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Management Options for Multiple Herbicide–Resistant Corn Poppy (*Papaver rhoeas*) in Spain

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Corn poppy is the most widespread broadleaf weed infesting winter cereals in Europe. Biotypes that are resistant (R) to both 2,4-D and tribenuron-methyl have evolved in recent decades, thus complicating their chemical control. In this study, field experiments at two locations over three seasons were conducted to evaluate the effects of different weed management strategies on corn poppy resistant to 2,4-D and tribenuron-methyl, including crop rotations, delayed sowing and different herbicide programs. After 3 yr, all integrated weed management (IWM) strategies reduced the initial density of corn poppy, although the most successful strategies were those which either included a suitable crop rotation (sunflower or field peas), or had a variation in the herbicide application timing (early POST or combining PRE or early POST and POST). The efficacy of IWM strategies differed between both locations, possibly due to different population dynamics and the genetic basis of herbicide resistance. Integrated management of multiple herbicide–resistant corn poppy is necessary in order to reduce selection pressure by herbicides, mitigate the evolution of new R biotypes, and reduce the weed density in highly infested fields.

Nomenclature: 2,4-D; tribenuron-methyl; corn poppy, *Papaver rhoeas* L. PAPRH; sunflower, *Helianthus annuus* L.; field pea, *Pisum sativum* L.

Key words: Chemical program, crop rotation, herbicide management, integrated weed management strategy.

Weeds are a major cause of yield losses because they compete with crops for nutrients, water, and light (Oerke 2006). Herbicides are the principal tool used for weed control in modern agriculture, and they are highly effective on most weeds, but are not a complete solution to the complex challenge that weeds represent (Harker and O'Donovan 2013). The overuse of herbicides imposes strong selection for any trait enabling plant populations to survive and reproduce under recurrent herbicide pressure. This has contributed to the worldwide evolution of herbicide resistance in weeds. Herbicide resistance causes higher crop yield losses, weed-seed contamination, reduced land values, increased mechanical and cultural weed management costs, and additional expense of eventual alternative herbicides and/or cropping systems for managing herbicide-resistant populations (Norsworthy et al. 2012). The best way to prevent the evolution of herbicide-resistant weeds is to implement diversified cropping systems with less frequent herbicide use by employing nonchemical weed management practices (Beckie 2006).

Corn poppy is a major weed of arable crops in southern Europe (Délye et al. 2011; Torra et al. 2011). Its competitive nature, which can decrease cereal yields up to 32% (Torra and Recasens 2008), makes it especially troublesome in winter cereals. The ability of this species to invade, grow, and remain in arable fields can be attributed to several factors; the development of a persistent seedbank, an extended germination period, and high seed production (Torra and Recasens 2008). Corn poppy is a growing problem due to the appearance of herbicide-resistant biotypes to synthetic auxins and/or to acetolactate synthase (ALS) inhibitors. In Spain, poor corn poppy control with 2,4-D was first reported in 1992 (Taberner et al. 1992), and then a biotype resistant to both 2,4-D and tribenuron-methyl was reported in 1998 (Claude et al. 1998). Resistance to ALS inhibitors was initially attributed exclusively to mutant ALS alleles (Délye et al. 2011; Kaloumenos et al. 2009; Marshall et al. 2010), though recently the presence of non–target site resistance mechanisms has been demonstrated for some biotypes (Délye et al. 2011). In Spain, the resistance to tribenuron-methyl is due to a single point substitution of Pro by Ala, Arg, His, Leu, Thr, and Ser in codon 197 of the ALS gene (Durán-Prado et al. 2004; Rey-Caballero et al. in press). Reduced 2,4-D translocation in resistant (R)

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corn poppy plants has most recently been described as the resistance mechanism in this species (Rey-Caballero et al. 2016).

