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Exoferality in sunflower (*Helianthus annuus* L.): A case study of intraspecific/interbiotype interference promoted by human activity

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ABSTRACT

Helianthus annuus subsp. *annuus*, a biotype of the same species of the domesticated sunflower *H. annuus* var. *macrocarpus*, is an emergent noxious weed in several regions of the world. The frequent hybridization that occurs between the two taxa could explain its diffusion in agricultural fields. The invasive dynamics of a weedy biotype was studied in a recently invaded field during four successive seasons. The weedy biotype was grown in an experimental plot and was characterized phenotypically. Competition between weedy and cultivated sunflower was studied at both the experimental plot and crop field levels under a wide range of weed densities. The weedy biotype shows evidence of crop introgression, with high morphological variability and intermediate traits between the crop and wild or ruderal sunflower. After four years under a sunflower–soybean summer crop rotation, the population was reproduced in the 75% of the field. The sunflower crop yield was reduced by more than 50% with >4 weeds m^{-2}. The weedy sunflower achenes are similar in size to the crop and so can be harvested, adding over 300 kg ha^{-1} to the harvested yield in crops with >4 weeds m^{-2}. However this contribution was not enough to balance the loss (1919 kg ha^{-1}) in the crop yield. Weedy sunflower has lower oil content and different fatty acid composition than the crop and therefore it could affect the oil quantity and quality of the harvested grains, by physical contamination. It was shown that weedy sunflower invades and remains in agricultural fields, causing up to 74% loss in sunflower crop yield, which emphasizes the need to prevent weedy sunflower colonization and invasion in sunflower fields. In order to prevent the introduction of weedy biotypes into non-invaded areas seed purity and thorough cleaning of agricultural machinery are of utmost importance. This is the first study of weedy sunflower density effect on sunflower yield loss.

1. Introduction

Several notable biological invasions have occurred around the world due to increased trade of agricultural products. Plant propagules are transported from one place to another, intentionally or inadvertently, as a result of human activities and so they become widespread and abundant outside their native habitat (Mashhadi and Radosevich, 2004). Some biological invasion hypotheses focusing on different mechanisms and scales of interaction are under continual discussion (Inderjit et al., 2005).

The number of species that cause economic loss in agriculture increases every year (Vitousek et al., 1997). Cropland ecosystems are extremely susceptible to biological invasions because the successional phase is continuously interrupted by agricultural operations (Baker, 1986). Sometimes agricultural operations promote the emergence of new and more complex weeds, causing important crop yield losses (Labrada-Romero, 2009). A plant is considered a "weed" when it "interferes with the activities of humans" regardless of its botanical identity (Booth et al., 2003).

The sunflower, *Helianthus annuus*, taxonomic group is a peculiar botanical complex since it includes wild, domesticated (crop) and weedy (weed or agrestal) biotypes, which sometimes share time and space (Heiser, 1978). In this botanical complex, volunteers that emerge from fallen crop seed are also noxious weeds (Håkansson, 2003), but they lack the capacity of self perpetuation and so their population depends on continued input to the soil seed bank (Gillespie and Miller, 1984).

In North America, the centre of origin of the genus, the weedy *H. annuus* biotype is the most noxious of the complex and is reported as a weed in several crops (Schweizer and Bridge, 1982; Geier et al., 1996; Villaseñor Ríos and Espinosa García, 1998; Allen et al., 2000; Rosales-Robles et al., 2002; Mesbah et al., 2004; Deines et al., 2004). However, the relationship between weedy sunflower densities and sunflower crop yield is unknown and this would be useful information for developing weed management strategies.

The wild or ruderal (RUD) sunflower, *H. annuus*, is an invasive non-native species included in the natural flora of central Argentina (Poverene et al., 2002). The RUD biotype has moved in

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successive steps across biotic and abiotic gradients, promoted by anthropogenic activity along the main roads due to grain transportation (Cantamutto et al., 2010). As the geographic distribution of the wild species overlaps the sunflower crop region extensively, hybridization between the two taxa is frequent (Ureta et al., 2008) resulting in a high risk of emergence of new biotypes. Up until the 2008 growing season only off-type crop plants were found in central Argentina, but no weedy *H. annuus* populations were detected (Poverene et al., 2008).

