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REDUCING SUGARBEET LOSSES TO *APHANOMYCES* ROOT ROT USING BIOLOGICAL CONTROL AND INDUCED RESISTANCE.

Michael S. Metzger[1] and John J. Weiland[2]

[1]Department of Plant Pathology, North Dakota State University, Fargo,
and Minn-Dak Farmers Cooperative, Wahpeton.
[2]USDA, Agricultural Research Service, Northern Crop Science Laboratory, Fargo.

Introduction:

Aphanomyces cochlioides is an oomycete organism, distantly related to members in the genera *Pythium* and *Phytophthora*. It is considered to be a water mold that affects sugarbeets in both the seedling and adult stages of the plant. During its life cycle, *A. cochlioides* produces two very different types of spores – motile zoospores and resting oospores. Zoospores are considered to be the infectious entity produced by the pathogen, whereas oospores are the stable, overwintering form of the organism, capable of surviving many years in the infested soil.

Seedling root rot disease caused by *A. cochlioides* is favored by warm, wet soils; hence the improved stand establishment offered in soils known to contain the pathogen when seed is sown as early in cooler soils. Chronic root rot typically occurs in June and July in Minnesota and North Dakota following wet, warm periods. The disease has been particularly severe in recent years in this production region due to unusually wet summers.

Over the last 5 years, American Crystal Sugar Company (ACSC) shareholders have destroyed an average of 12,761 acres of sugarbeets principally due to root rot disease caused by Aphanomyces. This has reduced the tons delivered to ACSC by an average of 262,748 tons per year. In addition to the acres that are actually destroyed, there are many acres that are infected with *A. cochlioides* that are not destroyed - these beets can drastically reduce on-farm profit to the grower by reducing the overall tonnage and the quality of their beet crop. Many of these infected roots also can cause harvest difficulties for the grower as well as storage complications for the cooperatives.

Heritable resistance is available to the chronic phase of *A. cochlioides* root rot and presently is being incorporated into commercial sugarbeet varieties. However, the yield of sugarbeet varieties characterized by tolerance to *A. cochlioides* currently is lower in comparison to top producing varieties under disease free conditions, thereby decreasing their attractiveness to growers. Treatment of sugarbeet seed with hymexazol (Tachigaren™) can offer protection to emerging seedlings. The effectiveness of hymexazol, however, is reduced or absent during the chronic phase of the disease and protection relies more upon conventional cultural practices. Combined, these facts reveal a need for alternative or complementing methods to control this disease of sugarbeet.

Objectives:

The present work was undertaken to determine if biocontrol approaches would yield new technologies for the control of *A. cochlioides* in sugarbeet in the Red River Valley of Minnesota and North Dakota. To this end a commercially-available inducer of systemic resistance (harpin protein formulated as Messenger™) and the isolates AMMDR1 of *Burkholderia cepia* and PRA25rifz of *Pseudomonas fluorescens* previously demonstrated to reduce *Aphanomyces euteiches* root rot of pea were all tested for the ability to reduce stand and yield impacts of *A. cochlioides* in sugarbeet. The results suggest that induced resistance and the application of biocontrol bacteria might complement the use of chemical fungicides and genetic resistance in the protection of sugarbeet from root rot.

Materials and Methods:

In both 2001 and 2002, plots were established within commercial fields located near Hillsboro, ND and Perley, MN contracted to American Crystal Sugar Company. Medium-sized seed of Maribo 9369 (a susceptible variety to *A. cochlioides*) was treated with the bacterium *Burkholderia cepacia* AMMDR1 at approximately 7.5 log CFU per seed. The same treatment was applied for *Pseudomonas fluorescens* PRA25rifz. The bacterium used for the treatments was cultured in standard nutrient broth medium and sprayed onto the seeds (to reduce damage to the fungicide coatings). During the 2001 growing season, the control seed was not treated with any bacterium nor was any of the seed treated with Apron™ (Metalaxyl), Thiram™ (Thiram), or Tachigaren™ (hymexazol). In 2002, however, three different seed variables were used including all three of the above seed fungicides to control other damping-off pathogens.

Root rot index values (0-100 scale, 0=Healthy, 100=Total Mortality) of the fields averaged 72 and 68 respectively for 2001 and 88 and 64 for 2002. Seeds were planted to stand (4.5 inches between seeds) with a modified John Deere Maxi-Emerge row crop planter in 4-row plots (22 inches between rows, 50 feet long). Planting of the Hillsboro and Perley sites was on May 18th and May 20th, 2001, and May 18th and May 7th, 2002, respectively. Plots were arranged in a complete randomized block design with four replications in 2001 and three replications in 2002. Herbicides, insecticides, and fungicides (to control Cercospora leaf spot) were applied as necessary for each individual location.

Stand counts were taken on the entire plot at 15, 30, and 45 days after planting, as well as a final count (on the center two rows) at harvest. In 2001, foliar applications of formulated harpin protein (Messenger^{TM}) were applied weekly beginning immediately after seedling emergence (and continued for 12 consecutive weeks) using a 4-row boom sprayer and Tee-Jet^{TM} 8002 flat fan nozzles. A rate of 4.5 grams per acre of Messenger^{TM} was applied along with 10 gallons per acre of distilled-deionized water in keeping with the recommendations of the manufacturer (Eden Bioscience, Bothell, WA). During the 2002 season, Messenger^{TM} was applied in the same manner as 2001, varying only in the time intervals of application: 4, 8, and 12 consecutive weeks after seedling emergence.