Herbicides alone are not always enough to control herbicide-resistant corn poppy populations; therefore, the development of new management tools is required. Chemical control strategies should be combined with nonchemical ones in an integrated weed management (IWM) program. Furthermore, this program should then be specifically designed and tested for each region (Powles and Bowran 2000). Various chemical and nonchemical tools have been analyzed to control herbicide-resistant weeds. Crop rotation is stated to be one of the best management options for preventing the evolution of herbicide-resistant weeds, because it allows for the introduction of herbicides having different modes of action (MOAs) (Vencill et al. 2012). This option provides farmers with opportunities to employ variable crop life cycles, sowing dates, harvest dates, and tillage and weed management practices to restrict the evolution of weeds adapted to monocultures (Liebman and Staver 2001). Specific crop rotations have been proposed to manage several herbicide-resistant weeds like blackgrass (*Alopecurus myosuroides* Huds.) (Moss et al. 2007), rigid ryegrass (*Lolium rigidum* Gaud.) (Busi and Powles 2013), or wild oat (*Avena fatua* L.) (Harker et al. 2009). Mechanical control by plowing is generally considered to be an effective method for displacing a proportion of the seeds to nonoptimal germination conditions, but this method should not be repeated for a few years for corn poppy, because seeds moving back up through the soil strata could germinate due to their high capacity for-survival (Cirujeda et al. 2003). Harrowing is also a good technique for the management of corn poppy, but its efficacy is highly dependent on the initial plant densities (Cirujeda et al. 2003; Torra et al. 2011). Delayed sowing (by 3 mo) and different fallows (physical and chemical) conducted in Spain showed their effectiveness in reducing corn poppy densities, but only when combined with other control methods like chemical control or cultivation (Torra et al. 2011). The results observed in Spanish winter cereals indicate that corn poppy populations resistant to 2,4-D and/or tribenuron-methyl can be controlled by application of PRE or POST herbicides with alternative MOAs (Torra et al. 2010); however, their long-term effects on corn poppy in an IWM strategy have not yet been researched. Moreover, crop rotations or variation of herbicide application timings between years still remain to be studied. Such knowledge is necessary to implement and design effective IWM strategies, particularly in the context of the present scenario, with no new MOA discovered in recent decades (Duke 2012) and considering that some of the herbicides that are currently successful in controlling corn poppy will not be available in the future. Furthermore, these studies are relevant to the European Directive 128/2009 (applicable in Spain since 2014), which obligates farmers to implement the principles of integrated pest management.

This study was thus conducted in order to: (1) characterize the herbicide resistance patterns of the corn poppy populations researched, and (2) study the effectiveness of several IWM strategies (with different crop rotations, sowing dates, and herbicide programs) over 3 yr to control corn poppy herbicide-resistant populations in winter cereals, while providing new data on the effects of individual methods that can later be combined in IWM programs.

Materials and Methods

Sites Description. Field trials were established in two commercial winter cereal fields with high corn poppy infestations in the province of Lleida in northeastern Spain. The first site was in Baldomar (Location 1, L-1) (41°54′N, 1°0′E), at an elevation of 334 m. The soil was silty clay loam (48.2% sand, 15% clay, and 36.8% silt), with a pH of 8.2 and organic matter content of 2.5%. The second site was in Sant Antolí (Location 2, L-2) (41°37′N, 1°19′E), at 581 m. The soil was silty clay loam (25.2% sand, 23.4% clay, and 51.4% silt), with a pH of 8.1 and organic matter content of 2.8%. In the years preceding these trials, the fields were under a monocrop of winter cereals, managed with minimum tillage (one or two cultivator passes). Selective POST herbicides (florasulam + 2,4-D in L-1; iodosulfuron-methyl + mesosulfuron-methyl alternating with florasulam + 2,4-D in L-2) had been employed for weed control during recent years at both sites.

Characterization of the Herbicide Resistance. Seeds from the two experimental sites were collected and stored during summer 2012. In autumn, dose–response experiments were conducted with L-1 and L-2 populations together with one susceptible (SC) population from a seed dealer (Herbiseed, Twyford, UK). Seeds were sterilized in a 30% hypochlorite solution and sown in petri dishes with 1.4% agar supplemented with 0.2% KNO_3 and 0.02% gibberellin. Petri dishes were placed in a growth chamber at 20/10 C day/night and a 16-h photoperiod under 350 μmol photosynthetic photon-flux density $m^{-2}s^{-1}$.

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After 14 d, seedlings were transplanted to 8 by 8 by 8 cm plastic pots filled with a mixture of silty loam soil 40% (w/v), sand 30% (w/v), and peat 30% (w/v). Five seedlings were transplanted per pot, and were later thinned to three per pot. In the potentially R populations, at the 5- to 6-leaf stage (5 to 6 cm), the ALS inhibitors tribenuron-methyl (tribenuron-methyl 500 g ai kg^{-1}, WSG) and florasulam (florasulam 22.8 g ai L^{-1}, WG) were applied at 0, 4.6, 9.3, 18.7 (1× the field rate), 37.5, 75, 150, 600, and 1,200 g ai ha^{-1}, and 0, 0.9, 1.8, 3.7, 7.5 (1×), 15, 60, 240, and 480 g ai ha^{-1}, respectively. The 2,4-D (2,4-D ethyl-hexyl 600 g ai L^{-1}, EC) was applied at 0, 75, 150, 300, 600 (1×), 1,200, and 4,800 g ai ha^{-1}. SC plants were sprayed at the same growth stages at 0, 0.25, 0.5, 1.1, 2.3, 4.6, 9.3, and 18.7 g ai ha^{-1} of tribenuron-methyl; 0, 0.1, 0.2, 0.4, 0.9, 1.8, 3.7, and 7.5 g ai ha^{-1} of florasulam; or 0, 9.3, 18.7, 37.5, 75, 125, 150, 300, and 600 g ai ha^{-1} of 2,4-D. Four replicates were included for each dose. Herbicides were applied using a precision bench sprayer delivering 200 L ha^{-1} at a pressure of 215 kPa. Pots were placed in a greenhouse at University of Lleida, Spain (41°37′43.1″N, 0°35′52.6″E) and were watered regularly. At 4 wk after treatment, plants from each dose were harvested (aboveground). Samples were dried at 65 C for 48 h, and the dry weights were measured. The experiment was repeated twice.