Noxious weed species can arise as a result of evolution of wild plant colonizers, by selection and adaptation to continual habitat disturbance in agricultural lands (Warwick and Stewart, 2005). Other weed biotypes can arise from crop species as feral forms capable of producing self-maintained populations (Martinez-Ghersa et al., 2000; Gressel, 2005). When feral populations originate from crop escapes, the process is known as endo-ferality, but if a biotype emerges after hybridization between the crop and wild or weedy parents, the process is called exo-ferality. It is considered that the latter process originated the weedy sunflower populations in France and Spain, from contaminated seed imported from USA (Muller et al., 2009).

Rice is another crop among the ten main grain crops, which coexists with a related noxious weed like sunflower. There are several weedy biotypes of the rice crop (*Oryza sativa*), which are extremely difficult to control in paddy fields (Estorninos et al., 2005). These weedy strains show early dehiscence before the crop is harvested, seed dormancy and they have several defense mechanisms (Gealy et al., 2003). Even though rice is a self-pollinated species, several crop traits were found in the weedy form, suggesting that hybridization with the crop might lead to the dramatic distribution of this weed around the world.

The invasive capacity of weedy sunflowers may be due to a wild-crop gene flow. It is unknown whether weedy biotypes of *H. annuus* were present in agricultural fields in Argentina, causing grain and oil losses by competition. If the weedy *H. annuus* biotypes were exoferal forms that arose from wild-crop gene flow, they would show variability in the intermediate morphological traits between the two taxa. The objective of this study was to detect and characterize the *H. annuus* invasion process and to estimate the reduction caused in crop yield and quality in sunflower.

2. Materials and methods

2.1. Agro-ecological characterization

The exploration field trips were undertaken following the procedure of previous *Helianthus* surveys (Poverene et al., 2008). To be considered as weedy (AGR), a *H. annuus* plant should be found within an agricultural field but outside the crop rows and must show at least two of the following traits; anthocyanin presence (stem, petiole), full branching (Hockett and Knowles, 1970), small head diameter (<5 cm) with a red disc. Areas occupied by crop and weeds were calculated using a personal GPS navigator (Garmin eTrex Vista® HCx). Weed density estimation was made in 2 m radius circles around the observer ($n = 10$), along a "W" transect arranged in the invaded area (Cantamutto et al., 2010).

A population of AGR *H. annuus* biotype was found in central Argentina (S 38° 16′ W 60° 07′), in a region without any previous records of RUD sunflower (Pablo Errazu, pers. com.). It was located in a crop field of 65 ha in a sector cultivated with sunflower, at a recommended stand (5.0 ± 1.2 plants m^{-2}). The soil texture was loamy (32.8% sand, 42.5% silt and 25.0% clay) with 4.98% organic matter and pH 6.5. Other weeds species of low prevalence were *Datura ferox* "Jimson weed", *Chenopodium album* "Lamb's quarters", *Xanthium spinosum* "Spiny cocklebur" *Cynodon dactylon* "Bermuda grass" and *Solanum sisymbrifolium* "Sticky nightshade".

2.2. Phenotypic characterization

The AGR *H. annuus* biotype was characterized in the Agronomy Department experimental field during three successive years. A wild or ruderal biotype and a cultivated sunflower (SUN) were used as controls. The RUD was collected in a road ditch outside cropland in La Pampa province. The SUN biotype was represented by three commercial hybrids from Syngenta: Dekalb 4050, Dekalb 3820, Dekalb 3845 OIL PLUS, and the open-pollinated variety Antilcó from El Cencerro. The seeds of the AGR and RUD biotypes were collected from a representative sample of plants ($n > 100$).

Biotypes were sown in 28 cm × 54 cm 100 cell plastic trays containing commercial substrate and the seedlings were grown for 30 days in a greenhouse under natural light at 20–25 °C. AGR and RUD seeds were stratified at 5 °C for 1 week to overcome dormancy (ISTA, 2004). Plants were transplanted in the experimental plot at the V4 stage (Schneiter and Miller, 1981). The phenotype was characterized at the R6 stage by means of 27 descriptors (GRIN Germplasm Resources Information Network, USDA, 2012). The evaluation comprised 42 RUD, 36 AGR and 40 SUN plants.

