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DIFFERENTIAL REACTION OF SUNFLOWER GENOTYPES TO INFECTION BY *Botrytis cinerea* Pers.

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SUMMARY

Several hybrids of sunflower (*Helianthus annuus* L.) were evaluated for resistance to grey mould, caused by *Botrytis cinerea* Pers., and their potentials as possible sources of resistance were determined. Cultivars were subjected to natural infection and inoculation in field and growth chamber experiments, respectively. The results obtained showed highly significant ($P<0.001$) differences in disease severity between cultivars. However, no genotype was completely resistant. A number of cultivars express low levels of susceptibility, particularly the hybrid 'NS-H-45'. The evaluation of S_1 progeny and single-plant-progenies of this cultivar revealed that selection for low level of susceptibility and hence breeding for resistance is possible.

Key words: *Botrytis cinerea*, cultivars, grey mould, *Helianthus annuus*, resistance, sunflower.

INTRODUCTION

Last two decades have seen an explosive expansion of sunflower production. This expansion has been stimulated by a number of factors. The most important has been the discovery of cytoplasmic male sterility (CMS) in interspecific crosses between *Helianthus petiolaris* and *H. annuus* by Leclercq (1969) and the subsequent identification of restorer lines (cf. Enns *et al.*, 1970; Kinman 1970; Leclercq 1971; Vranceanu & Stoenescu 1971; Velkov & Stoyanova 1974).

Simultaneously with this development, however, an immense increase in phytopathological problems, particularly due to fungal diseases, has been observed, Diseases, especially those caused by the pathogens *Bortytis cinerea, Phomopsis helianthi,* and *Sclerotinia sclerotiorum*, are still limiting factors for higher productivity in most sunflower growing areas (Seiler 1988).

Grey mould, caused by *Botrytis cinerea* Pers., is an important disease of sunflower and many other major crops (Moore 1959; Jarvis 1977). In Europe, the incidence and severity of grey mould on sunflower has attained a (high) magnitude over last few years, especially in Northern France and Germany, due to cool and wet climate. The consequences of this disease are reflected in severe yield and quality reductions. Although exact estimations of yield losses are still lacking, they may be as high as 30-40% (Kufner 1987).

Attempts to control grey mould by fungicide application have not been successful, generally due to a great variability of the fungus (Coley-Smith *et al.*, 1980) induced by heterokaryosis (Hansen & Smith 1932) and the effects of cultural and environmental conditions (Menzinger 1966), which can produce misleading results (Kovacs and Tüske

1980; Grindle 1981). In addition, fungicide-resistant strains of *B. cinerea* are not uncommon. Therefore, emphasis is currently laid on host plant resistance as the most effective method of control. The objective of this study lies in identifying useful breeding material for use in resistance breeding programmes. The present paper summarizes the progress achieved so far.

MATERIALS AND METHODS

Plant material

The cultivars used in this study represent a random sample from the collection of the Institute of Agronomy and Plant Breeding I, Justus-Liebig University, Giessen.

Isolate and inoculum preparation

A monoconidial isolate of *B. cinerea* obtained in 1987 from naturally infected sunflower plants was used in the inoculation experiment. Excised infected plant pieces were surface disinfected by dipping in 70% ethanol for 15 sec, then into a 1% solution of sodium hypochlorite for 15 sec, and finally into sterile distilled water. The collected strain was isolated by placing surface disinfected pieces on potato-dextrose-agar (PDA) plus streptomycin. For inoculation of plants, the isolate was grown on V-8 juice agar, at 22°C, in the dark. To maintain germination and infection capacity, conidia from 10-12 days old cultures were used (Clark & Lorbeer 1976; Blakeman 1980). Conidial suspension was prepared by flooding the cultures with distilled water and then rubbing the culture surface with a glass rod to dislodge spores.

The conidial suspension obtained was filtered through sterile muslin to remove hyphal fragments, followed by washing and centrifugation. Conidia were resuspended in distilled water and then adjusted to required concentration ($1x10^6$ spores ml^{-1}). Inoculum concentration was determined by haemocytometer counts.

Plant inoculation

Sunflower heads at full flowering stage were subjected to inoculation and sprayed until complete wetness with a suspension of inoculum using a hand-operated sprayer.

Disease measurement and observation

Disease severity was used as a measure of sunflower reaction. Individual plants in plots were rated for head rot symptoms on a scale of (1) no symptoms, (2) slight infection of epidermal cells; sporadic distribution of necrotic lesions, (3) moderate infection; large area of tissue show discolloration; no sporulation, (4) severe head rotting; intact tissues with *Botrytis* sporulating profusely, (5) extreme head rotting; tissues disintegrating, (6) decayed tissues. Plants in disease categories 1 and 2 are considered to be resistant, 3 moderately susceptible, and 4 to 6 susceptible. Cultivars were routinely monitored for disease symptoms starting with the anthesis stage. However, disease assessment began 129 days after sowing and was conducted three times at weekly intervals.

