Infection of oilseed rape (*Brassica napus*) by petals containing ascospores of *Sclerotinia sclerotiorum*

H. Alastair McCartney[1], Arwinderpal Heran[1], Qiangsheng Li[2] and Simon J Foster[1]
[1]Plant Pathogen Interactions, Rothamsted Research, Harpenden, AL5 2JQ, UK.
alastair.mccartney@bbsrc.ac.uk
[2] Anhui Academy of Agricultural Science, Hefei, Anhui, People's Republic of China.

ABSTRACT

In oilseed rape crops infection by *Sclerotinia sclerotiorum* is usually via ascospore-bearing petals that stick to leaves. In controlled environment experiments ascospores germinated on petals within a few hours of deposition, providing the petals were wet. The petals could infect plants for at least 24h. The effectiveness of infection depended on the number of ascospores on the leaf, up to about 100, above this, infection appeared to be independent of ascospore numbers. Similarly infection efficiency increased with the number of petals up to about 4 per leaf. Petal age influenced the effectiveness of infection, petals from flowers that had partially formed pods, produced larger lesions than younger petals. Lesions were produced at all test temperatures (between 15 and 30°C) when the humidity was close to saturation. Lesions were initiated in 2-4 days at temperatures between 20 and 25°C, but it took longer at 15 and 30ºC. Free water was not needed for infection, provided that humidity was high. Between 24 and 48h of continuous high humidity were needed for lesion formation. However, petals that dried and were then re-wetted were still be able to cause infections.

Key words: *Sclerotinia sclerotiorum*, stem rot, oilseed rape petals, infection processes

INTRODUCTION

Stem rot, caused by *Sclerotinia sclerotiorum*, is an economically damaging disease of rapeseed crops in many parts of the World. The disease is monocyclic and symptoms are usually only apparent four to six weeks after initial infection, when significant damage has already been done to the crop. In oilseed rape the main route of infection is via ascospores deposited on oilseed rape petals. The petals act as a substrate for ascospore germination. At petal-fall ascospore-infected petals stick to leaves and can initiate infections that can develop into stem rot lesions. The effectiveness of infection and disease development depends on environmental factors and on the ascospore load on the petals. We report the results of studies on the effects of ascospore numbers and environmental conditions needed for infection of oilseed rape leaves by petals.

MATERIALS AND METHODS

Ascospore production: Sclerotia of *S. sclerotiorum*, produced by the wheat-Perlite method (Sansford & Coley-Smith, 1992), were placed in Magenta vessels (Sigma Chemicals, Poole, UK) containing sterilised coarse grade Perlite (Silvaperl Products Ltd., Harrogate, UK) moistened with distilled water. A 37mm cellulose filter holder (Millipore (UK) Ltd., Watford, UK) was fixed to the lid of each vessel. The cultures were kept at 15°C until the appearance of stipes, after which they were placed under near-UV radiation to encourage apothecia growth (Mylchreest & Wheeler, 1987). Ascospores released from the apothecia were harvested onto the cellulose filters by drawing air through the Magenta vessel using a vacuum pump.

Petal inoculation: Sterile aqueous suspensions of ascospores were produced from ascospore-baring cellulose filters. Petals were inoculated by placing them in ascospore suspensions of known concentrations. The germination of ascospores on the petals was studied by clearing and staining petals using alcoholic lactophenol solution containing Trypan blue (18mL, 95% Ethanol: 6mL, Lactophenol: 1mL, Trypan blue) and examining them under a light microscope. Plants were inoculated by placing petals that had been placed in ascospore

suspensions for 3h, on leaf surfaces. The numbers of ascospores per petal were estimated by clearing and staining the petals and counting the ascospores under a light microscope.

Controlled environment experiments: Experiments were done in a CE chamber where the temperature, humidity and lighting conditions could be controlled. Most experiments were done with the spring type cultivar *Rebel* and the same *S. sclerotiorum* isolate (Great Harpenden). Lesion size was assessed using the length plus width as a lesion index (LI). Experiments were done on the time needed for ascospore germination and infection of petals; the effect of ascospore and petal number on infection; the requirements of water for infection; the effect of petal age on infection; and humidity requirements for infection.

RESULTS

Ascospores and infection: Ascospores germinated on petals within a few hours of deposition, providing the petals were wet and did not dry out. The petals could infect plants for at least 24h after inoculation. The effectiveness of infection, as determined by lesion size, depended on the ascospore numbers on the leaf, up to about 100 ascospores per leaf, above this, infection appeared to be independent of ascospore numbers (Figure 1). Similarly infection efficiency increased with the number of petals up to about 4 per leaf. It appeared to make little difference whether the petals were overlapping, touching or spaced out on the leaf. Petal age also influenced the effectiveness of infection, "old" petals, that is ones from flowers that had partially formed pods, produced larger (approximately 3 times bigger) and less variable lesions than younger petals. "Young" petals that had been frozen or freeze dried, and thus probably damaged, produce larger lesions than fresh "young" petals.

