Epidemiology and global distribution of A-group and B-group *Leptosphaeria maculans* on oilseed rape

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ABSTRACT

Ascospores of both A-group and B-group *L. maculans* germinated on oilseed rape leaves at 5 to 20°C. The percentage of A-group/B-group ascospores that had germinated after 24 h of incubation increased with increasing temperature from 5 to 20ºC. Germ tubes from B-group ascospores were longer than germ tubes from A-group ascospores at all temperatures. Hyphae from both A-group and B-group ascospores penetrated leaves mainly through stomata, but appresorium-like structures were observed with the A-group and not the B-group. The percentage of germinating ascospores which penetrated stomata was greater for the A-group than B-group. After burial in sand for 2, 4, 6, 8, 10 or 12 months, ascospores were still produced on stem base debris but not on upper stem debris. A-group *L. maculans* was isolated from buried debris but B-group was not. A-group ascospores exposed to different temperatures in dry conditions survived longer than B-group ascospores. At Rothamsted, a large proportion of the leaf spot lesions in early autumn were caused by the A-group, but the proportion of B-group isolates obtained from upper stems increased during spring; A-group was predominant in the pith and cortex of the stem base, while B-group was predominant in the cortex of the upper stem. The global distribution of A-group and B-group *L. maculans* differs between geographical locations. Only the B-group has been found in China and only the A-group in Australia. The B-group predominates in eastern Europe, while the A-group predominates in western Europe and North America.

Key words: Epidemiology, distribution, A/B-group *Leptosphaeria maculans*

INTRODUCTION

Leptosphaeria maculans is a serious pathogen of oilseed rape (*Brassica napus*), causing phoma stem canker (blackleg) worldwide (West *et al*., 2001). Populations of *L. maculans* can be divided into at least two main groups, which have been described by a number of terms, including A-group and B-group (Williams & Fitt, 1999). Recently these groups have been named as *Leptosphaeria maculans* (A-group) and *Leptosphaeria biglobosa* (B-group), on the basis of the length of the neck of pseudothecia (Shoemaker & Brun, 2001). Both A-group and B-group are present in the UK, and the A-group is more damaging than the B-group (West *et al*., 2001). Previous epidemiology work has mainly been on the A-group, with little information about the B-group. Controlled environment experiments have mainly used conidia of *L. maculans* as inoculum, rather than the ascospores, which play the major role in the epidemiology of the disease. This paper reports a comparison of key stages (e.g. ascospore germination and penetration of leaf surfaces, survival of pathogen after harvest) in the epidemiology of A-group and B-group *L. maculans* and their global distribution.

MATERIALS AND METHODS

A-group and B-group ascospore suspensions were made with ascospores harvested from pseudothecia produced on naturally infected oilseed rape debris. Suspensions of A-group or B-group ascospores were inoculated onto oilseed rape leaf surfaces. Germination of ascospores and penetration of leaf tissues were investigated using light microscopy (Huang *et al*., 2001; Huang *et al*., 2003). Oilseed rape debris naturally infected by *L. maculans* was buried in sand or exposed on the sand surface; survival of *L. maculans* on the buried or unburied debris was investigated by assessing the presence of mature pseudothecia with viable ascospores at

intervals of 2 months during a period of 1 year. Survival of ascospores was investigated by assessing their ability to germinate after exposure to different temperatures under dry conditions. Seasonal changes in proportions of A-group and B-group *L. maculans* in untreated winter oilseed rape crops and on their debris were investigated in 1999/2000, 2000/2001 and 2001/2002 experiments at Rothamsted. Changes in proportions of the A-group and B-group were investigated by visual assessments of leaf lesion appearance (large pale lesions with abundant pycnidia were classified as A-group and small dark lesions with few or no pycnidia as B-group) and by isolation from leaf lesions, stem base cankers (both cortex and pith) and upper stem lesions. After harvest, changes in proportions of A-group and B-group *L. maculans* on oilseed rape debris were examined by single ascospore isolation.