The AGR plants were classified with a hybrid index based on numerical scores of categorized traits (Carney et al., 2000): branching type (0 = no branching; 1 = apical branching; 2 = full branching), disc color (0 = yellow; 1 = red), anthocyanin presence (0–1) and principal head presence (0–1). The index of each plant was the sum of the scores for the four traits. The highest score was assigned to the RUD biotype and the lowest score corresponded to the SUN.

2.3. Competition study

2.3.1. Crop field

During three growing seasons, competition over SUN, at farm level, was estimated at the R8 stage by means of 1 m^2 plots ($n = 30$) randomly located along two parallels, in 200 m transects 5 m apart, at 30° to the planted rows, NE–SW. Measurements of plant density, head diameter, number of heads per plant and grain number per plant were taken in both SUN and AGR. Dry heads of SUN and AGR biotypes were harvested and manually threshed. The SUN grain biomass was estimated by a representative fraction (>20 g). The AGR achenes that were similar size to the crop seed, and so would be harvested with the crop, were separated using a manually operated sieve with rectangular holes (2 mm × 20 mm). The "crop yield" (g m^{-2}) involves only the SUN yield, whereas the "harvested yield" (g m^{-2}) represents the total yield, including both the crop yield and the AGR achenes possibly collected by the harvesting machine due to their size.

For the data analysis, the crop field plots were grouped into four categories according to the weed density: 0, 1, 2–3 and >4 plants per m^{-2}. Grain samples (25 g) of SUN and AGR (thicker >2 mm) from each density interval were analyzed by nuclear magnetic resonance and gas chromatography (Cámara Arbitral de Cereales Bahía Blanca) to estimate the quality change due to competition. The oil content and fatty acid profile were measured in each fraction. The harvested grain quality was estimated by considering the contribution of each fraction at any given weed density interval.

2.3.2. Experimental plot

The competitive effect of AGR on the SUN crop was evaluated in an experimental plot (38°41′46″S, 62°14′55″W) during two growing seasons. The soil has a well-drained loamy sand texture and pH 7.7, with 1.1% organic matter. Sunflower hybrids (DK 3880CL and Nidera P104 CL) were cultivated in soil with adequate fertility level and drip irrigation. Weed seed dormancy was overcome before sowing, as explained in the previous section. Both crop and weed were manually sown in excess at the same time. AGR seeds were placed equidistant between two SUN plants in the same row,

of no more than 10 cm width. At the V4 stage, the desired AGR and SUN stand (7.1 plants m^{-2}) was adjusted by hand thinning. The AGR density treatments studied varied between 0 (control), 0.2, 2.4, 7.1 and 10.7 plants m^{-2}. The field was kept weed free by hand weeding so that only the effect of the weed under study would be observed. The design was a randomized complete block with four replications. Experimental units were composed of three rows, each 2 m long and spaced at 0.7 m.

Weed competition was evaluated at the R6 stage in central sunflower crop plants ($n = 4$) of each experimental unit. Heads were manually harvested at the R8 stage and dried under laboratory conditions, until they were threshed. Measurements were taken of head diameter, grain number and yield per plant. Crop yield (g m^{-2}) was estimated as a product of plant density and yield per plant.

2.4. Statistical analysis

The morphology of the AGR biotype was characterized by principal component analysis (PCA) of plant height, head number, phyllary number, length and width, ray number, ray length and head diameter. Hybrid indexes were graphically represented as histograms, showing the percentage of plants with each index score.

The relationship between reproductive traits and AGR density was analyzed by PCA. The relationship of sunflower crop yield and AGR density was also analyzed by fitting the rectangular hyperbolic yield loss function described by Cousens (1985). The equation (Eq. (1)) used was:

$$Y = Y_{wf} - Y_{wf} \times I \left(\frac{Wd}{100 \times (1 + I \times Wd/A)} \right) \quad (1)$$

Y being the observed crop yield (g m^{-2}), Y_{wf}, I and A are model parameters estimated from the data, and Wd is weed density (plants m^{-2}). Y_{wf} is the weed-free crop yield, I and A are percent crop yield loss as weed density approaches zero and infinity, respectively. Parameters were determined for the five experiments (crop or experimental plot) and year. Using Eq. (1), the crop yield loss (%) was estimated for a range of weed densities and the means were compared by ANOVA considering two groups (crop field for three seasons, experimental field for two seasons).

Data were analyzed using the statistical packages NLREG (2008) and InfoStat (2010).

