# *SCLEROTINIA SCLEROTIORUM* CAPITULUM RESISTANCE TESTS USING ASCOSPORES: RESULTS OVER THE PERIOD 1991- 2003

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**Abstract**

Tests measuring sunflower capitulum resistance to *Sclerotinia sclerotiorum* are made by ascospore infections at the beginning of flowering. Comparison of the reactions of two control genotypes over 13 years has shown that, although there may be differences in percentage attack and delay in symptom appearance between years, the first are small and for the second, the results of the two lines are significantly correlated. These differences are not related to mean temperature or to rainfall, but improved regularity of irrigation has reduced mean delay in symptom appearance by 15% over 13 years (6 days for a mean of 36 days). Observations of other lines show that there may be considerable differences in percentage attack between genotypes in most years but that in some years, nearly all plants show symptoms. In this case, the latency index (delay in symptom appearance compared with that of the controls) provides a satisfactory measurement of resistance.

**Résumé**

Nous avons analysé la réponse de deux lignées de tournesol de référence (SD et GU) à un test mesurant la résistance du capitule à la pourriture blanche. L'analyse a porté sur 13 années d'observations. Le test consiste à pulvériser une suspension d'ascospore sur la face fleurie des capitules en début floraison et à observer les symptômes sur la face stérile. Les analyses portent sur les taux de plantes montrant des symptômes de pourriture et les temps d'apparitions des premiers symptômes=durée de latence. Ces observations sont comparées avec les données climatiques: températures et pluviométrie. Les comportements de nos génotypes de références sont conformes quelles que soient les années d'expérimentation (SD est un génotype résistant qui montre une longue période de latence alors que GU est passablement sensible avec une période de latence deux fois moins longue). Les taux d'attaque observés sur nos deux génotypes de référence fluctuent peu et sont indépendants des conditions climatiques. Par contre une meilleure maîtrise du rythme d'irrigation a permis de réduire de 15 % la durée de latence. L'analyse réalisée sur d'autres génotypes montre que si les taux de plantes montrant des symptômes fluctuent de façon importante selon les années, l'indice de latence (= durée de latence / moyenne de la durée de latence des deux lignées de référence infectée le même jour) est un bon critère de mesure de la résistance du tournesol au *Sclerotinia* du capitule.

## Introduction

Sunflower is an important crop in France, especially in the south, with a total of about 700,000 ha in 2003. In this last year, the abnormally hot and dry conditions during its cropping period made evident its ability to produce a reasonable yield in conditions of agronomic stress (Sebillotte et al., 2003). However, in years when rainfall is normal for France in spring and summer, diseases can cause economic yield loss if susceptible varieties are grown: high rainfall in April-May is favourable for downy mildew attack whereas in June and, more particularly July, Phomopsis and then Sclerotinia attacks may occur. *Sclerotinia sclerotiorum* (Lib.) and de Bary, which causes rot of sunflower roots, leaves, terminal bud and capitulum has an extremely wide host range and has always been known on the sunflower crop. No complete resistance has been observed and partial resistance is under quantitative control (Bert at al., 2002). Breeding for resistance to each type of attack requires tests on the plant parts in the field (Castaño et al., 1993). It was shown by Says-Lesage and Tourvieille (1988) that capitulum attack resulted from infection of florets at the anther stage by ascospores. The symptoms appear as the capitulum matures. To be able to make comparisons between years and between genotypes with different flowering dates, control genotypes are necessary. This paper reports on 13 years of tests, genotype stability and environmental effects.

## Materials and Methods

***Sunflower Genotypes.*** All the inbred lines were bred by INRA, France. The two main controls were SD, selected from a breeding population containing old genetic male-sterile hybrids and GU, a line of Rumanian origin. SD is the more resistant control, which shows as a high level of symptoms that take a long time to appear, whereas GU shows a high level of symptoms quite rapidly after infection. Both these lines are unbranched, male-sterility maintainers. Of the other lines whose reactions are reported, CP73, FU and GH are similarly unbranched maintainers, whereas PAC1, PAZ2 and PSC8 are apically branched male-fertility restorers.

***Ascospore Test.*** This was described by Vear and Tourvieille (1991). *Sclerotinia sclerotiorum* ascospores were obtained from sclerotia collected in several infected fields in previous years. The spores were generally kept dry, at -20C until required, for several years if necessary (Tourvieille, 1988). At the beginning of flowering of each plant, the open florets were sprayed with 5 ml of a suspension containing 5 spores/mm cubed. The capitula were covered with greaseproof paper bags to maintain sufficient humidity. To cover the different flowering dates of the material in the trial, tests were made twice a week for a period of about 4 weeks. To be able to make comparisons between these tests, the two control lines were sown at staggered sowing dates, so that at each infection date, 10-50 plants which were beginning to flower could be infected also. Irrigation or rainfall provided at least 50 mm each week, so that the plants matured slowly. From two weeks after infection, the first appearance of symptoms on the dorsal surface of the capitulum was observed. Final results were the percentage of plants showing symptoms, for the controls the mean duration of the latent period, and for the other lines, a latency index, which is the number of days between infection and symptom appearance of each plant, divided by the mean delay for the control plants infected at the same time.

