

**SUNFLOWER WHITE ROT INCIDENCE: COMBINING ABILITY OF INBRED  
LINES AND RELATIVE PERFORMANCE OF HYBRIDS IN TWO  
ENVIRONMENTS**

**Marcela Godoy**, Escuela de Postgrado en Producción Vegetal, UNMdP-INTA,  
CC 276, RA 7620 Balcarce, Argentina  
Fax: 54 22 66 42 17 56; e-mail: [claugo@yahoo.com](mailto:claugo@yahoo.com)

**Fernando Castaño**, Unidad Integrada Balcarce,  
CC 276, RA 7620 Balcarce, Argentina  
Fax: 54 22 66 42 17 56; e-mail: [fcastanio@balcarce.inta.gov.ar](mailto:fcastanio@balcarce.inta.gov.ar)

**José Ré**, Monsanto Global Seed Group,  
Estación Experimental Camet, RN 226, Km 7, RA 7600 Mar del Plata, Argentina  
Fax: 54 22 34 64 28 27; e-mail: [JOSE.V.RE@la2.monsanto.com](mailto:JOSE.V.RE@la2.monsanto.com)

**Raúl Rodríguez**, Unidad Integrada Balcarce,  
CC 276, RA 7620 Balcarce, Argentina  
Fax: 54 22 66 42 17 56; e-mail: [rhrdriguez@balcarce.inta.gov.ar](mailto:rhrdriguez@balcarce.inta.gov.ar)

**Alberto Escande**, Unidad Integrada Balcarce,  
CC 276, RA 7620 Balcarce, Argentina  
Fax: 54 22 66 42 17 56; e-mail: [aescande@mdp.edu.ar](mailto:aescande@mdp.edu.ar)

**Summary:**

This study shows the variation in performance of sunflower F1 crosses in relation to white rot incidence produced by *Sclerotinia sclerotiorum* inoculations on capitula across two environments. The combining ability of 14 parent inbred lines was also studied. Trials were conducted at two locations. After inoculation, at each location, sunflower capitula were treated differently. Analysis of variance over locations detected highly significant differences in hybrid performance and hybrid x location interaction. Thirty-four percent of the F1 crosses was placed in the same group of resistance level in both locations. When compared with a resistant check, good levels of resistance were observed in three F1 crosses across environments. Analysis of variance detected highly significant differences between combining abilities effects. General combining ability estimated values varied by 34% for female lines and 38% for male lines, whilst the range between specific combining ability values was 53%. It was possible to identify some inbred lines, which produced F1 crosses with a relative superior performance (i.e. low incidence of white rot), their direct use in sunflower cultivar development programs could be considered. However, further studies must be conducted to make a more detailed examination of the environmental factors responsible for the hybrid x location interaction. The effect of other independent components of partial resistance, such as the incubation period, need also to be studied further. The identification of genotypes presenting different behaviour to each component of resistance may allow combining resistance factors in commercial hybrids that could present improved levels of disease resistance across environments.

## Introduction

*Sclerotinia sclerotiorum* can restrict the productivity of sunflower (*Helianthus annuus* L.), especially when this oil crop is grown in humid and temperate environments. These environments often provide conditions favourable to both infection and disease development (Sackston, 1992). Although *S. sclerotiorum* parasites different organs of the sunflower plant, the infections on the capitula produce the greatest loss in the main sunflower-growing regions (e.g. Argentina, Europe, China). This type of attack is commonly known as white rot disease (Vear and Tourvieille de Labrouhe, 1984). Available fungicide products are ineffective against white rot disease. Resistant hybrids remain as the main tool for farmers that have to manage this particular pathosystem (Alvarez *et al.*, 1999; Godoy *et al.*, 1999). Therefore, breeding programs are actively working on developing such genotypes. In development programs of sunflower cultivars the assessment of the performance of parental inbred lines in hybrid combination and the evaluation of these F1 crosses in different environments are needed to detect inbred lines producing by crossing good levels of white rot resistance and gain a better understanding of the factors controlling resistance. This is the aim of this work.

