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### Project title
Understanding risks of severe phoma stem canker caused by *Leptosphaeria biglobosa* on winter oilseed rape in the UK

### Project number
RD- 2140021105 / 21120036

### Start date
1 April 2015

### End date
31 March 2019

### Project aim and objectives
The aim of this project is to understand phoma stem canker epidemics caused by *L. biglobosa* and to improve control of phoma stem canker by targeting both causal pathogens (*L. biglobosa* and *L. maculans*). There are four related objectives:

- **Objective 1.** To determine regional differences in proportions of *L. biglobosa* and *L. maculans*.
- **Objective 2.** To investigate cultivar resistance against *L. biglobosa*.
- **Objective 3.** To determine fungicide sensitivity of *L. biglobosa* compared to *L. maculans*.
- **Objective 4.** To make new recommendations to farmers for better control of phoma stem canker by targeting both *L. biglobosa* and *L. maculans* (knowledge transfer).

### Key messages emerging from the project
1. There are differences between regions in the pattern and timing of ascospore release. Epidemics of phoma stem canker are initiated by airborne ascospores; the information on timing of ascospore release can be used to guide timing of fungicide applications.
2. There are differences between regions in the pattern and timing of *L. biglobosa* and *L. maculans* ascospore release; more *L. biglobosa* ascospores observed in 2016/2017 than in 2015/2016 season.
3. The two causal pathogens of phoma stem canker (*L. biglobosa* and *L. maculans*) responded differently to triazole and non-triazole fungicides. The information on proportions of these two pathogens in local populations can be used to guide choice of fungicides.
4. There are differences between cultivars in severity of phoma leaf spot and phoma stem canker caused by *L. biglobosa* and *L. maculans*. The information on proportions of these two pathogens in local populations can be used to guide choice of cultivars for effective control of phoma stem canker.

### Summary of results from the reporting year
**Task 1. Determine regional differences in proportions of *L. biglobosa* in pathogen populations**

DNA was extracted from air samples collected from Sept 2016 to Feb 2017 at four sites. Results of qPCR showed that there were differences in patterns and proportions of *L. biglobosa* (Lb) and *L. maculans* (Lm) ascospores released between the four sites. In general, there were more Lb ascospores than Lm ascospores released in the 2016/2017 growing season.
Air samples were collected from Sept 2017 to Feb 2018 at four sites. There were differences in numbers and patterns of ascospores released between the four sites. Compared with the 2016/2017 growing season, ascospores were released earlier in 2017/2018, leading to early phoma leaf spotting.

**Task 2. Investigate cultivar resistance against *L. biglobosa***.

2.1 Winter oilseed rape field experiments. Six cultivars with different levels of ‘field’ phoma stem canker resistance (AHDB resistance rating) were used in the 2016/2017 field experiment at Boxworth. Severity of phoma stem canker was assessed in July 2017. Stems with phoma stem cankers were sampled for DNA extraction and qPCR. There were differences between the six cultivars in severity of phoma canker; Fencer and Harper had less severe stem canker than the other cultivars, and had more Lb DNA in their stems than Lb DNA in stems of other cultivars (except Incentive and Quartz).

The 2017/2018 field experiment was set up at Terrington. Severity of phoma leaf spot on those cultivars was assessed in Dec 2017. There were differences between cultivars in severity of Lm and Lb leaf spots. Fungicide application reduced number of Lm leaf spots for all cultivars but reduced number of Lb leaf spots only for three cultivars.

2.2. Five cultivars were tested for resistance against new Lm and new Lb isolates in controlled conditions. There were differences between the five cultivars in resistance against Lm and Lb isolates.

2.3. AHDB RL Disease Observation Plots (DOP) (extra work). Stems of 17 cultivars sampled from AHDB RL DOP in July 2016 from Morley in Norfolk were processed for DNA extraction and qPCR to investigate whether the stem cankers on those cultivars were caused by Lm or Lb.

**Task 3. Determine fungicide sensitivity of *L. biglobosa* compared to *L. maculans***.

3.1. *In vitro* test. Seven new Lm isolates and 12 new Lb isolates were tested with the fungicide prothioconazole-destho. Those isolates were tested with five different concentrations of prothioconazole-destho and a no fungicide control. The fungicide prevented the growth of Lm and Lb isolates when the concentration is > 0.5µg/ml. Isolates of Lm and Lb differed in their sensitivity to this fungicide at concentrations 0.05µg – 0.25µg/ml. In general, Lb isolates were less sensitive to this fungicide than Lm isolates. There were differences between Lm isolates in their sensitivity to this fungicide.

