Effects of plant age and inoculum concentration on light leaf spot disease phenotypes on oilseed rape

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Summary

Light leaf spot is caused by the fungal pathogen *Pyrenopeziza brassicae* and is the most economically damaging disease of oilseed rape (*Brassica napus*) in the UK. Current control relies on repeated fungicide applications; however, pathogen fungicide-insensitivity development highlights the need for non-chemical controls like host resistance. A study was done to assess light leaf spot disease phenotype on the susceptible *B. napus* cultivar Charger in different treatment conditions; factors studied included plant age and inoculum concentration. Results showed that older plants grown in a controlled-environment cabinet produced the most visible symptoms. Plants that received a greater inoculum concentration (10^5 spores/ml) were significantly shorter by 5 cm than those inoculated with a smaller inoculum concentration (10^4 spores/ml), suggesting possible correlations between fungal inoculum concentration and plant growth. Additionally, > 20 *P. brassicae* field isolates were collected from leaf samples across England through single-spore isolation and will be screened for virulence.

Introduction

Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae*, is the most economically damaging disease of winter oilseed rape (*Brassica napus*) in the UK, with annual yield losses of > £100M (CropMonitor, 2021; Karandeni Dewage *et al*., 2017). Control of light leaf spot is challenging because it is a polycyclic disease, with epidemics started in autumn by ascospores released from apothecia on infected crop debris from the previous season. Subsequently, conidia produced by asexual sporulation on infected leaves cause secondary infections on all parts of the plant (Boys *et al*., 2007; Evans *et al*., 2003; Fitt *et al*, 1998). Current control relies on fungicides; however, insensitivity development in *P. brassicae* isolates highlights the need for non-chemical controls like host resistance (Carter *et al*., 2014; Huang *et al*., 2006). There is currently little information about pathogenic *P. brassicae* populations and host resistance mechanisms, highlighting a need for new research. There is a need to improve our current knowledge about host resistance of winter oilseed rape against *P.*

brassicae by studying virulent races in pathogen populations, identifying candidate resistance genes and investigating mechanisms of host resistance.

Materials and methods

Results of a preliminary experiment to produce *P. brassicae* conidial inoculum were expanded to investigate the light leaf spot disease development on the *B. napus* susceptible cultivar Charger in different treatment conditions. Plants of cultivar Charger were grown in a controlled-environment cabinet and inoculated with *P. brassicae* conidial suspensions with 10^4 or 10^5 spores/ml when the plants were 4, 5, 6 or 7 weeks old. Severity of light leaf spot and plant height on inoculated plants were assessed at 23 days post-inoculation (dpi) and compared between different treatments to identify the effects of inoculum concentration and growth stage of plants on light leaf spot severity.

To establish a collection of *P. brassicae* isolates, leaves with light leaf spot symptoms were sampled from winter oilseed rape crops and fungal isolates for further study were obtained through single-spore isolation and subculturing on malt extract agar plates (Fig. 1).

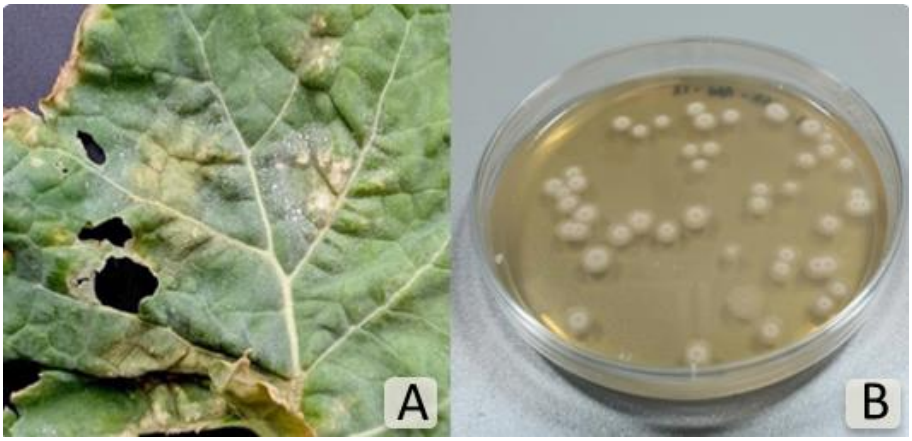

Fig. 1. Leaf of oilseed rape cv. Charger with light leaf spot (showing white *P. brassicae* acervuli containing conidia) (A). Single-conidial *P. brassicae* colonies derived from the same acervulus grown on a malt extract agar plate (B).

