Running title: Management of herbicide resistant corn poppy

Management options for multiple herbicide resistant corn poppy (*Papaver rhoeas* L.) in Spain

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Corn poppy is the most widespread broadleaved 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 *P. rhoeas* resistant to 2,4-D and tribenuron-methyl, including crop rotations, delayed sowing and different herbicide programs. After three years, all integrated weed management (IWM) strategies reduced the initial density of *P. rhoeas.* 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 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: corn poppy, *Papaver rhoeas* L. PAPRH.

Keywords: integrated weed management strategy, 2,4-D, tribenuron-methyl, crop rotation, herbicide management, chemical program.

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 non-chemical weed management practices (Beckie 2006).

Corn poppy (*Papaver rhoeas* L.) 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 seed bank, 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 later in 1998, a biotype resistant to both 2,4-D and tribenuron-methyl (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 R 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, thus, the development of new management tools is required. Chemical control strategies should be combined with non-chemical 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 non-chemical tools have been analyzed to control herbicide-resistant weeds. Crop rotation is stated as one of the best management options for preventing the evolution of herbicide-resistant weeds, because it allows for the introduction of herbicides having different MOAs (Vencill et al. 2012). This option provides farmers with opportunities to employ variable crop life cycles, sowing dates, harvest dates, 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* L.) (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 ploughing is generally considered to be an effective method for displacing a proportion of the seeds to non-optimal germination conditions, but this method should not be repeated for a few years for corn poppy because seeds would move back upwards in the soil strata due to their high capacity for survival (Cirujeda et al. 2003). Harrowing is also a good technique for the management of corn poppy, but is it highly dependent on the initial plant densities (Cirujeda et al. 2003; Torra et al. 2011). Delayed sowing (by three months) 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 2,4-D and/or tribenuron-methyl R corn poppy populations 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 in order to implement and design effective integrated weed management strategies, particularly in context of the present scenario where no new MOA has been discovered in recent decades (Duke 2012) and considering that some of the herbicides which 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), that obligate farmers to implement the principles of integrated pest management.

This study was thus conducted in order to: (a) characterize the herbicide resistance patterns of the corn poppy populations researched, and (b) study the effectiveness of several IWM strategies (with different crop rotations, sowing dates, and herbicide programs) over three years to control corn poppy herbicide-resistant populations in winter cereals, whilst providing new data on the effects of individual methods, which 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 North-Eastern Spain. The first site was in Baldomar (Location 1, L-1) (41° 54'N, 1° 0'E), at an elevation of 334 m elevation. 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 and 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 $m^{-2}s^{-1}$. After 14 days, seedlings were transplanted to 8 x 8 x8cm 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, which were later thinned to three per pot. In the potentially R populations, at the 5- to 6-leaf stage (5-6 cm), ALS inhibitors tribenuron-methyl (tribenuron-methyl 500 g a.i. Kg^{-1}, SG) and florasulam (florasulam 22.8 g a.i. L^{-1}, WG) were applied at 0, 4.6, 9.3, 18.7 (1x the field rate), 37.5, 75, 150, 600 and 1200 g a.i. ha^{-1}, and 0, 0.9, 1.8, 3.7, 7.5 (1x), 15, 60, 240 and 480 g a.i. ha^{-1}, respectively. 2,4-D (2,4-D ethyl-hexyl 600 g a.i. L^{-1}, EC) was applied at 0, 75, 150, 300, 600 (1x), 1200 and 4800 g a.i. 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 a.i. ha^{-1} of tribenuron-methyl; 0, 0.1, 0.2, 0.4, 0.9, 1.8, 3.7 and 7.5 g a.i. ha^{-1} of florasulam or 0, 9.3, 18.7, 37.5, 75, 125, 150, 300 and 600 g a.i. ha^{-1} of 2,4-D. A total of 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. Four weeks after treatment (WAT), plants from each dose were harvested (above ground). Samples were dried at 65°C for 48h, 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 (2011–12, 2012–13 and 2013–14) 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 × 10 m). In each locality the eight management strategies implemented were: 1-Traditional (TRA), wheat 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+POST, and the third season, early POST + POST, with active ingredient rotation and application timing rotation); 5-Oilseed rape rotation (OSR), wheat–Oilseed rape–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 one month) and chemical control (active ingredient rotation). A 4 m corridor was left between plots. Sowing doses for wheat cv. 'Berdún' was 200 kg ha^{-1}, 4 kg ha^{-1} for oilseed rape cv. 'Arsenal', 180 kg ha^{-1} for field peas cv. 'Enduro', and 9 kg ha^{-1} for sunflower cv. 'Limasun'. In 2011-12, 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 2012-13, wheat was sown on October 25 and 30 in L-1 and L-2, respectively, while oilseed rape on October 10, field peas on November 15, and sunflower on April 29 in both localities; in 2013-14, 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. Each season fertiliser was applied before sowing at 70 and 100 UPN for cereals and oilseed rape, 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-year experiment ended in June 2014 (2013-14 season), but *P. rhoeas* densities were also counted at the beginning of the 2014-15 season in January 2015. This sampling was considered as a proxy of the overall cumulative effect of the three years of management strategy application on the corn poppy population.