Integrated Weed Management Assessments. Field experiments were carried out during three consecutive cropping seasons (2011 to 2012, 2012 to 2013, and 2013 to 2014) to evaluate the effect of eight different weed management strategies on two herbicide-resistant corn poppy populations.

The experimental design was a complete randomized block with three replicates and eight plots (10 by 10 m). In each locality, the eight management strategies implemented were: (1) traditional (TRAD), wheat (*Triticum aestivum* L.) monocrop with POST chemical control; (2) herbicide rotation (HROT), wheat monocrop with POST chemical control (active ingredient rotation); (3) early POST (EAPOST), wheat monocrop with chemical control (active ingredient rotation and application timing rotation); (4) two herbicide applications (2APPL), wheat monocrop with chemical control (two herbicide applications: the first, PRE and POST, and the third season, early POST and POST, with active ingredient rotation and application timing rotation); (5) rapeseed (*Brassica napus* L.) rotation (OSR), wheat–rapeseed–wheat with chemical control; (6) field pea rotation (FPR), wheat–field pea–wheat with chemical control; (7) sunflower rotation (SFLR), wheat–sunflower–wheat with chemical control; (8) seed delay (DLY), wheat monocrop with seed delay in the first and third seasons (almost 1 mo) and chemical control (active ingredient rotation). A 4-m corridor was left between plots. Sowing rates were 200 kg ha^{-1} for wheat 'Berdún,' 4 kg ha^{-1} for rapeseed 'Arsenal,' 180 kg ha^{-1} for field peas 'Enduro,' and 9 kg ha^{-1} for sunflower 'Limasun.' In 2011 to 2012, the sowing dates for each crop were October 26 and 30 for wheat, and November 30 and 28 for wheat under the DLY strategy in L-1 and L-2, respectively; in 2012 to 2013, wheat was sown on October 25 and 30 in L-1 and L-2, respectively, while rapeseed was sown on October 10, field peas on November 15, and sunflower on April 29 in both localities; in 2013 to 2014, wheat was sown on October 22 and November 4, and November 26 and December 26 for wheat under the DLY strategy in L-1 and L-2, respectively. Herbicide applications were applied with a backpack plot sprayer using a 2-m-wide boom calibrated to deliver 300 L ha^{-1} of water at 253-kPa pressure. All details about the herbicide applications are summarized in Table 1. Agronomic practices were the usual for each crop in the area of study. For all crops, seedbed preparation was done with one or two cultivator passes. For each season, fertilizer was applied before sowing at 70 and 100 UPN (units of fertilizer, N or P) for cereals and rapeseed, respectively, and at 100 UPN in February.

Corn poppy density was counted monthly, from sowing to harvest, by randomly throwing ten 0.10-m^2 frames into each plot. Depending on the crop sowing date of each treatment, initial densities were estimated between December and February in each season. These estimations were proxies of the management effects of the preceding season on the corn poppy populations. The 3-yr experiment ended in June 2014 (2013 to 2014 season), but corn poppy densities were also counted at the beginning of the 2014 to 2015 season in January 2015. This sampling was considered as a proxy of the overall cumulative effect of the 3 yr of management strategy application on the corn poppy population.

Statistical Analysis. Data from dose–response experiments were analyzed using a nonlinear regression model. The GR_{50} of plants was calculated using a type 1 four-parameter logistic curve (Seefeldt et al. 1995):

$$y = c + \frac{(d - c)}{1 + \exp[b(\log(x) - \log(GR_{50}))]} \qquad [1]$$

Where c is the lower limit, d is the upper limit, GR_{50} is the herbicide rate required for 50% growth reduction, and b is the slope at GR_{50}. In this equation, the

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Table 1. Herbicide application date, herbicide management, active ingredient (with HRAC group), and rate (g ai ha^{-1}) used for different management strategies in 2011 to 2012, 2012 to 2013 and 2013 to 2014 seasons at Baldomar (L-1) and Sant Antolí (L-2).

