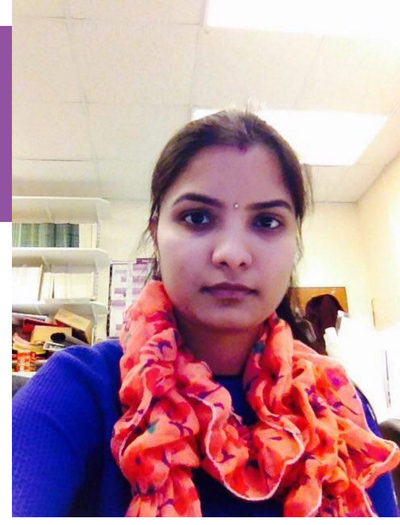
Monitoring the regional distribution of races of *Leptosphaeria maculans* populations in the UK



Gajula, L.H., Javaid, A., Fitt, B.D.L. & Huang, Y.
School of Life and Medical Sciences, University of Hertfordshire, UK

INTRODUCTION

- Phoma stem canker, caused by the fungus *Leptosphaeria maculans*, is an important disease of oilseed rape.
- Use of host resistance is the most important and effective way to control phoma stem canker.
- Two types of resistance to *L. maculans* are qualitative resistance (*R*-gene resistance) and quantitative resistance (QR).
- *R* gene-resistance against *L. maculans* is race-specific and is associated with a gene-for-gene interaction.
- *R* gene-mediated resistance is often rendered ineffective in 2-3 years due to *L. maculans* population changes from avirulent to virulent.
- This work aims to monitor virulent races of *L. maculans* both in air (ascospores) and in winter oilseed rape crops that is crucial for the effective deployment of *R* gene-mediated resistance in the UK.

MATERIALS & METHODS

In this study, the release of ascospores in the air was monitored by using Burkard spore samplers at four different sites in the UK and the frequencies of avirulent *AvrLm1* and *AvrLm6* in the *L. maculans* ascospore populations were identified by qPCR.

Figure 1: Burkard spore sampler set up at Bayfordbury site in Hertfordshire (2015/16 season). Burkard sampler surrounded by stem debris placed in free-draining plastic trays. Weather station located nearby to collect weather data can be seen in background.

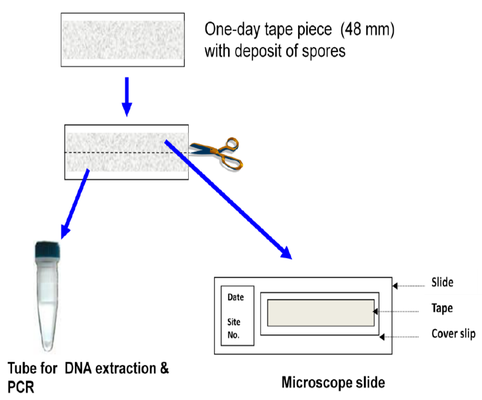


Figure 2: Processing of the spore tape from Burkard spore samplers. The 48 mm piece of spore tape was cut longitudinally and the piece on the top was placed on a microscopic slide, mounted with trypan blue and covered with a cover slip for counting the number of *Leptosphaeria* ascospores. The bottom piece was stored in a 2 ml screw cap tube for DNA extraction.

Winter oilseed rape field experiments were set up at five sites. Single pycnidial isolates were obtained from leaf lesions on cultivar Drakkar from all the sites and pathogen identification was done by morphology on PDA and confirmed by species-specific PCR. Changes in the frequencies of avirulent *AvrLm1*, *AvrLm4*, *AvrLm6* or *AvrLm7* alleles in *L. maculans* populations at different sites in the UK were investigated by inoculation of conidial suspensions on the cotyledons of a differential set of cultivars.

