Available online at www.sciencedirect.com

ScienceDirect

Agriculture, Ecosystems and Environment 123 (2008) 343–349

Agriculture Ecosystems & Environment

www.elsevier.com/locate/agee

Gene flow among wild and cultivated sunflower, *Helianthus annuus* in Argentina

M.S. Ureta [a,b,*], A.D. Carrera [b], M.A. Cantamutto [b,c], M.M. Poverene [a,b]

[a] *Centro de Recursos Naturales Renovables de la Zona Semiarida, CERZOS-CONICET, Argentina*
[b] *Department of Agronomy, Universidad Nacional del Sur, Argentina*
[c] *Centro UdL-Irta, Lleida, Cataluna, Spain*

Received 1 March 2007; received in revised form 26 June 2007; accepted 30 July 2007
Available online 6 September 2007

Abstract

Naturalized populations of wild sunflower, *Helianthus annnus*, occur in six provinces of central Argentina, usually near sunflower crops. Plants of intermediate morphology indicate that gene flow might take place in both senses. In order to quantify gene flow between cultivated and wild sunflower, an experimental stand of sunflower was sown surrounded by plots of wild plants at increasing distances. Hybridization rate was estimated using a crop specific isozyme marker, and a mean of 7% progenies were crop-wild hybrids. The nearest wild plants (3 m from the cultivar) showed the highest percentage (18%) of gene flow, which was found to decrease with distance, up to 500 m. Pollen flow from wild plants to crop, evaluated through morphological characters in the progenies of cultivated plants from a stand invaded by wild sunflowers, produced 3.75% intermediate plants.

Keywords: Gene flow; Wild *Helianthus*; Sunflower; Volunteers; Hybridization

1. Introduction

Agricultural biotechnology was massively adopted by farmers in Argentina in the last 10 years. At present, 40% cotton, 60% corn and almost all soybean production is transgenic over a total acreage of 18 million hectare. Argentina is among the main world producers of sunflower, following Russia and Ukraine, and is the first sunflower oil exporter (SAGPyA, 2006). About 77 genetically modified (GM) sunflower varieties have been authorized for field trials, but none of them has been released yet. Whereas in Argentina there is no wild species genetically related to the commercially approved GM crops, sunflower has flowering relatives growing synchronously in areas of commercial production. Wild *Helianthus annuus* and *H. petiolaris*, native to North America established about 60 years ago and occur in patches over seven central provinces (Poverene et al., 2002).

In the agro-ecological landscape other potential recipients of pollen from crop are the volunteers, plants spontaneously originated from seed of cultivated sunflower, which are frequently found in roadsides and fallow fields following sunflower cropping. Off-type volunteers arise from wild pollen contamination in sunflower seed production fields (Reagon and Snow, 2006). As a pollen source, such feral plants carrying GM traits could contaminate conventional crops and affect the non-GM seeds commercialization.

Wild *H. annuus* is considered the ancestor of the cultivated sunflower, *H. annuus* var. *macrocarpus*, and despite many phenotypic differences between them, they are interfertile (Heiser, 1954; Rieseberg and Seiler, 1990; Burke et al., 2002a). Hybridization reaches 42% and alleles from cultivated sunflower persist in frequencies up to 38% in wild sympatric populations (Arias and Rieseberg, 1994; Whitton et al., 1997; Linder et al., 1998).

---

* Corresponding author at: Department of Agronomy, UNS, 8000 Bahia Blanca, Argentina. Tel.: +54 291 4595102; fax: +54 291 4595127.
E-mail address: msureta@uns.edu.ar (M.S. Ureta).

0167-8809/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.agee.2007.07.006

Morphologically intermediate forms were found among wild typical plants in several locations in central Argentina (Poverene et al., 2004). Agro-ecological conditions in Argentina differ from those in North America, and probably determine different relationships between sunflower crop and wild *Helianthus* populations. Therefore, their geographical overlapping (Burke et al., 2002b) was first investigated and an experimental method based on genetic markers (Arias and Rieseberg, 1994) was used in order to estimate crop-wild gene flow. Also, wild-crop gene flow was estimated using a morphological characterization approach.

