Running title: Managing R poppy in no-till

Management of herbicide resistant corn poppy (*Papaver rhoeas*) under different tillage systems does not change the frequency of resistant plants

Joel Torra[1]*, Aritz Royo-Esnal[1], Jordi Rey-Caballero[2], Jordi Recasens[3], and Marisa Salas[4]

[1]Researcher, Department of Hortofructicultura, Botànica i Jardineria, Agrotecnio, Universitat de Lleida, Lleida, Spain; [2]Researcher, FTS AgroConsulting, Salteras, Spain; [3]Full Professor, Department of Hortofructicultura, Botànica i Jardineria, Agrotecnio, Universitat de Lleida, Lleida, Spain; [4]Researcher, DuPont de Nemours, Paris, France

Author for correspondence: J. Torra, Department of Hortofructicultura, Botànica i Jardineria, Agrotecnio, Universitat de Lleida, Alcalde Rovira Roure 191, 25198 Lleida, Spain. (Email: joel@hbj.udl.cat)

Corn poppy (*Papaver rhoeas* L.) is the most widespread broadleaf weed species infesting winter cereals in Europe. Biotypes that are ~~multiple~~ resistant ~~(R)~~ to both 2,4-D and tribenuron-methyl, an acetolactate synthase (ALS)-inhibitor, have evolved in recent decades, thus narrowing the options for effective chemical control. Though the effectiveness of several integrated weed management (IWM) strategies have been confirmed, none of such strategies has been tested to manage multiple herbicide-resistant *P. rhoeas* under no-till planting. With the expansion of no-till systems, it is important to prove the effectiveness of such strategies. In this study, a field experiment over three consecutive seasons was conducted to evaluate and compare the effects of different weed management strategies, both under direct drilling (i.e. no-till) and intensive tillage, on a multiple herbicide-resistant *P. rhoeas* population. Moreover, evaluations were carried out as to whether the proportions of ALS-inhibitor resistant individuals were affected by the tillage systems for each IWM strategy at the end of the three-year period. The IWM strategies tested in this research included crop rotation, delayed sowing and different herbicide programs such as PRE plus POST or POST. All IWM strategies greatly reduced the initial density of *P. rhoeas* each season (≥ 95%), both under direct drilling and intensive tillage. After three years, the IWM strategies were very effective in both tillage systems, though the effects were stronger under direct drilling (~95%) compared to intensive tillage (~86%). At the end, the proportion of ALS-inhibitor resistant plants was not different between the IWM strategies in both tillage systems (94% on average). Therefore, crop rotation (with sunflower (*Helianthus annuus* L.)), delayed sowing, or a variation in the herbicide application timing are ~~also~~ effective under direct drilling to manage herbicide-resistant *P. rhoeas*. Adoption of IWM strategies is necessary in order to mitigate the evolution of resistance in both conventional and no-till systems.

~~**Nomenclature:** corn poppy, *Papaver rhoeas* L. PAPRH.~~

Keywords: crop rotation; delayed sowing; direct drilling; integrated weed management strategy; intensive tillage; multiple resistance.

Since the advent of agriculture, ~~soil~~ tillage has been used to provide suitable soil conditions for crop establishment and growth (Cannell, 1985). Additionally, cultivation is a useful pre-sowing weed control strategy that has a major influence on the vertical weed seed distribution in arable soils (Cousens and Moss, 1990), a critical factor affecting weed survival, germination and emergence, and thus ~~enhancing~~ the effectiveness of weed management tactics (Mohler, 1993).

Under direct drilling (also called no-till or zero-till), where the soil is left intact and the only disturbance is a narrow slot of few centimeters wide created at sowing, the use of broad-spectrum herbicides such as glyphosate~~,~~ is recommended (Kleemann and Gill~~,~~ 2009). Therefore, the greater reliance of direct drilling systems on herbicides due to the absence of tillage can complicate the management of herbicide resistant ~~weeds~~ (Renton and Flower 2015) and other problematic weeds (García et al. 2014).

The adoption of conservation tillage systems is increasing throughout Spain in rain-fed arable crops because of environmental benefits~~–~~ and savings in time and economic inputs (Holland~~,~~ 2004; Sánchez-Girón et al.~~,~~ 2007). In these areas, corn poppy (*Papaver rhoeas* L.) is the most widespread broadleaf weed species (Torra et al.~~,~~ 2011). The ability of this species to invade and persist in arable fields can be attributed to the development of a persistent seedbank, an extended germination period, and high fecundity (Torra and Recasens 2008). Furthermore, *P. rhoeas* is a growing problem due to the appearance of ~~herbicide-resistant~~ biotypes resistant to synthetic auxins and/or ~~to~~ acetolactate synthase (ALS) inhibitors (Rey-Caballero et al. 2017a).

