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Smaller agricultural fields, more edges, and natural habitats reduce herbicide-resistant weeds

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ABSTRACT

The exponential growth of herbicide-resistant weeds poses enormous challenges to the sustainability of food systems. While great efforts in weed management are being performed at the plot level, the influence of the landscape context on the presence of herbicide-resistant weeds remains largely unknown. We tested these ideas through a large-scale sampling on two of the most important crops globally: maize and soybean. In Argentina, we co-developed with farmers the sampling of 2846 soybean and 1539 maize fields (covering an area of 159 million ha) and measured the presence of herbicide-resistant weeds, landscape context (field size, edge density, natural habitat size), management variables (e.g. fertilization), crop variety, farm identity and region. We found that smaller fields, with higher edge density, and neighboring larger natural habitats were associated to a lower presence of herbicide-resistant weeds. These results were not confounded with the influence of some other management variables (e.g. fertilization), crop variety, farm or region. Landscape design is an important, but underrepresented, management tool that could help to achieve a sustainable control of weeds.

1. Introduction

Herbicide-resistant weeds have spread worldwide at exponential rates and present one of the most critical challenges for extensive agriculture nowadays (Heap, 2014; Heap and Duke, 2018; Scursoni et al., 2019). Synthetic herbicides were introduced into agroecosystems 70 years ago and continue today as the main strategy to control weeds (Heap, 2014; Heap and Duke, 2018; Vila-Aiub, 2019). Herbicide resistance emerges as predictable result of selection by repeated and intense use of herbicides (Dixon et al., 2021; Heap, 2014; Hicks et al., 2018). This is the case for large-scale monocultures, which have dominated agricultural landscapes, replacing more diverse farming systems and relying on high amounts of herbicides (Gage et al., 2019; Ramankutty et al., 2018).

There are many alternatives to herbicides by which farmers may reduce the spread of herbicide-resistant weeds (Beckie, 2006; Heap, 2014; Scursoni et al., 2019). Solutions at the landscape scale belong to the least studied (Seppelt et al., 2020), but have enormous potential (Dauer et al., 2009). Since agricultural landscapes are being designed with increasing size of crop fields, they may enhance the spread of herbicide resistance in comparison to more diverse and complex landscapes. This could be explained by multiple hypotheses. For example, as resistance-inducing mutations are often linked to fitness costs in herbicide-untreated conditions (e.g., diversion of resources from reproduction to defense; Vila-Aiub, 2019), more diverse and complex landscapes could promote the outcross of weeds inside crop fields with

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those outside crop fields and thus reduce the spread of herbicide-resistant traits. Also, as weed community composition inside a crop field changes with distance to field edge (Bourgeois et al., 2020), smaller fields neighboring large natural and semi-natural habitats can act as barriers to the spread of herbicide-resistant traits. Here, we tested these ideas through a large-scale sampling on two of the most important crops globally: maize and soybean.

2. Methods

In Argentina, we performed an extensive, standardized protocol on 1539 maize and 2846 soybean fields covering an area of almost 1.6 million km^2 (159 million ha; Fig. 1). The data were gathered and systematized in collaboration with CREA (https://www.crea.org.ar/), a non-profit civil association integrated by more than 2000 farming companies that share farming experiences and knowledge. The data are stored as CREA DAT (https://www.crea.org.ar/dat-crea/), a unified agricultural database to analyze the main productive variables. For each field, we gathered data on the presence of herbicide-resistant weeds (two categories: present or absent), field size (ha), spatial location (latitude and longitude), region (11 regions were considered according to CREA database), farm identity, crop variety, N fertilization (kg ha^{-1}), and P fertilization (kg ha^{-1}). When working with such a large number of sampling sites, collection of seeds and assaying of resistance frequency is impossible. Therefore, herbicide-resistant weeds were classified as "present" when at least one dominant and uncontrolled weed population of the herbicide-targeted species has been documented to evolve resistance. This is a valid estimate because samplings were performed after herbicide applications and because, where herbicide-resistant weeds were present, we commonly observed more than one species of herbicide-resistant weeds. Sampling effort was the same in small and large fields. Most growers use similar standard methods of weed control based on the use of herbicides (and no tillage), mainly glyphosate, irrespective of field size. However, we could not measure the amount of glyphosate used in each of the 4385 sampled fields. The historical land use and management intensity was accounted for in the statistical analyses through several other proxies (see below).

