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USING SOWING DATE MODIFICATION AND GENETIC RESISTANCE TO MANAGE SUNFLOWER BROOMRAPE (*Orobanche cumana* Wallr.)*

Akhtouch, B.[1], Molinero-Ruiz, L.[1], Dominguez, J.[2], Melero-Vara, J.M.[1] and Fernández-Martínez, J.M.[1**]

[1] *Institute for Sustainable Agriculture, CSIC, Apdo. 4084, 14080 Córdoba, Spain*
[2] *IFAPA Centro Alameda del Obispo, CAPMA (Junta de Andalucía), Apdo 3092, 14080 Córdoba, Spain*

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SUMMARY

The parasitic weed *Orobanche cumana* Wallr. (broomrape) constraints sunflower production in eastern and southern Europe and in the Middle East. Resistance of sunflower hybrids to *O. cumana* race F, which is widespread in the main sunflower growing countries including Spain, is not complete. The infection of six populations of *O. cumana* (races B and F) in four sunflower genotypes in greenhouse (10 to 32ºC) and in growth chamber (20 to 25ºC) was studied. Also the effect of four sowing dates (SD) on the intensity of the attack of sunflower genotypes by *O. cumana* race F at three inoculum densities was investigated in an irrigated field in 2000 and 2001. Greenhouse was more favorable than growth chamber for *O. cumana* infection, which was highest by race F populations. In the field experiment, the reduction of the attack in the moderately resistant hybrid was significant at all SD and higher at late SD as compared to early sowings in both growing seasons. Late sowings (from the end of March until the beginning of April) favor an enhanced expression of the resistance of sunflower to *O. cumana* race F irrespective of seedbank, and can be therefore recommended, under irrigation and together with the use of moderately resistant sunflower hybrids, as part of an efficient strategy on the control of this parasitic weed.

Key words: **broomrape races, cultural control methods, genes of resistance, *Helianthus annuus*, parasitic plants, sunflower protection**

INTRODUCTION

The chlorophyll-lacking parasitic weed *Orobanche cumana* Wallr. infects sunflower (*Helianthus annuus* L.) roots and depletes the plant of nutrients and water,

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** Corresponding author: jfernandezm@ias.csic.es

causing severe crop losses in most countries of the Middle East and eastern and southern Europe. Within *Orobanche* spp., *O. cumana* is the only species that exhibits a clear race structure with respect to sunflower genotypes. Single major genes (Or_1 to Or_5) were reported to confer resistance to races A to E of *O. cumana* (Vrânceanu *et al.,* 1980). Parasite populations which overcome the resistance gene Or_5 (race F) were identified in Spain in the mid 90's (Saavedra del Río *et al.,* 1994). Race F is widespread in the main sunflower growing areas of the country since then (Molinero-Ruiz *et al.,* 2009). Genetic resistance is the most effective and feasible control method against *O. cumana*, but complete immunity of sunflower hybrids to race F is infrequently expressed in the field; instead moderate resistance of the hybrids is observed (Molinero-Ruiz *et al.,* 2008; 2009). Also imidazolinone herbicides can be very effective, but they must be applied to imidazolinone-resistant sunflowers (Clearfield production system) (Tan *et al.,* 2005). Therefore, strategies for the control of *O. cumana* race F must be implemented aiming at improving sunflower performance in infested areas of the Mediterranean Basin (Fernández-Martínez *et al.,* 2009).

Field studies on winter legumes in the Mediterranean area showed that a delay of the sowing date (from October and until January) was related to a decrease of the number of attached parasite plants of the crenate broomrape (*O. crenata*) (Mesa-García and García-Torres, 1986; Rubiales *et al.,* 2003). Drought is a limiting factor for crop production under the semiarid Mediterranean climatic conditions of southern Spain. Therefore, early sunflower sowings (before February) yield better than late sowings (April) due to an optimization of crop water use efficiency and to the escape of the plants from high temperatures at the end of the growth cycle (Gimeno *et al.,* 1989). As for the effect of the sowing date on the attacks of sunflower by *O. cumana* in this area, contradictory results have been reported. While Castejón-Muñoz *et al.* (1993) observed low levels of infection of confectionary sunflower in early sowing dates, other authors suggested that early sowing favored both the incidence of *O. cumana* and the degree of broomrape attack in sunflower genotypes (Alvarado-Aldea *et al.,* 1998).

Modifications of the sowing date cause altered environmental conditions that affect *Orobanche* spp. development and its establishment in the host plant. A preconditioning period in a warm moist environment is required before dormancy of *Orobanche* spp. seeds is broken and they become responsive to germination stimulants (Joel *et al.,* 1995; Song *et al.,* 2005). Late sowing dates were associated to a reduction of broomrape infection in winter legumes because low temperatures delayed the end of seed dormancy and decreased germination of *O. crenata* (Mesa-García and García-Torres, 1986; Van Hezewijk *et al.,* 1994; López-Granados and García-Torres, 1996). Seeds of *O. cumana* stored outside the host rhizosphere may remain dormant and infective in the absence of any pre-conditioning for at least 17 years (Molinero-Ruiz *et al.,* 2008). Also, the different effect of thermal regimes on the infection of genotypes of sunflower by *O. cumana* under controlled conditions

has been reported (Sukno *et al.*, 2001). Most of the sunflower genotypes tested by Sukno *et al.* (2001) had highest degree of attack by broomrape when the incubation temperature was 19ºC. Infections consisted on small nodules which did not develop to emerged broomrapes at 23 and 27ºC, and a delay in the emergence of broomrape was observed at 15ºC (Sukno *et al.*, 2001).