3.2. Field experiments. For the 2016/2017 field experiment, severity of phoma stem canker was assessed in July 2017. Treatment with fungicides Proline or Refinzar reduced the severity of phoma stem canker. There were differences between cultivars in response to fungicides Proline (Prothioconazole) and Refinzar (Penthiopyrad + picoxystrobin). Results of qPCR showed that more Lm DNA was detected in stems of the two susceptible cultivars (RL rating 3-4) than in those of the resistant cultivars (RL rating 8-9). Fungicides reduced both Lm and Lb DNA in stems of five out of six cultivars.
In the 2017/2018 field experiment, severity of phoma leaf spot was assessed in Dec 2017. Both Proline and Refinzar reduced the number of Lm phoma leaf spots on all cultivars but reduced the number of Lb phoma leaf spots on only four cultivars.

**Task 4. Make new recommendations to farmers for better control of phoma stem canker.**

Results of the field experiments and the RL DOP trials suggest that it is possible to select cultivars with resistance against both Lm and Lb. Results of winter oilseed rape fungicide experiments suggest that the fungicides Proline and Refinzar have similar effects on control of phoma stem canker caused by Lm and Lb. However, there were differences between cultivars in terms of effectiveness of fungicide treatments. Results of *in vitro* tests and field experiments suggest that there is a need to combine the information on cultivar resistance and pathogen populations to achieve effective control of phoma stem canker.

**Key issues to be addressed in the next year**

**Task 1. *L. maculans* and *L. biglobosa* in air samples.** Air samples are being collected at four sites in the 2017/2018 growing season. Finish counting the numbers of ascospores for the 2017/2018 spore samples from the four sites. DNA will be extracted from the 2017/2018 spore samples for qPCR to determine the ratios of Lm/Lb in pathogen populations.

**Task 2 Cultivar resistance against *L. biglobosa*.** Severity of phoma stem canker will be assessed in June/July 2018 in the field experiment at Terrington. Stem samples will be taken for DNA extraction and qPCR. Four cultivars with different RL ratings will be selected for testing for resistance against Lm and Lb in controlled environments with different isolates.

**Task 3 Fungicide sensitivity of *L. biglobosa* and *L. maculans*.** New isolates are being collected from the 2017/2018 field experiment and will be tested *in vitro* with different fungicides. Severity of phoma stem canker on cultivars treated with different fungicides will be assessed in June/July 2018 in the field experiment. Yield will be harvested from each plot to examine the effects of fungicide on yield.

**Task 4. Knowledge transfer.** New information from this project will be disseminated to AHDB at project meetings and to farmers at the Cereals 2018. Results of the project will be also disseminated to a wider audience in conferences and seminars or workshops.

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<tr>
<th>Lead partner</th>
<th>Dr Yongju Huang, University of Hertfordshire</th>
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<tbody>
<tr>
<td>Scientific partners</td>
<td>None (sub-contracted field experiment to ADAS)</td>
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<td>Industry partners</td>
<td>DuPont</td>
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<td>Government sponsor</td>
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Has your project featured in any of the following in the last year?

<table>
<thead>
<tr>
<th>Events</th>
<th>Press articles</th>
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<tbody>
<tr>
<td>Hertfordshire County Show, 27-28 May 2017, in Dunstable Road, Rebourn. University of Hertfordshire (UH) stand. Demonstration of phoma leaf spot symptoms caused by Lm and Lb. Talked to visitors about our research projects on control of diseases of oilseed rape. Cereals 2017, 14th – 15th June, Lincolnshire. Hutchinsons Winter Farmer Technical Conference, 15 Nov 2017, Peterborough. Posters and leaflets at the University of Hertfordshire stand. Talked to visitors (including farmers) about our research projects and the new agriculture degree at UH.</td>
<td>None</td>
</tr>
<tr>
<td>Conference presentations, papers or posters</td>
<td>Scientific papers</td>
</tr>
<tr>
<td>1. School of Life and Medical Sciences, research conference, April 2017, College Lane Campus. Poster presentation and interactions with other researchers and students in the School. Abstract in conference proceedings.</td>
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<th>canker) in UK winter oilseed rape cultivars. Plant Pathology in press.</th>
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<td>Other</td>
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