Title: A survey of *Lolium rigidum* populations in citrus orchards: factors explaining infestation levels

Running title: *Lolium rigidum* in citrus orchards

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This word has 5 supplementary tables that do not appear in the printed article but that do accompany the online paper.

Abstract

Herbicide resistant *L. rigidum* in Mediterranean (Spanish) citrus orchards was reported in 2005, and it poses a serious threat to crop management. The main objective of this research was to investigate which components could be responsible for the persistence of annual ryegrass populations in Mediterranean mandarin and orange orchards. This is the first study regarding *L. rigidum* populations in Mediterranean citrus orchards.

Surveys were conducted in 55 commercial citrus orchards in eastern Spain in 2013 by interviewing cooperative technicians about crop management. Infestation by *L. rigidum* and the presence of harvester ants (*Messor barbarus*) were then estimated in the same orchards. Variables were subjected to two-dimensional analysis, and both univariate and multivariate logistic regression models were fitted for each of the three *L. rigidum* density levels established.

Multivariate models showed the significant factors associated with various *L. rigidum* densities: (1) at low densities, herbicides applied, number of applications in 2013, and the type of irrigation (flood or drip); (2) at medium densities, the presence of harvester ants; and (3) at high densities, herbicides applied (in 2013). Results indicated that drip irrigation and one application of glyphosate mixed with other herbicides (or herbicides other than glyphosate) were associated with lower *L. rigidum* densities. Alternative management options presented here should help farmers reduce weed problems in Mediterranean citrus orchards. Future research is required to better understand the presence of herbicide resistant populations as well as the possible beneficial presence of granivorous ant species.

Keywords: rigid ryegrass, seed predation, glyphosate, herbicide resistance, weed management.

Introduction

Citrus crops are widespread in the Mediterranean region. Although it is a geographical region with frequent water shortages, the surface area of land under citrus fruit cultivation has been expanding (Rana *et al.* 2005). As citrus grows during the dry season, irrigation is therefore essential to obtain yields (Tejero *et al.* 2011). The two methods of supplying water to citrus orchards are flood and drip irrigation (MAPA 2012). In flood irrigation systems, water is transported to the field in canals and spread among the trees via lateral surface flow. Drip irrigation increases water and fertilizer use efficiency by using localized irrigation, allowing better field access for equipment and decreasing spread of weeds (Fereres *et al.* 2003). The frequency of fertilization depends on the irrigation system. Flood systems are not suited for frequent fertilizer applications, while drip irrigation allows for more frequent applications (Quiñones *et al.* 2007).

Conventional weed management practices in Spanish citrus orchards rely on annual applications of herbicides, including non-selective and residual herbicides, while non-chemical strategies (i.e. cultivation or mowing) are not common practices (Verdú & Mas 2007). However, repeated applications of herbicides accompanied by the absence of other control tactics may lead to the development of weed resistance (Shaner *et al.* 2012). The first report on herbicide resistant annual ryegrass (*Lolium rigidum* Gaud.) in Spanish citrus orchards was in the region of Castellon (Fernández-Anero *et al.* 2005). This scenario poses a threat to the sustainability of citrus orchards, because grasses can reduce vegetative growth and yield in tree crops without an adequate weed management. It has been proven that grass competition affects growth, leaf N, and leaf water potential of peach orchards, reducing yield up to 37% (Tworkoski & Glenn 2012). In citrus crops, other weed control techniques should be developed to avoid overreliance on a single herbicide (i.e., glyphosate), to decrease development of resistant species and to preserve the sustainable use of glyphosate (González-Torralva *et al.* 2013). Integrated weed management strategies are recommended, and various scientific studies suggest different methods, such as the application of glyphosate in mixtures of herbicides with different modes of action (Beckie & Reboud 2009), seed predation (Westerman *et al.* 2003), mechanical control, tillage, or mowing (Verdú & Mas 2007). In order to improve current weed control measures, farmers must be offered packages of integrated farming systems.

Seed predation is a plant-animal interaction in which granivores (seed predators) feed on plant seeds as a main food source (Hulme & Bekman 2002). Granivorous ants are important seed predators that cause high seed losses in Mediterranean agro-ecosystems (Plowes *et al.* 2013). The harvester ant *Messor barbarus* L. causes 46 to 100 % of the seed losses in arable fields in NE Spain (Westerman *et al.* 2012). This suggests that harvester ants are able to eliminate a significant number of seeds of troublesome weeds. The *Messor* species that are found in citrus orchards in NE Spain forage on the ground beneath trees and between the rows, and feed on many nearby plant species (Platner *et al.* 2012). In irrigated fields in Valencia, the harvester ant *M. barbarus* represents a majority of the ant populations, so they may be an important factor in biological weed control (Urbaneja *et al.* 2006). Little is known, however, whether this ant species influences the presence of weed species in citrus orchards, if it has a role in weed control, or if it hinders the spread of *L. rigidum* populations, especially seeds of glyphosate resistant plants.

