

## QUANTIFYING THE RELATIONSHIP BETWEEN VERTICILLIUM WILT INTENSITY AND YIELD LOSS OF SUNFLOWER (*Helianthus annuus* L.), AND FEASIBILITY OF UTILIZING THE HYPODERMIC INOCULATION TECHNIQUE AS A SELECTION METHOD

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### SUMMARY

The objectives of this report were (a) to determine the amount of yield loss of 20 sunflower hybrid varieties in relation to their relative susceptibility to *Verticillium* wilt under field conditions, and, (b) to associate this loss with the relative susceptibility of the same genotypes evaluated by the hypodermic inoculation technique.

Significant differences among hybrids were detected for *Verticillium* wilt score (VWS) evaluated under field conditions, yield (kg/ha) and oil percent in two localities (Henderson and Ballenera). The VWS was negatively correlated with yield in both environments ( $r = -0.68^{**}$  and  $-0.44^*$ ) but not with oil percent. Depending on the environment evaluated, yield loss per point of the VWS can range from 6.5% to 12.2% if it is taken as percentages over the means of the trials. On the other hand, if it is taking into account the extreme values of the VWS at each locality, yield loss for a given hybrid can range from 12 to ca. 50% at Henderson and from 0 to 20% at Ballenera, depending on the degree of susceptibility of the genotype considered.

*Verticillium* wilt scores recorded under greenhouse conditions were positively correlated with the scores obtained under field conditions ( $r = 0.56^*$  and  $0.45$  for each locality) and negatively correlated with yield ( $r = -0.56^*$  and  $-0.55^*$ ). Both facts indicate that the hypodermic method permits to predict the degree of susceptibility (and its associated reduction in yield) of a given genotype when exposed to this disease under field conditions.

**Key Words:** *Verticillium dahliae* Kleb, sunflower, yield loss assessment, resistance breeding.

### INTRODUCTION

*Verticillium* wilt of sunflower, caused by the soilborne fungus *Verticillium dahliae* Kleb., is known to be present in most sunflower producing areas of the world, especially in temperate zones (Aćimović, 1984, 1988; Škorić, 1988). In Argentina, it is considered as a major disease since it occurs at about 2,000,000 hectares (Bertero de Romano *et al.*, 1992).

Breeding for resistance is the most effective means of control for *Verticillium* wilt (Zimmer & Hoes, 1978). It was reported that there exists genetic variability for resistance to this disease (Putt, 1964; see Sackston, 1992) and materials have been created (for

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example the inbred line HA 89) whose resistance is based on one dominant allele (Fick & Zimmer, 1974). However, under the conditions of Argentina, these materials are susceptibles because the race (or races) of *V.dahliae* present in this country is (are) different from those of North America (Bertero de Romano & Vázquez, 1982). So, under such conditions losses from Verticillium wilt can be very significant.

In order to assess the relative resistance of a genotype to a given pathogen strain it is necessary to have a disease measurement method which expresses the quantitative relationship between the degree of infection, or severity, and the amount of yield loss. In this sense, Bertero de Romano and Vázquez (1985) have presented and evaluated a disease measurement method to determine resistance levels of sunflower to Verticillium wilt. For all the above, the objectives of this report were (a) to determine the amount of yield loss of 20 sunflower hybrid varieties in relation to their relative susceptibility to Verticillium wilt under field conditions, and, (b) to associate this loss with the relative susceptibility of the same genotypes evaluated by the hypodermic inoculation technique.

#### MATERIALS AND METHODS

Four trials were designed to evaluate the effect of Verticillium wilt on yield. Two of them were conducted at Henderson, and the others were planted at Ballenera, province of Buenos Aires (Argentina), during the 1990 and 1991 growing seasons. These trials, part of the Nidera Sunflower Performance trial, contained 20 commercial hybrids grown in a randomized block design with three replications. The utilized hybrids were: Contiflor 3, Contiflor 7, Contiflor 9, Contiflor 15, Contioil 1000 (ICI Argentina SAIC.), Paraíso, Paraíso 2, Asgrow 548 (Nidera S.A.), ACA 871, ACA 882 (Asociación Cooperativas Argentinas), Pioneer 6440, Pioneer 6448 (Pioneer Overseas Corporation), Dekalb G100, Dekalb G103, Dekalb G105 (Dekalb Argentina S.A.); Morgan 731, Morgan 733, Morgan 734, sungro 380 (Morgan) and SPS 3130 (SPS). Experimental plots consisted of three rows 6m in length spaced 70cm apart. These were overplanted, then thinned by hand to a final density of ca. 48000 plants ha<sup>-1</sup>. The incidence of Verticillium wilt was scored from 0 to 4, following the description given by Bertero de Romano and Vázquez (1985): 0: no symptoms, 1: chlorotic leaf spots with incipient necrotic halo on the lower leaves, 2: chlorosis with a pronounced necrotic halo on the middle leaves, 3: chlorosis, necrosis and leaf roll in all leaves, 4: necrotic leaves, stems striated and twisted.

