Development of *Pyrenopeziza brassicae* on oilseed rape leaves at different temperatures and wetness durations

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Abstract

Light leaf spot (caused by *Pyrenopeziza brassicae*) is a disease that infects oilseed rape leaves, stems, flowers and pods. The fungus produces ascospores, formed in apothecia on dead tissue, and conidia, formed in acervuli on living tissue. Ascospores initiate epidemics in autumn, whilst conidia are responsible for secondary spread of the disease during autumn and winter. Infection of oilseed rape leaves by *P. brassicae* spores is influenced by temperature and leaf wetness duration. Controlled environment experiments were done to investigate the effects of temperature and leaf wetness duration on development of *P. brassicae* on oilseed rape leaves inoculated with ascospores or conidia. Little difference in infection criteria between ascospores and conidia was found. Both types of spores were able to infect oilseed rape leaves at temperatures from 8 to 20°C with an optimum temperature at *c*. 16°C. The level of infection for both kinds of spores increased with increasing wetness duration from 6-72h. Evidence from these experiments with *P. brassicae* suggests that the optimum temperature for development of light leaf spot epidemics in winter oilseed rape crops is in the range 12-16ºC and 48-72h wetness duration.

Key words: *Pyrenopeziza brassicae*, infection criteria, winter oilseed rape

INTRODUCTION

Light leaf spot (caused by *Pyrenopeziza brassicae*) is a disease that infects leaves, stems, flowers and pods of oilseed rape and other *Brassica* crops – cauliflower, Brussels sprouts, turnip, broccoli (Rawlinson *et al*., 1978; Maddock *et al*., 1981). Although both spore types, ascospores and conidia, produced by fungus are morphologically similar, they play different roles in the UK life cycle. Epidemics of light leaf spot in winter oilseed rape are initiated in autumn by air-borne ascospores, produced in apothecia on oilseed rape debris and dispersed by wind (McCartney and Lacey, 1990; Gilles *et al*., 2001). Conidia formed in acervuli are splashed by rain and they are responsible for secondary spread of the disease (Evans *et al*., 1999).

Infection criteria for conidia of UK *P. brassicae* isolates on oilseed rape leaves have been established in controlled environment experiments, with an optimum temperature of *c.*16ºC. At 12°C and 18°C, chlorotic areas developed on leaves (cv. Cobra) inoculated with suspensions of conidia when leaf wetness duration after inoculation was 16 to 48 h but not 0 to 13 h (Figueroa *et al*., 1995). On leaves (cv. Bristol) inoculated with suspensions of conidia, *P. brassicae* sporulated when leaf wetness duration was > 6 h at 20, 16 or 12 °C and > 10h at 8, 6 or 4°C (Gilles *et al.*, 2000). Ascospores can infect leaves at 16°C after 48 h wetness duration (Gilles *et al*., 2001) but there is little information about other infection criteria. Controlled environment experiments were done to investigate the effects of temperature and leaf wetness duration on development of *P. brassicae* on oilseed rape leaves inoculated with ascospores or conidia.

MATERIALS AND METHODS

Field *P. brassicae* isolates were obtained from sporulating leaves of winter oilseed rape at Rothamsted. Conidia were washed off from the surface of leaves using distilled water and then spores were subsequently maintained on oilseed rape plants cv. Bristol. To produce conidia for inoculation, leaves of oilseed rape (cv. Bristol) at GS 1,6-1,7 were sprayed with a suspension of conidia (0.5×10^6 conidia ml^{-1}) using an aerosol sprayer (Chrom Atomiser, Camlab, Cambridge, UK). Directly after inoculation, plants were covered with polyethylene

