A FIELD STUDY OF RAPESEED (*BRASSICA NAPUS*) RESISTANCE TO *SCLEROTINIA SCLEROTIORUM*

BRUN Hortense[1], TRIBODET M.[1], RENARD M.[2], PLESSIS J.[1] TANGUY X. [2] .

(1) - Station de Pathologie Végétale - (2) Station d'Amélioration des Plantes I.N.R.A. - Centre de Recherches de RENNES - BP 29 - 35650 - LE RHEU - FRANCE .

INTRODUCTION.

Sclerotinia sclerotiorum is an important disease agent for oilseed rape grown in France. Increasingly intensive exploitation of this crop, and the introduction into the rotation of others also susceptible such as sunflower, faba beans, peas, soya beans and beans, may favour increase in sclerotes abundance in soil, thus risk of epidemics and gravity of attack. A certain number of fungicides can be used to appreciably reduce parasite incidence (CETIOM, 1987). However, the sporadicity in disease outbreak and difficulties in establishment of an efficient predicting system, result in a certain amount of useless applications. Furthermore, increase, in the practise of applying treatments at the onset of flowering may favour appearance of strains resistant to fungicides. Commercialisation of oilseed rape cultivars resistant to the fungus may usefully offset these problems, and provide a means of combatting the disease. The existence of genotypes having an appreciable potential for resistance to *S. sclerotiorum* has been demonstrated in a variety of species (*e.g. Phaseolus vulgaris* var. *Ex. Rico* (TU and BEVERSDORF, 1982) and *Helianthus annuus* (TOURVIELLE and VEAR, 1984). The aim of the present study was to study genetic variablity in oilseed rape resistance to *S. sclerotiorum* with different innoculation conditions.

MATERIALS AND METHODS.

Natural and artificial innoculation techniques were used to detect oilseed genotypes resistant to *S. sclerotiorum*.

1. Natural innoculation in field crops : Different oilseed genotypes were planted in a complete block design in plots (four blocks, variable treatment number) which had previously carried bean crops, sensitive to and attacked by *S. sclerotiorum* . Experimental plots were located in the department of Finistere, where climate is oceanic, and springs usually moist and mild. Most data is presented as percentage plants showing symptoms of fungus attack.

2. Artificial innoculation in field crops : Several methods were compared. Only those which resulted in high infection levels and were practically easy to apply were retained.

a) Innoculation with ascospores under aluminium paper.

Sclerotes were obtained from infected oilseed plants, or from sunflower heads or sliced carrot. They were placed in pots with slightly humid perlite, at 2 cm depth, and at 10°C . Two months later, sclerotes

were extracted, stipes removed, and they could then be kept for up to two years at 4°C . They were placed on wet cotton in 5 cm diameter covered glass dishes. Apothecia produced the first ascospores at 15 days, in daylight, and at 20°C, and these were recovered and stored following (STEADMAN, 1974). An ascospore suspension in sterile water was prepared by scraping on 2 µ micropore filter, then ultrason treatment for 7 seconds.

Innoculation flower (BRUN *and al.*, 1981) involved insertion of a 5 mm diameter filter paper pastel laid on a flower petal to ensure infection at the base of a leaf. Pastels were wetted with 30 µl of a 5.10^5 spore/ml suspension, prior to insertion. They were covered with a slightly moistened pad of cotton wool, and the whole envelopped in aluminium paper.

b) Innoculation with mycelium in matchstick fragments.

Short fragments of specially furnished crude matchsticks (5 cm) were sterilised twice at 120°C at a 24 hr interval, in a 2 % malt solution. They were then placed in 14 cm diameter petri dishes, after slight drying on filter paper. Dishes contained 4-day cultures of *S. sclerotiorum* on a 2 % malt / 2 % gelose water medium, and cultures then held at 20°C for eight days. Match fragments were then inserted in oilseed rape stems in a cut at 30 cm height (fig. 1) .

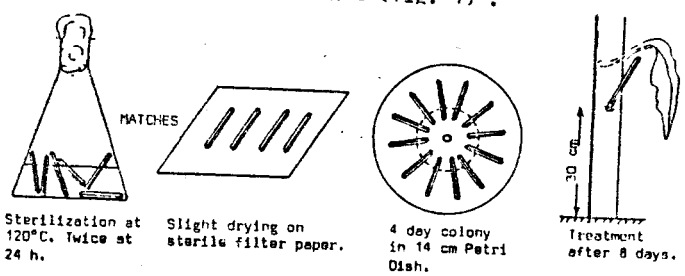

Fig. 1 : INNOCULATION USING "MATCHES" IMPREGNATED WITH *S. SCLEROTIORUM* MYCELIUM.

c) Experimental apparatus and scoring.