Results

Older plants of cv. Charger (7 weeks old at time of inoculation) grown in a controlled-environment cabinet that received the greater inoculum concentration (10^5 spores/ml) produced the most severe light leaf spot symptoms. Plants inoculated with the greater inoculum concentration were significantly shorter (by up to 5 cm) than those inoculated with the smaller inoculum concentration (10^4 spores/ml) (Figs 2 & 3).

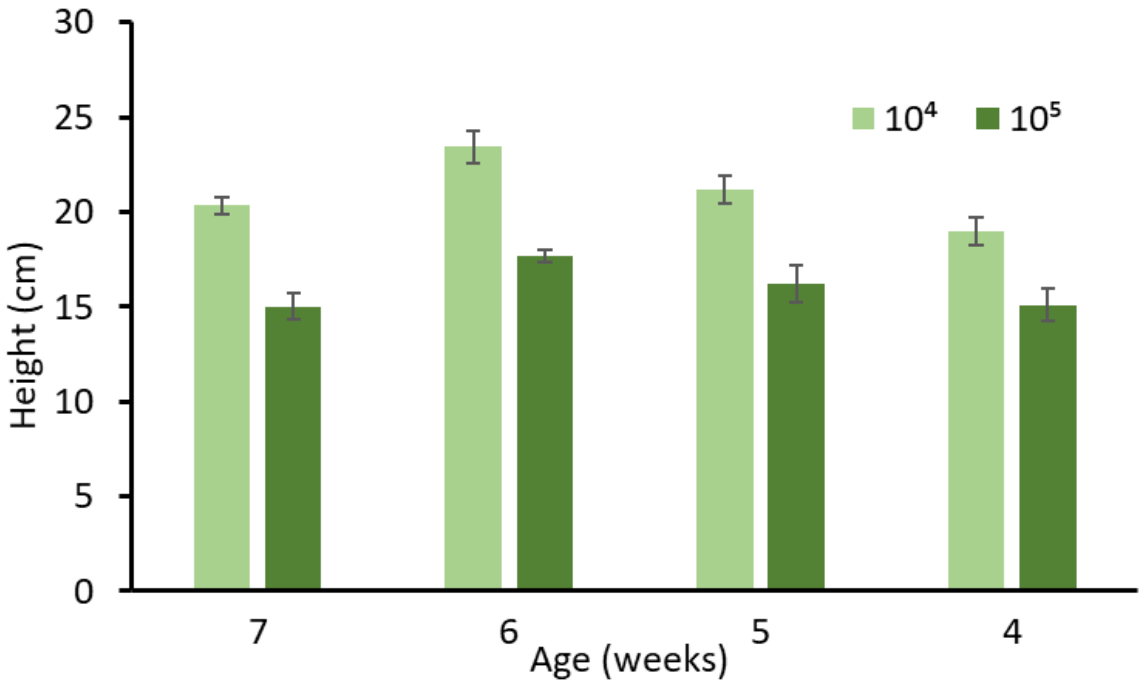

Fig. 2. Height of plants inoculated with different *P. brassicae* conidial concentrations (10^4 or 10^5 spores/ml) at 23 days post-inoculation (dpi). Error bars are standard error of the means (DF = 3).

Fig. 3. Height of plant (aged 6 weeks at time of inoculation) that received 10^4 spores/ml (1) or 10^5 spores/ml (2) of *P. brassicae* inoculum, assessed at 23 dpi.

Discussion

Greater fungal inoculum concentration (10^5 spores/ml) produced a greater disease severity and reduced height of plants, suggesting a possible correlation between inoculum concentration and plant growth. Observations of plant growth stunting on light leaf spot-infected plants have been reported previously in field trials (Karandeni Dewage *et al*., 2018), with no prior reports in cabinet-grown plants. These results will be investigated further in future experiments.

More than 20 *P. brassicae* field isolates have been collected from oilseed rape and kale cultivars across England and will be further screened for virulence.

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