INVESTIGATION OF INTERACTION BETWEEN *CERCOSPORA BETICOLA* AND *PYRENOPHORA TERES* AS BASIS FOR IMPACT ON THEIR POTENTIAL FOR SURVIVAL

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Introduction

Sugarbeet and barley are major crops in the Northern Plains Area including MonDak (Eastern Montana and Western North Dakota) area of the USA. The two crops are often rotated and also frequently seen growing near each other (Figure 1)

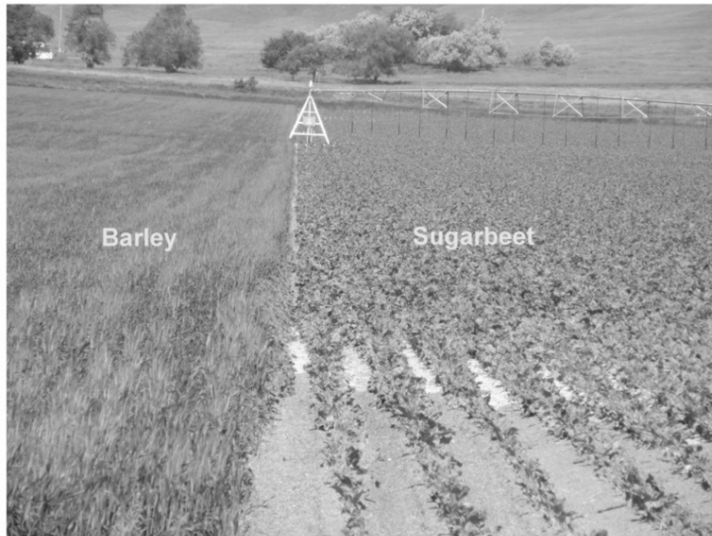
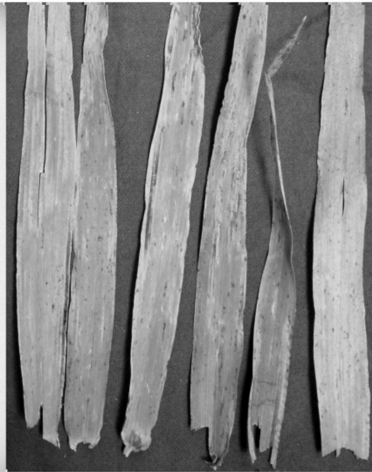


Figure 1. Barley and Sugar beet fields adjacent to each other near Sidney MT.

Cercospora leaf spot (CLS) of sugar beet (Figures and Net blotch of barley (Figure 2), are major diseases of the two crops. CLS caused by *C. beticola* is a major disease that has been reported worldwide (Bleiholder and Weltzien 1972) including the MonDak area. Under optimal condition CLS incidence results in significant economic loss of beet yield,



Cercospora leaf spot of Sugarbeet Net blotch of Barley

sugar content and recoverable sugar (Smith and Ruppel 1973). Net blotch caused by the ascomycete *Pyrenophora teres* Drechs. (anamorph: *Drechslera teres* (Sacc.) is a widely distributed foliar disease of barley (*Hordeum vulgare* L emend Bowden). Occurrence of net blotch disease could result in yield losses between 10 and 40%, but can be as high as

Figure 2. Cercospora leaf spot of sugar beet and net blotch of barley.

100% under heavy disease pressure. The frequent rotation of the two crops raises a question as to whether the two pathogens may antagonize each other and potentially impact incidence of respective disease in a growing season.

To understand the potential impact of these two pathogens on each other, we initiated and examined their antagonistic interactions. As a follow up we utilized scanning electron microscopy to examine the interaction between *C. beticola* and *P. teres* to study the basis for previously observed inhibition. We present here results on basic antagonism and observation of electron microscopic mechanism of antagonism between the two pathogens. This may serve as basis for future research on manipulation of the two pathogens to manage the two diseases

Objectives

The objective of this research is to investigate antibiosis interaction between *C. beticola* and *P. teres.* The research focused on standard antibiosis test and scanning electron microscopic examination to elucidate mechanisms of antagonism between the two pathogens as basis for understanding the potential impact on each other and disease incidence.

