Maturation of *Leptosphaeria maculans* pseudothecia in relation to forecasting phoma leaf spots (stem canker) on oilseed rape

Yongju Huang[1], Bruce Fitt[1], Julie Steed[1], Avice Hall[2]
[1] Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK
Email: Yong-ju.huang@bbsrc.ac.uk, Bruce.fitt@bbsrc.ac.uk
[2] University of Hertfordshire, Hatfield, Herts, AL10 9AB, UK
Email: A.M.Hall@herts.ac.uk

ABSTRACT

At Rothamsted in 2000/2001 and 2001/2002, pseudothecia on oilseed rape stem bases matured earlier than those on upper stems. The first release of ascospores in 2001 was 6 days earlier than in 2000, but the majority of ascospores were released between October and late December in both seasons. Phoma leaf spotting appeared 8-15 days after first release of ascospores. Phoma leaf spotting and stem canker were 13 days earlier in 2001 than in 2000. Seasonal differences in maturation of pseudothecia were related to differences in summer rainfall; with more rainfall in summer maturation of pseudothecia, release of ascospores, and subsequently leaf infection and appearance of stem canker were earlier.

Key words: Maturation, pseudothecia, *Leptosphaeria maculans*, forecast

INTRODUCTION

Phoma stem canker (blackleg), caused by *Leptosphaeria maculans* (anamorph *Phoma lingam*), is a damaging disease of winter oilseed rape (*Brassica napus* ssp*. oleifera*) in the UK (Fitt *et al*., 1997). Stem canker epidemics are initiated by air-borne ascospore produced in pseudothecia on infected crop debris. Ascospores land on the leaf surfaces, germinate and infect the leaf under favourable conditions to cause leaf lesions (Toscano-Underwood *et al*., 2001). The fungus can then grow down the petiole to reach the stem, causing stem canker (Hammond & Lewis, 1987). Infections on leaves early in the season are associated with the most damaging stem cankers at the stem base, whereas later infections produce upper stem lesions. The oilseed rape crops are considered to be more susceptible to the disease at early growth stages (West *et al*., 2001). Early appearance of leaf lesions may lead to early appearance of stem cankers and development of severe symptoms by harvest, resulting in yield loss (Sun *et al*., 2000). In the UK, control of severe stem canker epidemics depends on use of fungicides. However, fungicides cannot control *L. maculans* once it has reached the stem. Therefore, the timing of fungicide applications is crucial for effective control of the disease. There is thus a need to develop accurate methods for forecasting the risk of severe epidemics, to optimise the timing of fungicide sprays. Since ascospores released from pseudothecia produced on infected debris are the main source of inoculum, monitoring maturation of pseudothecia and release of ascospores offers a direct measure of the risk of leaf infection. This paper reports a study of pseudothecial maturation in relation to forecasting timing of phoma leaf spotting.

MATERIALS AND METHODS

In 2000/2001 and 2001/2002, infected UK oilseed rape stem base and upper stem (> 10 cm above the ground) debris were collected 2 weeks after harvest. Stems were placed in freely draining plastic trays and incubated outdoors to let pseudothecia mature in natural conditions at Rothamsted. Ten stem bases and ten upper stems were sampled weekly at random to monitor formation of pseudothecia and developmental stage of the ascospores. Temperature and rainfall were recorded by a weather station near the incubation site. A Burkard spore sampler was used to monitor release of air-borne ascospores of *L. maculans*. To study relationships between timing of ascospore release and timing of phoma leaf spot development, field experiments were done at Rothamsted in 2000/2001 and 2001/2002. No fungicides were applied. In both seasons, after the first release of ascospores was observed, 30 plants (cv. Apex) were sampled weekly (October to November) and then monthly (December to July) at random from each untreated plot to assess incidence and severity of phoma leaf spots and stem canker.

RESULTS

Maturation of pseudothecia on stem bases and upper stems

In both 2000/2001 and 2001/2002, pseudothecia were observed on stems under natural conditions from autumn to spring. After harvest in July, in both seasons, pseudothecia on stem bases matured earlier than those on upper stems (Table 1). The percentage of mature pseudothecia on stem bases and on upper stems differed between the two seasons. In 2001, the time until the first mature pseudothecia were observed on upper stems was 9 days earlier than in 2000; the time until the first mature pseudothecia were observed on stem bases was only 1 day earlier than in 2000, but the percentage of pseudothecia which were mature was higher (14%) than in 2000 (1%). Furthermore, on stem bases the time until 50% of pseudothecia were mature was 22 days earlier in 2001 than in 2000 (Tables 1). The time until 90% pseudothecia were mature was 36 days earlier in 2001 than 2000.