The following questions were posed: Is there overlap between wild populations and sunflower cultivation? Do volunteer sunflowers increase the risk for gene flow? How readily are wild populations pollinated by crop plants? How readily are crop plants pollinated by wild populations?

2. Materials and methods

Exploration for wild sunflower populations was carried out in eight provinces in the central part of the country. Heads were collected at random along one or several transects depending on the size of each population and they included dry, senescent heads and mature heads with still green bracts (phyllaries) to avoid achene loss due to shattering. Evidence of crop-wild hybridization was assessed through the observation of a number of phenotypic traits, mainly branching type, head number and size, disc and seed color, anthocyanin presence, leaf, phyllary (bract) and seed size.

Volunteers were surveyed along 30 km of Hwy 51, which connects western Buenos Aires province with Bahia Blanca city port. No wild sunflower plants were found in this area. Volunteers were very abundant, as they came from seeds fallen from trucks. Plants growing on the roadside were directly examined and screened for two traits: male sterility and branching (Miller and Fick, 1997). Male parents of commercial hybrids usually carry the dominant male-fertility restorer gene and the recessive gene for branching, while females carry the alternative alleles, so F1 hybrids are heterozygous for both traits. In order to evaluate the genetic constitution of volunteers, chi-square statistics was used in F2 and F3 generation to test the goodness-of-fit of the observed phenotypic classes to expected ratios under single gene hypothesis.

3. Pollen flow from crop to wild plants

A field experiment comprising cultivated and wild materials was planned to simulate natural flow conditions. Isozyme data were used to obtain a more accurate estimation of the gene flow. The isozyme marker was selected based on results from a molecular screening of 13 wild populations of *H. annuus*, four commercial hybrids (DK 3881, 3900, 3915, and 4033) and the inbred line HA89. The study was intended to assess genetic variability of the wild species and to search for specific crop markers (unpublished data). Upon this analysis, hybrid DK3881 and a wild population collected in Cordoba province that differed in the acid phosphatase enzyme locus *Acp-1*, were chosen for the field experiment.

The experimental field design was similar to that used by Arias and Rieseberg (1994). A 25 m × 25 m sunflower plot was sown in the center of a field covering 60 ha, with a commercial planting density. Sowing was done weekly to ensure pollen production during the wild sunflower flowering. About 1000 to 1250 sunflower plants were in anthesis each week. Honeybees from hives in the vicinity ensured pollination.

Around the cultivated stand, 16 plots of 4 m^2, each comprising at least 10 wild *H. annuus* individuals were established along four rays oriented according to cardinal points, at distances of 3 m, 100 m, 300 m and 500 m, plus two more plots located at 1000 m and 1200 m. Wild *H. annuus* seeds were germinated in plastic trays and planted into pots in the greenhouse. Seedlings at four to six leaf stage were transplanted to the experimental field. No other wild, volunteers or cultivated sunflowers were present in the surrounding area. After flowering was complete, heads of wild sunflower plants were protected with plastic mesh to prevent bird predation and shattering. Mature heads were harvested and bagged by plot and by plant. For isozyme analysis, 40–45 seeds were taken at random from a seed pool within each wild plot, and evaluated for *Acp-1* marker. Enzymes were extracted from seeds and resolved on horizontal starch gels (Carrera and Poverene, 1995). Wild-crop hybrid frequency was calculated by counting heterozygous individuals over the total of individuals analyzed at each distance. The rate of pollen dispersal was defined as the total pollen flow expected at each distance assuming a concentric, circular arrangement of wild plants around crop. Therefore, it was estimated by multiplying hybrid frequencies by π (*pi*) d (diameter). Analysis of variance was carried out to study the effect of ray orientation and distance on hybrid frequency. Hybrid frequency values were arcsine square-root transformed.

