The Interaction of a Biological Control Agent (*Trichoderma harzianum*) and the Spot Blotch Pathogen *Bipolaris sorokiniana* in Barley Leaves

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Introduction

There is a need for sustainable methods of controlling plant diseases. One promising approach is biological control by microorganisms that are antagonistic to pathogens. Recently, it has been shown that biological control agents can reduce the severity of spot blotch, an important disease of cereals in many developing countries, but we do not know what the mechanisms are (Aada, 2014). Thus, this research was carried out in order to investigate how an effective biological control agent (*Trichoderma harzianum* T-22) and the pathogen (*Bipolaris sorokiniana)* interact while growing on barley leaves. DNA extractions were completed as well as microscopic analysis by clearing and staining in lactophenol blue.

Methods and Results

For microscopic germination counts, barley plant leaves were cut into 1cm pieces and inoculated with 20 µl of 10^{-1} Trichoderma and Bipolaris spore suspensions, both separately and together. Tissue pieces were left for 48 hours on water agar at 28 °C, periodically removed, cleared using Farmer's Fluid and stained in Lactophenol Cotton Blue (Fig. 1). To further investigate the bio-control agent, the experiment was repeated as above, except using *Trichoderma* spore supernatant, produced by centrifuging spore suspension. This latter experiment yielded a result of 86% germination of *Bipolaris* spores in the absence of Trichoderma supernatant, and 27% in presence (P-value 0.0001 from Fisher's Exact Test).

CTAB DNA extractions using liquid nitrogen were completed on barley plants that had been inoculated with combinations of spore suspensions and left for 10 days (Fig. 4).

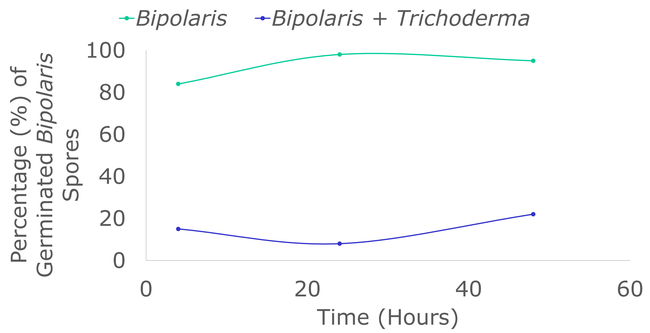

Fig. 1. The percentage of Bipolaris spores that germinated over a period of 48 hours, when grown on barley leaf tissue alone and with Trichoderma T-22 (P Value <0.0001 from Fisher's Exact Test).

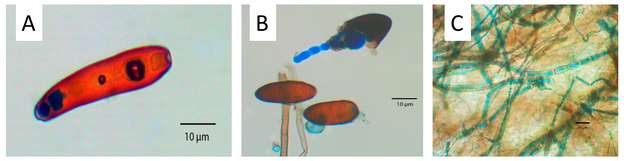

Fig.2. Sequential spore germination of Bipolaris over 96 hours. (A) 4 Hours; no germination, (B) 48 hours, (C) 96 hours.

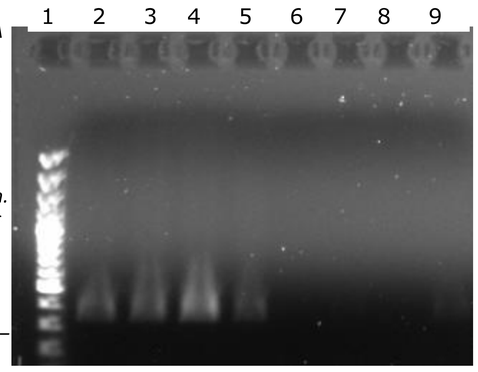

Fig. 4. Bipolaris DNA with COCH-F and COCH-R primers. Lane 1 – 100bp Plus DNA Ladder. 2, 3, 4, and 5 – Repeats of Bipolaris inoculation. 6 and 7 – repeats of Bipolaris combined with Trichoderma inoculation. 8 – - negative control. 9 – positive control.

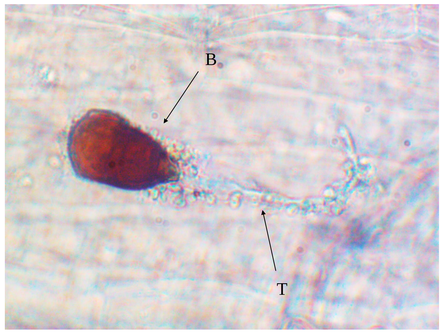

Fig. 3. Interaction between Bipolaris (B) and Trichoderma (T) spores on Barley leaf surface (24 hours). Bipolaris germination sequence seems to have been halted by the presence of Trichoderma.

Discussion

The interaction of *Trichoderma* species with *Bipolaris* in this study supports the potential of *Trichoderma* as a bio-control agent against spot blotch. There is a significant reduction in *Bipolaris* spore germination (Fig. 1) when *Trichoderma* is present. A similar reduction occurs from repeating the experiment with *Trichoderma* spore supernatant and no spores. This suggests the release of an antifungal metabolite from the control agent spores as the cause of the inhibition. Figure 3 shows a collection of *Trichoderma* spores around *Bipolaris*; this suggests a mechanism of attraction between the two species leads to a prevention of germination. Normal *Bipolaris* germination occurs as shown in Figure 2 for reference. DNA work showed successful amplification of *Bipolaris* DNA in the absence of *Trichoderma*, yet no *Bipolaris* DNA could be amplified with *Trichoderma*. This suggests the control agent has successfully reduced the presence of the pathogen (Fig. 4).

Further Research

Further DNA and microscopic analysis will be helpful in determining a relationship between the two fungi, as well as aiding to identify the potential antifungal metabolite mentioned above. This will be beneficial for control of spot blotch in barley by *Trichoderma.*

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Aada AMMA (2014) Identification of pathogens and control of spot blotch disease of barley (*Hordeum vulgare*) by combining plant resistance and biological control. PhD thesis, Newcastle University.