The main objective of this research was to investigate which components could be responsible for the persistence of *L. rigidum* populations in mandarin and orange orchards in Spain. Citrus fields were surveyed by personally interviewing managers, and the densities of the weed *L. rigidum* were estimated (heads per m^{-2}). Various factors were considered such as weed management (chemical vs. non-chemical), crop management and the presence of harvester ants in these fields.

Material and methods

Surveyed area

Research was conducted in Eastern Spain in July and August 2013 and was based on interviews regarding agricultural management (see below) and surveys which were conducted to identify management techniques used for each separate field. Surveys were carried out in 55 commercial citrus orchards in five municipalities, namely Castellon de la Plana, Onda, Nules, Villareal and Burriana (see supporting material Table 1). The interview consisted of questions about crop type and agricultural management techniques: age of citrus crop, type of citrus crop, variety, spacing between trees, irrigation system, presence of weed species, opinion about possible herbicide resistance, herbicides used and number of applications, timing of herbicide applications, history of herbicide applications, mechanical operations, and timing of the mechanical operations and fertilization. All fields were located using the coordinates from SIGPAC (2013). Most fields were managed by technicians from cooperatives, except for a few orchards that were partially managed by the owners. The technicians were from nine different cooperatives in the area (see supporting material Table 1). According to the main cooperative center (INTERCOOP), farmers rarely manage their orchards themselves.

Orchard management

A total of 46 fields were planted with mandarin trees (*Citrus reticulata* Blanco), and nine were planted with different varieties of orange trees (*Citrus maxima* x *Citrus sinensis* (L.) Osbeck). The majority of mandarin fields (28) were planted with 'clemenules' variety, while the remainder of the fields was planted with 18 other varieties (supporting material in Table 1). Fields were surrounded by other irrigated mandarin and orange orchards. The trees were planted in a regular square pattern, with spacing between the trees ranging from 2 to 6 m (supporting material in Table 2). Out of a total 55 fields, only one orchard was not in production. The age of trees ranged from 2 to 25 years (supporting material in Table 2) and field size was from 0.5 to 2 ha.

Herbicide applications

During the 2012/13 season, herbicides were sprayed in the orchards from October 2012 until end of July 2013 (supporting material in Table 3). Herbicide information regarding applications prior to the autumn of 2012 was also included in the analysis (supporting material in Table 3). In one orchard, herbicides were not sprayed due to certain experimental management practices incurred over the last two years, and another orchard had been neglected during the previous season. In 47 orchards the technicians used glyphosate or a mixture of glyphosate and other herbicides. Technicians mostly used 36% glyphosate formulations (at 0.72-1.8 kg ai per ha); 36% glyphosate was mixed with other herbicides such as terbuthylazine, oxyfluorfen, quizalofop, MCPA, glufosinate, flazasulfuron, fluroxypyr, and sometimes adjuvant was added to the mixtures. Neither glyphosate nor any mixtures were applied in six orchards in the late summer of 2012 and nine orchards during 2013. Instead, these applications were completely replaced with amitrol, flazasulfuron or quizalofop.

The mixtures of glyphosate and other herbicides differed between fields, seasons and applications, so the numerous combinations of herbicides and their mixtures would make further analysis difficult. The herbicide applications were therefore classified into four groups: 1) glyphosate, 2) glyphosate mixed with other herbicides, 3) other herbicides 4) no herbicide application.

Irrigation, fertilization system and mechanical operations

The citrus crop was maintained in well watered conditions, either with flood or drip irrigation. NPK fertilizer was used. Each year, several applications of NPK were administered via fertigation (supporting material in Table 1). In drip irrigated fields (Figure 3), fertilization ranged from 180 to 220 kg N ha^{-1}, while the flood irrigated fields were fertilized with higher amounts of NPK, according to the technicians (data unavailable). In order to achieve maximum yield, fertilizer and irrigation dates and rates varied between the fields because they were modified by each cooperative. According to the data from Sindicato de Riego de Castellon, Castellon de la Plana drip irrigated fields received an avergae of 12 m^3 ha^{-1} of water per hour, at a frequency of 2.5 hours per day (supporting material in Table 4). Unfortunately, these data are unavailable for orchards with flood irrigation systems. Regarding mechanical operations, a rotavator was employed in only one young orchard, specifically when the pre-emergence herbicide was applied. The other orchards were not tilled. Mechanical operations involved blade mowing of the spontaneous cover ground, mowing using a flail mower or flail crusher, or tractor tilling plants whenever they reached a height of 50 cm (supporting material in Table 2). Spontaneous flora was mowed two times per year, in April and July.