Aiming a more effective and sustainable control of *O. cumana* in sunflower, new strategies must be explored. Among them, and because immunity of commercial hybrids to race F of *O. cumana* is uncommon (Molinero-Ruiz *et al.*, 2009; 2014), the combination of genetic resistance together with the shift of sowing date may have an effect on the reduction of the crop infection. Our work hypothesis was that the modification of the date of sowing can influence the establishment and development of *O. cumana* in moderately resistant sunflower. The objectives of this work were:

1) to compare the effect of different environments (growth chamber and greenhouse) on the degree of the attack of resistant and susceptible sunflower genotypes by *O. cumana* populations, and
2) to study the effect of different sowing dates on the infection of *O. cumana* race F in different genotypes of sunflower genotypes under field conditions.

MATERIALS AND METHODS

Infection of sunflower by *O. cumana* under controlled conditions

The effect of different environments on the infection of sunflower genotypes carrying different sources of resistance to *O. cumana* were investigated in one experiment that was twice conducted in greenhouse and in a growth chamber with controlled conditions. Seed from six populations of *O. cumana* were collected in the main growing areas of sunflower in central (CU996) and southern (CO197, SE395, SE296, SE198 and SE498) Spain between 1995 and 1998, and yearly increased on the susceptible confectionary inbred line B117 (Molinero-Ruiz *et al.*, 2008). *Orobanche cumana* seed was collected at full maturity of broomrape plants, air-dried, separated from plant debris with a 500 μm sieve and recovered on a 200 μm sieve, and stored in glass jars kept in the dark at room temperature (15 to 26ºC) until used.

Four sunflower genotypes were used: Kruglik A-41, J8281, Turbo and P96. The inbred lines Kruglik A-41 and J8281 carry the genes Or_1 (resistance to race A) and Or_2 (resistance to race B) respectively, and are used as tester lines for races A and B of *O. cumana* (differentials) (Vrânceanu *et al.*, 1980). The hybrid Turbo carries the Or_5 gene conferring resistance to race E of the parasite and is susceptible to race F (García-Ruiz, 2000). The inbred line P96 is the differential for race G, since it is resistant to races A to F and susceptible to race G (Molinero-Ruiz *et al.*, 2008). Table 1 summarizes the sunflower genotypes used in this work as well as their reaction to races of *O. cumana* and the experiments in which they were included.

Table 1: Genotypes of sunflower used in this work, their reaction to races of *Orobanche cumana* and experiments in which they were included

Name (type)	Races of *O. cumana*							Experiment
	A	B	C	D	E	F	G	
Kruglik A-41 (inbred line)	R[a]	S	S	S	S	S	S	Controlled conditions
J8281 (inbred line)	R	R	S	S	S	S	S	Controlled conditions
NR5 (inbred line)	R	R	R	R	R	S	S	Field
P96 (inbred line)	R	R	R	R	R	R	S	Controlled conditions & field
Turbo (hybrid)	R	R	R	R	R	S	S	Controlled conditions
Rodrigo (hybrid)	R	R	R	R	R	R*	S	Field

[a] R=resistance, S=susceptibility, R*=moderate resistance

Sunflower seeds were surface-sterilized by immersing them in 10% sodium hypochlorite for 5 to 10 minutes, then thoroughly rinsed in deionized water and incubated in the dark at saturation humidity in a germinator at 24 to 28°C until radicles were 2 to 5 mm long. Five seedlings (replications) of each of the genotypes were inoculated with each of the populations of *O. cumana* and arranged in a split-split-plot completely randomized design with one plant as experimental unit. Environment (growth chamber or greenhouse) was assigned to main factor, and populations and genotypes were assigned to secondary and tertiary factors respectively. Inoculations were performed by transplanting individual sunflower seedlings to pots with 80 g of perlite uniformly infested with 18 mg of parasite seed and grown in chamber of controlled conditions set at 20 to 25°C or in greenhouse at 10 to 32°C (both with photoperiod of 14 hours/day). After growing for two weeks, plants were transplanted, with the infested soil, to 5-L pots containing SSPM soil mixture (sand : silt : peat moss, 2 : 1 : 2, V/V) and grown under the same conditions (growth chamber or greenhouse) until physiological maturity (10 to 12 weeks). Plants were fertilized three times a week with 500 ml of complete nutrient solution (Hoagland and Arnon, 1950) per pot, and watered as required.

Reactions of sunflower were assessed at the end of the experiment by recording the final degree of attack (FDA) as the average final number of emerged *O. cumana* stems per sunflower plant. As no significant differences were found for FDA, data were pooled across experiments. Analysis of variance (ANOVA) was conducted on data of FDA transformed according to Ln (FDA+1). When significant differences were found for environment, populations, genotypes or for their interactions, comparisons were performed by means of Fisher's protected least significant difference (LSD) tests (P=0.05). Data were statistically analyzed using Statistix v. 8.0 (Analytical Software, Tallahassee, FL, USA).

