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Resistance of Sunflowers to Extension of *Sclerotinia sclerotiorum* Mycelium on Leaves and Capitula.

Emmanuelle MESTRIES., Felicity VEAR and Denis TOURVIEILLE de LABROUHE
I.N.R.A., G.R.E.A.T., Centre de Recherches Agronomiques, Domaine de Crouelle,
63039 Clermont-Ferrand cedex 2, France.

Abstract

Sunflower resistance to *Sclerotinia sclerotiorum* was studied on the F2 plants and F3 and F4 families from a cross between GH, a susceptible line and PAC2, a line with a good level of resistance. Mycelium tests were applied on the leaves and capitula of 161 F2 plants in 1993. In 1994 and 1995, the same tests were applied on 10 F3 or F4 plants of each family. The leaf test gave a better precision than the capitulum test and, for the 3 generations, there was no correlation between the results of the two tests, indicating that sunflower resistance to *S. sclerotiorum* varies according to the plant part attacked. There were highly significant correlations between the results for the F2 plants and their F3 families and the results of the F3 and F4 families for both tests (leaf test r=0.28 and r=0.42 respectively ; capitulum test r=0.25 and r=0.45 respectively). Parent-offspring regressions were used to estimate narrow-sense heritabilities and showed that heritability estimates for the F3-F4 regression were higher than for the F2-F3 regression for both tests (leaf test : h^2_{F2-F3}=0.12 and h^2_{F3-F4}=0.28 ; capitulum test : h^2_{F2-F3}=0.08 and h^2_{F3-F4}=0.30). Testing for *Sclerotinia sclerotiorum* resistance on leaves and capitula on families at this stage is thus possible and probably more efficient than on individual plants.

Introduction

Sclerotinia sclerotiorum is one of the most important pathogens of sunflower in France, causing white rot and wilt. It is particularly serious because it can attack all parts of the plant : roots, stem base, leaves, stems, terminal bud and capitulum.

Chemical control is difficult and not economic, so the development of resistant varieties is an important breeding objective. However, resistance is polygenic and for a given genotype, the level of resistance of each plant part may be quite different (Tourvieille and Vear, 1984). It is thus necessary to consider each form of attack as a different disease and to breed simultaneously for the resistance of each plant part in order to obtain varieties with a satisfactory level of overall resistance. Castaño *et al.* (1993) showed two types of resistance, the first to entry of the fungus into the plant and the second, resistance to mycelial extension in the tissues.

This paper presents studies on the second type of *Sclerotinia* resistance in leaves and capitula. Our aim was to follow the heritability of this type of resistance in three generations from a cross between two inbred lines with different levels of resistance.

Materials and methodes

Sunflower genotypes: 161 plants of an F2 progeny from a cross between two inbred lines, GH and PAC2, both bred by I.N.R.A., were studied. GH selected from a Rumanian population, is a male sterility maintainer and rather susceptible to *Sclerotinia* attacks on leaves and capitulum whereas the restorer line PAC2 comes from the cross (Peredovik x wild *H. annuus*) x *H. petiolaris* restorer and shows good resistance to *Sclerotinia*. The plants were grown in the field at Clermont-Ferrand in 1993. They were selfed by covering the capitula with grease-proof paper bags just before flowering.

The seeds of each F2 plant were sown in 1994 to obtain 161 F3 families. Six plants of each F3 family were selfed and the seeds from each F3 family were bulked and sown in 1995 to study the F4 families.

Sclerotinia sclerotiorum isolates : In 1993, the isolate used was SS40, isolated at Clermont-Ferrand on sunflower cotyledons. In 1994, it was SS41 and in 1995, SS44. The mycelium explants used as inoculum in resistance tests were cut from the edges of fungal cultures in Petri dishes on 1% malt agar, incubated for 3 days at 23±1°C in the dark.