4. Pollen flow from wild plants to crop

A commercial field planting of sunflower located in La Pampa province was found invaded by wild sunflower plants. Heads of cultivated plants were collected at regular intervals and a quarter section of each was sampled. A subset of achenes was sown in the experimental field and the plants obtained were screened for wild-crop intermediate phenotypes. Achenes from the same wild population collected two years before together with a commercial sunflower hybrid (cv. DK 3881) were also included in the experimental field. In order to assess hybridization, 28 morphological traits (18 continuous: plant height, stem diameter, head position and number, leaf width, length, and size (WxL), leaf number, petiole length, blade/petiole index, ray width, length and number, phyllary width, length, and L/W ratio, phyllary number, disc diameter; 10 categorical: branching type, presence of main head, leaf base, margin and surface, leaves at head back, phylary disposition, disc flower color, pale and stigma anthocyanins) were registered on each plant. Differences among plants were compared through Kruskal–Wallis non-parametric analysis and discriminant analysis of continuous variables, and cluster analysis based on categorical variables using the InfoStat program (2002).

5. Results

Wild *H. annuus* populations occurred in six central provinces in Argentina, from eastern Entre Rios (32°03′S, 60°38′W) to western Mendoza and San Juan, being the latter the northwestern border for the species (31°20′S, 68°32′W). The southern border was placed in Buenos Aires (37°36′S, 62°53′W). The largest populations were found in Cordoba and Entre Rios. Isolated plants were found in three localities of San Luis. The populations were patchily distributed along roadsides and on disturbed soils. The crop area mainly comprised the western part of Buenos Aires, northeast of La Pampa, and central and southern Cordoba provinces. Besides, there were areas devoted to seed production between 31°30′S and 35°20′S in the same provinces, including Mendoza. During the field exploration and collection, evidence of crop-wild hybridization was apparent in some populations because of intermediacy in morphology traits. Intermediate phenotypes were found in populations of La Pampa, Córdoba, Buenos Aires, and Mendoza provinces, in 21 out of 90 sites where seed was collected. Some off-type individuals were observed within cultivated lots in Buenos Aires and La Pampa, although no large wild population was present in the area. A number of crop-wild hybridization events are detailed in Table 1.

Table 2
Expected and observed numbers of fertile and male-sterile plants among volunteers, considering the natural progeny of commercial hybrids (equivalent to F2) and progeny of the year-before volunteers (F3)

Cross	Fertile progeny	Male-sterile progeny
F2		
Rr × Rr	3/4R-	1/4rr
Observed	453	129
Expected	436.5	145.5
χ^2 goodness-of-fit	$0.20 > P > 0.10$	
F3		
1/12 RR × RR	1/12RR	
4/12 RR × Rr	4/12R-	
4/12 Rr × Rr	3/12R-	1/12rr
1/12 rr × RR	1/12R-	
2/12 rr × Rr	1/12R-	1/12rr
Total	10/12R-	2/12 rr
Observed	453	129
Expected	485	97
χ^2 goodness-of-fit	$P < 0.001$	

Volunteers were present along roadsides in all the sunflower cultivation area. In the studied area, out of 582 screened volunteers, 351 were fertile and unbranched, 102 were fertile and branched, 108 were male-sterile and unbranched, and 21 were male-sterile and branched. This represents a ratio of 77.8% fertile:22.2% male-sterile, and a 78.8% unbranched:21.1% branched ratio. If volunteers were mostly the first generation progeny of sunflower commercial hybrids, a ratio of 75% fertile (R-) and 25% sterile (rr) would be expected. If all volunteers were F3 plants, a ratio of 83% fertile:17% sterile would be expected (Table 2). Male-fertile:sterile ratios were in agreement with an F2 generation but the presence of some F3 individuals could not be discarded.