Infection of sunflower by O. cumana under field conditions

The assessment of the effect of the date of sowing on the infection of sunflower by the race F of *O. cumana* was performed in one experiment that was conducted in 2000 and 2001. In both seasons the experiment site was established in southern

Spain (Córdoba) (37º51'42" N and 04º48'00" W). Four sowing dates (SD), approximately every 20 days, were compared in both years: February 17 (SD1), March 6 (SD2), March 27 (SD3) and April 6 (SD4). Population SE296 was used at three inoculum densities (ID) (12.5, 25 and 50 mg of broomrape seeds per sunflower plant). Three sunflower genotypes were used: the inbred lines NR5 and P96, which are susceptible and resistant differentials for *O. cumana* race F respectively (Fernández-Martínez *et al.*, 2004; Molinero-Ruiz *et al.*, 2006) and Rodrigo, a commercial hybrid which is moderately resistant to race F (García-Ruiz, 2001). Sunflower seeds were surface-sterilized and induced to germinate following the methodology described in the previous subsection. Small pots (7 × 7 × 8) were filled with 180 g of SSPM that had been homogeneously infested with broomrape seeds at the corresponding ID. Sunflower seedlings were individually planted in each of the pots, grown in shadehouse for 15 days and then transplanted with the soil mixture to the experimental field. Soil was previously tilled and prepared for furrow irrigation. Hand-weeding was performed when necessary for weed control in both experimental seasons.

Each year the experiment was set up as a split-split-plot arranged in a randomized complete block design with four blocks, SD as main factor, genotype as secondary factor and ID as tertiary factor. The design was combined over years as random effect, with SD, genotype and ID fixed effects (McIntosh, 1983). The experimental unit consisted of one row of 15 plants 0.2 m apart. The FDA as well as the incidence of broomrape (BI, percentage of sunflower plants with emerged stems of *O. cumana*) were recorded at the end of the crop cycle. The inbred lines NR5 and P96 had BI values of 100 (complete susceptibility) and 0% (complete resistance) respectively, but high BI of slightly infected sunflower plants were observed in Rodrigo. Since no replicated data of BI were available, and trying to correctly characterize and assess the moderate resistance of Rodrigo, the intensity of the attack (IA) was statistically analyzed, being IA=(BI/100) × FDA. No yield data were recorded because productions of the genotypes were not comparable - Rodrigo is a productive and commercial hybrid while NR5 and P96 are inbred lines used as donors of resistance to race F.

Data transformation of IA according to Log (IA+1) was performed prior to ANOVA. When significant differences were found for the ID, values of IA were subjected to multiple regression analysis. Fisher's protected LSD tests (P=0.05) were used for comparisons of SD, genotypes, years and their interactions. Statistical analyses of data were performed using Statistix v. 9.0.

RESULTS

Infection of sunflower by *O. cumana* under controlled conditions

The infection (FDA) of sunflower by *O. cumana* depended significantly on environment (P=0.0001), sunflower genotype and *O. cumana* population (P<0.0001

both). Also the interactions environment × genotype, environment × population and genotype × population had a significant effect on FDA ($P \leq 0.0031$).

Greenhouse was more favorable for *O. cumana* infection than controlled conditions in growth chamber (4.8 and 1.9 broomrape stems per sunflower plant respectively, averaged across genotypes and populations) (Figures 1a, b). The inbred line P96 was fully resistant to all the populations of *O. cumana* in both environments, and genotypes J8281 and Turbo had significantly higher values of infection than Kruglik A-41 (5.1 and 4.7, and 3.5 broomrape stems per sunflower plant respectively, averaged for environments and for populations) (Figures 1a, b).

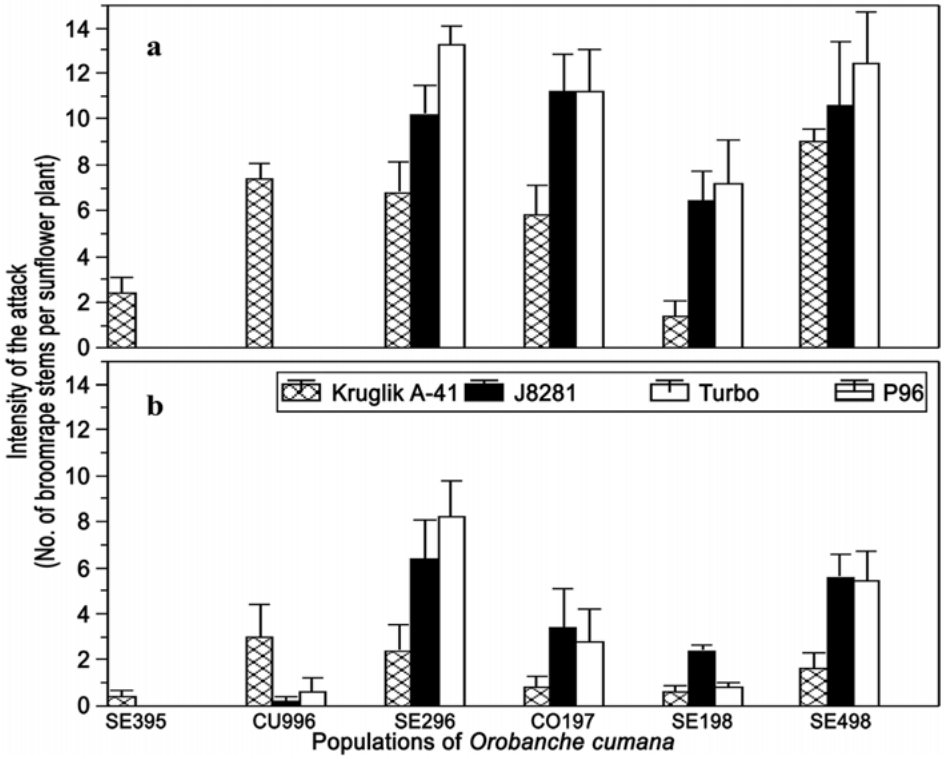

Figure 1: Final degree of attack by six populations of Orobanche cumana in four sunflower genotypes with resistance to the parasite under two different environments: greenhouse at 10 to 32ºC (a) and chamber of controlled conditions at 20 to 25ºC (b), both with photoperiod of 14 hours/day. Vertical upper bars represent the standard error of the mean of five replications over two experiments (n=10).