Materials and Methods

Antagonism test between *Cercospora beticola* and *Pyrenophora teres*. In preliminary experiments, petri dish tests (Johnson and Curl,1972) were conducted to evaluate antagonism between *C. beticola* and *P. teres*. Mycelial discs of the two pathogens were each transferred simultaneously and placed approximately 6 cm apart on the PDA plates. In parallel tests, the mycelial discs of control *C. beticola* and *P. teres* were transferred to fresh agar plates and remained unchallenged. Half of each experimental treatment were incubated under 12h photoperiod at 25ºC. The other half were incubated at 15ºC under

uninterrupted darkness to evaluate antagonistic interaction without impact of active cercosporin. The cultures were observed daily for antagonism and their visual effect on the two fungi.

Microbial growth on *C. beticola* cultures under light. Cercosporin is a *Cercospora* spp producing toxin with broad activity against wide range of organisms including fungi, non-host plants, viruses, fungi, bacteria animal cells cultures and animals such as mice and is activated under light. (Chung et al. 1999). To determine impact of cercosporin on other microbes, four culture of *C. beticola* were incubated under light at 12 h photoperiod. Other four cultures were incubated under continuous darkness. The cultures were observed daily for microbial growth.

Scanning Electron microscopy. To understand mechanism for interactive antagonism between *C. beticola* and *P. teres* the cultures from the standard antagonism study were examine by scanning electron microscopy. Hyphal tissue samples from the junction of two interacting fungi *C. beticola* and *P. teres,* were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, overnight at 4ºC. When fixed, the samples were rinsed with 0.1 M cacodylate five times, for two minutes each. The samples were postfixed in cacodylate-buffered (pH 7.4) 1% osmium tetroxide, dehydrated in a graded series of ethanol (50%, 70%, 80%, 95%, and 100%) for 10-15 minutes each. After dehydration, the samples were air dried, coated with gold using a Denton Desk II Sputter Coater and examined under a Zeiss Ultraplus Field Emission SEM. Images were captured digitally using PCI Quartz image acquisition software and saved as JPEG format in Photoshop.

Results and Discussions

Antagonism between *C. beticola* and *P. teres* Results of standard antagonism between *C. beticola* and *P. teres* are presented in Figure 2

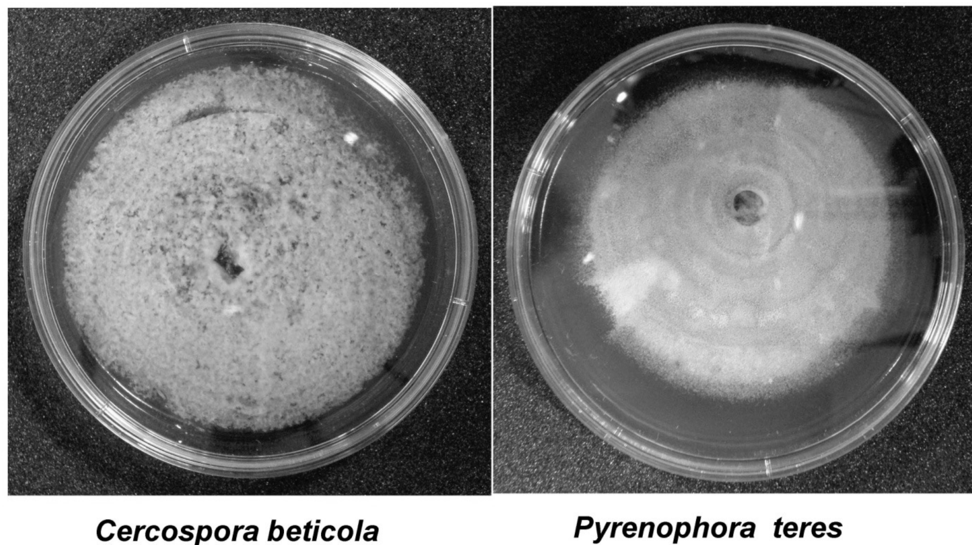


Figure 2a. Unchallenged, both fungal pathogens *C. beticola* and *P. teres* grew and eventually covered the plate.