3. Results

3.1. Agro-ecological characterization

An increase in the invaded area of the crop field associated with sunflower crop was observed (Table 1). The crop field was managed by the farmer as two subplots, the SE (46 ha) and the NW (19 ha), following an alternating sunflower and transgenic soybean summer crop rotation system. Initially the patch invaded by AGR sunflower covered more than 15% of the SE subplot, cultivated with sunflower. The AGR population was at a density of 4.2 ± 3.0 plants m^{-2}, reaching up to 11 plants m^{-2} in the denser patches. In 2010, the AGR population size was small ($n < 200$) because transgenic soybean had been cultivated in this sector and it had been sprayed with glyphosate. However, several plants were located outside the SE subplot, distributed in three small patches (<100 m^2), more than 400 m away from the initial site. In the following season (2011) the weedy population was in the SE subplot, cultivated with sunflower. The invaded area increased to 26 ha, although on average the density was lower (1.8 ± 1.6 weeds m^{-2}) than in 2009. In 2012, the SE sector was again free of AGR plants as it had been cultivated with transgenic soybean and sprayed with glyphosate. However in this season, the weedy population had moved to the NW subplot invading an area of 10 ha where a patch with 4.8 ± 4.7 weeds m^{-2} density was detected. After four years under a sunflower–soybean rotation the area invaded by the AGR biotype had increased and it was reproduced in 75% of the field under study.

Table 1
Progression of weedy sunflower (AGR) invasion in the crop field during four years of observations, associated with summer crops planted by the farmer.

Observation	Subplot	
	SE (46 ha)	NW (19 ha)
2009		
Crop	Sunflower	Soybean
AGR population size (n)	3.4×10^5	0
AGR invaded area (ha)	8	0
2010		
Crop	Soybean	Sunflower
AGR population size (n)	0	2.0×10^2
AGR invaded area (ha)	0	<1
2011		
Crop	Sunflower	Soybean
AGR population size (n)	4.7×10^5	0
AGR invaded area (ha)	26	0
2012		
Crop	Soybean	Sunflower
AGR population size (n)	0	4.8×10^5
AGR invaded area (ha)	0	10

3.2. Phenotypic characterization

AGR showed an intermediate morphology, resembling the RUD biotype, which was 35% taller than SUN (Table 2). AGR had 6 and 13 more leaves per main stem than RUD and SUN, and they were larger. The AGR had 29 ± 13 heads per plant whereas the RUD had 110 ± 57. The heads diameter of AGR was between RUD and SUN in size. Also the ray number per head, ray length, and phyllary number of AGR were intermediate between RUD and SUN. Moreover, the principal component analysis clearly showed an intermediate morphology between RUD and SUN of the AGR biotype (Fig. 1).

The hybrid index based on four categorical traits also showed that AGR had an intermediate morphology (score) between RUD and SUN (Fig. 2). SUN plants had no branching or anthocyanin and a yellow disk color. On the other hand, RUD sunflower plants had full branching and red disk color. However, some AGR plants (<5%) showed a main head and anthocyanin absence (<15%). AGR plants had an intermediate score, with a slight overlap with RUD. Plants were more similar morphologically to RUD than to SUN plants, showing 64% of the plants as fully branched, 80% with red disk color, 70% with anthocyanin in stem and petioles. The presence of a main head in 94% of the plants was the trait most associated with the SUN.

3.3. Competition study

The correlation matrix obtained from principal component analysis showed that an increase in the weed plant density decreased the head diameter ($r = -0.52^{**}$), grains per plant ($r = -0.54^{**}$), grain biomass ($r = -0.49^{**}$) and crop yield ($r = -0.59^{**}$) (Fig. 3). Traits that mainly explained 69.8% of component 1 were the yield per plant (0.97), head diameter (0.91) and grain biomass (0.81). On the other hand, component 2 was mainly explained by grain biomass (0.62) and grains per plant (0.60).

Estimated weed-free crop yield (Y_{wf}) for the five experiments did not differ from the observed weed-free crop yield (Table 3). The rectangular hyperbola yield loss model showed that when weed density tended to zero (parameter I), AGR caused an average yield loss of 51.7%. However, at a very high weed density (parameter A), yield loss due to AGR reached 80.6%. Based on the estimated crop yield loss, differences were only found with 1 weed m^{-2} (Fig. 4).