	2011–2012		2012–2013		2013–2014	
Management strategy	L-1	L-2	L-1	L-2	L-1	L-2
1. Traditional (TRAD)	January 5	January 9	February 5	February 20	February 18	February 19
	POST Bromoxynil C3 + Ioxynil C3 + MCPP O 210 + 210 + 630		POST Bromoxynil C3 + Ioxynil C3 + MCPP O 210 + 210 + 630		POST Bromoxynil C3 + Ioxynil C3 + MCPP O 210 + 210 + 630	
2. Herbicide rotation (HROT)	January 5	January 9	February 5	February 20	February 18	February 19
	POST Aminopyralid O + Florasulam B 10 + 4.5		POST Bromoxynil C3 + Ioxynil C3 + MCPP O 210 + 210 + 630		POST Aminopyralid O + Florasulam B 10 + 4.5	
3. Early POST application. (EAPOST)	December 5	December 20	February 5	February 20	January 21	February 1
	EAPOST O + O[a] 6 + 5		POST Bromoxynil C3 + Ioxynil C3 + MCPP O 210 + 210 + 630		EAPOST [c] O + O 6 + 5	
4. Two herbicide applications (2APPL)	November 2	November 1	February 5	February 20	December 18	December 18
	PRE Isoxaben L 125				PRE Bifenox E + Isorpoturon C2 680 + 1200	
	January 5	January 9			February 18	February 19
	POST Aminopyralid O + Florasulam B 10 + 4.5		POST Bromoxynil C3 + Ioxynil C3 + MCPP O 210 + 210 + 630		POST Aminopyralid O + Florasulam B 10 + 4.5	
5. Rapeseed rotation (OSR)	January 5	January 9	November 5	October 25	February 18	February 19
			PRE Propyzamide K1 700			
			February 1	February 1		
	POST Aminopyralid O + Florasulam B 10 + 4.5		POST Aminopyralid O + Clopyralid O 6.25 + 127		POST Aminopyralid O + Florasulam B 10 + 4.5	
6. Field pea rotation (FPR)	January 5	January 9	November 15	November 15	February 18	February 19
	POST Aminopyralid O + Florasulam B 10 + 4.5		PRE Pendimenthalin K1 1,365		POST Aminopyralid O + Florasulam B 10 + 4.5	
7. Sunflower rotation (SFLR)	January 5	January 9	April 29	April 29	February 18	February 19
	POST Aminopyralid O + Florasulam B 10 + 4.5		PRE Benfluralin K1 990		POST Aminopyralid O + Florasulam B 10 + 4.5	
8. Seed sowing delay (DLY)	January 5	January 9	February 5	February 20	February 18	February 19
	POST Aminopyralid O + Florasulam B 10 + 4.5		POST Bromoxynil C3 + Ioxynil C3 + MCPP O 210 + 210 + 630		POST Aminopyralid O + Florasulam B 10 + 4.5	

[a] Hormonal mixture containing a new synthetic auxin.

herbicide rate (g ai ha^{-1}) was the independent variable (x) and the dry weight (percentage of the untreated control for each population) was the dependent variable (y). The resistance index (RI) was computed as $GR_{50}(R)/GR_{50}(SC)$.

For the field experiment, the effect of treatments on both initial and final corn poppy densities in each season was tested with linear mixed-effects models (LMM). A preliminary analysis using locality and strategy as fixed factors and repetitions as random factor revealed differences between localities ($P > 0.01$) for initial and final densities. Therefore, these densities were analyzed and presented separately for each location. Within localities, the strategies were established as fixed factors and repetitions as random factors. Corn poppy density data were transformed as needed (log ($x + 1$) or $\sqrt{(x + 0.5)}$) prior to the analysis, because exploratory analysis revealed some nonnormal data distributions and heterogeneity of variances (Zuur et al. 2010). Only in two cases (final densities of L-2 in 2011 to 2012 and 2012 to 2013) were these assumptions not met, so nonparametrical tests (Kruskal-Wallis) were employed. Finally, a post hoc Tukey's pairwise comparison was employed to test differences between strategy means (at $P < 0.05$). Data were back-transformed to the original scale for presentation. Data from management involving PRE treatments or seeding delay were not included in initial corn poppy density analysis, because these interventions disturbed the natural germination pattern of corn poppy seedlings.

The reduction in initial corn poppy densities (seedlings m^{-2}) between 2011 and 2015 (DR) was calculated as:

$$DR = 100 - \left[\frac{(\text{initial density in 2015} \times 100)}{(\text{initial density in 2011})}\right] \quad [2]$$

LMM were conducted with DR values as described above. Data were transformed as needed (arcs[$\sqrt{(x + 0.5)}$]) when normal assumptions were not met. Data were then back-transformed for presentation.

All statistical analyses were carried out with the use of the R programming language (R Development Core Team 2013). The 'drc' package was used for the nonlinear regression (Knezevic et al. 2007), while the 'LME4' (Bates et al. 2014) and 'nlme' (Pinheiro et al. 2014) packages were employed for the LMM analysis. For comparison of weed densities between sampling dates for each cropping system each season, strategy was held as the single factor and the repeated statement option was used in SAS v. 9.0 (PROC NLIN; SAS Institute, Cary, NC).

