THIRD INTERNATIONAL SYMPOSIUM ON BROOMRAPE (*Orobanche* spp.) IN SUNFLOWER

Córdoba, Spain, June 3rd to 6th, 2014

PROCEEDINGS

Proceedings of the

Third International Symposium on Broomrape (*Orobanche* spp.) in Sunflower

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Dedicated to the memory of Carlos Alberto Sala and Alexandru Viorel Vrânceanu

Dr. Carlos Alberto Sala (Argentina; 1961-2013) leaded the Biotechnology Department of Nidera for two decades, where he made significant contributions to the development and genetic characterization of new herbicide tolerance technologies for weed and broomrape control in sunflower, particularly CLPlus imidazolinone resistance technology. He was an active member of the Scientific Committee of this Symposium until his final days.

Prof. Alexandru Viorel Vrânceanu (Romania; 1927-2014) is one of the fathers of modern sunflower breeding. He contributed greatly to the commercial introduction of hybrid technology in sunflower and to the development of many improved cultivars. He also developed a set of differential lines to identify sunflower broomrape races and laid the basis for understanding the inheritance of broomrape resistance in sunflower. His crucial contribution to sunflower improvement was recognized by the International Sunflower Association with the V.S. Pustovoit Award.

If I have seen further it is by standing on the shoulders of Giants
Isaac Newton

Foreword

Broomrape (*Orobanche* spp.) is a major constraint for sunflower production in most production areas around the world, particularly in Europe and Asia. The most widespread broomrape species attacking sunflower is *O. cumana* Wallr. The development of resistant cultivars as well as optimized managing strategies is a high priority in sunflower breeding programs all over the world.

The Board of the International Sunflower Association (ISA) proposed in their meeting held in Buenos Aires in February 2012 to celebrate an International Symposium on Broomrape in Sunflower in June 2014 in Spain. This is the third specific symposium on broomrape in sunflower after those held in Turkey in 2008 and Moldova in 2011.

With around 200 participants from 24 countries, and 46 scientific and technical communications, the Symposium represents a unique opportunity to get a complete picture of sunflower broomrape situation around the world, to evaluate the validity of current strategies of control, and to discuss new strategies for the future. The contents of the manuscripts included in these proceedings are the responsibility of the authors and their edition by the Scientific Committee has been reduced to formatting issues and misspellings. They should be considered as being privileged communications that require the express consent of the authors to be reprinted in part or as a whole.

The Symposium has been organized by the Spanish Sunflower Association (Asociación Española del Girasol), which is formed by sunflower researchers and managers from public institutions and private companies involved in the sunflower sector. We are indebted to many individuals, institutions, and companies that have contributed to the success of this event.

Finally, we would like to thank the Scientific Committee for the advice in preparing the scientific sessions and workshops, and all the participants for their stimulating discussions throughout the Symposium. We hope you acquired some valuable information from our speakers, benefited from fruitful discussions with other colleagues, and returned home with new ideas to be developed in the next years. If you also found a friendly environment and enjoyed the social events we prepared, then our efforts in organizing this Symposium will have been worthwhile.

The Organizing Committee

The Spanish Sunflower Association

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Session 1: *Current situation of sunflower broomrape around the world*

Session 2: *Knowing the parasite: Biology and genetics of* Orobanche

Broomrape occurrence in natural populations of annual *Helianthus sp.*

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ABSTRACT

Wild annual *Helianthus* species native to North America have demonstrated to be a valuable genetic resource for sunflower crop improvement. The search of genes for broomrape (*Orobanche cumana* Wallr.) resistance demonstrated a natural resistance to this parasitic weed in a wild sunflower species, *H. petiolaris*. This species along with the wild *H. annuus* form natural populations in extended areas of Argentina. In Europe, mostly the wild *H. annuus* is found across the main sunflower crop production areas. This species seems to be susceptible to the specialized parasitic weed, native from Europe. The environment and plant phenotypes of five wild *H. annuus* populations in their natural habitats in Argentina were compared to agrestal populations of *H. annuus* attacked by broomrape, located in Fernan Nuñez, Spain, and Kovilovo, Serbia. The study comprised four susceptible *H. annuus* and five resistant *H. petiolaris* populations. The goal was to realize if wild populations in Argentina could be in risk to be invaded by the parasitic weed. The plant morphology was not affected by the broomrape attack in Spain. Cluster analysis based on morphology showed differences among the two annual species but grouped *H. annuus* natural populations from Argentina and Europe. Clustering of ecological variables did not separate species and geographical localization. No differences in the environment for broomrape attacked and susceptible populations were found. Natural *H. petiolaris* populations could be considered a genetic resource for resistance against broomrape but natural *H. annuus* populations should be considered as a potential reservoir of the parasitic weed.