In addition, we used Argentina's national Crop Data Layer (de Abelleyra et al., 2019) to quantify the landscape composition and configuration in circular sectors of a 1500 m radius around each field, as many weed seeds disperse at least 500 m from source populations (e.g., Dauer et al., 2007). The average distance between maize fields was 551.5 km (SD 321.78 km). Of the total number of point pairs (1,332, 528), only 0.26% were less than 3 km apart. In the case of soybean fields, the average distance was 506.8 km (SD 335.4 km). Of the total number of point pairs (4,131,375), only 1.1% were less than 3 km apart.

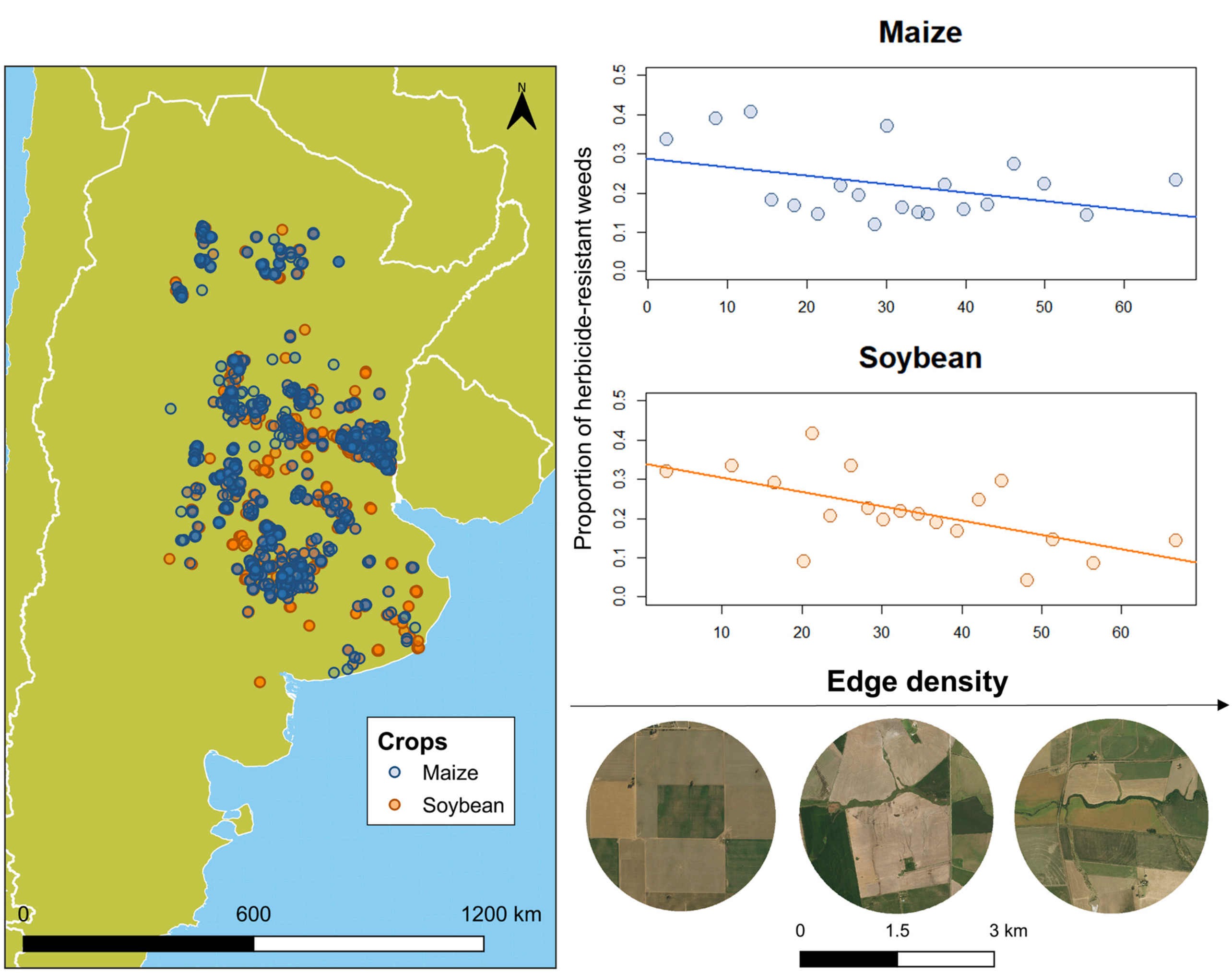

Fig. 1. Herbicide-resistant weeds are influenced by the landscape context. The presence of herbicide-resistant weeds was surveyed in 1539 maize and 2846 soybean fields across the extensive agricultural region of Argentina (left side). Edge density was associated with a lower presence of herbicide-resistant weeds in both maize and soybean fields (right side). The dispersion plots on the right side show the proportion of fields with herbicide-resistant weeds calculated at an interval of 5 m ha^{-1} of edge density (this was performed just for graphical purposes, the mixed-effects models focus on the presence of herbicide-resistant weeds at each field, see Methods). The satellite images on the bottom right are centered on soybean fields and visualize a gradient of edge density.

For the landscape composition, we quantified the mean size of all patch areas of natural and semi-natural habitats ("natural habitats" hereafter), which include grasslands, wetlands, shrublands and forests. For the landscape configuration, we quantified edge density (m ha^{-1}) as the sum of the lengths (m) of all edge segments in the landscape, divided by the total landscape area (ha).

