University of Hertfordshire UH

School of Life and Medical Sciences

Leptosphaeria biglobosa inhibits production of secondary metabolite sirodesmin PL by *L. maculans in planta*

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Introduction

Phoma stem canker is an economically damaging disease of oilseed rape *(Brassica napus)*, caused by two co-existing pathogens *Leptosphaeria maculans* and *L. biglobosa*, with annual yield losses > £80M, despite use of resistant cultivars and fungicides.

L. maculans produces a non-host selective epipolythiodioxopiperazine (ETP) phytotoxin called sirodesmin PL with an *m/z* of 487.2 and causes more severe cankers (Fig.1). *L. biglobosa* does not produce sirodesmin PL.

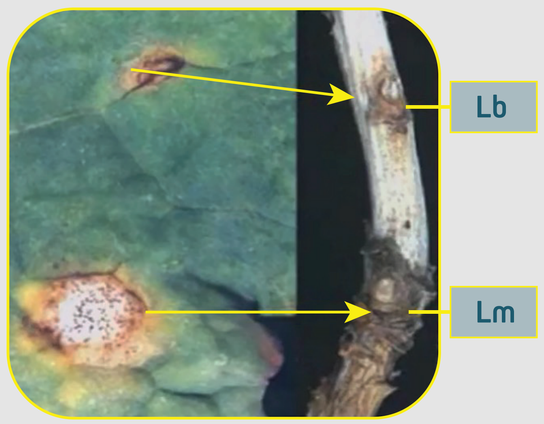

Figure 1: Phoma leaf spots and stem canker caused by *Leptosphaeria maculans* (Lm) and *L. biglobosa* (Lb).

In vitro studies indicated that simultaneous inoculation with *L. biglobosa* inhibits production of sirodesmin PL by *L. maculans*. To investigate novel strategies to improve control of phoma stem canker, this study aimed to determine whether *L. biglobosa* inhibits production of sirodesmin PL by *L. maculans in planta* and reduces severity of disease caused by *L. maculans*.

Materials and Methods

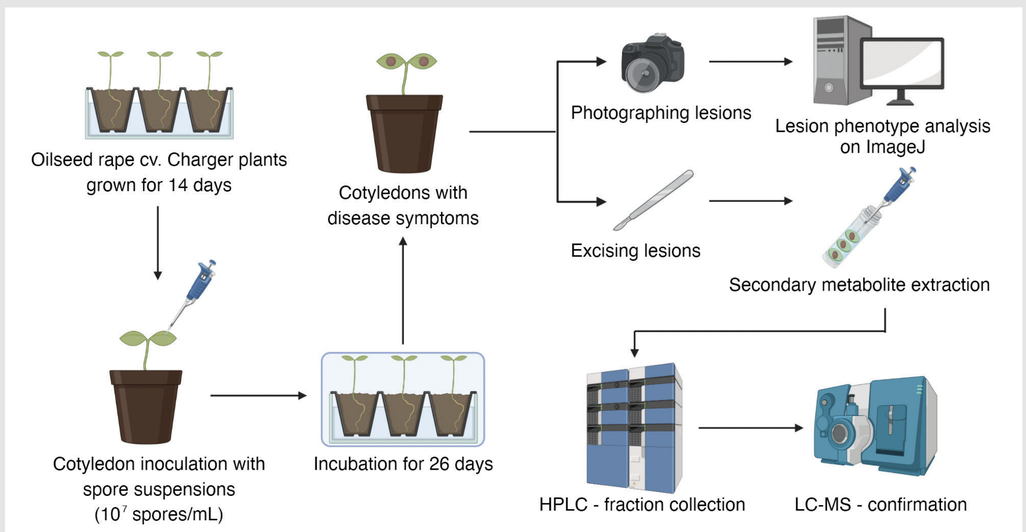

Figure 2: Illustration of method used in this work.

- Treatments were SDW, Lm only, Lb only, and Lm&Lb.

Results

- Plants with 'Lm only' treatment produced large grey lesions, while plants with 'Lm&Lb' produced small, dark lesions, which were similar to 'Lb only' treatment (Fig.3).
- The LC-MS chromatograms for *m/z* 487.2 showed unique maxima in 'Lm only' (Fig.4).
- At retention time 5.11 min, the LC-MS positive ion spectra indicated presence of ions corresponding to sirodesmin PL and several of its adducts and molecular ions (Fig.5).
- Presence of sirodesmin PL was confirmed only in 'Lm only' treatment.

Acknowledgements

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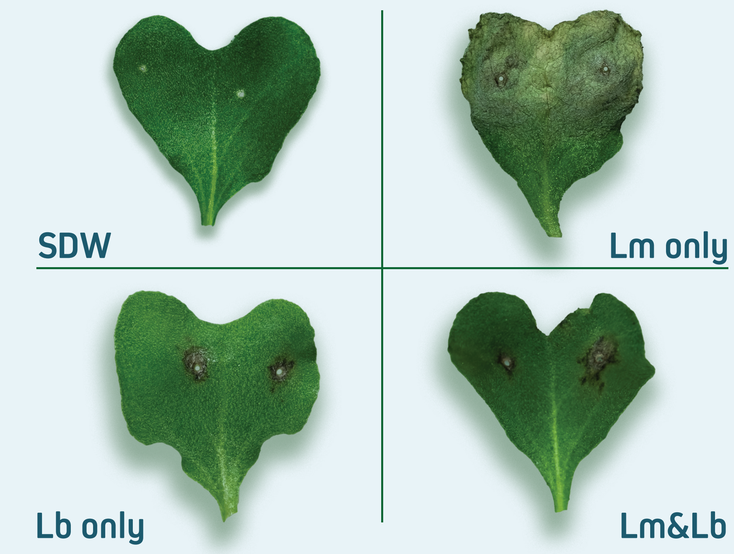