RESULTS

Ascospore germination and penetration of oilseed rape leaves

Ascospores of both A-group and B-group *L. maculans* germinated at 5 to 20ºC on detached oilseed rape leaves by producing germ tubes. After 2 h of incubation, >5% of A-group ascospores had germinated at 15-20ºC and >5% of B-group ascospores had germinated at 5 to 20ºC. Both % A-group/B-group ascospores that had germinated after 24 h of incubation and germ tube length increased with increasing temperature from 5 to 20ºC. At all temperatures, B-group ascospores produced longer germ tubes than A-group ascospores. Hyphae from ascospores of both A-group and B-group *L. maculans* penetrated leaf tissues predominantly through stomata, at temperatures from 5 to 20ºC. The percentage of germinating ascospores which penetrated stomata was greater for the A-group (18%) than the B-group (14%). Furthermore, appresorium-like structures were observed with the A-group and not the B-group.

Survival of *L. maculans* on debris and survival of ascospores under dry condition

In 2000/2001 and 2001/2002, no pseudothecia were observed on stem debris buried for 2, 4, 6, 8, 10 or 12 months immediately after they were dug up. After subsequent exposure to natural conditions for 2-4 months, mature pseudothecia were produced. However, the percentage of buried stem debris which produced pseudothecia and the pseudothecial density decreased with increasing burial time. In both seasons, all ascospores produced on the buried debris were A-group and no B-group *L. maculans* ascospores were produced. Pseudothecia with viable ascospores were observed on stem debris exposed unburied on the sand surface at the time of sampling, and both A-group and B-group ascospores were produced.

After exposure in dry conditions at 5 to 20ºC, ascospores of both A-group and B-group could germinate on distilled water agar by producing germ tubes. At all temperatures, the mean percentage of ascospores surviving after exposure to dry conditions was greater for A-group (18.2%) than for B-group (5.6%) *L. maculans*. The percentages of both A-group and B-group ascospores that survived decreased with increasing temperature and increasing exposure time. After 35 days of exposure, for example, the percentage of viable ascospores decreased from 37% (A-group) or 31% (B-group) at 5ºC to 10% (A-group) or 2% (B-group) at 20ºC.

Changes in proportions of A-group and B-group *L. maculans*

The ratio of B-group to A-group infections on leaves differed between months of sampling and between seasons. In the autumn/winter, most (75%) leaf lesions assessed were classified as A-group; 213 out of 220 large pale lesions produced A-group colonies and 157 out of 184 small dark lesions produced B-group colonies. There were more A-group than B-group isolates obtained from the stem bases. When isolations were made from tissues across the stem base, A-group isolates were obtained from samples taken from the cortex (71%) and pith (91%), while B-group isolates were rarely obtained from pith tissues (9%), but were more frequently isolated from the cortex (29%). B-group isolates were more frequently isolated from symptomless stem tissue (74%) 10 cm above ground. The proportions of B-group ascospores released from stem base pieces were generally <10% (and sometimes 0%) and there was no evidence that this proportion changed with time during the autumn/winter period (October to March). However, the proportion of B-group ascospores released from upper stem pieces increased with time and was generally >50% from November onwards.

DISCUSSION

The differences between A-group and B-group *L. maculans* in the key stages of stem canker epidemiology provide further evidence that the two groups are actually different species. 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. A-group *L. maculans* survived longer than B-group *L. maculans* on buried stem 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 the next season. That A-group ascospores survived longer than B-group ascospores suggests there is higher risk of A-group spread to a new area through air-borne ascospores. Studies on populations of A-group and B-group *L. maculans* have shown that relative proportions of A-group and B-group change, both on winter oilseed rape crops during their growing season and on stem debris after harvest during saprophytic growth.

The global distribution of A-group and B-group *L. maculans* differs between geographical locations. Only the B-group has been found in China and only A-group has been found in Australia. Both the A-group and B-group are present in Europe, USA and Canada. However, over the last decade, the relative proportions of A-group and B-group have changed in some regions of the world. In Poland, where the B-group is predominant, the proportion of A-group has been increasing (Jedryczka *et al*., 2000). Similarly, in Canada, the proportion of A-group has gradually increased after the A-group was first found in 1975. Recently, the A-group has spread into Mexico (Moreno-Rico *et al*., 2002). It is necessary to develop strategies to prevent the more damaging A-group from spreading into areas where it is not already present (e.g. China).

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