When the interaction genotype × population was analyzed, J8281 and Turbo showed a higher susceptibility to SE296, CO197, SE198 and SE498 as compared to Kruglik A-41 in both greenhouse (Figure 1a) and growth chamber (Figure 1b). Populations CO197, SE198 and SE498 were identified as race F because they were not controlled by Or_5 in Turbo. Population SE296, previously identified as race F

(Akhtouch *et al.*, 2002), was selected for the subsequent field experiment because it exhibited a good infectivity in both environments (Figure 2b). Populations SE395 and CU996 only infected Kruglik A-41 (1.4 and 5.2 broomrape stems per sunflower plant averaged across environments, respectively). Therefore they were characterized as race B. Infections by these populations were moderate in greenhouse (Figure 1a) and low in growth chamber (Figure 1b).

Concerning the interaction genotype × environment, no significant differences of FDA between Turbo, J8281 and Kruglik A-41 were obtained in greenhouse (average 6.4 broomrape stems per sunflower plant across populations and genotypes). Kruglik A-41 had a lower susceptibility in the chamber (1.5 broomrape stems per sunflower plant averaged across populations) as compared to the one of Turbo and J8281 in this same environment (3 broomrape stems per sunflower plant averaged across populations and genotypes) (Figure 2a). As previously mentioned, P96 was not infected neither in greenhouse nor in growth chamber.

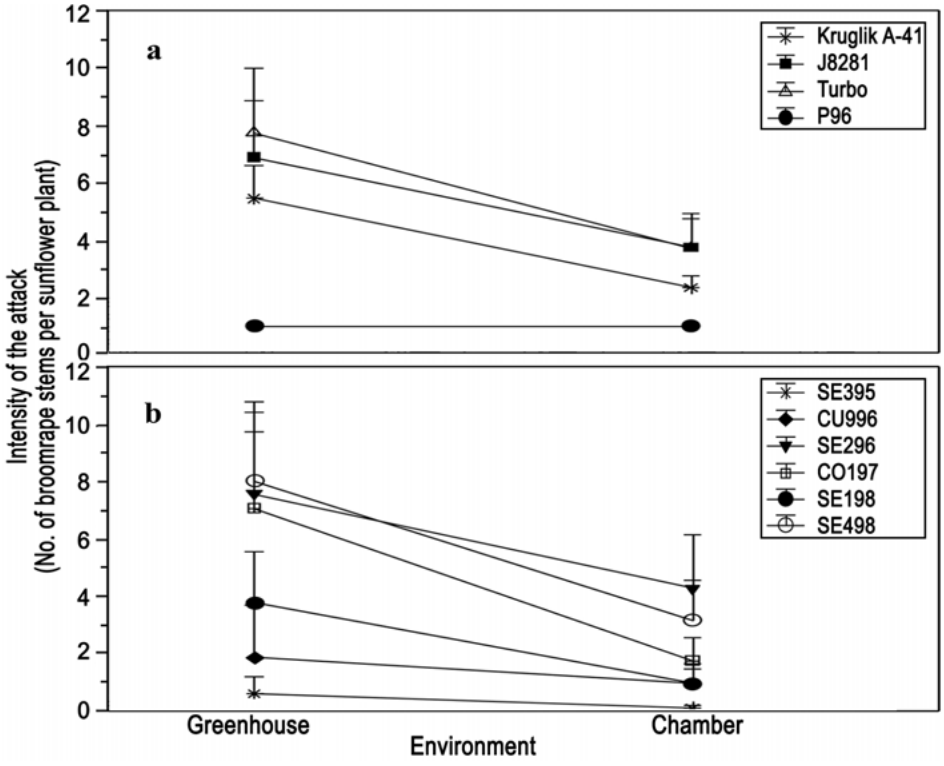

Figure 2: Effect of the environment (greenhouse or chamber of controlled conditions) on the infection of four sunflower genotypes (critical least significant difference value for environment × genotype=0.5) (a), and caused by six populations of Orobanche cumana (critical least significant difference value for environment × population=0.8) (b), expressed as final degree of attack.

Finally, when the effect of the environment on the infectivity of *O. cumana* was considered (environment × population), SE296, CO197 and SE498 showed similar and high values of FDA in greenhouse (average 7.5 *O. cumana* stems per sunflower plant across genotypes), and moderate (SE296 and SE498) or low (CO197) levels in growth chamber (averages 3.7 and 1.7 *O. cumana* stems per sunflower plant respectively, across genotypes) (Figure 2b). Statistical differences were also found between the infectivity of SE198 in both environments: 3.7 and 0.9 *O. cumana* stems per sunflower plant averaged across genotypes, in greenhouse and growth chamber respectively (Figure 2b).