A single inoculation was made per plant, and 30 plants innoculated per block (four replicates per block). At three weeks percentage plants with overt lesions and length of apparent mould were scored.

3. Artificial contamination in green houses.

Overwintered plants (rosettes) were taken from field plots. Collection was staggered to obtain regrowth at different times. Gelose discs were extracted from the edges of experimental cultures of *S. sclerotiorum*. These were placed on subject stems (one per stem) at 30 cm height and covered with a strip of parafilm.

Twelve plants were innoculated per growth stage, with three replications per growth stage. Scores concerned percent infected plants, and lengths of apparent stem necrosis.

RESULTS.

1. Natural infections.

Plant resistance machanisms are best understood under natural conditions without artificial intervention. However, the limits of natural infection as a means of studying oilseed resistance to *S. sclerotiorum* are now apparent after eight years of study. With high infection levels,

varietal ranking is not consistent from year to year (fig. 2). The reason for this may be the high intervarietal variability in degree of early flowering for oilseed cultivars. Because of this, all genotypes are not necessarily or invariably subjected to the same number of contaminatory phases (conjunction of ascospores, petals, favorable climatic conditions). Moreover, despite being in an area that is generally humid, infection levels have been too low in some years (attributable to particularly dry spells) to allow varietal ranking.

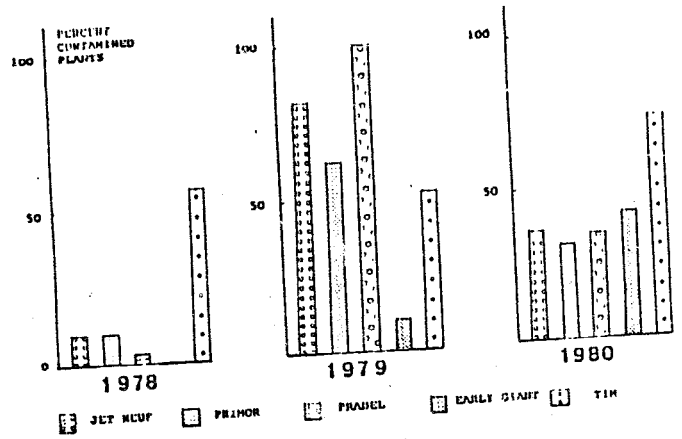

Fig. 2 : VARIETAL RANKING OF FIVE GENOTYPES UNDER NATURAL INFECTION IN THE FIELD.

2. Artificial infection.

The two artificial innoculation techniques confirm low variability in resistance to *S. sclerotiorum* of oilseed rape varieties currently grown in France (or being introduced to the french catalogue CTPS) (Table 1). This contrasts with major differences between genotypes of asiatic origin (Table 2).

Good performance of Norin 9 has been confirmed over several years of experimental study, both in terms of percent infection and length of apparent necrosis. However, for some genotypes there may be a correlation between resistance performance and flowering precocity. This possibility was tested by simultaneous innoculation of Norin 9, Jet Neuf, and Bienvenu in green house trials. Results (Table 3) indicate that growth phase of Norin 9 was not a factor affecting fungus development in stems.

DISCUSSION.

Results of the present study indicate the need for artificial innoculation techniques in study of oilseed rape cultivar variability in resistance to *S. sclerotiorum*, and search for resistance factors. The two artificial innoculation techniques suggest a possible difference in resistance potential of asiatic oilseed rape strains in comparison to those currently grown in France. Innoculation using matchstick fragments has proved more rapid in application and production of research results, than that using ascospores, and may be more useful in selection studies. Further work will be required to determine the nature of Norin 9 resistance, and possibilities for its transfer to winter varieties.