Considering the branching trait the 78.8% unbranched to 21.1% branched ratio was in accordance to the recessive

Table 1
Morphological evidence and site characteristics of wild-crop hybridization events observed along 4 years in central Argentina

County and province	Morphological evidence of hybridization	Site characteristics	Year
Evidence in wild *H. annuus* populations			
J. Celman, Cordoba	Head and leaf size, branching	Roadsides and fallow lands along Hwy 4 and 24	2000–2003
A. Alsina, Buenos Aires	Yellow convex discs, single large head on top	Roadsides of Hwy 60 and lateral dust roads	2002
Rancul, La Pampa	Yellow discs, wide bracts, anthocyanins	Roadsides of Hwy 188	2002
San Rafael, Mendoza	Single head, yellow discs, wide bracts and leaves	Roadsides of Hwy 179 and dust roads, border of irrigation ditches, farm limits	2002
Albardon, Caucete, San Juan	Yellow discs, long bracts, variable branching and cycle	Farm limits and within vineyards	2002
Quemu, Quemu, La Pampa	Single large head on top, striate pericarp	Roadsides of Hwy 10	2002
Diamante, Entre Ríos	Yellow discs, variable color in pericarp	Access to port roads, riverside and city limits	2003
Rio Cuarto, Cordoba	Disc size, single large head on top	Roadsides of Hwy 35 and lateral dust roads	2003
Evidence in sunflower crops			
Trenque Lauquen, Buenos Aires	Numerous small heads, anthocyanin in disc and stem, pubescent achenes	Commercial field lots	2001
Realico, La Pampa	Tall, numerous heads, wide discs, variable color in pericarp	Isolate plants in fallow lands, city limits	2001

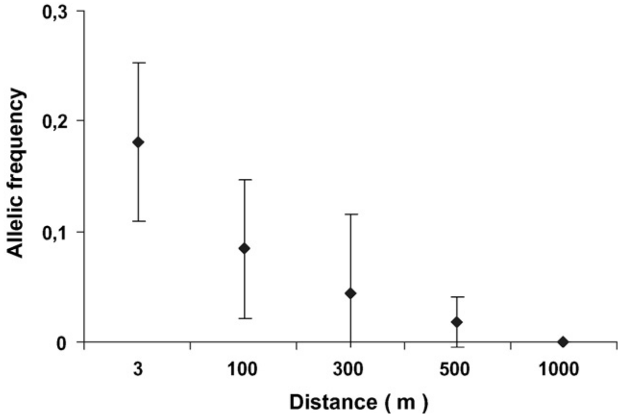

Fig. 1. Frequency of sunflower cultivar marker among progeny of wild plants. Values represent the mean and standard deviation at each distance ($n = 40–45$).

character b1 described by Putt (1964). This trait was analyzed under the same assumptions as the fertility trait and the χ^2 goodness-of-fit values were $0.20 > P > 0.10$ for the F2 ratio expectation and $P < 0.001$ for the F3 ratio expectation.

6. Gene flow

It was not possible to identify crop-specific alleles to assess gene flow within the whole collection. However, DK3881 showed homozygosis for the slowest acid phosphatase allele *Acp-1-e*, which was rare in wild populations and completely absent in the analyzed accession of Cordoba province. Hybrid progeny was easily identified by the presence of bands that were absent in maternal plants, displaying a typical heterozygous three-banded pattern attributed to a dimeric structure of the active enzyme. Fifty-three out of 760 analyzed seeds (7%) were crop-wild hybrids. Frequency values per stand ranged from 0 to 0.275. Crop-wild hybrids were found up to 500 m from the cultivated pollen source. Mean values and standard deviation for each distance are shown in Fig. 1. Significant differences in hybrid frequency were observed between distances ($P < 0.009$). No significant differences in pollen flow were detected for the same distance among the four rays. No evidence of gene flow was detected at the 1000 m and 1200 m sites. The maximum pollen dispersal rate was found at 300 m, being the distance at which pollen was transported with the highest relative amount (Fig. 2). This means that the relative amount of pollen required to produce a given hybrid frequency was greater at 300 m than at 3 m.