Table 2
Descriptors used to characterize the weedy sunflower population in a common garden study.

Acronym	Descriptors (categories, units)	Biotype		
		RUD	AGR	SUN
Plant characters				
BRAT	Branching type (NO = no branching; A = apical branching; F = full branching)	F	A–F	NO
PHT	Plant height (cm)	198 ± 52	200 ± 56	128 ± 23
SDI	Stem diameter at mid-height (cm)	2.1 ± 0.5	2.3 ± 0.8	2.5 ± 0.4
MHD	Presence of main head (P = presence, A = absence)	A	P–A	P
HNU	Head number (*n*)	110 ± 57	29 ± 13	1 ± 0
ANTP	Anthocyanin in stem and petioles (P = presence, A = absence)	P–A	P–A	A
Leaf characters				
LWI	Leaf width (cm)	19.5 ± 3.1	21.8 ± 5.3	28.0 ± 4.8
LLE	Leaf length (cm)	23.4 ± 3.6	22.0 ± 4.9	25.9 ± 3.7
PLG	Petiole length (cm)	17.5 ± 3.4	16.9 ± 5.6	16.1 ± 2.9
LBAS	Leaf base (CU = cuneate, CO = cordate)	CU–CO	CO	CO
LSUR	Leaf surface (F = flat, W = waxy, C = curled)	F–W	W–C	C
LMAR	Leaf margin (S = smooth, SE = serrate, DS = deeply serrate)	SE–DS	SE–DS	DS
LNU	Leaf number (*n*)	23 ± 6	29 ± 11	16 ± 3
HLF	Heads with leaf on the back (P = presence, A = absence)	P–A	P–A	A
RFNU	Ray floret number (*n*)	24 ± 4	35 ± 6	47 ± 7
Disk characters				
RWI	Ray width (mm)	11.8 ± 1.6	13.2 ± 2.2	22.2 ± 3.8
RLE	Ray length (mm)	37.2 ± 5.0	39.7 ± 16.4	76.7 ± 12.6
PNU	Phyllary (bract) number (*n*)	30 ± 4	41 ± 9	44 ± 9
PLE	Phyllary length (mm)	21.1 ± 3.2	36.4 ± 10.3	61.1 ± 9.1
PWI	Phyllary width (mm)	8.2 ± 1.4	15.1 ± 3.2	35.7 ± 11.5
HDI	Head diameter (cm)	4.0 ± 0.4	7.0 ± 2.6	18.3 ± 4.2
DFC	Disk flower color (Y = yellow, R = red)	R	Y–R	Y

With >4 weeds m^{-2} the yield loss was over 50%, and there was a tendency to stabilize.

The differences in SUN and AGR oil content (44.7% vs 38.7%), oleic fatty acids (27.6% vs 22.7%) and linoleic fatty acids (61.0% vs 64.9%) were highly significant between the two biotypes, however no significant differences were found in palmitic, stearic and linolenic fatty acid composition. The weed density did not affect the SUN oil content or fatty acid profile. When the quality of harvested grain including the AGR achenes (SUN + AGR achenes >2 mm) was estimated, the oil content, linoleic acid and oleic acid were significantly less ($p < 0.05$), however the change was practically irrelevant (<1%).

A high proportion of AGR plants were still alive at sunflower crop harvest. The difference in cycle duration with SUN may make the mechanical harvest operation difficult, as it has to be delayed until the weedy plants have dried. Although under field crop conditions the estimated weed seeds contribution to the harvested yield reached 324 kg ha^{-1} when >4 AGR plants m^{-2} were present it was not enough to balance the loss in the sunflower crop (1919 kg ha^{-1}). Moreover, the AGR contribution to the soil seed bank reached