## Materials and Methods

Fourteen sunflower inbred lines (Tables 1 and 2) of different origins, of which 7 were cytoplasmic male-sterile (females) and 7 fertility-restorer (males), were utilised as parents in a genetic factorial mating design. Twelve of them (6 females and 6 males) had good agronomic characteristics and came from the sunflower-breeding program of Dekalb Argentina (now Monsanto Group). The other two, SD (female) and PAC1 (male), were obtained from INRA, Clermont Ferrand, France. SD x PAC1 cross could be considered as a resistance check because it has a high performance against white rot (Castaño *et al.*, 1992, 1993; Godoy *et al.*, 1999). The 49 F1 crosses were sown in the Experimental Station of Monsanto in Camet, Mar del Plata, and in the "Unidad Integrada" in Balcarce, Argentina, according to a randomised complete block design with three replications. Each experimental plot had at least 15 plants. Due to seed problems two hybrids (ADK3 x R200; ADK5 x R199) were excluded from inoculations.

Inoculation was made following the French protocol (Vear and Tourvieille de Labrouhe, 1984). All capitula were inoculated when approximately three external rows of hermaphrodite disk flowers were in pistillate stage [R5.3 stage of sunflower development (Schneider and Miller, 1981) or its homologous E3 (Cetiom, 1992)]. The floral surface of each capitulum was sprayed with 5 ml of an aqueous suspension containing approximately 5000 ascospores/ml. Inoculations were made twice a week, each plant being infected once at the correct stage. Environment at each location differed mainly in the way that sunflower capitula were treated after inoculation and in the irrigation procedure. At Balcarce the inoculated capitula were immediately covered with sulphured paper bags in order to avoid drying and a commercial sprinkler overhead irrigation system was utilised to apply water at the rate of 5 mm, twice per week, until the end of the experiment. At Camet the inoculated capitula were kept uncovered and an overhead micro sprinkler system was utilised to irrigate two or three times a day keeping a high humidity level throughout the day. After two weeks, each capitulum was observed twice a week to detect first symptoms of white rot on the back of it. The disease incidence, as a percentage, was calculated as in earlier work (Alvarez *et al.*, 1999; Castaño *et al.*, 1993; Godoy *et al.*, 1999) for each F1 cross at maturity as: [the number of capitula showing symptoms after inoculation/number of inoculated capitula] x 100.

Nested analysis of variance combined over locations and least significance difference test (LSD) were made using the MSTATC software. The analysis of Model I (where parental

inbred lines are the genotypes under consideration) provided two independent estimates of General Combining Ability (GCA) effects, one for females and one for males, and one estimate for Specific Combining Ability (SCA) effects (males x females). A F-test was used to detect if differences between effects were significantly different.

The values for the two missing hybrids were estimated using the missing data formula currently utilised to allow estimations of GCA for each parental line. The relative magnitude and sign of the effect for each female and male inbred line and for each cross was made as previously reported (Castaño 1992; Castaño et al, 1992). For each F1 cross, the centred value  $V_c$  was calculated (data not shown) from the difference between its observed mean value across locations and the general mean of the two trials ( $\mu$ ). The GCA values for every male and female inbred line (GCAM and GCAf) were obtained by subtracting " $\mu$ " from the mean value of their respective F1 crosses in the two experiments. GCA value refers to the average performance of the line in crosses with other parents. The SCA values for each cross was obtained as  $SCA_{mf} = V_c - GCAM - GCAf$ . SCA value is the performance of the line in a cross with a specific parent. In all cases a negative sign indicates favourable effects related to the decrease of white rot incidence.

