Understanding symptomless growth of *Leptosphaeria maculans* in *Brassica napus* (oilseed rape) to manage phoma stem canker

Yongju Huang[1], Elizabeth Pirie[1], Neal Evans[1], Regine Delourme[2], Graham J. King[1], Bruce DL Fitt[1]

[1] *Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK*
[2] *INRA, UMR, APBV, BP 35327, 35653 Le Rheu Cedex, France Email:yong-ju.huang@bbsrc.ac.uk*

Abstract

Two winter oilseed rape cultivars differing in quantitative resistance to *L. maculans* (Darmor resistant, Eurol susceptible) were used to investigate relationships between symptomless growth of *L. maculans* in autumn (October/November) and severity of stem canker at harvest (July). In field experiments at Rothamsted, Darmor had at least as many phoma leaf lesions as Eurol at the initial leaf infection stage in autumn. However, at harvest in summer Darmor had less severe cankers than Eurol in both the 2003/04 and 2004/05 growing seasons. To visualise symptomless growth of *L. maculans* from leaf lesions to stems, leaves of Darmor and Eurol were inoculated with a GFP-expressing isolate in controlled conditions. No significant difference between Darmor and Eurol was observed in growth rate of *L. maculans* in petioles, and stem cankers developed on both cultivars. However, *L. maculans* was often confined to the stem cortex of Darmor but penetrated to the stem pith of Eurol. These results indicate that understanding symptomless growth of *L. maculans* along leaf petioles and within stem tissues is crucial for understanding quantitative resistance to *L. maculans*.

Key words: blackleg, canola, quantitative resistance, GFP-expressing *L. maculans*

Introduction

Phoma stem canker (blackleg), caused by *Leptosphaeria maculans*, is the major disease problem for *Brassica napus* (oilseed rape, canola) in Europe, Australia and North America (Fitt *et al*., 2006). Epidemics of phoma stem canker are initiated by air-borne *L. maculans* ascospores (West *et al*., 1999; Huang *et al*., 2005), which infect the leaves of winter oilseed rape in autumn (October/November), causing leaf lesions. From these leaf lesions the pathogen grows without symptoms along the petiole to reach the stem where, in summer (May-July), it causes stem cankers and resulting yield loss (Hammond *et al*., 1985; West *et al.,* 1999). Two types of resistance to *L. maculans* in *B. napus* have been identified: qualitative (major gene mediated) complete resistance expressed in leaves and quantitative partial resistance expressed later in petioles and stems (after initial leaf infection) (Delourme *et al.*, 2006). Qualitative resistance to *L. maculans* is race-specific and is effective in protecting plants only if the corresponding avirulent allele is predominant in the local pathogen population (Balesdent *et al*., 2001). Qualitative resistance usually loses its effectiveness within three seasons of widespread use in commercial cultivars because of selection for virulence within the variable *L. maculans* population. Quantitative resistance is considered to be race non-specific and more durable than qualitative resistance (Delourme *et al*., 2006). Quantitative resistance to *L. maculans* is thought to operate by decreasing pathogen growth in leaf petiole and plant stem tissues. However, after initial infection in autumn (October/November), *L. maculans* has a long period of symptomless growth from leaf lesions along the leaf petiole and into stem tissues. It has been difficult to study this symptomless growth (Hammond *et al*., 1985). However, the availability of green fluorescent protein (GFP) expressing isolates of *L. maculans* (Eckert *et al.*, 2005; Huang *et al*., 2006) now provides the opportunity to study this symptomless growth in detail. This paper describes experiments using two oilseed rape cultivars differing in quantitative resistance to *L. maculans* to investigate relationships between symptomless growth of *L. maculans* in autumn (October/November) and severity of stem canker at harvest (July).

Materials and methods

Field experiments

Winter oilseed rape cvs Darmor (quantitative resistance) and Eurol (susceptible) were grown in field plots (15 × 3 m) with three replicate plots arranged in randomised block design at Rothamsted (N51°48', W0°21') over two seasons (2003/04 and 2004/05). Plants were inoculated in autumn with crop debris from the previous season to provide a high ascospore inoculum concentration. No fungicide treatments were applied to the plants. Phoma leaf spot lesions were counted in the field on ten tagged plants per plot, at approximately weekly intervals throughout the growing season. Basal stem cankers were assessed from late spring, every 2 weeks until harvest on a 0-4 severity scale based on external symptoms (where 0 = healthy; 1 ≤ 50% stem circumference girdled; 2 = 51%−75% girdled, stem firm; 3 >75% girdled, stem weak; 4 = plant dead or lodged) (Zhou *et al*., 1999). Before harvest, these tagged plants were pulled up and cut through the crown to assess the internal severity of stem canker on a 0-4 scale (where 0 = healthy; 1 ≤ 50% stem cross-section affected; 2 = 51−75% stem cross-section affected; 3 >75% stem cross-section affected; 4 = 100% stem cross- section affected).

