Effects of Temperature on Germination of UK and Polish *Leptosphaeria maculans* Ascospores and on Phoma Leaf Spot Development

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INTRODUCTION

Leptosphaeria maculans (Desm.) Ces. & de Not. (anamorph *Phoma lingam* (Tode ex Fr. Desm.) causes phoma stem canker (blackleg) of oilseed rape (*Brassica napus* ssp. *oleifera*), a damaging disease of economic importance in the main oilseed rape growing areas of Europe, Canada and Australia (West *et al*., 2001). There are two genetically distinct sub-populations of the fungus, the A and B groups, which may be different species (Williams & Fitt, 1999). A-group isolates are characterised by slow growth *in vitro*, absence of water-soluble pigments in liquid culture and ability to synthesise a group of phytotoxins named sirodesmins. B-group isolates grow more rapidly *in vitro*, accumulate a yellow-brown pigment in culture and are unable to produce sirodesmins (Brun *et al.*, 1997). The A-group of *L. maculans* causes damaging crown cankers at the base of plants, whereas the B-group more often affects upper parts of the stems (Johnson & Lewis, 1994). In Western Europe, where the epidemics are severe, the A-group is usually predominant (West *et al*., 2001). In contrast, the majority of *L. maculans* isolates collected in Poland belonged to the B-group (Jedryczka *et al*., 1999; Karolewski, 1999). There is a close relationship between the incidences of infection on leaves in autumn/winter and on stems in spring/summer (Sun *et al*., 2000), because the fungus colonises the stems through leaf petioles (Hammond *et al*., 1985). This paper reports work to compare the germination of ascospores of UK A-group and Polish B-group *L. maculans* and the infection of oilseed rape leaves by ascospores of A-group and B-group *L. maculans*.

MATERIALS AND METHODS

Germination experiment

The experiment, replicated three times, was done at 5, 10, 15 and 20^{o}C. Ascospores of A-type *L. maculans* were obtained from the stem debris collected in the UK (Rothamsted) and B-type ascospores were obtained from debris collected in Poland (Poznan). Water agar slides were inoculated by applying two drops (*c.* 60 μL) of fresh ascospore suspension (containing 5 x 10^{3} ascospores mL^{-1}) onto each slide using a glass Pasteur pipette. After inoculation, all slides were put into the Petri dishes lined with moistened filter paper and the insides of the dishes were sprayed with distilled water to maintain 100% relative humidity. The dishes were then placed in incubators at the desired temperatures and germination parameters were measured after incubation times of 2, 4, 6, 8, 10, 12, 14 and 24 h. After the designated incubation time, agar slides were removed from the incubators and three drops of tryphan blue (0.1% w/v in lactophenol) were added. The percentages of ascospores of A-group or B-group *L. maculans* which had germinated were assessed by the observation of 100 ascospores per slide. The lengths of the germ tubes of 20 A-group or B-group ascospores per slide were also assessed, for each temperature and incubation time tested.

Phoma leaf spot experiment

The experiment was done in controlled-environment cabinets, with four replicates. The inoculation of plants was done at GS 1,4, when four true leaves were present. Winter oilseed rape stem debris infected by *L. maculans* were collected after harvest from fields at Rothamsted, UK (A-type) and from Poznan, Poland (B-type). Oilseed rape plants cv. Lipton (National Institute of Agricultural Botany resistance score 5, Anonymous, 1997) were inoculated with suspensions of ascospores (10^{3} mL^{-1}) using an aerosol sprayer (Chromatomiser, Camlab Ltd, Cambridge, UK). Directly after inoculation, the plants were enclosed in polyethylene bags to maintain continuous leaf wetness. After 8, 16, 24 or 72 h at temperatures of 15 and 20^{o}C and 16, 24, 48 or 72 h at 5 and 10^{o}C, the polyethylene bags were removed. The numbers of phoma leaf lesions were assessed daily on four leaves per plant until the leaves died or no new lesions appeared.

RESULTS AND DISCUSSION

Germination experiment

Ascospores of both UK A-group and Polish B-group *L. maculans* germinated at temperatures from 5 to 20ºC on distilled water agar by producing germ tubes. After 2 h of incubation, >5% of A-group ascospores had germinated at 10 to 20ºC and >5% of B-group ascospores had germinated at 5 to 20ºC. At all temperatures, the percentage germination had almost reached its maximum after 14 h of incubation. Germ tube length increased with increasing temperature from 5 to 20ºC for both ascospore groups. Germ tubes from Polish B-group ascospores were longer than germ tubes from UK A-group ascospores at all temperatures tested, but the mean diameter of germ tubes from A-group ascospores (1.8 μm) was greater than that of those from B-group ascospores (1.2 μm) at 15 and 20ºC. These results agree with those reported by Petrie (1988), who found that conidia of B-group isolates germinating on distilled water agar produced significantly longer germ tubes than did conidia of A-group isolates. Wittern & Krüger (1985) had shown that ascospores of *L. maculans* are able to germinate in distilled water at temperatures from 4 to 28ºC. Data from these experiments suggest that B-group ascospores germinate more rapidly than do A-group ascospores on water agar. The time elapsed from inoculation on water agar to the germination of 50% of viable B-group ascospores (Ve_{50}) was shorter than that for A-group ascospores.

Phoma leaf spot experiment

The symptoms formed on leaves inoculated by Polish or UK ascospores were different. Lesions caused by the B-group were smaller, often with dark margins and light brown centres, containing fewer pycnidia than A-group lesions (UK ascospores caused grey-green spots with many pycnidia). Similar differences in symptoms on leaves between the two groups were found by Johnson & Lewis (1994) and Brun *et al*. (1997). The results of controlled-environment experiments indicated that UK (A-type) of *L. maculans* were able to infect oilseed rape leaves over the wide range of temperatures (Biddulph *et al.*, 1999). This experiment suggests that Polish (B-type) isolates can also cause lesions on oilseed rape leaves over the wide range of temperatures (5-20^{o}C) and leaf wetness durations (8-72 h). The maximum number of

leaf lesions produced by both groups of *L. maculans* was greatest at 15-20^{o}C and when the wetness duration was >48 h. The number of leaf lesions caused by UK ascospores was greater than produced by Polish ascospores for all treatments. This confirms the conclusions of Jedryczka *et al*. (1999) that UK A-type isolates were more pathogenic to oilseed rape than Polish B-type isolates. The incubation period (time from inoculation to the appearance of the first lesions) was usually shorter for Polish ascospores than for UK ascospores. Johnson & Lewis (1994) suggested that A and B-type of *L. maculans* had similar infection pathways but the infection caused by B-type developed more rapidly. Isolates belonging to A-type were generally characterised by slower growth in vitro and germ tube extension than B-type isolates (Williams & Fitt, 1999).

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