SPLASH DISPERSAL OF PSEUDOCERCOSPORELLA CAPSELLAE, CAUSE OF WHITE LEAF SPOT OF OILSEED RAPE

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INTRODUCTION

White leaf spot of oilseed rape, caused by Pseudocercosporella capsellae (Ell. & Ev.) Deighton, which has a Mycosphaerella teleomorph (Inman et al. 1991), has been reported on leaves at all heights and on stems and pods in UK crops (Hardwick et al. 1989) and has caused serious epidemics in Canada (Petrie and Vanterpool 1978) and France (Penaud 1987). On leaves the disease typically produces grey-white spots with brown margins. Stem lesions become grey and pod lesions are initially brown, becoming white with age. In experimental plots of turnips, Crossan (1954) observed that, during a period of dry weather, white leaf spot spread only in irrigated plots and he suggested that the spores of P. capsellae were likely to be splash-dispersed.

Evidence that the position and nature of the host surface greatly affect the process of spore dispersal by rain-splash has come from experiments with artificial targets, with infected strawberry fruit or with leaves of cereals, field beans (Vicia faba L.) and brassicas (Stedman 1980; Macdonald and McCartney 1988; Reynolds et al. 1987). In crops of wheat, splash droplets were dispersed further from sources at the top of the crop canopy than from ground level (Stedman 1980). Furthermore, in laboratory experiments the volume of liquid splashed decreased as the angle of inclination of the target increased from 0 to 60^0, but more liquid was splashed from rigid Brussels sprout leaves than from flexible leaves (Stedman 1979). The texture of the host surface greatly influences the formation of splash droplets and hence the dispersal of spores by rain-splash (Macdonald and McCartney 1988). Pathogens themselves can affect the splash process because they can modify the surface of the host, for example by rupturing leaf cuticles and causing premature senescence. Thus, the dispersal of P. capsellae spores from infected leaves is likely to be affected, not only by changes in leaf position and age as the crop grows, but also by changes in leaf texture and flexibility. This paper describes rain tower and field experiments to study the dispersal in splash droplets of *P. capsellae* spores from infected oilseed rape.

MATERIALS AND METHODS

Inoculation

Oilseed rape (cv. Cobra) was grown in compost in a glasshouse in pots or trays. Pseudocercosporella capsellae was grown on maize meal (40 g l^{-1}) agar (20 g l^{-1}) and sporulation was induced under near-ultra-violet (350 nm) light at 10^oC. Plants with three expanded leaves were inoculated with spore suspensions from infected plants containing >50,000 spores ml^{-1} and were immediately placed in a sealed polythene-sided chamber to maintain 100% r.h. for 2-3 days. White leaf spot lesions appeared 7-10 days after inoculation. To induce sporulation, infected plants were returned to the chamber at 100% r.h. for 2 days. Further experimental details are described by Fitt et al. (1989b, 1991).

Field experiments

Winter oilseed rape (cv. Cobra) was sown at 8 kg ha^{-1} on 26 August 1988 (area 25 x 25 m) and four sites ($1m^2$) were each inoculated on 13 December with pots of infected plants. Numbers of P. capsellae conidia on plants in these sites were estimated from April to July on leaf samples taken at heights of 0, 20, 40 and 80 cm, and numbers of spores dispersed weekly were estimated using microscope slides as samplers. The experiment was repeated in 1989 when the crop (area 130 x 210 m) was sown on 22 August at 8 kg ha^{-1} and each of four 1 m^2 sites was inoculated with infected plants on 20 November. A further group of uninfected plants was inoculated with a spore suspension (2 x 10^4 spores ml^{-1}) on 20 March when the crop was almost in flower (growth stage 3.4, Sylvester-Bradley and Makepeace 1984). Lesions developed by 12 April on all leaves of the plants which had been covered with polythene bags for 3 days but did not develop on uncovered plants.

Raintower experiments

Simulated rain (volume mean drop diameter 3 mm), produced by a rain generator at the top of a rain tower (Fitt et al. 1986), fell a height of 11 m for 15-20 min. on to oilseed rape leaves infected by P. capsellae and the distribution of spore-carrying splash droplets was measured. Experiment 1 compared horizontal detached leaves of different ages: a) older, senescent (yellow) leaves; b) younger, green leaves. Experiment 2 compared attached leaves of different ages and/or positions: a) plants with lower leaves attached (upper leaves removed); b) plants with upper leaves attached (lower leaves removed); c) plants with both upper and lower leaves attached. Experiment 3 compared leaves of a similar age in different positions: a) horizontal leaves; b) leaves inclined at 30^0 to the horizontal; c) flexible leaves with petioles held between two strips of wood at an angle of c. 20^0 so that each leaf lamina was approximately horizontal but unsupported.