Inoculations were performed under greenhouse conditions with the hypodermic method (Moser & Sackston, 1973). Four weeks after the day of inoculation, the incidence of Verticillium wilt was scored from 0 to 4 following the score proposed by Bertero de Romano & Vázquez (1985): 0: no symptoms, 1: chlorosis, 2: chlorosis and incipient necrosis, 3: necrosis and necrotic halo, 4: necrosis, necrotic halo and leaf roll.

Data were also recorded for yield (kg/ha) and oil percent. Correlation and regression coefficients between Verticillium wilt score (VWS) recorded under field infection and greenhouse inoculations on one side and these agronomic characters on the other were determined for each trial.

RESULTS

Two out of the four trials presented a generalized attack of *Verticillium*: Henderson in 1990 and Ballenera in 1991. The other trials were discarded for subsequent analyses.

Means, standard deviations, minimum and maximum values for the variables recorded at each environment are shown in Table 1. The 1991 trial at Ballenera presented greater values for yield and oil percent than the 1990 trial at Henderson and also, a greater incidence of the disease. Significant differences among hybrids were detected for all these variables (results not shown).

Table 1. Means, standard deviations, minimum and maximum values for disease score, yield and oil percent in each of the two environments tested.

	Environment							
	Henderson				Ballenera			
	$\bar{X}$	S.D.	MIN	MAX	$\bar{X}$	S.D.	MIN	MAX
Disease score	1.65	1.09	1.0	4.0	2.64	1.07	0.0	3.0
Yield (kg/ha)	2312	701	1022	3728	2993	305	2465	3530
Oil percent	49.1	3.05	43.3	54.1	50.1	2.46	45.3	53.6

Correlation coefficients between the VWS and the other traits recorded are shown in Table 2. Disease score was negatively correlated with yield in both environments. Oil percent and disease score were not mutually associated in any environment.

Table 2. Correlation coefficients between *Verticillium* wilt scores, yield (kg/ha) and oil percent in each of the two environments tested.

	Verticillium wilt score	
	Field infections	Greenhouse inoculations
Yield		
Ballenera	-0.68**	-0.56*
Henderson	-0.44*	-0.55*
Oil percent		
Ballenera	0.36	-0.25
Henderson	-0.18	-0.28
Verticillium wilt		
Score (natural infections)		
Ballenera		0.56*
Henderson		0.45*

\*: Significant at P=0.05; \*\*: Significant at P=0.01.

The dependence of yield on the VWS was evident from the significant linear relationship obtained from regression analysis (Table 3). The amount of yield loss per point of the disease score ranged from 194 kg/ha at Ballenera to 282 kg/ha at Henderson.

Table 3. Regression equations for the relationship between Verticillium wilt score (VWS) and yield (kg/ha) in each of the two environments tested.

	ENVIRONMENT	
	HENDERSON 1990	BALLENERA 1991
Yield=	2778** - 282*xVWS	3505** - 194**xVWS
	(267) (136)	(141) (49)
*: Significant at P=0.05; **: Significant at P=0.01.		
Standard deviations of estimators are given in brackets.		

All the inoculated hybrids developed leaf symptoms, although there were conspicuous differences among materials for their the VWS recorded under these conditions. Correlation coefficients between the VWS evaluated by the hypodermic method and the VWS recorded under field conditions are shown in Table 2. As can be seen, both scores were associated ( $r=0.45$  and  $0.56$  for Henderson and Ballenera, respectively;  $P<0.05$ ). In addition, the score obtained under greenhouse conditions showed a negative correlation with yield in both environments ( $r=-0.55$  and  $-0.56$ , for Henderson and Ballenera, respectively;  $P<0.05$ ). No significant correlation was obtained between this score and oil percent in any environment.

## DISCUSSION

There are many reports in the literature of studies that were conducted to demonstrate the nature of the relationship between different levels of a disease and the yield of a particular crop (v.g.: Ashwort *et al.*, 1981; Backman & Crawford, 1984; among others).

To gather such information, many different methods have been developed for field plot experimentation (James, 1974). The main goal of any of these methodologies is to generate different levels of disease and then use regression analysis to evaluate statically the relationship between disease severity and crop yield (Teng, 1987). In this paper we have used the genetic differences for resistance to Verticillium wilt, known to be present among cultivars, to study the effects of disease severity on yield.

The obtained results demonstrate that Verticillium wilt causes yield losses directly by affecting yield components (i.e., number of seeds per head and/or 1000 seed weight). Depending on the environment evaluated, yield loss per point of the VWS can range from 194 kg/ha to 282 kg/ha, or, in other words, it can range from 6.5% to 12.2% if it is taken as percentage over the means of the trials. On the other hand, if extreme values of the VWS at each environment are taken into account, yield loss for a given hybrid can range from 12% to ca. 50% at Henderson and from 0% to ca. 20% at Ballenera depending on the degree of susceptibility of the genotype considered.