INNOCULATION WITH ASCOSPORE SUSPENSION					
	Genotypes	% plants attacked (arc sin %)	Stages •	Newman and Keuls Test for homogeneity	
1	NORIN 9	16,09	G3	A	
2	KID	47,91	G2	B	
10	PR 18	52,72	G1	B	C
12	DORAL	56,44	F2 (G1)	B	C
5	JET NEUF	58,09	G1	B	C
8	MIKADO	58,68	G2	B	C
9	EGK 1002	62,79	F2 (G1)	B	C
4	DARMOR	64,02	F2	B	C
3	BIENVENU	68,74	G2	B	C
11	PERLE	72,06	F2	B	C
7	BELINDA	74,17	F2 (G1)	B	C
6	KORINA	76,49	G1 (G2)		C
	CVM %	18,9			

INNOCULATION WITH MYCELIUM ON MATCHES					
	Genotypes	% plants attacked (arc sin %)	Stages •	Newman and Keuls Test for homogeneity	
1	NORIN 9	10,59	G3	A	
8	MIKADO	52,20	G2	B	
2	KID	54,21	G2	B	
3	BIENVENU	59,29	G2	B	
12	DORAL	62,82	F2 (G1)	B	
9	EGK 1002	63,54	F2 (G1)	B	
4	DARMOR	65,89	F2	B	
10	PR 18	66,18	G1	B	
11	PERLE	68,14	F2	B	
5	JET NEUF	71,51	G1	B	
7	BELINDA	71,52	F2 (G1)	B	
6	KORINA	80,70	G1 (G2)	B	
	CVM %	21,2			

• CETIOM, 1987

TABLE 1 : BEHAVIOUR OF CULTIVATED VARIETIES OR THOSE BEING INTRODUCED TO THE FRENCH CATALOGUE.

ASCOSPORE INNOCULATION UNDER ALUMINIUM								
Genotypes		% plants attacked (arc sin %)	Stages •	Newman and Keuls Test for homogeneity				
5	GENKAI	29,99	G2	A				
4	MIYUKI	30,32	G2	A				
6	ISUZU	33,90	G2	A	B			
1	NORIN 9	34,92	G2	A	B			
7	KOGANE	38,75	G2	A	B			
3	NORIN 16	39,67	G3	A	B			
9	TOWADA	60,90	F2	A	B	C		
10	TAISETSU	68,63	F1		B	C		
8	HOKKAIDO	79,16	G1			C		
2	KID	80,80	F2			C		
CVM %		32,8						

INNOCULATION WITH MYCELIUM ON MATCHES									
Genotypes		% plants attacked (arc sin %)	Stages •	Newman and Keuls Test for homogeneity					
1	NORIN 9	2,66	G2	A					
3	NORIN 16	8,36	G2	A	E				
5	GENKAI	9,94	G2	A	B				
6	ISUZU	13,04	G2	A	B				
7	KOGANE	18,08	G2	A	B	C			
4	MIYUKI	19,69	G2	A	B	C			
8	HOKKAIDO	21,69	G1		B	C	D		
2	KID	32,25	F2			C	D	E	
9	TOWADA	35,30	F2				D	E	
10	TAISETSU	41,09	F1					E	
CVM %		39,2							

• CETIOM, 1987

TABLE 2 : BEHAVIOUR OF ASIATIC GENOTYPES UNDER ARTIFICIAL INFECTION.

Genotypes	Growth Stages	Mean necrosis length (cm)	Newman and Keuls test for homogeneity	CMV %
NORIN 9	E	3,8	A	
NORIN 9	G3	4,7	A	
NORIN 9	G1	5,3	A	17,4
JET NEUF	G3	7,6	B	
JET NEUF	G1	8,0	B	
BIENVENU	G3	8,2	B	
BIENVENU	G1	8,3	B	

TABLE 3 : INFLUENCE OF GROWTH STAGE ON MEAN NECROSES LENGHT ON OILSEED STEMS IN GREENHOUSE.

REFERENCES.

BRUN H. et M. RENARD, 1982. *Sclerotinia sclerotiorum* : techniques de contamination - Agronomie (3), 1, 93.

CETIOM, 1987. Colza d'hiver - Les maladies - Cahiers techniques CETIOM.

STEADMAN J.R. and G.E. COOK, 1974. A simple method for collecting ascospores of *Whetzelinia sclerotiorum*. Plant Dis. Reptr. 58 (2), 190.

TOURVIEILLE de LABROUHE, D. and F. VEAR, 1984. Comparaison de méthodes d'estimation de la résistance du Tournesol à *Sclerotinia sclerotiorum* (Lib.) de Bary - Agronomie, 4 (6) 517-525.

TU J.C. and BEVERSDORF W.D., 1982. Tolerance to white mold (*Sclerotinia sclerotiorum* (Lib.) de Bary) in EX RICO 23, a cultivar of white bean (*Phaseolus vulgaris* L.). Can. J. Plant Sci. 62, 65-69.