Key words: Argentina – ecology – Europe – morphology – *Orobanche* – wild sunflower

INTRODUCTION

The sunflower/broomrape (*Helianthus annuus/Orobanche cumana*) complex is one of the most intriguing biological relationships in the modern crop production systems. Broomrape is native to the Caucasus region, where it develops on natural vegetation. After the discovery of the New World, the introduction of sunflower crop and gradually migration to Eastern Europe brought together these two taxa in the present Russian territory (Acimovic, 1998). In Krasnodar, Russia, Dr. Pustovoit devoted much of his breeding activity to create sunflower varieties with genetic resistance to broomrape (Pustovoit, 1967).

In the century following to Pustovoit's work, there is still continued effort to search and generate new sources of genetic resistance to broomrape. In Southern and Eastern Europe broomrape causes considerable yield losses and reduces sunflower seed quality. Genetic resistance had proved the most efficient method for avoiding severe broomrape attacks in the field, but natural selection promotes the emergence of new and more aggressive races of the parasite (Kaya et al., 2012).

Annual and perennial wild *Helianthus* species have been used as a source of resistance genes against broomrape (Terzic et al., 2010; Petcu and Pacureanu, 2011). The wild *H. annuus* is a successful plant invader all over the world (Cantamutto and Poverene, 2010). Extended populations of these taxa, growing in ruderal and agrestal habits, are present in South America and Europe (Stankovic-Kalezic et al., 2007; Muller et al., 2009; Cantamutto et al., 2010). Natural populations of the wild *H. annuus* met the parasitic weed only in Europe, because broomrape is absent in the sunflower cultivation region from America (Cantamutto et al., 2012).

The *H. annuus* populations of Argentina are susceptible to broomrape attack (Miladinovic et al., 2013), but the wild species *H. petiolaris*, also present in Argentina (Poverene et al., 2008), is resistant to the parasitic weed. The resistance profile of the *H. annuus* populations of Europe has not been described. Poverene and Cantamutto (2010) observed the presence of broomrape in an agrestal *H. annuus* population growing within a sunflower crop in Spain. Broomrape infection in wild *H. annuus* has also been detected in a ruderal population of Serbia (Dr. Dragana Miladinovic, personal communication).

Broomrape infection in *H. annuus* natural populations has not been reported in the modern literature. The objectives of this work were to communicate the observations on *H. annuus* natural populations attacked by broomrape and to answer the following questions: 1- Are Argentinian populations susceptible to broomrape morphologically different from the infected populations of Europe? 2- Do these European populations develop in a different environment than Argentinian populations? 3- Could *H. petiolaris* resistance be related to the environmental conditions?

The absence of morphological and environmental differences between infected and not infected populations entails a high risk for wild populations to be reservoir of broomrape populations for sunflower attack. The goal of this study was to compare morphological traits and environmental factors of wild populations infected and not infected by the parasitic weed in Europe and America.

MATERIALS AND METHODS

Materials comprised four wild *Helianthus annuus* and five *H. petiolaris* populations sampled in Argentina, one *H. annuus* population from Spain, and one from Serbia. These latter two accessions were broomrape infected (Poverene and Cantamutto, 2010, Miladinovic personal communication), with more than 30% incidence. Population data included botanical name, collection site (district, province, latitude, longitude, and altitude), estimated population area and size, and morphological variation registered according to Poverene et al. (2008). Morphological data consisted of plant height, head number, head angle, leaf shape, leaf margin, petiole/leaf rate, phyllary (bract) length, disc color, branching, and the presence of a main head. These traits were registered in the natural site. Broomrape presence was registered for each plant if tassels were visible in the stem base.

The average annual rainfall, and mean temperature of the hottest and coolest month were obtained from the nearest locality (web data). The population site was classified as roadside, riparian or crop when plants were found growing adjacent to cultivated lands (sunflower, maize, or wheat), within crop if plants were growing in the same row and/or between sunflower plants, and sunflower volunteers were recorded if present.

Morphological and ecological data were subjected to ANOVA and cluster analysis with the Infostat program (Di Rienzo et al., 2013).

RESULTS

Table 1 presents information about the surveyed populations. Site data corresponded to temperate lowlands. Population data were variable but similar in both hemispheres. The *H. annuus* (ANN) populations from Spain and from Serbia were broomrape infected at the moment of data collection. The *H. annuus* and *H. petiolaris* (PET) population from Argentina were free from the parasite.

Table 1. Spontaneous *Helianthus* populations studied in Argentina and Europe.