Herbicides alone are not usually enough to control herbicide-resistant *P. rhoeas* populations (Rey-Caballero et al. 2017a). Therefore, ~~the development of~~ integrated weed management (IWM) programs needs to be developed for ~~for~~ this species ~~is required~~ (Torra et al.~~,~~ 2010b). Various chemical and non-chemical tools have been used to control herbicide-resistant *P. rhoeas* populations. Those included crop rotations, herbicide programs, late sowing, mechanical control or different types of fallow management, as a part of different IWM programs (Rey-Caballero et al. 2017a; Torra et al.~~,~~ 2011; Torra et al.~~,~~ 2010a; Cirujeda et al.~~,~~ 2003). However, though most of them were adequate to manage herbicide-resistant *P. rhoeas*, especially under ~~different tillage systems, such as~~ intensive tillage, none have been tested under direct drilling. So far, there are no studies on the long-term effects of an IWM program on herbicide-resistant *P. rhoeas* populations under direct drilling situations. Additionally, it is unknown if the proportion of resistant plants might change with time depending on the management strategies, such as tillage regimes. No changes would be expected if herbicide-resistant plants do not carry a fitness penalty compared to the susceptible ones (Panozzo et al. 2017). However, no fitness studies have been carried out ~~there are not fitness cost studies~~ on herbicide-resistant *P. rhoeas*.

Several studies have shown that *P. rhoeas* is better adapted to direct drilled, rather than conventionally-tilled cropping systems (Dorado and López-Fando~~,~~ 2006; Dorado et al.~~,~~ 1999). Non-inversion tillage such as direct drilling allows the weed seeds to remain mainly in the 0–5 cm soil layer (Scherner et al. 2016), and weed species with small-sized seeds, such as *P. rhoeas* (≈1 mm), are able to emerge from this soil profile (Froud-Williams et al. 1984). Therefore, with the expansion of direct drilling, it is important to develop and test IWM programs for herbicide-resistant *P. rhoeas* adapted to rain-fed cropping systems ~~under these reduced tillage systems~~. Considering that enhanced metabolism to ALS inhibitors or synthetic auxins can evolve in *P. rhoeas* (Rey-Caballero et al.~~,~~ 2017b; Torra et al.~~,~~ 2017), it is even more crucial to improve IWM programs that are more effective on sustainable control of such biotypes than using herbicides alone (Rey-Caballero et al. 2017a). Enhanced detoxification poses a great threat to agriculture because multi-herbicide resistance to unexpected modes of action can occur involving multi-genes ~~in the mechanisms~~ (Yuan et al.~~,~~ 2007), threatening the design and development of herbicide programs.

This study was, thus, conducted ~~in order~~ to: (1) test and compare the effectiveness of crop rotation, sowing date, and herbicide programs under two contrasting tillage systems, direct drilling and intensive tillage, in controlling herbicide-resistant *P. rhoeas* populations in winter cereals, (2) determine the impact of IWM strategies on the frequency of ALS-inhibitor resistant individuals in the population under both tillage systems, and (3) characterize the herbicide resistance status to different ALS-inhibitor families and to 2,4-D with dose-response experiments.

Materials and Methods

Site description. A field trial was established in a commercial winter cereal field with high *P. rhoeas* infestations in the province of Lleida in North-Eastern Spain. The field was in Cubells (41° 52'N, 0° 56'E), at an elevation of 465 m. The soil was silty–clay loam (11% sand, 33% clay, and 56% silt), pH was 8.1, and organic matter content was 2.5%. In the preceding years, the field was under a winter cereal monocropping system, managed with intensive tillage. Selective POST herbicides (florasulam + 2,4-D, Mustang® from Dow; iodosulfuron-methyl + mesosulfuron-methyl, Atlantis® from Bayer) had been employed at recommended label rates for weed control the last five years. Precipitation and temperature were recorded at a meteorological station located 9.5 km away from the experimental field (41º55′N, 1º10′E).

Integrated weed management assessments. A field experiment with a split-plot design with three replicates was conducted during three consecutive cropping seasons (2013 to 2016) to evaluate the effect of three different IWM strategies under different tillage systems on a multiple herbicide-resistant *P. rhoeas* population. Main plot treatments consisted of two tillage systems, direct drilling and intensive tillage, whereas the subplot treatments (plot size: 9 x 10 m) consisted of three IWM strategies in randomized arrangement, including: 1) a barley (*Hordeum vulgare* L.) monocrop with normal seeding date (November) wherein weed control was carried out by chemicals only, namely Chemical; 2) a sunflower-barley-barley rotation with delayed sowing (December) for barley in both seasons, which includes PRE and POST applications, namely Rotation PRE; 3) a sunflower-barley-barley rotation with delayed sowing (December) for barley in both seasons, but only POST herbicide applications in all years, namely Rotation POST. Sowing dates, rates, crops and varieties for each IWM strategy in each tillage system are specified in Table 1. The main plots (direct drilling, intensive tillage) were separated by a corridor of 10 m wide. Tillage included a single pass in late summer and another in early autumn with a chisel plow for seedbed preparation. Herbicide treatments were applied using a backpack sprayer with a 3-m-wide boom, calibrated to deliver 300 L ha^{-1} of spray liquid at a pressure of 253 kPa. All details on the herbicide applications are summarized in Table 2. Agronomic practices were the usual for each crop in the area of study. In each season, fertiliser was applied prior to sowing at 70 units of phosphorus nitrogen fertilizer (UPN) and again at 100 UPN in February.

