Seed bank dynamics of an invasive alien species, *Helianthus annuus* L.

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Abstract

Aims

The ability to form persistent seed banks is one of the best predictors of species´ potential to establish in new ranges. Wild sunflower is native to North America where the formation of persistent seed banks is promoted by disturbance and it plays a key role on the establishment and persistence of native populations. However, the role of the seed banks on the establishment and persistence of invasive populations has not been studied. Here, we evaluated the role of seed bank and disturbance on the establishment and fitness, and seed persistence in the soil in several sunflower biotypes collected in ruderal (wild *H. annuus*) and agrestal (natural crop-wild hybrid) habitats of Argentina as well as volunteer populations (progeny of commercial cultivars).

Methods

In a seed-bank experiment, we evaluated emergence, survival to reproduction, survival of emerged seedlings, inflorescences per plant and per plot under disturbed and undisturbed conditions over two years; in a seed-burial experiment, we evaluated seed persistence in the soil over four springs (6, 18, 30 and 42 months).

Important Findings

Overall, seedling emergence was early in the growing season (during winter), and it was promoted by disturbance, especially in the first year. Despite this, the number of inflorescences per plot was similar under both conditions, especially in ruderals. In the second year, emergence from the seed bank was much lower, but the survival rate was higher. In the seed-burial experiment, genetic differences were observed but seeds of

ruderals and agrestals persisted up to 42 months while seeds of the volunteer did not

persist longer than 6 months. The agrestal biotype showed an intermediate behavior

between ruderals and volunteers in both experiments. Our findings showed that wild

and crop-wild sunflower can form persistent seed banks outside its native range and that

disturbance may facilitate its establishment in new areas.

Keywords: seed bank, seed persistence, wild sunflower, invasive alien species, ruderal,

agrestal

Introduction

Invasive alien plant species represent a major threat to native plants causing ecological and economic impacts across the globe (Mooney and Cleland 2001; Leger and Espeland 2010; Vilà et al. 2011). Increasing transport networks and demand for commodities have increased the risk of biological invasions because non-native species can enter as contaminants in traded goods (Hulme 2009). The invasive process begins once propagules arrive at a new site, beyond the limits of their native range (Richardson et al. 2000; Blackburn et al. 2011).

In short-lived species, soil seed banks (hereafter seed banks) are a reserve of viable seeds present in the soil which are crucial for the growth and maintenance of existing populations and for the establishment of alien species (Chauhan et al. 2006; Radosevich et al. 2007). Seed banks have been classified into three categories: transient -when no viable seeds persist for more than one year-, short-term persistent -when seeds persist no longer than five years- and long-term persistent -when seeds persist longer than five years- (Thompson et al. 1997). Persistent seed banks represent the main source of genetic variability, enabling a range of responses to environmental variability and buffering populations against changes in genetic composition that may occur after fluctuations in population size (Levin 1990; McCue and Holtsford 1998; Gioria and Pyšek 2016; Schulz et al. 2018). In addition, the ability to form a persistent seed bank of an alien species is considered an important attribute for the establishment and naturalization in new ranges (Pyšek et al. 2015; Gioria et al. 2019).

Seed persistence is influenced by seed traits (e.g. size, shape, dormancy, longevity) and environmental characteristics (e.g. temperature, humidity, disturbance) (Long et al. 2015). Among the seed characteristics, seed dormancy -defined as the failure of seeds to

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germinate under environmental conditions that are favorable for germination- is an important seed feature because it regulates the timing of germination so that the environmental conditions are favorable for seedling survival and the seed is preserved from accelerated ageing (Baskin and Baskin 2004; Finch-Savage and Leubner-Metzger 2006; Saatkamp et al. 2011; Long et al. 2015). However, dormancy is not strictly a condition for seed persistence because non-dormant seeds are also able to form persistence seed banks, e.g., buried seeds that have light requirements (Thompson et al. 2003; Ooi et al. 2007; Saatkamp et al. 2011). Also, seed size and shape can influence seed persistence, although not necessarily in the same direction in all environments (Thompson et al. 1993; Bekker et al. 1998; Funes et al. 1999; Moles et al. 2000). The environment after propagule arrival is crucial for the success of an introduction, e.g. the place where seeds are located may result in different patterns of emergence but also affect seed predation, which is one of the most important causes of seed loss in the soil once initial dispersal has taken place (Westerman et al. 2003). In this regard, human disturbance (e.g. agricultural machinery) can place the seed in an environment of more uniform temperature, humidity and less light exposure (i.e. buried seeds) than in one with small or no disturbance, where most seeds are in the top 10 mm of the soil profile (Benech-Arnold et al. 2000). Disturbed environments may protect the seeds and increase the probability of germination by reducing post-dispersal predation and ageing (Fenner and Thompson 2005). However, mortality between germination and seedling establishment is probably high, especially if the seeds are emerging from any depth (Zhang and Maun 1990; Chen and Maun 1999; Tobe et al. 2005).

Besides the ability to form persistent seed banks, there are several traits considered important for explaining invasiveness, such as early and rapid germination during the

growing season (Pyšek and Richardson 2007; Gioria and Pyšek 2017; Gioria et al. 2018), faster growth, and/or high seed production (van Kleunen et al. 2010; Godoy et al. 2012). Alien species may acquire these traits from preadaptation prior to introduction (van Kleunen et al. 2011), rapid evolution in novel environments (Oduor et al. 2016; Boheemen et al. 2019), and selection following hybridization between species/populations (Ellstrand et al. 2010; Ellstrand and Rieseberg 2016). Crop to wild hybridization can drive plant evolution, including seed persistence, in neighboring populations of wild relatives (Rieseberg et al. 1993; Whitton et al. 1997; Warwick et al. 2008; Snow et al. 2010). While invasive populations may have pre-adapted traits (Hernández et al. 2019), crop volunteers, defined as those plants deriving from unharvested seeds of crops, often shows maladaptive traits for persisting in nature, such as low seed dormancy, large seed size, and out-of-season germination (Ellstrand et al. 2010; Alexander et al. 2014; Hernández et al. 2017). In addition, these early life-history traits are mostly influenced by the maternal parent (Lemontey et al. 2000; Pace et al. 2015; Singh et al. 2017). So, in crop-wild hybrids, the fate of seed in the soil will probably depend on the maternal parent, the wild-like maternal parent (i.e. small seeds, strong dormancy) having greater likelihood to persist (Pace et al. 2015).