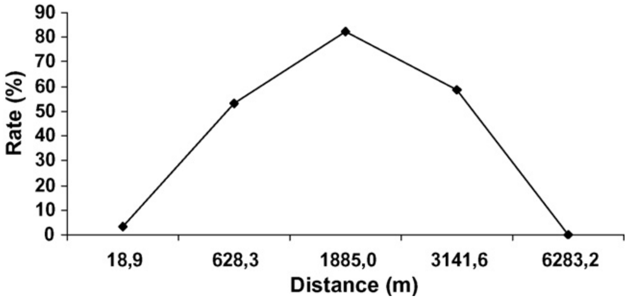

Fig. 2. Crop pollen dispersal rate estimation into wild plants situated at five distances from a central sunflower pollen source.

Regarding gene flow from wild plants to crop, three morphologically intermediate plants out of 80 were found (3.7%), obtained from the heads collected in a field invaded with wild sunflowers. The intermediate plants differed from cultivated sunflower in eight morphological characters but none from the wild plants. Stem diameter had an intermediate value between wild plants and crop (Table 3).

Discriminant analysis based on 18 continuous traits confirmed that the three intermediate plants grown from seeds harvested on crop heads displayed a different morphology compared to wild or cultivated sunflower. The variables which most contributed to discriminate between cultivated and wild sunflower (canonical axis 1 in Fig. 3) were ray and phyllary width and head number, whereas those which best explained differences among intermediate and true type plants (canonical axis 2) were phyllary length, ray width, and head number. Cluster analysis based on 10 categorical traits showed that intermediate plants were more similar to wild than to cultivated plants (Fig. 4).

Table 3
Morphological traits of hybrid plants from sunflower crop pollinated by wild sunflower and representative parental phenotypes

Character	Wild *H. annuus* (male phenotype)	Intermediate plants (hybrids)	Cultivated sunflower (female phenotype)	Significance
Plant height (cm)	157.80a	134.33b	108.80c	***
Stem diameter (cm)	1.69b	1.93ab	2.36a	***
Head number (*n*)	75.60a	33.67b	1.00c	**
Leaf width (cm)	18.00b	19.03b	25.60a	**
Ray length (cm)	4.13b	4.73b	8.72a	***
Ray flower number (*n*)	24.60b	22.67b	41.20a	***
Bract number (*n*)	27.67b	26.90b	33.70a	***
Bract L/W ratio	2.96a	2.95a	2.02b	***
Disc diameter (cm)	3.77b	4.23b	19.00a	***

Means are significantly different at $P < 0.01$ (**) or $P < 0.001$ (***). Values followed by the same letter within a column are not significantly different at $P = 0.05$.

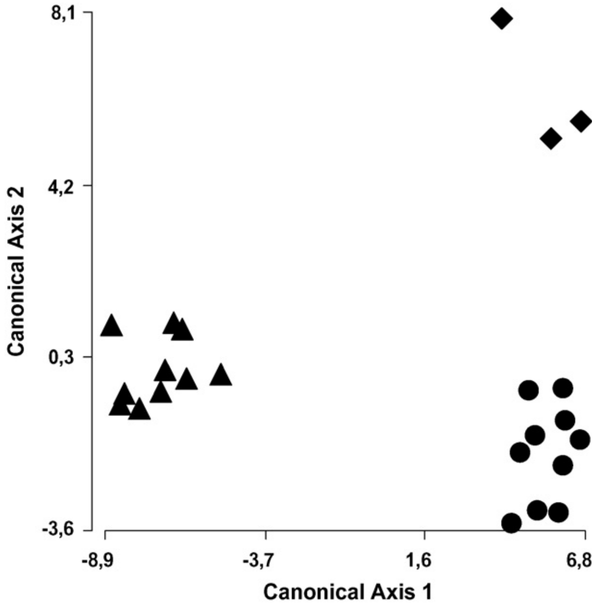

Fig. 3. Discriminant analysis of progenies from heads harvested in a crop field invaded by wild sunflowers, based on 18 metric morphological traits. Each point represents the score for an individual. Crop-like plants (triangles) and intermediate plants (diamonds) were found among progenies; wild plants (circles) were included as controls.