Data collection. *Papaver rhoeas* density was counted twice each year, at the beginning and at the end of each season, by randomly placing 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 *P. rhoeas* populations. The 3-year experiment ended in June 2016 (2015-16 season), but *P. rhoeas* densities were also counted at the beginning of the 2016-17 season in December 2016. This sampling was considered as a proxy of the overall cumulative effect of the different IWM strategies tested after three years of application on the *P. rhoeas* population.

Winter barley yield was measured (kg-ha^{-1}) using a commercial combine harvester at the end of the season, usually beginning of July. Sunflower was not harvested.

DNA extraction, ALS gene sequencing and restriction analysis. To evaluate if the three different IWM strategies applied during three consecutive seasons, both under direct drilling and intensive tillage, affected the frequency of plants resistant to the ALS-inhibitor tribenuron-methyl, the frequency of resistant plants was estimated in each IWM strategy in both tillage systems. ~~Plants from the SC population used in the dose-response experiments were not included in this experiment, but results from previous work did not detect any mutation among sixty plants (Rey-Caballero et al. 2017).~~ For the herbicide-resistant field population, two samplings were carried out: the first at the beginning of the first season (2013-14) at the end of autumn 2013, and the second at the beginning of the ~~theoretical~~ fourth season (2016-17) in end of autumn 2016. In each sampling date, leaf fragments (~50 mg) from 25 different plants per subplot were taken and frozen for subsequent molecular analyses. DNA from the leaf fragment was extracted using the Speedtools Plant DNA Extraction Kit (Biotools B&M Labs S.A., Valle de Tobalina, Madrid, Spain) and the DNA sample concentration was measured in a NANODROP Thermoscientific spectrophotometer (ThermoFisher, Nano-Drop Products, Wilmington, DE). Each DNA sample was diluted to a final concentration of 10 ng µl^{-1}, which was immediately used for the polymerase chain reaction (PCR) test or stored at -20ºC until use.

Mutations conferring ALS-inhibitor resistance in *P. rhoeas* at Pro197 and Trp574 codons were first analyzed in all the samples. Fragments of the ALS gene that included the regions of those codons were amplified using primers described in a previous work (Kaloumenos et al. 2009). The amplification was accomplished following the procedures described in the above mentioned work (Kaloumenos et al. 2009). PCR amplification products were separated in a 1.5% agarose gel. Gels were then observed under ultraviolet light (320 nm; ALPHA DIGI DOC Pro instrument, Alpha Innotec Corporation, Johannesburg, South Africa) and images recorded with gel photography. Amplified DNA fragments were purified using the Speed tools PCR Clean-Up Kit (Biotools, B&M Labs, Madrid, Spain), then sequenced. Restriction analyses were conducted to define double-peaks detected in the sequence chromatograms. For this analysis, primers and procedures were utilized as described by Kaloumenos et al. (2009). The resulting electrophoresis bands were visualized under UV light after staining with GelRed (Biptium, California, USA). The digestion profile for each population was compared with its respective, non-digested control profile as well as the susceptible-control digestion profile. Haplotype inference was determined by comparing sequences obtained from the other samples within the same population.

Dose-response experiments. Seeds from the experimental site were collected and stored during summer 2012. In autumn, dose response experiments were conducted 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 x 8 cm plastic pots filled with a mixture of silty loam soil, sand, and peat (1.3:1:1 by vol). Five seedlings were transplanted per pot, which were later thinned to three. In the putative herbicide-resistant population and the susceptible one, at the 5- to 6-leaf stage (5-6 cm), ALS-inhibitors tribenuron-methyl, florasulam and imazamox, and the synthetic auxin 2,4-D (2,4-D ethyl-hexyl) were applied at the rates detailed in Table 3. A total of four replicates (pots) were included for each dose in a complete randomized design. 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 the University of Lleida, Spain (41°37'43.1" N, 0°35'52.6" E) and were watered regularly. Four weeks after treatment (WAT), ~~the~~ plant mortality from each dose was evaluated in each pot. Plants without green tissues were classified as dead. The experiment was repeated twice.

Statistical analysis. Data from dose-response experiments were analyzed using a non-linear regression model. The herbicide rate causing 50% of plant mortality (LD_{50}) was calculated using a four-parameter logistic curve of the type 1 (Seefeldt et al. 1995):

$$y = \mathrm{c} + \frac{(d - c)}{1 + \mathrm{EXP}[b(\log(x) - \log(LD50))]}$$

Where *c* is the lower limit, *d* is the upper limit, LD_{50} is the herbicide rate required for 50% growth reduction and *b*, the slope at LD_{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 LD_{50}(herbicide-resistant) / LD_{50}(susceptible).