Wild *H. annuus* is an excellent model species for studying the role of seed banks in the invasion process because it is an invasive alien species, found in ruderal and agrestal habitats in several regions of the world, in some of which it interacts with the domesticated sunflower (Dry and Burdon 1986; Poverene et al. 2009; Ribeiro et al. 2010; Muller et al. 2011). In its native range, large post-dispersal disturbances resulted in greater seedling emergence and reproduction than small disturbances and there was a negative density-dependence in seedling mortality and reproduction (Moody-Weis and

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Alexander 2007). Seed persistence on the soil surface was much lower than if buried, probably due to strong seed predation on the soil surface (Alexander and Schrag 2003). In addition, early emerged seedlings had a greater probability of surviving to reproduction (Mercer et al. 2011). However, the ability of *H. annuus* to form persistent seed banks outside its native range has not been studied. The aim of the present study was to evaluate the role of the seed bank in invasive populations of *H. annuus* adapted to ruderal and agrestal habitats as well as in crop volunteers, under two different scenarios (with and without disturbance), and to determine the seed persistence in the soil. For these purposes, we evaluated five variables related to invasion success: cumulative emergence, survival to reproduction, survival of emerged seedlings, inflorescences per plant and inflorescences per plot in different sunflower biotypes, under disturbed and undisturbed conditions for two years. In an independent experiment, we also recorded seed germination, mortality and dormancy in different biotypes buried in the soil for 42 months. The information presented here is essential for better understanding the mechanisms behind the successful invasion of *H. annuus* in central Argentina, which could increase our ability to manage the invasion of this species in ruderal and agrestal habitats.

Materials and methods

Study system and site

Wild sunflower, *H. annuus*, is native to North America where it commonly grows in habitats that receive frequent disturbance, including roadsides, open areas in prairies, crops and crop margins (Heiser 1978). Wild *H. annuus* was introduced and has become invasive in several regions of the world, such as South America, Europe, Africa, Australia (Dry and Burdon 1986; Muller et al. 2009; Poverene et al. 2009; Ribeiro et al.

2010). The propagules (achenes) have non-deep physiological dormancy governed by the maternal pericarp and intrinsic hormone regulation. In addition, light stimulation increases germination in the wild sunflower but has no effect on the domesticated sunflower (Seiler 1998; Weiss et al. 2013; Presotto et al. 2014; Hernández et al. 2017). The study was conducted in an Entic Haplustoll soil (CIRN 1989) at the Agronomy Department, Universidad Nacional del Sur, Bahia Blanca, Argentina (S 38°41, W 62°14). The Agronomy Department is located in the Espinal eco-region, where several wild populations are established (Poverene et al. 2009). The predominant landscape is a dry, thorny forest, savanna and steppe (Burkart et al. 1999). The climate of Bahia Blanca is semiarid temperate (mean average temperature, 15°C; mean average precipitation, 651.4 mm (www.smn.gob.ar). The coolest month is July (mean average temperature, 7.9°C) and the hottest month is January (mean average temperature, 23.0°C) (www.smn.gob.ar).

Plant material

Eight biotypes of *H. annuus* were evaluated: four ruderal populations, one agrestal population and three sunflower crop volunteers. Ruderal and the agrestal populations were collected in central Argentina: Adolfo Alsina (AAL, S 37°16′, W 62° 59′), Barrow (BRW; S 38°16′, W 60°07′), Colonia Baron (BAR; S 34°47, W 68°15), Diamante (DIA; S 32°03′, W 60°38′) and Río Cuarto (RCU; S 33°09, W 64°20). The agrestal population (BRW) is considered an agrestal biotype because it has been growing in an agricultural field for at least 10 years (Casquero et al., 2013; Presotto et al., 2017) while the remaining populations are considered ruderals because they have been growing in patches in disturbed habitats, such as roadsides, ditches, and fence rows (Poverene et al. 2009). Ruderal biotypes were introduced from multiple wild populations from the

native range (Hernández et al. 2019) while the agrestal biotype is a natural crop-wild hybrid (Casquero et al. 2013; Presotto et al. 2017). In experiment 1 "seeding emergence and reproduction", we used four ruderal biotypes (AAL, BAR, DIA, RCU), the agrestal BRW and the volunteer biotype was the progeny of CAC CL cultivar. In experiment 2 "seed persistence in the soil", we used AAL, BAR, RCU and BRW and the volunteer biotypes were the progenies of the DK3880 CL and DK4000 CL cultivars.

To minimize the environmental effects and to explore the maximum primary seed dormancy, all the achenes (hereafter seeds) used for the experiments were produced in a common garden the previous season. The seeds of ruderal and agrestal biotypes were produced under controlled pollination of the heads of 20 – 30 plants covered with paper bags at the pre-flowering stage. At the flowering stage, the heads were pollinated with pollen of sibling plants, 3 – 4 times per head. On the other hand, heads of the cultivars were self-pollinated by covering the heads with paper bags at the R4 stage (Schneiter and Miller 1981) and their seed (F2 seeds) constituted the volunteer biotype.