The numbers of spores in each source were estimated by counting the numbers washed from each treatment and calculating the numbers of spores g^{-1} dry weight of leaves. Spore-carrying splash droplets were collected at distances of 10-70 cm and numbers of P. capsellae spores and spore-carrying droplets were counted. Regressions of ln(number of spores) on distance were calculated for all experiments and regressions of ln (number of spores) on time were calculated for experiment 3. Analyses of position and parallelism were done to assess whether the data for each experiment were fitted best by single regression lines, sets of parallel lines or sets of non-parallel lines. The exponential model has the property that the number (of spores) decreases by a half as the distance/time increases by a constant increment, so values of the half-distance (experiments 1-3) or half-life (experiment 3) were estimated as 0.693/b or 0.693/d where b and d are, respectively, the slopes of the regression lines for distance and time.

RESULTS

Field experiments

In both years white leaf spot developed on plants near to the sources of infection but, after April, the weather became hot and dry and there was little further disease spread. Diagnosis was hindered by difficulty in distinguishing symptoms of white leaf spot from those of other diseases. In 1989, conidia were recovered from leaves up to 80 cm above the ground; the greatest numbers were recovered in early May but some were recovered until July (Table 1). Conidia were detected on the exposed slides only during the week 5-12 July when there was heavy rain (34.2 cm) and these were collected at up to 80 cm height. In 1990, conidia were produced on lesions which developed on plants in the crop but

Table 1. Numbers of Pseudocercosporella capsellae spores on leaves of oilseed rape at different heights in the crop canopy in 1989.

Sample date	Number of conidia (g^{-1} dry weight) ($x10^{-4}$)			
Height (cm)	0	20	40	80
12 April	9.1	3.0	*	*
3 May	3.6	70.6	28.0	*
24 May	8.1	52.5	23.2	9.0
14 June	15.6	37.2	31.1	7.4
5 July	12.3	6.5	7.5	1.3

* no leaves present

those recovered from plants exposed to hot dry weather in April were no longer viable and no further disease spread occurred.

Rain tower experiments

The decreases in numbers of spores collected with distance from the source (Fig.1) were fitted well by exponential models for all treatments in all three rain tower experiments. Gradients were steepest, with the smallest half-distances (5.0-5.5 cm), when the sources were younger detached leaves inclined at 30^0 to the horizontal or older, detached, horizontal leaves, and were shallowest for upper or lower leaves remaining attached to plants (half-distances 8.7-9.8 cm). However, the analyses of position and parallelism suggested that these data were best fitted by two parallel lines with the same slope and different intercepts in experiment 1 (older v. younger leaves), by three parallel lines in experiment 2 (lower v. upper v. upper + lower leaves) and by a single line in experiment 3 (horizontal v. inclined v. flexible leaves).

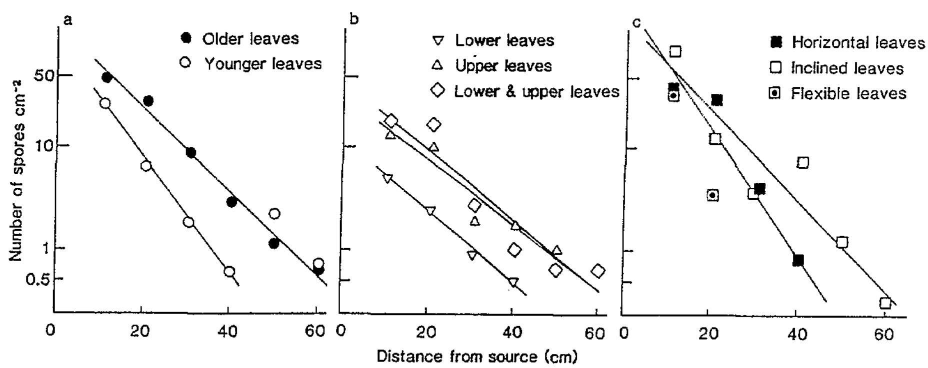

Fig. 1. Numbers of Pseudocercosporella capsellae spores (ln-scale) (y) dispersed to different distances (x) from infected oilseed rape leaves on to which simulated rain fell in three experiments: a) older v. younger detached leaves; b) lower v. upper v. upper and lower attached leaves; c) horizontal v. inclined v. flexible detached leaves. Lines calculated by regression of y on x.

Droplet size categories with the greatest numbers of spore-carrying droplets dispersed from a source were 0-200μm diameter (43% of the spore-carrying droplets) and 200-400μm (30%) respectively, for the younger and older horizontal detached leaves in experiment 1. For both sources <10% of the droplets were in each of the categories 600-800, 800-1000 and >1000μm. The number of spores dispersed decreased rapidly with time for all three treatments in experiment 3 (Fig.2). The half-lives were estimated as 2.4, 5.3 and 3.3 min. respectively for the horizontal, inclined and flexible leaves. The analysis of position and parallelism suggested that the data for the three treatments was fitted best by a single regression line.