The effectiveness of the IWM strategies within season was estimated (% of Density Reduction or DR) between the initial and final densities (seedlings m^{-2}). Moreover, the reduction in *P. rhoeas* densities after 3 years of management was calculated by comparing weed densities between the seasons 2013-14 and 2016-17 (3-years-% Density Reduction or 3-yr DR) as (2):

$$3 - \text{yr DR} = 100 - \left[\frac{(\text{Initial Density in season } 2016/17 * 100)}{\text{Initial Density in season } 2013/14}\right] \qquad (2)$$

From analyses of ALS gene sequence at position 197, the percentage of wild type plants (Pro/Pro) and the percentage of mutant plants (heterozygous plus homozygous plants) were estimated for the two sampling dates in each subplot. Afterwards, mean values for each IWM strategy and tillage system were calculated. ~~No mutations in position 574 of the ALS gene were found in >900 sequenced plants.~~

For the field experiment, three-way ANOVA were performed on six data sets: initial *P. rhoeas* density, final *P. rhoeas* density, DR, crop yields, % of wild plants and % of mutant plants at positions (Pro197), with season, tillage type and IWM strategy as the fixed factors. If year*treatment interactions were statistically ~~different~~significant, then data ~~was~~were analyzed and presented separately for each year ~~with two-way ANOVA.~~ If new interactions were found, a new separation of factors was done. Data were transformed as needed (log (x+1) or √(x+0.5)) prior to the analysis because exploratory analysis revealed some non-normal data distributions and heterogeneity of variances (Zuur et al. 2010). Finally, a post-hoc Tukey´s pairwise comparison was employed to test differences between IWM strategy means (at $P<0.05$). For~~,~~ the 3-yr DR a two-way ANOVA was performed considering tillage type and IWM strategy as factors. For the initial density in the fourth season, a two-way ANCOVA was carried out considering the same previous factors and the initial density in the first season as a covariate. ~~If interactions were found between factors, one-way ANOVA was performed to separate the respective factors.~~

Dose-response ~~curves~~ analyses were carried out with the use of the R programming language (R, 2013). The ~~Drc~~ *drc* package was used for the non-linear regression (Ritz and Streibig 2005) while the *LME4* (Bates et al. 2014) and *nlme* (Pinheiro et al. 2009) packages were employed for the LMM analysis. The rest of the statistical analyses were carried out with the use of the software Sigmaplot 11.0 (San Jose, California, USA). When necessary, data were back-transformed to the original scale for presentation.

Results and discussion

***Papaver rhoeas* density changes.** At the beginning of the first season (2013-14), the densities were not homogenous, with statistical differences detected between both tillage systems. Initial *P. rhoeas* density ~~reached~~ in direct drilling was on average 525 seedlings m^{-2}, higher than in intensive tilled plots, where there was an average of 80 seedlings m^{-2} (Table 4). All three management systems significantly reduced (≥99%) ~~the~~ *P. rhoeas* density at the end of the season irrespective of the tillage system, but the Chemical system resulted in the highest weed densities under direct drilling, with 1.3 plants m^{-2} (Table 4).

Initial weed densities in the second season (2014-15) were significantly lower than those observed in the preceding season (Table 4). Nevertheless, densities were higher under direct drilling (216 seedlings m^{-2}) than that of intensive tillage (46 seedlings m^{-2}). At the end of the season, all management systems were equally effective in reducing *P. rhoeas* densities (<0.4 plants m-2 with ≥99%). Applications including K1 herbicides in PRE or C3 herbicides in POST are good chemical options to manage herbicide-resistant *P. rhoeas* populations (Torra et al. 2010a; Rey-Caballero et al. 2017a), as observed in the second season in this research.

Initial *P. rhoeas* densities in the third season (2015-16)~~,~~ were the lowest (only 9 seedlings m^{-2} irrespective of tillage and management system) compared to preceding seasons (Table 4). This was due to the cumulative effect of management systems, but also because that season was the driest in autumn (Table 5). At the end of this season, densities were significantly reduced in all plots, revealing that the Rotation PRE system was the least efficient (93%) system compared to others systems (≥99%).

The initial density evaluated in 2016 before any herbicide application reflects the cumulative effect of the three preceding seasons for the different IWM strategies evaluated. Data showed that all three management systems were able to greatly reduce *P. rhoeas* densities in both tillage systems, with 23 and 10 seedlings m^{-2} on average, in direct drilling and intensive tillage, respectively (Table 4). The density achieved during the fourth season in each management system was independent of the different initial densities in the first season in each tillage system, as evident from insignificant ANCOVA results when the initial densities were used as a covariate in the analysis~~because when analyzing it with the initial density the first season with an ANCOVA, this covariable was not significant~~ (data not shown). Previous studies have shown that within 3-~~y~~ to 5-yr of proper IWM practices, it is possible to significantly reduce populations of herbicide resistant *P. rhoeas* (Torra et al. 2011; Rey-Caballero et al. 2017a). The main non-chemical cultural practices successfully incorporated here were tillage system, delayed sowing and rotation of winter cereals and summer crops. These cultural practices are considered among the most efficient in managing the worst herbicide-resistant weeds worldwide, such as *Lolium rigidum* or *Alopecurus myosuroides* (Gill and Holmes 1997; Gerhards et al. 2016).