Results and Discussion

Herbicide Resistance of the Corn Poppy Populations. The presence of multiple herbicide–resistant biotypes was confirmed in both localities. There was no population mortality from L-1 and L-2 at the commercial label rates for the herbicides (Figure 1). The GR_{50} for tribenuron-methyl was 320 and 392 times higher in plants from L-1 and L-2 than in the SC population (Table 2). In addition, cross-resistance between sulfonylureas and triazolopyrimidines was observed in plants at both locations, and L-1 and L-2 biotypes were 24 and 18 times more resistant to florasulam than SC plants (Table 2). High tribenuron-methyl resistance levels and cross-resistance to triazolopyrimidines were also found in Greek corn poppy populations (Kaloumenos et al. 2011). Furthermore, resistance to 2,4-D was confirmed, and plants from L-1 and L-2 were 12 and 13 times more resistant to

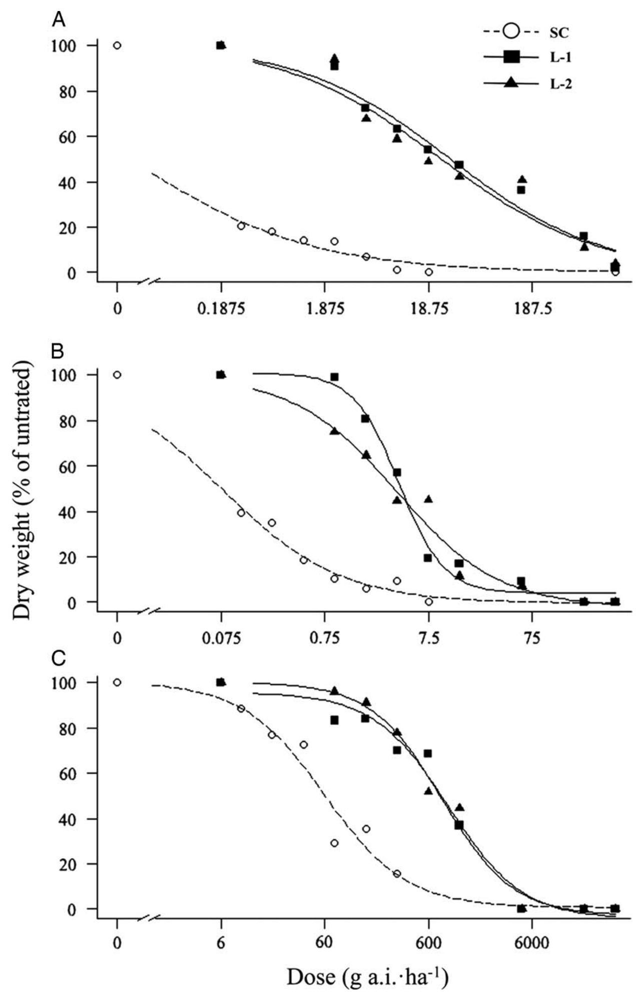

Figure 1. Dose–response regression curves of Baldomar (L-1), Sant Antolí (L-2), and susceptible (SC) corn poppy populations to tribenuron-methyl (A), florasulam (B), and 2,4-D (C) (log scale). Data were expressed as percentage of the mean dry weight of untreated control plants.

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Table 2. Estimated GR_{50}, slope at GR_{50}, and resistance factor (RF) values for Baldomar (L-1), Sant Antolí (L-2), and susceptible (SC) corn poppy populations when sprayed with tribenuron-methyl, florasulam, and 2,4-D.[a]

Population	$GR_{50} \pm SE$ (g ai ha^{-1})	Slope ± SE[b]	Res SS	RI
	Tribenuron-methyl			
L-1	25.2 ± 6.4	0.6 ± 0.1	10,084	320
L-2	30.9 ± 8.1	0.6 ± 0.1	10,609	392
SC	0.1 ± 0.0	0.4 ± 0.1	4,894	—
	Florasulam			
L-1	3.9 ± 0.4	2.0 ± 0.4	3,899	24
L-2	2.9 ± 0.3	0.9 ± 0.1	1,529	18
SC	0.2 ± 0.0	0.7 ± 0.1	21,738	—
	2,4-D			
L-1	816.6 ± 96.0	1.3 ± 0.2	2,872	12
L-2	925.8 ± 156.0	1.0 ± 0.3	5,038	13
SC	68.6 ± 10.2	1.2 ± 0.2	23,693	—

[a] Abbreviations: GR_{50}, ALS inhibitor concentration for 50% reduction of corn poppy dry weight biomass; Res SS, residual sum of square; RI, resistance index.