## Results and Discussion

### 1.- Hybrid performance

In Table 1, the mean values of *S. sclerotiorum* incidence over two environments are shown for all F1 crosses. The general mean was 62 %. The estimation of coefficient of variation (CV= 26 %) indicates an acceptable precision in the observed data.

**Table 1.** Average of *S. sclerotiorum* incidence in 49 F1 crosses over two environments.

<i>Females</i>	8-ADK1	9-ADK2	10-ADK3	11-ADK4	12-ADK5	13-ADK6	14-SD	Mean
<i>Males</i>								
1- R 122	85	93	78	84	90	63	69	80
2- R 152	91	62	40	64	87	67	34	64
3- R 161	71	52	37	58	76	40	57	56
4- R 167	79	71	46	81	82	46	61	67
5- R 199	59	34	21	71	( 61 )	27	21	42
6- R 200	75	68	( 53 )	81	79	64	55	68
7- PAC1	79	53	59	35	90	65	34	59
<b>Mean</b>	77	62	48	68	81	53	47	62

CV = 26 %.

F-hybrids = 9.13\*\*, highly significant ( $P \leq 0.001$ ).

F-hybrid x location interaction = 4.23\*\*, highly significant ( $P \leq 0.001$ ).

(...): estimated values.

Analysis of variance did not detect any significant difference between the mean values of the two locations. This result indicates that neither the different management practices followed at each site nor the differences in other environmental variables that could exist between Camet and Balcarce produced significant variation between environmental means. Highly significant differences ( $P \leq 0.001$ ) between the hybrid responses to *S. sclerotiorum* inoculations were found. The hybrid x location interaction was also highly significant ( $P \leq 0.001$ ); therefore the relative performance of hybrids across environments was statistically different. Then it was necessary to make a complementary analysis in order to study the hybrid behaviour in each location.

In Camet, the estimated general mean was 67 % with a range between the most and the least diseased hybrids of 93 %. The calculated coefficient of variation was 21 %. The analysis of variance allowed detection of highly significant ( $P \leq 0.001$ ) differences between F1 crosses. By utilising the estimated LSD ( $P = 0.05$ ) value of 23 %, F1 crosses were placed in three groups (G1, G2, G3) according to similar levels of resistance. The 20 hybrids included in the G1 group had the greatest proportion of diseased capitula (low-resistance level group). G3 was made up by 5 hybrids that had a disease incidence similar to that one estimated for the SD x R152 cross, the hybrid with the lowest proportion of diseased capitula in Camet (high-resistance level group). Meanwhile the rest of the hybrids were included in G2, an intermediate group between G1 and G3.

In Balcarce, the general incidence mean was 58 % with a difference between extreme hybrids of 73 %. The CV calculated for this site was 30 %. The analysis of variance for this trial also detects highly significant differences ( $P \leq 0.001$ ) between F1 crosses. The LSD ( $P = 0.05$ ) was 28 %, value utilised to classify crosses into the three groups G1, G2 and G3. Twenty hybrids were grouped in G1. The 13 hybrids placed in the G3 group were those showing no differences with the ADK3 x R 199 cross which had the lowest incidence in this location. The rest of the hybrids were included in G2.

In both locations four hybrids (ADK3 x R199; ADK6 x R199; SD x R199; SD x PAC1) were placed in the same G3 group. These four crosses did not interact with the environment and displayed always the highest level of resistance. Whereas 12 F1 crosses (ADK1 x R122; ADK1 x R152; ADK1 x R167; ADK1 x PAC1; ADK2 x R122; ADK3 x R122; ADK4 x R122; ADK4 x R200; ADK5 x R122; ADK5 x R152; ADK5 x R167; ADK5 x PAC1) were located in the group showing high incidence (G1). Therefore, more than a quarter of the evaluated F1 crosses showed the lowest level of resistance regarding location or environment management practices. By conducting this type of evaluations it is possible to identify genotypes that show superior or inferior performance across environments. Thus it is a useful tool to utilise in selection programs for white rot resistance. The larger range of diseased capitula found in Camet suggests that in this location utilising the environmental-managing-practices described, the genotypic differences among hybrids could be enhanced. For example in Camet 5 hybrids (ADK5 x R122; ADK5 x R167; ADK5 x R200; ADK1 x R152; ADK1 x R200) had the maximal incidence (100%) and the hybrid SD x R152 showed only 7 % of diseased capitula. The appearance of some crossed results, the most prominent one happened with the ADK1 x R199 cross (G1 in Camet and in G3 in Balcarce), suggest that more environments may be needed to safely classify some particular genotypes. A piece of information that could help to decide the value of a genotype could be the estimation of the relationship between artificial and natural infections.