On the other hand, after 3-yr of management, all management systems were more efficient in reducing densities under direct drilling than in intensive tillage. A possible reason to explain these results could be that higher soil water contents in direct drilled plots might have promoted a more early *P. rhoeas* emergence compared to tilled plots, making ~~more effective~~ delayed sowings in Rotation PRE and Rotation POST systems, or pre-sowing treatment with glyphosate in the Chemical system, a more effective strategy. In fact, the Chemical system under intensive tillage was the least effective after the 3-yr (without delayed sowing or pre-sowing treatments). Lampurlanés et al. (2002), comparing different tillage systems in the region, confirmed that direct drilling favored greater and deeper water accumulation in the soil profile. This is in accordance with higher mean yields under direct drilling than in intensive tillage observed in these trials.

The Rotation POST system was overall the most efficient (Table 4). All cereal seasons included later POST herbicide applications. *Papaver rhoeas* has an extended emergence periodicity and seedlings still can emerge in spring (Cirujeda et al. 2008), if rainy, like the third growing season in this study (Table 5). These results highlight the relevance of herbicide application timing with regard to *P. rhoeas* emergence, and the importance of avoiding the incorporation of new seeds into the soil, both from early and late emerging plants, to achieve an effective management in the mid- to long-term for herbicide-resistant populations (Norsworthy et al. 2012).

Winter barley yields. Significant differences were observed between the yields of the three management systems in 2014–15 and in 2015–16. In both of these seasons, barley yields were higher in the Chemical system compared to the other two systems with delayed sowings (Table 6). Sunflower was not harvested in the first season (2013–14), and therefore yield was only estimated in the Chemical system. No significant differences were found between both tillage systems the three seasons. Yields in these trials were normal for the study area, which usually can substantially change from season to season (García et al. 2014, Torra et al. 2011).

It has been shown, in the absence of weeds, that a delayed sowing reduces cereal yields in rain-fed cropping systems because the crop cannot reach the optimal or potential development in a shorter growing period (Spink et al. 2005). However, other studies have shown that delayed sowings can avoid autumn–winter annual weeds~~'~~ competition, thus obtaining higher yields (Singh et al. 1995; García et al. 2014). However, those studies were on grass weeds, such as *Bromus diandrus*, which can emerge in early autumn (García et al. 2014; Recasens et al.~~,~~ 2016). For broadleaved weed species such as *P. rhoeas* which have a more extended and delayed emergence period, benefits from delayed sowings in terms of yield were not observed here, hindering the implementation of this cultural strategy by farmers to manage herbicide-resistant populations.

Changes in the *Papaver rhoeas* resistance status to ALS-inhibitors. The initial proportions of susceptible and resistant plants (homozygous plus heterozygous) in position 197 were equal and homogenous between both tillage and management systems (Table 7). Overall averages of 4~~%~~ and 96% of susceptible and resistant plants were estimated. After three years of management, these proportions did not change, with averages of 6~~%~~ and 94%. No mutations in position 574 of the ALS gene were found in >900 sequenced plants. Recent studies on fitness costs of several ALS resistance alleles (for Ala-122, Pro-197r, Asp-376 or Trp-574) in grass species rigid ryegrass (*Lolium rigidum* Gaudin) or dicot species (wild radish (*Raphanus raphanistrum* L.) or kochia [Bassia~~*Kochia*~~ *scoparia* (L.) A.J. Scott]) demonstrated a negligible effect on ALS enzyme kinetics, plant growth and competitiveness (Yu and

Powles 2014). Therefore, ALS resistance alleles, once selected in the field, are likely to remain unchanged in the population. In the absence of selection pressure by ALS-inhibitors for several years, these resistance mutants will persist in the population and not decline with time. This study showed that ceasing to use the selection agent did not significantly modify the ratio between resistant and susceptible plants when the *P. rhoeas* seed bank initially had more than 90% of resistant seeds. The long-lived seeds, such as those of *P. rhoeas*, impose long-term resistance management strategies when the seed bank contains predominantly resistant individuals (Panozzo et al. 2017).