Seedling emergence and reproduction

To simulate initial invasion of a biotype in a new environment, we established two levels of disturbance: disturbed and undisturbed, both under rainfed conditions. Seeds in the undisturbed habitat were distributed on the soil surface whereas in the disturbed habitat, the soil was carefully removed with a shovel (10 cm-deep) before seed distribution and then the soil was turned over. The design was completely randomized with four replicates of 620 seeds of each biotype (AAL, BAR, DIA, RCU, BRW, VOL) distributed in treatment plots (disturbed vs undisturbed) in the experiment field. The experimental field was located 50 m away from the common garden plot and it had not been cultivated for at least 10 years and it had never been cultivated with sunflower

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before. Seeds were sown once in autumn 2014, and the seedling emergence was
monitored for two years, in 0.5 m^2 (0.7 m x 0.7 m) plots with 1 m space (aisle) around
each plot to minimize uncontrolled movement of seeds between plots. The seed addition
density was 1242 seeds m^{-2} (620 seeds per plot), resembling a low plant density (0.25-
0.50 plants m^{-2}) in their natural environment (Poverene et al. 2009) and, a seed
production (~3000 seeds per plant) similar to that found under semiarid conditions
(Presotto et al. 2017). Seeds were dispersed in May 2014, in accordance with the
shattering period.

Pests (if present) were not controlled within the plots, only the aisles were maintained
clean. Seedlings were counted monthly during two growing cycles (2014 and 2015)
distinguishing between new seedlings and established seedlings/plants. This allowed us
to determine the cumulative emergence, as the sum of emerged seedlings; survival to
reproduction, as the number of plants that reached the reproductive stage; survival by
emerged seedlings, as the plants that reached the reproductive stage relative to the
emerged seedlings. At the end of each growing cycle, we counted the number of
inflorescences per plot, and we estimated the number of inflorescences per plant, as the
number of inflorescences per plot divided by the number of adult plants in that plot. To
avoid refilling the seed bank in the second year, the immature inflorescences of the first
cycle were removed.

Minimum and maximum temperatures and rainfall were recorded daily at a nearby
weather station (Davis Vantage Pro 2) at CCT-Bahía Blanca and the historical weather
data was obtained from the national weather service (www.smn.gob.ar).

Seed persistence in the soil

Seed germination, dormancy and mortality in the seed bank of several *H. annuus*
biotypes from Argentina (BAR, AAL, RCU, BRW, VOL1 and VOL2; details presented
in plant material section) were studied in a four-year trial.

Four repetitions of 50 seeds of each biotype, grown under controlled conditions in a
common garden, were buried at 10 cm in depth, in 0.10 x 0.15 m permeable
polyethylene bags (mesh = 21 x 42), in autumn 2010. The design was a randomized
complete block with four replicates, where each biotype (bag=replicate) was randomly
distributed in each block and the blocks were nested in each storage time. Each
exhumation period (storage time) was kept physically separated from each other in the
soil. At the beginning of the following four springs (6, 18, 30 and 42 months) the
samples (a set of 6 biotypes x 4 blocks x 1 replicate block^{-1} x 50 seeds replicate^{-1}) were
exhumed and washed with water. We also measured the germination, dormancy and
mortality of the seeds after harvest (time 0). The non-germinated seeds were placed in
plastic trays with moist paper at 20 °C, and neutral photoperiod (12 hours of light and
12 hours of darkness) (ISTA 2004) to discriminate between the viable and dead seeds,
and dormant and non-dormant in the viable seeds. The number of germinated seeds was
counted every three days for 17 days. Seeds were considered germinated when the
radicle length was ≥ 5 mm (Seiler 2010). At the end of each germination test, the
viability of non-germinated seeds was determined by the tetrazolium staining test (ISTA
2004). The pericarp and seed coat were removed manually, and the seeds were placed in
a 0.5% 2,3,5-trifenyl-tetrazolium chloride solution in the dark, at room temperature for
24 hours. Dormancy was estimated as the proportion of viable seeds (stained) that did
not germinate. The fraction of viable seeds in each period was obtained from the sum of

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the germinated and dormant seeds (non-germinated but stained with tetrazolium). The

seeds were classified into the following categories (1) dead seeds -seeds that germinated

prior to the exhumation or were degraded by the action of microorganisms within the

soil and seeds with embryos that showed no evidence of enzymatic function with

tetrazolium testing-; (2) non-dormant seeds -germinated in the laboratory at 20 °C

during 17 days-; (3) dormant -stained seeds that did not germinate in the laboratory-.

Under both field and laboratory conditions a completely randomized block design was

used, with four replicates.

Statistical analysis

I. Seeding emergence and reproduction

We used a general linear model (GLM) with PROC GLM in SAS (SAS University

Edition, SAS Institute Inc., Cary, NC, USA) to evaluate differences in five response

variables (cumulative emergence, survival to reproduction, survival by emerged

seedlings, inflorescences per plant and inflorescences per plot) between the sunflower

biotypes (AAL, BAR, DIA, RCU, BRW, VOL) under two disturbance treatments

(disturbed vs. undisturbed) in two growing seasons (year 1 and year 2). All response

variables were square-root transformed to improve homoscedasticity, but data are

shown with back-transformed least squares means and 95% confidence intervals (in

brackets). The effects were year, disturbance, biotype and the interactions, and all the

factors were considered fixed. We also performed analyses of covariance (ANCOVA)

within each year with PROC GLM in SAS (SAS University Edition, SAS Institute Inc.,

Cary, NC, USA) to test whether the differences observed in survival to reproduction and

inflorescences per plot were explained by differences in earlier life-history traits

(cumulative emergence and survival to reproduction, respectively). The coefficient of

determination (R^2) was used to compare models with and without covariates. If a covariate is mediating the main response, then the inclusion of the covariate will reduce the sum of squares explained by the main effect. For survival to reproduction, disturbance, biotype and their interactions were considered as fixed, whereas cumulative emergence was included as a covariate. For inflorescences per plot, disturbance, biotype and their interactions were considered as fixed, whereas survival to reproduction was included as a covariate.