7. Discussion

Wild *H. annuus* populations were found forming extensive clumps from the warm riverside in Entre Rios, to the rather xeric environments of San Juan. Populations varied in size and had a patchily distribution. Based on acreage of provincial counties, overlapping between wild sunflower distribution and cropped area, which was 1.96 million ha in 2005 (SAGPyA, 2006) was estimated in 37%. This included the area devoted to seed production where gene flow could affect hybrid seed purity. Observed off-type individuals within cultivated lots in Buenos Aires and La Pampa, probably originated from wild pollen flow in the seed production fields. A similar situation is of concern also in the U.S. and France (Faure et al., 2002; Reagon and Snow, 2006).

Plants with intermediate morphology were found in 23% of the collecting sites, so hybridization and gene flow between wild and cultivated sunflower seemed a frequent event, as observed in six provinces. Gene flow is expected to occur in the whole range of sunflower cultivation in the central part of the country. Wild *H. annuus* has also established in Spain and Italy and interferes with crop (Faure et al., 2002).

Volunteers were found as feral plants growing in the whole crop area, as in the U.S. (Reagon and Snow, 2006) and in France (Faure et al., 2002). The phenotypic ratios found among volunteers confirmed their origin from seeds of commercial hybrids (F1) lost during transport. It cannot be discarded that at least some of these plants came from seeds of volunteers grown the year before (F2:F3). One out of five individuals was male-sterile or branched, becoming an extremely vulnerable plant susceptible to gene flow, thus increasing the chance for novel traits dissemination. Volunteers could act as a genetic bridge through which transgenes would spread to wild or cultivated plants (Reagon and Snow, 2006). If GM sunflowers were released in Argentina, this would certainly take place over the whole cropping area.