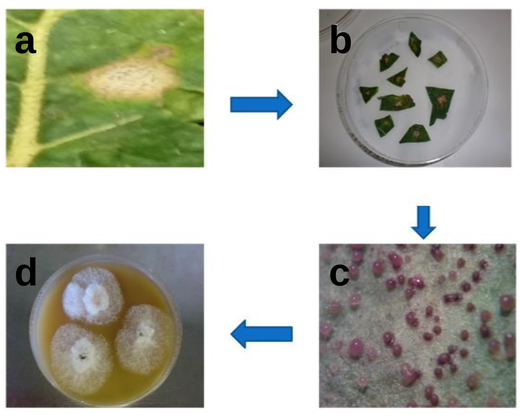


Figure 3: The procedure for the single pycnidial isolation to obtain isolates of *Leptosphaeria* species. Leaf lesions caused by *L. maculans* (a) were incubated in a Petri dish lined with Whatmann filter paper (b). After 2-3 days of incubation the cirrhus produced from a pycnidium (c) was mixed with sterile distilled water and pipetted on a PDA agar media plate. *L. maculans* cultures were observed after 4-5 days of incubation (d).

Figure 4: Tray with 14 day old seedlings of the *Brassica* cultivars or lines from the differential set immediately after inoculation with the spore suspensions of *Leptosphaeria maculans*

RESULTS

- **Differences between the four sites in patterns of ascospore release and in dates of first major ascospore release.**
- **Differences between sites in the timing of release of ascospores with avirulent *AvrLm1* and *AvrLm6* alleles.**
- ***AvrLm6* alleles were detected more frequently from spore samples compared to *AvrLm1* alleles.**
- **All the isolates tested from different sites were avirulent against *Rlm7*. There were variations between sites in the frequencies of avirulent *AvrLm1* and *AvrLm4* alleles.**
- **None of the isolates from different sites were avirulent against *Rlm3* (*AvrLm3*) or *Rlm9* (*AvrLm9*).**

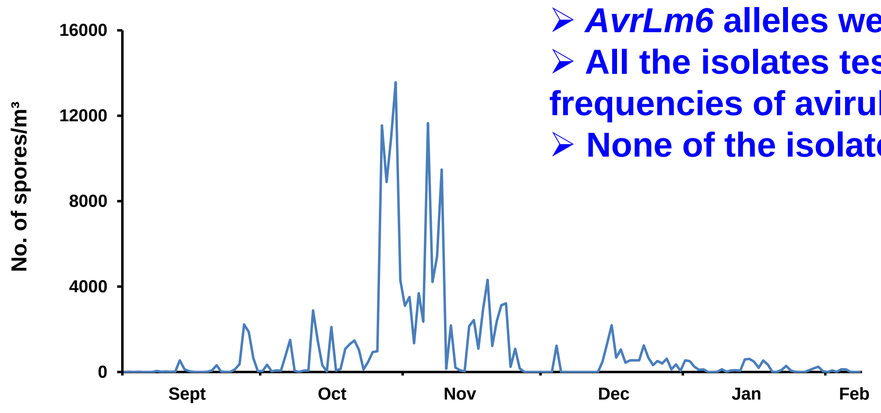


Figure 5: Patterns of *Leptosphaeria* ascospore release at the Bayfordbury site (2015/16 season). Daily counts of ascospores released in the period from 9 September 2015 to 16 February 2016 from winter oilseed rape debris placed around a Burkard spore sampler at this site.

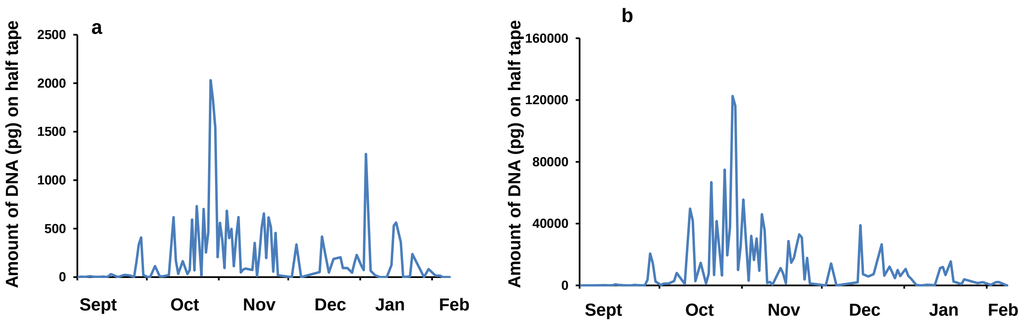


Figure 6: Patterns of *AvrLm1* (a) and *AvrLm6* (b) alleles in the spore samples at Bayfordbury site (2015/16 season). The DNA was extracted on half tape by CTAB method and qPCR was used for detecting the amount of *AvrLm1* and *AvrLm6* DNA present on the half tape.