Population	Species	Locality	Province*	Altitude	Area m^2	Plants (n)
AAL	ANN	Puan	B. Aires	240	6600	6000
DIA	ANN	Diamante	Entre Rios	14	35000	12000
LMA	ANN	Malvinas	Mendoza	609	1680	5000
RCU	ANN	Rio Cuarto	Córdoba	366	30000	20000
FNU	ANN	Fernan Nuñez	Andalusia	125	1200	200
FK	ANN	Kovilovo	Vojvodina	77	50000	60000
CAT	PET	Q. Quemú	La Pampa	117	12000	10500
HLA	PET	H. Lagos	B. Aires	124	560	200
NGA	PET	N. Galia	San Luis	306	2750	2000
PEL	PET	La Zanja	B. Aires	101	1320	4300
SRO	PET	S. Rosa	La Pampa	183	1350	4050

*Except for Andalusia (Spain) and Vojvodina (Serbia) the remaining are provinces of Argentina

All morphological traits but three were polymorphic and were included in the analyses. All surveyed plants had red discs, total branching from the bottom, and none of them had a main head. Cluster analysis based on morphological traits comparing *H. annuus* and *H. petiolaris* populations is shown in Fig. 1 (Gower distance, cophenetic correlation 0.967). There were two main clusters (A and B), one for each

species. Plant position, branching type, anthocyanin pigment, head diameter and red disk of Spanish and Serbian populations corresponded to the wild or weedy *H. annuus* taxonomic descriptors. In Spain, plant height, stem diameter, leaf large, petiole length, phylary size, and head diameter of parasitized plants (n=4) did not differ from the healthy plants (n=6).

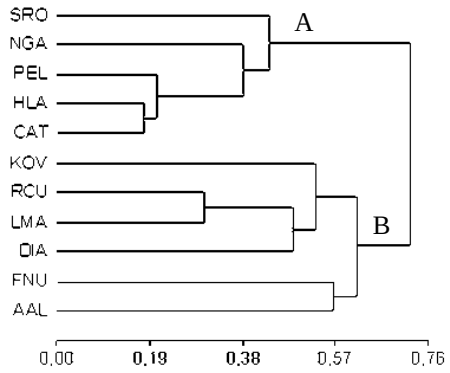

Fig 1. Cluster analysis of *Helianthus annuus* and *H. petiolaris* populations based on morphological traits.

When ecological variables were taken in consideration both species and origins failed to separate broomrape resistant from susceptible populations (Fig. 2, Gower distance, cophenetic correlation 0.882).

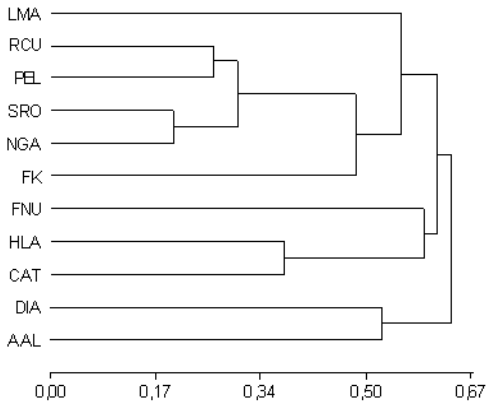

Fig. 2. Cluster analysis of *H. annuus* and *H. petiolaris* populations based on ecological variables.

DISCUSSION

Wild *Helianthus annuus* and *H. petiolaris* are important germplasm sources for sunflower breeding. These species have been established in central Argentina as feral populations since at least 60 years ago, when they were unintentionally introduced from the centre of origin (Cantamutto et al., 2010). Nine populations were chosen so as to cover the whole territory. None of them were invaded by broomrape, but artificial inoculations in Serbia proved that *H. annuus* accessions were susceptible whereas *H. petiolaris* accessions were resistant to broomrape (Miladinovic et al., 2013). Both *H. annuus* populations from Spain and Serbia were infected by broomrape at the moment of sampling. A former morphological characterization showed that Argentinian *H. petiolaris* populations were similar to those of the central part of the US, Texas, New Mexico and Nebraska (Salomón et al., 2008). Three among five *H. petiolaris* accessions with resistance to the parasite found by Terzic et al. (2010) come from the same US states (http://www.ars-grin.gov).

Cluster analysis based on morphological traits clearly differentiated between both species, showing differences among populations. Diversity level was higher among *H. annuus* populations than among *H. petiolaris* populations. There was no clear cut separation between susceptible *H. annuus* populations and those attacked form broomrape. The Argentinian population AAL, free from the parasite, was more similar to Spanish population than to the remaining populations from Argentina. Clustering did not reveal correspondence with geographical distribution within the species.

Cluster analysis based on ecological variables failed to discriminate *Helianthus* species from Argentina. RCU, PEL SRO, and NGA populations clustered close to Serbian *H. annuus* population (FK). Spanish FNU population was close to *H. petiolaris* populations HLA and CAT. As the European FK and FNU populations were invaded by the parasite, it could be concluded that there were no differences in the environment for broomrape resistant and susceptible materials. DIA and AAL *H. annuus* populations formed another cluster and LMA was the most dissimilar accession, probably because rainfall in Malvinas location is very low and there is a need for irrigation.

The wild *H. annuus* populations from Europe infected by broomrape and the susceptible populations from South America where the weed is absent showed a high morphological similarity and developed in similar environmental conditions. The ruderal and agrestal *H. annuus* populations should be considered a potential reservoir of the parasitic weed, and thus very vulnerable to broomrape invasion, while natural populations of *H. petiolaris* could be of a high value as genetic resource for resistance against this parasitic weed.

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