Resistance tests : **a) Mycelium test on leaves** : The protocol of Castaño *et al.* (1992) was applied on 161 F2 plants, infected all at one date in 1993 and 10 plants of the F3 and F4 families in 1994 and 1995, respectively. Mycelium explants measuring 8 mm in diameter were placed on the upper surface of the extremities of two young but fully grown leaves on each plant when the flower bud measured about 5 cm in diameter. The explants were covered with aluminium foil to prevent drying and the plants were irrigated to maintain humid conditions until the observations, 7 days later. Measurements were made on the lower surface of leaves of the length of lesion along the main vein. After these measurements, the infected leaves were removed in order to maintain normal plant development.

b) Mycelium test on capitulum : This test was carried out on the 161 plants F2 wich have been previously tested for resistance on leaves. It was not the case for the other generations and 10 plants were tested for each F3 (in 1994) and F4 (in 1995) family. The method used is that of Vear and Guillaumin (1977), at physiological maturity (yellowing capitulum), 4 to 6 weeks after flowering. In this case, 3 explants, 10 mm in diameter, were placed on the dorsal surface of each capitulum, which had been harvested but kept, with its stem soaking in water, in a growth chamber at 18°C. After 3 days, the lesion areas were measured. These areas were compared with those observed on a control variety infected on the same day, to give a disease index.

Oil content : Seed oil content was measuered on 2g samples from each capitulum by Nuclear Magnetic Resonance (Bruker Mini-Spec 10).

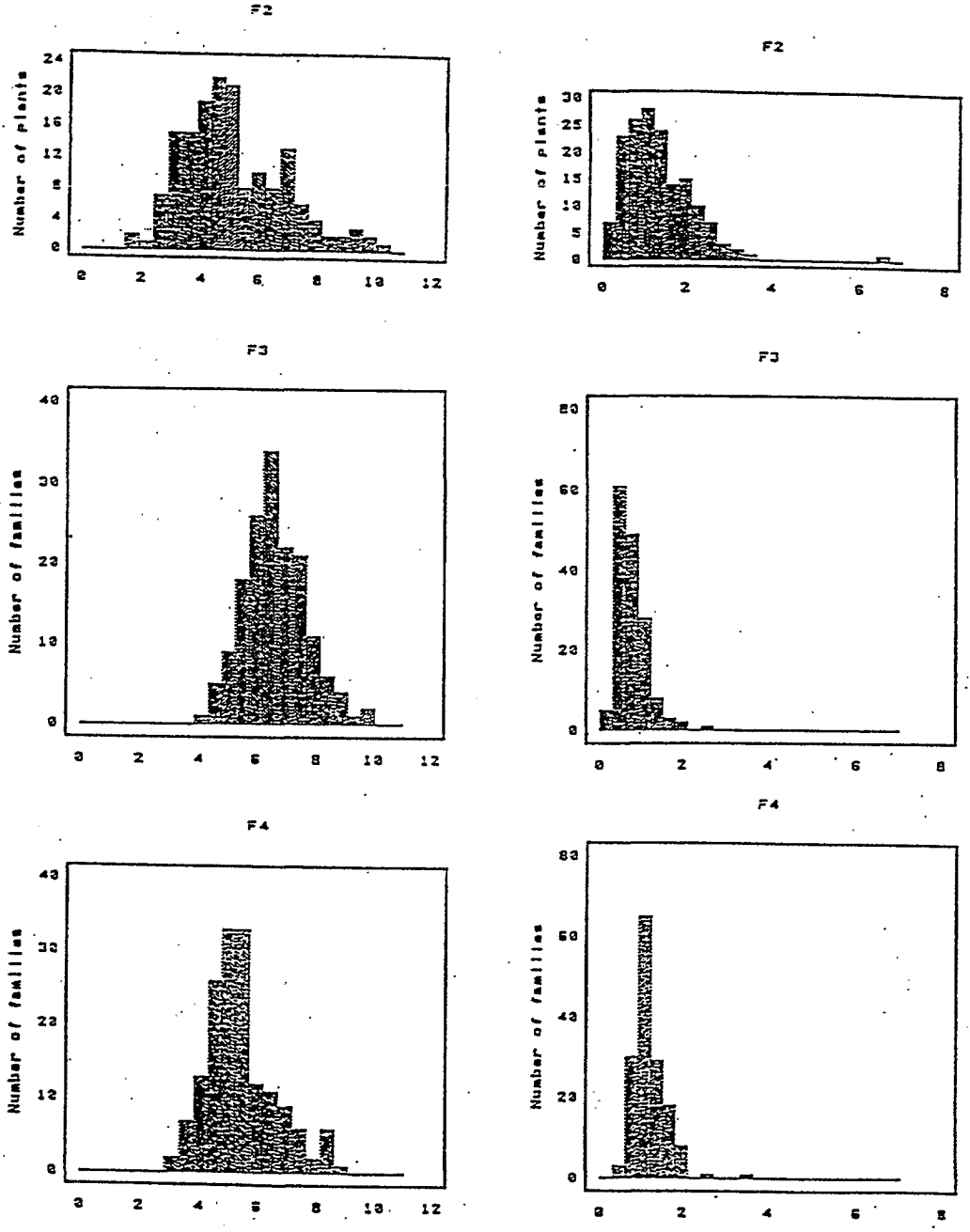
Statistical Analyses : The means, variances and coefficients of variation were calculated for each character. Pearson correlation coefficients were calculated between the 3 traits. Narrow-sense heritabilities (h^2) were estimated by parent-offspring regression, with adjustement for parental degree of inbreeding described by Smith and Kinman (1965). The software Statgraphics Plus (version 7.1) was used for these analyses.