More studies on the importance of the genotype x environment interaction for the sunflower-*S. sclerotiorum* pathosystem could permit to determine whether it is necessary to divide the large sunflower geographical area into smaller sub areas with the purpose of improving the white rot resistance evaluation. Consequently, this could allow improvement of the efficiency of allocating resources dedicated to cultivar evaluation. Complementary studies on the stability of cultivars for white rot resistance should be carried out in order to enhance the understanding of the suitability of hybrids for the different growing conditions that they are like to encounter.

## 2.- Combining Ability Studies

Table 2 shows GCA and SCA estimated values for the 14 sunflower inbred lines. Negative values in GCA indicate that these mean parental values are lower than the general mean; these lines produce an offspring average value smaller than the mean incidence of all F1 crosses evaluated. Negative values in SCA describe those F1 combinations showing a proportion of diseased capitula inferior to that expected of the utilization of parental GCA values.

**Table 2.** General (GCA) and specific (SCA) combining abilities values estimated from 14 inbred lines

<i>Females</i>	8-ADK1	9-ADK2	10-ADK3	11-ADK4	12-ADK5	13-ADK6	14-SD	<b>GCA<sup>(#)</sup></b>
<i>Males</i>								
1- R 122	<sup>(§)</sup> -10	13	12	-2	-9	-8	4	18
2- R 152	12	-2	-10	-6	4	12	-15	2
3- R 161	0	-4	-5	-4	1	-7	16	-6
4- R 167	-3	4	-7	8	-4	-12	9	5
5- R 199	2	-8	-7	23	<sup>(*)</sup> //	-6	-6	-20
6- R 200	-8	0	<sup>(*)</sup> //	7	-8	5	2	6
7- PAC1	5	-6	14	-30	12	15	-10	-3
<b>GCA<sup>(+)</sup></b>	15	0	-14	6	19	-9	-15	

<sup>(#)</sup> = Male line GCA values; <sup>(+)</sup> = Female line GCA values; <sup>(§)</sup> = Male x female SCA values;

<sup>(\*)</sup> = Non-estimated.

The estimated values of GCA for males varied from -20 % to 18 %. Inbred lines R161, R199 and PAC1 showed favourable values when they were used as males in crosses. GCA estimated values for females varied from -15 % to 19 %. In this case, the inbred lines ADK3, ADK6 and SD were those showing the favourable values. Therefore if the main objective of a sunflower breeding program is to develop hybrids with good performance against white rot, the male inbred lines R161, R199 and PAC1 and the female inbred lines ADK3, ADK6 and SD could be considered as good combiners.

The calculated SCA values varied from -30 % to 23 %. The F1 hybrid obtained by crossing ADK4 x PAC1 showed the most favourable SCA estimated value and it was 30 % more resistant than expected from the parental GCA values. On the other hand, the hybrid ADK4 x R199 had the highest and unfavourable SCA value (23 %). Both hybrids with extreme SCA values have the female line ADK4 in common. It appears that ADK4 has resistance genes that may augment the reaction type (i.e. favourable or not) of the resulting specific cross when they are combined with those of PAC1 or R199.