Field experiments

In the 1987 growing season, a preliminary survey of 49 sunflower genotypes was carried out at two locations (Gross-Gerau and Rauischholzhausen) under natural field

infection. In order to gain further information on the reaction of these cultivars, a selection of five hybrids ('Alphasol', 'Flamme', 'Frankasol', 'NS-H-45', and 'Primasol') chosen on the basis of their disease reaction, were subjected to inoculation in another field experiment performed at Gross-Gerau in 1988.

Growth chamber experiment

An experiment with inoculation was also carried out under controlled environmetal conditions in 1988 with the hybrids'Alphasol', 'Frankasol', 'NS-H-45' and 'Primasol'. Plants were raised in Mitscherlich-vessels (1 plant/vessel) under the following climatic conditions: photoperiod 16hrs, temperature at germination 20°C, until the 4rd-true-leaf-stage 20°C day/12°C night, until the 6th-leaf-stage 22°C day/14°C night, from the 6th-leaf-stage to the end of the experiment 24°C day/16°C night. Relative humidity was maintained at 30% and raised to 98% immediately after inoculation to maintain moist condition.

Progeny evaluation

Of particular interest was the perfomance of the cvs. 'NS-H-45' and 'Flamme'. The susceptible cultivars were also included in the experiment to assure that adequate disease pressure was present. An experiment was therefore conducted in 1989 to evaluate selfed progeny (S_1) of the (selected) F_1 hybrids 'Alphasol', 'Flamme', 'Frankasol', 'NS-H-45' and 'Primasol'. The S_1 progeny of each hybrid was obtained by bulking seeds from single-plant-progenies produced by self-pollination of resistant plants. Both the F_1 hybrids and their S_1 progenies were grown under natural infection in the field. The trial consisted of 10 plants of each F_1 hybrid and 25 plants of each S_1 progeny. In addition, the 20 single-plant-progenies (with 52 individual plants) of the cultivars 'NS-H-45' and 'Flamme' were also evaluated.

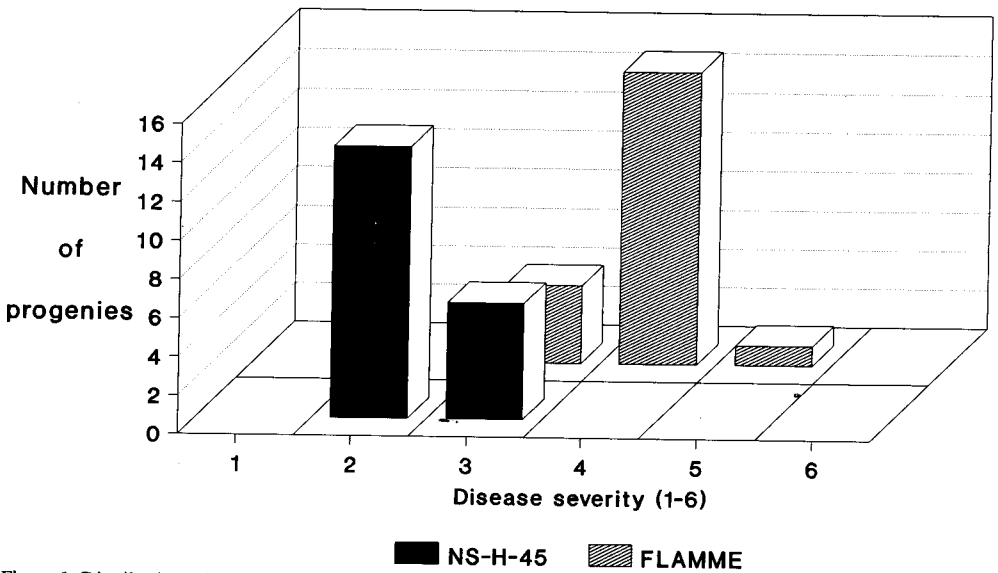

Figure 1. Distribution of single-plant-progenies of two sunflower cultivars with low susceptibility to B. cinerea

Table 1: Disease severity differences of sunflower cultivars observed under natural conditions at two locations in 1987

	Disease Severity (mean)[1]	
	Gross-Gerau 1987	Rauischholzhausen 1987
Cultivar***	Mean and LSD Group#	Mean and LSD Group#
NS-H-45	2.18ab	1.94ab
Primasol	4.69d	3.53d
Cargisol	2.01a	2.25ac
Flamme	2.26ab	1.41b
Mirasol	2.61ab	3.45d
Frankasol	3.39bc	2.74cd
NS-H-27	3.15bc	3.14cd
Alphasol	1.78a	2.78cd
Cerflor	3.80cd	3.81d
Kaliflor	4.16cd	3.96d
Average	3.16	2.72