Weather conditions

Maximal, minimal and mean daily air temperatures from mid February to the end of May were in the range of the long-term regional average during both growing seasons, although milder conditions occurred in 2000 as compared to 2001. Average maximal temperature was 0.4ºC higher in 2001 as compared to 2000. On the contrary, average minimal temperature was 0.4ºC higher in 2000 than in 2001. A slight difference of 0.2ºC was recorded between averaged mean temperatures along the period in both seasons (Figure 3). Rainfall measured from September 1999 to June 2000 was 313 mm. The experiment received 469 mm of rainfall along the same monthly period in the following season. Two furrow irrigations of 500 mm each were applied to sunflower before flowering and at maturity in both seasons. Therefore the total water input (rainfall plus irrigation) amounted to 1313 mm in 2000 and 1469 mm in 2001.

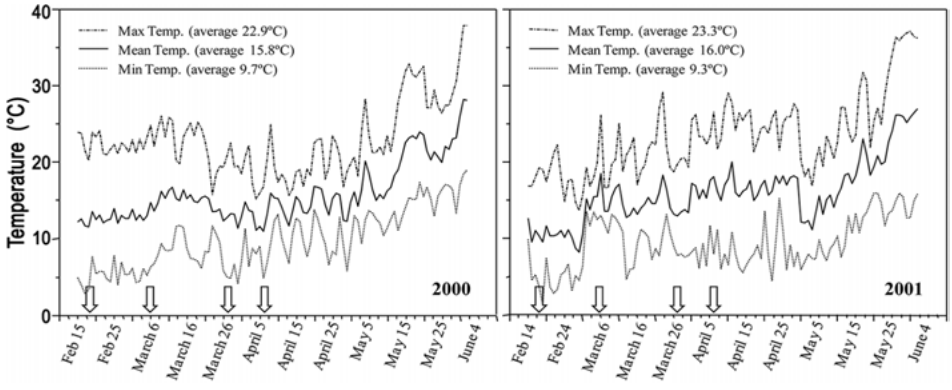

Figure 3: Maximal, minimal and mean daily air temperatures from Feb 15 and 14, to June 4 in 2000 and 2001 respectively. The four sowing dates in each season are indicated with arrows.

Infection of sunflower by *O. cumana* under field conditions

Significant effects of the sources of variation in the ANOVA model for the combined experiment are presented in Table 2. The IA by *O. cumana* for years, SD, ID and genotypes is presented in Table 3.

Broomrape attacks were significantly (P=0.0072) more intense in 2001 as compared to 2000. Concerning the inoculum densities, a significant (P=0.0404) effect on the occurred. Since significant (P=0.0008) differences of IA were also found for genotypes, the continuous course of IA with ID was independently analyzed in NR5 and Rodrigo. The attack of *O. cumana* in P96 was zero irrespective of ID and SD. The best fits of data were obtained as quadratic functions of IA with ID (Figure 4).

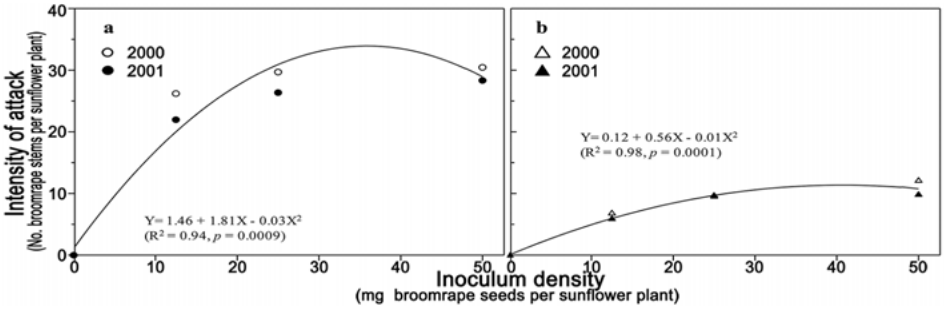

Figure 4: Intensity of the attack of sunflower inbred line NR5 (a) and hybrid Rodrigo (b) by Orobanche cumana race F in field experiments conducted in 2000 and 2001 as a function of inoculum density.

Table 2: Analysis of variance to test the effect of year, sowing date, and inoculum density of *Orobanche cumana* on the intensity of the attack by the parasite in three genotypes of sunflower

Sources of variation [a]	df	MS	F		P
Year (Y)	1	M1	M1/M2	15.8900	0.0072
Year / Block (Y / B)	6	M2			
Sowing date (SD)	3	M3	M3/M4	9.2961	0.0459
Y × SD	3	M4	M4/M5	7.9336	0.0014
Y × B × SD	18	M5			
Genotype (G)	2	M6	M6/M7	1237.4271	0.0008
Y × G	2	M7	M7/M9	4.1458	0.0423
Y × B × G	12	M9			
SD × G	6	M8	M8/M11	45.3228	0.0000
Y × SD × G	6	M10	M10/M11	5.3307	0.0005
Y × B × SD × G	36	M11			
Inoculum density (ID)	2	M12	M12/M13	23.7686	0.0404
Y × ID	2	M13	M13/M20	1.4565	0.2365
SD × ID	6	M14	M14/M16	1.1068	0.4526
G × ID	4	M15	M15/M17	5.3054	0.0675
Y × SD × ID	6	M16	M16/M20	2.2391	0.0427
Y × G × ID	4	M17	M17/M20	2.5978	0.0387
SD × G × ID	12	M18	M18/M19	0.4512	0.9088
Y × SD × G × ID	12	M19	M19/M20	2.6739	0.0028
Pooled error b	144	M20			
Pooled error	287				

[a] The field experiment was designed as a split-split-plot arranged in a randomised complete block design combined over years (random effect) with sowing date, genotype and inoculum density (fixed effects) as treatments (McIntosh, 1983).