Figure 3: Symptoms on cotyledons of oilseed rape cv. Charger inoculated with sterilised distilled water (SDW), *Leptosphaeria maculans* (Lm only), *L. biglobosa* (Lb only) or *L. maculans* and *L. biglobosa* simultaneously (Lm&Lb) at 26 days post inoculation (dpi).

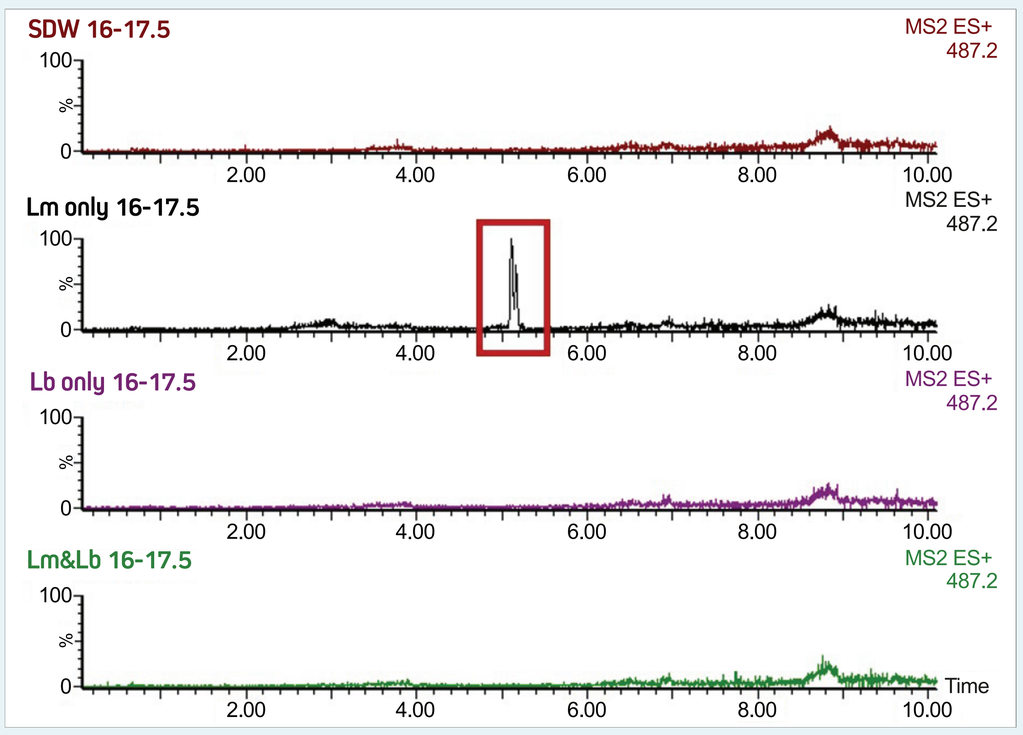

Figure 4: LC-MS chromatograms for *m/z* 487.2 of HPLC fractions at retention time 16-17.5 min for all treatments. Unique maxima found only in Lm only 16-17.5 sample is highlighted.

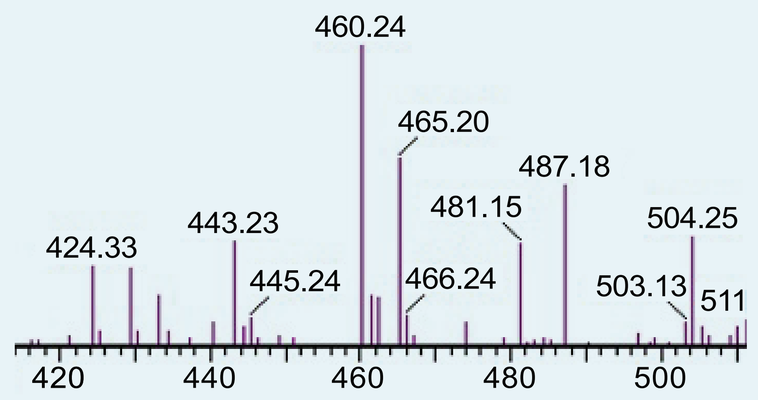

Figure 5: LC-MS positive ion spectra of Lm only 16-17.5 sample between *m/z* 420 and 510. Sirodesmin PL (*m/z* 487.18) and several of its adducts and molecular ions (*m/z* 424.33, 445,24, 460.64, 465.20 and 504.25) are identified.

Discussion

- *L. biglobosa* inhibits the production of sirodesmin PL by *L. maculans in planta* when both are inoculated simultaneously. There is a need to further investigate the mechanisms of this inhibition by *L. biglobosa*.
- Understanding the interactions between *L. maculans* and *L. biglobosa* can provide new strategies for effective control of phoma stem canker, alongside resistant cultivars and fungicide applications.