Because of the absence of two F1 crosses two different factorial analyses needed to be made in order to distinguish statistic differences between the parental average performances. Also this type of analysis allows evaluation of whether the observed deviation of individual F1 crosses is significantly different from the average performance of each common parent. The results of these analyses are shown in Table 3. All F1 crosses were used in Analysis 1, except those formed by the inbred lines 6 (male) and 12 (female), while for Analysis 2, the cross of lines 5 (male) and 10 (female) were not included.

**Table 3.** Factorial analysis including 47 F1 crosses across two environments

Analysis	Males (m) <sup>#</sup>	Females (f) <sup>#</sup>	F-males	F-females	F- m x f
1	1,2,3,4,5, ,7	8,9,10,11, ,13,14	24.1**	20.8**	3.7**
2	1,2,3,4, ,6,7	8,9, ,11,12, 13,14	10.8**	26.2**	3.5**

# : the identification of males, numbered 1 to 7, and females, numbered 8 to 14, is given in Tables 1 and 2; \*\* : highly significant ( $P \leq 0.001$ ).

In both analyses highly significant ( $P \leq 0.001$ ) male, female and male x female effects were detected. Therefore, the presence of significant variability among the inbred lines could make it possible to develop F1 crosses with low *S. sclerotiorum* incidence on capitula for diverse environmental conditions. For males, F-test value in Analysis 1 was much higher than in Analysis 2. This could be explained because Analysis 1 involved all hybrids in which the inbred line R 199 was present in the cross. This line showed the most favourable (-20 %) GCA value (Table 2). Thus, the participation of R 199 inbred line produced a high variability of the male line effects and a range of incidence of 72 % in the 36 hybrids included in this first analysis. In Analysis 2, the absence of F1 crosses having R 199 meant that the range of symptoms was smaller (59 %) and that the variability of male lines effects was also lower than that observed in Analysis 1. On the other hand, the F-test for female effects showed similar values in both analyses. Therefore, the substitution of different female inbred lines did not produce any changes in the genotypic variability among F1 crosses. F-test for male x female interaction had also similar values in the two analyses. Although these F-tests were statistically different from one, the expected deviation of SCA from parental GCA values could be considered of low importance.

The average value of combined inbred lines was estimated through their offspring, this value estimates the additive gene effect of these lines. Since the F-test values for males and females were much higher than F values for the interaction in both analyses, it may be suggested that in this group of inbred lines the additive gene effects are more important than those corresponding to the dominance gene effects. Although it is necessary to carry out other complementary analyses utilising other components of resistance (i.e. incubation period), this interpretation agrees with earlier ones (Castaño, 1992; Castaño *et al.*, 1992) studying different sunflower genotypes.

## References

- Álvarez, M.; Castaño, F.; Rodríguez, R. 1999. *Annals of Applied Biology* **134** (Supplement): 46-47.
- Castaño, F. 1992. *Thesis*. ENSA Rennes. 152 p.
- Castaño, F.; Hémerly-Tardin, M.; Tourvieille de Labrouhe, D.; Vear, F. 1992. *Euphytica*, **58**: 209-219.
- Castaño, F.; Vear, F.; Tourvieille de Labrouhe, D. 1993. *Euphytica*, **68**: 85-98.
- Cetiom (Ed). 1992. *La culture du tournesol. Guide cultural*. Paris.
- Godoy, M. ; Castaño, F. ; Ré, J. ; Rodríguez, R. ; Escande, A. 1999. *29º Congreso Argentino de Genética*, Rosario. p.401.
- Sackston, W. 1992 *Annual Review of Phytopathology*, **30**: 529-551.
- Schneiter, A., Miller, J. 1981. *Crop Science*, **21**: 901-903.
- Vear, F.; Tourvieille de Labrouhe, D. 1984. *INRA*, 106p.

## Acknowledgements

This work was supported by UNMdP (Project AGR93/99), INTA (Project 62:9514) and Monsanto (Argentina).