II. Seed persistence in the soil

To investigate the effects of soil storage time on the germinated, dormant, and dead seeds in six sunflower biotypes (AAL, BAR, RCU, BRW, VOL1 and VOL2) we used a generalized linear mixed model (GLMM) with PROC GLIMMIX to perform a maximum-likelihood analysis with a multinomial response distribution. Seeds from each bag in the three seed categories -germinated seed, dormant seed or dead seed- were analyzed jointly, based on their frequencies in the bag. The biotype, storage time and the interaction between biotype and storage time effects were considered as fixed effects, whereas block nested in time was a random effect. As the volunteer biotypes (VOL1 and VOL2) showed 100% seed germinated after harvest (time 0), which probably inflated the interaction between biotype and storage time, we ran the analysis with and without the volunteer biotypes.

Because we also found a significant interaction between biotype and storage time without volunteer biotypes, we used generalized linear mixed models (GLMM) based on a restricted maximum likelihood estimation (REML) with PROC GLIMMIX to evaluate the differences between the sunflower biotypes (AAL, BAR, RCU and BRW) in the non-dormant, dormant, and dead seeds at each storage time (0, 6, 18, 30 and 42

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months). We evaluated the three variables using a binomial distribution: the proportion of viable (non-dormant plus dormant seeds) over total (viable and dead seeds) seeds, the proportion of dormant over viable seeds, and the proportion of dormant over total seeds. Within each time, biotypes were compared using a Tukey–Kramer adjustment for multiple comparisons. The block*biotype interaction was used as an error to test the biotype. Biotype was considered as a fixed effect, whereas block and the block*biotype interaction were considered as random effects.

Results

Seedling emergence and reproduction

In the first year, the monthly temperatures were within the historical weather averages, but wetter, especially during autumn, winter and spring (Table S1). In the second year, the monthly temperatures were also similar to the historical weather averages, except for May which was hotter, and September and October that were cooler. In addition, year 2 was drier than the historical weather, especially during winter and spring (Table S1). In both years, freezing temperatures were concentrated in the late autumn-early winter (June-middle of July) plus a couple of days during August and September and in the second year, there were also freezing days during early spring (Fig. S1). Surprisingly, emergence was mostly concentrated in winter (July and August) in both years and in both habitats (Fig. 1).

Under disturbance, the total cumulative emergence (year 1 and year 2) was 27.3%, on average, mostly concentrated during the first year; whereas in the undisturbed habitat, the total cumulative emergence was 3.7%, on average, and more balanced between the years (Table 1). In year 1, the seed bank emergence ranged between 116.6 [95% CI: 51.9, 207.3] seeds in agrestal BRW and 200.2 [126.1, 291.3] seeds in ruderal AAL in

the disturbed habitat, while being 1.2 [0.1, 5.9] in the volunteer and 24.3 [10.5, 43.9] in ruderal BAR in the undisturbed habitat (Fig. 1). In year 2, the seed bank emergence ranged between 0.6 [0.7, 6.1] seeds in the volunteer and 12.0 [5.5, 21.0] seeds in ruderal RCU in the disturbed habitat, while being 0.72 [0.01, 3.2] in the volunteer and 16.2 [12.0, 21.0] in ruderal RCU in the undisturbed habitat (Fig. 1).

In all traits, except for inflorescences per plant, we observed significant main effects of the year, disturbance and biotype (Table S3), and a significant year*disturbance interaction (Table S3). We observed no significant biotype interaction effects in any trait (Table S3) with only one exception (the year*biotype interaction in survival by emerged seedlings; Table S3). In general, in year 1, disturbance increased the emergence, survival to reproduction and inflorescences per plot, whereas the proportion of survival by emerged seedlings decreased. In year 2, emergence, survival to reproduction, and inflorescences per plot were lower compared to year 1, whereas the proportion of survival by emerged seedlings was higher (Fig. 2). To unravel the strong year*disturbance interaction and to explore biotype differences within each year, we performed GLMs for years 1 and 2, which are shown in Fig. 2.

In year 1, emerged seedlings varied, on average, from 11.9 [7.5, 17.2] plants in the undisturbed habitat to 155.5 [133.7, 178.9] under disturbance. We also observed significant biotype effect and biotype*disturbance interaction on emergence (Fig. 2); most of the biotypes showed high values for emergence under disturbance (Table 1), but in the undisturbed habitat, emergence was dramatically affected, especially in the volunteer and agrestal BRW biotypes (Table S2; Fig. 2). Survival to reproduction was significantly affected by disturbance and varied, on average, from 5.1 [2.9, 8.0] to 15.4 [11.0, 20.7] plants per plot, in the undisturbed and disturbed habitats, respectively. We

also found a biotype effect, but the biotype*disturbance interaction was not significant (Fig 2). Under disturbance, survival to reproduction of the wild biotypes varied from 14.1 [6.1, 25.3] in ruderal AAL to 25.3 [3.2, 68.5] in ruderal BAR but it was lower in the volunteer: 5.5 [1.4, 12.2] reproductive plants plot^{-1} (Table S2). Similarly, in the undisturbed habitat, survival to reproduction of wild biotypes varied from 4.8 [0.5, 13.5] in ruderal AAL to 14.2 [4.2, 30.4] in ruderal RCU, was lower in the volunteer: 0.4 [0.3, 3.0] reproductive plants plot^{-1} and intermediate in agrestal BRW: 1.6 [0.1, 8.4] reproductive plants plot^{-1} (Table S2).

Survival by emerged seedlings showed a significant disturbance effect, but the biotype effect and biotype*disturbance interaction were not significant (Fig 2). Even though the survival to reproduction was significantly higher in the disturbed habitat than in the undisturbed, the survival by emerged seedlings was higher in the undisturbed habitat (Fig. 2). This reduced the differences observed in emergence between the disturbance treatments, i.e. emergence in disturbed habitats was 10.8-fold times higher than in undisturbed habitats, but survival to reproduction was only 2.5-fold times higher than for undisturbed habitats (Table 1). Finally, we observed significant main effects of the biotype and disturbance on inflorescences per plot, but no significant biotype*disturbance interaction (Fig. 2). Under disturbance, the inflorescences per plot varied from 26.0 [6.9, 57.3] in the volunteer to 98.0 [38.3, 185.2] in ruderal BAR, whereas in the undisturbed habitat, it varied from 2.7 [2.0, 22.1] in the volunteer to 84.6 [57.4, 117.2] in ruderal DIA. The largest decrease in inflorescences per plot was observed in agrestal BRW and volunteer biotypes (Fig. 2).