Weed survey

The 20-m-distance method was elected for estimating the density of *L. rigidum* (Colbach *et al.* 2000). This method was modified, however, by using the diagonal line between the field corners. After herbicide treatments in July, heads of *L. rigidum* were counted in a 1 m^2 frame that was placed every 20 m along the transect. The distance was introduced to limit dependence between samples. The number of samples ranged from five to eleven frames per transect, due to unequal distribution of weeds in the plot and variable field size. The same number of frames in all fields would have caused some weed patches to be missed. A total of three *L. rigidum* density levels were considered to classify each frame sampling: less than 1 head per m^{-2}, 1-10 heads per m^{-2} and >10 heads per m^{-2}. Other weeds were found in the orchards, but they were not dominant species (supporting material in Table 2).

Presence of ant nests

The presence of harvester ants was determined in two ways. In the first method, we used the same transects staked for weed sampling to determine the presence of *Messor barbarus* nests in each 20 m section. It is known, however, that harvester ant nests can have a clumped distribution that may cause an absence of ant nests in some areas of the field (Blanco-Moreno *et al.* 2014). For this reason, an additional counting method was included. It involved walking between the tree rows and along the field edge to record the evidence of nest presence with an approximate nest number. When evidence of short-term predation activity by harvester ants was observed, predated weed seeds were then identified. Seed predation percentages were not measured.

Statistical analysis

Univariate analysis

Two-dimensional analysis of the variables was performed in order to explore the relationships between each of the independent variables and three densities of *L. rigidum* (<1 heads per m^{-2}; 1-10 heads per m^{-2} and >10 heads per m^{-2}). Contingency tables were constructed for qualitative variables and the chi-square test was used for each of them separately (supporting material in Table 4). The non parametric Kruskal-Wallis test was performed for quantitative variables since the variables did not have normal distribution (confirmed by the Shapiro-Wilk test) (Table 1).

Furthermore, a logistic regression model was fitted for each independent variable at each *L. rigidum* density (<1 heads per m^{-2}, 1-10 heads per m^{-2} and >10 heads per m^{-2}*). L. rigidum* density was defined as the binary dependent variable. Each defined level of density was coded as binary (1 when the weed was present at the level of density studied and 0 when it was present at the rest of levels, so as to serve as the reference category). To test the statistical significance of each independent variable, each model was compared with the corresponding null model using the conditional Likelihood Ratio Test (LRT). The proportion of 'variance explained' for each model was evaluated using Nagelkerke pseudo-R^2 determination coefficient (analogous to R^2 coefficient of determination of the linear regression) (Nagelkerke, 1991) and the misclassification error.

Multivariate analysis

To assess the combined effect of all the variables on each density level, we obtained a Multiple Logistic Regression model, using a stepwise procedure based on Likelihood Ratio Test for each *L. rigidum* density. Akaike Information Criterion (AIC) was used to select the best model. In order to assess how well the model described the data, analysis of deviance was performed. The fit of the model was assessed using the same parameters as in the case of the univariate models.

Results

Weed survey

Lolium sp. was the main weed species in all orchards, in particular *L. rigidum*, though *L. multiflorum* was also present in some fields. Other spontaneous weeds were found between the tree rows, such as *Conyza* sp., *Solanum* sp., *Convonvulus arvensis*, *Equisetum* sp., *Amaranthus* sp., *Asparagus* sp., *Diplotaxis erucoides* and *Parietaria judaica* (supporting material in Table 2).

Harvester ants

M. barbarus was the only granivorous species in the surveyed orchards. *M. barbarus* nests were found in field edges and between the tree rows in the field. No nests were observed under the trees, which is most likely due to the lack of insulation. The nests were large in size (quantified by having several nest entrances and a high number of workers), while small colonies were detected in only a few fields. The number of nests was approximated to be 0-5 nests per field, based on observations from the diagonal transect as well as between the rows (supporting material in Table 2). Seed predation was observed in almost all fields with *M. barbarus*. The harvester ants mostly removed *L. rigidum* and *Solanum* sp. seeds, though in one orchard, *Conyza* spp. seeds were observed surrounding nest entrances.

Statistical analysis

The Chi square test showed that of all qualitative variables, only the 2013 herbicide treatment significantly influenced (p = 0.0421) the three levels of defined density of *L. rigidum* (supporting material in Table 5). None of the quantitative variables showed significant differences (p > 0.05, Table 1).

