



Research Paper

Crop-wild sunflower hybridization can mediate weediness throughout growth-stress tolerance trade-offs



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ABSTRACT

Agricultural weeds are plants well-adapted to agricultural environments interfering directly and indirectly with crop production and causing important economic losses worldwide. Crop-wild hybridization is one of the main forces that have ruled weed evolution along with adaptation to agricultural (or benign) environments. Considering the competing demands for resources in any plant, adaptation to agricultural environments might result in an increase in growth but with lower tolerance to stress. In Argentina, most of the non-native *H. annuus* populations grow on roadsides, ditches, fences, hedgerows (ruderals), but there are also a few cases of *H. annuus* growing in agricultural field as weeds (agrestals). We assume that weediness of these agrestal biotypes came after crop hybridization as result of growth-stress tolerance trade-offs. Ruderal, agrestal (with evidence of crop introgression), and crop biotypes were contrasted under studies of drought and defoliation stresses, as well as for plant growth under non-stressful conditions and sequences of stress-related genes. The agrestal biotype was less tolerant to defoliation and drought than the ruderal biotype. Drought tolerance variation was largely explained by plant height rate (growth) and defoliation tolerance variation was mainly explained by biomass accumulation (resource allocation). Agrestal biotype sequences of two genes encoding transcription factors involved in stress response, *DREB2* and *NAC*, showed evidence of positive selection in the crop direction. Therefore, selection in the agricultural environment combined with crop hybridization driver the evolution of a well-adapted genetic variant of *H. annuus* with fast growth but reduced stress tolerance.

1. Introduction

Agricultural weeds impact crop production through direct competition for nutrients, moisture and light, and they cause the higher potential losses (34%) than animal pests and pathogens (losses of 18 and 16% respectively) (Heap, 2014; Oerke, 2006). Weeds can originate in three ways: 1) from wild populations, where selection acts on standing variation; 2) from hybrids between wild and crop taxa (exofertility), by which segregation produces extreme and intermediate phenotypes on which selection acts; and 3) from plants directly descended from crop varieties (endofertility) (Bagavathiannan and Acker, 2008; De Wet and Harlan, 1975; Ellstrand et al., 2010, 2013).

Crop-wild hybridization is the first step in the flow of novel crop alleles into native or wild populations (Ellstrand et al., 2013). After

that, these novel crop alleles can be introgressed (permanent incorporation of genes from one set of differentiated populations into another) in the recipient population (Stewart et al., 2003). Hybridization can be responsible for the formation of new hybrid lineages (Abbott et al., 2013) or can cause the extinction of populations or species (Todesco et al., 2016). Crop-wild hybridization has played an important role in the adaptive evolution of weeds like weedy *Brassica rapa* (Warwick et al., 2008), weed beet (Arnaud et al., 2010), California wild radish (Campbell et al., 2006) and weedy rice (Xia et al., 2011). Despite some maladaptive traits stemming from the crop (e.g. lack of seed dormancy and seed shattering), which may prevent introgression, under selection in agricultural environments (e.g. by herbicides or competition with crops) some crop traits, such as rapid growth, herbicide tolerance and early flowering, may enhance hybrid fitness,

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increasing the risk of introgression (Mercer et al., 2014, 2007). Moreover, under wild-like conditions (e.g. low water availability) some crop traits (e.g. head diameter, petiole length and leaf size) could be favored in crop-wild advanced generations (Owatt et al., 2014).

Agriculture produces relatively stable environments, through practices such as conservation tillage, fertilization, herbicide use, monoculture or short-term rotations, which exert high selected pressure on the weedy community (Ghersa and Satorre, 2000; Neve et al., 2009; Poggio et al., 2013). If agricultural selection pressures are consistently maintained and there is a genetic basis for beneficial traits, then the weed populations found in the fields will have been enriched in alleles that allow the plants to survive under conditions associated with cropping practices (Owen et al., 2015). Some of the well-documented evolutionary changes observed in weeds are traits related to seed germination, leaf shape, flowering pattern, seed shattering, seed size and shape, and herbicide resistance. Also, stem and leaf growth rates, plant height, and branching habit contribute to the architecture of the plant canopy, and consequently determine the timing and effectiveness of the shading of weeds over crop plants (Ghersa and Satorre, 2000; Murphy and Lemerle, 2006; Vigueira et al., 2013). Furthermore, the plant strategy to adapt to different levels of productivity or disturbance, for instance, can result in a resource use trade-offs that ensure rapid growth at the expense of lower stress tolerance (Agrawal et al., 2015; Grime, 1977; Koehler et al., 2012; Liancourt et al., 2005). For example, in alien environments as compared to native environments, plants tend to have improved competitive abilities, but reduced resource allocation to herbivore defenses due to the absence of specialist herbivores (Blossey and Notzold, 1995). In agricultural environments, individuals of the weed *Ipomoea purpurea* resistant to glyphosate showed a trade-off between resistance and fitness, which was evident in the absence of the herbicide (Debban et al., 2015). According to this concept, in environments where rapid growth is positively selected as agroecosystems, a trade-off between growth and stress tolerance may constrain the evolution or maintenance of stress tolerance in agroecosystems, or environments where rapid growth is positive selected (Grime, 1977; Vila-Aiub et al., 2015). There is also strong evidence that genetic differences between populations subjected to selection in contrasting environments, play a central role in these growth-stress tolerance responses. In particular, some transcription factors play a key role producing phenotypic variation and hence are considered quite important in adaptation (Fan et al., 2014; Kane and Rieseberg, 2007; Mayrose et al., 2011). So, crop-wild hybrids may evolve into genetic variants that have adaptive traits inherited from the crop parent as well as traits that favor proliferation and persistence inherited from the wild parent, thereby creating a weedy population (Vigueira et al., 2013).

Helianthus annuus L. is native to North America but nowadays it is widely distributed throughout several continents with reports from North and South America, Europe, Africa, and Australia (Dry and Burdon, 1986; Heiser, 1954; Muller et al., 2009; Poverene et al., 2002; Ribeiro et al., 2010). Natural hybrids between wild and crop sunflower have been recorded in their native and non-native area (Arias and Rieseberg, 1994; Ureta et al., 2008) and crop genes may contribute to increasing weediness (Muller et al., 2011; Casquero et al., 2013). In the US, weedy *H. annuus* populations were not highly differentiated from geographically proximal wild *H. annuus* populations, indicating that the formers were more closely related to nearby wild populations than to other weedy populations, which may have evolved multiple times within *H. annuus* (Kane and Rieseberg, 2008). However, an analysis of the set of common weedy genes revealed parallel shifts in gene expression across different weedy populations, indicating parallel adaptation to agricultural conditions (Lai et al., 2008). In addition, when a wide range of weedy populations from different countries were analyzed and compared with native *H. annuus* populations, a trade-off between growth and stress tolerance was found (Koziol et al., 2012; Mayrose et al., 2011). The European weedy populations were genetically different from wild US populations and they exhibited an

intermediate variability between wild US populations and crop varieties (Muller et al., 2011). Thus, weedy European populations probably originated from the introduction of crop-wild hybrids into the farmers' fields (Faure et al., 2002; Muller et al., 2011). In Argentina, *H. annuus* populations are mainly found in roadsides, ditches, fences, hedgerows and field margins (hereafter, ruderal biotype) (Poverene et al., 2009) and these are morphologically similar to native US populations (Cantamutto et al., 2010a; Garayalde et al., 2011). However, recently a few cases of *H. annuus* populations were found in an agricultural field as a weed (hereafter, agrestal biotype) (Casquero et al., 2013). A case in the Buenos Aires province reduced the sunflower crop yield by more than 50% with > 4 weeds m^{-2} . This weed showed strong evidence of crop introgression, with intermediate traits between the crop and ruderal sunflower. This population was more aggressive with the crop than the ruderal plants due to their faster initial growth (Casquero and Cantamutto, 2016). Based on our observations, it is highly likely that weediness of this agrestal biotype evolved after crop hybridization because invasive *H. annuus* populations, which have been naturalized in Argentina for at least 70 years (Poverene et al., 2002), were never before found in agricultural fields as weeds. Moreover, this biotype was established in a different environment (with different climatic, soil and human intervention features) and isolated from ruderal populations, making it an ideal scenario for studying the evolution of weediness under agricultural selection. Here, we focus on three groups of plants (hereafter biotypes): ruderal, agrestal and crop plants, which where contrasted under drought and defoliation stresses, plant growth under non-stressful conditions, and variability in sequences of stress-related genes. Our hypothesis is that the rapid adaptation of the agrestal biotype to agricultural environments came after crop-wild hybridization as result of growth-stress tolerance trade-offs.

2. Materials and methods

2.1. Plant material

Three biotypes of *H. annuus* were evaluated: ruderal (RUD), agrestal (AGR) and crop sunflower (CROP). The RUD biotype was represented by two *H. annuus* accessions collected in central Argentina (Cantamutto et al., 2010a): Río Cuarto (RCU; S 33°09, W 64°20) and Colonia Baron (BAR; S 36°10, W 63°52). The AGR biotype was represented by one *H. annuus* accession collected in central Argentina (BRW; S 38°16, W 60°07) (Fig. 1). The agrestal population showed clear evidence of crop introgression, like plants with absence of anthocyanin, apical branching, presence of a main head, head diameter greater than 6 cm, male sterility, and an oil content of 38.7% (Casquero et al., 2013). We only included one agrestal accession because it is the only stable population found since 2000. While we have found some wild plants in agricultural fields these could not be consider agrestal populations because there were in low quantity along field margins and/or "sporadic appearances" during these years. The CROP biotype was represented by two cultivars (Paraíso 104 CL from Nidera company, and VDH 487 from Advanta company) and two inbred lines (HA89 and B71). These cultivars and inbred lines were also considered as accessions.