2001 plots were harvested September 25 at Hillsboro and September 28 at Perley and September 17 at Perley and September 24 at Hillsboro during 2002. Each plant was harvested and topped by hand. In order to reduce the effects of bordering plots, only beets in the center 2 rows were harvested for yield analysis. Each sugarbeet root was visually rated for Aphanomyces Root Rot (0-4 Scale, 0=Clean Root, 4=Completely Rotted) before begin bagged and transported to the Minn-Dak Farmers Cooperative Tare Lab (Wahpeton, N.D.) for quality and purity analysis. Samples were rated for root yield, percent tare, sugar content, and impurity level (sugar loss to molasses).

Results and Discussion:

At both locations and in both years, weather conditions were favorable for infection of sugarbeet seedlings and roots by *A. cochlioides*. However, during the 2001 season, the disease was somewhat more noticeable at the Hillsboro location than at the Perley site. Established stands and the emergence rate for the experiment were stronger at both locations during the 2001 season compared to 2002 possibly due to unseasonably cool temperatures in the early weeks of the 2002 growing season.

For both years, stand counts began to decrease at 15 days after planting and continued a downward trend at the thirty-day count as the disease pressure began increase. Counts were taken at 45 days post planting for the treated replications at both locations and for both seasons showed an improvement over the untreated checks (Figure 1 & 2). For the 2001 season (Hillsboro location), the average stand count for the *B. cepacia* AMMDR1 treated seed was 29 plants lower than its 15-day count – a 20.35% reduction in established stand. Whereas the untreated check averaged 63 plants less than its previous count resulting in a 37.67% stand reduction. The Messenger^{TM} treated plants also exhibited a stronger stand by only losing an average of 30.5 plants to the

disease, amounting to a 20.13% reduction (Figure 3). Stand count data for the 2002 season at the Perley location revealed a similar trend. The *B. cepacia* AMMDR1 and Messenger™ treated replications lost an average of 22.5 (35.71%) and 29 (25.64%) plants, respectively, while the Untreated Check experienced a 35.92% stand reduction losing an average of 63 plants (Figure 4). The seed pelleted with Tachigaren™ showed an 18.52% and 15.52% reduction for the Hillsboro and Perley sites for the 2002 season. Data sets for both years revealed a decrease in initial emergence of seedlings for treated versus untreated seed (Figures 3 & 4). A reduced emergence effect has been observed for many types of industry seed treatments, both those that do and do not involve seed pelleting.

During harvest, lesions and scars caused by *A. cochlioides* were evident on most roots. This was not observed to vary between roots of treated versus untreated replicates. Yield components, however, were seen to vary at the Hillsboro location in 2001 (Figure 5). When analyzing the 2001 yield data it was found that both the Messenger™ and bacterial treatments yielded beets that out-produced the untreated check for the Hillsboro location. The *B. cepacia* AMMDR1 treated seed produced a yield of 10.95 tons per acre with 1656.11 pounds of recoverable sugar per acre. The plots that received weekly applications of Messenger™ yielded 13.98 tons per acre and 2070.77 pounds of recoverable sugar per acre while the untreated check was only able to yield 7.81 ton per acre with a total of 1185.03 pounds of recoverable sugar (Figures 5 & 7). Similar trends could be observed in the 2002 Hillsboro trial as well. Messenger™ (applied to seed treated with Apron/Thiram™) foliar applied immediately after a herbicide micro-rate application yielded 11.46 tons per acre with 1,659.36 pounds of recoverable sugar per acre compared to the Untreated Check yielding 6.43 tons per acre and only 945.85 pounds of recoverable sugar per acre (Figures 5 & 7). It is also interesting to note that seed treated with *P. fluorescens* PRA25rifz yielded higher than the Untreated Check in both the Apron/Thiram™ and Tachigaren™ seed variables. The standard deviations expressed for the Hillsboro locations are higher than those from the Perley location due to the uneven plot terrain, causing a soil moisture gradient across the plot at this location. Thus, some of the replicates in the experiment showed significantly higher losses in the same plot than did other replicates within the same treatment.

By contrast with data from the Hillsboro location, 2001 treatments at the Perley site provided little control of *Aphanomyces* root rot (Figure 6). It is noteworthy that the Perley site did not receive any foliar treatments for 3 weeks post planting due to saturated soil conditions and continuous heavy rainfall. 2002 data collected from Perley revealed a notable increase in tons per acre and sugar per acre when pelleting seed with Tachigaren™. Messenger™ foliar applied after a micro-rate herbicide application and *P. fluorescens* PRA25rifz applied as a seed treatment yielded 2.96 and 1.12 tons per acre better than the Untreated Check, respectively (Figure 6). The Messenger™ Micro-Rate application also exhibited a significant increase in recoverable sugar per acre 917.2 pounds over the Untreated Check (Figure 8). Although stand counts still revealed a decrease in the loss of seedlings over the 45-day interval at this location, yield data did not support a significant improvement in yield with the treatments used. Furthermore, at both locations, the parameter of percent stand loss was not as significantly affected by treatments in 2002 as it was in 2001. This may have been influenced by the cool wet weather in April and May of 2002 that left plot soils saturated for several weeks. The delay resulted in the planting of the trial at a time when the higher temperatures are particularly conducive to the development of seedling diseases caused by *Aphanomyces* as well as other seedling damping-off organisms. The inclusion in 2002 of a standard seed treatment of Apron™, Thiram™, and Tachigaren™ (45g rate), illustrates the control that is typically afforded by this chemical treatment.

Summary:

Currently, producers turn to cultural control methods and tolerant varieties pelleted with Tachigaren™ to obtain acceptable to high yields with disease suppression in Aphanomyces infested soils. Tachigaren™ is the only chemical control method labeled for disease control. Unfortunately, the seasonable warm soil temperatures and wet spring conditions speed up the degradation of the product giving the plant 5 – 6 weeks protection. By using

biological treatments alone and in unison with chemical treatments, both yield and quality were increased on several of the replications, potentially resulting in a higher per acre return to the grower.

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