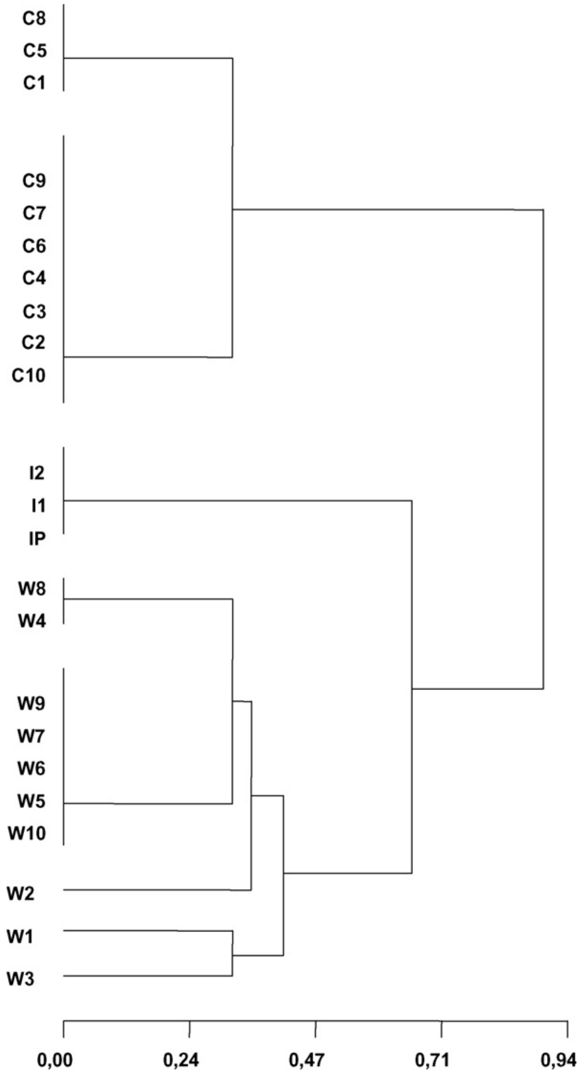

Fig. 4. Cluster of crop-like (C), wild *H. annuus* (W), and intermediate (I) plants based on categorical morphological traits (standardized variables). UPGMA clustering on the basis of Gower's distance matrix (Cophenetic correlation 0.992).

8. Wild populations pollinated by crop plants

The occurrence of crop-wild hybridization was confirmed using isozyme markers, a reliable approach for the detection of such events. The overall mean frequency of hybrids (7%) was comparable to that obtained by Arias and Rieseberg (1994) in Mexico (10%). The region where the gene flow experiment was conducted displays warmer temperatures and lower humidity compared with the core sunflower production zone where higher hybridization rates could be expected. Arias and Rieseberg (1994) also found significant differences in gene flow rates between localities.

It was possible to track the distance and direction of pollen movement from source plants. No significant differences between cardinal rays mean that there was no effect of wind direction on pollinator activity. A leptokurtic distribution of pollen dispersal was observed, similar to that observed in North America. Average hybrid frequency half-declined from one distance to the next, but no hybrids were found at 1000 m. This finding and the maximum pollen dispersal rate sustain the isolation distance of 3000 m recommended by The National Committee for Agricultural Biotechnology in Argentina for GM sunflowers management. Wild sunflower carrying the transgene that confers white mould resistance did not show a significant increase in fitness measured by seed output (Burke and Rieseberg, 2003) while plants with Bt transgene produced more seeds than non-trangenic individuals (Snow et al., 2003). In Argentina, traits under field experimentation in sunflower include tolerance to herbicides (glyphosate, glufosinate), insect tolerance (lepidoptera, coleoptera), fungal resistance, and other fitness-enhancing traits such as increased nitrogen assimilation (Cantamutto and Poverene, 2007). These transgenes are expected to be neutral or beneficial in wild sunflower populations and may spread quickly, but this does not mean that they will result in more invasive weeds (Snow et al., 2005).

9. Crop plants pollinated by wild populations

Gene flow also occurs from the wild species to the cultivated sunflower. This was demonstrated by intermediate morphological traits in plants grown from seeds of sunflower heads collected in a field invaded by wild *H. annuus*. Frequency of intermediate plants was 3.75 %, in a random sample of such seeds. The plants resulted morphologically more similar to the wild parent and this was considered enough evidence of gene flow from wild plants to crop, because a molecular screening would have been impractical.

One caveat regarding this experiment was the low number of plants examined, even though the finding of three intermediate-type plants out of 80 was enough evidence of wild-crop gene flow. Furthermore, this pollen flow was expected to be lower than crop-wild pollen flow for several reasons. First, in the Argentine landscape the number of cultivated plants is several times higher than wild plants, which usually occur in small patches. In the 2005/2006 season, sunflower acreage in the invaded provinces was of 1.7 million hectares. Considering about 40,000 plants per ha, this means over 70 billion cultivated plants. Second, big sunflower heads attract more insects because of larger nectaries and anthers than the small wild heads, therefore crop pollen could represent the majority of the pollen load in the insects. Finally, during crop flowering time (7–10 days) the amount of available wild pollen is much lower, because heads are small, with 3-4 cm discs, even though wild plants flower for a longer time (20–40 days).

Gene flow in Argentina was demonstrated by the extent of overlapping areas of wild and cultivated sunflower, hybridizing frequencies, and recurrence of wild-crop hybrids. Sunflower can be considered a crop of high probability of transgene and herbicide tolerance transference.