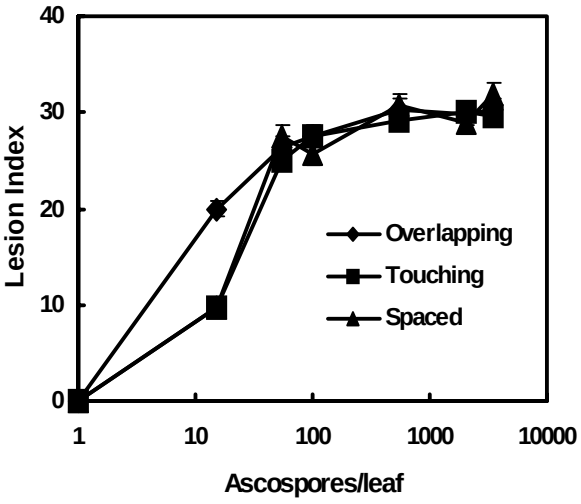

Figure 1: Ascospore numbers and lesion size (length + width). ◆ - overlapping petals; ■ - touching petals; ▲ - spaced petals.

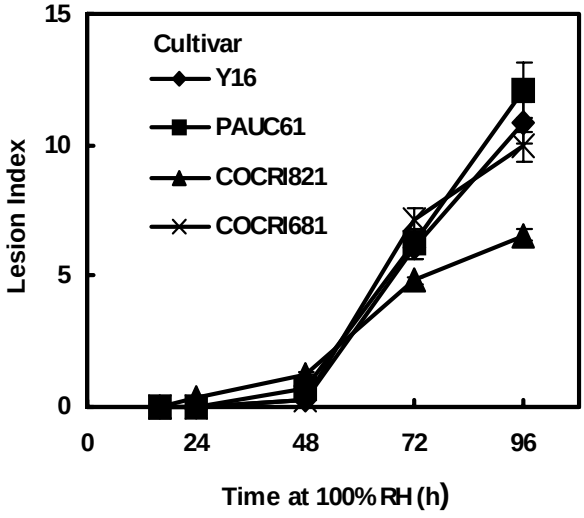

Figure 2. Effect of humidity period on infection. Plants were kept at 100% RH for different periods, then removed to low humidity. Lesion index = length + width.

Environmental effects on infection: Lesions were produced at all test temperatures (between 15 and 30°C) when the humidity was kept continuously close to saturation. Lesions were initiated in 2-4 days at temperatures between 20 and 25°C. But it took longer to initiate lesions at both 15 (4-5 days) and 30ºC (3-4 days). Free water was not needed for infection, provided that humidity was high (near saturation) and the petals did not dry out. However, lesion sizes were two or three times larger when water was present. Between 24 and 48h of

continuous high humidity were needed to initiate lesion formation from ascospores on petals placed on leaves. However, lesions size, assessed 6 days after inoculation, increased significantly as the period of continuous humidity increased (Figure 2). In these experiments the plants were kept at high humidity for different periods of time then maintained at low humidity where the petals eventually dried. Petals which dried and were then re-wetted were still be able to cause infections: in one experiment lesions formed even when ascospore-bearing petals were dried only 4h after being placed on the leaf and re-wetted 24h later (Table 1). How long they can remain dry before re-wetting and still infect is not know.

Table 1: Lesion index, 6 days after inoculation, on plants inoculated with ascospore-baring petals. The plants were kept in high humidity for different lengths of time followed by 24h in low humidity; the petals were re-wetted and returned to high humidity.

	Length of initial period at high humidity (h)						
	4	6	16	24	48	72	control
Mean LI*	19.5	17.5	16.0	20.2	17.5	17.0	18.7

*LI = lesion index (length = width), mean of 3 replicates.

DISCUSSION

Petals containing ascospores are effective sources of leaf infections provided about 100 or more ascospores are associated with the petals. Infection appears to be more effective at temperatures around 20°C. At optimum temperatures, if the petals are wet ascospores will germinate on them in about 4h. These results suggest that to infect leaves ascospore-baring petals need to be wetted and remain “wet” (either in water or in high humidity to prevent them drying) for a period of at least 48h. However, the petals may remain infective after wetting and drying, but this will increase the time needed for infection. Thus, in crops continuous high humidity for 24-48h may not be a strict requirement for infection. Further work is clearly needed to determine the dynamics of infection under the variable environmental conditions encountered in the field. Old petals seem to infect more efficient than young petals; thus petals that are ready to fall are likely to be more effective sources of inoculum. Humidity and leaf wetness clearly plays an important role in oilseed rape infection via petals, therefore knowledge of crop microclimate and the effects of intermittent wetting and dying of petals on infection are needed to understand and assess the risk of stem development in rapeseed crops.

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