2.2. Defoliation tolerance study

The defoliation tolerance study was designed to simulate biotic stress like that of defoliation caused by Lepidoptera. RUD, AGR and CROP biotypes were grown in a common garden and were irrigated. The rows were separated at 1.40 m and plants at 0.30 m apart in the row. At the R3 stage (Schneiter and Miller, 1981), four defoliation treatments (non-defoliated control, 33, 66 and 100% defoliation treatments) were applied to the all active leaves on the main stem of each plant (Muro et al., 2001). In defoliation treatments, a portion of each of the active leaves on the main stem were cut-off according to

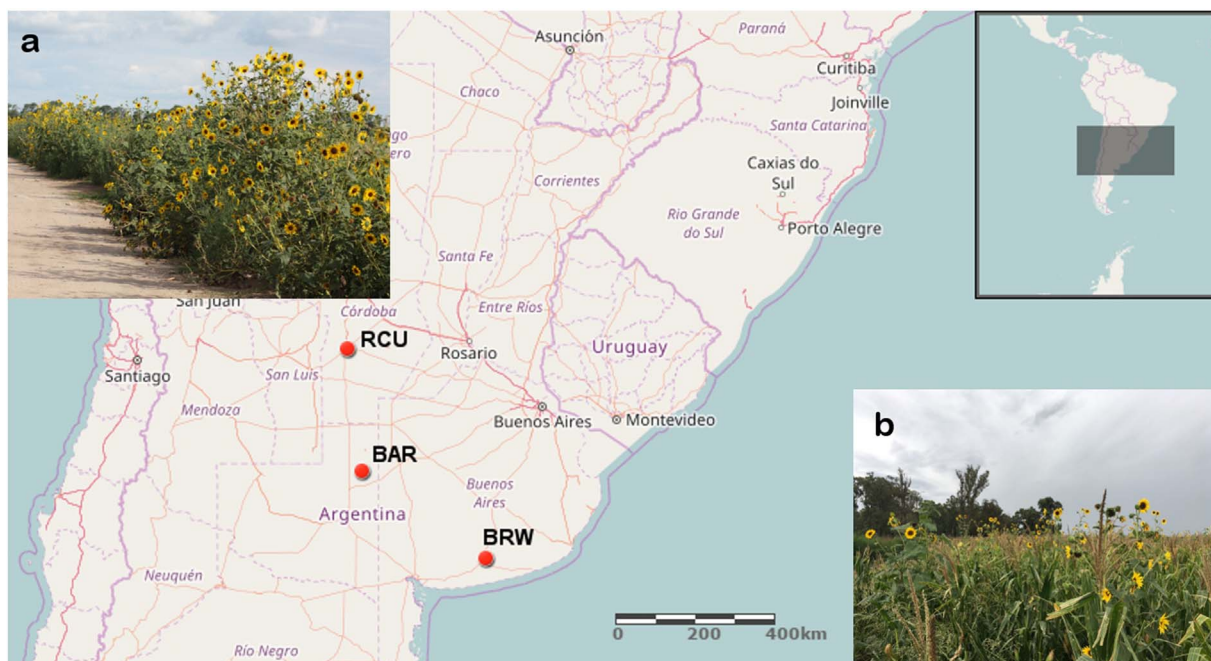


Fig. 1. Geographic location of invasive *Helianthus annuus* populations employed in this study. Ruderal biotype (a) growing on a roadside near to Río Cuarto (RCU; Córdoba province). Agrestal biotype (b) growing in a maize field near to Tres Arroyos (BRW; Buenos Aires province). *H. annuus* biotypes were last surveyed in March 2017.

each treatment. For instance, in 33% defoliation treatment, two-thirds of each leaf of the plant remains and one-third was removed. Populations and treatments were randomly arranged within the rows in groups of 10–15 individuals. At physiological maturity, the number of heads per plant was measured on 10 plants per treatment. After harvest, the head diameter, number of achenes (hereafter referred as seeds) per head and the seed weight were measured on one representative head from each plant and treatment. Seeds per plant were estimated by multiplying the number of heads by the average number of filled seeds per head. Plots (with 10–15 individuals per plot) were randomly distributed by accession and defoliation treatment between the rows, thus each plant within treatment was considered a replicate. Sources of variations were biotype, accessions, defoliation and their interactions.

2.3. Drought tolerance study

A trial was conducted in a greenhouse in 101 pots to evaluate drought tolerance during tubular disc flower formation. Once the plants have reached the floral initiation (this stage was determined in a preliminary test for each biotype, by dissecting the apex at different stages) -0.8 MPa water stress was induced until they reached the R1 stage (Schneider and Miller, 1981). Stress was achieved by prior determination of the water retention curve of the soil. Pots were weighed daily and then the necessary water to reach -0.8 MPa was added. Under drought stress, each accession was represented by 10–15 individuals, and another 10–15 individuals per accession were maintained without limited water during this period (control treatment). Pots were randomly arranged on the greenhouse benches. Each pot was considered as a replicate.

At the R2-stage (Schneider and Miller, 1981), plant height, leaf width and length were measured. Then, the main head of each plant was dissected, fixed in an ethanol: acetic acid (3:1) solution and stored in 70% alcohol. Subsequently, the flowers were counted on quarter discs under a Nikon SMZ 745T magnifying glass ($7.5\times$) and the number of flowers per head was estimated. Experiment was conducted as a complete randomized design. Sources of variations were biotype, accessions, drought and their interactions. Each accession (within each biotype and treatment) was represented by 10–15 replicates (plants).

2.4. Plant growth study

To estimate the plant growth in the RUD, AGR and CROP biotypes, the plant height and above-ground biomass were measured in an irrigated common garden. The rows were separated at 1.40 m and plants at 0.30 m apart in the row. Every 15 days, the height of two plants of each accession was measured and then the plants were dissected and dried at 60°C for 7 days and weighed. Plant growth was expressed as the increment of plant height or above-ground biomass per growing degree-day. The growing degree-days were calculated as the difference between the mean daily temperature (provided by CCT-Bahía Blanca meteorological station) and a basal temperature for sunflower of 4°C (Villalobos et al., 1996).

2.5. Gene sequencing

DNA fragments of four stress-related genes were isolated by PCR on genomic DNA from young leaves of individuals of each category (two ruderal RCU plants, two agrestal BRW plants, and two crop plants: HA89 and B71) using a CTAB method (CIMMYT, 2006). The amplified fragments correspond to partial sequences of each gene (Exon 1 for *DREB2* gene and Exon 1 and 2 for *NAC*, *DHN*, and *LTP* genes; Giordani et al., 2011). The ruderal RCU was chosen due to its higher allele richness and also because this population is thought to be the entry point of the species into Argentina (Cantamutto et al., 2010b; Garayalde et al., 2011). Within these four genes (Table S1), two are transcriptional regulators of stress-related genes (*DREB2*, *NAC*; hereafter TF group), and two of them are directly involved in stress protection (*DHN*, *LTP*; hereafter non-TF group) (Giordani et al., 2011). Within the eight genes sequenced in sunflower (four TF and four non-TF) by Giordani et al. (2011), we chose one transcription factor involved in the ABA independent pathway (*DREB2*) and one gene involved in the ABA dependent pathway (*NAC*). Both genes activates the transcription of genes under drought or high-salt stresses (Shinozaki and Yamaguchi-Shinozaki, 2007). Within the non-TF group, we chose the *DHN* gene which produce proteins associated to inhibition of the coagulation of macromolecules and preserve their structural integrity and the *LTP* gene which produce proteins, related to lipid metabolism, that might be

involved in secretion or deposition of extracellular lipophilic material (Natali et al., 2003; Ouvrard et al., 1996). This four genes were previously validated in sunflower (Díaz-Martín et al., 2005; Giordani et al., 2011; Liu and Baird, 2003; Natali et al., 2003; Ouvrard et al., 1996). PCR was performed according to Presotto et al. (2012) and PCR products were cloned into pGEM-T Easy vector (Promega) following the manufacturer's instructions. Competent *E. coli* cells (strain DH5 α) were transformed with the recombinant vector and plated onto LB-agar-ampicillin-X-Gal-IPTG plates. White colonies were picked and amplified in liquid LB with ampicillin, and plasmids including inserts were purified using the Wizard Plus SV Minipreps DNA Purification System (Promega). At least three colonies were sequenced for each plant to construct a consensus sequence. Each amplicon was sequenced on both strands at SIGYSA (EEA INTA-Castelar, Argentina) using an ABI 3130XL Genetic Analyzer (Applied Biosystems, USA). In addition consensus sequences of three inbred lines (D8, L72 and C1) of each gene obtained by Giordani et al. (2011) and available in the National Center of Biotechnology Information (<https://www.ncbi.nlm.nih.gov/>) were added to the statistical analysis to improve the statistical power.