[b] The slope at GR_{50}.

this herbicide, respectively, than the SC plants (Figure 1; Table 2). Results obtained for a multiple herbicide–resistant Greek biotype established a GR_{50} for 2,4-D of 1,127 g ai ha^{-1} (Kati et al. 2014). In our experiment, these values were 816 and 925 g ai ha^{-1} for L-1 and L-2, respectively.

Corn Poppy Density Changes. At the beginning of the first season (2011 to 2012), the densities within each location were homogenous, and no statistical differences were detected between plots. Initial corn poppy density reached in L-1 was on average 326 seedlings m^{-2}, lower than in L-2, where there was an average of 622 seedlings m^{-2} (Appendix; Tables 3A,B). In this first season, three herbicide management strategies were used (PRE, EAPOST, and POST), and only one cultural management (DLY) was performed. All these treatments significantly reduced the corn poppy density at the end of this season, but the strategy that resulted in the lowest density in both locations was 2APPL, with 3 and <1 plants m^{-2} in L-1 and L-2, respectively (Tables 3A,B). Differences were also found between sampling dates for each system (unpublished data).

Overall, initial density in the second season (2012 to 2013) was lower than initial densities observed in the preceding season (Tables 3A,B; Appendix). In L-2 the 2APPL strategy resulted in a significantly lower density (37 seedlings m^{-2}) than the other management strategies (ranging from 84 to 120 seedlings m^{-2}) (Table 3B). Similarly, in L-1 the 2APPL strategy also resulted in a lower initial density (49 seedlings m^{-2}), but it was not different from densities obtained by other strategies such as DLY, EAPOST, and HROT (54, 66, and 77 seedlings m^{-2}, respectively) (Table 3A). In the second season, one herbicide management strategy was used in cereals (POST), reducing the corn poppy density at the end of the season to an average of 11 plants m^{-2} in L-1 and <1 plant m^{-2} in L-2. The results for the crop rotations at the end of this second season were unequal, FPR (3 and <1 plants m^{-2} in L-1 and L-2, respectively) and SFLR (1 and <1 plants m^{-2} in L-1 and L-2,

Table 3A. Initial and final corn poppy densities means (plants m^{-2}) under different management strategies in 2011 to 2012, 2012 to 2013, 2013 to 2014 and 2015 for data collected at Baldomar (L-1).[a]

	2011–2012		2012–2013		2013–2014		2015	
Management strategy	Initial density	Final density	Initial density	Final density	Initial density	Final density	Initial density	DR[b]
1. TRAD	320 (a)	27 (cb)	144 (a)	18 (a)	367 (a)	29 (a)	276 (ab)	33.3 (a)
2. HROT	275 (a)	29 (a)	77 (bc)	10 (cba)	207 (b)	12 (b)	183 (b)	41.7 (a)
3. EAPOST	285 (a)	20 (cb)	67 (b)	10 (cba)	141 (bc)	11 (b)	72 (dc)	74.6 (b)
4. 2APPL	—[c]	3 (d)	49 (b)	7 (db)	—	0.2 (d)	42 (d)	81.9 (b)
5. OSR	267 (a)	27 (a)	—	13 (ba)	612 (d)	4 (dc)	294 (a)	20.6 (a)
6. FPR	281 (a)	20 (ba)	—	3 (dc)	175 (bc)	2 (dc)	98 (dc)	65.7 (b)
7. SFLR	295 (a)	38 (a)	115 (ac)	1 (d)	102 (c)	0.5 (d)	82 (dc)	72.1 (b)
8. DLY	—	9 (dc)	55 (b)	11 (ba)	—	6 (cb)	102 (c)	65.8 (b)

[a] Data are back-transformed means used for the LMM. Sampling dates included in the statistical analysis. Initial density: season 2011–2012: December 20, 2011; season 2012–2013: January 9, 2013; season 2013–2014: January 21, 2014; and in 2015: January 15, 2015. Final density: season 2011–2012: May 3, 2012; season 2012–2013: May 8, 2013; season 2013–2014: May 27, 2014.Means within a column followed by the same letter indicate that no significant difference ($P < 0.05$) was detected by means of the Tukey (HSD) test at the 5% level of probability

[b] DR: reduction (%) in corn poppy densities between December 2011 and January 2015 for the different management strategies.

[c] Initial density data from those strategies with any intervention that avoided the natural germination pattern of corn poppy seedlings (seed sowing delay and PRE treatments) were not included in the analysis.