Logistic models

Low density (<1 heads m^{-2})

Univariate log models: Logistic regression models fit for each variable (Table 2a), showed significant associations between low density of *L. rigidum* and (1) the herbicide application types in 2013 (p = 0.003) and (2) the irrigation system (p = 0.033). In 2013, the Nagelkerke R^2 determination coefficient was around 32% for herbicides and 12% for the irrigation system, thus explaining 44% of the variance.

Multiple log models: The best multiple logistic regression model (p = 0.008) included two variables: herbicide application types in 2013 and the number of applications (Table 2b). This model explained 35% of the variability in the response, according to Naglekerke's determination coefficient and it had a misclassification error of 23%. In 2013, the application of herbicides, other than glyphosate was significantly related to low density level of *L. rigidum* (estimate = 2.55; pr(>Chi) < 0.01). For the same year, a low number of herbicide applications was related to the samples with low density level of *L. rigidum* (estimate = -1.03; *Pr*(>|z|) < 0.05).

Medium density (1-10 heads m^{-2})

Univariate log models: Regarding medium density of *L. rigidum*, logistic regression models fitted for each variable (Table 3a) showed that two variables were significantly associated: *M. barbarus* presence (p = 0.036) and herbicide application types in 2012 (p = 0.043). Nagelkerke R^2 determination coefficient was around 16% for *M. barbarus* and and 20% for herbicides in 2012, thus explaining 36% of the variance.

Multiple log models: The most accurate multiple logistic regression model related *M. barbarus* to the increasing of the frequency of *L. rigidum* medium density samples (Table 3b). According to Naglekerke's determination coefficient, the response had only 16% variability, which is the lowest variation amongst the three densities. The misclassification error was higher than estimated for low density (27%). Absence of *M. barbarus* was related to the medium density (estimate = 0.56; *Pr*(|z|) = 0.43).

High density (> 10 heads m^{-2})

Univariate log models: Univariate log models for the high density value of *L. rigidum* (Table 4a) showed that herbicide application types in 2013 were almost significant associated (p = 0.051). Nagelkerke R^2 determination coefficient was around 17% and misclassification error was 33%.

Multiple log models: The most accurate multiple logistic regression model (p = 0.023) included herbicide application types in 2013 and presence of other weed species (Table 4b). According to Naglekerke's determination coefficient, the response had only 31% variability and the misclassification error was 33%. The application of glyphosate combined with other herbicides (estimate = -2.32; *Pr*(>|z|) = 0.02) or an application of any other herbicide (estimate = -3.71; *Pr*(>|z|) = 0.01) were related with the decrease of *L. rigidum* high density samples. Presence of other weed species in the plot was significantly associated with *L. rigidum* high density samples (estimate = -1.67; *Pr*(>|z|) = 0.06).

Discussion

Results showed that herbicides were the most important factor explaining infestations levels of *L. rigidum* in Mediterranean citrus orchards. The herbicides applied, particularly in 2013, were the main factor affecting all densities of *L. rigidum*. Other significant associated factors were: the type of irrigation system and the number of herbicide applications at low *L. rigidum* densities, the presence of harvester ants at medium densities and the presence of other weed species at high densities.

Most of the orchards surveyed had been herbicide treated during the past 25 years (supporting material in Table 3). The timing and number of applications, however, were not uniform and ranged from one to six times per year. Glyphosate was the primary tool for chemical weed control and was often mixed with other herbicides. The herbicide mixtures were used because the technicians observed overall improvement in *Lolium* control, compared to glyphosate alone, as occurred in other Mediterranean perennial crops (Urbano *et al.* 2007; Sansom *et al.* 2013). No information is available on whether *L. rigidum* surveyed populations are glyphosate resistant, but according to the observations by technicians, it is hypothesized that most populations have developed some level of resistance over the past six years. In 2013, the herbicide mixtures for nine fields did not include glyphosate.

According to multivariate models, the application of herbicides with glyphosate exclusion was significantly correlated with low *L. rigidum* densities in 2013. In the same year, less frequent herbicide application was also related to lower *L. rigidum* density. In contrast, application of the same types of herbicides with several annual repetitions was associated with higher *L. rigidum* density. During the interview, few technicians recognized that herbicides were sprayed more times than recommended. Unfortunately, this datum is unavailable.

