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METHODS OF STUDYING THE REACTION OF SOME CULTIVARS AND WILD SPECIES OF SUNFLOWER TO INFECTION BY *SCLEROTINIA SCLEROTIORUM.*

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ABSTRACT

The work carried out at the Experimental Station of C.E.T.I.O.M. in Saint-Pathus was aimed at studying semi-artificial inoculation of sunflower and cultivar response to inoculation, with *Sclerotinia sclerotiorum.* The trial was carried out in the field, partially under a roof. The results showed that *S. sclerotiorum* developed more slowly on two cultivars, 9SC-69(4) and Stepniak 1-2, than on all other cultivars tested. Rates of disease development differed, depending on which inoculation technique was used. The two techniques which produced different rates of disease development were: (1) a spore suspension of 40 ascospores mm^{-3} and; (2) wet confetti with 20 to 50 ascospores placed between the bracts and tubular flowers. We concluded that there were differences in the rate of disease development in sunflower heads which could not be related to resistance or tolerance.

SUMMARY (in French)

Les orientations du travail du Centre expérimental du C.E.T.I.O.M. à Saint-Pathus ont porté sur l'étude de la contamination semi-artificielle du Tournesol et la réponse des cultivars à cette inoculation. L'essai se déroule au champ, en partie sous un toit.

Des résultats on tire que: deux cultivars 9SC69(4) et Stepniak 1-2 présentent des taux d'attaque moins élevés que tous les autres; un accroissement relatif des taux d'attaque variable selon les formes est observé pour les plantes inoculées par rapport aux plantes témoins non inoculées (mais placées en zone polluée); les deux techniques permettant ces accroissements sont une suspension de spores à concentration de 40 ascospores par mm3 d'une part, et des confetti humides porteurs de 20 à 50 ascospores, disposés entre les bractées des fleurs tubulées fleuries d'autre part.

Sur capitule on conclut à des différences de taux d'expression des symptômes, ce qui ne rend pas compte de la résistance ou de la tolérance, celle-ci ne pouvant être dégagée que par comparaison sur plusieurs années.

INTRODUCTION

C.E.T.I.O.M. and I.N.R.A. have been studying possible ways of controlling *Sclerotinia* in sunflower. Among them, cultivar resistance and tolerance were studied in detail (Pierre and Regnault, 1978, 1979). We know that the normally grown cultivars do not seem to have any resistance to the disease, but disease development depends on climatic conditions and consequently varies in different years and localities. Hence, it was important to study the response of lines, hybrids, parental lines, foreign varieties and a wild species to different methods of inoculation with *S. sclerotiorum* under fairly uniform conditions.

MATERIALS AND METHODS

The following lines, cultivars and species were used:

Source	Line	Code Used in Tables
I.N.R.A. Clermont-Ferrand (Mr Leclercq)	9SC-69(4)	1
	9SC-28(4)	2
	GHP	3
	9SC-10(3)	4
	9SC-2(7)	5
	PAC 1	6
Hungary	GAHIB 7 apavonal 1980	7
	GAHIB 12 apavonal 1980	8
I.N.R.A. Montpellier (Mr Tersac)	H9P3	9
(Mr Piquemal)	STEPNIAK 1-2	10
(Mr Serieys)	9SC120-2	11
I.N.R.A. Montpellier Wild Species (Mr Serieys)	*H. rigidus* x H. annuus C G 60sib	18

Plants were grown under a semi-transparent roof which opened automatically at 21°C. This allowed temperature conditions to remain near normal while protecting the trial from rain. Cultivars were grown in plots (2 rows each 4.5 m long with 8 to 9 plants per row) randomized within each of 4 blocks. The wild species was grown at some distance to avoid any interplot interference with other cultivars. The whole trial was surrounded by at least eight rows of sunflower, variety Mirasol, in order to standardize microclimatic conditions in the experimental plots.

Inoculation Methods.

Inoculations were conducted with ascospores trapped from apothecia produced from sclerotes under room conditions. Fresh ascospores were rinsed from the trapping cup with water at room temperature. They were counted in a Thomas-cell and the desired spore concentration obtained. Uniform inoculations were carried out at flowering on the same date in each block. Every second plant was inoculated and immediately covered with a plastic bag fastened around the stem. The adjacent non-inoculated plant was sprayed with water and also covered with a plastic bag in order to prevent other inoculations. Plastic bags remained on the heads for 60 — 100 hours. It is worth repeating that these plants were grown under a roof, they could be inoculated either naturally (by ascospores present in the air and coming from apothecia present in the neighbouring fields), or semi-artificially (by spores emitted from apothecia produced by sclerotia placed in cultivated varieties).