Statistical analysis. Data from dose-response experiments were analyzed using a non-linear regression model. The GR$_{50}$ of plants was calculated using a four parameter logistic curve of the type 1 (Seefeldt et al. 1995):

$$y = \mathrm{c} + \frac{(d - c)}{1 + \mathrm{EXP}[b(\log(x) - \log(GR50)]}$$

Where c is the lower limit, d is the upper limit, GR$_{50}$ is the herbicide rate required for 50% growth reduction and b, the slope at GR$_{50}$. In this equation, the herbicide rate (g a.i. 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). In 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 non-normal data distributions and heterogeneity of variances (Zuur et al. 2010). Only in two cases (2011/12 and 2012/13 final densities of L-2) were these assumptions not met, so non-parametrical 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 was back-transformed to the original scale for presentation. Data from management involving PRE treatments or a seed 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 (2):

$$\mathrm{DR} = 100 - \left[\frac{(\text{Initial Density in 2015} * 100)}{\text{Initial Density in 2011}}\right] \quad (2)$$

LMM were conducted with DR values as described above. Data were transformed as needed (arcs[√(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, 2013). The *Drc* package was used for the non-linear regression (Knezevic et al. 2007) while the *LME4* (Bates et al. 2014) and *nlme* (Pinheiro et al. 2009) packages were employed for the LMM analysis. To compare 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 9.0 (PROC NLIN; SAS Institute, Cary, NC, USA).

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 were 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 R 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 R to 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 1127 g a.i. ha^{-1} (Kati et al. 2014). In our experiment, these values were 816 and 925 g a.i. ha^{-1} for L-1 and L-2, respectively.

Corn poppy density changes. At the beginning of the first season (2011-12), 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, and Tables 3a-b). In this first season, three herbicide management strategies were used (PRE, Early POST 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 plant(s) m^{-2} in L-1 and L-2, respectively (Tables 3a-b). Differences were also found between sampling dates for each system (data not shown).

Overall, initial density in the second season (2012-13) was lower than initial densities observed in the preceding season (Tables 3a-b and Appendix). In L-2 the strategy 2APPL 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 strategy 2APPL 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(s) m^{-2} in L-2. The results for the crop rotations at the end of this second season were unequal, FPR (3 and < 1 plant(s) m^{-2} in L-1 and L-2, respectively) and SFLR (1 and < 1 plant(s) m^{-2} in L-1 and 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 (data not shown).

The analysis of the initial corn poppy density in the third season (2013-14), revealed that in L-1 the OSR rotation resulted in the highest density, ranging between 540 and 686 seedlings m^{-2}. On the contrary, 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 the 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 (data not shown).

With < 1 plant(s) 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 population 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) corded the lowest initial corn poppy densities after three years (Tables 3a-b). The favorable results observed for SFLR can be explained by the sowing date used in 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 North-Eastern Spain hinders the integration of sunflower into the rotation. However, in other areas of Spain with higher rainfall, and where herbicide-resistant corn poppy is present, this crop rotation would be a viable option for implementation. Introducing crop rotations 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 by 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 HR corn poppy control in Spanish dry land areas (Torra et al. 2010). The use of a FPR could be improved using spring varieties of field peas, which again would allow for breaking 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 early POST) provide better control because variability in weed phenology at application time can be avoided compared to 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). 2APPL results reveal this strategy as a serious option in such fields where corn poppy densities are very high and difficult to control.

A sowing delay of one month did not improve corn poppy control within a season when compared to 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 three months was proposed as a management option for improving the corn poppy seed bank 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 2013-14, especially in L-1 where an average of 294 seedlings m^{-2} were present (Table 3a). Oilseed rape requires early sowing in September, extending the emergence period of corn poppy, and thus not breaking its life cycle. Moreover, oilseed rape 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, 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 three years of management, it was possible to reduce corn poppy infestations 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 three years (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 R to florasulam compared to 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 to L-2. Moreover, higher frequencies of Pro_{197} to Ser mutants were found in L-1 (Rey-Cabellero et al. in press). This could explain why the first season herbicide treatments achieved much lower densities in L-2 than in L-1 despite of having two-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 pl/m^2) than in L-1, particularly in those strategies with cereal where bromoxynil plus ioxynil plus MCPP was applied in POST. Therefore, the seed bank would have been more replenished in L-1 than in L-2 during the first two seasons, explaining why the effectiveness these strategies were 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 MOA 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 MOA and application timings, and reduce reliance on high resistance-risk modes of action.

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Table 1. Herbicide application date, herbicide management, active ingredient (with HRAC group) and rate (g a.i. ha^{-1}) used for different management strategies in 2011-12, 2012-13 and 2013-14 seasons at Baldomar (L-1) and Sant Antolí (L-2).