[1] calculated from untransformed data
*** significant at $P \leq 0.001$
# within column means suffixed by the same letter are not significantly different at 5% level according to Kruskal-Wallis test (LSD grouping based on ranked data)

Table 2: Cultivar reaction to inoculation with *B. cinerea* under field and controlled conditions in 1988

Cultivar	Field 1988 Mean values/ LSD Group#	Growth chamber 1988, Mean values/ LSD Group#	Class
NS-H-45	2.23a	1.89a	part.resistant
Flamme	2.25a	-	part.resistant
Frankasol	3.17b	3.17b	mod.susceptible
Primasol	4.32c	3.44b	susceptible
Alphasol	4.37c	3.56b	susceptible

*** significant at $P \leq 0.001$
# within column means suffixed by the same letter are not significantly different at 5% level according to Kruskal-Wallis test (LSD grouping based on ranked data)
mod = moderately, part = partially

Table 3: The reaction of five sunflower cultivars in three different environmental conditions in 1987 and 1988

	Cultivar***					
	Alphasol	Flamme	Frankasol	NS-H-45	Primasol	
Location**	(Disease severity)					Average#
GG'87	1.78	2.26	3.39	2.18	4.69	2.95a
RH'87	2.77	1.41	2.74	1.94	3.53	2.49a
GG'88	4.37	2.25	3.17	2.23	4.32	3.27b
Average***z	3.61b	2.09a	3.13b	2.16a	4.24c	

, * significant at $0.01 \geq P > 0.001$ and $P \leq 0.001$, respectively
GG=Gross-Gerau, RH=Rauischholzhausen
# within column (z within row) means suffixed by the same letter are not significantly different at 5% level according to Kruskal-Wallis test (LSD grouping based on ranked data)

Experimental design and data analyses

In 1987, the experimental design was a 7x7 triple lattice. Cultivars were grown in plots consisting of three rows with 15 plants each. In the experiment of 1988, cultivars were grown in double-row-plots with 10 plants per row, in a completely randomised block design with three replications. In both trials plants were spaced 0.4m within and 0.5m between rows.

Statistical analyses were performed on a CDC Cyber 180/860 using the statistical program packages SPSS^x and BMDP (Pfeifer 1988). Due to the absence of homoscedasticity (equality of variances among samples) and normality of the residuals in the data obtained, analyses of variance were carried out and significant differences between means determined using Kruskal-Wallis multiple comparison test (Diehl&Kohr 1977; Siegel 1985; Thöni 1985), the non-parametric equivalent of a one-way analysis of variance. The null hypothesis tested is that the population distributions are identical. The progress of the disease (repeated sampling) was determined using the SPSS^q procedure reliability-Friedman's analysis of variance for ranked data (Siegel 1956).

RESULTS

Field experiment 1987

Most sunflower cultivars were susceptible to *Botrytis*. Within cultivars, plants with different disease levels were observed. In Table 1, disease severity means of 10 sunflower cultivars are summarized. As can be seen, cultivar reactions were generally inconsistent at the two locations. However, the hybrids 'Cerflor' and 'NS-H-27' exhibited a consistent pattern of reaction at both locations. Differences in disease severity means among cultivars were highly significant ($P<0.001$). For example, in Gross-Gerau, the hybrids 'Alphasol' (1.78), 'Cargisol' (2.01), 'NS-H-45' (2.18), and 'Flamme' (2.26) exhibited low levels of disease, while 'Kaliflor' (4.16) and 'Primasol' (4.69) had the highest disease severity levels. In Rauischholzhausen, the cultivars showed lower susceptibility in general, with 'Flamme' (1.41), 'NS-H-45' (1.94) and 'Cargisol' (2.25) being comparatively resistant and 'Primasol' (3.53) and 'Kalifor' (3.96) showing greater susceptibility.

The Friedman's test indicated high significance ($P<0.01$) between the different sampling dates, indicating an increase in disease level with each disease assessment.

Field experiment 1988

The results of cultivar reaction to inoculation are presented in Table 2. Significant differences among cultivars were also observed under inoculation. 'NS-H-45' and 'Flamme' were the most resistant cultivars, whereas 'Frankasol' exhibited moderate susceptibility. The disease reaction patterns of the cultivars were generally unaffected by inoculation when compared with the 1987 results, especially with those observed in Gross-Gerau in 1987, except 'Alphasol', which had a higher disease value in 1988.

Growth chamber experiment

In the growth chamber, despite high relative humidity, cultivar susceptibility was less pronounced than in the field study (Table 2). Cultivar reaction was significant, whereby, 'NS-H-45' varied markedly from the other cultivars. According to their reaction to

inoculation, the hybrids 'Flamme' and 'NS-H-45' were considered partially resistant, 'Frankasol' moderately susceptible, and 'Alphasol' and 'Primasol' susceptible.

