Chemical warfare: the fungal quest to conquer oilseed rape

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Introduction

Phoma stem canker, caused by *Leptosphaeria maculans* and *L. biglobosa*, causes an average yield loss of > £70M annually in UK oilseed rape (www.cropmonitor.co.uk) (Zhang *et al*., 2014). Previous studies had shown that *L. biglobosa* ascospores were released later than those of *L. maculans* (Huang *et al*., 2011). However, more recent investigations that have used qPCR analysis have reported that ascospores of both species are more frequently released at similar times (Javaid *et al*., 2018). *L. maculans* produces sirodesmin PL, a non-host selective epipolythiodioxopiperazine; *L. biglobosa* does not (Pedras & Yu, 2009). Sirodesmin PL has an inhibitory effect on *L. biglobosa* (Elliott *et al*., 2007). There has been limited work investigating the interaction between *L. maculans* and *L. biglobosa* at key stages of their life cycles. Therefore, this study aims to provide a better understanding of the unknown interactions between *L. maculans* and *L. biglobosa* and investigate the changes in phytotoxin production as a result of increased interspecific competition.

Materials and methods

L. maculans and *L. biglobosa* were cultured in liquid culture, either individually or dual cultured with a competing pathogen. After 14 days, a secondary metabolites ethyl acetate extraction was done for each treatment, to investigate the effect of secondary metabolites on the colony growth of *L. maculans* and *L. biglobosa*. Fungal plugs (8mm diameter) of *L. maculans* or *L. biglobosa* were inoculated onto clarified V8 juice agar plates. Each fungal plug was inoculated with the corresponding secondary metabolite extract from each treatment or ethyl acetate. Each treatment was replicated five times; the ethyl acetate control was replicated three times. Colony diameters for *L. maculans* and *L. biglobosa* were recorded at 7 days post inoculation and converted to colony areas. To investigate the changes in phytotoxin production, the secondary metabolites extracted from each treatment were analysed to identify differences in composition using HPLC and LC-MS.

Results

Analysis of interspecific interactions between the pathogens *in vitro* confirmed that different mechanisms of interspecific competition were used to out-compete each other. The secondary metabolites produced by *L. maculans* inhibited *L. biglobosa* colony growth. This inhibition was not observed when *L. biglobosa* was inoculated with secondary metabolites extracted from

the co-culture of *L. maculans* and *L. biglobosa*. There were three unique maxima found only in the secondary metabolite extracts that inhibited *L. biglobosa* colony growth. Using HPLC and LC-MS, these maxima were identified as sirodesmin PL precursors deacetylsirodesmin PL and phomamide, sirodesmin PL and an unknown compound. When *L. maculans* and *L. biglobosa* were co-inoculated, sirodesmin PL and its precursors were not produced. Additional maxima on the HPLC chromatograph were not found. Results of this study suggest that *L. biglobosa* must inhibit the formation of sirodesmin-precursor. Due to sirodesmin having an antagonistic effect on *L. biglobosa*, it is thought that this interference must happen very early in *L. maculans-L. biglobosa* interactions, before the production of sirodesmin. Considering application of the results for control of phoma stem canker in field conditions, if *L. maculans* and *L. biglobosa* ascospores are released at the same time, phoma leaf spot lesions may appear later or be smaller, allowing fungicides to be applied later.

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