Table 3B. Initial and final corn poppy densities means (plants m^{-2}) under different management strategies in 2011 to 2012, 2012 to 2013, 2013 to 2014, and 2015 for data collected at Sant Antolí (L-2).[a]

	2011–2012		2012–2013		2013–2014		2015	
Management strategy	Initial density	Final density	Initial density	Final density	Initial density	Final density	Initial density	DR
1. TRAD	617 (a)	1.5 (ba)	84 (a)	0.3 (a)	201 (a)	11 (ba)	57 (ab)	90.5 (ab)
2. HROT	666 (a)	1.2 (ba)	99 (a)	0.9 (a)	229 (ab)	11 (ba)	56 (ab)	91.7 (ab)
3. EAPOST	617 (a)	0.9 (b)	94 (a)	0.3 (a)	118 (c)	12 (ba)	57 (ab)	90.2 (b)
4. 2APPL	—	0.3 (b)	37 (b)	0.3 (a)	—	1 (c)	24 (c)	95.7 (a)
5. OSR	627 (a)	2.1 (ba)	—	8.9 (b)	320 (b)	14 (a)	87 (a)	84.9 (c)
6. FPR	621 (a)	1.8 (ba)	—	0.3 (a)	128 (c)	4 (cb)	44 (bc)	92.6 (ab)
7. SFLR	588 (a)	1.5 (ba)	92 (a)	0.3 (a)	233 (ab)	12 (ba)	57 (ab)	89.4 (bc)
8. DLY	—	8.3 (a)	120 (a)	0.3 (a)	—	12 (a)	82 (a)	87.9 (bc)

[a] Data are back-transformed means used for the LMM. DR: reduction (%) in corn poppy densities between December 2011 and January 2015 for the different management strategies. Sampling dates included in the statistical analysis. Initial density: season 2011–2012: December 20, 2011; season 2012–2013: January 9, 2013; season 2013–2014: February 10, 2014; and in 2015: January 15, 2015. Final density: season 2011–2012: May 3, 2012; season 2012–2013: May 8, 2013; season 2013–2014: May 27, 2014. Means within a column followed by the same letter indicate that no significant difference ($P < 0.05$) was detected by means of the Tukey (HSD) test at the 5% level of probability

[c] Initial density data from those strategies with any intervention that avoid the natural germination pattern of corn poppy seedlings (seed sowing delay and PRE treatments) were not included in the analysis.

[d] Due to the abundance of zeros, nonparametric tests were conducted with 2011–2012 and 2012–2013 final density data in L-2.

respectively) also significantly reduced the number of plants, while OSR was the management strategy that resulted in the highest densities in May 2013 (13 and 9 plants m^{-2} in L-1 and L-2, respectively) (Tables 3A,B). Significant differences in plant densities were found between sampling dates for each strategy (unpublished data).

The analysis of the initial corn poppy density in the third season (2013 to 2014), revealed that in L-1, the OSR rotation resulted in the highest density, ranging between 540 and 686 seedlings m^{-2}. In contrast, the SFLR strategy was the management strategy that resulted in the lowest initial corn poppy density (102 seedlings m^{-2}), although this was not statistically different from that observed in other management strategies (FPR and EAPOST) (Table 3A). In L-2 the strategies that had a lower initial density were EAPOST and FPR, with mean values of 118 and 128 seedlings m^{-2}, respectively. OSR was the strategy with the highest number of seedlings (320 seedlings m^{-2}) at the beginning of the third season, and no significant differences were found between this management strategy and others (Table 3B). These results highlight the relevance of crop management with regard to corn poppy and the importance of avoiding incorporation of seeds into the soil so as to achieve effective management in the mid- to long-term for herbicide-resistant weeds (Norsworthy et al. 2012). Finally, significant differences in plant densities were found between sampling dates for each IWM strategy (unpublished data).

With <1 plants m^{-2} in both locations, the 2APPL strategy had the lowest densities at the end of the third season. TRAD in L-1 and OSR in L-2 were the management strategies with the highest populations in May 2014: 29 and 14 plants m^{-2}, respectively (Tables 3A,B).

Three-Year Assessment of Weed Management Strategies. The initial density evaluated in 2015 before any herbicide application reflects the cumulative effect of the three preceding seasons for the different management strategies evaluated. Data collected in both locations showed that of all the different management strategies, those that included suitable crop rotation, such as SFLR or FPR, or those that introduced a modification to herbicide timing (2APPL and EAPOST), recorded the lowest initial corn poppy densities after 3 yr (Tables 3A,B). The favorable results observed for SFLR can be explained by the sowing date used for this crop, which contributed to the suppression of the emergence of a great number of corn poppy plants. Sunflower sowing begins in April, and corn poppy emergence in semi-arid Mediterranean conditions occurs mainly in autumn and winter (Cirujeda et al. 2008). For this reason, seedbed preparation and crop sowing break the weed life cycle, thus eliminating almost all corn poppy plants. Despite the significant reduction in corn poppy density, limited rainfall in dryland fields of northeastern Spain hinders the integration of sunflower into the rotation. However, in other areas of Spain with higher rainfall

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and where herbicide-resistant corn poppy is present, this crop rotation would be a viable option for implementation. Introducing crop rotation is the first cultural practice that should be considered for farmers managing herbicide-resistant weeds regardless of the cropping system (i.e., Harker et al. 2009; Moss et al. 2007). Results obtained with the FPR strategy were achieved mainly due to the use of pendimethalin in PRE. This herbicide has been proposed as one of the best chemical options for herbicide-resistant corn poppy control in Spanish dryland areas (Torra et al. 2010). The use of an FPR could be improved using spring varieties of field peas, which again would disrupt the corn poppy life cycle.

