Phenotypic characterization of *Helianthus annuus* and *H. petiolaris* agrestal biotypes

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ABSTRACT

- Both wild *H. annuus* (ANN) and *H. petiolaris* (PET) are annual exotic plant invaders of the natural flora of Argentina. They usually grow in uncultivated habitats as ruderal species, but they are also present in uncultivated lands as agrestal weeds. The differentiation between agrestal and ruderal biotypes is of extreme importance, because agrestal biotypes might limit the adoption certain herbicides resistance, since gene flow could promote the occurrence of herbicide resistant weeds. The objective of this experiment was to detect phenotypic differences between *Helianthus* biotypes, which could be useful to detect them in the first invasive steps.
- We characterized four agrestal PET and two agrestal ANN biotypes collected in Argentina, growing as weeds under natural conditions in the agroecosystems. Five PET and six ANN biotypes classified as ruderal in a common garden were used as controls. At R6 stage plants were characterized by 24 metric and 14 categoric traits.
- Agrestal ANN biotypes had fewer leaves and heads per plant than ruderal ANN biotypes. These genotypes also showed wider heads and larger leave petioles, more and bigger bracts (phyllaries), more and wider rays flowers and reached the R1 stage 13 days before ruderal ANN biotypes. Agrestal PET showed longer petioles, more anthocyanin content and narrower phyllaries than ruderal biotypes. Although the agrestal PET biotype reached earlier the R1 stage, the life cycle duration did not differ from ruderal PET biotypes. In both annual *Helianthus* the agrestal biotypes produced greater seed number per head than the ruderal ones; however, they produced low seed number per plant because of lesser branching. While both PET biotypes did not differ in seed biomass, the seeds of agrestal ANN were twice heavier than those of ruderal ANN biotypes.
- Agrestal biotypes had overall bigger reproductive structures than ruderal biotypes. Main head predominance and determinate habit growth of ruderal ANN biotopes suggested sunflower crop introgression. It is noteworthy that agrestal PET biotypes, once reached the reproductive stage, fast produced a first cohort of seeds, and after this continued their reproductive process with a slow develop of several secondary heads. This reproductive mechanism would perpetuate the species for the following seasons, even under adverse conditions, avoiding a large period of seeds production.
- This findings alert about the possible diffusion of annual *Helianthus* agrestal biotypes in Argentina. Due to their difficult morphological differentiation from ruderal biotypes , it is extremely important to raise awareness among producers about the emerging problem to prevent their development and subsequent invasion.

Key words: Invasive, ruderal, agrestal, sunflower

INTRODUCTION

The genus *Helianthus* belongs to the family Asteraceae, is native to North America, where more than 50 species including annuals and perennials, are adapted to various natural and agricultural habitats. Some of them are classified as crop weeds (Seiler and Rieseberg, 1997).

Heiser (1978) recognizes the existence of at least four subspecies that would interfere with crops, *H. annuus* ssp *jaegeri*, *H. annuus* ssp *lenticularis*, *H. annuus* ssp *texanus* and *H. annuus* ssp *annuus*. This author also indicates that the morphological differentiation between them is very complex. The genus also include other six species considered as weeds in 12 states of North America continent (USDA, 2011).

Two species of this genus are included in the Argentina flora, *H. annuus* and *H. petiolaris* (Zuloaga and Morrone, 1999). Both are annual, recently naturalized and behave as invaders. The most extended populations are widespread in Buenos Aires, La Pampa, Córdoba, San Luis, Mendoza, Entre Rios, and San Juan provinces (Poverene et al., 2002). Argentine wild *Helianthus* merit interest because their adaptation covers a wide range of agro-ecological conditions (Cantamutto et al., 2008). The geographic distribution of both invasive species overlaps with the main summer crop regions in Argentina.

However they usually grow in uncultivated habitats as ruderal species, they are also present in cultivated lands, as agrestal weeds. The phenotypic differentiation between both biotypes is of extreme importance, due to the risk emerged from the adoption of new biotechnologies, as genetic resistance to herbicide. Agrestal biotypes limit the adoption of this technology because gene flow could promote the occurrence of herbicide resistant weeds (Poverene et al., 2004).

Agrestal *H. annuus* has been identified as an important weed in many crops (Gillespie and Miller, 1984; Geier et al., 1996; Villaseñor and Espinosa, 1998; Allen et al., 2000; Rosales-Robles et al., 2002; Deines et al., 2004; Mesbah et al., 2004). The agrestal biotype related with IMI sunflower might not be controlled by imidazolinone herbicides (IMI) and so causing economic crop losses. The prevention of agrestal biotype invasions could be facilitated if it were easily differenced by phenotypic traits.