2.6. Statistical analyses

Drought and defoliation tolerance studies were analyzed using linear mixed models, with biotype and treatments and their interaction as fixed effects and accessions within the biotypes, and all of their interactions as random effects, using Proc Mixed in SAS (SAS University Edition; SAS Institute Inc., Cary, NC). Because each plant, randomly arranged, was considered as a replicate we could not test for replicate nor interaction by replicate effects. First, the drought stress traits (plant height, leaf length and width, and flowers per head) and defoliation tolerance traits (head diameter, seeds per head, seed weight and seed yield per plant) were analyzed across the treatments (well-watered vs. water stress or 0, 33, 66, 100% of leaf defoliation) and the variation in the interaction term of treatments by biotype was tested. In this analysis, the main effects were biotype, treatment and the biotype by treatment interaction. Next, the traits with a significant biotype by treatment interaction were analyzed as a proportion of the treatment according to the control (well-watered and without defoliation). To relativize the data, we used the ratio between the value of each individual (in each treatment and accession) and the average of the control in each accession. In these analyses, the main effects were biotype in the drought stress experiment and biotype, treatment (33, 66 and 100% of leaf defoliation) and biotype by treatment interaction in the defoliation tolerance experiment. Input data were from 0 to 1, indicating susceptibility or tolerance, respectively, and they were analyzed using generalized linear mixed models. On account of the natural non-normal distribution of the proportion, all the models were adjusted using Beta distribution. When the main fixed effect was significant, the least square means of the biotypes were compared using orthogonal contrast.

Plant growth data (vegetative biomass and plant height) were fitted to a logistic model with three parameters according to the following equation:

$$Y \text{ (g or cm)} = d/[1 + (x/GD50)^b]$$

where Y is the aboveground biomass or plant height (%) at growing degree-day x , d is the maximum aboveground biomass or plant height value (g or cm), $GD50$ is the number of growing degree-days required to reach 50% of maximum above-ground biomass or plant height, and b indicates the slope around $GD50$. The logistic dose-response curves were fitted using the *drc* package of the R version 3.2.2 statistical software. The function *compParm* was used to compare the relative differences between the parameters of the biotype curves (AGR, CROP and RUD).

In order to test whether the differences between biotypes for stress

response are explained by differences in growth traits, an analysis of covariance (ANCOVA) of the drought and defoliation tolerance was run using Proc Glimmix in SAS. Both final biomass and plant growth rate under non-stressful conditions were included in the model as covariates. Akaike's information criterion (AIC) was used to compare models with and without covariates. Covariates that mediate the differences in drought or defoliation tolerance between biotypes will improve the model (smaller AIC value) and reduce the sums of squares explained by the biotype effect and by the three orthogonal contrasts between AGR vs. CROP, AGR vs. RUD, and CROP vs. RUD biotypes. Such reduction in the sums of squares indicate a trade-off between growth (covariates) and stress tolerance (main effects) (Kozioł et al., 2012). Percent of variation explained by covariates was calculated using F values from the models with and without covariates.

The sequences were analysed by multiple sequence alignments using the Clustal Omega Multiple Sequence Alignment tool (McWilliam et al., 2013). The coding and non-coding regions of each gene were then identified by aligning the target sequences with ESTs via BLAST searches. Estimates of nucleotide diversity (S , π , Θ and K), population differentiation (F_{ST} and pairwise distance-PD- between each of three biotypes) and Tajima's D were obtained using the software package DnaSP 5.10.1 (Librado and Rozas, 2009). Nucleotide diversity (π) was calculated for the overall sequence but also for synonymous and non-synonymous substitutions. To better assign the synonymous and non-synonymous substitutions, the best open reading frame was predicted by using the translate tool of ExpAsy (Gasteiger et al., 2003). We ran a clustering method for each of the four genes to investigate the relationship of AGR plants alleles with their CROP and RUD ancestors. The neighbour-joining algorithm was used with MEGA software package (Kumar et al., 2016), distances were computed using the number of differences method, all three codon positions and the non-coding positions were included whereas all the positions containing gaps and missing data were eliminated. Branch support was estimated based on 1000 bootstrap replicates of the data. The outgroup sequences were *Lactuca sativa* for DREB2 gene, *Vitis vinifera* for NAC gene, *H. petiolaris* for DHN gene and *H. annuus* spp. *annuus* for LTP gene.

3. Results

3.1. Response to drought and defoliation stresses

All the traits measured in the defoliation experiment showed significant biotype, treatment, and biotype by treatment interaction effects (Table S2). When the traits were analyzed as a proportion of the treatment without defoliation, the biotype effect was not significant whereas the treatment and biotype by treatment interaction effects were highly significant (Table 1). An increase in defoliation tends to decrease the relative values of each trait but the orthogonal contrasts were only statistically significant at 100% defoliation. Head diameter, seeds per head, and seeds per plant were much more reduced in CROP and AGR than in RUD and seed weight decreased in CROP (Table 1; Fig. 2). In general, head diameter was the trait least affected by total defoliation, being reduced by 39% in AGR, 54% in CROP, but only 7% in RUD. On the other hand, seeds per plant was the trait most affected, falling by 96% in AGR, 94% in CROP, and 75% in RUD (Fig. 2).

In the drought experiment, the treatment effect was significant for all traits, while the biotype by treatment interaction was significant for leaf length and width, and flowers per head. The biotype effect was only significant for plant height (Table S2). When the traits were analyzed as a proportion (plant height was not included in this analysis because the biotype by treatment interaction was not significant), the biotype effect was significant for leaf length and flowers per head but not significant for leaf width (Table 2). CROP and AGR were affected by drought stress, reducing their leaf length by 10 and 6%, and the number of flowers per head by 23 and 32%, respectively. RUD were less affected in both traits, falling by 2% in leaf length and 16% in flowers per head (Fig. 3).

Table 1

Statistics (*F*-value) with their respective *p*-value for the trait evaluated in the defoliation experiment. The main effects were: biotype (AGR, CROP, RUD), defoliation (33, 66 and 100%) and the interaction biotype by defoliation. The orthogonal contrasts (at 100% defoliation) are shown below the ANOVA parameters. Data were analyzed as a proportion of the treatment according to the control (without defoliation) with generalized linear mixed models, models were adjusted using a Beta distribution. All the main effects (biotype, defoliation and biotype by defoliation interaction) were considered as fixed, whereas the accessions were considered as random.

Effect	Head diameter		Seeds per head		Seed weight		Seeds per plant	
	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value
Biotype (B)	6.7	0.13	4.9	0.17	1.1	0.47	1.8	0.35
Defoliation (D)	33.5	< 0.0001	44.4	< 0.0001	29.5	< 0.0001	89.1	< 0.0001
B × D	7.8	< 0.0001	6.6	< 0.001	7.7	< 0.0001	12.4	< 0.0001
Orthogonal contrast (at 100% defoliation)								
AGR vs. CROP	1.61	0.21	0.87	0.35	1.56	0.22	0.13	0.72
AGR vs. RUD	17.70	< 0.0001	11.16	< 0.01	1.12	0.29	8.25	< 0.01
CROP vs. RUD	44.60	< 0.0001	33.31	< 0.0001	8.05	< 0.01	14.83	< 0.001

3.2. Plant growth in common garden

Plant height and above-ground biomass of the three biotypes, grown under well-watered common garden conditions (hereafter, non-stressful conditions), fitted to a logistic model with three parameters. RUD were 1.12 and 1.46-fold taller than AGR and CROP, respectively ($d_{RUD} = 265.0$ vs. $d_{AGR} = 236.7$; $t = 3.34$ $p = 0.001$ and $d_{RUD} = 265.0$ vs. $d_{AGR} = 180.9$; $t = 11.92$ $p < 0.001$). However, AGR were significantly faster than RUD in reaching the maximum plant height ($b_{AGR} = 5.8$ vs. $b_{RUD} = 4.2$; $t = 2.13$ $p = 0.03$). CROP showed an intermediate, but not significantly different, slope to AGR and RUD

(Fig. 4a). On the other hand, the above-ground biomass of RUD was 1.82 and 2.59- times higher than that of AGR and CROP, respectively ($d_{RUD} = 620.2$ vs. $d_{AGR} = 339.1$; $t = 9.28$ $p < 0.001$ and $d_{RUD} = 620.2$ vs. $d_{AGR} = 239.4$; $t = 15.26$ $p < 0.001$). However, AGR, CROP and RUD showed similar rates (b parameter) of reaching maximum biomass accumulation (Fig. 4b).

3.3. Trade-off between stress tolerance and growth traits

There was evidence of a trade-off between growth and stress tolerance traits across the biotypes. ANCOVA provided evidence that