Dose- response experiments. The presence of a multiple herbicide- resistance in the experimental *P. rhoeas* population was confirmed initially. There was no mortality at the commercial label rates for the herbicides. The LD_{50} for tribenuron-methyl was 2311 times higher in the –herbicide-resistant population compared to the susceptible ~~one~~ standard (Table 8). In addition, cross-resistance to triazolopyrimidines (florasulam) and imidazolinones (imazamox), with resistance factors (RF) of 9.3 and 7.2, respectively, was observed in this population (Table 8). High tribenuron-methyl resistance levels and cross-resistance to triazolopyrimidines or imidazolinones were also found in Spanish *P. rhoeas* populations from the studied area (Rey-Caballero et al. 2017). Furthermore, multiple resistance to 2,4-D was confirmed, with a RF around 15 (Table 8). Previous studies have also confirmed the presence of multiple herbicide-resistant *P. rhoeas* populations in Spain (Rey-Caballero et al. 2016; 2017).

Conclusions. This research demonstrates that it is possible to manage herbicide-resistant *P. rhoeas* populations under different tillage systems, such as direct drilling or intensive tillage. Crop rotation (with sunflower), delayed sowings and robust herbicide programs (inclusion of PRE) were successful options in both tillage systems. Several IWM strategies, including different herbicide programs, have shown to be successful in managing herbicide-resistant weeds under different tillage systems, such as no-till (e.g. Norsworthy et al. 2016). This research highlights that IWM tactics can be equally effective in no-till as they are in conventional till systems~~the effectiveness of the different management strategies need to be adapted and tested depending on the soil management implemented~~. ~~Delayed sowings and later POST applications can be more effective under direct drilling with lower yield penalties.~~ Therefore, farmers are encouraged to diversify strategies at all levels to manage herbicide-resistant *P. rhoeas* populations in direct-seeded systems where tillage is no longer a weed control option: crop rotations, ~~soil tillage management when possible,~~ sowing dates, herbicide sites of action, ~~and herbicide MoA's~~ and application timings. Finally, this research demonstrates that the rapid worldwide adoption of no-till in rain-fed cropping systems can be accompanied with suitable IWM strategies to manage herbicide-resistant weeds.

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Table 1. Crops (and varieties) and cultural practices (dates and sowing densities) for three different weed management strategies, both under direct drilling and intensive tillage, in 2013-14, 2014-15 and 2015-16 seasons at Cubells (Spain).

	2013-14		**2014-15**		**2015-16**	
IWM Strategy	*Direct drilling*	*Intensive tillage*	*Direct drilling*	*Intensive tillage*	*Direct drilling*	*Intensive tillage*
Chemical	November 9 Winter Barley Meseta 200 kg ha^{-1}	November 25	November 16 Winter Barley Meseta 200 kg ha^{-1}	November 16	November 17 Winter Barley Meseta 200 kg ha^{-1}	November 15
Rotation PRE	April 9 Sunflower ExpressSun® 5 kg ha^{-1}	April 9	December 13 Winter Barley Graphic 200 kg ha^{-1}	December 14 Gustav	December 10 Winter Barley Graphic 200 kg ha^{-1}	December 16 Meseta
Rotation POST	April 9 Sunflower ExpressSun® 5 kg ha^{-1}	April 9	December 13 Winter Barley Graphic 200 kg ha^{-1}	December 14 Gustav	December 10 Winter Barley Graphic 200 kg ha^{-1}	December 16 Meseta

Table 2. Herbicide management, application date, active ingredient (with HRAC group) and rate (g ai ha^{-1}) used for three different management strategies, both under direct drilling and minimum tillage, in 2013-14, 2014-15 and 2015-16 seasons at Cubells (Spain).

	2013-14		2014-15		2015-2016	
IWM strategy	Direct drilling	Intensive tillage	Direct drilling	Intensive tillage	Direct drilling	Intensive tillage
CHEMICAL	November 7 PRE-sowing Glyphosate (M) - 900		November 14 PRE-sowing Glyphosate (M) - 900		November 15 PRE-sowing Glyphosate (M) - 900	
			January 17 PRE Pendimenthaline (K1) - 1800	January 17 PRE		
	March 5 POST Nitrile (C3) + Sulfonylurea (B) [a] - 334	March 5 POST	March 4 POST Tribenuron-methyl (B) - 38	March 4 POST	March 29 POST Nitrile (C3) + Sulfonylurea (B) [a] - 334	March 29 POST
ROTATION PRE	April 8 PRE-sowing Glyphosate (M) - 900		December 11 PRE-sowing Glyphosate (M) - 900		December 8 PRE-sowing Glyphosate (M) - 900	
			January 17 PRE Pendimenthaline (K1) - 1800	January 17 PRE		
	May 30 POST Tribenuron-methyl (B) - 38	May 30 POST	January 1 POST Tribenuron-methyl (B) - 38	January 1 POST	January 19 POST Nitrile (C3) + Sulfonylurea (B) [a] - 334	February 1 POST
ROTATION POST	April 8 PRE-sowing Glyphosate (M) - 900		December 11 PRE-sowing Glyphosate (M) - 900		December 8 PRE-sowing Glyphosate (M) - 900	
	May 30 POST Tribenuron-methyl (B) - 38	May 30 POST	March 4 POST Nitrile (C3) + Sulfonylurea (B) [a] - 334	March 4 POST	March 29 POST Nitrile (C3) + Sulfonylurea (B) [a] - 334	March 29 POST

[a] Experimental mixture.