The obtained results clearly show that *Verticillium dahliae* is a pathogen of great importance for sunflower, at least under the Argentine conditions, and that there exists a considerable genetic variability among high yielding genotypes for resistance to this disease. To capitalize this variability in a breeding program, it is necessary to develop a selection method in order to efficiently screen a great number of genetic materials. The hypodermic inoculation of sunflower with *Verticillium dahliae* was recognized as a succesful method of inoculation, since this technique gave more uniform and predictable

infection than root dip inoculation (Moser & Sackston, 1973). Hoes (1966) found that the hypodermic inoculation permitted differentiation of high, moderate and low resistance of sunflower to *Verticillium*, whereas root dip distinguished only between high and low levels. In addition with this advantage, the positive correlations found between *Verticillium* wilt scores under artificial and natural conditions for both years of experimentation, indicate that the hypodermic method permits to predict the degree of susceptibility (and its associated reduction in yield) of a given genotype when exposed to this disease under field conditions.

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**CUANTIFICACION DE LA RELACION ENTRE INTENSIDAD DE MARCHITAMIENTO POR VERTICILIOSIS Y PERDIDA DE RENDIMIENTO EN GIRASOL (*Helianthus annuus* L.) Y POSIBILIDAD DE UTILIZAR LA TECNICA DE LA INOCULACION HIPODERMICA COMO METODO DE SELECCION**

**RESUMEN**

Los objetivos de este trabajo fueron a) determinar la perdida de rendimiento de 20 variedades hibridas en relación a su susceptibilidad de los mismo genotipos evaluados por la tecnica de la inoculacion hipodermica.

Diferencias significativas entre hibridos fueron detectadas para la medida del grado de verticiliosis (VWS) evaluado en para la medida del grado de verticiliosis (VWS) evaluado en condiciones de campo, el rendimiento (kg/ha) y el porcentaje de aceite en dos localidades (Henderson y Ballenera). El VWS estuvo correlacionado negativamente con el rendimiento en ambos ambientes ( $r = -0.68^{**}$  y  $0.44^{**}$ ) pero no con el porcentaje en aceite. Dependiendo del ambiente evaluado, la perdida de rendimiento por punto de VWS puede oscilar de 6.5% a 12.2% si son tomadas como porcentaje de media de experimento. Por otro lado si se toman en cuenta los valores extremos de VWS en cada localidad, la perdida de rendimiento para un hibrido dado puede variar de 12 a 50% en Henderson y de 0 a 20% en Ballenera dependiendo del grado de susceptibilidad del genotipo considerado.

La medida del grado de verticiliosis medido en condiciones de invernadero estuvo positivamente correlacionada con las obtenidas en condiciones de campo ( $r = 0.56^*$  y  $0.45^*$ ) para cada localidad y negativamente correlacionadas con el rendimiento ( $r = -0.56^*$  y  $-0.55^*$ ). Ambos factores indican que el método hipodermico permite predecir el grado de susceptibilidad y su reducción asociada del rendimiento de un genotipo dado cuando se expone a esta enfermedad bajo condiciones de campo.

**QUANTIFICATION DES PERTES DE RENDEMENT PROVOQUÉES PAR *Verticillium* SUR LE TOURNESOL (*Helianthus annuus* L.), POSSIBILITÉ D'UTILISER UNE INOCULATION DE TYPE "HYPODERMIQUE" COMME MÉTHODE DE SÉLECTION**

**RÉSUMÉ**

Les objectifs de cette publication sont premièrement de déterminer les pertes de rendement de 20 hybrides de tournesol en fonction de leur sensibilité à *Verticillium* en champ et dans un second temps, de chercher à corrélér ces résultats à la sensibilité des mêmes génotypes soumis à une inoculation de type "hypodermique".

En champ, des différences significatives entre hybrides ont été mises en évidence concernant les niveaux d'attaque par *Verticillium*, le rendement et la teneur en huile. Les niveaux d'attaque étaient négativement corrélés au rendement pour les deux sites d'expérimentation ( $r = 0.68^{**}$  et  $r = 0.44^{**}$ ) mais pas au pourcentage d'huile. Selon la localité, les pertes de rendement par niveau d'attaque de *Verticillium* variaient de 6.5 à 12,2% si ils sont considérés par rapport au pourcentage moyen des essais. D'un autre coté, si les pertes de rendement sont estimées par rapport aux valeurs extrêmes d'attaques pour chaque localité, ils varient de 12 à 50% à Henderson et de 0 à 20% à Ballenera selon la sensibilité des génotypes.

Les niveaux d'attaque enregistrés sous serre sont corrélés positivement avec les notations en champ ( $r = 0.56^*$  et  $r = 0.45^*$  pour chacune des localités) et négativement avec le rendement ( $r = -0.56^*$  et  $r = -0.55^*$ ). Ces deux observations indiquent que la technique d'inoculation "hypodermique" permet de prédire le degré de sensibilité (et les perte de rendement associées) pour un génotype donné exposé à une infection en champ.