Cultivar performance over three environments

The Kruskal-Wallis variance analysis emphasized highly significant effects ($P<0.001$) of cultivar and environment (Table 3). In the three environments, 'Flamme' and 'NS-H-45' exhibited an average disease severity of 2.09 and 2.16, respectively. In comparison, the cultivars 'Alphasol' (3.61), 'Frankasol' (3.13), and 'Primasol' (4.24) showed a more susceptible reaction. Environmental effects were also significant, between Gross-Gerau 1987 and 1988, and between Rauischholzhausen 1987 and Gross-Gerau 1988.

Progeny evaluation

Interestingly, the S_1 progeny of the cultivar 'NS-H-45' showed, despite segregation, no profound increase in disease level as compared with the parent reaction. By contrast, the S_1 progeny of 'Flamme' were susceptible (Table 4). The performance of the single-plant-progenies showed most progenies of the cv. 'NS-H-45' falling into disease severity class 2 and 3, while a greater number of progenies of the cv. 'Flamme' was susceptible (Figure 1). The high frequency of resistant progenies, especially of 'NS-H-45', indicates that selection for low susceptibility is possible and, therefore, an improvement of resistance of sunflower to *B.cinerea* should be feasible.

Table 4: The mean disease values of the F_1 hybrids and their S_1 progeny (field experiment Gross-Gerau 1989)

Cultivar***	F_1 hybrid#	S_1 progeny#
Alphasol	4.63c	4.94c
Flamme	2.22ab	3.26b
Frankasol	3.05b	3.46b
NS-H-45	1.67a	2.14a
Primasol	4.56c	4.79c

*** significant at $P \leq 0.001$
#within column means suffixed by the same letter are not significantly different at 5% level according to Kruskal-Wallis test (LSD grouping based on ranked data)

DISCUSSION

The screening of a collection of sunflower hybrids indicates genotypic variation for susceptibility to the pathogen. Most cultivars evaluated were susceptible to the disease. However, substantital differences among sunflower genotypes in resistance to infection by *B.cinerea* were detected. Several cultivars with considerable degree of resistance to the pathogen were identified with 'NS-H-45' being the most promising. 'NS-H-45' showed a comparatively stable disease reaction over the environments and low levels of susceptibility were also exhibited by the progenies of this cultivar, illustrating that the reaction may be of a strong genetic background and there is therefore variation upon which selection can act. The present state of knowledge indicates resistance to many diseases of sunflower, including those caused by *Botrytis cinerea, Macrophomina phaseoli, Phomopsis helianthi, Sclerotinia sclerotiorum,* and *Verticilium dahliae* to be partial and polygenically controlled. However, the chance of finding complete resistance to unspe-

cialized pathogens like *Bothytis cinerea* are slim. Although complete resistance to *B. cinerea* may never be achieved, any increase in the level of resistance would be beneficial for successful sunflower cultivation.

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REACCION DIFERENCIAL DE GENOTIPOS DE GIRASOL A INFECCION DE *Botritis cinerea* PERS

RESUMEN

Varios hibridos de girasol (*Helianthus annuus* L.) fueron evaluadas para resistencia a podredumbre gris causadas por *Botritis cinerea* Pers y su potencial como posibles fuentes de resistencia fueron determinados. Los cultivares fueron expuestos a infección natural e incubación artificial en experimentos en campo y cámara de crecimiento respectivamente. Los resultados obtenidos mostraron unas diferencias significativas ($P<0.001$) en severidad de las enfermedad entre cultivares. Sin embargo ningún genotipo fué completa–mente resistente. Un número de cultivares expresa bajos niveles de susceptibilidad particularmente el hibrido NS-H-45. La evaluación de la progemie S1 u progemies de plantas individuales de este cultivar reveló que la selección para bajo nivel de susceptibilidad y por tanto la mejora para resistencia es posible.

RÉACTION DE GÉNOTYPES DE TOURNESOL À UNE INFECTION PAR *Botritys cinerea*

RÉSUMÉ

Plusieurs hybrides de tournesol (*Helianthus annuus* L.) ont été testés d'une part pour leur résistance à la pourriture grise provoqué par *B. cinerea* et d'autre part pour leur utilisation comme sources de résistance. Les cultivars ont été soumis à des inoculations naturelles en champ et artificielles en chambre de culture. Les résultats obtenus montrent des différences hautement significatives ($P<0.001$) concernant la sévérité d' attaque selon les cultivars. Cependant, aucun génotype n'était totalement résistant. Un certain nombre d'entre eux ont experimé un faible niveau de sensibilité, en particulier l'hybrides NS–H 45. L'évaluation de la descendance S1 et de la single plant progenies de cet hybride révéle que la sélection pour un faible niveau de sensibilité, et de ce fait pour la résistance, est possible.