Friendly Fungi Help in War against *Cercospora*

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The following is the latest offering in a monthly series discussing ongoing research efforts at the USDA-ARS Northern Plains Agricultural Research Laboratory (NPARL) in Sidney, Mont.

Like sugar beet growers elsewhere, producers in Eastern Montana and Western North Dakota are becoming all too familiar with *Cercospora* leaf spot of sugar beet, a fungal disease that can lead to yield and other losses totaling as much as 30 percent of the crop's value. Financial loss could be as high as $1,500 per hectare. The disease is found wherever sugar beets are grown and is one of the most important foliar diseases of that crop. The disease can produce significant root yield losses, as well as reduce sugar content, extraction and root storage life.

As with any disease, effective management of *Cercospora* leaf spot depends upon a rapid and accurate diagnosis. In this instance, *Cercospora* leaf spot can be identified with the aid of a microscope to determine the presence of conidiophores and conidia in the leaf lesions. Without the microscope to discern the tell-tale conidia, even an experienced person can sometimes confuse the symptoms of *Cercospora* leaf spot with other "spots" or leaf blotches produced by other agents.

Current Management of Cercospora Leaf Spot

Cercospora leaf spot is caused by the fungus *Cercospora beticola* Sacc. and is primarily controlled with a combination of cultural practices, the use of resistant varieties and the application of fungicides. Crop rotations are also varied to curb the disease with rotations of sugar beets and non-host crops routinely carried out in two- to four-year cycles. While these approaches can help, producers are sometimes reluctant to use certain tools, such as resistant varieties, because of lower yields, and even when they do use these practices *Cercospora* leaf spot can still occur.

A sugar beet leaf infected with *Cercospora* leaf spot.

Other problems, too, can limit a producer's control options. In the Northern Plains area, different classes of fungicides – such as benzimidazoles (Topsin M), sterol biosynthesis inhibitors (Eminent), protectant fungicides (SuperTin, AgriTin, Dithane M45, Penncozeb), or stobilurin fungicides (Gem and Quadris) – are alternately applied to manage the disease. But while these fungicides have proven effective in managing *Cercospora* leaf spot in the short run, the pathogen frequently develops a resistance to them over time. In addition, concern for the environment has led to the banning of several other effective fungicides.

Taking a second look

Given the limitations of existing control options, we need to examine new ways for identifying and managing *Cercospora* leaf spot. Alternatives being studied at NPARL include the use of

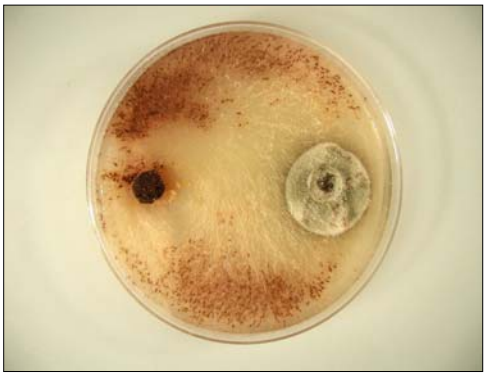
biological control and new strategies designed to identify and exclude the pathogen from alternate host plants which act as "carriers" of the disease.

Over the past two and half years, we have been examining new approaches to combat *Cercospora beticola* based first on the fact that it survives mostly in infected crop debris in the soil, and secondly on the premise that the disease-causing fungus may have a wider host range than previously thought. In either instance, surviving conidia and other disease-causing factors found in soil debris or in alternate host plants initiate infection in the following sugar beet crop by germinating and producing spores that are spread by wind, water, rain or insects. Secondary spores from those newly infected plants then further spread the disease.