Herbicide rotation is one possible solution for avoiding multiple applications of herbicides with the same modes of action (Beckie & Reboud 2009). In Spanish perennial crops, it is known that multiple applications of glyphosate with wrong timing and poor application techniques may lead to resistance (Shaner *et al.* 2012; Sansom *et al.* 2013). In this study, applications based only on glyphosate were related to higher *L. rigidum* densities. Herbicides applied in 2012 (Table 3a) were related to a medium density of *L. rigidum*. These increased populations of *L. rigidum* occurred, however, during the autumn crop harvest and could thus be attributed to the fact that this is the period when herbicides were usually not sprayed. This seasonal period was not considered for herbicides applied in 2013. It is likely that if the corresponding data had been obtained in 2013, they would have shown similar results to 2012.

Data show that the flood irrigation system is related to high densities of *L. rigidum*. Fields with flood irrigation generally have higher infestation levels due to the more resources available. Not only is the water input on the soil surface always higher compared to drip irrigation, but the addition of fertilizers in flood water may also increase the growth and abundance of weeds. The amount of nitrate retained in the soil profile above 90 cm of depth is greater under flood irrigation than under drip irrigation (Quiñones *et al.* 2007). It is known that nitrogen fertilizer promotes growth and the number of *L. rigidum* ears, thus increasing competition (Ponce 1998). In our study, data referring to N doses applied are not available. The technicians explained their efforts to improve the irrigation system and replace flood with drip irrigation. Neither they, nor the growers, however, had the perception that *L. rigidum* was expanding under flood irrigation.

Harvester ants (*M. barbarus*) were the main ant species observed in the fields, as confirmed by previous studies in this area (Monzó *et al.* 2013). They are present in citrus orchards during most of the year, except in the winter months November to April (Urbaneja *et al.* 2006). *M. barbarus* never climbs on trees and forages on the soil surface, therefore it is a species that does not damage citrus fruits (Platner *et al.* 2012). In surveyed fields, ant nests were located both in the field and on the borders. This differed from drip irrigated citrus orchards in Valencia, where ants were settled only along the edge (Monzó *et al.* 2013). Cerdà *et al.* (2009) did not find differences in nest numbers between the margins and the inner part of the citrus orchards.

In our study, the role of harvester ants was a significant factor at medium densities of *L. rigidum*. The actual effect of these ants on the density of *L. rigidum* was, however, unclear because misclassification error was high (27%) and only 16% of the variance was explained by the model. Moreover, the absence of *M. barbarus* was only significantly related with medium plant density; it is unknown what can happen at other infestation levels, especially high. During the survey, predation activity was observed in many fields.

It is therefore likely that in this agro-ecosystem, harvester ants can play an ecological role with the possibility to influence weed infestation levels. *Lolium rigidum* is a favorable species for ants (Westerman *et al.* 2012) and they are likely to retrieve most seeds in the field where weed patches are found (Torra *et al.*, accepted). In possible future studies, fields with both high *L. rigidum* and high ant densities could be monitored to understand how ants affect weed infestation levels in citrus orchards.

In this study, the presence of nests was considerably low at approximately 5 nests per field (rough estimation). Similarly, very few ant nests were found in Valencian orchards (Cerdà & Jungersn 2008). Despite their low abundance, all nests surveyed were considered to be large in size because they had several entrances (Baraibar *et al.* 2011). Large nests are known to have a higher number of ants available to forage, which can extend their range of influence up to 30 m (Azcárate & Peco 2003). Mechanical operations did not include soil disturbance so they would not have disturbed the existence of the nests.

Considering these results, there are some management recommendations that can be made to farmers in order to improve weed management in citrus orchards. First, implement alternative weed management options to avoid overreliance on herbicides (Shaner *et al.* 2012) and decrease the number of annual chemical treatments. Among these non-chemical tactics mechanical control, cultivation or mowing should be considered. Mulches are also promising alternatives for managing weeds in the citrus orchards (Verdú & Mas 2007). When herbicides are applied, rotation or mixtures with different modes of action should always be used to reduce the development of resistance (González-Torralva *et al.* 2013). Finally, it is recommended to convert irrigation systems from flood to drip irrigation. Drip irrigation avoids the movement of seeds that can occur when flood irrigation is used (Juárez *et al*., 2010) and improves the crop yield (Bravdo & Proebsting, 1993). In addition, drip irrigation optimises the efficiency of fertilizers applied by direct injection (Fereres *et al*, 2003).

It was not possible to incorporate seedbank data in this study, but its impact on the results is thought to be minor. For this species, only 20-30% of the seedbank can persist to the next season (Chauan *et al.*, 2006). So, the seedbank alone cannot be responsible for the persistence of *L. rigidum* populations, while some of the management factors identified would contribute to persistence by failing to control the species. Therefore, 2-3 years of proper weed management, thus preventing seed replenishment in the soil, may be sufficient to practically eradicate *L. rigidum*, considering its annual seed decay (70-80%).