## Results

Table 1 presents the mean percentage attack of the two control lines over 13 years, from 1991 to 2003. Both lines showed high mean percentages of plants with symptoms (SD: 91.7%, GU: 99.3%) but there was a significant difference between them. There was no significant year effect, and the percentages for the two lines were not significantly correlated (r=0.320) because GU showed little variation whereas some years SD showed 20-30% of plants without symptoms, in particular in 1996 and 1999.

Table 1. Mean percentages of plants of control inbred sunflower lines SD and GU showing symptoms after capitulum infection with *Sclerotinia sclerotiorum* ascospores, from 1991 to 2003. (F genotypes: 11.14**, F years: 1.66ns).

| | 1991 | 1992 | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SD** | 83 | 85 | 100 | 100 | 94 | 77 | 97 | 100 | 67 | 98 | 99 | 100 | 92 | 92 |
| **GU** | 100 | 99 | 100 | 100 | 99 | 100 | 96 | 100 | 97 | 100 | 100 | 100 | 100 | 99 |
| **Mean** | 92 | 92 | 100 | 100 | 97 | 88 | 96 | 100 | 82 | 99 | 99 | 100 | 96 | 96 |

Table 2 presents the mean delays in symptom appearance for the two lines from 1991 to 2003. In this case the genotypic difference was very large (SD: 46 days, GU: 26 days), but there was also a highly significant year effect, the shortest mean latency period being 29-30 days, in 1996 and 1994, whereas the longest was 44 days in 1992 (LSD: 8 days). In this case the variability concerned the two lines with a significant correlation between their rates of symptom appearance (r = 0.608). Over the 13 years of trials, there was a general tendency for the latency period to be reduced by about 6 days overall, although there was no significant correlation between year and delay in symptom appearance (r = 0.319). Mean percentage attack was not correlated with latency period (r = 0.329) for the two lines, over the 13 years.

Table 2. Mean numbers of days between capitulum infection with *Sclerotinia sclerotiorum* ascospores and symptom appearance for control inbred sunflower lines SD and GU from 1991 to 2003. (F genotypes: 201.4**, F years: 3.28**).

| | 1991 | 1992 | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SD** | 54 | 54 | 49 | 38 | 55 | 39 | 39 | 43 | 54 | 41 | 46 | 41 | 43 | 46 |
| **GU** | 26 | 34 | 27 | 21 | 29 | 20 | 28 | 23 | 25 | 23 | 24 | 28 | 22 | 26 |
| **mean** | 40 | 44 | 38 | 30 | 42 | 30 | 34 | 33 | 40 | 32 | 35 | 34 | 33 | 36 |

To determine whether the variations in percentage attack and latency period were determined by any simple environmental factors, comparisons were made with the mean temperatures, and sums of temperatures and rainfall during the incubation period. These are presented in Table 3. There was absolutely no correlation between mean delay in symptom appearance and mean temperature (Table 3) and it was not significant for rainfall. The close correlation with sum of temperatures simply indicates that there was no great variation in this sum/day.

Table 3. Correlations between environmental conditions and percentage attack and latency duration of control inbred sunflower lines after capitulum infection with *Sclerotinia sclerotiorum* ascospores, from 1991 to 2003. (**: p<0.01).

| | SD | GU | (SD+GU)/2 |
|---|---|---|---|
| Total sum of temperatures during latency period | 0.887** | 0.943** | 0.896** |
| Mean daily temperature | - 0.287(ns) | 0.113(ns) | 0.047(ns) |
| Rainfall | 0.457(ns) | 0.219(ns) | 0.373(ns) |

The percentage attacks and latency indices for some other lines which were tested over several years are presented in Table 4. The lines CP73 and GH generally appear as susceptible, although in 1992 only about half the plants showed symptoms. The other lines, both unbranched, FU and branched (PAC1, PAZ2, PSC8), showed much lower levels of attack in some or all years tested. Their relative reactions appear quite constant: PAC1 and PSC8 showed their highest levels of infection in 1996, but PSC8 always had less infection than PAC1. FU and PAZ2 were both infected at about 40% in 1997 but at more than 90% in 2000. However, in years such as 1996 and 2000, it was not possible to distinguish most of these quite resistant genotypes from the susceptible ones. For those years, the latency index provides a measure of resistance which does not differ from those of years with lower levels of attack (e.g., FU: latency index 1.15 in 2000, a mean of 1.21 for 1997-1998; PSC8: 1.28 in 1996, a mean of 1.40 for 1992-1997-2001).