Controlled environment experiments

Based on development of leaf lesions in the field experiments, controlled environment experiments were done with GFP-expressing conidia of *L. maculans* to investigate whether quantitative resistance in *B. napus* operates during *L. maculans* symptomless growth from leaf lesions along leaf petioles towards the stem. The conidial suspension of GFP-expressing ME24/3.13 (Huang *et al*., 2006) was prepared from a 12-day-old culture on V8 agar and the concentration of conidia was adjusted to 10^6 conidia ml^{-1} using a haemocytometer slide. Plants of Darmor and Eurol were grown either in pots (7 cm diameter) at 18°C, or in pairs of Magenta vessels (77 mm × 77 mm × 97 mm) connected with Magenta couplers (one plant per pair of vessels) at 25°C. Plants were inoculated when each had three fully expanded leaves (GS1,3). For inoculation, the basal part of laminas (i.e. 1 cm from the petiole) of three leaves from each plant were wounded using a sterile pin, and a 10 µl droplet of conidial suspension was placed on the wound. Each leaf had two inoculation sites on each side of the midrib. For plants grown in Magenta vessels, the inoculated leaves were detached 16 days after inoculation. For plants grown in pots, inoculated leaves were detached 22 days after inoculation. Leaves were observed using a Leica MZ FLIII stereo-microscope equipped with a mercury lamp. For detection of GFP fluorescence, filter GFP2 from Leica Microsystems (Milton Keynes, UK) was used. The distance from the inoculation site along the leaf petiole to the leading GFP hypal tips was measured on each leaf. The experiment was repeated once.

Results

In 2003/2004, there was no difference in number of phoma leaf spot lesions between Darmor and Eurol in autumn and winter, but more leaf lesions developed on Eurol than Darmor in spring (Figure 1). In 2004/2005, more leaf lesions developed on Darmor than Eurol in autumn, but there was no difference in number of phoma leaf spot lesions between Darmor and Eurol in winter. More leaf lesions developed on Eurol than on Darmor in spring, as in 2003/2004 (Figure 1). In field conditions, both cultivars had similar leaf areas and numbers of leaves (data not presented). In both seasons, external stem canker was more severe on Eurol than Darmor (Figure 1) and the internal severity of stem canker before harvest was greater on Eurol than Darmor (data not presented).