In summary, agricultural practices that were related to low *L. rigidum* infestation levels in citrus orchards were: (1) fewer applications of glyphosate and other herbicide mixtures and (2) the utilization of a drip irrigation system. Harvester ants were the only significant factor at medium infestation levels. All these factors could have great potential to benefit weed control. Adoption of integrated practices and non-chemical methods in commercial orchards may prevent weed expansion and help to manage the development and spread of glyphosate resistance (Sansom *et al.* 2013). Glyphosate must be preserved as a tool for chemical weed control in Mediterranean citrus orchards. Overall, results should promote improved farming practices, leading to more sustainable agriculture in this perennial cropping system.

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References

Azcárate F.M. and Peco B. 2003. Spatial patterns of seed predation by harvester ants (*Messor* Forel) in Mediterranean grassland and scrubland. *Insectes Soc.* **50**, 120-126.

Baraibar B., Torra J. and Westerman P.R. 2011. Harvester ant (*Messor barbarus* (L.)) density as related to soil properties, topography and management in semi-arid cereals. *Appl. Soil Ecol.* **51**, 60-65.

Beckie H.J. and Reboud X. 2009. Selecting for Weed Resistance: Herbicide Rotation and Mixture. *Weed Tech.* **23**, 363-370.

Blanco-Moreno J.M., Westerman P.R., Atanackovic V. and Torra J. 2014. The spatial distribution of nests of harvester ants (*Messor barbarus* L) in dryland cereals. *Insectes Soc.* **61**, 145-152.

Bravdo B. and Proebsting E.L. 1993. Use of drip irrigation in orchards. *Hort. Tech*. **3**, 44-49.

Cerdà X., Palacios R. and Retana J. 2009. Ant community structure in citrus orchards in the mediterranean basin: Impoverishment as a consequence of habitat homogeneity. *Environ. Entomol.* **38**, 317-324.

Cerdà A. and Jurgensen M.F. 2008. The influence of ants on soil and water losses from an orange orchard in eastern Spain. *J. Appl. Entomol.* **132**, 306-314.

Chauan B.S., Gill G., Preston C. 2006. Influence of tillage systems on vertical distribution, seedling recruitment and persistence of rigid ryegrass (*Lolium rigidum*) seed bank. Weed Sci. **54**, 669-676.

Colbach N., Dessaint F., and Forcella F. 2000. Evaluating field-scale sampling methods for the estimation of mean plant densities of weeds. *Weed Res.* **40**, 411-430.

Fereres E., Goldhamer D.A. and Parsons L.R. 2003. Irrigation water management of horticultural crops. *Hort. Sci.* **38**, 1036-1042.

Fernández-Anero J., Costa J., Plancke M.P., Garnett R. and Starke M. 2005. 30 años de eficacia con Roundup. Visión general y situación en los casos de resistencia a glifosato. *Phytoma España* **173**, 119-125.

González-Torralva F., Gil-Humanes J., Barro F., Domínguez-Valenzuela J. and Prado R. 2013. First evidence for a target site mutation in the EPSPS2 gene in glyphosate-resistant Sumatran fleabane from citrus orchards. *Agron. Sustain. Dev.* **34**, 553-560.

Heap I. 2013. The International Survey of Herbicide Resistant Weeds (on line). Available at: http://www.weedscience.com (last accessed 23 September 2013).

Hulme P.E. and Benkman C.W. 2002. Granivory. In: *Plant-animal interactions. An evolutionary approach* (ed. by Herrera C.M. and Pellmyr O.). Blackwell Science Ltd, Oxford, 132-154.

Juárez A., Solé X and Conesa J.A. 2010. Diversity and richness of exotic weeds in fruit tree orchards in relation to irrigation management. *Asp. Appl. Biol.* **104**, 79–87.

M.A.P.A. 2012. Ministerio de Agricultura, Alimentacion y Medio Ambiente. http/www.magrama.gob.es/es (last accessed 15 September 2013).

Monzó C., Juan-Blasco M., Pekár S., Mollá Ó., Castañera P. and Urbaneja A. 2013. Pre-adaptive shift of a native predator (Araneae, Zodariidae) to an abundant invasive ant species (Hymenoptera, Formicidae). *Biol. Invasions* **15**, 89-100.

Nagelkerke, N.J.D. 1991. A note on a general definition of the coefficient of determination. *Biometrika* **78**, 691-692.

Ponce R.G. 1998. Competition between barley and *Lolium rigidum* for nitrate. *Weed Res.* **38**, 453-460.

Platner C., Piñol J., Sanders D. and Espadaler X. 2012. Trophic diversity in a Mediterranean food web-Stable isotope analysis of an ant community of an organic citrus grove. *Basic Appl. Ecol.* **13**, 587-596.

