Influence of host seedling exudates on germination of sclerotia of Sclerotinia sclerotiorum

S.R. RIMMER and J.G. MENZIES. University of Manitoba, Department of Plant Science, Winnipeg, Canada R3T 2N2

Introduction

Sclerotinia sclerotiorum (Lib) de Bary is an important pathogen of rape (Brassica napus L.), sunflower (Helianthus annus L.) and many other crops. Huang and Dueck (1980) observed that sclerotinia-wilted sunflowers were attacked below the soil line and that wilt resulted from infections derived from sclerotia which had germinated myceliogenically. However stem rot of rape and head rot in sunflowers result from infections above the soil surface and these infections are initiated from ascospores released from apothecia produced by carpogenic germination of sclerotia (Kruger, 1975; McLean, 1958; Morrall and Dueck, 1982).

The influence of root exudates on the germination of propagules of soil-borne plant pathogens is well known (e.g. Coley-Smith and Cooke, 1971). One explanation of the different behaviour of Sclerotina sclerotia when associated with these two hosts may be that sclerotia are affected in their type of germination by host root exudation. To investigate this hypothesis we studied the germination of sclerotia of isolates of S. sclerotiorum as influenced by the proximity of different hosts and by exudates obtained from seedlings of rape, sunflower and corn.

Materials and Methods

Sclerotia of six single spore isolates of \underline{S} . $\underline{sclerotiorum}$ grown on PDA were used in germination experiments.

 $\underline{\text{Experiment 1}}$. Exudates of rape, sunflowers and corn were collected in glass petri dishes containing 10 ml of water and

sterile seedlings of the crop species. Exudates from each host were bulked, freeze dried and stored at -5° C. A mixture of 0.05 g of exudate and 100 ml of double distilled water was used in germination experiments. Double distilled water was used as a control solution. Sclerotia were preconditioned for germination for six weeks at 5° C in moist sand.

To examine the effects of exudates on germination of sclerotia glass fibre discs were placed in the centre of glass petri dishes and surrounded with washed sand. Ten ml of double distilled water were gently pipetted onto the glass fibre discs and then either 5 ml of seedling exudates or double distilled water was added. Preconditioned sclerotia were then placed in two concentric circles of ten sclerotia on the sand at 1.5 and 4 cm respectively from the centre of the plate. An additional 3 ml and 2 ml of the original treatments were added at 2 and 4 weeks respectively to replenish the exudates in the dish. The plates were examined each week and the amount and type of germination was recorded. Germinated sclerotia were removed to prevent possible effects on other sclerotia. The experiment was terminated after 8 weeks.

Experiment 2. Sunflowers (cv 894) and rape (cv Regent) were grown in soil in pots in the greenhouse. Preconditioned sclerotia of two isolates of \underline{S} . sclerotiorum in nylon mesh bags were placed in the soil 1.5 cm and 5 cm from the stem when the plants were 15-20 cm tall. The amount and type of germination was recorded each week for 7 weeks.

Results

Experiment 1. Three types of sclerotial germination were observed in response to seedling exudates, viz. eruptive mycelial germination (EMG) consisting of a mass of hyphae arising from one or more locations on the sclerotium, general myceliogenic germination (GMG) involving diffuse single hyphal strands and carpogenic germination (CG). The analysis of variance for germination of sclerotia of the six isolates of S. sclerotiorum after eight weeks as influenced by seedling exudates is presented in Table 1. All of the six isolates used in the study showed significant differences for rates and types of germination (Table 2). GMG of all the isolates except isolate SSO7 was affected by the seedling exudates. Two of the isolates were stimulated for GMG by the exudates whereas three of the isolates were inhibited by rape and corn exudates. Two of the isolates were affected by seedling exudates for EMG. Isolate SSO3 had a higher rate of EMG when treated with corn and rape exudates compared to sunflower and the water control, whereas SSO7 had a higher rate of EMG with corn than with rape

exudate. Generally there was no effect of the seedling exudates on CG of the isolates, but SS22 had a higher rate of CG with sunflower exudate than with rape or corn exudates.

Experiment 2. The data for this experiment are presented in Table 3. Significant differences between isolates SS03 amd SS22 were observed for both mycelial and carpogenic germination. For both isolates there was a tendency for more carpogenic germination to occur in association with sunflowers but this was not significant. Virtually all the sclerotia of isolate SS03 germinated carpogenically regardless of host and at a higher level than with isolate SS22.

Mycelial type of germination was higher for SS22 than with SS03, and was higher in association with sunflower than with rape (P=.05). As can be seen from the data there is a significant host x isolate interaction for mycelial germination.