Acknowledgements

Authors want to thank National Research Council of Argentina (CONICET) for a fellowship to MSU. Technical assistance from A. Garayalde and A. Gutierrez, and statistical help from S. Luis and A. Hernandez are greatly appreciated. This research was supported by grants ANPCYT-PICT 08-9881 and UNS-PGI 24A106.

References

Arias, D.M., Rieseberg, L.H., 1994. Gene flow between cultivated and wild sunflowers. Theor. Appl. Genet. 89, 660–665.

Burke, J.M., Gardner, K.A., Rieseberg, L.H., 2002a. The potential for gene flow between cultivated and wild sunflower (*Helianthus annuus*) in the United States. Am. J. Bot. 89, 1550–1552.

Burke, J.M., Rieseberg, L.H., 2003. Fitness effects of transgenic disease resistance in sunflowers. Science 300, 1250.

Burke, J.M., Tang, S., Knapp, S.J., Rieseberg, L.H., 2002b. Genetic analysis of sunflower domestication. Genetics 161, 1257–1267.

Cantamutto, M., Poverene, M., 2007. Genetically modified sunflower release: opportunities and risks. Field Crops Res. 101, 133–144.

Carrera, A., Poverene, M., 1995. Isozyme variation in *Helianthus petiolaris* and sunflower, *H. annuus*. Euphytica 81, 251–257.

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.

Heiser Jr., C.B., 1954. Variation and subspeciation in the common sunflower, *Helianthus annuus*. Am. Mid. Nat. 51, 287–305.

InfoStat 2002. InfoStat Profesional, Versión 1.1. Universidad Nacional de Córdoba, Estadística y Diseño, F.C.A. www.infostat.com.ar.

Linder, C.R., Taha, I., Seiler, G.J., Snow, A.A., Rieseberg, L.H., 1998. Long-term introgression of crop genes into wild sunflower populations. Theor. Appl. Genet. 96, 339–347.

Miller, J.F., Fick, G.N., 1997. The genetics of sunflower. In: Schneiter, A.A. (Ed.), Sunflower Technology and Production, Agronomy Series 35. American Society of Agronomy, Inc., Madison, Wisconsin, pp. 441–495.

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. RIA (Arg.) 31, 97–116.

Poverene, M., Carrera, A., Ureta, S., Cantamutto, M., 2004. Wild *Helianthus* species and wild-sunflower hybridization in Argentina. Helia 27, 133–142.

Putt, E.D., 1964. Recessive branching in sunflowers. Crop Sci. 4, 444–445.

Reagon, M., Snow, A.A., 2006. Cultivated *Helianthus annuus* (Asteraceae) volunteers as a genetic "bridge" to weedy sunflower populations in North America. Am. J. Bot. 93, 127–133.

Rieseberg, L.H., Seiler, G.J., 1990. Molecular evidence and the origin and development of the domesticated sunflower (*Helianthus annuus*, Asteraceae). Econ. Bot. 44 (supplement), 79–91.

SAGPyA (Secretaría de Agricultura, Ganadería, Pesca y Alimentos, Argentina) 2006. www.sagpya.mecon.gov.ar/biotecnologia.

Snow, A.A., Andow, D.A., Gepts, P., Hallerman, E.M., Power, A., Tiedje, J.M., Wolfenbarger, L.L., 2005. Genetically engineered organisms and the environment: current status and recommendations. Ecol. Appl. 15, 377–404.

Snow, A.A., Pilson, D., Rieseberg, L.H., Paulsen, M.J., Pleskac, N., Reagon, M.R., Wolf, D.E., Selbo, S.M., 2003. A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. Ecol. Appl. 13, 279–286.

Whitton, J., Wolf, D.E., Arias, D.M., Snow, A.A., Rieseberg, L.H., 1997. The persistence of cultivar alleles in wild populations of sunflowers five generations after hybridization. Theor. Appl. Genet. 95, 33–40.