Table 3: Intensity of the Attack by *Orobanche cumana* race F in three genotypes of sunflower sown in four dates. Three densities of inoculum of the parasite were used for sunflower infection and the experiment was conducted under field conditions in 2000 and 2001

Sowing date	Density of inoculums (mg seed per sunflower plant)	IA [a, b]					
		2000			2001		
		NR5	Rodrigo	P96	NR5	Rodrigo	P96
SD1	ID1 (12.5)	25.3 ± 1.31 [c]	9.3 ± 1.32	0 ± 0	34.6 ± 1.04	12.2 ± 2.37	0 ± 0
	ID2 (25)	33.5 ± 2.64	14.3 ± 2.70	0 ± 0	38.6 ± 0.64	17.9 ± 2.72	0 ± 0
	ID3 (50)	37.0 ± 0.61	16.4 ± 2.26	0 ± 0	39.1 ± 0.83	18.6 ± 3.22	0 ± 0
	Mean	31.9 ± 1.73	13.3 ± 1.45	0 ± 0	37.5 ± 0.75	16.2 ± 1.69	0 ± 0
SD2	ID1 (12.5)	31.7 ± 2.04	10.7 ± 1.85	0 ± 0	31.1 ± 1.56	10.4 ± 1.98	0 ± 0
	ID2 (25)	38.0 ± 0.73	16.3 ± 0.68	0 ± 0	33.1 ± 1.06	12.3 ± 2.37	0 ± 0
	ID3 (50)	36.2 ± 0.43	14.9 ± 1.85	0 ± 0	32.9 ± 3.69	11.1 ± 1.98	0 ± 0
	Mean	35.3 ± 1.04	14.0 ± 1.09	0 ± 0	32.4 ± 1.27	11.3 ± 1.13	0 ± 0
SD3	ID1 (12.5)	21.7 ± 1.55	2.5 ± 0.66	0 ± 0	23.3 ± 1.84	1.9 ± 0.37	0 ± 0
	ID2 (25)	21.9 ± 1.64	5.0 ± 1.22	0 ± 0	25.9 ± 2.76	3.7 ± 0.81	0 ± 0
	ID3 (50)	24.0 ± 5.50	5.7 ± 1.31	0 ± 0	27.3 ± 4.31	5.2 ± 1.03	0 ± 0
	Mean	22.6 ± 1.82	4.4 ± 0.71	0 ± 0	25.5 ± 1.71	3.6 ± 0.58	0 ± 0
SD4	ID1 (12.5)	9.1 ± 1.67	1.0 ± 0.36	0 ± 0	11.1 ± 1.37	2.7 ± 1.19	0 ± 0
	ID2 (25)	12.2 ± 0.78	2.7 ± 0.83	0 ± 0	20.7 ± 0.92	3.8 ± 0.87	0 ± 0
	ID3 (50)	16.0 ± 2.71	1.8 ± 0.83	0 ± 0	22.4 ± 3.02	13.2 ± 1.50	0 ± 0
	Mean	12.4 ± 1.31	1.9 ± 0.43	0 ± 0	19.4 ± 1.40	6.6 ± 1.56	0 ± 0
Mean		25.5 ± 1.49	8.4 ± 0.91	0 ± 0	28.7 ± 1.19	9.4 ± 0.94	0 ± 0

[a] IA = (BI/100) * DA at the end of the crop cycle, being BI = percentage of sunflower plants with emerged broomrape stems and DA = number of broomrape stems per sunflower plant.

[b] Least significant differences for comparing the intensity of the attack were: 0.3, 0.9, 1.2, 0.5, 0.7 and 1.2, for year, SD, year × SD, genotype, year × genotype and SD × genotype respectively.

[c] Data are the means ± standard error (SE) of four replications of 15 plants.

Significant differences of IA were found for SD as well (P=0.0459), but most interesting were the significant interactions between years, SD, and/or genotypes ($P \leq 0.0427$) (Table 2).

When the interaction year × SD was considered, early sowings (SD1 and SD2) were associated to the highest IA in both years (15.7 and 16.2 broomrape stems per sunflower plant averaged across ID, genotypes and SD for 2000 and 2001 respectively) (Table 3). In contrast, the lowest broomrape attacks were recorded for the latest sowing date (SD4) in 2000 (4.8 broomrape stems per sunflower averaged for ID and genotypes) (Table 3 and Figure 5).