Table 4. Percentage attack (%) and latency indices (Il) of six sunflower lines after capitulum infection with *Sclerotinia sclerotiorum* ascospores between 1992 and 2001.

| Lines | CP73 | | FU | | GH | | PAC1 | | PAZ2 | | PSC8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Years | % | Il | % | Il | % | Il | % | Il | % | Il | % | Il |
| 1992 | 52 | 69 | - | - | 46 | 83 | 17 | 125 | - | - | 1 | 149 |
| 1995 | 97 | 69 | - | - | 86 | 89 | 64 | 94 | - | - | - | - |
| 1996 | 100 | 64 | - | - | 100 | 72 | 100 | 106 | - | - | 47 | 128 |
| 1997 | 100 | 72 | 40 | 122 | 100 | 83 | 66 | 108 | 42 | 132 | 14 | 102 |
| 1998 | - | - | 61 | 119 | 100 | 65 | - | - | - | - | - | - |
| 1999 | - | - | - | - | 70 | 84 | - | - | 18 | 111 | - | - |
| 2000 | - | - | 98 | 115 | 100 | 80 | - | - | 96 | 146 | - | - |
| 2001 | - | - | - | - | - | - | - | - | - | - | 13 | 170 |

(-) = line not tested

**Discussion**

Tests of resistance to Sclerotinia head rot using ascospore infections at flowering under programmed irrigation over the last 13 years with the control lines SD and GU have shown that these infections are repeatable whatever the climatic conditions of the year. The percentage attack of these lines varies little from year to year, whereas latency periods vary, but in the same direction for the two lines. Simple environmental effects, mean temperature and rainfall do not appear to play a large role. The general reduction in latency period of 6 days in 13 years is probably related to increased regularity of irrigation, which also limits the possible effect of rainfall. Ascospore suspensions were always made with mixtures of spores obtained from several different origins, so it is unlikely that variations between years were due to differences in aggressiveness. In addition, Vear et al. (2004) reported that there are no interactions between *S. sclerotiorum* isolates and sunflower genotype in the reaction to the ascospore test. Thus, the conclusion is that some short term environmental conditions play a role in the rate of *S. sclerotiorum* development in the capitulum. Vear and Guillaumin (1977) found that the temperature the day after infection influenced results of a mycelium test in the field. The use of two control lines with very different reactions to *S. sclerotiorum* for latency duration is important in judging new material. The use of a mean of two lines showing symptoms at different dates and so possibly slightly different conditions, provides a better base than would a single control genotype.

The importance of control genotypes is also shown by the comparison of some other sunflower lines whose resistance in many years is apparent from their differences in percentage attack. In years when all lines show a high percentage of plants with symptoms, a comparison with SD will show whether the lines are susceptible or whether they have the same reaction as this resistant control. In this case, the latency index provides a good indication of resistance, calculated according to the delay in symptom appearance of the two controls infected under the same conditions. This index remains quite stable whatever the percentage attack.

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**References**

Bert, P.F., Jouan, I., Tourvieille de Labrouhe, D., Serre, F., Nicolas, P. and Vear, F. 2002. Comparative genetic analysis of quantitative traits in sunflower (*Helianthus annuus* L.).1. QTL involved in resistance to *Sclerotinia sclerotiorum* and *Diaporthe helianthi*. Theor. Appl. Genet. 105:985-993.

Castaño, F., Vear, F. and Tourvieille de Labrouhe, D. 1993. Resistance of sunflowers to different forms of attack by *Sclerotinia sclerotiorum* and relations with some morphological characters. Euphytica. 68:85-98.

Says-Lesage, V. and Tourvieille de Labrouhe, D. 1988. Recherche des sites de pollution et d'infection des fleurons de tournesol, *in situ,* par les spores de *Sclerotinia sclerotiorum.* nformations Techniques CETIOM. 102:3-13.

Sebillotte, C., Ruck L. and Messéan, A. 2002. Les systèmes de culture avec oléagineux in “Prospective et compétitivité des oléagineux dans l’avenir”. CETIOM, 12 av. Georges V, Paris. Tome II. p. 128-159

Tourvieille de Labrouhe, D. 1988. Conservation of *Sclerotinia sclerotiorum* ascospores. Sclerotinia Newsletter. 1:7.

Tourvieille de Labrouhe, D. and Vear, F. 1984. Comparaison de méthodes d'estimation de la résistance du tournesol à *Sclerotinia sclerotiorum* (Lib.) de Bary. Agronomie. 4:517-525.

Vear, F. and Guillaumin, J.J. 1977. Etude de méthodes d'inoculation du tournesol par *Sclerotinia sclerotiorum* et application à la sélection. Ann. Amélior. Plantes. 27:523-537.

Vear, F. and Tourvieille de Labrouhe, D. 1988. Heredity of resistance to *Sclerotinia sclerotiorum* in sunflower. II. Study of capitulum resistance to natural and artificial ascospore infections. Agronomie. 8:503-508.

Vear, F., Willefert, D., Walser, P., Serre, F. and Tourvieille de Labrouhe, D. 2004. Reaction of sunflower lines to a series of S*clerotinia sclerotiorum* isolates. Proc. 16th Int. Sunf. Conf., Fargo, USA. International Sunflower Association, Paris, France. (In press).