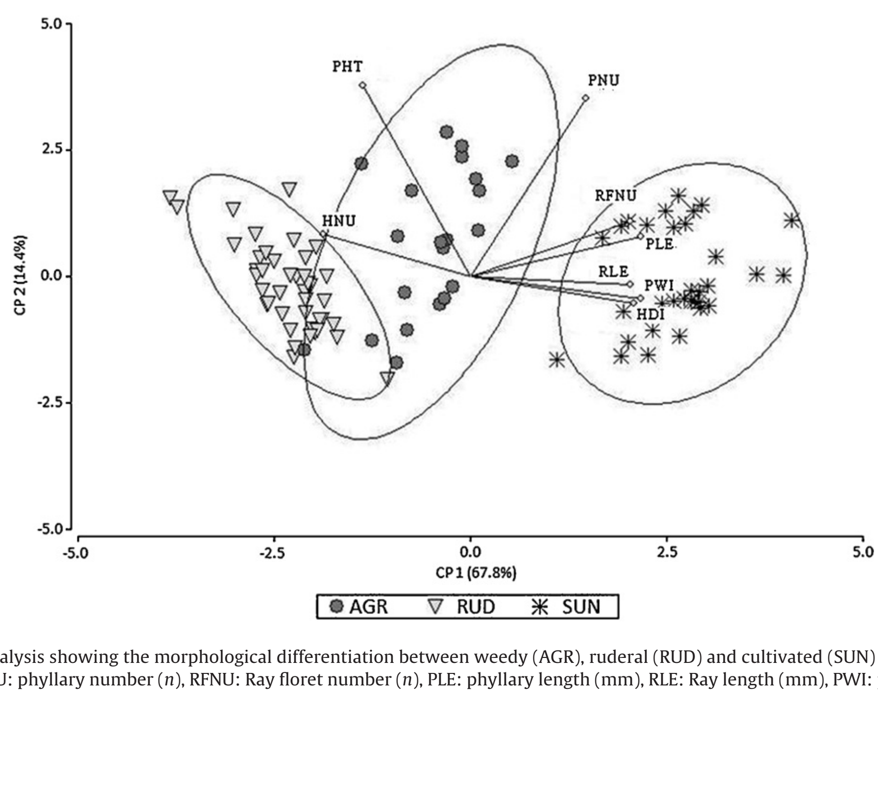

Fig. 1. Principal component analysis showing the morphological differentiation between weedy (AGR), ruderal (RUD) and cultivated (SUN) sunflowers. HNU: head number (*n*), PHT: plant height (cm), PNU: phyllary number (*n*), RFNU: Ray floret number (*n*), PLE: phyllary length (mm), RLE: Ray length (mm), PWI: phyllary width (mm), HDI: head diameter (cm).

Table 3
Weed-free sunflower crop yield and parameter estimates (±SE) for each environment studied.

Year	Weed-free SUN crop yield (g m^{-2})	R^2 (%)	Parameter estimates		
			Y_{wf} (g m^{-2})	I (%)	A (%)
Farmer field					
2009	370.8 (103.4)	39.0	368.1 (44.8)	79.5 (71.2)	60.6 (11.1)
2011	372.7 (61.4)	61.3	372.1 (26.3)	70.1 (35.0)	73.7 (16.1)
2012	229.8 (28.1)	84.0	229.0 (10.3)	50.7 (14.3)	70.5 (5.3)
Experimental field					
2010	431.0 (30.8)	84.2	371.5 (21.6)	34.1 (21.6)	94.4 (16.7)
2012	343.3 (32.6)	84.9	306.0 (16.5)	24.3 (10.3)	103.8 (23.6)

$Y_{wf:}$ weed-free crop yield; I: percent crop yield loss as weed density approaches zero; and A: is percent crop yield loss as weed density approaches infinity.

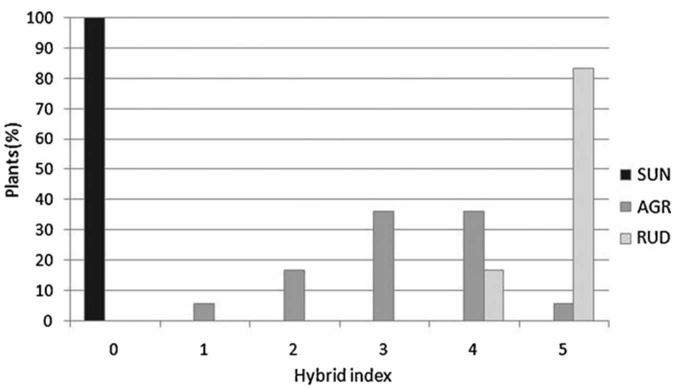

Fig. 2. Hybrid index based on categorical traits of 42 ruderal (RUD), 36 weedy (AGR) and 40 cultivated (SUN) sunflower plants.

around 909 ± 733 seeds m^{-2}, a number more than a hundred times higher than a stand capable of producing economic loss.