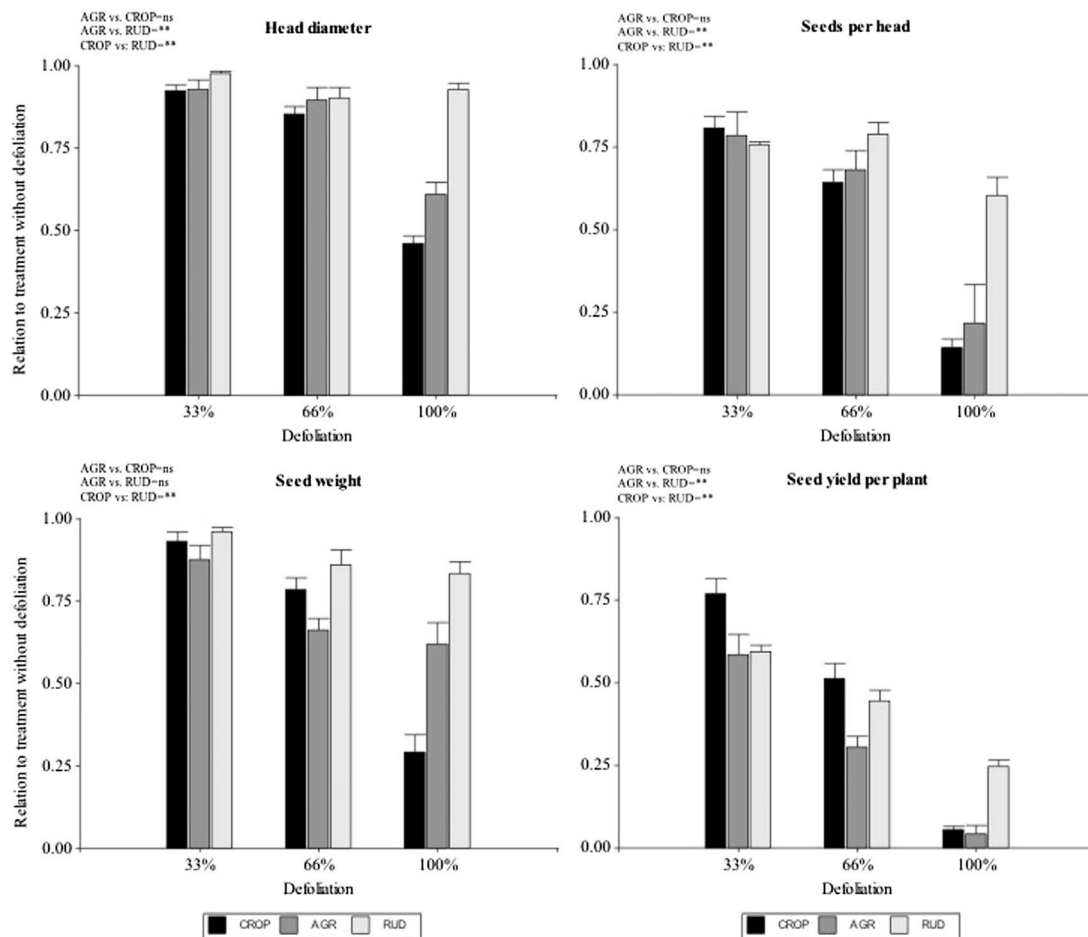


Fig. 2. Effect of three levels of leaf defoliation (33, 66 and 100%) on head diameter, seeds per head, seed weight and seeds per plant in agrestal (AGR), domesticated (CROP) and ruderal (RUD) sunflower biotypes. Bars show the proportion (\pm SE) of each trait (in each treatment and biotype) in relation to plants without defoliation treatment. Orthogonal contrasts between biotypes (at 100% defoliation) on each trait are shown in the figure. ** = $p < 0.01$; * = $p < 0.05$; ns = $p > 0.05$. For more details see Table 1.

Table 2

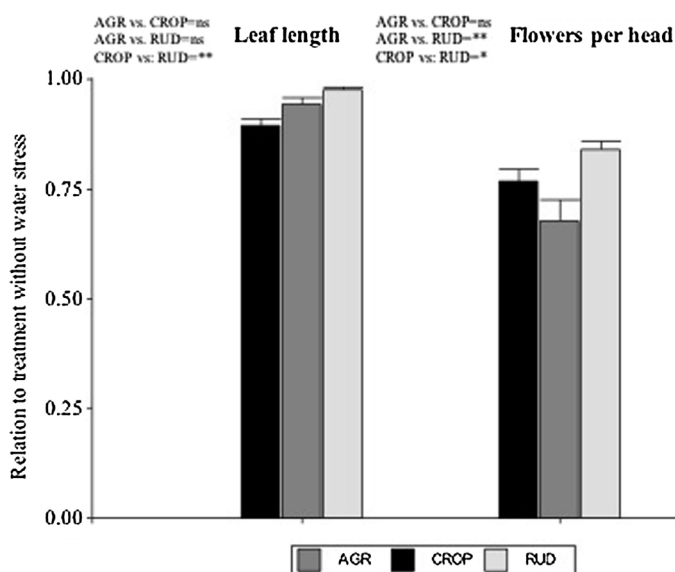
Statistics (*F*-value) with their respective *p*-value for the trait evaluated in the drought experiment. The main effect was biotype (AGR, CROP, RUD). The orthogonal contrasts are shown below the ANOVA parameters. Data were analyzed as a proportion of the treatment according to the control (well-watered) with generalized linear mixed models, models were adjusted using a Beta distribution. The main effect (biotype) was considered as fixed, whereas the accessions were considered as random.

Effect	Leaf width		Leaf length		Flowers per head	
	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value
Biotype	1.75	0.36	13.4	< 0.0001	7.0	0.002
Orthogonal contrast						
AGR vs. CROP	0.01	0.92	3.46	0.07	2.48	0.12
AGR vs. RUD	3.10	0.22	3.75	0.06	12.18	< 0.001
CROP vs. RUD	1.81	0.31	26.85	< 0.0001	6.07	0.02

selection on growth rate (plant height growth rate as covariate) and above-ground biomass accumulation (final biomass as covariate) contributed to the reduction of sum of squares by biotype effect in drought (flowers per head as response variable) and defoliation (seeds per plant as response variable) tolerances (Tables S3 and S4). The final biomass explained 35% and 80% of the variation in drought and defoliation tolerance between biotypes, respectively. Likewise, plant height growth rate explained approximately 65% of the variation in both drought and defoliation tolerance (Tables S3 and S4). Interestingly, the final biomass explained up to 80% of the differences between CROP and RUD in the defoliation tolerance and plant height growth rate was involved in both of the stresses, explaining up to 70% and 82% of the differences found between AGR and RUD, in drought and defoliation tolerance, respectively (Tables S3 and S4). When both final biomass and plant height growth rate were included as covariates, they explained most of the variation (~90%) in drought and defoliation tolerance (Tables S3 and S4).

3.4. Nucleotide diversity in four stress-related genes

To investigate whether differences in the stress response of the AGR, RUD and CROP biotypes may be explained by allelic differences, the sequence of four stress-related genes was analysed (Table 3). As expected, genes belonging to the TF group (DREB2 and NAC) exhibited a much lower nucleotide diversity (lower values of S , π , Θ and K) than genes belonging to the non-TF group (DHN and LTP). In addition, the former group showed both lower $\pi_{\text{syn}}/\pi_{\text{non}}$ (0.370 ± 0.047 vs.



1.019 ± 0.343 for TF and non-TF, respectively) and Tajima's D than the latter group (-0.84 ± 0.48 vs. 0.00 ± 0.23 for TF and non-TF, respectively). In terms of the extent and direction of divergence between categories, F_{ST} was higher for AGR vs. RUD than AGR vs. CROP comparisons in DREB2 ($F_{\text{AGR-RUD}} = 0.286$; $F_{\text{AGR-CROP}} = 0.000$) and NAC ($F_{\text{AGR-RUD}} = 0.909$; $F_{\text{AGR-CROP}} = 0.128$). For DHN and LTP, the high variability observed within each biotype results in a low differentiation level between biotypes (all the $F_{\text{ST}} < 0.128$). Similarly, pairwise distances between groups were higher in AGR vs. RUD than in AGR vs. CROP comparisons for DREB2 ($PD_{\text{AGR-RUD}} = 6.67 \pm 1.95$; $PD_{\text{AGR-CROP}} = 0.92 \pm 0.51$), NAC ($PD_{\text{AGR-RUD}} = 6.5 \pm 2.3$; $PD_{\text{AGR-CROP}} = 5.5 \pm 1.2$) and DHN ($PD = 11.5 \pm 2.28$; $PD_{\text{AGR-CROP}} = 7.60 \pm 1.69$) genes. Thus, for the two TF-coding genes (DREB2 and NAC) a clustering was made using the neighbour-joining method. The clustering method of the DREB2 and NAC genes reveals that AGR and CROP are part of the same group whereas RUD forms a separate group (Fig. 5) showing crop-like alleles in the AGR biotype.

4. Discussion

We found evidence of a trade-off between growth and stress tolerance involved in the evolution of the agrestal *H. annuus* biotype. This biotype has greater weediness than the ruderal biotype, and shows evidence of crop introgression (Casquero et al., 2013; Casquero and Cantamutto, 2016). This increase in weediness in AGR linked to faster growth (plant height), which may determine the effectiveness of shading on crop plants (Weinig, 2000), was associated with reduced drought and defoliation tolerance. AGR and CROP were more affected by defoliation and drought than RUD, as seen by stress-induced reductions in plant height, leaf length, head diameter, flowers per head, seeds per head, seed weight and seeds per plant (Figs. 2 and 3). Furthermore, AGR showed faster growth (plant height) than RUD, but a significant reduction in above-ground biomass (Fig. 4).

Variation in defoliation tolerance was mainly explained by above-ground biomass under non-stressful conditions (Table S3) which could indicate that RUD, with higher biomass, were less sensitive because of a greater carbohydrate pool prior to defoliation –fully branched plants- than AGR plants -apical branched plants- and CROP plants –no branching-. The allocation of carbon, nitrogen, and presumably other resources from branches to defoliated stem, may provide a potential mechanism of herbivory tolerance by growth following defoliation (Caldwell et al., 1981; Mabry and Wayne, 1997; Richards and Caldwell, 1985; Stevens et al., 2008) and allow RUD plants to maintain

Fig. 3. Effect of drought stress on leaf length and flowers per head in agrestal (AGR), domesticated (CROP) and ruderal (RUD) sunflower biotypes. Bars show the proportion (\pm SE) of each trait (in each biotype) in relation to plants without defoliation treatment. Orthogonal contrasts between biotypes on each trait are shown in the figure. ** = $p < 0.01$; * = $p < 0.05$; ns = $p > 0.05$. For more details see Table 2.