MANAGEMENT STRATEGY	**2011-12**		**2012-13**		**2013-14**	
	(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^{b}		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	December 5	December 20	February 5	February 20	January 21	February 1

(EAPOST)	[a] EAPOST [c] O + O 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 PRE Isoxaben L 125 January 5 January 9 POST Aminopyralid O + Florasulam B 10 + 4.5	February 5 February 20 POST Bromoxynil C3 + Ioxynil C3 + MCPP O 210 + 210 + 630	December 18 December 18 PRE Bifenox E + Isorpoturon C2 680 + 1200 February 18 February 19 POST Aminopyralid O + Florasulam B 10 + 4.5
5- Oilseed rape rotation (OSR)	January 5 January 9 POST Aminopyralid O + Florasulam B 10 + 4.5	November 5 October 25 PRE Propyzamide K1 700 February 1 February 1 POST Aminopyralid O + Clopyralid O 6.25 + 127	February 18 February 19 POST Aminopyralid O + Florasulam B 10 + 4.5
6- Field peas rotation (FPR)	January 5 January 9 POST Aminopyralid O + Florasulam B 10 + 4.5	November 15 November 15 PRE Pendimenthalin K1 1,365	February 18 February 19 POST Aminopyralid O + Florasulam B 10 + 4.5
7- Sunflower rotation (SFLR)	January 5 January 9 POST Aminopyralid O + Florasulam B 10 + 4.5	April 29 April 29 PRE Benfluralin K1 990	February 18 February 19 POST Aminopyralid O + Florasulam B 10 + 4.5
8- Seed delay (DLY)	January 5 January 9 POST Aminopyralid O + Florasulam B 10 + 4.5	February 5 February 20 POST Bromoxynil C3 + Ioxynil C3 + MCPP O 210 + 210 + 630	February 18 February 19 POST Aminopyralid O + Florasulam B 10 + 4.5

[a] EAPOST: Early Post-emergence application.

[b] g a.i. ha^{-1}.

[c] Hormonal mixture containing a new synthetic auxin.

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.

Population	GR$_{50}$ ± SE (g a.i. ha^{-1})[a]	Slope ± SE[b]	Res SS[c]	RI[d]
	Tribenuron-methyl			
L-1	25.2 ± 6.4	0.6 ± 0.1	10084	320
L-2	30.9 ± 8.1	0.6 ± 0.1	10609	392
SC	0.1 ± 0.0	0.4 ± 0.1	4894	--
	Florasulam			

L-1	3.9 ± 0.4	2.0 ± 0.4	3899	24
L-2	2.9 ± 0.3	0.9 ± 0.1	1529	18
SC	0.2 ± 0.0	0.7 ± 0.1	21738	--
2,4-D				
L-1	816.6 ± 96.0	1.3 ± 0.2	2872	12
L-2	925.8 ± 156.0	1.0 ± 0.3	5038	13
SC	68.6 ± 10.2	1.2 ± 0.2	23693	--

[a] GR$_{50}$, ALS inhibitor concentration for 50% reduction of corn poppy dry weight biomass.

[b] The slope at GR$_{50}$.

[c] Res SS, residual sum of square.

[d] RI, Resistance Index.

Table 3a. Initial and final corn poppy densities means (plants m^{-2}) under different management strategies in 2011-12, 2012-13, 2013-14 and 2015 for data collected at Baldomar (L-1). 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.

Management strategy	[a]**2011-12**		**2012-13**		**2013-14**		**2015**	*DR*
	Initial Density	*Final Density*	*Initial Density*	*Final Density*	*Initial Density*	*Final Density*	*Initial Density*	
1-TRAD	320 ([b]*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] Sampling dates included in the statistical analysis. Initial density: Season 2011-12: 20/12/2011 Season 2012-13: 09/01/2013; Season 2013-14: 21/01/2014 and in 2015: 15/01/2015. Final density: Season 2011-12: 03/05/2012; Season 2012-13: 08/05/2013; Season 2013-14: 27/05/2014.

[b] 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 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 2011-12, 2012-13, 2013-14 and 2015 for data collected at Sant Antolí (L-2). 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.

Management strategy	[a]**2011-12**		**2012-13**		**2013-14**		**2015**	*DR*
	Initial Density	*Final Density*	*Initial Density*	*Final Density*	*Initial Density*	*Final Density*	*Initial Density*	
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] Sampling dates included in the statistical analysis. Initial density: Season 2011-12: 20/12/2011 Season 2012-13: 09/01/2013; Season 2013-14: 10/02/2014 and in 2015: 15/01/2015. Final density: Season 2011-12: 03/05/2012; Season 2012-13: 08/05/2013; Season 2013-14: 27/05/2014.

[b] 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 delay and PRE treatments) were not included in the analysis.

[d] Due to the abundance of zeros non parametric test were conducted with 2011-12 and 2012-13 final density data in L-2.