The presence of herbicide-resistant weeds was modeled through a generalized linear mixed-effects approach in R assuming a binomial error distribution (R version 3.6.3, glmmTMB package, glmmTMB function Brooks et al., 2017, R Core Team, 2020). We established separate models for maize and soybean. All models considered region, farm identity, and crop variety as non-nested random-effects (i.e. random intercepts) to account for spatial, environmental, genetic, and management influence on weed resistance. For fixed-effects, we estimated two models. The first included field size, edge density, natural habitat size, and their interactions as fixed-effects. From this model, we performed multi-model inference based on Akaike's Information Criterion (AIC) (Harrison et al., 2018). Minimum adequate models were selected after evaluating the models resulting from all possible combinations of the predicting variables and their interactions (MuMIn package, dredge function) (Bartón, 2019). Relative importance values were calculated for each predictor by summing the Akaike weights over all models that include the predictor (MuMIn package, importance function). The predictor variable with the largest relative importance value is estimated to be the most important for explaining variance in the response variable.

For the second model, fixed-effects included the predictors of the minimum adequate model plus N fertilization and P fertilization, which are important co-variables related to management and environmental conditions. Similar parameter estimates of our focused predictors (i.e. field size, edge density, and natural habitat size) between the first and the second model imply that their impacts are not confounded by other management and environmental variables (note that all models included the above-described random effects as a complementary way to account for spatial, environmental, genetic, and management variables). We tested statistical model assumptions using the DHARMa package (Hartig, 2021). No spatial autocorrelation was found in the residuals of the models (gstat package, variogram function). Variance inflation factors (VIFs) among all predictors (field size, habitat size, edge density, N fertilization, P fertilization) were always lower than 1.8 in both maize and soybean databases.