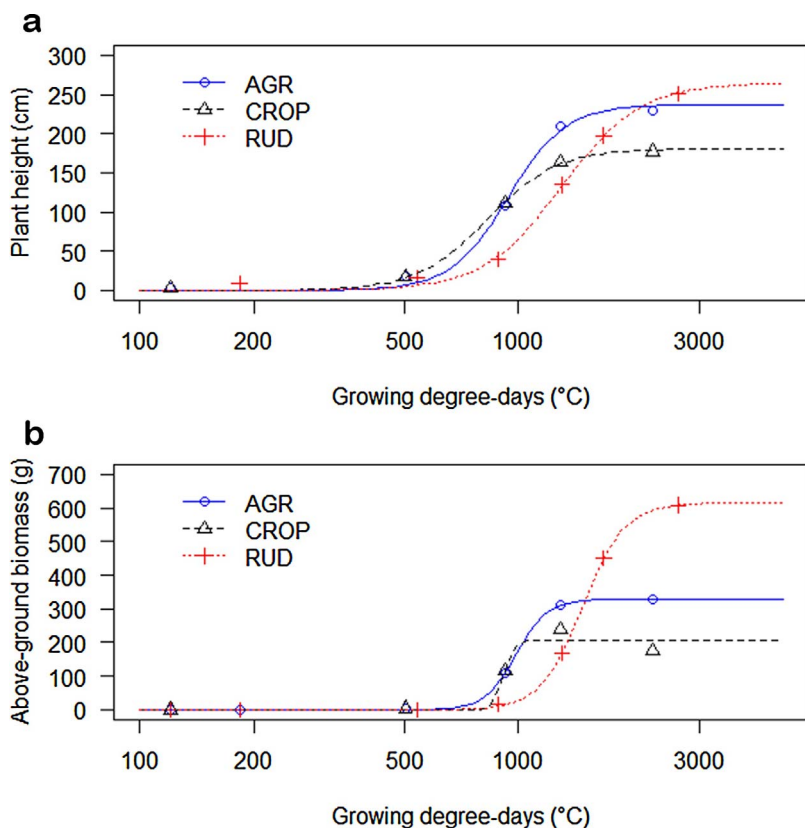


Fig. 4. Dynamic of plant height (a) and above-ground biomass (b) as a function of the growing degree-day in agrestal (AGR), domesticated (CROP) and ruderal (RUD) sunflower biotypes. Data were fitted to a 3-parameters logistic model as follows: a. $Y_{AGR} = 236.7/[1 + (x/937.9)^{-5.8}]$; $Y_{CROP} = 180.9/[1 + (x/827.5)^{-4.7}]$; $Y_{RUD} = 265.0/[1 + (x/1301.2)^{-4.2}]$ b. $Y_{AGR} = 339.1/[1 + (x/990.5)^{-7.2}]$; $Y_{CROP} = 239.4/[1 + (x/891.6)^{-6.3}]$; $Y_{RUD} = 620.2/[1 + (x/1502.1)^{-7.0}]$.

significantly more seeds per plant than CROP and AGR plants (Table 1). Moreover, drought tolerance variation was mainly explained by the plant height rate under non-stressful conditions meaning that RUD plants, with a lower growth rate (Fig. 4), were less sensitive to drought than the AGR and CROP plants, which showed faster growth rate (Kozioł et al., 2012; Mayrose et al., 2011). This lower growth rate in RUD than in AGR and CROP may be the result of the more conservative water-use strategy of the former (Brouillette et al., 2014; Donovan et al., 2007) which impedes fast growth rates under non-stressful conditions but allows a better use of water under drought stress conditions. Although the timing and severity of the stress may alter the responses observed here, and other stresses as the induced by herbicide application or competition with the crop were not accounted for, we provided evidence of the existence of trade-offs between growth under non-stressful conditions and both drought and defoliation stress tolerance involved in the evolution of weediness in sunflower.

Three of the four stress-related genes diverged between RUD and AGR. Gene sequence analysis revealed higher diversity in the two genes encoding enzyme or defense protein (*DHN*, *LTP*) than in those genes involved in transcriptional regulation (*DREB2*, *NAC*), which is in agreement with a previous report on inbred lines of sunflower (Giordani et al., 2011). Values of π_{syn}/π_{non} closer to zero and negative Tajima's D in the *NAC* gene, and especially in the *DREB2* gene, suggest that nucleotide diversity at these two loci is governed by purifying

selection, indicating past selection on these loci. For *DHN*, less evidence of purifying selection was found due to π_{syn}/π_{non} being closer to one and Tajima's D closer to zero (Table 3). On the contrary, for *LTP*, the higher π_{syn}/π_{non} may indicate a positive selection on this locus or a relaxation of selection, however the high variability and the positive Tajima's D suggest the latter at this locus (Table 3). In addition, the alleles in the TFs of AGR plants were similar to those found in CROP plants (Fig. 5) suggesting that crop alleles introgression in these loci are may be potentially important for rapid adaptation to agricultural environments (Kane and Rieseberg, 2007). *DREB2* and *NAC* family genes are induced by drought, salt and heat stress and are also associated with plant immune response (Nuruzzaman et al., 2013; Shinozaki and Yamaguchi-Shinozaki, 2007). In addition, overexpression of the *DREB2* gene also resulted in growth retardation (Sakuma et al., 2006). This dual function in growth and stress response of upstream transcriptional regulators may partially explain the trade-offs involved in the adaptation to agricultural environments (Kozioł et al., 2012; Mayrose et al., 2011). It is possible that during the adaptation to agricultural environments, crop alleles had been selected for, favoring resource allocation to growth (Bakker et al., 2008; Blair and Wolfe, 2004; Zhen and Ungerer, 2008).

Adaptive evolution after introduction to a novel environment (Prentis et al., 2008) may be one of the causes of the morphological, physiological, and allelic divergence between AGR and RUD biotypes

Table 3

Summary of nucleotide diversity variables. n: number of sequences used in the analysis; size: number of sites excluding gaps and missing data; S: number of segregating sites; π : average number of nucleotide differences per site (π), for synonymous (π_{syn}) and non-synonymous sites (π_{non}); Θ : theta per site from S; K: average number of nucleotide differences; Tajima's D statistic.

Gene	n	Size (bp)	S	π	Θ	K	π_{syn}	π_{non}	π_{syn}/π_{non}	Tajima's D
DREB2	9	588	17	0.0077	0.0106	4.6	0.0163	0.0053	0.322	-1.32
NAC	9	592	15	0.0086	0.0093	5.1	0.0185	0.0077	0.417	-0.36
DHN	9	815	28	0.0121	0.0126	9.8	0.0091	0.0062	0.676	-0.23
LTP	9	448	109	0.0987	0.0945	44.2	0.0737	0.1004	1.362	0.23

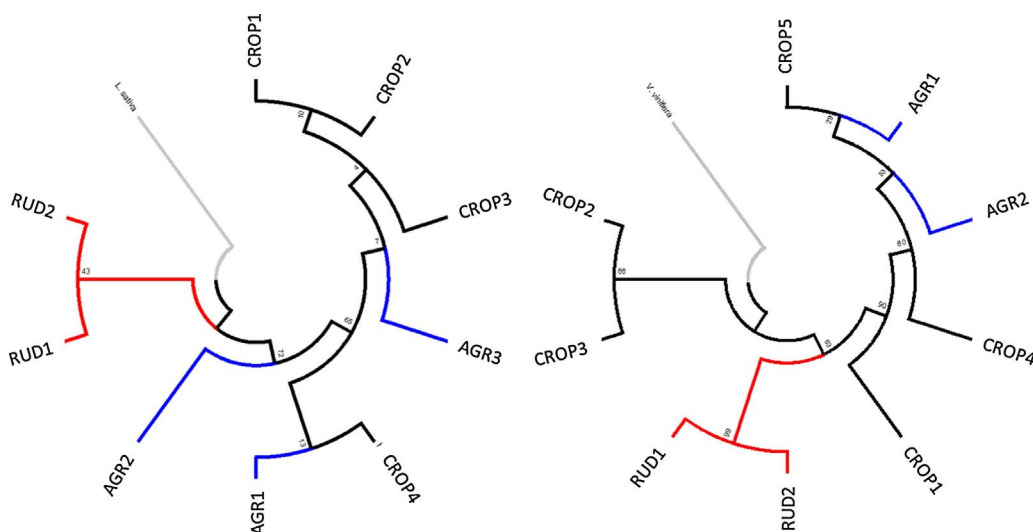


Fig. 5. Relationship between AGR, CROP and RUD sequences of two transcriptional regulators of stress-related genes: DREB2 (left) and NAC (right). The relationships between biotypes were inferred using the neighbour-joining method. The analysis involved 9 nucleotide sequences for each gene. CROP1 = inbred line D8, CROP2 = inbred line L72, CROP3 = inbred line C1, CROP4 = inbred line HA89 and CROP5 = inbred line B71.

and, given the recent origin of the agrestal biotype, the shift can occur in a few generations (Franks et al., 2016; Sultan et al., 2013). Most RUD populations are located in the central or central-west regions of Argentina in disturbed habitats, such as roadsides, ditches, fences, hedgerows and field margins (Poverene et al., 2009). Conversely, the AGR biotype is located in the central-east region of Argentina in a crop field with low/medium human input (Young, 2006). This biotype is established in an environment without any previous records of RUD populations (Casquero et al., 2013; Poverene et al., 2009). Based on our research, including farmer interviews, recurrent trips in the area, and previous knowledge, it is thought that the initial field invasion was initiated from RUD seed carried by hired agricultural machinery, frequently used in Argentina (Casquero et al., 2013). From our study, we cannot split the effects of non-agricultural and agricultural conditions on the adaptation of the AGR biotype. However, the absence of plants outside the field margins observed in periodic monitoring, the greater competitive ability of the AGR biotype compared to the RUD biotype (Casquero and Cantamutto, 2016) and the similar phenotypic changes observed in AGR biotype from another continent (Muller et al., 2009) strongly suggest that the adaptation of this AGR biotype is to agricultural conditions.