Regarding the management strategies that introduced an herbicide timing modification (2APPL and EAPOST), it is hypothesized that early applications (both PRE and EAPOST) provide better control because variability in weed phenology at application time can be avoided compared with POST treatments, when densities are high. Finally, it was proposed that drastic measures may be necessary in fields that are highly infested with herbicide-resistant weeds (Cirujeda and Taberner 2009). The 2APPL results reveal this strategy as a serious option in fields where corn poppy densities are very high and difficult to control.

A sowing delay of 1 mo did not improve corn poppy control within a season when compared with the other strategies with normal sowing dates (Tables 3A,B). An extended sowing delay is most likely necessary for improving the management of this weed due to its broad emergence, which can last from December to March (Cirujeda et al. 2008). The use of cereal varieties with short life cycles and delaying the sowing time by 3 mo was proposed as a management option for increasing corn poppy seedbank depletion (Torra et al. 2011). OSR was also inefficient in the management of corn poppy in this study. This strategy resulted in higher initial densities in 2015, as in 2013 to 2014, especially in L-1, where an average of 294 seedlings m^{-2} were present (Table 3A). Rapeseed requires early sowing in September, extending the emergence period of corn poppy, and thus not disrupting its life cycle. Moreover, rapeseed is not a competitive crop in its early life stages, and a small number of herbicides are available for the control of dicotyledonous weeds, especially POST. Finally, the TRAD management strategy did not provide effective control (276 and 57 seedlings m^{-2} in L-1 and L-2, respectively), especially in L-1 (Table 3A). At high corn poppy densities, even if the timing of POST application is optimal, some large corn poppy individuals will survive the herbicide application. These few surviving plants can be enough to replenish the seedbank due to their high fecundity (Torra and Recasens 2008).

After 3 yr of management, it was possible to reduce corn poppy infestation levels in both locations (from the end of 2011 until early 2015). Depletion by 57% on average was observed in L-1, and 90% in L-2 (Tables 3A,B). In L-1, 2APPL (81%), EAPOST (74%), SFLR (72%), FPR (65%), and DLY (65%) were the strategies that led to a more drastic reduction of the initial corn poppy densities, and these percentages were significantly different from those obtained by the other management strategies: HROT (41%), TRAD (33%), or OSR (20%) (Table 3A). In L-2, 2APPL obtained the highest percentages of initial corn poppy DR after 3 yr (95%), being significantly different from the management strategies EAPOST, SFLR, DLY, and OSR (90, 89, 88 and 84%, respectively) (Table 3B).

Applications of florasulam (ALS inhibitor) plus aminopyralid (synthetic auxin) in the first and third years were done in all management strategies except TRAD (Table 1). Recent studies have shown that only plants carrying a Ser-197 ALS allele were moderately resistant to florasulam compared with plants carrying ALS alleles with other substitutions, which can be SC (Délye et al. 2011). In this study, the RI for florasulam was higher in L-1 compared with L-2. Moreover, higher frequencies of Pro-197-Ser mutants were found in L-1 (Rey-Cabellero et al. in press). This could explain why the first season of herbicide treatments achieved much lower densities in L-2 than in L-1 despite having 2-fold higher initial densities. The same occurred in the second season; when starting from similar infestation levels, final densities were again much lower in L-2 (<1 plants m^{-2}) than in L-1, particularly with those strategies with cereal where bromoxynil plus ioxynil plus MCPP was applied POST. Therefore, the seedbank would have been more replenished in L-1 than in L-2 during the first two seasons, explaining why the effectiveness these strategies was better in L-2 at the end. It may be that the type of herbicide resistance in corn poppy was different between both localities.

Conclusions. To manage herbicide-resistant corn poppy populations, crop rotation with (spring) field peas is a successful option, and in those areas where rainfall is not restrictive, summer crops such as sunflower are very promising alternatives. PRE or early POST plus POST interventions with different

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MOAs provided a significant depletion of the soil seedbank and could be an option in highly infested fields. Effectiveness of the IWM strategies is more dependent on the locality; consequently, this study also highlights that complete knowledge of the population dynamics and the genetic basis of resistance are important in designing better chemical programs adapted to local populations. To prevent and manage herbicide-resistant corn poppy, farmers are encouraged to implement crop rotations, use sequences of herbicides with different MOAs and application timings, and reduce reliance on high resistance–risk MOAs.

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Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/wsc.2016.38

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