bags for 48 h. After 18 days from inoculation, conidia were washed from the leaves by shaking them in distilled water and conidial suspensions were adjusted to the desired concentration using a haemocytometer slide (Weber Scientific International Limited, Teddington, UK) for inoculating plants (GS 1,6-1,7). Inoculation of plants with conidia was done by spraying the leaves with a conidial suspension (0.5 x 10^6 conidia ml^{-1}) with an aerosol sprayer. To produce a source of ascospores for the infection experiment, leaves of oilseed rape (cv. Bristol) were initially inoculated with a suspension of conidia (Gilles et al., 2001). Inoculation with ascospores was done by attaching five 10 cm lengths of the senescent leaf petioles with mature apothecia (*c*. 150 apothecia per petiole) to the underside of the lid of each plastic propagator (22 cm x 36.5 cm) containing five plants (GS 1,6-1,7) in pots (diameter 9 cm). To stimulate natural release of ascospores by decreasing humidity, a 2 cm gap was left between propagator trays and their lids. The petioles were removed after 24h and the inoculated leaves were sprayed with distilled water. After inoculation with ascospores or conidia, the plants were enclosed in polyethylene bags for 6, 10, 16, 24, 48 or 72h to obtain appropriate wetness durations. Assessment of the severity of light leaf spot was done by a visual estimation of percentage leaf area with sporulation (pustules of conidia) each day from inoculation until the percentage leaf area with sporulation did not increase further.

RESULTS

Little difference in infection criteria between ascospores and conidia was found. Symptoms of light leaf spot (acervuli) were observed on oilseed rape leaves inoculated with ascospores or conidia at temperatures from 8 to 20^{o}C after leaf wetness durations from 6 to 72h, except after 6h leaf wetness at 8^{o}C (Tab. 1). For both ascospore and conidial inoculum, the percentage leaf area with sporulation increased with temperature from 8 to 16 ^{o}C, but was less at 20^{o}C. The percentage area with sporulation increased with increasing wetness duration from 6 to 72h. Time from inoculation to the first appearance of sporulation observed (latent period) was shortest at 16^{o}C and 48h wetness duration for both ascospores (12 days) and conidia (11 days). At all wetness durations latent period decreased as temperature increased from 8 to 16^{o}C for both types of spores and slightly increased as temperature increased from 16 to 20^{o}C. Generally latent period decreased when leaf wetness duration increased from 6 to 48h.

DISCUSSION

Previous work with conidia (Figueroa *et al*., 1995; Gilles *et al*., 2000) showed similar infection criteria to those obtained for ascospore and conidial inoculum. The observed decreases in latent period with increasing leaf wetness duration for ascospores and conidia were similar to those obtained with conidia (*Gilles et al*., 2000). Results from this controlled environment experiments suggest that ascospores and conidia are able to infect winter oilseed rape over a wide range of temperatures (8-20^{o}C) and wetness durations (6-72h). The optimum conditions for development of light leaf spot in winter oilseed rape is in the range 12-16 ^{o}C and 48-72h wetness duration. In the UK these temperatures often occur in autumn when ascospores initiate epidemics (Gilles *et al*., 2001). The secondary spread of conidia in winter and early spring can proceed because infection is possible at 8 and even at 4^{o}C (Gilles *et al*., 2000).

Table 1. Effects of temperature and wetness duration on maximum percentage of leaf area with sporulation observed on leaves of oilseed rape (cv. Bristol) inoculated with ascospores or conidia of *Pyrenopeziza brassicae.*

Temperature (°C)	Wetness duration (h)	% leaf area affected[a]	
		Ascospores	Conidia
8	6	0	0.1
	10	0.7	0.8
	16	4.2	6.3
	24	13.8	16.7
	48	40.5	47.3
	72	46.0	48.0
12	6	0.5	0.2
	10	19.5	23.0
	16	47.5	43.5
	24	71.3	67.5
	48	90.5	89.3
	72	89.0	88.8
16	6	4.2	3.4
	10	27.0	36.3
	16	63.3	69.5
	24	71.8	81.5
	48	85.5	94.8
	72	81.5	92.0
20	6	6.6	1.9
	10	16.3	12.8
	16	27.0	31.0
	24	41.3	46.5
	48	49.5	62.3
	72	52.8	57.5

[a]Maximum percentage of leaf area with sporulation (two leaves per plant, five plants per treatment), 21-31 days after inoculation (depending on temperature).

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