In year 2, there were significant biotype effects in all the traits (Fig. 2), but no disturbance effect or biotype*disturbance interaction (Fig. 2). Ruderal biotypes (AAL,

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BAR, DIA and RCU) showed higher values in all traits than the agrestal BRW and

volunteer biotypes (Fig. 2). On average, cumulative emergence and survival to

reproduction varied from 0.7 [0.03, 2.2] and 0.25 [0.0, 0.9] plants in the volunteer to

14.3 [11.2, 17.8] and 8.4 [6.6, 10.4] plants in ruderal RCU, respectively (Fig. 2).

Inflorescences per plot varied from 0.7 [0.0, 2.7] in volunteer to 44.5 [32.4, 58.5] in

ruderal RCU (Fig. 2).

Finally, ANCOVA was used to test whether differences observed in survival to

reproduction and inflorescences per plot were explained by differences in earlier life-

history traits. In year 1, when cumulative emergence was included as a covariate, it did

not explain the differences in survival to reproduction (F= 0.83; P=0.37). However,

increased survival to reproduction observed under disturbance explained most of the

variation observed in inflorescences per plot (covariate explained ~88% of the total

variation; F=50.5; P<0.0001). In year 2, survival to reproduction was mostly explained

by differences in the cumulative emergence (covariate explained ~92% of the total

variation; F=84.3; P<0.0001), whereas the former variable explained most of the

variation in inflorescences per plot (covariate explained ~94% of the total variation;

F=28.4; P<0.0001).

Seed persistence in the soil

Multinomial analysis of non-dormant, dormant and dead seeds in the six sunflower

biotypes (AAL, BAR, RCU, BRW, VOL1 and VOL2) revealed a significant biotype

effect (F=41.74; P<0.0001) and biotype*storage time interaction (F=30.86; P<0.0001),

but storage time was not significant (F=2.29; P=0.107). To better understand this

interaction and because all the volunteer seeds germinated or died within six months of

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storage in the soil (Fig. 3a and b), we re-ran the analysis without the VOL biotypes. In the second analysis, we found significant biotype (F=63.03; P<0.0001), storage time (F=125.37; P<0.0001) and biotype*storage time (F=43.06; P<0.0001) effects. Due to the significant biotype*storage time interaction, we compared BAR, AAL, RCU and BRW biotypes within each storage time using the three binomial models (the proportion of viable over total seeds, the proportion of dormant over viable seeds and the proportion of dormant over total seeds).

Dormant seeds varied, on average, between 48% at the beginning and 10% at the end of the experiment (42 months) (Fig. 3). The three binomial models revealed significant biotype effects in all the storage times, except for 42 months (Table S4). At the beginning of the experiment, the dormant fraction was significantly different between the biotypes (Fig. 3a), ruderal BAR had the highest value of dormant seeds (76%), whereas agrestal BRW had the lowest (20%). Ruderals AAL and RCU biotypes showed intermediate values (51% and 43%, respectively) (Fig. 3a). After 18 months of soil storage, the viable seeds varied from 40% to 52% in the ruderal populations (AAL, BAR and RCU) but they were much lower in the agrestal biotype (BRW; 14 %). Of these viable seeds, the dormant fraction varied from 22% in ruderal AAL to 55% in ruderal BAR (Fig. 3c). At the end of the experiment (42 months), 26%, 19%, 14% and 8% of seeds remained viable in ruderal AAL, RCU and BAR and agrestal BRW biotypes, respectively. At the same time, 20% of viable seeds in ruderal AAL and between 6% and 9% of viable seeds in ruderal BAR and RCU and agrestal BRW biotypes were still dormant (Fig. 3e).

Discussion

Here, we evaluated the seed emergence and reproduction, and seed persistence in the soil, in several sunflower biotypes (ruderal, agrestal and volunteer). We found that the seed emergence was early, mostly concentrated in July and August (winter), especially during the first year. Seed bank emergence was highly promoted by disturbance, being at least 10 times higher than the average emergence in the undisturbed habitat. In spite of the dramatic effect of disturbance on plant emergence and survival to reproduction, the inflorescences per plot (our fitness proxy) was similar under both conditions and in most of the biotypes, showing that phenotypic plasticity can largely compensate for lower emergence, at least in ruderal biotypes. In the seed-burial experiment, we found that ruderal and agrestal seeds can persist for up to 42 months in the soil, forming persistent seed banks. On the other hand, all the volunteer seeds died after 6 months in the soil; this lack of ability to form persistent seed banks could be the main constraint to forming self-perpetuating populations of volunteer sunflower and it highlights the importance of crop-wild hybridization in escaped cultivated varieties (ferality) observed in Argentina (Casquero et al., 2013) and in southern Europe (Muller et al. 2011). We also observed a significant effect of genetic background on plant and seed dynamics. The agrestal BRW (a natural crop-wild hybrid), in general, showed intermediate behavior between the ruderal biotypes and the volunteer in both experiments, which agrees with our previous findings (Casquero et al., 2013; Presotto et al., 2017).

Early emergence strategy

Despite that freezing days may have affected plant survival, especially under disturbance, early emergence (July-August) was the rule in all biotypes and in both habitats, suggesting an adaptive importance of this trait. Early emerged plants may

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experience reduced competition and may accumulate more biomass, increasing the

probability to survive and reproduce (Verdú and Traveset 2005; Mercer et al. 2011).