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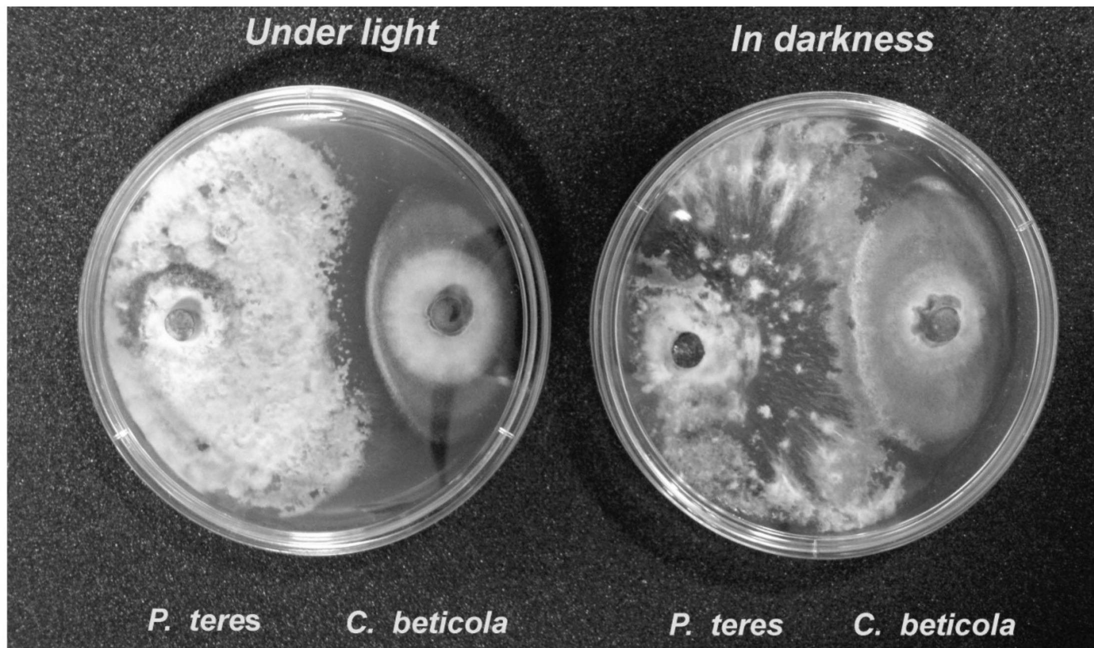

Figure 2b. Challenged under light, physical contact between the two fungi was severely limited and damage of *P. teres* at the site of contact with the red cercosporin pigment was obvious. Under darkness of *C. beticola* was severely inhibited and was eventually overwhelmed by *P. teres*.

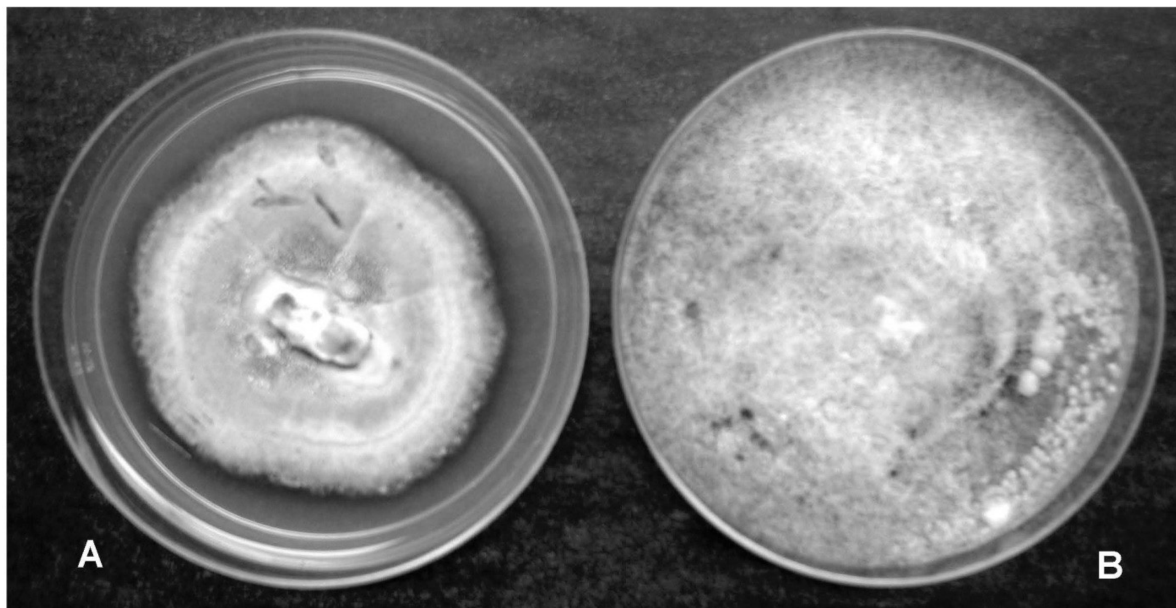

Figure. 4. Growth of microorganisms on *C. beticola* cultures.