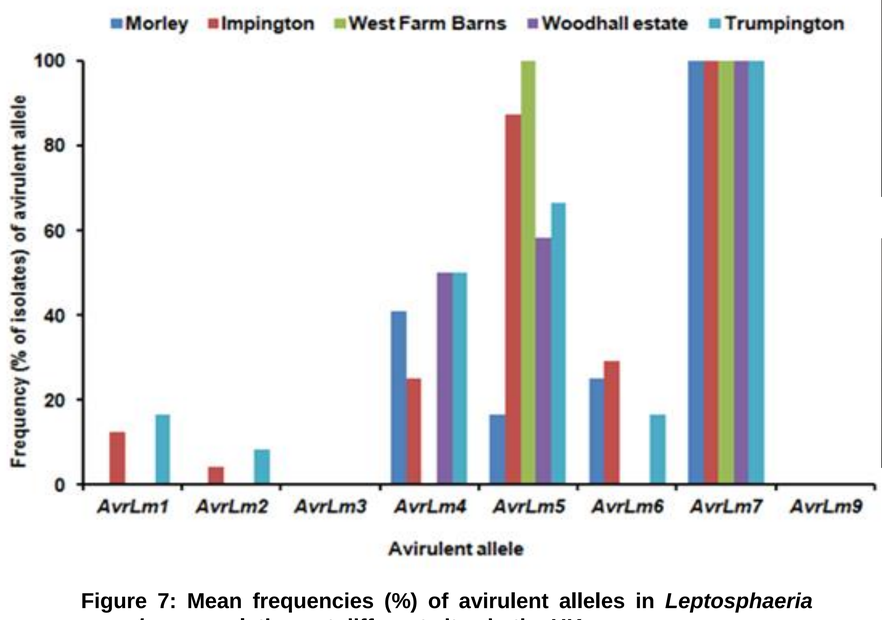


Figure 7: Mean frequencies (%) of avirulent alleles in *Leptosphaeria maculans* populations at different sites in the UK.

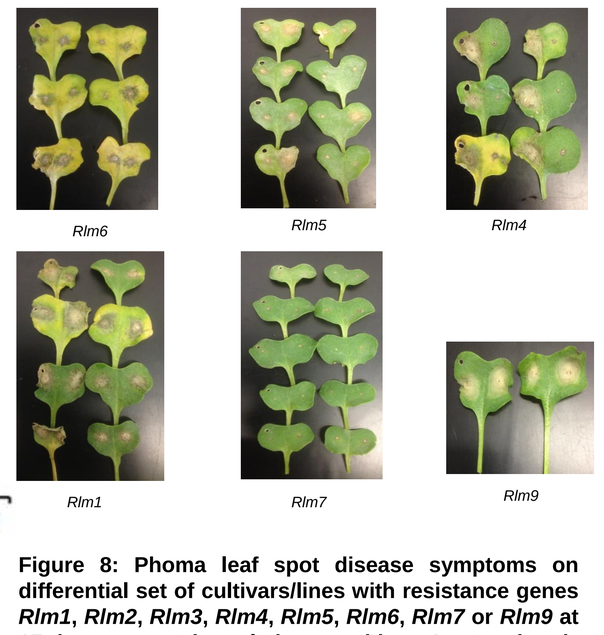


Figure 8: Phoma leaf spot disease symptoms on differential set of cultivars/lines with resistance genes *Rlm1*, *Rlm2*, *Rlm3*, *Rlm4*, *Rlm5*, *Rlm6*, *Rlm7* or *Rlm9* at 17-days post-inoculation with *Leptosphaeria maculans* isolates.

CONCLUSION

The *AvrLm7* allele is predominant in the UK *L. maculans* populations suggesting that the corresponding *Rlm7* resistance gene is still effective. Virulent *avrLm3* and *avrLm9* alleles are predominant in the UK *L. maculans* populations suggesting that *Rlm3* and *Rlm9* resistance genes are no longer effective in the UK. There is a need to continue the monitoring the regional distribution of *L. maculans* populations in the UK for the effective deployment of *R* genes.

ACKNOWLEDGEMENTS