Interactions between *Leptosphaeria maculans*, *L. biglobosa* and fungicides in oilseed rape

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Abstract

Phoma stem canker is a damaging disease of oilseed rape caused by a *Leptosphaeria* species complex, which consists of the related pathogen species *L. maculans* and *L. biglobosa*. Stem cankers caused by *L. maculans* are more damaging than those caused by *L. biglobosa*. The main fungicide used to control phoma stem canker in the UK is flusilazole, which is often applied in autumn during development of phoma leaf spots from wind-dispersed ascospores which land on the leaves. Interactions between *L. maculans* and *L. biglobosa* are being investigated. A winter oilseed rape field experiment to investigate effects of early and late applications of flusilazole on the disease was done in the 2005/06 growing season. In December, early-sprayed (29 October) plots had a lower incidence of phoma leaf spot than late-sprayed (29 November) plots. However, severity of phoma stem canker in July was decreased more and yield was increased more by the late spray than the early spray in this experiment.

Key words: Blackleg, *Brassica napus*, canola, flusilazole, *Phoma lingam*, Punch C

Introduction

One of the most damaging diseases of oilseed rape (canola) is 'phoma stem canker', otherwise known as 'blackleg', which is caused by a *Leptosphaeria* species complex (Fitt *et al.*, 2006a). The *Leptosphaeria* species complex consists of two morphologically similar pathogens, *L. maculans* and *L. biglobosa*. The disease cycle of the *Leptosphaeria* species complex has been studied in detail. Primary infection of the host plant is caused by wind-dispersed ascospores released from *Leptosphaeria* resting structures surviving on the debris of the previous crop (Huang *et al*., 2005). The first visual symptoms of infection are circular leaf lesions. *L. maculans* lesions are populated with small black pycnidia but *L. biglobosa* lesions have few or no pycnidia. Following leaf lesion development, systemic symptomless colonisation of the leaf petiole occurs, with the leading hyphae extending through xylem vessels (Howlett *et al*., 2001). *L. maculans* infections produce lesions at the root collar (crown) of the plant that cause necrosis of the stem cortex, leading to cracking of the stem and formation of cankers (Williams and Fitt, 1999). Infection with *L. biglobosa* results in less damaging upper stem lesions (Williams and Fitt, 1999). Differences in infection strategy and final disposition of the pathogens allow the two species to co-exist as they exist in separate (although similar) ecological niches (Fitt *et al.*, 2006b).

There are several methods for controlling fungal pathogens in oilseed rape, the most important being fungicides, cultural practices, crop management and breeding resistance to the fungal pathogens into the host plants (West *et al*., 2001). A number of fungicides are effective against oilseed rape pathogens; the manufacturers produce recommendations on timing and rate of application, although advisors and growers modify them to fit their own requirements. Fungicides can be applied as a seed, fertiliser or foliar spray treatment. Decisions about application of fungicide are based on a number of factors including economics, the need to control several pathogens, location, climate and epidemiology of the diseases. In crops with a low yield potential, for example, fungicide could be applied with the fertiliser to protect the young plants at a vulnerable stage of growth (West *et al*., 2001). Ideally, fungicides should be applied to crops with some genetic resistance to the pathogen, as the combination of fungicide and resistance provides improved protection against the pathogen. Sprays of protectant fungicides have a limited period of efficacy and should be timed to coincide with ascospore release and early phoma leaf spot epidemics (West *et al.*, 2001). This paper describes a field experiment to investigate the effect of spray timing on the severity of phoma leaf spot and stem canker.

Materials and methods

The experiment involved twelve plots of winter oilseed rape cultivar Apex and treatment with flusilazole (0.8L/hectare) plus carbendazim (250 g/L flusilazole: 125g/L carbendazim) as Punch C. Plots were arranged in a randomised block experiment with three treatments (unsprayed control and two fungicide timings) and four replicate blocks. The two fungicide timings were 29 October 2005 and 29 November 2005. The phoma leaf spot epidemic on each plot was assessed monthly using samples of ten plants per plot collected by sampling in a W-shaped pattern along the edges of the plot. The proportion of plants affected with phoma leaf spot lesions (disease incidence), the growth stage of the crop (Sylvester-Bradley and Makepeace, 1985) and the percentage leaf area affected (until December) or the number of leaves affected with phoma leaf spotting (from January) (disease severity) were recorded. Concentrations of ascospores were determined using a Burkard spore sampler. In June/July, the incidence and severity of stem canker in each plot were assessed by collecting 30 stems from

each plot and recording the severity of stem canker on each stem. A severity scale of 0 (healthy stem) to 6 (dead) based on internal symptoms was used and the results were averaged to give a disease incidence and severity score for each plot (Newman and Bailey, 1987). Yield data were collected by Rothamsted farm at harvest. Incidence, severity and yield data were analysed by analysis of variance using Genstat 8.2.