This strategy has generally been found in invasive species, which may benefit from

reduced competition and the possibility of using resources before other species, even

with detrimental effects on the establishment (Gioria and Pyšek 2017). We recently

demonstrated that invasive sunflower presents higher seed germination than the native

sunflower, probably in response to the warmer environments found in Argentina,

especially due to the warmer winters found there (Hernández et al. 2019). Therefore, it

is possible that this milder winter in Argentina may have allowed germination before

the onset of the spring. In addition, these early emergence peaks have probably faced

strong directional selection (Mercer et al. 2011) after introduction which would account

for the tolerance to freezing during the early stages found in invasive populations

(Hernández 2019). Also, in both years, emergence after October (mid spring) was

negligible (Fig. 1), possibly due to the induction of secondary dormancy by higher

temperatures (Corbineau et al. 1990; Presotto et al. 2014; Dominguez et al. 2016;

Hernández et al. 2017). Low seed germination under high temperatures has been

observed in seeds from native and invasive sunflower (Hernández et al., 2019),

suggesting that this mechanism to avoid summer germination/emergence of invasive

populations is a pre-adapted trait.

Seed emergence increase with disturbance

Seed emergence was strongly modified by disturbance, being similar to the behavior of

native populations (Moody-Weis and Alexander 2007), suggesting that invasive

populations from Argentina are pre-adapted to disturbance. This was evident in the first

year but also in the second year, because the habitats were similar when disturbance was

not recent. In the undisturbed habitat, a low proportion of the seed bank emerged (<4%), whereas under disturbance this proportion increased to ~20%. In undisturbed habitats, seeds may suffer from greater predation that could limit establishment in suitable habitats (Alexander and Schrag 2003; Chauhan et al. 2006; Kröel-Dulay et al. 2019). In our conditions, birds were possibly the main seed predators (Presotto et al. 2016) and they were also seen on the plots after the initiation of the experiment (first author´s observation). Previous studies have found differential sunflower predation associated with seed size (i.e. larger seeds were eaten preferentially) (Alexander et al., 2001; Presotto et al., 2016) and/or oil content (i.e. seed with more oil content were eaten preferentially) (Dechaine et al. 2010). Interestingly, the biotypes with less cumulative emergence, in the undisturbed habitat, were BRW and the volunteer (Fig. 2; Table S2); both have larger seeds and higher oil content than ruderal populations (Casquero and Cantamutto 2016).

Persistence of buried seeds

Seed burial can ameliorate environmental effects and increase seed persistence (Thompson et al. 1998; Wijayratne and Pyke 2012). We found that ruderal and agrestal seeds can persist in the soil for at least 42 months, but volunteer seeds died after 6 months. Depending on the biotype, between 8 and 26% of seeds remained viable in the soil at the end of the experiment whereas 6 to 20% remained dormant. Similar results were reported in the native environment (Alexander and Schrag 2003), where at least 16% of the seeds remained viable after 3 years in the soil. Previous results have also shown that light stimulation increased germination in the wild populations but not in the crop (Presotto et al. 2014). Hence, the lack of this stimulus is probably preserving non-dormant seeds in the seed bank. Surprisingly, the agrestal BRW showed viable and

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dormant seeds at the end of the experiment, indicating that crop-wild hybrids have substantial phenotypic variation in seed traits on which selection can act. On the other hand, if the seeds are deposited on the soil surface, seed survival would be much lower due to high temperature and humidity fluctuations (Alexander and Schrag 2003; Wijayratne and Pyke 2012). The increased performance of ruderal and agrestal vs. volunteer sunflower in the seed bank suggests that pre-adaptation to disturbed habitats has probably contributed to the successful naturalization of wild *H. annuus* in Argentina.

In the seed-burial experiment, we also observed genetic differences in dormancy levels, which were consistent with those observed previously, under controlled conditions (Presotto et al. 2014; Hernández et al. 2019). We could expect that genetic differences in dormancy might result in differences in the timing of emergence and/or the number of emerged seeds at the onset of the spring (i.e. the lower the seed dormancy the earlier the emergence and/or the higher the number of emerged plants), however we observed no relationship between seed dormancy (measured in the seed-burial experiment) and the timing of emergence or the number of emerged plants (both from the seed-bank experiment). Indeed, all biotypes (including the non-dormant volunteer) showed similar emergence patterns in both years. Germination/emergence timing is strongly influenced by the interaction between the genetic and environmental factors (especially temperature) during pre- and post-dispersal (Chiang et al. 2011; Burghardt et al. 2016), it being possible that these interactions (not evaluated here) explain the absence of a relationship between dormancy and timing of emergence. On the other hand, seed dormancy and light stimulation of germination observed in the wild populations (Presotto et al. 2014), probably contributed to the persistence of viable seeds in the seed

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bank (Gioria and Pyšek 2017). Further experiments, manipulating the degree of seed dormancy, light stimulation together with controlled ageing tests are needed to better understand the role of these factors on seed persistence in the soil.

Similar fitness in both habitats

Although seedling emergence increased under disturbance, the inflorescences per plot were similar in both habitats, which can be explained by density-dependent processes (Mercer et al. 2014) and the high plasticity of wild sunflower, expressed as the number of inflorescences per plant (Alexander and Schrag 2003; McSteen and Leyser, 2005). Inflorescences per plant is highly correlated with branching, one of the traits with a dramatic morphological change due to domestication (Burke et al. 2005; Wills and Burke 2007). Branching is a plastic trait that can respond to external factors (i.e. intra-specific competition) (McSteen and Leyser 2005), modifying the number of branches and consequently, the number of inflorescences. In fact, our results showed that when the number of adult plants was low (i.e. in the undisturbed habitat, in the first year), the number of inflorescences per plant increased. However, this was not the case in the volunteer and BRW, which were severely affected when the number of established plants was reduced. It is possible that the adaptation of BRW to agricultural environments has reduced its phenotypic plasticity as a result of growth-stress tolerance trade-offs (Casquero et al., 2013; Presotto et al., 2017).