During the period between harvest and the time when the first mature pseudothecia were observed, there was no difference in the average temperature between 2000 and 2001, but there were differences in total rainfall and rain days (days with rainfall > 0.5 mm). Total rainfall during this period was 54.2 mm more in 2001 than 2000 and there were 5 more rain days in 2001 than 2000; consequently stem wetness duration was longer (Table 1).

Table 1 Effects of temperature and rainfall on maturation of pseudothecia of *L. maculans* on oilseed rape stem base and upper stem debris under natural conditions at Rothamsted

		2000		2001	
		Stem base	Upper stem	Stem base	Upper stem
Date first mature pseudothecia observed		11 Sep.	2 Oct.	10 Sep.	24 Sep.
Date > 50% pseudothecia mature		23 Oct.	23 Oct.	1 Oct.	1 Oct.
Days to first mature pseudothecia		40	62	39	53
Average temperature (°C)[a]		16.7	15.9	16.4	15.4
Rainfall	Total rain(mm)[b]	77.7	172.3	131.2	165
	Rain days[c]	15	27	20	29
Stem wetness (h)[e]		456[f]	829	489[f]	658

[a]Average temperature, [b]total rainfall, and [c]number of days with rainfall > 0.5 mm, from the day when the stem debris was collected and incubated outside to the day when the first mature pseudothecia were observed.
[d]Total number of hours when the stem debris was wet, monitored using a stem debris wetness sensor, from the day when the debris was collected to the day when the first mature pseudothecia were observed.

Seasonal periodicity in release of ascospores

There were differences in the numbers and patterns of ascospore release between the two seasons. At the start of each season, few or no ascospores were detected before September; the majority of ascospores were released during October to late December in both seasons. The first days with > 20 ascospores per day detected were 30 September in 2000 and 18 September in 2001 and ascospores continued to be released until late spring in each season, with ascospore release associated with rainfall.

Timing of phoma leaf spots and stem canker development

Phoma leaf spotting was first observed on 9 October in 2000 and 26 September in 2001. The times from the first observed release of ascospores to the first observed leaf spots on plants in were 9 and 8 days in 2000 and 2001, respectively. In 2000/2001, the epidemic was severe, and the incidence of affected plants reached 36% in late October with an incidence of 100% in late November; the severity of leaf infection was 5.5% in late October and increased rapidly to a maximum of 34% in mid-November. In contrast, in

2001/2002 the epidemic was early but less severe, with an incidence of 4% plants affected in late September and a maximum incidence of 70% in late October; the severity of leaf spotting was only 0.6% leaf area affected in late September and increased to a maximum of 16% in late October. Phoma stem canker appeared from late March or April onwards in the two seasons. The incidence of crown cankers in untreated plots differed between seasons. Differences between seasons in the incidence reflected earlier differences in the phoma leaf spot epidemics. In 2000/2001, a high incidence of phoma leaf spots (100%) in winter was associated with a high incidence of stem canker (100%) in spring/summer. In early May (8 May 2001), the incidence of stem canker reached 90%, and the stem canker severity score reached 1.1. In 2001/2002, following earlier appearance of leaf spotting than in 2000/2001, stem canker development started 13 days earlier.

DISCUSSION

Results of pseudothecial maturation and ascospore release experiments in natural conditions suggest that weather factors (temperature and rainfall) affected maturation of pseudothecia and the timing of the first major ascospore release and subsequent phoma leaf spot development. In 2001/2002, there was more rain during the period from harvest until the first mature pseudothecia were observed than in 2000/2001, and pseudothecia matured earlier than in 2000/2001. Subsequently ascospores were released 1 week earlier and the phoma leaf spots were observed 2 weeks earlier than in 2000/2001. Results of this study suggest that the first release of ascospores can be predicted by monitoring weather conditions and maturation of ascospores. Accurate forecasting of severe epidemics can improve disease control and decrease fungicide use when the risk of crop damage is low.

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