Results

The summer before the 2005/2006 growing season had an average amount of rainfall (monthly average 54 mm over July, August and September) at Rothamsted, which resulted in an early ascospore release in October. In December, the October-sprayed plots had a significantly lower incidence and severity of phoma leaf spot than the unsprayed and November-sprayed plots. In January, the November-sprayed plots had similar incidence and severity of phoma leaf spot to October-sprayed plots (Figure 1, Figure 2). The maximum ascospore release occurred towards the end of October, before the November assessment.

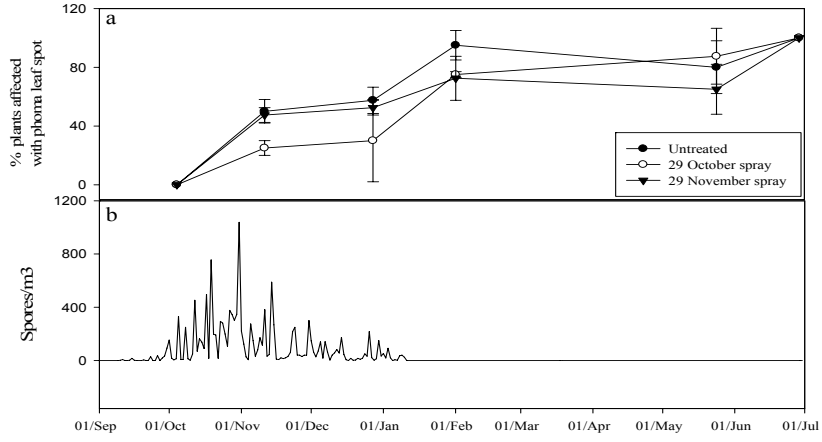

Figure 1: Changes in incidence of phoma leaf spot on winter oilseed rape cv. Apex (% plants affected) (a) in relation to numbers of air-borne *Leptosphaeria maculans* and *L. biglobosa* ascospores (b) in the 2005/2006 growing season at Rothamsted. Vertical bars are standard deviations (2 d.f.). The spray timings (29 October, 29 November) are marked with arrows.

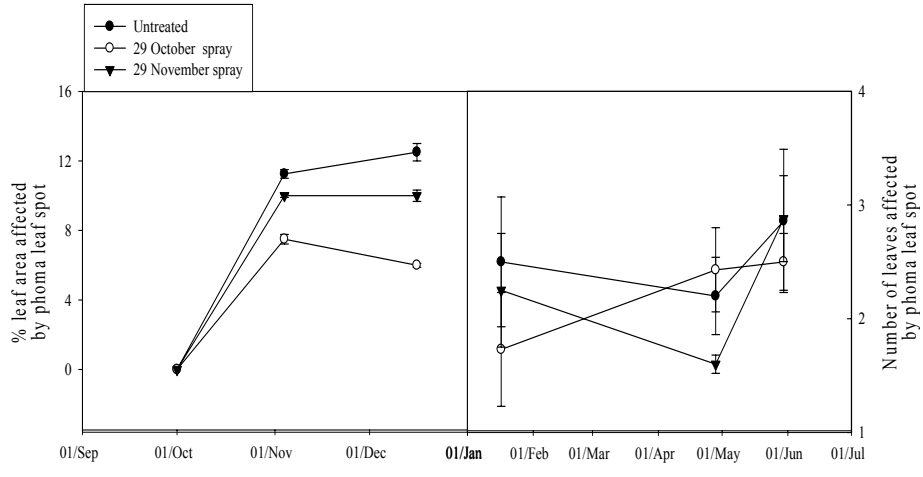

Figure 2: Changes in phoma leaf spot lesion area per leaf on winter oilseed rape cv. Apex in the 2005/2006 growing season at Rothamsted. Vertical bars are standard deviation (2 d.f.).

Severity and incidence of phoma leaf spot increased steadily in all plots between October and January during the period of ascospore release. Disease severity was initially lower in the October sprayed plots. By the May assessment, the November sprayed plots had the lowest leaf spot severity. There was an observable difference in the internal and external severity of stem canker between the October and November sprays, but it was not statistically significant (external severity [P=0.130, 2 d.f., SED 0.351], internal severity [P= 0.150, 2 d.f., SED 0.417])(Table 1). The data suggest that the October spray was more effective against leaf spotting, but the November spray was more effective in decreasing the canker severity and yield loss.

Discussion

A maximum ascospore release in October (Figure 1) suggests that plants in the early and late sprayed plots are likely to have been infected before the first fungicide application. Incidence of phoma leaf spot increased in all plots up until late January (Figure 1), with consistently lower percentages of plants affected in the October sprayed plots. The decrease in

incidence of phoma leaf spot (% plants affected) in the early sprayed plots assessed in November may have occurred either because the October spray prevented ascospores from germinating or there was a curative effect of flusilazole (Figure 1). Consequently, in December the disease severity (% area affected) was less in the 29 October sprayed plots than the November sprayed plots (Figure 2). The results suggest that the November spray was less effective in decreasing leaf spotting in this growing season. In October sprayed plots, there was evidence of control of phoma leaf spot, with lower numbers of leaves affected, smaller areas with lesions and a lower percentage of plants affected than in both the untreated control and the November sprayed plots. The effectiveness of the October spray was likely to have been because of the timing of application.