Overall, our data showed early seed emergence, mostly concentrated during winter, which played a key role in the establishment and fecundity of the wild sunflower biotypes. Seed bank emergence was highly promoted by disturbance, but the fitness was similar under both conditions and especially in ruderal biotypes, indicating that phenotypic plasticity can largely compensate the lower number of established plants. In

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the seed-burial experiment, ruderal and agrestal seeds persisted at least for 42 months in

the soil while all the volunteer seeds died after 6 months in the soil. The agrestal BRW

(a natural crop-wild hybrid) showed intermediate behavior between the ruderal biotypes

and the volunteer in both experiments. These results highlight the ability of wild *H.*

annuus to form persistent seed banks outside its native range which may explain their

successful naturalization in several regions of the world. In addition, our findings

reinforce the key role of anthropogenic activities -e.g. soil disturbance with agricultural

machines- promoting the establishment and spread of wild *H. annuus*. Furthermore, this

study provides information that could help to develop strategies for the management

and control of invasive *H. annuus*, which clearly should include long-term practices.

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Table 1: Back-transformed least square means and 95% confidence intervals of our five response variables under two disturbance treatments (disturbed vs. undisturbed) in two growing seasons (year 1 and year 2). Mean values of each biotype are shown in Table S2.

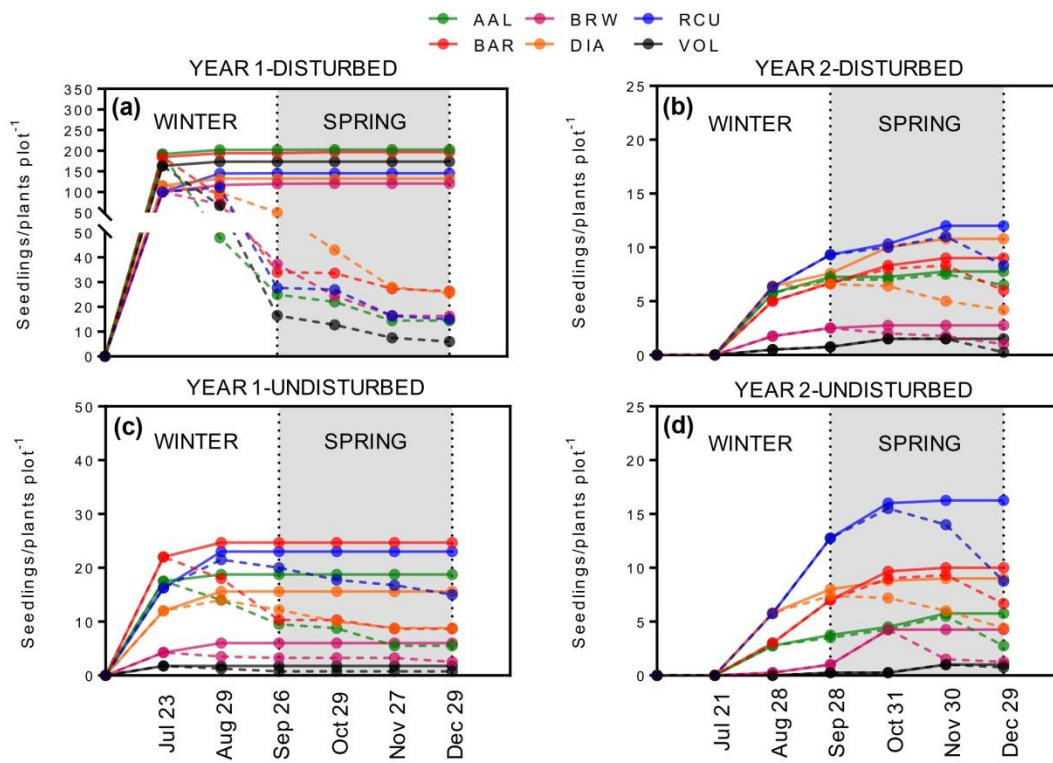| Year	Disturbance	Cumulative emergence (seedlings plot^{-1})	Survival to reproduction (reproductive plants plot^{-1})	Survival by emerged seedling	Inflorescences plot^{-1}	Inflorescences plant^{-1}
1	Disturbed	155.5 [133.7, 178.9]	15.4 [11.0, 20.7]	0.11 [0.07, 0.15]	65.6 [50.4, 82.8]	4.4 [3.8, 5.1]
	Undisturbed	11.9 [7.5, 17.2]	5.1 [2.9, 8.0]	0.38 [0.24, 0.55]	37.8 [22.1, 57.6]	6.3 [3.9, 9.2]
2	Disturbed	5.6 [3.3, 8.5]	2.8 [1.4, 4.7]	0.33 [0.18, 0.53]	16.7 [8.8, 27.1]	3.8 [2.0, 6.0]
	Undisturbed	5.7 [3.2, 8.9]	3.1 [1.8, 4.6]	0.46 [0.31, 0.65]	17.7 [10.2, 27.2]	4.5 [2.7, 6.7]

Figure 1: Monthly emergence of seedlings (solid lines) and survival plants (dashed lines) of six sunflower biotypes under disturbed (**a** and **b**) and undisturbed (**c** and **d**) habitats, during year 1 (**a** and **c**) and year 2 (**b** and **d**). Sunflower biotypes: ruderals AAL, BAR, DIA, RCU, the agrestal BRW and the volunteer VOL. Note the variation in the scale of the y-axis in **a** to show large differences between emerged and survived plants and also the variation in the scale of the y-axes between **a** and **b** and **c** and **d**.

Figure 2: Effect of disturbance, in six biotypes, on cumulative emergence, survival to reproduction, survival by emerged seedlings and inflorescences per plot for two years. Least square means (square-root transformed data) and standard errors are shown. F and *P* values by year and trait for biotype (B), disturbance (D) and biotype by disturbance interaction (B*D) are shown on the upper part of each chart. Sunflower biotypes: ruderals AAL, BAR, DIA, RCU, the agrestal BRW and the volunteer (VOL).