The location of the sclerotia, either $1.5\ \mathrm{or}\ 5\ \mathrm{cm}$ from the host stem did not affect the amount or type of germination.

Discussion

These experiments provide evidence that certain isolates of Sclerotinia sclerotiorum may be influenced in their type of germination by the proximity of different crop plants. The results of the experiment 1 in relation to the effects of seedling exudates on myceliogenic types of germination are rather inconclusive and the major effects seemed to be inhibition of mycelial types of germination by rape and corn exudates rather than the stimulation of myceliogenic germination as observed with sunflowers in the second experiment. One difficulty with studies on the effects of plant exudates is determining the appropriate concentrations to employ to simulate natural exudation in the rhizosphere. The data indicate that the type of germination that occurs depends greatly on the isolates of pathogen and their genetic makeup and that environmental influences can modify this to a certain extent. are encouraged by these preliminary experiments that sunflowers do influence germination of sclerotia in the root environment and further studies are in progress to clarify this issue.

References

- Coley-Smith, J.R. and R.C. Cooke. 1971. Survival and germination of fungal sclerotia. Ann. Rev. Phytopathol. 9:65-92.
- Huang, H.C. and J. Dueck. 1980. Wilt of sunflower from infection by mycelial germinating sclerotia of <u>Sclerotinia sclerotiorum</u>. Can. J. Pl. Path 2:47-52.

- Kruger, W. 1975. Die Beinflussung der Apothezien- und Ascosporen-Entwicklung des Rapskrebserregers <u>Sclerotinia sclerotiorum</u> (Lib) de Bary durch Umweltfaktoren. 2. Pflanzenkr. Pflanzenschutz. 82:101-108.
- McLean, D.M. 1958. Role of dead flower parts in infection of certain crucifers by <u>Sclerotinia</u> <u>sclerotiorum</u> (Lib. de Bary. Plant Dis. Reptr. 42:663-666.
- Morrall, R.A.A. and J. Dueck. 1982. Epidemiology of sclerotinia stem rot of rapeseed in Saskatchewan. Can. J. Pl. Path. 4: 161-168.

TABLE 1

The analysis of variance for germination of isolates of Sclerotinia sclerotiorum in response to seedling exudates.

Source of Variation	Degrees	Mean Square			
	of	Eruptive	General	Carpogenic	
	Freedom	Mycelial	Mycelial	Germin-	
		Germin-	Germin-	ation	
		ation	ation		
Replicate Number	2	0.16	2.93*	0.14	
Isolate	5	6.17**	34.81**	95.60**	
Treatment	3	1.02*	4.32**	0.10	
Isolate x Treatment	15	0.72*	3.50**	1.80	
Location	1	0.02	47.94**	3.78**	
Location * Isolate	5	0.58*	1.77*	1.15	
Location * Treatment	3	0.30	0.32	1.16	
Location * Isolate * Treatment	15	0.16	1.56	0.59	

^{*} significant (P = .05)

^{**} significant (P =.01)

TABLE 2

The manner of germination of six isolates of Sclerotinia sclerotiorum.

Manner of				a
Germination	Isolate	N	Mean	Grouping ^a
			0 500	•
EMG	SS03	192	0.599	A
	SS10	192	0.297	В
	SS13	192	0.281	В
	SS07	192	0.167	BC
	SS02	192	0.120	BC
	SS22	192	0.052	С
	5522	1,2	5,000	-
GMG	SS10	192	1.646	Α
GriG	SS03	192	1.547	Α
	SS07	192	1.359	A
	SS22	192	0.813	В
		- -	0.755	В
	SS02	192		
	SS13	192	0.750	В
			0.017	
CG	SS02	192	2.047	Α
	SS13	192	1.740	В
	SS22	192	1.740	В
	SS10	192	1.016	С
	SS03	192	0.516	D
	\$\$07	192	0.349	D
	5507	- / -	3.0.7	

a Determined using an Alpha = 0.01.

TABLE 3

Influence of host plants on mode of germination of isolates of <u>Sclerotinia sclerotiorum</u>.

Isolate	Host				
	Sunflower		Rap	Rapeseed	
	Inner	Outer	Inner	Outer	
SS03					
Carpogenic	43	47	39	36	
Mycelial	0	0	0	4	
Total Germination (%)	86	94	78	80	
SS-22					
Carpogenic	30	32	26	28	
Mycelial	8	10	1	3	
Total Germination (%)	76	84	54	62	

a Period of Experiment - 7 weeks.