Inoculation Conditions.

Block 1: 10 ascospores mm^{-3} date 1st of August.

Block 2: No artificial inoculation, therefore no bagged controls as described above. This block was fogged for 45 hours on the 29th of July and the 7th of August.

Block 3: 40 ascospores mm^{-3} date 4th of August.

Block 4: The method used here was that of Mrs Lamarque (1978, 1980) who works with ascospore deposits on dry confetti. The confetti was soaked in a suspension containing about 50 ascospores mm^{-3}. After determining the amount of water absorbed by the confetti and counting the ascospores remaining in suspension we concluded that each piece of confetti carried between 20 and 50 ascospores.

RESULTS

Plants were inspected for disease development at several stage of plant growth. The stages were: F4 end of flowering — wilted ligule flowers; M1a beginning of maturity — the back of the head is yellow — green or yellow; M1b beginning of maturity — the first leaf on the stem near the crook has dried; M2a end of maturity — leaves on the upper half of the stem are dried in 50% of cases; M2b all leaves are dried with harvesting often possible at this stage.

A) Observations on disease development — meaned data from all inoculation treatments.

Growth stage when disease symptoms appeared.

Observations showed that symptoms appeared from flowering to stage M1b (once reached) i.e. for a period of 3 weeks. After that disease levels became constant which indicated that infection had taken place at a definite point in plant growth.

Final levels of disease development are listed in Table 1. Data collected included the number of plants infected by *Sclerotinia* only or by both *Sclerotinia* and *Botrytis*. Plants infected by *Botrytis* also were not included in the calculation.

There were quite different cultivar responses to inoculation. The lowest level of disease development occurred in the following cultivars: 9SC69(4), STEPNIAK 1-2 and PAC 1.

Table 1. Final level of Sclerotinia in Primary Head. (mean of all methods)

		Cultivar (Number)												Mean	Percent increase
		1	2	3	4	5	6	7	8	9	10	11	18		
Plants infected with Sclerotinia %	Inoculated	4	50	48	21	12	7	39	59	34	3	15	35	29	81
	Non-inoculated	1.5	24	39	18	1.5	9	33	35	32	0	11	27	16	95
	Percent increase	62	108	23	17	700	−22	18	69	6	(+)	36	30		
Proportion of Healthy plants present	Inoculated	0	0	0	0	8	0	0	0	0	0	0	0		
	Non-inoculated	4	0	0	0	16	0	0	0	0	14	0	0		
Stage at inoculation time		F4	F2 to F4	F3 to F4	F4 to F2	F1 to F2	F3 to M1a	F3 to F4	F4	F2 to F4	F2	F2	F2 to F4		
Stage at reading time		M2a	M2a	M2b	M1b	M2a	M2a	M2a	M2b	M2b	M2a	M2b	M2b		

B) **Effect of different methods of inoculation.**

These are listed in Table 2. It appears that the inoculation method used in block 4 is the most effective with a 118 percent increase in disease where plants were inoculated. However, the natural level of disease in the control plants in this block was very low when compared to the uninoculated control in other blocks. In block 3, artificial inoculation increased the percentage of infected plants by 31%.

It would appear (Table 2) that certain cultivars are infected at a particular time. For example: 9SC-69(4) either on the 29th of July or the 7th of August, PAC 1 only on the 30th or 31st of August; Stepniak 1-2 on the 4th of August and; 9SC-2(7) on the 29th of July or the 7th of August.

Table 2. Final level of Sclerontinia according to the different inoculation rates.

Plots	1		2	3		4		
Varieties	I	NI	NI	I	NI	I	NI	% max I
1	0	0	6	11	0	0	0	11
2	33	12	23	75	12	43	50	75
3	37	37	18	70	85	37	16	85
4	14	11	23	17	20	33	17	33
5	0	0	6	0	0	37	0	37
6	22	37	0	0	0	0	0	37
7	22	33	35	50	63	44	0	63
8	37	20	23	80	62	—	—	80
9	44	50	39	37	33	22	7	50
10	0	0	0	8	0	—	—	8
11	33	25	11	12	9	0	0	33
18	33	30	28	50	28	22	22	50
Mean	22	21	18	34	26	24	11	
Percent increase	5		—	31		118		
inoculation dates	30-31/7		29/7 and 7/8	4/8		8/8		

I = inoculated
NI = non-inoculated

The percentage of plants infected is highly variable — low for 9SC-69(4) and Stepniak 1-2 (11% maximum) and higher for 9SC-2(7) and PAC 1 (37% maximum). The levels of infection for the other cultivars were highly variable in each plot. Hence, from a breeding point of view, we consider it better to remove cultivars which have a high level of infection, even if this only occurs occasionally.