Table 3. Herbicides used in dose-response experiments and dosage for the susceptible (S) and resistant (R) populations.

Herbicide active ingredient.	Commercial product	Field rate(g ai ha^{-1})	Manufacture		Dose ~~rate~~ used (g ai ha^{-1})
Tribenuron-methyl	Granstar 50 SX	18.7	DuPont	*R*	1200, 600, 150, 75, 37.5, 18.7, 9.3, 4.6 and 0
				S	18.7, 9.3, 4.6, 2.3, 1.1, 0.5, 0.25 and 0
Florasulam	Nikos	7.5	Dow AgrosciencesIberica	*R*	480, 240, 60, 15, 7.5, 3.7, 1.8, 0.9 and 0
				S	7.5, 3.7, 1.8, 0.9, 0.4, 0.2, 0.1 and 0
Imazamox	Pulsar 40	50	BASF España	*R*	3200, 1600, 400, 100, 50, 25, 12.5, 6,2 and 0
				S	50, 25, 12.5, 6.2, 3.1, 1.5, 0.7 and 0
2,4-D	Esteron 60	600	Dow AgrosciencesIberica	*R*	4800, 1200, 600, 300, 150, 75 and 0
				S	600, 300, 150, 75, 37.5, 18.7, 9.3 and 0

Table 4. Initial and final *Papaver rhoeas* densities (plants m^{-2} ± SE), density reduction each season (% ± SE), and density reduction after 3-years or 3-yr DR (% ± SE) under three management strategies, both under direct drilling or intensive tillage, during three seasons at Cubells (Spain). 3-yr DR: see text for calculation details.

Soil Tillage	Management System	**2013-14**			**2014-15**			**2015-16**			**2016**	3-yr DR
		Initial Density	*Final Density*	*% Densit. Reduction*	*Initial Density*	*Final Density*	*% Densit. Reduction*	*Initial Density*	*Final Density*	*% Densit. Reduction*	*Initial Density*[c]	
Direct drilling	Chemical	278 ± 129 b[a]	1.3 ± 0.8 a	99 ± 0 b	17 ± 12 b[b]	0.0 ± 0.0 b	100 ± 0 a	3 ± 1 b	0.1 ± 0.0 a	97 ± 2 a	7 ± 3 b	97 ± 0 a
	Rotation PRE	652 ± 184 a	0.3 ± 0.1 b	100 ± 0 a	355 ± 70 a	0.1 ± 0.1 ab	100 ± 0 a	12 ± 3 a	0.1 ± 0.0 a	99 ± 0 a	40 ± 6 a	93 ± 1 a
	Rotation POST	644 ± 197 a	0.2 ± 0.1 b	100 ± 0 a	276 ± 70 a	0.4 ± 0.2 a	100 ± 0 a	11 ± 4 ab	0.2 ± 0.1 a	99 ± 1 a	21 ± 5 a	96 ± 2 a
Intensive tillage	Chemical	51 ± 18 a	0.4 ± 0.2 a	99 ± 0 b	3 ± 2 b[b]	0.0 ± 0.0 b	100 ± 0 a	5 ± 2 a	0.0 ± 0.0 a	98 ± 2 ab	5 ± 1 b	89 ± 3 ab
	Rotation PRE	74 ± 20 a	0.0 ± 0.0 a	100 ± 0 a	27 ± 18 ab	0.0 ± 0.0 ab	100 ± 0 a	10 ± 4 a	0.5 ± 0.2 b	95 ± 0 b	19 ± 8 a	74 ± 13 b
	Rotation POST	114 ± 34 a	0.0 ± 0.0 a	100 ± 0 a	107 ± 65a	0.3 ± 0.3 a	99 ± 1 a	12 ± 0 a	0.0 ± 0.0 a	100 ± 0 a	7 ± 2 ab	94 ± 1 a

	Initial Density	*Final Density*	*% Densit. Reduction*	*3-years % Densit. Reduction*
Season	<0.001	0.026	<0.001	-
Tillage	<0.001	NS	NS	0.032
Management	<0.001	NS	NS	NS
Season x Tillage	<0.001	0.031	NS	-
Tillage x Management	0.002	NS	0.022	NS
Season x Management	<0.001	<0.001	0.017	-
Season x Tillage x Management	NS	NS	0.003	-

[a] Means within a column (year factor) and Soil Tillage (tillage factor) followed by the same letter indicate that no significant difference ($P < 0.05$) was detected by means of Tukey (HSD) test at the 5% level of probability

[b] Initial density data with PRE treatments that avoid the natural germination pattern of *P. rhoeas* seedlings were included in the analysis.