Rapid detection of Cercospora in other weeds and crops

Our first strategy for controlling the disease is to rapidly identify these alternate weed and crop hosts in which *Cercospora* hides. This will allow us to destroy these plants, where possible, and interrupt the pathogen's life cycle, ultimately killing it. To this end, we have developed a molecular technique (PCR) that amplifies extremely small amounts of suspected *Cercospora beticola* DNA segments directly from infected plant tissues, allowing us to readily compare them with known DNA samples from pure *C. beticola* cultures. If necessary, any "matches" can be confirmed through DNA sequencing.

All this can be achieved without the need to purify the DNA from infected plant tissues, enabling us to screen a wide range of potential secondary hosts, even those which show no outward symptoms of the disease. By identifying these secondary hosts, we can develop a management system to either remove the "carrier" plant or eliminate the *C. beticola* pathogen harbored in it.



This "friendly fungus" (on left) is overtaking a *Cercospora beticola* sample.

A new approach to biological control of Cercospora

There have been earlier, limited studies of potential biological controls for *Cercospora beticola*. But because the disease is considered a foliar disease, the previous attempts at biocontrol have relied on the use of foliar antagonists such as the bacteria *Bacillus subtilis*. However, at NPARL we've taken a new approach by soliciting help from soil-inhabiting fungi to combat the pathogen in its survival (overwintering) stage, during which it resides primarily on soil debris. Over the past two years, we have been evaluating four such promising soil fungi and their ability to combat *C. beticola* in the lab. They include *Laetisaria arvalis, Trichoderma harzianum, Trichoderma aureoviride* and *Trichoderma virens*.

Control with *Laetisaria arvalis*

Laetisaria arvalis is a soil-inhabiting fungus originally isolated from a sugar beet farm in Nebraska. Because this fungus has been shown to suppress several plant pathogens, it is considered a likely candidate to also attack *Cercospora* – and it does. In laboratory culture experiments, growth of *C. beticola* was significantly repressed when it was paired against isolates of *Laetisaria*.

It does so by degrading cercosporin, a toxin produced by *C. beticola* and implicated in the development of disease symptoms in infected plants. Our research indicates that cercosporin may also act to protect *C. beticola* against potential natural enemies. Working together with NPARL microbiologist Dr. TheCan Caesar-TonThat, we have shown that *L. arvalis* produces an enzyme(s) which degrade this toxin, thus exposing *Cercospora* to attack. In addition, *L. arvalis* produce metabolites which kill *Cercospora* prior to physical contact of the two fungi. Whether the death is caused by the same enzyme or by other product(s) of *L. arvalis* remains to be established. It is now obvious, however, that *L. arvalis* is capable of antagonizing *Cercospora* and reducing its ability to cause disease. Our results suggest a need for further research into the use of soil-inhabiting agents to combat foliar pathogens like *C. beticola* when they are found in the soil.

Control with *Trichoderma*

The other friendly fungi we have been evaluating belong to the *Trichoderma* group, which has received the most attention for its biological control potential against a large number of plant pathogens. The *Trichoderma* fungi antagonize pathogens using a variety of methods such as producing toxins which inhibit or destroy the pathogens or by physically coiling around the pathogens and killing them. In our evaluation in cultural media, it appears that the three Trichoderma species studied – *Trichoderma harzianum, Trichoderma aureoviride* and *Trichoderma virens* – all destroy *Cercospora* by producing toxins that kill it prior to physical contact. Exactly how the three *Trichoderma* species accomplish that task will be determined in future research at NPARL.

Spraying a Montana sugar beet field for *Cercospora* leaf spot disease. *Cercospora beticola*, the fungus causing the disease, is rapidly developing resistance to some of the fungicides being used to fight it.

The next step

All four fungi studied have been tested extensively at NPARL for their potential to antagonize *Cercospora*. With the successful completion of this preliminary work, we will be expanding our research this year to greenhouse trials to determine if these friendly fungi can actually destroy *Cercospora* in the soil, not just on test plates, and what size population is needed to accomplish that task. If these greenhouse tests subsequently prove successful, we will expand our trials further to include large field tests next year in preparation for the future adoption of this biocontrol strategy by area sugar beet producers.