4. Discussion

Even though RUD plants are often observed in areas where crops and wild relatives occur together, the AGR biotype was found in a region without any naturalized *H. annuus* populations (Poverene et al., 2002, 2008). The AGR biotype clearly fits the definition of a feral form of sunflower. It has the capacity to reproduce successfully and forms a self-perpetuating population without human aid (Bagavathiannan and Van Acker, 2008). Due the presence of several wild and crop traits (Table 2), an exoferality origin of this AGR biotype could not be discarded. The AGR invasion might have been initiated from contaminated sunflower seed (as in Europe, Muller et al., 2009), or from RUD seed carried by hired agricultural machinery, frequently used in Argentina (Piñeiro and Villarreal, 2005). Trading of agricultural products has been recognized as the main means of weed dispersal (Kawata et al., 2009).

This is the first report of the AGR density effect on a sunflower crop. Our model predicts a crop yield loss of over 50% when weed density is >4 plants m^{-2}. This fact agrees with Muller et al. (2009), who found a similar yield loss under a single density of between 12 and 15 weedy sunflower plants m^{-2} in four naturally invaded fields in France. Our findings contribute a better understanding of the interference mechanism over a wide range of weed densities. In densities greater than 4 weeds m^{-2}, the largest AGR achenes (>2 mm) could become mixed with the sunflower grains adding over 300 kg ha^{-1} to the harvested yield, but this contribution would not be enough to balance the estimated crop yield losses (1919 kg ha^{-1}). The AGR grains showed lower oil content and a different fatty acid composition to SUN and therefore the oil quantity and quality of the mixed grains might be lower because of the physical contamination. It was proposed that sunflower embryo could changes its fatty acid profile by the effect of the male parent (Bervillé, 2010). This fact was not found in the present study, probably because of the differences in oil composition between AGR and SUN biotype were small. Thus, the AGR biotype can decrease SUN grain yield by competition and oil quality through physical contamination.

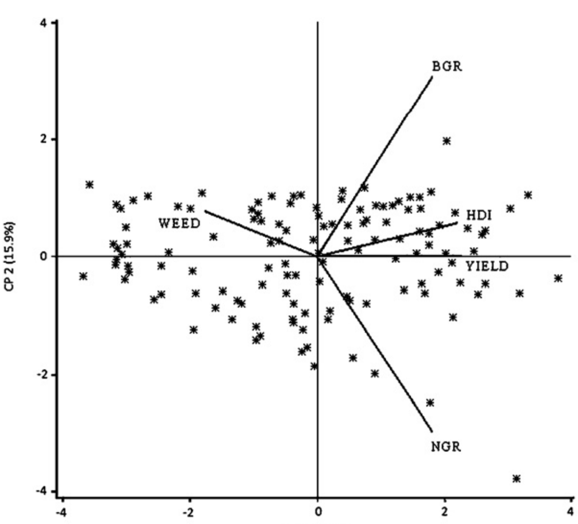

Fig. 3. Principal component analysis showing weed density and reproductive traits in five experiments. Vectors correspond to the most descriptive variables. WEED: weeds per m^2; BGR: grain biomass (mg); HDI: head diameter (cm); YIELD: crop yield (g m^{-2}); NGR: grains per plant (n).

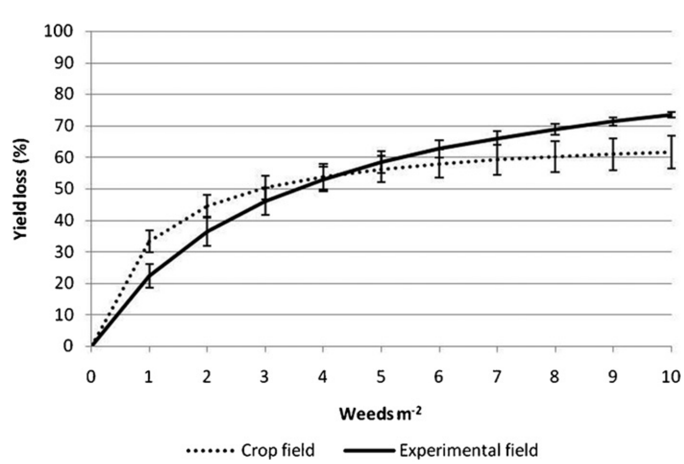

Fig. 4. Crop yield loss caused by increasing density of weedy sunflower, estimated using the formula provided by the NLREG program.