Figure 3: Dynamics of non-dormant, dormant and dead seed of six sunflower biotypes for different storage times in the soil. **a**: after harvest; **b**: 6 months; **c**: 18 months; **d**: 30 months; **e**: 42 months. Sunflower biotypes: ruderals AAL, BAR, and RCU, the agrestal BRW and the volunteers (VOL1 and VOL2).
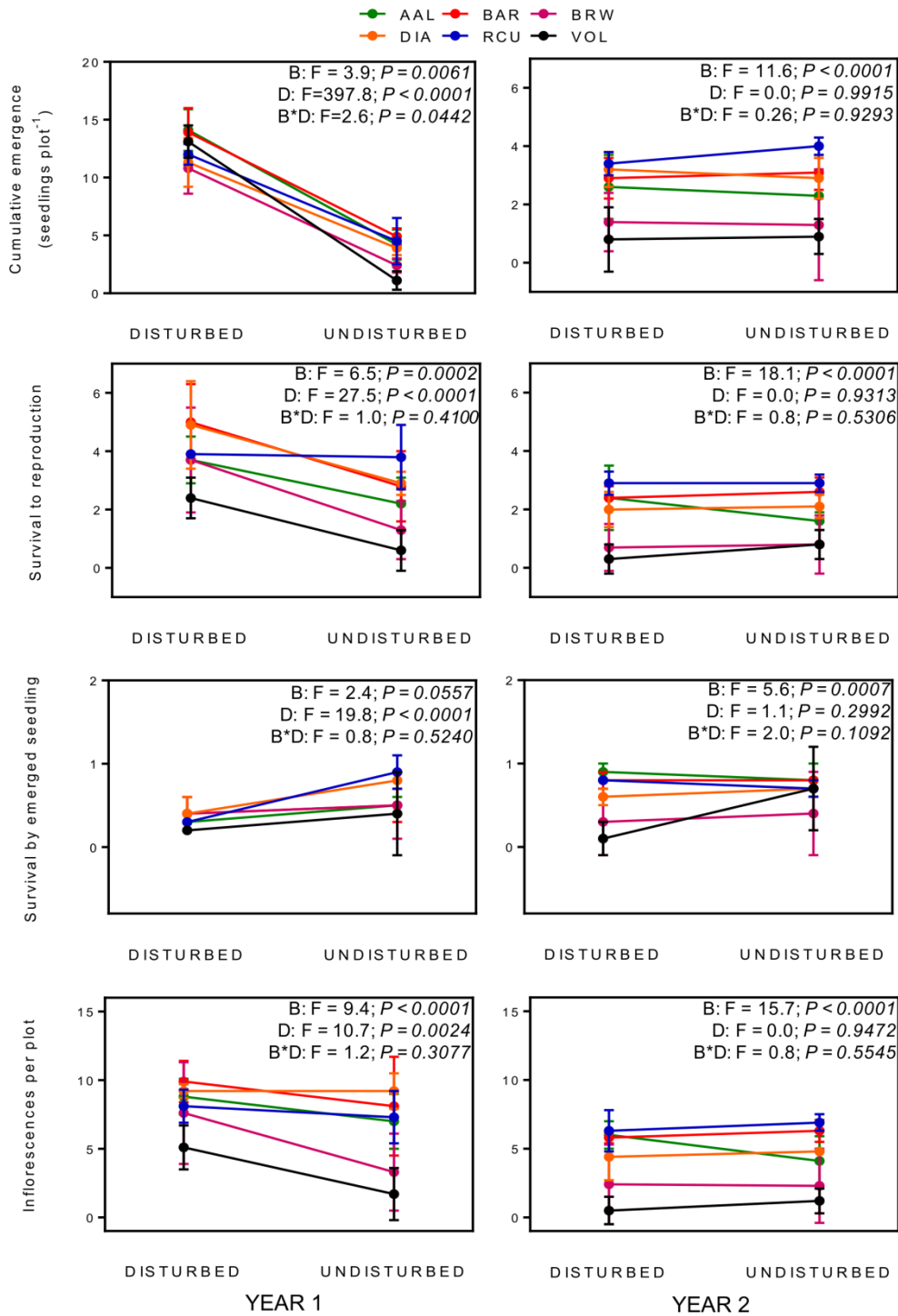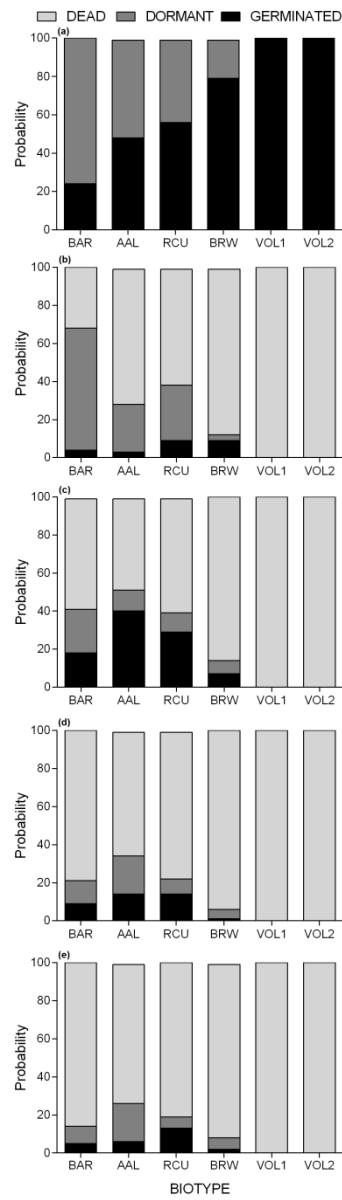
Figure 1

Figure 2

Figure 3