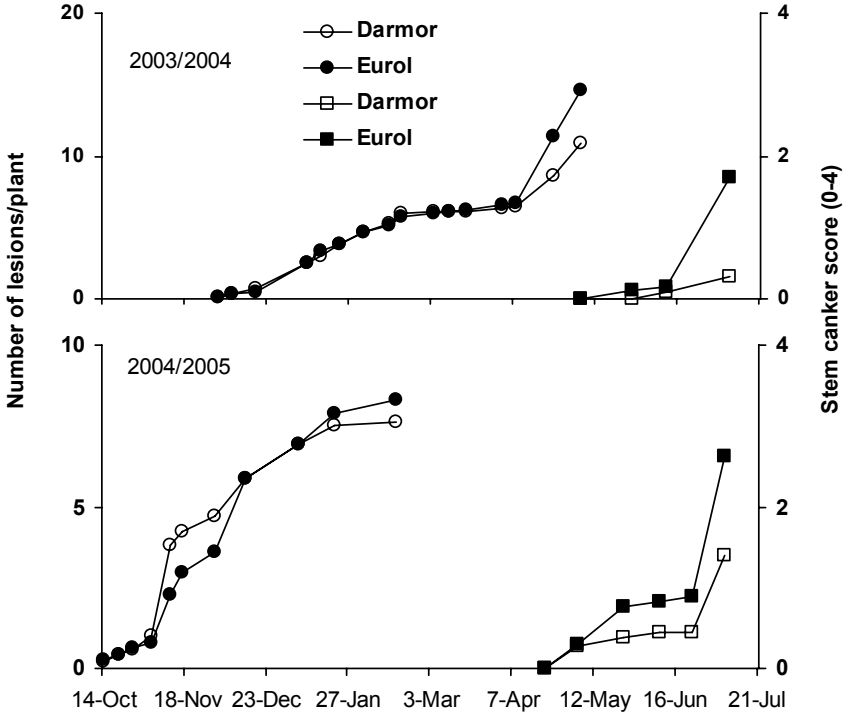

Figure 1 Cumulative numbers of phoma leaf spot lesions per plant on winter oilseed rape (○, Darmor; ●, Eurol) and mean stem canker scores for the same plants before harvest (□, Darmor; ■, Eurol) on a 0-4 scale (Zhou *et al.*, 1999). Each point represents the mean of 30 plants.

In controlled environment experiments, leaves of Darmor and Eurol inoculated with conidia of GFP-expressing *L. maculans* ME24/3.13 at 18°C and 25°C produced necrotic lesions with pycnidia. Growth of GFP-expressing hyphae along the leaf petiole towards the stem was observed, primarily through xylem vessels or between cells of the xylem parenchyma and cortex. There was no significant difference between Darmor and Eurol in the distance grown from the inoculation site along the petiole towards the stem at 18°C or 25°C (Figure 2). However, *L. maculans* was often confined to the stem cortex of Darmor but penetrated to the stem pith of Eurol.

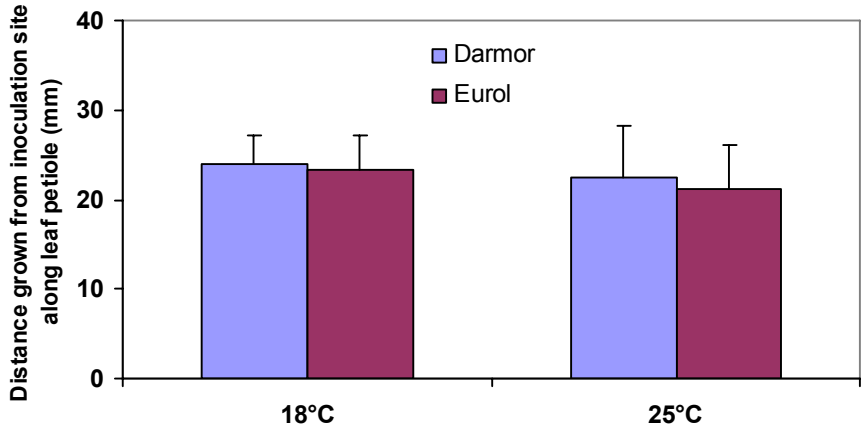

Figure 2 Distance grown by GFP-expressing *L. maculans* from the inoculation site along leaf petioles of Darmor and Eurol in controlled environments at 18 and 25°C. Data presented were the mean of 21 leaves of each cultivar.

Discussion

Results of field and controlled environment experiments suggest that understanding symptomless growth of *L. maculans* from leaf lesions along leaf petioles and within the stems before the appearance of stem canker is crucial for understanding quantitative resistance to *L. maculans* in oilseed rape. In field experiments at Rothamsted in England, there was little difference between Darmor (quantitative resistance) and Eurol (susceptible) in the number of lesions on leaves but there were large differences between Darmor and Eurol in stem canker severity on stems before harvest. Similar results were observed in field experiments on these cultivars at Rennes in France (personal communication, Dr H Brun, INRA) and at Boxworth in England (personal communication, Dr P Gladders, ADAS). These results suggest that quantitative resistance to *L. maculans* may operate by decreasing the growth or accumulation of *L. maculans* biomass in stem tissues. Although there was no difference between Darmor and Eurol in the growth rate of *L. maculans* along leaf petioles in controlled conditions, there were differences between Darmor and Eurol in *L. maculans* distribution within the stem. Additionally, there may have been differences between Darmor and Eurol in *L. maculans* biomass in petioles. Growth of *L. maculans* in petiole and stem tissues of Darmor and Eurol requires further investigation using quantitative PCR and GFP-labelled *L. maculans*.

Acknowledgements

We thank the Biotechnology and Biological Sciences Research Council (BBSRC), the European Union (QLRT-2001-01813, SECURE project) and DuPont for supporting the work. All work involving the use and storage of genetically modified plant pathogens was done under Defra licence no. PHL 174B/4650(11/2003).

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