Plowes N.J.R., Johnson R.A. and Hölldobler B. 2013. Foraging behavior in the ant genus *Messor* (Hymenoptera: Formicidae: Myrmicinae). Myrmecol. *News* **18**, 33-49.

Quiñones A., Martínez-Alcántara B. and Legaz F. 2007. Influence of irrigation system and fertilization management on seasonal distribution of N in the soil profile and on N-uptake by citrus trees. *Agric. Ecosyst. Environ.* **122**, 399-409.

Rana G., Katerji N. and de Lorenzi F. 2005. Measurement and modelling of evapotranspiration of irrigated citrus orchard under Mediterranean conditions. *Agric. For. Meteorol.* **128**, 199-209.

Sansom M., Saborido A.A. and Dubois M., 2013. Control of *Conyza* spp. with glyphosate - A review of the situation in Europe. *Plant Protect. Sci.* **49**, 44-53.

Shaner D.L., Lindenmeyer R.B. and Ostlie M.H. 2012. What have the mechanisms of resistance to glyphosate taught us?. *Pest. Manag. Sci.* **68**, 3-9.

Sistema de Informacion Geografica de Parcelas Agricolas (SIGPAC) http://www.magrama.gob.es/es/agricultura/temas/sistema-de-informacion-geografica-de-parcelas-agricolas-sigpac-/ (last accessed 20 September 2013).

Tejero I.G., Zuazo V.H.D., Bocanegra J.A.J. and Fernández J.L.M. 2011. Improved water-use efficiency by deficit-irrigation programmes: Implications for saving water in citrus orchards. *Sci. Hortic.* **128**, 274-282.

Torra J., Atanackovic V., Royo-Esnal A. and Westerman P.R. accepted. Effect of patch size on seed removal by harvester ants. *Weed Res*.

Tworkoski T.J. and Glenn D.M. (2012) Weed suppression by grasses for orchard floor management. *Weed Tech.* **26**, 559-565.

Urbaneja A., Marí F., Tortosa D., Navarro C., Vanaclocha P., Bargues L. and Castañera P. 2006. Influence of Ground Predators on the Survival of the Mediterranean Fruit Fly Pupae, *Ceratitis capitata*, in Spanish Citrus Orchards. *BioControl* **51**, 611-626.

Urbano J.M., Borrego A., Torres V., Leon J.M., Jimenez C., Dinelli G. and Barnes J. 2007. Glyphosate-resistant Hairy Fleabane (*Conyza Bonariensis*) in Spain. *Weed Tech.* **21**, 396-401.

Verdú A.M. and Mas M.T. 2007. Mulching as an alternative technique for weed management in mandarin orchard tree rows. *Agron. Sustain. Dev.* **27**, 367-375.

Westerman P., Atanackovic V., Royo-Esnal A. and Torra J. 2012. Differential weed seed removal in dryland cereals. *Arthropod. Plant Interact.* **6**, 591-599.

Westerman P.R., Wes J.S., Kropff M.J. and Van Der Werf W. 2003. Annual losses of weed seeds due to predation in organic cereal fields. *J. Appl. Ecol.* **40**, 824-836.

Tables

Table 1. Effect of age crop, rows between the trees, number of herbicides applications in 2012 and 2013 and timing of mechanical operations (means with standard errors) on the three densities of *Lolium rigidum* (heads m^{-2}). (Kruskal-Wallis Chi-square test) *significance <0.05.

	Density of *Lolium rigidum*			Kruskal-Wallis Chi-square	*P**
	<1 heads m^{-2}	1-10 heads m^{-2}	>10 heads m^{-2}		
Age crop	16 ± 6.63	15.53 ± 10.66	14.66 ± 6.81	18.947	0.3316
Rows	4.75 ± 0.66	4.57 ± 0.90	4.33 ± 1.03	7.2231	0.406
Number of applications in 2012	1.31 ± 0.95	1.53 ± 1.16	1.91 ± 1.18	2.9115	0.8199
Number of applications in 2013	2.31 ± 0.95	2.73 ± 1.16	2.92 ± 1.18	4.2116	0.6481
Timing of mechanical operations	1 ± 0.82	1 ± 0.84	1.25 ± 0.74	2.4337	0.4874

Table 2a. Low density of *Lolium rigidum* results from the logistic regression models fitted for each variable separately. Likelihood ratio test (LRT) *P*-value, Naglekerke's *R*2 determination coefficient and misclassification error obtained when using the models for prediction are shown