Although wild sunflower has been naturalized in Argentina for 60 years (Poverene et al., 2002), it has never been found as a weed in agricultural fields. The new noxious weedy sunflower biotype showed some weedy attributes that can provide competitive advantage not present in the wild (RUD) relative. Lai et al. (2008) found that the expression of genes turned out to be different in AGR and RUD *H. annuus* populations. The growth rate of AGR biotypes doubled that of the RUD biotypes. However, they did not find a common pattern for all AGR biotypes, due to the differing environmental conditions (climate, soil, pathogens and management) that have been involved in their evolution.

The AGR plants, detected for the first time in Argentina, showed intermediate traits between the RUD and SUN *H. annuus* biotypes. Several morphological traits, such as full branching, small heads, anthocyanin in stems and flower structures, agree with the taxonomic description of RUD *H. annuus* (Seiler and Rieseberg, 1997). However, the absence of anthocyanin, apical branching, main disk, head diameter greater than 5 cm and male sterility present in several plants, show evidence of hybridization with SUN (Figs. 1 and 2). In addition, the oil content of the AGR biotype (38.7%) is higher than the range found in RUD populations in Argentina (21.4–28.2%; Cantamutto et al., 2008) which also indicates the crop genes effect.

The AGR was more heterogeneous than the RUD biotype, showing evidence of crop hybridization. Hybridization between RUD biotypes and SUN had previously been found by Ureta et al. (2008). Our results are the first to show crop introgression in a weedy population in Argentina. The frequent hybridization of wild and crop sunflower in Argentina shows a high risk of emergence of new biotypes. This current process might make the weedy sunflower biotype as noxious as weedy rice, which might be introgressed by the same crop (Chin, 2001).

In some cases, hybrids between wild and crop species have led to increased invasiveness, e.g. *Sorghum halepense* that seems to have increased its aggressiveness due to the introgression of cultivated sorghum traits (Harlan, 1992). Gene flow from the crop has increased aggressiveness in wild relatives of seven of the most important crops in the world (Ellstrand, 2003). In these cases, the evolution of wild species was subject to hybridization with the crop, the persistence and reproduction of hybrids and alleles of cultivated species that conferred an adaptive advantage to agriculture lands. In sunflower, the hybridization of AGR biotypes with imidazolinone-tolerant SUN cultivars could facilitate the acquisition of herbicide resistance in the first generation (Presotto et al., 2012). For this reason, this technology is not recommended for the control of AGR populations.

Soybean, sunflower, wheat, and corn are the dominant grain crops in central Argentina (MinAgri, 2012). The area of transgenic soybean is increasing due to its economic profitability, displacing other crops and endangering the stability of the sunflower production area (Coll et al., 2012). In this region, the emergence of the AGR biotype represents a new challenge for SUN crops. Even though it can be controlled by glyphosate in transgenic soybean, our results showed that this technique applied alone was not sufficient to limit the AGR invasion. Careful cleaning of agricultural machinery is recommended to prevent AGR propagule dispersion.

Our data provide a robust estimation of sunflower crop yield losses under different crop conditions and weedy sunflower densities. Our findings agree with the "human commensal hypothesis", where humans play a significant role in the introduction and spread of the invader (Inderjit et al., 2005). In future research it would be necessary to characterize the invasive ability of the AGR biotype by means of phenotypic and genetic studies in a common garden study. In the worst scenario, under minimal seed production of 176 seed m^{-2} measured under low AGR densities, a survival over 2.3% of the seeds in the soil bank would be enough to generate an AGR stand ($\geq$4 weeds m^{-2}) capable of reducing the SUN crop yield by more than 50%.

The plant invasive process is highly dependent on propagule pressure (Martínez-Ghersa and Ghersa, 2006). Although weedy sunflower seems to be harmless in the early successional stages, it can invade agricultural fields and become harmful in a few generations on account of its high fitness. At low densities, farmers might allow them to grow in their fields, as crop yield loss is not perceived and so their achenes might be harvested. However, the AGR biotype is of great concern because it can invade sunflower fields quickly, resulting in up to 74% of crop yield loss and making the harvesting operation difficult when high densities are reached. Therefore, it is extremely important to prevent the introduction and establishment of weedy sunflower in agricultural fields.

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