Results

Table 1 presents the main results and statistical parameters for each of the 3 quantitative traits studied. For the F2 (1993), the results are for individual plants, whereas for the F3 (1994) and F4 (1995) generations, results are family means. The parental lines GH and PAC2 showed the following *Sclerotinia* reactions :

GH : Leaf test : 7.21 cm in 1994 and 6.81 cm in 1995
Capitulum index : 1.47 in 1994 and 0.96 in 1995
PAC2 : Leaf test : 4.30 cm in 1994 and 3.52 cm in 1995
Capitulum index : 0.43 in 1994 and 0.64 in 1995.

Figure 1 shows the distributions of reactions to the 2 resistance tests in the 3 generations studied. For both *Sclerotinia* resistance tests, the range of response of F2 plants was very large, but the differences and variances are smaller for the means of F3 and F4 families.



a) Mycelium test on leaves

b) Mycelium test on capitulum

Fig.1. Distribution histograms of reactions to two *Sclerotinia* tests in F2, F3 and F4 progenies for the sunflower cross GH x PAC2.

Table 1. Results of the two *Sclerotinia* resistance tests and oil contents studied on the F2, F3 and F4 generations of the sunflower progeny GH x PAC2

Quantitative trait	Plant or family number	Mean	Variance	Minimum	Maximum	CV
Leaf test (cm)						
F2	161	5.12	3.08	1.75	10.25	34.24
F3	160	6.60	1.16	4.29	9.85	16.34
F4	161	5.44	1.36	3.00	8.78	21.47
Capitulum index						
F2	161	1.33	0.68	0.23	6.60	61.72
F3	157	0.75	0.13	0.25	2.62	48.67
F4	155	1.19	0.16	0.53	3.62	33.46
Oil content (%)						
F2	161	37.79	19.92	27.3	49.7	11.81
F3	154	37.11	24.22	26.2	52.1	13.26
F4	155	36.06	21.05	23.3	53.9	12.72

For the leaf test, there was an effect of year, mean lesion length being greater in 1994 (F3), in agreement with the results on the parental lines. For the capitulum index, in spite of use of a control, the F3 showed less mycelial growth than the F2 or F4. The results of the leaf test were distributed normally whereas those of the capitulum test showed excesses of more resistant plants. Mean oil content varied little between generations.

For all three generations, there were no significant phenotypic correlations between the results of the two *Sclerotinia* tests (F2 : r=0.011; F3 : r=0.075; F4 : r=0.029). This was also the case for *Sclerotinia* resistance and oil content (F2 : r=-0.109; F3 : r=-0.077; F4 : r=-0.145). For all characters (table 2), the results of the three generations of each family were correlated.

Table 2. Pearson correlation coefficients for two *Sclerotinia* resistance tests and oil contents in F2, F3 and F4 generations of the sunflower progeny GH x PAC2

	Leaf test	Capitulum index	Oil content
F2-F3	0.279^{***}	0.254^{***}	0.555^{***}
F3-F4	0.416^{***}	0.454^{***}	0.575^{***}

*** significant at P=0.001

The regression coefficients and the estimates of heritabilities for each of the 3 traits are given in Table 3. For both *Sclerotinia* tests, the slope of the regression, and thus the estimates of heritability, were greater when calculated on F3-F4 generations than when calculated on the F2-F3. They were quite similar, 0.28 and 0.30 for F3-F4 leaf and capitulum tests respectively. In contrast, heritability of oil content, which was relatively high, 0.40, in F2-F3, was only 0.30 in F3-F4.