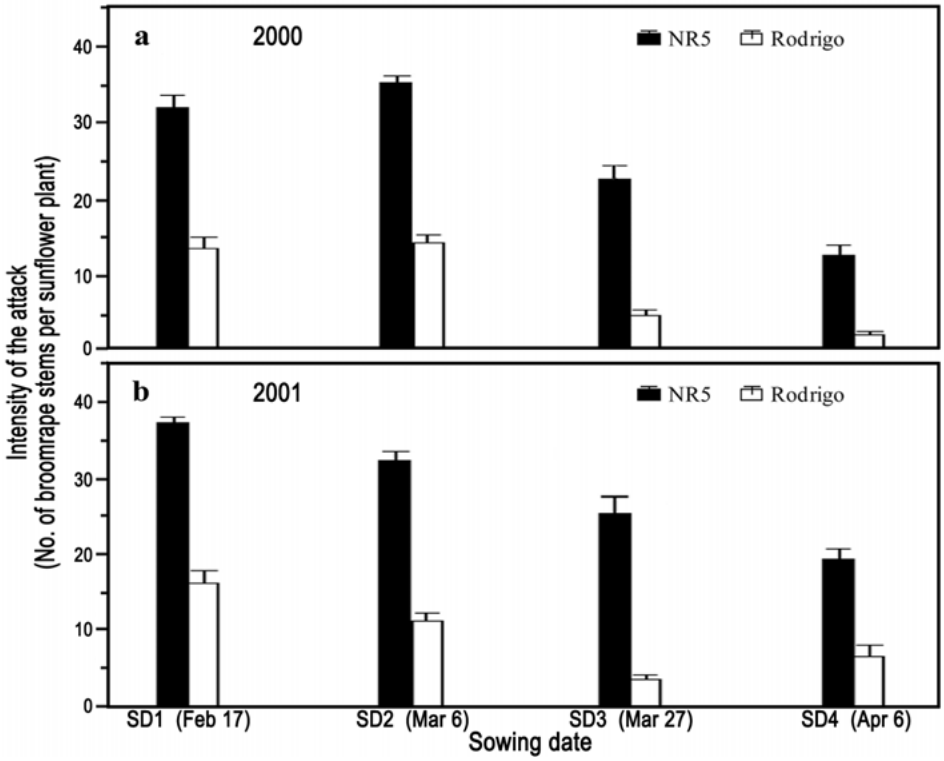

Figure 5: Effect of four different sowing dates on the intensity of the attack (IA) by Orobanche cumana race F in sunflower genotypes with resistance to the parasite grown in irrigated field experiments. Since a significant effect of the interaction year × sowing date was obtained, the data are presented by years. The IA was calculated as IA=(broomrape incidence/100)× final degree of attack. Vertical upper bars represent the standard error of the mean of four replications of 15 plants and across the three ID (n=12).

Concerning the interaction year × genotype, in both years the highest IA occurred on NR5, and IA was zero in P96 (Table 3). The moderate resistance of Rodrigo was shown by intermediate and significantly (P=0.0423) different values of IA in 2000 and 2001 - 8.4 and 9.4 broomrape stems per sunflower plant averaged across ID and SD, respectively (Table 3).

Most interesting was the significant ($P<0.0001$) interaction SD × genotype. The highest infections occurred in NR5, although SD3, and mainly SD4, were related to low values of IA in this line (averages 24.0 and 15.9 broomrape stems per sunflower plant across years, respectively) (Figure 5).

The moderate resistance of Rodrigo was expressed through a significantly lower IA than the one in NR5 irrespective of SD. Even when Rodrigo was sown at the most favorable time for *O. cumana* infection (SD1 or SD2) the IA by the parasite was lower as compared to the one in NR5 (13.7 and 34.3 broomrape stems per sunflower plant averaged for SD1 and SD2 across ID and years, respectively) (Table 3). The level of control achieved by the moderate resistance of Rodrigo was highest at late sowing dates SD3 and SD4 (4.0 and 4.2 broomrape stems per sunflower plant averaged across ID and years, respectively) (Table 3).

DISCUSSION AND CONCLUSIONS

Delayed sowings have been recommended in order to prevent severe attacks of *O. crenata* in susceptible cultivars of winter legumes (Mesa-García and García-Torres, 1986; Arjona-Berral *et al.,* 1987; Rubiales *et al.,* 2003), but early sowings are considered as part of an integrated control strategy of *O. crenata* when partially resistant cultivars are sown (Pérez-de-Luque *et al.,* 2004). Under Mediterranean conditions, early sowing of spring sunflower was recommended for growing a very susceptible confectionary cultivar in moderately broomrape-infested areas (Castejón-Muñoz *et al.,* 1993). Similarly, a delayed sowing date was found to be associated with increases in *O. cumana* infestation and yield losses in oilseed sunflower in Romania (Grenz *et al.,* 2008). However, when oilseed cultivars of sunflower with resistance to *O. cumana* were early planted in Israel, their resistance was not expressed (Ish-Shalom-Gordon *et al.,* 1994). The results of our work, in agreement with those of Alvarado-Aldea *et al.* (1998), suggest that the modification of the SD affects differently the natural infection by *O. cumana* in susceptible and moderately resistant sunflower. While high and statistically similar attacks were observed in the susceptible genotype NR5 irrespective of the SD and of the ID in both growing seasons, the reduction of the attack in Rodrigo was significant at all SD and higher at late SD as compared to early sowings. The horizontal resistance of crops to parasites is highly affected by environmental conditions (Simmonds, 1991; McDonald and Linde, 2002; Stuthman *et al.,* 2007; Wallwork, 2009). In fact, the strong effect of the environment on the expression of the resistance of sunflower hybris to *O. cumana* race F has been reported, with these hybrids yielding twice as much as susceptible ones under low water availability and high intensities of *O. cumana* attack (Molinero-Ruiz *et al.,* 2009). From our results, we can conclude that a delay of sowing causes a shift of environmental conditions that can be associated to an enhanced expression of the moderate resistance of hybrids of sunflower against the race F of *O. cumana* under our conditions of sufficient water availability. The effect

of a shift of sowing date on their performance in fields infested by *O. cumana* and under drought conditions as compared to irrigation might be investigated in the future.