3. Results and discussion

We found that 22% of the 1539 maize fields and 20% of the 2846 soybean fields had herbicide-resistant weeds. Such weeds have been a reality for farmers for decades. Associated yield reductions have been successfully overcome because the chemical industry provided until the late 80′s a steady supply of new herbicide sites of action to combat resistant weeds (Heap, 2014). However, this is no longer the case, as no new herbicide sites of action have been delivered to the market in over 30 years (Heap, 2014; Heap and Duke, 2018). In particular, glyphosate resistance evolution has shown an alarming increase among weeds in recent years (Gage et al., 2019; Heap and Duke, 2018; Vila-Aiub, 2019). Genetically-modified glyphosate-resistant crops have enabled farmers to use glyphosate in broadcast post-emergence applications in maize, soybean, cotton, canola, sugar beet and alfalfa, making glyphosate the most widely used herbicide globally (Gage et al., 2019; Heap and Duke, 2018).

In our study, the main resistant weeds reported for maize and soybean were *Amaranthus* sp., *Conyza* sp., *Echinocloa* sp., *Chloris* sp., *Trichloris* sp., and *Sorghum halepense* (Table 1). These six genii account for 63.8% and 72.6% of all records of main resistance occurrence in maize and soybean, respectively (Table 1). In total, Argentina has almost 30 weed species with resistance to different herbicides (http://www.weedscience.org/). The majority is resistant to glyphosate, due to the high dependence of maize and soybean crop systems on this herbicide (Scursoni et al., 2019).

Table 1
Frequency of weed species reported as the most dominant, second-most dominant, and third-most dominant in maize and soybean fields of Argentina.

Weed species	Dominant	Second-most dominant	Third-most dominant	Overall presence
Maize fields				
Amaranthus sp.	24.7%	16.1%	24.1%	64.9%
Conyza sp.	9.7%	26.3%	31.0%	67.0%
Echinocloa sp.	11.2%	4.2%	6.9%	22.3%
Chloris sp./ Trichloris sp.	9.4%	14.4%	3.4%	27.2%
Sorghum halepense	8.8%	3.4%	13.8%	26.0%
Others	36.2%	35.6%	20.8%	
Soybean fields				
Amaranthus sp.	40.7%	12.2%	4.3%	57.2%
Conyza sp.	11.5%	36.7%	25.7%	73.9%
Echinocloa sp.	8.6%	10.0%	4.3%	22.9%
Chloris sp./ Trichloris sp.	6.0%	9.6%	17.1%	32.7%
Sorghum halepense	5.8%	7.8%	2.9%	16.5%
Others	27.4%	23.7%	45.7%	

Mixed-effects models showed that maize fields in landscapes with higher edge density and larger adjacent natural habitats had a lower presence of herbicide-resistant weeds (Fig. 1, Table 2). On the contrary, larger field sizes were associated with a greater presence of herbicide-resistant weeds (Table 2). These effects were consistent between models with and without co-variables reflecting the independent (not confounded) effects of edge density, natural habitat size, and field size from other spatial, environmental, genetic and management variables relevant to weed management (Table 2). Soybean fields showed similar results (Fig. 1, Table 2): the presence of herbicide-resistant weeds was lower in landscapes with higher edge density but increased with field size. However, in this case no association with natural habitat size was found. Again, co-variable inclusion did not alter effects for edge density and field size substantially (Table 2).