Table 1: Effects of timing of flusilazole plus carbendazim spray (in autumn 2005) on severity of phoma stem canker and yield of winter oilseed rape (cv. Apex) in July 2006 at Rothamsted

Fungicide spray date	Severity of phoma stem canker (July) 0-6 Scale*		Yield (t/ha)
	Internal	External	
Untreated	3.14±0.61	2.99±0.67	2.6±0.47
29 October	2.47±0.27	2.42±0.48	2.98±0.26
29 November	1.87±0.54	2.01±0.13	3.20±0.40
SED (d.f.)	0.417 (2)	0.351 (2)	0.330 (6)

***Internal severity:** 0 - a healthy stem with no affected tissue, 1 - 1-25% of cross section (internal assessment) affected, 2 - 26-50%, 3 - 51-75%, 4 - 76-99%, 5 - 100% (plant still alive) and 6 - dead stem with hollow or severely necrotic pith (100% affected)

External Severity: 0 - a healthy stem with no affected tissue, 1 - 1-25% of stem circumference covered by lesions, 2 - 26-50%, 3 - 51-75%, 4 - 76-99%, 5 - 100% (stem weak and discoloured, but alive) and 6 - dead stem (100% affected)

There was negative relationship between yield and severity in the untreated, October- and November-treated plots. As severity increased, yield decreased. There was a significant difference in internal severity, external severity and yield between fungicide treated plots and the untreated control. The untreated plots had the lowest yield and the greatest stem canker severity and the plots sprayed in November had the lowest severity score and the greatest yield.

The poor relationship between severity of leaf spotting and stem canker severity was unexpected. Given the greater incidence and severity of phoma leaf spot in the November sprayed plots it might suggest that there would also be a greater severity of stem canker. The ideal growth stage for application of flusilazole plus carbendazim is between stages 1,7 to 1,11, and the October spray may have been too early for optimum protection, but there is insufficient data to prove this. Furthermore, where the epidemic starts early a two-spray programme is optimal for control of stem canker; if a single spray is applied too early or too late it may not control the pathogen in leaves which are important for subsequent development of stem canker. The experiment is being repeated with a greater sampling frequency, a wider range of cultivars and with qPCR analysis of pathogen colonisation behaviour to identify the cause of the lack of correlation in results.

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References

Fitt, B. D. L., Evans, N., Howlett, B. J., & Cooke, B. M. (2006a). *Sustainable strategies for managing Brassica napus (oilseed rape) resistance to Leptosphaeria maculans (phoma stem canker)*. 126 pp. Springer, Dordrecht, The Netherlands.

Fitt, B. D. L., Huang, Y. J., van den Bosch, F., & West, J. S. (2006b). Coexistence of related pathogen species on arable crops in space and time. *annual Review of Phytopathology* **44**, 8.1-8.20.

Howlett, B. J., Idnurm, A., & Pedras, M. S. C. (2001). *Leptosphaeria maculans*, the causal agent of blackleg disease of Brassicas. *Fungal Genetics and Biology* **33**, 1-14.

Huang, Y.-J., Fitt, B. D. L., Jedryczka, M., Dakowska, S., West, J. S., Gladders, P., Steed, J. M., & Li, Z.-Q. (2005). Patterns of ascospore release in relation to phoma stem canker epidemiology in England (*Leptosphaeria maculans*) and Poland (*Leptosphaeria biglobosa*). *European Journal of Plant Pathology* **111**, 263-277.

Newman, P. L., & Bailey, D. J. (1987). Screening for Resistance to Canker (*Leptosphaeria maculans*) in Winter Oilseed Rape (*Brassica napus Ssp Oleifera*). *Plant Pathology* **36**, 346-354.

Sylvester-Bradley, R., & Makepeace, R. J. (1985). Revision of a code for stages of development in oilseed rape (*Brassica napus* L.). *Aspects of Applied Biology* **10**, 395-400.

Toscano-Underwood, C., Huang, Y. J., Fitt, B. D. L., & Hall, A. M. (2003). Effects of temperature on maturation of pseudothecia of *Leptosphaeria maculans* and *L. biglobosa* on oilseed rape stem debris. *Plant Pathology* **52**, 726-736.

West, J. S., Kharbanda, P. D., Barbetti, M. J., & Fitt, B. D. L. (2001). Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. *Plant Pathology* **50**, 10-27.

Williams, R. H., & Fitt, B. D. L. (1999). Differentiating A and B groups of *Leptosphaeria maculans*, causal agent of stem canker (blackleg) of oilseed rape. *Plant Pathology* **48**, 161-175.