[c] This data was analyzed with an ANCOVA using initial density the first season as covariate; please check text for details.

Table 5. Average season maximum, mean and minimum temperature (°C), and rainfall (mm) over the trial period, from October 2013 to December 2016. Autumn: October to December; winter: January to March; spring: April to June

Campaign	Season	Temperature (ºC)			Cumulative Precipitation (mm)
		Maximum	Mean	Minimum	
2013-14	Autumn	23	8	-5	75
	Winter	20	6	-4	94
	Spring	29	17	4	239
2014-15	Autumn	22	10	-2	109
	Winter	19	5	-7	30
	Spring	33	18	4	63
2015-16	Autumn	22	9	-4	58
	Winter	20	6	-7	97
	Spring	29	16	1	153
2016-17	Autumn	22	8	-2	115

Table 6. Cereal yields in kg ha^{-1} (mean ± SE) for three management systems, each under direct drilling and intensive tillage, during three seasons in Cubells (Spain). Means within a column (year factor) followed by the same letter indicate that no significant difference ($P < 0.05$) was detected by means of the Tukey's test at the 5% level of probability.

Soil Tillage	Management system	2013/14	2014/15	2015/16
Direct drilling	Chemical	1801 ± 141 a	2258 ± 626 a	5171 ± 578 a
	Rotation PRE	--[b]	1052 ± 222 b	2854 ± 319 b
	Rotation POST	--[b]	1361 ± 275 ab	3398 ± 356 ab
Intensive tillage	Chemical	1410 ± 142 a	1844 ± 191 a	6816 ± 535 a
	Rotation PRE	--[b]	1179 ± 236 b	3462 ± 978 ab
	Rotation POST	--[b]	1028 ± 214 b	3753 ± 419 ab
Season	<0.001	--	--	--
Tillage	NS[a]	NS	NS	NS
Management	<0.001	NS	0.015	<0.001
Tillage x Management	NS	NS	NS	NS
Season x Tillage	NS	--	--	--
Season x Management	NS	--	--	--
Season x Tillage x Management	NS	--	--	--

[a] NS: not significant.

[b] Sunflower was not harvested.

Table 7. Percentage of wild type plants at position 197 of ALS gene (Pro/Pro) and mutant plants with any substitution in this position for any of the two alleles (X/Pro, Pro/X or X/X) for three management systems, each under direct drilling and intensive tillage, in two years in Cubells (Spain). No mutant plants were found out of 900 in position Trp574 of the ALS gene. Means within a column (year factor) followed by the same letter indicate that no significant difference (P < 0.05) was detected by means of the Tukey's test at the 5% level of probability.

Soil Tillage	Management system	2013		2016	
		Wild type	Mutants	Wild type	Mutants
Direct drilling	Chemical	1 ± 1 a	99 ± 1 a	4 ± 3 a	96 ± 4 a
	Rotation PRE	3 ± 2 a	97 ± 1 a	1 ± 2 a	99 ± 3 a
	Rotation POST	5 ± 5 a	95 ± 2 a	7 ± 6 a	93 ± 1 a
Intensive tillage	Chemical	2 ± 2 a	98 ± 2 b	3 ± 4 a	97 ± 4 a
	Rotation PRE	6 ± 1 a	94 ± 1 b	13 ± 5 a	87 ± 1 a
	Rotation POST	7 ± 4 a	93 ± 4 b	6 ± 2 a	94 ± 2 a

	Wild type	Mutants
Season	NS	NS
Tillage	NS	NS
Management	NS	NS
Tillage x Management	NS	NS
Season x Tillage	NS	NS
Season x Management	NS	NS
Season x Tillage x Management	NS	NS

[a] NS: not significant.

Table 8. Estimated LD_{50}, slope at LD_{50} and resistance factor (RF) values for Cubells (herbicide-resistant) and a susceptible *Papaver rhoeas* populations when sprayed with tribenuron-methyl, florasulam, imazamox and 2,4-D.

Population	LD_{50} ± SE (g ai ha^{-1})[a]	Slope ± SE[b]	RI[d]
	Tribenuron-methyl		
herbicide-resistant	130 ± 43	-0.45 ±0.1	2311
susceptible	0.1 ± 0.0	0.75 ± 0.0	1
	Florasulam		
herbicide-resistant	0.1 ± 0.0	0.75 ± 0.0	7.2
susceptible	0.2 ± 0.0	2.0 ± 0.6	1
	Imazamox		
herbicide-resistant	8.8 ± 1.5	-1.0 ± 2.0	9.3
susceptible	1.0 ± 0.1	-2.0 ± 0.5	1
	2,4-D		
herbicide-resistant	1266 ± 192	-2.8 ± 2.7	14.6
susceptible	87 ± 12	-2.0 ± 0.6	1

[a] LD_{50}, ALS-inhibitor concentration for 50% reduction of survival.

[b] The slope at $LD_{50.}$

[c] RI, Resistance Index.