The objective of this experiment was to detect phenotypic differences between *Helianthus* biotypes, which could be useful to detect them in the first invasive steps.

MATERIALS AND METHODS

In a common garden study we characterized four agrestal PET and two agrestal ANN collected in Argentina under natural agroecosystem conditions. Five PET and six ANN classified as ruderal were used as controls (Table 1). Agrestal biotypes were collected within crop fields, behaving as weeds. Meanwhile ruderal biotypes were collected outside crop fields, established in road sides.

Tabla 1: Collection data of *Helianthus* biotypes characterized in a common garden study.

Acronym	Origin[1]	Recollection date	Observations
			RUDERAL *Helianthus petiolaris*
RIV	Rivera (LP)	2009	Wild population, without history of crop gene flow.
TRE	Cuero de Zorro (BA)	2009	Population on an area studied by possible sunflower crop introgression.
SRO	Santa Rosa (LP)	2009	Population growing on a Hwy 35 shoulder, far of crop sunflower fields.
LON-R	Lonquimay (LP)	2010	Plants outside a sunflower crop field
PETDA	DA-UNS	2010	Plants with the UNS campus forest, possibly dispersed by birds.
			AGRESTAL *Helianthus petiolaris*
LON-A	Lonquimay (LP)	2010	Population in a rangeland intensively invaded
PAS	Catriló (LP)	2010	Invasive plants within a soybean field
PAG	Rivera (LP)	2010	Invasive population within a sunflower crop, cultivated on the shoulder.
RELF	Relmó (LP)	2010	Invasive population within a forest. The oldest invasive process in Argentina
			RUDERAL *Helianthus annuus*
RCU	Río Cuarto (COR)	2009	Big and stable population next to the Air club.
AAL	Puan (BA)	2009	Stable population next to the drain channel of Maltería Puán
TOL	Toledo (COR)		Small population, with old records of herbarium.
BAR-R	Colonia Barón (LP)	2009	Dense population. On the road perpendicular Hwy 10.
AWDA	DA-UNS	2010	Plants within the UNS campus forest, possibly dispersed by birds.
ACDA	DA-UNS	2010	Plants within the UNS campus forest, possibly dispersed by birds.
			AGRESTAL *Helianthus annuus*
BRW	Barrow (BA)	2009	Invasive population within a sunflower crop.
BAR-A	Colonia Barón (LP)	2009	Invasive population within a sunflower crop.

[1] LP= La Pampa, BA= Buenos Aires, COR= Córdoba, DA-UNS= Departamento de Agronomía UNS

The evaluation was performed at the Agronomy Department (UNS), Universidad Nacional del Sur, Argentina, experimental field (38°41'46"S, 62°14'55"W) during 2010-11 summer. Before sowing, seeds were maintaining over a wet paper at 5 ºC during one week to overcome dormancy. Seeds were sown in 28 x 54 cm 100 cell plastic trays containing commercial substrate and grown 30 days in the greenhouse under natural light at 20-25 °C.

In V4 stage (Schneiter and Miller, 1981), plants were transplanted to the experimental field under 2 x 0.25 m arrangement. At R6 stage plants were characterized by 24 quantitative and 14 qualitative traits (Table 2). At R9 stage, a sample of 20 to 111 heads per biotype was harvested and air dried. I was determined head diameter, grain number and seed weight per head. The number of seeds per plant was estimated multiplying the number of seeds per head by the number of heads per plant.

Field was hand weeded and water requirements were supplied by drip irrigation. It was used a complete randomized design, with 10 repetitions. Data were analyzed by ANOVA and means were compared using the LSD Fisher test ($p < 0.05$).

Table 2: Descriptors used for characterization *Helianthus* biotypes in a common garden. Adapted from Presotto et al. (2009).