On the other hand, we found that the response of IA to ID followed a quadratic response in both susceptible and moderately resistant genotypes. Previous field works have determined that the intensity of the attachment of *O. cumana* to susceptible sunflower versus ID fitted a rectangular hyperbolic function (Grentz *et al.,* 2008). The different fitting obtained in both works could be due to differences in methodologies for host plants inoculation and ID quantification, as well as to different ranges of ID in each work. In any case, and in agreement Grenz et al. (2008), the negative response of IA to high ID could be due to competition between parasite seeds for the successful establishment in the host. According to our results in pot experiments (unpublished data), the competition between seeds of *O. cumana* at very high ID can importantly weaken the root system of the host at early growth stages, decreasing its vigor and therefore its potential to sustain a high number of full-grown plants of *O. cumana*. Of particular importance is the fact that in our work a quadratic equation fitted the response of IA to ID in the case of the moderately resistant genotype Rodrigo as well.

As mentioned, the modification of the SD is related to environmental changes under which the establishment and development of *O. cumana* in sunflower take place. Since our field experiments were conducted under irrigation, a shift of temperature conditions seem to be the major factor for the decrease of broomrape infection in the crop as SD is delayed. Moreover, it could also be related to the differential effect of the SD on the final broomrape infection in susceptible and resistant genotypes (significant interaction SD × genotype). In fact, Eizenberg *et al.* (2012) have recently validated a thermal time model based on the main role of temperature on the parasitism of *O. cumana* in susceptible irrigated sunflower in Israel. Therefore the relationship between SD, temperature conditions (*i.e.* thermal time) and their effect on establishment, development and final field infection by *O. cumana* in moderately resistant sunflower should be explored in the future.

The strong influence of the temperature on different stages of the biological development of *O. cumana* under controlled conditions is widely documented (Nandula *et al.,* 1996; Sukno *et al.,* 2001; Eizenberg *et al.,* 2003a, b; Ephrat and Eizenberg, 2010). In our work, temperatures of 10 to 32ºC in the greenhouse provided a more conducive environment for *O. cumana* infection than 20 to 25ºC in chamber. Since plants were watered as needed and a 14-h photoperiod was used in both environments, differences of FDA between both environments show, in accordance with our results from the field experiments, that temperature played the major role in the successful establishment and subsequent emergence of the parasite in the host. Surprisingly, greenhouse conditions favored the infection of populations of *O. cumana* belonging to race F but not the one of populations of the less virulent race B. Future research should explore the hypothesis that a fitness cost of the virulence

is related to a low adaptability of *O. cumana* to environmental changes, as reported for other parasites (Zhan and McDonald, 2013).

Under Mediterranean conditions, early sunflower sowings are recommended in order to obtain yield increases provided by high water availability (Gimeno *et al.*, 1989). However, the management of the crop might be different when *O. cumana* race F exists in the field. The planting of moderately resistant sunflower in highly infested fields and under dryland conditions has been reported, in spite of the development of some *O. cumana* plants in them, as a good control measure because of the good performance of these hybrids with low water availability and high parasite pressure (Molinero-Ruiz *et al.*, 2009). Other authors have mentioned the benefit of using crop cultivars with moderate resistance to *Orobanche* spp. because they can slow down parasite development and prevent seed production (Pérez-de-Luque *et al.*, 2004). The results of our work show that, under irrigation, late sowings (from the end of March until the beginning of April) of sunflower hybrids with moderate resistance to *O. cumana* race F hinder the establishment of the parasite irrespective of the infestation level, and can be therefore recommended as an efficient control method. Future works should address the advisability of late sowings when moderately resistant hybrids are grown under dryland conditions. Alternatively, and when levels of *O. cumana* infestation are low, growers might give priority to the increase of yield through high water availability. In this case very early or even winter sunflower planting might be convenient, but then the control of broomrape infections should be achieved with imidazolinone herbicides that must be applied to imidazolinone-resistant sunflowers. As a conclusion, our work shows that *O. cumana* race F can be efficiently controlled in irrigated sunflower through the use of moderately resistant hybrids and the delay of the sowing date, and constitutes a good example of combination of genetic and cultural methods in crop protection.

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UTILIZACIÓN DE LA MODIFICACIÓN DE LA FECHA DE SIEMBRA Y LA RESISTENCIA GENÉTICA EN EL MANEJO DEL JOPO DE GIRASOL (*Orobanche cumana* Wallr.)

RESUMEN

La mala hierba parásita *Orobanche cumana* Wallr. (jopo) limita la producción de girasol en el este y sur de Europa y en el Medio Este. La resistencia de los híbridos de girasol a la raza F de *O. cumana*, que está ampliamente distribuida en los países productores de girasol, incluido España, no es completa. Se estudió, en invernadero (10 a 32ºC) y en cámara de crecimiento (20 a 25ºC), la infección de seis poblaciones de *O. cumana* (razas B y F) en cuatro genotipos de girasol. También se investigó el efecto de cuatro fechas de siembra (SD) sobre la intensidad del ataque de genotipos de girasol por la raza F de *O. cumana* a tres densidades de inóculo, en campo y con riego, en 2000 y 2001. El invernadero fue más favorable que la cámara de crecimiento para la infección por *O. cumana*, la cual fue máxima en el caso de las poblaciones de raza F. En el experimento de campo se obtuvo una reducción significativa de la intensidad del ataque en el híbrido moderadamente resistente con todas las fechas de siembra y, en ambos años, la reducción fue mayor con siembras tardías en comparación a las siembras tempranas. Las siembras tardías (desde finales de marzo hasta principios de abril) favorecen un incremento en la expresión de la resistencia de girasol a la raza F de *O. cumana* independientemente de la cantidad de inóculo en el suelo, y pueden por lo tanto recomendarse, en condiciones de riego y junto con la siembra de híbridos de girasol moderadamente resistentes, como parte de una estrategia efectiva para controlar esta mala hierba parásita.