Comparative Biology and Epidemiology of A-group and B-group *Leptosphaeria maculans* on Winter Oilseed Rape

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INTRODUCTION

Stem canker (blackleg), caused by *Leptosphaeria maculans*, is a serious disease of oilseed rape in the UK, which caused losses of over £20 M per season since 1993 (DEFRA survey results; http://www.csl.gov.uk/prodserv/cons/crop/survey/osrintro.cfm). Populations of *L. maculans* can be divided into A-group and B-group (Williams & Fitt, 1999), which recently have been named as *Leptosphaeria maculans* (A-group) and *Leptosphaeria biglobosa* (B-group), on the basis of length of neck of pseudothecia (Shoemaker & Brun, 2001). Previous epidemiology work has mainly been on the A-group, with little information about the B-group. Controlled environment experiments have mainly used as inoculum conidia of *L. maculans*, rather than the ascospores, which play the major role in the epidemiology of the disease. The aim of this study was to compare the biology and epidemiology of A-group and B-group *L. maculans*.

MATERIALS AND METHODS

Suspensions of A-group or B-group ascospores were inoculated onto water agar or oilseed rape leaf surfaces. Germination of ascospores and penetration of leaf tissues were investigated using light microscopy (Huang, 2002). Oilseed rape debris naturally infected by A-group or B-group *L. maculans* was incubated at 5 – 20°C under continuous wetness. Maturation of pseudothecia was observed weekly. Oilseed rape debris naturally infected by *L. maculans* was buried or exposed on the soil surface; the presence of mature ascospores on this debris was investigated at intervals of 2 months during a period of 1 year. The ability of ascospores to germinate after exposure to different temperatures under dry conditions was investigated. Lengths of ascospores and lengths of necks of pseudothecia produced on naturally infected debris were measured.

RESULTS AND DISCUSION

The differences between A-group and B-group *L. maculans* provide further evidence that the two groups are actually different species (Table 1). The results increase our knowledge about the biology and epidemiology of A-group and B-group *L. maculans*. This will help in developing strategies to control phoma stem canker more effectively. Germination patterns of ascospores of A-group and B-group *L. maculans* were

different (Table 1), whether ascospores germinated on water agar or leaf surfaces (Huang *et al*., 2001). This could also be used to assess proportions of the two groups

Table 1 Comparative biology and epidemiology of A-group and B-group *L. maculans*, as shown by results of these experiments

Experiment	Parameter	A-group	B-group
Ascospore germination on water agar or leaf surfaces	Germ tube position	Originated from interstitial cells	Originated from terminal cells
	Germ tube length	Shorter (40 µm in 12h at 20°C)	Longer (131 µm in 12h at 20°C)
	Hyphal growth	Tortuous	In almost straight lines
	No. of germ tubes	More per ascospore (3.8)	Less per ascospore (3.1)
	Diameter of germ tubes	Thicker (1.8 µm)	Thinner (1.2 µm)
Penetration leaf tissues	Stomata	Mainly through stomata	Mainly through stomata
	Wounds	Through wounds	Through wounds
	Appressorium	Appressorium-like structure formed	No appressorium-like structure formed
Pseudothec. maturation	Maturation	Mature at 5-20°C, earlier than B-group at 5-10°C	Mature at 5-20°C
	Position of pseudothecia	Whole pseudothecia exposed on stem base surface	Only neck exposed; under epidermis of upper stem
Survival on buried or unburied debris	Survival on buried debris	Survived longer (1 year) on buried stem bases	Did not survive 2 months on buried stem bases or upper stems
	Survival on unburied debris	Survived 1 year on unburied stem base and upper stem debris	Survived 1 year on unburied stem base and upper stem debris
	Survival of ascospores	Survived longer (18-36% survived 50 days at 5-15°C; 10% survived 35 days at 20°C) at 5-20°C in dry conditions	Survived shorter (1.2-19% survived 50 days at 5-15°C; 17% survived 35 days at 20°C) at 5-20°C in dry conditions
Size of ascospores	Ascospore length	Similar length range (28-50 µm) to B-group	Similar length range (28-44 µm) to A-group
Size of pseudothe.	Neck length	Generally shorter neck (76.5 µm), but 50% had same neck length range as B-group (< 81 µm)	Generally longer neck (94.5 µm), but 50% had same neck length range as A-group (< 81 µm)

in populations in early autumn and to predict the severity of stem canker epidemics. The main mode of penetration of oilseed leaf tissue by hyphae from both A-group and B-group ascospores was through stomata, but the percentage of germinated ascospores that penetrated stomata was greater for A-group than for B-group *L. maculans*. Furthermore, appressorium-like structures were formed by A-group not B-group *L. maculans*. These results suggest that B-group may be less efficient than A-group *L. maculans* in infecting the host tissues under the same conditions. This may explain why the proportion of ascospores which established lesions for A-group *L. maculans* (12% infection efficiency) was higher than for B-group *L. maculans* (7% infection efficiency) after inoculation with ascospores in controlled conditions (Toscano-Underwood *et al.,* 2001).

The average length of A-group ascospores (38 µm) was longer than that of B-group ascospores (36µm), but they had the same length range (28-50 µm). A-group pseudothecia were mainly produced on stem bases and exposed to the air, while B-group pseudothecia were mainly produced on upper stems under the epidermis. However, the shape of B-group pseudothecia was similar to that of the A-group. Although the average length of pseudothecial neck of B-group *L. maculans* was longer than that of A-group *L. maculans*, 50% of A-group and B-group pseudothecia had neck lengths in the same range. This suggests that the two groups cannot be reliably differentiated by the length of ascospores or length of the neck of pseudothecia (Shoemaker & Brun, 2001).

A-group pseudothecia matured earlier than B-group pseudothecia at 5-10°C, but there were no differences between the two groups at 15-20°C. These different effects of temperature on maturation of A-group and B-group ascospores could explain why isolates from upper stems were mainly B-group, while isolates from stem bases were mainly A-group (West *et al*., 2002). Results suggest that in the UK low temperatures in winter can delay maturation of B-group ascospores more than that of A-group ascospores. In spring, as temperatures increase, maturation of B-group ascospores may start again and the number of air-borne B-group ascospores may increase. Subsequently, infection of upper stems is mainly associated with B-group *L. maculans.* The effects of temperature on maturation of pseudothecia of A-group and B-group *L. maculans* could also be used to forecast the first release of ascospores and timing of phoma leaf spotting, and subsequently to optimise the use of fungicides.

A-group *L. maculans* survived longer than B-group *L. maculans* on buried stem base debris, but both A-group and B-group *L. maculans* survived longer on unburied debris than on buried debris. These results suggest that deep ploughing immediately after harvest may help to decrease the severity of stem canker in crops planted the next season in the surrounding area. A-group ascospores survived longer than B-group ascospores in darkness in dry conditions at temperatures of 5-20°C. These results suggest that more A-group ascospores than B-group ascospores can survive after travelling a long distance. This suggests that there is higher risk of spread to a new area through air-borne ascospores for A-group than B-group.

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