Abbreviation	Descriptors (category, units)	Source	Type
	Plant Characters		
RAMI	Branching type (no branching, apical branching, full branching)	A	At
ALT	Plant height (cm)	A and M	Q
DIAMTA	Stem diameter at mid-height (cm)	M	Q
CAPRIN	Presence of main head (presence, absence)	A	At
NUCAP	Number of head (n)	A	Q
ANTALL	Anthocyanin in stem and petioles (presence, absence)	A	At
	Leaf Characters		
ANHOJ	Leaf width (cm)	M	Q
LARHOJ	Leaf length (cm)	A	Q
TAMHOJ	Leaf size (width x length, cm^2)	A	Q
LARPEC	Petiole length (cm)	A	Q
IANLAR	Width/length index (ratio)	A	Q
INLAM	Leaf blade/petiole index (ratio)	A	Q
BAHOJ	Leaf base (cuneate, cordate)	A	At
FORHOJ	Leaf shape (cordate, oblate, triangular, lance, round-shape)	A	At
SUHOJ	Leaf surface (flat, waxy, curled)	A	At
MAHOJ	Leaf margin (smooth, serrate, deeply serrate)	A	At
NUHOJ	Leaf number (n)	M	Q
DISPHOJ	Leaf arrangement (opposite, alternate)	A	At
HOJCAP	Leaves on back of head (presence, absence)	A	At
	Disk characters		
NUFLIG	Number of ray flower (n)	A	Q
ANFLIG	Ray width (cm)	A	Q
LARFLIG	Ray length (cm)	A	Q
PIGFLIG	Ray flower color (other than yellow)	A	At
NUFIL	Phyllary (bract) number (n)	A	Q
DISPFIL	Phyllary disposition (appressed, loose)	A	At
PUFIL	Phyllary tip (acute, acuminate)	A	At
LARFIL	Phyllary length (cm)	A	Q
ANFIL	Phyllary width (cm)	A	Q
RLARAN	Phyllary length/width (ratio)	A	Q
CODIS	Disk flower color (yellow, red)	A	At
PEBCAP	Disk white hair (presence or absence)	A	At
DIAMCAP	Head diameter (cm)	M	Q
NSPH	Number of seeds per head (n)	A	Q
NSPP	Number of seeds per plant (n)	A	Q
SBIO	Seed biomass (mg)	M	Q
	Life cycle characters		
DR1	Days from R1 stage (days)	M	Q
DR6	Days from R6 stage (days)	M	Q
DEND	Days from end of cycle (days)	M	Q

[1]M: descriptors used in INTA Manfredi Active Germplasm Bank; A: descriptors used in GRIN-Germplasm Resources Information Network (USDA 2007). [2]At=Attribute; Q=Quantitative.

RESULT AND DISCUSSION

Although ANN biotypes were similar in height, stem diameter, leaf width and length, presence of anthocyanin and ray length (Table 3) agrestal ANN biotypes had fewer leaves and heads per plant than ruderal biotypes. These genotypes also showed wider heads and larger leave petioles, more and bigger phyllaries, more and wider rays flowers and reached the R1 stage 13 days before ruderal ANN biotypes. The agrestal biotypes did not differ from ruderal PET biotypes in height, stem diameter, number and size of heads, leaves, phyllaries and rays flowers. However, agrestal PET showed longer petioles, more anthocyanins content and narrower phyllaries. Although the agrestal PET reached R1 stage earlier, their life cycle duration did not differ from that of ruderal PET. In both annual *Helianthus* the agrestal biotypes produced greater seed number per head than the ruderal ones; however, they produced low seed number per plant due to lesser branching. While both PET biotypes did not differ in seed biomass, agrestal ANN seeds were twice heavier than those of ruderal ANN biotypes.

Table 3: Metric traits of *Helianthus* invasive biotypes characterized in a common garden (Agronomy Department, UNS).

	Helianthus annuus			***Helianthus petiolaris***		
Trait	Biotype		ANOVA	Biotype		ANOVA
	Agrestal	**Ruderal**		**Agrestal**	**Ruderal**	
ALT (cm)	172,9 ± 38,8	163,9 ± 23,4	ns	131.5 ± 22,6	132.7 ± 14.3	ns
DIAMTA (cm)	1,86 ± 0,68	1,64 ± 0,29	ns	0.9 ± 0.2	0.9 ± 0.2	ns
ANTALL (1-5)	1,9 ± 1,15	2,0 ± 1,0	ns	1.6 ± 0.9	1.1 ± 0.4	ns
ANHOJ (cm)	18,9 ± 4,3	18,1 ± 2,9	ns	6.1 ± 1.1	6.5 ± 1.2	**
LARHOJ (cm)	20,3 ± 2,9	21,3 ± 2,9	ns	10.1 ± 1.5	10.0 ± 1.4	ns
NUHOJ (n)	21 ± 6	25 ± 5	**	18 ± 3	17 ± 3	ns
LARPEC (cm)	16,0 ± 4,7	13,5 ± 2,6	**	11.9 ± 2.9	10.2 ± 2.2	**
NUFLIG (n)	24 ± 7	21 ± 3	*	21 ± 2	22 ± 4	ns
ANFLIG (cm)	2,6 ± 1,3	1,8 ± 0,4	**	1.2 ± 0.2	1.2 ± 0.3	ns
LARFLIG (cm)	3,5 ± 1,0	3,6 ± 0,5	ns	3.2 ± 0.4	3.2 ± 0.4	ns
NUFIL (n)	36 ± 7	28 ± 3	**	31 ± 4	32 ± 4	ns
LARFIL (cm)	2,6 ± 1,3	1,8 ± 0,4	**	1.3 ± 0.3	1.2 ± 0.2	*
ANFIL (cm)	0,9 ± 0,3	1,2 ± 0,2	**	0.4 ± 0.1	0.5 ± 0.1	**
NUCAP (n)	35 ± 12	44 ± 18	*	140 ± 48	135 ± 47	ns
DIAMCAP (cm)	6,2 ± 2,1	4,12 ± 0,6	**	2.4 ± 0.2	2.4 ± 0.3	ns
NSPH (n)	210 ± 78	142 ± 50	**	119 ± 33	111 ± 30	ns
NSPP (nx100)	70 ± 26	63 ± 30	ns	166 ± 65	146 ± 46	ns
SBIO (mg)	24,8 ± 16,4	9,73 ± 2,7	**	4.6 ± 1.4	4.4 ± 0.7	ns
DR1 (days)	122 ± 8	135 ± 6	**	119 ± 8	113 ± 8	**
DR6 (days)	129 ± 8	141 ± 6	**	127 ± 8	127 ± 6	ns
DEND (days)	149 ± 11	168 ± 11	**	214 ± 14	216 ± 16	ns