In addition to selection in the novel agricultural environment, crop hybridization has probably played an important role in creating a genetic variant with traits already adapted to agricultural conditions (those inherited from the crop parent such as fast growth or early flowering) and traits that favor proliferation and persistence (those inherited from the wild parent, like branching or shattering) (Vigueira et al., 2013). Although standing variation is believed to be the more likely source of adaptive traits in US weedy sunflower populations (Kane and Rieseberg, 2008), this may not be our scenario because Argentine invasive *H. annuus* populations were never found in agricultural fields as weeds (Poverene et al., 2009). The origin of the Argentinean agrestal sunflower seems to be more related to European sunflower populations, which were originated from crop-wild hybrids (Muller et al., 2011), probably imported as impurities of cultivar seeds from the US (Faure et al., 2002; Muller et al., 2011). However, Argentina has been characterized for the production of their own seed since the 1930s (Bertero de Roman and Vázquez, 2003). Therefore, while crop-wild hybridization in the European case occurred in the native range (US), Argentinean agrestal population probably came after hybridization between crop cultivars and ruderal populations naturalized in Argentina.

It is possible that crop hybridization in ruderal populations has happened, because some sunflower crop areas in Argentina overlap with areas of ruderal population distribution and some morphological

traits have shown evidence of crop introgression (Cantamutto et al., 2010a). Despite that, under opposing selection (ruderal environment), some or all of the immigrant alleles in the recipient population eventually disappear (Ellstrand et al., 2013). Crop-like traits, such as larger leaves, heads and seeds, suggest that some cultivar alleles could spread into wild populations in ruderal environments (Baack et al., 2008; Dechaine et al., 2009; Mercer et al., 2007) but naturally occurring agents of selection (e.g. birds, lepidoptera, rodents) might have prevented the spread of other cultivar alleles into ruderal populations, retaining many wild traits even in the face of recurrent reproductive contact (Baack et al., 2008; Cummings et al., 1999; Dechaine et al., 2009; Presotto et al., 2016). Conversely, the agrestal biotype has followed a different pathway. A scenario where crop-wild hybrids were selected in the agricultural environment, where abiotic and biotic stresses are minimized (i.e. with agrochemical products, fertilizers, or tillage) to reach higher yields, and without the possibility of diluting crop alleles by backcrossing with neighbor ruderal plants (Ellstrand, 2003). This situation may have exacerbated crop introgression by combining favorable wild and crop alleles to evolve into a biotype with increased weediness.

Previous studies on sunflowers have found strong evidence of a trade-off between growth and stress tolerance in weedy populations (Kozioł et al., 2012; Mayrose et al., 2011). Although this adaptation was not directly linked to domesticated sunflower introgression, it is possible because of the multiple routes involved in weediness in this species (Kane and Rieseberg, 2008; Muller et al., 2011). However, Mayrose et al. (2011), within their extensive collection, evaluated a weedy population from Córdoba (Spain) which, together with the rest of the French and Spanish populations, most probably originated from crop-wild hybrids (Muller et al., 2011). In support of this idea, the crop-wild hybrid intensively selected for in agricultural environments and without any contact with nearby ruderal populations (except from the initial field invasion) was the probable mechanism that created this agrestal biotype in Argentina.

5. Conclusions

We found evidence of a trade-off between growth and stress tolerance involved in the adaptation of the agrestal *H. annuus* biotype to agricultural conditions. We also found that defoliation tolerance was mainly associated with the above-ground biomass, and drought tolerance was mainly related to the plant growth rate. The contrasting environment plus crop hybridization created a genetic variant adapted to field conditions due to fast growth rate but with reduced drought and defoliation tolerance. Our study contributes to a better understanding

of weed evolution in the context of human-caused selection and could help to design more effective weed management strategies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.agee.2017.08.003>.