Variable	LRT *p* value	Naglekerke's R^2	Missclasiffication error
Herbicide application types in 2013	0.003	0.323	0.22
Irrigation	0.033	0.113	0.29
Number of herbicide applications in 2013	0.099	0.069	0.29
Town	0.100	0.220	0.25
Crop	0.161	0.091	0.29
Rows	0.203	0.041	0.29
Spacing	0.214	0.516	0.22
Mechanical operations	0.228	0.168	0.29
Number of herbicide applications in 2012	0.233	0.036	0.29
Other weeds presence	0.494	0.012	0.29
Timing of mechanical operations	0.501	0.012	0.29
Age crop	0.666	0.005	0.29
Seed predation	0.833	0.009	0.29
Messor barbarus	0.849	0.009	0.29
Herbicide application types in 2012	0.864	0.019	0.29

Table 2b. Deviance table showing the significance of each of the variables included in the multivariate logistic regression model, fitted with a stepwise method. Naglekerke's *R*2 determination coefficient and misclassification error obtained when using the model for prediction are shown. Effect of herbicides applied in 2013, number of herbicides applications in 2013 and flood irrigation on the low density of *Lolium rigidum* (<1 heads m^{-2}).

Variable	Df	Deviance	Resident Df	Resident deviation	*p*
Null			54	62.480	
Herbicide application types in 2013	3	6.9020	51	55.578	0.008
Number of herbicide applications in 2013	1	5.1806	50	50.397	0.022
Irrigation	1	2.7274	49	47.670	0.09

Table 3a. Medium density of *Lolium rigidum*. Results from the logistic regression models fitted for each variable separately. Likelihood ratio test (LRT) *P*-value, Naglekerke's *R*2 determination coefficient and misclassification error obtained when using the models for prediction are shown.

Variable	LRT *p* value	Naglekerke's R^2	Missclasiffication error
Messor barbarus	0.036	0.165	0.27
Herbicide application types in 2012	0.044	0.199	0.25
Other weeds presence	0.160	0.051	0.27
Seed predation	0.215	0.079	0.27
Town	0.268	0.159	0.27
Mechanical operations	0.293	0.153	0.27
Irrigation	0.311	0.027	0.27
Spacing	0.492	0.431	0.24
Herbicide application types in 2013	0.505	0.060	0.27
Timing of mechanical operations	0.520	0.010	0.27
Crop	0.705	0.004	0.27
Number of herbicide applications in 2012	0.725	0.003	0.27
Rows	0.804	0.001	0.27
Number of herbicide applications in 2013	0.862	0.001	0.27
Age crop	0.888	0.001	0.27

Table 3b. Deviance table showing the significance of each of the variables included in the multivariate logistic regression model, fitted with a stepwise method. Naglekerke's *R*2 determination coefficient and misclassification error obtained when using the model for prediction are shown. Effect of *Messor barbarus* on medium density of *Lolium rigidum* (1-10 heads m^{-2}).

Variable	Df	Deviance	Resident Df	Resident deviation	*p*
Null			54	64.455	
Messor barbarus	2	6.6407	52	57.814	0.01

Table 4a. High density of *Lolium rigidum*. Results from the logistic regression models fitted for each variable separately. Likelihood ratio test (LRT) *P*-value, Naglekerke's *R*2 determination coefficient and misclassification error obtained when using the models for prediction are shown.

Variable	LRT *p* value	Naglekerke's R^2	Missclasiffication error
Herbicide application types in 2013	0.051	0.176	0.33
Herbicide application types in 2012	0.154	0.122	0.35
Spacing	0.164	0.506	0.27
Number of herbicide applications in 2012	0.171	0.045	0.44
Messor barbarus	0.176	0.082	0.38
Rows	0.176	0.044	0.42
Number of herbicide applications in 2013	0.180	0.043	0.42
Town	0.217	0.161	0.38
Timing of mechanical operations	0.236	0.034	0.47
Mechanical operations	0.376	0.124	0.42
Irrigation	0.397	0.017	0.42
Other weeds presence	0.514	0.010	0.44
Seed predation	0.529	0.031	0.44
Age crop	0.599	0.007	0.44
Crop	0.957	0.000	0.44

Table 4b. Deviance table showing the significance of each of the variables included in the multivariate logistic regression model, fitted with a stepwise method. Naglekerke's *R*2 determination coefficient and misclassification error obtained when using the model for prediction are shown. Effect of herbicides applied in 2013, the rows and presence of other weed species on the high density of *Lolium rigidum* (> 10 heads m^{-2}).

Variable	Df	Deviance	Resident Df	Resident deviation	*p*
Null			54	75.35	
Herbicide application types in 2013	3	9.5198	51	65.833	0.01
Rows	1	0.6549	50	65.178	1
Other weeds	1	4.2154	49	60.963	0.01