Microbial growth on *C. beticola* cultures under light. Under light, no microorganisms were observed growing on culture of *C. beticola* (Figure 4). Red cercosporin pigments were obvious on the cultures. In contrast, several unidentified microorganisms were

observed on *C. beticola* cultured under darkness. Cercosporin pigment was not observed on these cultures.

Scanning Electron microscopy. Our scanning microscopic data provided evidence for structural damages of both pathogens under selected condition and are presented in Figure 4

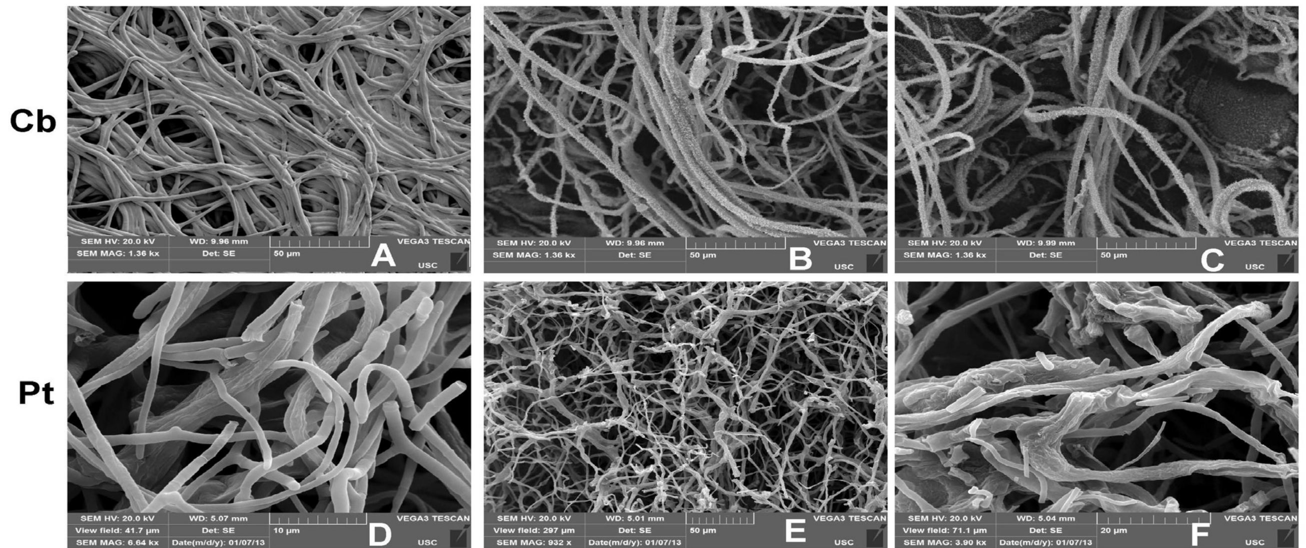

Figure 5. Under both light and unchallenged darkness *C. beticola* hyphae remained intact without any structural changes. However under darkness (B and C), structural changes characterized by developed puberulent (minutely pubescent fine short hairs) of the hyphae were observed in *C. beticola* cultures.

Structures of unchallenged under light and challenged under darkness *P. teres* hyphae (D) remained intact. However challenged under light loss of structural integrity in *P. teres* hyphae were observed. The *P. teres* hyphae over time showed an indication of possible degradation resulting from contact with *C. beticola*.

Discussions and Conclusion

We have evaluated antagonistic interaction between a *C. beticola* that causes CLS of sugar beet and *P. teres,* causal agent of net blotch of barley. Under darkness *C. beticola* was severely inhibited with loss hyphal structural integrity. Under light, *P. teres* in contrast was severely inhibited with loss hyphal structural integrity. Our results indicate that under certain condition, either of the pathogens can successfully antagonize the other. This could form basis for future research manipulate condition for either of the pathogens to manage disease incidence of the other.

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