References

- Abbott, R., Albach, D., Ansell, S., Arntzen, J.W., Baird, S.J.E., Bierne, N., Boughman, J., Brelsford, A., Buerkle, C.A., Buggs, R., Butlin, R.K., Dieckmann, U., Eroukhanoff, F., Grill, A., Cahan, S.H., Hermansen, J.S., Hewitt, G., Hudson, A.G., Jiggins, C., Jones, J., Keller, B., Marczewski, T., Mallet, J., Martinez-Rodriguez, P., Möst, M., Mullen, S., Nichols, R., Nolte, A.W., Parisod, C., Pfennig, K., Rice, A.M., Ritchie, M.G., Seifert, B., Smadja, C.M., Stelkens, R., Szymura, J.M., Väinölä, R., Wolf, J.B.W., Zinner, D., 2013. Hybridization and speciation. *J. Evol. Biol.* 26, 229–246. <http://dx.doi.org/10.1111/j.1420-9101.2012.02599.x>.
- Agrawal, A., Hastings, A., Bradburd, G., Woods, E., Züst, T., Harvey, J., Bukovinsky, T., 2015. Evolution of plant growth and defense in a continental introduction. *Am. Nat.* 186, E1–E15. <http://dx.doi.org/10.1086/681622>.
- Arias, D.M., Rieseberg, L.H., 1994. Gene flow between cultivated and wild sunflowers. *Theor. Appl. Genet.* 89, 655–660. <http://dx.doi.org/10.1007/BF00223700>.
- Arnaud, J.-F., Phane, S., Nart, F., Cordellier, M., Cuguen, J.L., 2010. Populations of weedy crop-wild hybrid beets show contrasting variation in mating system and population genetic structure. *Evol. Appl.* 3, 305–318. <http://dx.doi.org/10.1111/j.1752-4571.2010.00121.x>.
- Baack, E.J., Sapiro, Y., Chapman, M.A., Burke, J.M., Rieseberg, L.H., 2008. Selection on domestication traits and quantitative trait loci in crop-wild sunflower hybrids. *Mol. Ecol.* 17, 666–677. <http://dx.doi.org/10.1111/j.1365-294X.2007.03596.x>.
- Bagavathiannan, M., Van Acker, R.C., 2008. Crop ferality: implications for novel trait confinement. *Agric. Ecosyst. Environ.* 127, 1–6. <http://dx.doi.org/10.1016/j.agee.2008.03.009>.
- Bakker, E.G., Traw, M.B., Toomajian, C., Kreitman, M., Bergelson, J., 2008. Low levels of polymorphism in genes that control the activation of defense response in *Arabidopsis thaliana*. *Genetics* 178, 2031–2043. <http://dx.doi.org/10.1534/genetics.107.083279>.
- Bertero de Roman, A., Vázquez, N., 2003. Origin of the Argentine sunflower varieties. *Helia* 26, 127–136. <http://dx.doi.org/10.2298/HEL0338127d>.
- Blair, A.C., Wolfe, L.M., 2004. The evolution of an invasive plant: an experimental study with *Silene latifolia*. *Ecology* 85, 3035–3042. <http://dx.doi.org/10.1890/04-0341>.
- Blossey, B., Notzold, R., 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *J. Ecol.* 83, 887. <http://dx.doi.org/10.2307/2261425>.
- Brouillette, L.C., Mason, C.M., Shirk, R.Y., Donovan, L.A., 2014. Adaptive differentiation of traits related to resource use in a desert annual along a resource gradient. *New Phytol.* 201, 1316–1327. <http://dx.doi.org/10.1111/nph.12628>.
- CIMMYT, 2006. *Protocolos de laboratorio: Laboratorio de Genética Molecular Aplicada del CIMMYT, Tercera ed.* CIMMYT, México D.F.
- Caldwell, M.M., Richards, J.H., Johnson, D.A., Nowak, R.S., Dzurek, R.S., 1981. Coping with herbivory: photosynthetic capacity and resource allocation in two semiarid *Agropyron* bunchgrasses. *Oecologia* 50, 14–24. <http://dx.doi.org/10.1007/BF00378790>.
- Campbell, L.G., Snow, A.A., Ridley, C.E., 2006. Weed evolution after crop gene introgression: greater survival and fecundity of hybrids in a new environment. *Ecol. Lett.* 9, 1198–1209. <http://dx.doi.org/10.1111/j.1461-0248.2006.00974.x>.
- Cantamutto, M., Presotto, A., Fernandez Moroni, I., Alvarez, D., Poverene, M., Seiler, G., 2010a. High infraspecific diversity of wild sunflowers (*Helianthus annuus* L.) naturally developed in central Argentina. *Flora Morphol. Distrib. Funct. Ecol. Plants* 205, 306–312. <http://dx.doi.org/10.1016/j.flora.2009.12.009>.
- Cantamutto, M., Torres, L., Presotto, A., Gutierrez, A., 2010b. Migration pattern suggested by terrestrial proximity as possible origin of wild annual *Helianthus* populations in central Argentina. *Biol. Invasions* 12, 541–551. <http://dx.doi.org/10.1007/s10530-009-9458-8>.
- Casquero, M., Cantamutto, M., 2016. Interference of the agrastal *Helianthus annuus* biotype with sunflower growth. *Weed Res.* 56, 229–236. <http://dx.doi.org/10.1111/wre.12197>.
- Casquero, M., Presotto, A., Cantamutto, M., 2013. Exofertility in sunflower (*Helianthus annuus* L.): A case study of intraspecific/interspecific interference promoted by human activity. *Field Crops Res.* 142, 95–101. <http://dx.doi.org/10.1016/j.fcr.2012.11.022>.
- Cummings, C.L., Alexander, H.M., Snow, A.A., 1999. Increased pre-dispersal seed predation in sunflower crop-wild hybrids. *Oecologia* 121, 330–338.
- De Wet, J.M.J., Harlan, J.R., 1975. Weeds and Domesticates: evolution in the man-made habitat. *Econ. Bot.* 29, 99–108. <http://dx.doi.org/10.1007/BF02863309>.
- Debban, C.L., Okum, S., Pieper, K.E., Wilson, A., Baucom, R.S., 2015. An examination of fitness costs of glyphosate resistance in the common morning glory, *Ipomoea purpurea*. *Ecol. Evol.* 5, 5284–5294. <http://dx.doi.org/10.1002/ece3.1776>.
- Dechaine, J.M., Burger, J.C., Chapman, M.A., Seiler, G.J., Brunick, R., Knapp, S.J., Burke, J.M., 2009. Fitness effects and genetic architecture of plant-herbivore interactions in sunflower crop-wild hybrids. *New Phytol.* 184, 828–841. <http://dx.doi.org/10.1111/j.1469-8137.2009.02964.x>.
- Díaz-Martín, J., Almoguera, C., Prieto-Dapena, P., Espinosa, J.M., Jordano, J., 2005. Functional interaction between two transcription factors involved in the developmental regulation of a small heat stress protein gene promoter. *Plant Physiol.* 139, 1483–1494. <http://dx.doi.org/10.1104/pp.105.069963>.
- Donovan, L.A., Dudley, S.A., Rosenthal, D.M., Ludwig, F., 2007. Phenotypic selection on leaf water use efficiency and related ecophysiological traits for natural populations of desert sunflowers. *Oecologia* 152, 13–25. <http://dx.doi.org/10.1007/s00442-006-0627-5>.
- Dry, P., Burdon, J., 1986. Genetic structure of natural populations of wild sunflowers (*Helianthus annuus* L.) in Australia. *Aust. J. Biol. Sci.* 39, 255–270.
- Ellstrand, N.C., 2003. *Dangerous Liaisons?: When Cultivated Plants Mate with Their Wild Relatives.* JHU Press, Baltimore.
- Ellstrand, N.C., Heredia, S.M., Leak-Garcia, J.A., Heraty, J.M., Burger, J.C., Yao, L., Nohzadeh-Malakshah, S., Ridley, C.E., 2010. Crops gone wild: evolution of weeds and invasives from domesticated ancestors. *Evol. Appl.* 3, 494–504. <http://dx.doi.org/10.1111/j.1752-4571.2010.00140.x>.
- Ellstrand, N.C., Meirmans, P., Rong, J., Bartsch, D., Ghosh, A., de Jong, T.J., Haccou, P., Lu, B.-R., Snow, A.A., Neal Stewart, C., Strasburg, J.L., van Tienderen, P.H., Vrieling, K., Hooftman, D., 2013. Introgression of crop alleles into wild or weedy populations. *Ann. Rev. Ecol. Evol. Syst.* 44, 325–345. <http://dx.doi.org/10.1146/annurev-ecolsys-110512-135840>.
- Fan, M., Bai, M.-Y., Kim, J.-G., Wang, T., Oh, E., Chen, L., Park, C.H., Son, S.-H., Kim, S.-K., Mudgett, M.B., Wang, Z.-Y., 2014. The bHLH transcription factor HB11 mediates the trade-off between growth and pathogen-associated molecular pattern-triggered immunity in *Arabidopsis*. *Plant Cell* 26, 828–841. <http://dx.doi.org/10.1105/tpc.113.121111>.
- Faure, N., Serieys, H., Bervillé, A., 2002. Potential gene flow from cultivated sunflower to volunteer, wild *Helianthus* species in Europe. *Agric. Ecosyst. Environ.* 89, 183–190. [http://dx.doi.org/10.1016/S0167-8809\(01\)00338-3](http://dx.doi.org/10.1016/S0167-8809(01)00338-3).
- Franks, S.J., Kane, N.C., O'Hara, N.B., Tittes, S., Rest, J.S., 2016. Rapid genome-wide evolution in *Brassica rapa* populations following drought revealed by sequencing of ancestral and descendant gene pools. *Mol. Ecol.* 25, 3622–3631. <http://dx.doi.org/10.1111/mec.13615>.
- Garayalde, A.F., Poverene, M., Cantamutto, M., Carrera, A.D., 2011. Wild sunflower diversity in Argentina revealed by ISSR and SSR markers: an approach for conservation and breeding programmes. *Ann. Appl. Biol.* 158, 305–317. <http://dx.doi.org/10.1111/j.1744-7348.2011.00465.x>.
- Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R.D., Bairoch, A., 2003. ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Res.* 31, 3784–3788.
- Ghersa, C.M., Satorre, E.H., 2000. Coevolution of agricultural systems and their weed companions: implications for research. *Field Crops Res.* 67, 181–190.
- Giordani, T., Buti, M., Natali, L., Pugliesi, C., Cattonaro, F., Morgante, M., Cavallini, A., 2011. An analysis of sequence variability in eight genes putatively involved in drought response in sunflower (*Helianthus annuus* L.). *Theor. Appl. Genet.* 122, 1039–1049. <http://dx.doi.org/10.1007/s00122-010-1509-0>.
- Grime, J., 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am. Nat.* 111, 1169–1194.
- Heap, I., 2014. *Herbicide resistant weeds.* Integrated Pest Management. Springer Netherlands, Dordrecht, pp. 281–301. http://dx.doi.org/10.1007/978-94-007-7996-5_12.
- Heiser, C.B., 1954. Variation and subspeciation in the common sunflower, *Helianthus annuus*. *Am. Midl. Nat.* 51, 287–305. <http://dx.doi.org/10.2307/2422222>.
- Kane, N.C., Rieseberg, L.H., 2007. Selective sweeps reveal candidate genes for adaptation to drought and salt tolerance in common sunflower, *Helianthus annuus*. *Genetics* 175, 1823–1834. <http://dx.doi.org/10.1534/genetics.106.067728>.
- Kane, N.C., Rieseberg, L.H., 2008. Genetics and evolution of weedy *Helianthus annuus* populations: adaptation of an agricultural weed. *Mol. Ecol.* 17, 384–394. <http://dx.doi.org/10.1111/j.1365-294X.2007.03467.x>.
- Koehler, K., Center, A., Cavender-Bares, J., 2012. Evidence for a freezing tolerance-growth rate trade-off in the live oaks (*Quercus* series Virentes) across the tropical-temperate divide. *New Phytol.* 193, 730–744. <http://dx.doi.org/10.1111/j.1469-8137.2011.03992.x>.
- Kozioł, L., Rieseberg, L.H., Kane, N., Bever, J.D., 2012. Reduced drought tolerance during domestication and the evolution of weediness results from tolerance-growth trade-offs. *Evolution* 66, 3803–3814. <http://dx.doi.org/10.1111/j.1558-5646.2012.01718.x>.
- Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874. <http://dx.doi.org/10.1093/molbev/msw054>.
- Lai, Z., Kane, N.C., Zou, Y., Rieseberg, L.H., 2008. Natural variation in gene expression between wild and weedy populations of *Helianthus annuus*. *Genetics* 179, 1881–1890. <http://dx.doi.org/10.1534/genetics.108.091041>.
- Liancourt, P., Callaway, R.M., Michalet, R., 2005. Stress tolerance and competitive-response ability determine the outcome of biotic interactions. *Ecology* 86, 1611–1618. <http://dx.doi.org/10.1890/04-1398>.
- Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25, 1451–1452. <http://dx.doi.org/10.1093/>