All the *Helianthus* plants studied presented pubescence in the stem, full branching, alternate leaf arrangement, yellow ray flowers and absence of leaf on back of heads (Table 4). Generally, plants did not present main head, except two plants of BRW and two plants of AWDA. This plant structure (Full branching with main head) was observed by Terzić et al. (2006) in F1 plants from crosses of male-sterile lines with wild *H. annuus* plants.

ANN biotipes leaves shape was cordate (43%) or triangular (39%), meanwhile more than 90% of PET plants had lance or oblong-lance leaves. All PET biotypes had cuneate leaf base, except one plant of RIV that present cordate base, as the 43% of ANN plants.

Helianthus annuus can be clearly differentiated from *H. petiolaris* based on its wide acuminate phyllaries

and absence of white hairs in the apex of the main chaff lobe in the center of disks (Seiler and Rieseberg, 1997). Our experiment support this results, however 25% of agrestal *H. petiolaris* had acuminate and 3% had intermediate phyllary tip, could be attributed to sunflower crop introgression.
Although disk color was yellow and red to PET and ANN, respectively; 2 plants of BRW had yellow disk, being another trait that could be attributed to sunflower crop introgression.

Table 3: Categoric traits of *Helianthus* invasive biotypes characterized in a common garden (Agronomy Department, UNS).

	Helianthus annuus		***Helianthus petiolaris***	
Trait	Biotype		Biotype	
	Agrestal	**Ruderal**	**Agrestal**	**Ruderal**
PUBTA presence	1.00	1.00	1.00	1.00
RAMI full branching	1.00	1.00	1.00	1.00
CAPRIN absent	0.90	0.96	1.00	1.00
BAHOJ cordate	0.40	0.45	0.00	0.02
FORHOJ cordate	0.40	0.45	0.00	0.00
SUHOJ wavy/curled	0.30	0.55	0.05	0.00
MAHOJ deeply serrate	0.50	0.75	0.00	0.00
DISPHOJ alternate	1.00	1.00	1.00	1.00
ANTALL present	0.90	0.98	0.95	0.96
HOJCAP absent	1.00	1.00	1.00	1.00
PUFIL acuminate	1.00	1.00	0.25	0.00
ANEST absent	1.00	1.00	1.00	1.00
CODIS red	0.90	1.00	0.00	0.00
PEBCAP absent	1.00	1.00	0.00	0.00

CONCLUSIONS

These findings alert about the possible diffusion of annual *Helianthus* agrestal biotypes in Argentina. Because their morphological differentiation from ruderal biotypes appears to be difficult, it is extremely important to raise awareness among producers about the emerging problem to prevent their development and subsequent invasion.

Agrestal biotypes had generally bigger reproductive structures than ruderal biotypes. Main head predominance and determinate habit growth of ruderal ANN biotypes suggested sunflower crop introgression. On the other hand, ruderal and agrestal PET biotypes were more similar among them than ANN biotypes.

Agrestal PET biotypes produced more seeds in a shorter grain filling period than ruderal biotypes. It is noteworthy that agrestal PET biotypes, once reached the reproductive stage, fast produce a first cohort of seeds, and after this continue their reproductive process with a slow develop of several secondary heads. This reproductive mechanism would perpetuate the species for the following seasons, even under adverse conditions, avoiding a large period of seeds production.

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