DISCUSSION AND CONCLUSION

The data reported here does not claim to solve the problem of *S. sclerotiorum* but does provide data on inoculation techniques and variable reactions to the fungus. The following points can be made:

Undoubtedly inoculation techniques had some effect on disease expression as did the time of inoculation and the growth stage of the plant when inoculation occurred. Ascospore discharge occurred over something like a 10 day period, so cultivars with a wide flowering period may have avoided some infection. We might be able to overcome this by inoculating one head several times.

All things being equal, it seems that the best inoculation technique is to use an ascospore suspension with a concentration of 40 mm^{-3}. On some occasions, however, it may be useful to use the confetti method (although this wounds the head as we forced the plant microclimate interface) because the confetti can provide a nutritive medium which the fungus requires to develop its saprophytic phase (as reported by several authors).

High temperatures in the bugs may limit disease development and therefore, the success of a particular technique.

The low levels of infection obtained in the various cultivars do not indicate resistance but might be an expression of tolerance with the pathogen producing typical symptoms within a precise microclimate. High levels of infection indicate that these conditions existed. The expression of tolerance in a given cultivar under field conditions, would then depend on favourable enivronmental conditions occurring at the same time as the correct growth stage of the plant. Hence, tolerance could only be acquired in appropriate controlled conditions when one could be sure that climatic conditions necessary for infection occurred at the growth stage most conducive to infection.

From these observations it can be seen that further study on cultivar response to infection by *S. sclerotiorum* should be first be carried out with 9SC-69(4) and Stepniak 1-2 and then with PAC 1 and 9SC-2(7).

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FIELD INOCULATION OF SUNFLOWER FOR *SCLEROTINIA SCLEROTIORUM* BASAL STALK ROT AND VIRULENCE OF ISOLATES FROM VARIOUS HOSTS.

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ABSTRACT

Effective field screening of sunflower (*Helianthus annuus* L.) for resistance to basal stalk rot, caused by *Sclerotinia sclerotiorum*, can be difficult in naturally infested soils because of uneven inoculum distribution. In 1979 and 1980 field trials, an inoculation method was used to achieve uniform field infection. For inoculum, *S. sclerotiorum* was grown on auto-claved oats. The inoculum was placed 2 — 3 cm below the base of 6 week-old plants (6 ml/plant), then covered with soil. In 1979, certain genotypes had a lower percent of infected plants compared with susceptible genotypes. The survivors were selfed, then selfed again in a winter nursery, and the S_2 generation screened in the 1980 field nursery. Progeny from surviving plants had a significantly lower percent of basal stalk rot than susceptible material, indicating that the parental genotypes had some resistance to *S. sclerotiorum*, and that resistance was transferable to the S_2 generation. One isolate each of *S. sclerotiorum* from soybean, carrot, and snapbean, and two from sunflower, were evaluated in the field for virulence on two susceptible and two resistant sunflower genotypes. There were significant differences in virulence between the isolates, based on a percent of plants infected. A sunflower isolate which had been repeatedly subcultured in the lab was significantly less virulent than an isolate recently obtained fron sunflower.

INTRODUCTION

Basal stalk rot of sunflower, caused by *Sclerotinia sclerotiorum*, is a significant problem in many of the sunflower growing regions of North America (Gulya, 1981; Sackston, 1981). Resistance or tolerance in *H. annuus* to basal stalk rot has recently been reported by various workers (Dueck and Campbell, 1978 and Zimmer and Hoes, 1978). In 1979, we evaluated 85 lines for resistance to *S. sclerotiorum* in the field. Surviving plants were self-pollinated, the S_1 progeny self-pollinated in a winter nursery, and the S_2 generation evaluated for reaction to *S. sclerotiorum* in 1980. In addition, we compared isolates of *S. sclerotiorum* from sunflower, soybean, snapbean and carrot for virulence to sunflower genotypes differing in reaction to basal stalk rot. Results of these studies are presented in this paper.

MATERIALS AND METHODS

To obtain uniform field infection, a method similar to that described by Dueck and Campbell (1978) was used. All isolates of *S. sclerotiorum* were maintained on Difco potato-dextrose-agar (PDA) at 18 — 22°C under low light. The field inoculum was produced as follows. Oat seeds were steeped in hot water for six hours, soaked overnight under room conditions, then drained. The oat medium, in aliquots of 300 gms, was placed in 1 litre canning jars modified with a