Table 3. Regression coefficients and narrow-sense heritabilities obtained by parent-offspring regression for the two *Sclerotinia* resistance tests and oil contents studied on the F2, F3 and F4 generations of the sunflower progeny GH x PAC2

		Leaf test	Capitulum index	Oil content
F2-F3	b	0.18	0.12	0.60
	h^2	0.12	0.08	0.40
F3-F4	b	0.49	0.53	0.52
	h^2	0.28	0.30	0.30

Discussion

These studies on 161 F2 plants and their F3 and F4 families, have shown that observations of *Sclerotinia* resistance show a quite constant ranking of genotypes. Although year effects on lesion size were significant, correlation coefficients between years were significant also. For the leaf test, Castaño *et al.* (1992) observed a correlation coefficient of r=0.72 between the results of two years. The non-normal distribution of reaction to the capitulum test, with an excess of more resistant plants, was also reported by Vear and Guillaumin (1977). The variation in mean reaction between years, even when compared with a control, was reported by Castaño (1992) on F2 and F3 generations from a cross between two inbred sunflower lines different from those used in this study. The absence of correlation between the results of the two resistance tests agrees with the observations of Castaño *et al* (1992) on 20 hybrids from a factorial cross and indicates that sunflower resistance to *Sclerotinia sclerotiorum* varies according to the plant part attacked.
The negative correlation between oil content and capitulum resistance to *Sclerotinia* reported by Vear and Tourvieille (1988) is not apparent in the present results. This may come from the fact that the difference in oil content between the 2 parents is quite small (a mean 4.3% over 3 years) whereas the earlier results were drawn from observations of different lines and hybrids with highly different oil contents.

The estimations of heritability show considerable increases from F2-F3 to F3-F4, from 0.12 to 0.28 for the leaf test reaction and from 0.08 to 0.30 for the capitulum test. This result is probably due to the fact that measurements of the mean *Sclerotinia* resistance of families are more precise than those of individual plants. Miller and Brinkman (1983) made the same observation for the inheritance of spike nodding angle in spring barley, with greater heritabilities for F4-F5 than for F3-F4 generations. Uhr and Murphy (1992) reported that the results of heritability of oat mosaic resistance based on progeny means among F2-F3 lines in one year are a good predictor of F3-F4 lines in a subsequent year. Castaño *et al.* (1992) estimated the heritability of resistance to *Sclerotinia* leaf attack on a factorial cross, using the parent-progeny regression, and obtained a value of 0.61. Other studies on heritability of *Sclerotinia* capitulum resistance gave values of between 0.57 (F2-F3 regression) (Castaño, 1992) and 0.77 (Robert *et al.*, 1987) on F1 hybrids from a factorial cross. These authors reported that, in all cases, additive control was more important than dominance.
The present estimation of heritability of oil content in F2-F3 was slightly lower than that of Fick (1975) (h^2=0.52), calculated from a parent progeny regression, or of Marinkovic (1993) on a 7X7 diallel cross (h^2=0.53). As with *Sclerotinia* resistance, additivity is more important than dominance, but, for oil content, heritability of F3-F4 was lower than F2-F3 estimates. This could be due to quite rapid fixation of the relatively small number of genes controlling this character.

Parent-offspring regression is often recommended as an empirical method for estimating narrow-sense heritability that is relatively free of genetic assumptions (Dudley and Moll, 1969) and describes directly the similarity between generations (Uhr and Murphy, 1992) ; heritability in the narrow sense is important for the breeder because the effectiveness of selection depends on the proportion of additive genetic variance (Luciano *et al.*, 1965). It may be concluded that although the level of heritability for the F3-F4 generations is only moderate, testing for *Sclerotinia sclerotiorum* resistance on leaves and capitulum on families at this stage is possible and probably more efficient than on individual plants.

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