Diverse and complex landscapes could reduce the spread of herbicide-resistant weeds because of multiple reasons. For example, first, given that weed community composition inside a crop field changes with distance to field edge (Bourgeois et al., 2020), smaller fields neighboring large natural and semi-natural habitats can act as barriers to the spread of herbicide-resistant traits. Second, given that there are fitness costs of herbicide resistance mutations in the absence of herbicide applications (Vila-Aiub, 2019), greater outcross between weeds inside and outside crop fields may be promoted by more diverse landscapes with more edges and interactions with neighbor fields, thus reducing the spread of herbicide-resistant traits. If the amount of natural habitat is increased, weeds face more complex fitness landscapes with alternating selection targets. An implementation with a simultaneous reduction of field size could therefore provide an effective natural control mechanism for herbicide-resistant weeds. Third, it may be possible that doses of glyphosate are lower in more diverse and complex landscapes, however, we found no confounding effects with several other variables that might be associate to glyphosate use, including N fertilization, P fertilization, crop variety, farm or region. Overall, our results can be seen as a starting point for discussing how future studies could be targeted to elucidate alternative explanations for the reduction of herbicide-resistant weeds in more complex landscapes. These could include a population genomics study of species with contrasting biology (Dixon et al., 2021), sampling of vegetation in habitats neighboring fields with analysis of effects of landscape features at nested spatial scales (Bourgeois et al., 2020) or more comprehensive analysis of components of the interaction with field management (Hicks et al., 2018).

Table 2
Results from mixed-effects models for the influence of field size, natural habitat size, and edge density on the presence of herbicide-resistant weeds. The best models were derived from comparing the Akaike information criterion (AIC) values of all possible combinations of predicting variables without co-variables (see Methods). In bold, values for which the 95% confidence interval does not overlap with zero. Fixed-effect estimates and their standard errors for field size, natural habitat size, and edge density are very similar in models with and without co-variables showing their independent effects on the presence of herbicide-resistant weeds. The models also accounted for the variability in crop variety, management and environment by including variety, farm, and region as random-effects. The relative importance is the sum of the AIC weights of all the models with each predictor.

	Maize			Soybean		
	Model without co-variables		Model with co-variables	Model without co-variables		Model with co-variables
	Relative importance	Parameter estimate	Parameter estimate	Relative importance	Parameter estimate	Parameter estimate
Fixed effects (mean)						
Intercept	–	-11.0 (1.5)	-8.9 (1.6)	–	-10.0	-12.1 (1.1)
Field size	0.79	0.0040 (0.0030)	0.0043 (0.0030)	**0.97**	**0.0063 (0.0023)**	**0.0075 (0.0025)**
Habitat size	**0.99**	**-0.010 (0.0040)**	**-0.010 (0.0039)**	0.46		
Edge density	**0.98**	**-0.054 (0.018)**	**-0.058 (0.018)**	**0.97**	**-0.028 (0.011)**	**-0.024 (0.012)**
Field size • habitat size	0.48			0.13		
Field size • edge density	0.28			0.61		
Edge density • habitat size	0.35			0.13		
Co-variables						
N fertilization			0.00036 (0.0068)			-0.022 (0.088)
P fertilization			-0.064 (0.022)			0.16 (0.040)
Random effects (sd)						
Region		1.9	1.4		0.66	1.0
Variety		0.83	0.72		1.0	0.74
Farm		21.5	19.6		17.0	18.9
Delta AIC with null model		11	–		9	–

The increase in herbicide reliance over the last decades exerted one of the strongest selection pressures ever experienced by weeds, which has inevitably led to the evolution of herbicide-resistance in an increasing list of weed species (Dauer et al., 2009; Vila-Aiub, 2019). While herbicide mixtures and herbicide rotations may slow the evolution of herbicide resistance, these practices are only delaying the inevitable when herbicides are the sole weed control strategy (Gage et al., 2019; Hicks et al., 2018). Our results suggest that landscape design could be an important, complementary management tool to achieve a sustainable control of weeds. Agricultural landscapes could be designed with smaller agricultural fields, more edges, and natural habitats, with co-benefits for biodiversity and yield stability (Seppelt et al., 2020). Unfortunately, the opposite trend has been observed in most agricultural landscapes during the past decades (Ramankutty et al., 2018). The potential to control herbicide-resistant weeds might provide an important incentive to halt current destruction of natural habitats and design more diversified agricultural landscapes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The authors do not have permission to share data.

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