Techniques for artificially inoculating oilseed rape with *Sclerotinia sclerotiorum*.

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Two techniques were developed for artificially inoculating oilseed rape with *Sclerotinia sclerotiorum* (Lib.) de Bary, to use in screening plants for disease resistance. One method used an ascospore suspension sprayed on to flowering plants and the other method used mycelial inoculum attached to the plant stems.

Materials and methods

Mycelial inoculation. Pearl barley was soaked for 24 hours in distilled water before autoclaving at 28 psi for 40 minutes. The flasks of barley were inoculated with plugs of *S. sclerotiorum* growing on an acidified potato sucrose agar plate. The flasks were kept at 22°C for 6-10 days, so that the fungus colonized the grains but had not formed sclerotia. Stems of flowering rapeseed were inoculated between the 2nd and 3rd leaf from the base of the stem. One colonized grain was taped on the intact stem. Plants were maintained in the greenhouse at 30-50% RH, and visible symptoms occurred after 1 week. Typical, white, bleached lesions developed and sclerotia were sometimes formed in the stems. Disease reaction was assessed 3 weeks after inoculation by recording the % of plants developing lesions and the lesions lengths.

Ascospore inoculation. Sclerotia were cleaned from seed of naturally infected rapeseed crops and stored at 4°C for at least 2 months. Sclerotia were sown at a depth of 1-1.5 cm in 10 cm pots planted with barley seed. The pots were kept in a growth cabinet under conditions suitable for growth of the barley. Apothecia were normally produced in 4-8 weeks and production continued over several months. Sclerotia with apothecia were dug out of the pots and placed in glass dishes; high humidity was maintained by placing damp filter papers in the dishes.

Dish lids were removed once daily, and the change in atmosphere triggered puffing of spores which were collected, by suction, on Millipore filters (1.2 μ). Spore-laden filters were stored at 4°C in a dessicator, and spores remained viable for at least 6 months (Hunter, Steadman and Cigna, 1982). Flowering plants were sprayed to run-off with an ascospore suspension made by brushing spores from filters soaked in distilled water for 1/2 hr. Concentrations of approximately 6 X 10^4 spores/ml were used, with 1 drop of Tween-20 added per 100 ml of suspension. Sprayed plants were maintained in a dew chamber at 98-100% RH for 6 days and then transferred to a growth cabinet (RH ≈ 70%). Infections were noticeable when the plants left the dew chamber. Petals were colonized and infection spread to the stems via leaves and leaf axils. Assessments of the percentage of infected plants and disease reaction were made 3 weeks after inoculation.

Disease reaction in the field. The material to be evaluated was grown in 4 row plots, 6 m long, in an area where sclerotia of S. sclerotiorum were spread the previous autumn. Apothecia produced by the sclerotia provided a natural source of ascospore inoculum. Disease reaction was assessed at harvest time by the percentage of infected plants.

Rapeseed material evaluated. Populations 4, 6, 8, and 9 were the F1 seed from crosses between 4 Japanese selections of spring type B. napus and Altex, a Canadian B. napus cultivar. Populations 6-1 and 6-2 were single plant selections from population 6 grown in the field. Populations 4R and 6R were reciprocal crosses of 4 and 6. Reaction of the populations was compared with that of three Canadian B. napus cultivars, Altex, Andor and Westar.

Results and discussion

Results from preliminary tests using both inoculation techniques are given. Two stem inoculation tests were carried out. Test 1 compared populations 4, 6-1, 4R and 6R with Westar, Altex and Andor (Table 1). Both 4R and 6R appeared resistant to disease, and 6-1 and 4 were only slightly susceptible. In test 2 populations 6-2, 8, 9 and 6R were compared with Westar and Andor. Westar had the lowest percentage of infected plants but the plants infected were severely diseased and developed large lesions. Populations 9 and 6R were slightly less susceptible than Andor, and 6-2 and 8 were more susceptible. Population 6-2 had the highest percentage of infected plants but the average lesion length was low, possibly indicating a slow rate of disease progress.

Two ascospore inoculation tests were carried out on the same material tested by stem inoculation. In test 3, populations 4R, 6R, 6-1 and 4 all appeared less susceptible than the 3 standard cultivars, with 4R and 6R having the lowest percentage of infected plants. In test 4 all populations except 8 were less susceptible than the standard cultivars.

The results of the two types of inoculation test were in agreement except for the ranking of Westar in test 2. All populations except 8 were less susceptible than Altex and Andor. However, in the field plots Westar was the least susceptible and population 6 was the only population less susceptible than Andor (Table 3). Population 8 was very susceptible in the field, which agrees with the results of the inoculation tests. The populations tested were all at very early generations in the selection process and a large amount of variation might be expected. The value of the inoculation tests in determining disease reaction in the field would be better assessed with more homogeneous material.

Reference

Hunter, J.E., Steadman, J.R., and Cigna, J.A. 1982. Preservation of ascospores of Sclerotinia sclerotiorum on membrane filters. Phytopathology 72: 650-652.

Table 1. Mycelial inoculation of stems of oilseed rape with *S. sclerotiorum*.

Material evaluated	Total no. of plants inoculated	% of plants infected	average lesion length/infected plant (cm)
Test 1			
Popln. 6R	12	0	0
" 4R	12	0	0
" 6-1	12	8	1.0
" 4	12	8	2.0
Westar	12	25	6.0
Altex	11	33	2.5
Andor	12	33	8.1
Test 2			
Westar	9	22	12.0
Popln. 9	14	29	4.9
" 6R	14	29	7.7
Andor	14	36	6.8
Popln. 8	14	43	6.8
" 6-2	13	62	3.6

Table 2. Inoculation of oilseed rape with ascospores of *S. sclerotiorum*.

Material evaluated	Total no. of plant inoculated	% of plants infected
Test 3		
Popln. 4R	9	0
" 6R	10	10
" 4	10	20
" 6-1	8	33
Altex	11	36
Westar	10	50
Andor	12	75
Test 4		
Popln. 9	9	56
" 6R	10	60
" 6-2	14	71
Andor	11	100
Westar	12	100
Popln. 8	10	100

Table 3. Response of material evaluated to naturally-produced disease inoculum in field plots.

Material evaluated	% of plants infected (out of 40 assessed)
Westar	5
Popln. 6	20
Andor	36
Popln. 9	43
" 4	65
" 8	85