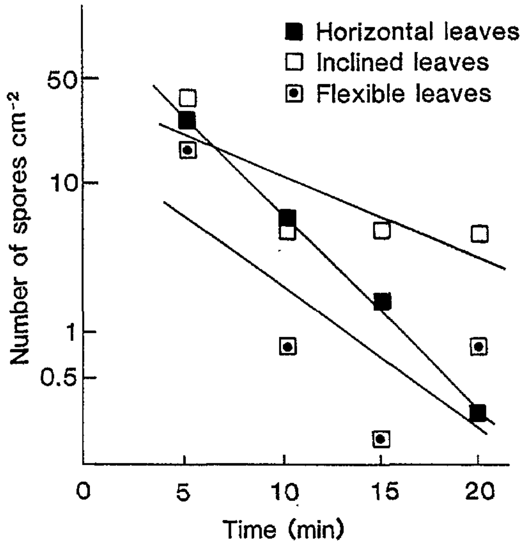

Fig. 2. Numbers of Pseudocercosporella capsellae spores (ln-scale) (y) dispersed in successive time periods (t) from infected oilseed rape leaves (horizontal, inclined or flexible) on to which simulated rain fell (experiment 3). Lines calculated by regression of y on t.

To estimate the numbers of spores dispersed in splash droplets from each source, we integrated under the negative exponential form of the equations calculated by regression of ln (number of spores) on distance (Fig.1), beyond a distance of 5 cm. Thus it was estimated that c. 2.3 M and 1.1 M spores were dispersed, respectively, from the older and younger leaves in experiment 1 (Table 2). When these figures are divided by the total numbers of spores available for dispersal, it can be shown that the efficiency of dispersal was greater (50%) for the younger than for the older (9%) leaves. Similarly, the efficiency of dispersal was greater from the upper (43%) than the lower (2%) attached leaves in experiment 2, and for the horizontal (78%) than the inclined (49%) or flexible (39%) detached leaves in experiment 3. In experiment 3 it was estimated that 5-10% of the spores were lost in run-off water and 15-40% of the spores available remained after 20 min. of simulated rainfall.

Table 2. Estimated numbers of Pseudocercosporella capsellae spores dispersed in splash droplets or run-off water when simulated rain fell on to infected oilseed rape leaves or remaining on leaves after the experiment. Numbers in parentheses indicate proportions (%) of the estimated spores available (source strength)

Treatment leaves	Number of spores (x 10^{-4})			
	Source	Splash droplets	Run-off water	Remaining
Expt. 1				
Older	2500	234 (9.4)		
Younger	210	105 (50.0)		
Expt. 2				
Lower	69	1.6 (2.2)		
Upper	20	8.6 (43.1)		
Lower & upper	81	15.1 (18.6)		
Expt. 3				
Horizontal	460	361 (78.4)	22.2 (4.8)	178 (38.7)
Inclined	460	223 (48.5)	11.5 (2.5)	73 (15.7)
Flexible	460	180 (39.1)	35.8 (7.8)	105 (22.8)

DISCUSSION

These experiments provide good evidence that spores of P. capsellae are dispersed by rain-splash, as suggested by Crossan (1954). Large numbers of spores were dispersed in splash droplets in rain tower experiments and in field experiments spores were collected only during periods when rain fell. Furthermore, most spores were carried in the largest droplets and spore dispersal gradients were fitted well by exponential models, as they are for other pathogens which are typically splash-dispersed (Fitt et al. 1989a). The rapid decline in the number of spores dispersed with time (Fig.2) suggests that short showers are likely to be particularly effective in spreading white leaf spot.

The results of the rain tower experiments suggest that the inclination, flexibility and age of infected leaves all affect the dispersal of P. capsellae spores in splash droplets and that the efficiency of dispersal may decrease as the angle of inclination increases from the horizontal (Table 2), possibly because the volume of liquid splashed decreases (Stedman 1979). Also, as the flexibility of leaves increases, the efficiency of spore dispersal decreases. Differences in flexibility may have played a role in the marked differences in efficiency between younger (50% of spores available in source dispersed in splash droplets) and older (10%) detached horizontal leaves (Table 2), since the younger leaves were turgid whereas the older leaves were flaccid and senescent.

The effect of source height accentuated the effect of leaf age in experiment 2, with 43% of spores dispersed from upper leaves as opposed to 2% for lower leaves and an intermediate value of 19% from upper and lower leaves. This effect was also noted by Stedman (1980) in wheat crops where the distance travelled by splash droplets increased as the source height increased. In a field crop the advantage of an elevated source over a ground level source would be further increased by wind. Our results suggest that, in theory, infected upper, younger leaves will be a more efficient source of inoculum than lower, senescent leaves.

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