- bioinformatics/btp187.
- Liu, X., Baird, W.V., 2003. Differential expression of genes regulated in response to drought or salinity stress in sunflower. *Crop Sci.* 43, 678–687.
- Mabry, C.M., Wayne, P.W., 1997. Defoliation of the annual herb *Abutilon theophrasti*: mechanisms underlying reproductive compensation. *Oecologia* 111, 225–232. <http://dx.doi.org/10.1007/s004420050229>.
- Mayrose, M., Kane, N.C., Mayrose, I., Dlugosch, K.M., Rieseberg, L.H., 2011. Increased growth in sunflower correlates with reduced defences and altered gene expression in response to biotic and abiotic stress. *Mol. Ecol.* 20, 4683–4694. <http://dx.doi.org/10.1111/j.1365-294X.2011.05301.x>.
- McWilliam, H., Li, W., Uludag, M., Squizzato, S., Park, Y.M., Buso, N., Cowley, A.P., Lopez, R., 2013. Analysis tool web services from the EMBL-EBI. *Nucleic Acids Res.* 41, W597–W600. <http://dx.doi.org/10.1093/nar/gkt376>.
- Merker, K.L., Andow, D.A., Wyse, D.L., Shaw, R.G., 2007. Stress and domestication traits increase the relative fitness of crop–wild hybrids in sunflower. *Ecol. Lett.* 10, 383–393. <http://dx.doi.org/10.1111/j.1461-0248.2007.01029.x>.
- Merker, K.L., Emry, D.J., Snow, A.A., Kost, M.A., Pace, B.A., Alexander, H.M., 2014. Fitness of crop-wild hybrid sunflower under competitive conditions: implications for crop-to-wild introgression. *PLoS One* 9. <http://dx.doi.org/10.1371/journal.pone.0109001>.
- Muller, M.-H., Délieux, F., Fernández-Martínez, J.M., Garric, B., Lecomte, V., Anglade, G., Leflon, M., Motard, C., Segura, R., 2009. Occurrence, distribution and distinctive morphological traits of weedy *Helianthus annuus* L. populations in Spain and France. *Genet. Resour. Crop Evol.* 56, 869–877. <http://dx.doi.org/10.1007/s10722-009-9409-3>.
- Muller, M.-H., Latreille, M., Tollon, C., 2011. The origin and evolution of a recent agricultural weed: population genetic diversity of weedy populations of sunflower (*Helianthus annuus* L.) in Spain and France. *Evol. Appl.* 4, 499–514. <http://dx.doi.org/10.1111/j.1752-4571.2010.00163.x>.
- Muro, J., Irigoyen, I., Militino, A.F., Lamsfus, C., 2001. Defoliation effects on sunflower yield reduction. *Agron. J.* 93, 634–637. <http://dx.doi.org/10.2134/agronj2001.933634x>.
- Murphy, C.E., Lemerle, D., 2006. Continuous cropping systems and weed selection. *Euphytica* 148, 61–73. <http://dx.doi.org/10.1007/s10681-006-5941-9>.
- Natali, L., Giordani, T., Cavallini, A., 2003. Sequence variability of a dehydrin gene within *Helianthus annuus*. *Theor. Appl. Genet.* 811–818. <http://dx.doi.org/10.1007/s00122-002-1093-z>.
- Neve, P., Vila-Aiub, M., Roux, F., 2009. Evolutionary-thinking in agricultural weed management. *New Phytol.* 184, 783–793. <http://dx.doi.org/10.1111/j.1469-8137.2009.03034.x>.
- Nuruzzaman, M., Sharoni, A.M., Kikuchi, S., 2013. Roles of NAC transcription factors in the regulation of biotic and abiotic stress responses in plants. *Front. Microbiol.* 4, 248. <http://dx.doi.org/10.3389/fmicb.2013.00248>.
- Oerke, E.-C., 2006. Crop losses to pests. *J. Agric. Sci.* 144, 31–43. <http://dx.doi.org/10.1017/S0021859605005708>.
- Ouvrard, O., Cellier, F., Ferrare, K., Tusch, D., Lamaze, T., Dupuis, J.-M.M., Casse-Delbart, F., 1996. Identification and expression of water stress- and abscisic acid-regulated genes in a drought-tolerant sunflower genotype. *Plant Mol. Biol.* 31, 819–829. <http://dx.doi.org/10.1007/BF00019469>.
- Owart, B.R., Corbi, J., Burke, J.M., Dechaine, J.M., 2014. Selection on crop-derived traits and QTL in sunflower (*Helianthus annuus*) crop-wild hybrids under water stress. *PLoS One* 9. <http://dx.doi.org/10.1371/journal.pone.0102717>.
- Owen, M.J., Goggin, D.E., Powles, S.B., 2015. Intensive cropping systems select for greater seed dormancy and increased herbicide resistance levels in *Lolium rigidum* (annual ryegrass). *Pest Manag. Sci.* 71, 966–971. <http://dx.doi.org/10.1002/ps.3874>.
- Poggio, S.L., Chaneton, E.J., Ghersa, C.M., 2013. The arable plant diversity of intensively managed farmland: effects of field position and crop type at local and landscape scales. *Agric. Ecosyst. Environ.* 166, 55–64. <http://dx.doi.org/10.1016/j.agee.2012.01.013>.
- Poverene, M.M., Cantamutto, M.A., Carrera, A.D., Ureta, M.S., Salaberry, M.T., Echeverría, M.M., Rodríguez, R.H., 2002. El girasol silvestre (*Helianthus* spp.) en la Argentina: Caracterización para la liberación de cultivares transgénicos. *Rev. Invest. Agropecu.* 31, 97–116.
- Poverene, M., Cantamutto, M., Seiler, G.J., 2009. Ecological characterization of wild *Helianthus annuus* and *Helianthus petiolaris* germplasm in Argentina. *Plant Genet. Resour.* 7, 42–49. <http://dx.doi.org/10.1017/S1479262108032048>.
- Prentis, P.J., Wilson, J.R.U., Dormontt, E.E., Richardson, D.M., Lowe, A.J., 2008. Adaptive evolution in invasive species. *Trends Plant Sci.* 13, 288–294. <http://dx.doi.org/10.1016/j.tplants.2008.03.004>.
- Presotto, A., Ureta, M.S., Cantamutto, M., Poverene, M., 2012. Effects of gene flow from IMI resistant sunflower crop to wild *Helianthus annuus* populations. *Agric. Ecosyst. Environ.* 146, 153–161. <http://dx.doi.org/10.1016/j.agee.2011.10.023>.
- Presotto, A., Pandolfo, C., Poverene, M., Cantamutto, M., 2016. Can achene selection in sunflower crop–wild hybrids by pre-dispersal seed predators hasten the return to phenotypically wild sunflowers? *Euphytica* 208, 453–462. <http://dx.doi.org/10.1007/s10681-015-1579-9>.
- Ribeiro, A., Gouveia, M., Bessa, A., Ferreira, A., Magumisse, A.T., Manjate, M., Faria, T., 2010. Population structure and genetic diversity of wild *Helianthus* species from Mozambique. *Russ. J. Genet.* 46, 967–975. <http://dx.doi.org/10.1134/S1022795410080089>.
- Richards, J.H., Caldwell, M.M., 1985. Soluble carbohydrates, concurrent photosynthesis and efficiency in regrowth following defoliation: a field study with *Agropyron* species. *J. Appl. Ecol.* 22, 907–920. <http://dx.doi.org/10.2307/2403239>.
- Sakuma, Y., Maruyama, K., Osakabe, Y., Qin, F., Seki, M., Shinozaki, K., Yamaguchi-Shinozaki, K., 2006. Functional analysis of an arabidopsis transcription factor, DREB2A, involved in drought-responsive gene expression. *Plant Cell Online* 18, 1292–1309. <http://dx.doi.org/10.1105/tpc.105.035881>.
- Schneiter, A.A., Miller, J.F., 1981. Description of sunflower growth stages. *Crop Sci.* 21, 901–903. <http://dx.doi.org/10.2135/cropsci1981.0011183X002100060024x>.
- Shinozaki, K., Yamaguchi-Shinozaki, K., 2007. Gene networks involved in drought stress response and tolerance. *J. Exp. Bot.* 58, 221–227. <http://dx.doi.org/10.1093/jxb/erl164>.
- Stevens, M.T., Kruger, E.L., Lindroth, R.L., 2008. Variation in tolerance to herbivory is mediated by differences in biomass allocation in aspen. *Funct. Ecol.* 22, 40–47. <http://dx.doi.org/10.1111/j.1365-2435.2007.01356.x>.
- Stewart, C.N., Halfhill, M.D., Warwick, S.I., 2003. Genetic modification: transgene introgression from genetically modified crops to their wild relatives. *Nat. Rev. Genet.* 4, 806–817. <http://dx.doi.org/10.1038/nrg1179>.
- Sultan, S.E., Horgan-Kobelski, T., Nichols, L.M., Riggs, C.E., Waples, R.K., 2013. A resurrection study reveals rapid adaptive evolution within populations of an invasive plant. *Evol. Appl.* 6, 266–278. <http://dx.doi.org/10.1111/j.1752-4571.2012.00287.x>.
- Todesco, M., Pascual, M.A., Owens, G.L., Ostevik, K.L., Moyers, B.T., Hübner, S., Heredia, S.M., Hahn, M.A., Caseys, C., Bock, D.G., Rieseberg, L.H., 2016. Hybridization and extinction. *Evol. Appl.* 9, 892–908. <http://dx.doi.org/10.1111/eva.12367>.
- Ureta, M.S., Carrera, A.D., Cantamutto, M.A., Poverene, M.M., 2008. Gene flow among wild and cultivated sunflower, *Helianthus annuus* in Argentina. *Agric. Ecosyst. Environ.* 123, 343–349. <http://dx.doi.org/10.1016/j.agee.2007.07.006>.
- Vigueira, C.C., Olsen, K.M., Caicedo, A.L., 2013. The red queen in the corn: agricultural weeds as models of rapid adaptive evolution. *Heredity (Edinb)* 110, 303–311. <http://dx.doi.org/10.1038/hdy.2012.104>.
- Vila-Aiub, M.M., Gundel, P.E., Preston, C., 2015. Experimental methods for estimation of plant fitness costs associated with herbicide-resistance genes. *Weed Sci.* 63, 203–216. <http://dx.doi.org/10.1614/WS-D-14-00062.1>.
- Villalobos, F.J., Hall, A.J., Ritchie, J.T., Orgaz, F., 1996. OILCROP-SUN: a development, growth, and yield model of the sunflower crop. *Agron. J.* 88, 403–415. <http://dx.doi.org/10.2134/agronj1996.00021962008800030008x>.
- Warwick, S.I., Légère, A., Simard, M.J., James, T., 2008. Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy Brassica rapa population. *Mol. Ecol.* 17, 1387–1395. <http://dx.doi.org/10.1111/j.1365-294X.2007.03567.x>.
- Weinig, C., 2000. Differing selection in alternative competitive environments: shade-avoidance responses and germination timing. *Evolution* 54, 124–136. [http://dx.doi.org/10.1554/0014-3820\(2000\)054\[0124:DSIACE\]2.0.CO;2](http://dx.doi.org/10.1554/0014-3820(2000)054[0124:DSIACE]2.0.CO;2).
- Xia, H.-B., Wang, W., Xia, H., Zhao, W., Lu, B.-R., 2011. Conspecific crop-weed introgression influences evolution of weedy rice (*Oryza sativa* f. *spontanea*) across a geographical range. *PLoS One* 6, e16189. <http://dx.doi.org/10.1371/journal.pone.0016189>.
- Young, S., 2006. Agriculturalization as a Syndrome: A Comparative Study of Agriculture in Argentina and Australia. Naciones Unidas, CEPAL, Sustainable Development and Human Settlements Division, Santiago de Chile.
- Zhen, Y., Ungerer, M.C., 2008. Relaxed selection on the CBF/DREB1 regulatory genes and reduced freezing tolerance in the southern range of arabidopsis thaliana. *Mol. Biol. Evol.* 25, 2547–2555. <http://dx.doi.org/10.1093/molbev/msn196>.