffective in NE Spain; removal rates by harvester ants were simply too high. Only in the case of *P. rhoeas* were seeds small enough that the burial rate could compete with the removal rate. A certain proportion of seeds of *P. rhoeas* could escape into the soil matrix before harvester ants could gather them. This too resulted in relatively high estimates of \bar{S}_h .

Given their size, the mean exposure time on the soil surface of *A. sterilis* seeds should at least be 4 w, which should result in complete removal by harvester ants. However, many grass species, including *A. sterilis*, have a hygroscopically active awn, which propels the seed into cracks and indentions in the soil (Peart 1979). With every wetting–drying cycle, such as those occurring during the early morning hours, a seed can move a centimetre or more from its original location. Once stuck in an indentation or crack, the seed is anchored firmly on the microsite (Peart 1979) and may even propel itself into a crack, if present (Westerman, pers. obs.). The hygroscopically active awn could, therefore, constitute another way that seeds could avoid removal by harvester ants, simply by disappearing into the subsoil. However, this possibility needs to be confirmed in the field.

Over all weed species, fields and years, the long-term weed seed removal rate, \bar{M}_h , averaged 72 % (range 46–100 %). With these values, seed removal in NE Spain is among the highest ever recorded on arable fields. Similar rates have only been obtained for harvester ants in natural systems, such as deserts (e.g. Whitford 1978). As suspected, weed species that were currently difficult to control chemically because of herbicide resistance or tolerance, such as *B. diandrus*, *P. rhoeas* and *L. rigidum*, also employed one or more strategies to avoid, escape or prevent removal by harvester ants. Nevertheless, estimates of \bar{M}_h still ranged from 46 to 85 % for *B. diandrus*, 40–75 % for *L. rigidum* and 51–67 % for *P. rhoeas*. This suggests that harvester ants will eliminate at least an important part of these troublesome weeds. Apart from *G. spurium*, not many ‘non-problematic’ weeds were included in this study. However, the estimate of \bar{M}_h for *G. spurium* (95–100 %) suggests that such species could be fully controlled by harvester ants and other granivores.

In general, weeds that have seeds larger than 1 mm, that are preferred by granivores that have the peak in seed dispersal some time before crop harvest run the biggest risk of losing all newly produced seeds to granivores in rain-fed cereals in NE Spain. Losing or eliminating harvester ants from these fields would increase problems with the control of *B. diandrus*, *L. rigidum* and *P. rhoeas* and would cause many more weed species to become problematic and difficult to control.

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