PROJECT PROPOSAL
SUGARBEET RESEARCH AND EDUCATION BOARD OF MINNESOTA AND NORTH DAKOTA
FY 2018 – 2019

Project Title:
SCREENING OF SUGAR BEET GERMPLASM FOR RESISTANCE TO FUSARIUM YELLOWING DECLINE

Project Number: Continuing project

Project Leader:
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Other Personnel Involved:
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Project Location: USDA-ARS, Crops Research Laboratory, Fort Collins, CO

Justification for Research: (*For new projects only)*

Fusarium yellowing decline caused by *Fusarium secorum* is a new and emerging disease in the sugar beet production region of Minnesota and North Dakota and has now been reported in Montana. While this disease is similar to the more common Fusarium yellows disease, little is known about the interaction of *F. secorum* with sugar beet, best management practices that will manage the disease, nor if any of the traditional agronomic recommendations for Fusarium yellows, will also be effective for Fusarium yellowing decline. Because fungicide treatments are limited for Fusarium yellows, current agronomic recommendations include using tolerant or resistant varieties for management. While there are some sources of resistance to Fusarium yellows in commercial varieties and USDA breeding populations it is unknown if these sources of resistance will also provide any control against the *F. secorum* pathogen. Therefore this project attempts to screen current sugar beet germplasm with known resistance(s) for Fusarium yellows with multiple isolates of *F. secorum* for their effectiveness in also managing Fusarium yellowing decline.

Summary of Literature Review: (*For new projects only)*

Fusarium spp. can lead to significant economic losses for sugar beet growers throughout the United States production region by causing reductions in yield from several associated diseases (Campbell, Fugate, and Niehaus 2011;Hanson and Hill 2004;Hanson and Jacobsen 2009;Stewart 1931) including Fusarium yellows (Stewart 1931) and Fusarium tip root (Harveson and Rush 1998;Martyn et al. 1989). In 2008, a new sugar beet disease was found in the Red River Valley of MN and ND which caused *Fusarium* yellows-like symptoms but turned out to be more aggressive than Fusarium yellows (Rivera et al. 2008). Symptoms differed from the traditional Fusarium yellows by causing discoloration of petiole vascular elements as well as seedling infection and rapid death of plants earlier in the season. Subsequent studies confirmed that the causal agent of this disease was different from any previously described *Fusarium* species and was therefore named *F. secorum* and the disease it causes as Fusarium yellowing decline (Secor et al. 2014). Fusarium yellowing decline also been reported outside of the Red River Valley production region for the first time in Montana (Arabiat et al. 2017).

F. secorum was shown to belong to the *Fusarium fujikuroi* species complex whereas Fusarium yellows is primarily caused by *Fusarium oxysporum* f. sp. *betae* (Ruppel 1991;Snyder and Hansen 1940) but can be caused by other *Fusarium* spp. including *F. acuminatum*, *F. avenaceaum*, *F. solani,* and *F. moniliforme* (Hanson and Hill 2004). Currently, the most effective management strategy for the more common Fusarium yellows is through the use of resistant cultivars and crop rotations with non-hosts (Harveson, Hanson, and Hein 2009) with several sugar beet germplasm being reported to have some resistance (Hanson et al. 2009). However it is unknown if the resistance found in sugar beet to the more common Fusarium yellows will provide any protection against the emerging Fusarium yellowing decline. Therefore this project proposes to screen multiple sugar beet germplasm for resistance against *F. secorum* which causes Fusarium yellowing decline.

Objectives:

Objective 1: Screen select USDA-ARS, Fort Collins Sugar beet breeding program sugar beet germplasm with known resistance for Fusarium yellows for resistance to Fusarium yellowing decline caused by *F. secorum.*

Year 1 (FY17-18): Screen susceptible sugar beet germplasm and lines with *F. secorum* and determine if differences in pathogen virulence and host susceptibility are prevalent in the population. **(Completed)**

Year 2 (FY18-19): Screen resistant sugar beet germplasm and lines with *F. secorum* and determine if resistance to Fusarium yellows also confers resistance to Fusarium yellowing decline. **(Proposed)**

Objective 2: Continue characterizing *F. secorum* population and evaluate phylogenetic relationship with current *F. oxysporum* f. sp. *betae* regional populations. **(Proposed)**

Materials and Methods: *(Briefly describe)*

Plant treatment(s). Fifteen sugar beet lines/germplasm will be provided by the breeding program of Dr. Leonard Panella, USDA-ARS, Fort Collins, CO. Additionally, three sugar beet germplasm (Monohikori; FC716; and USH20) will be included as Fusarium yellows susceptible controls. Additional sugar beet lines provided by commercial sugar beet seed companies will be included as requested through lifetime of project. Experiments will be performed as previously described by Secor et al. (2014). Briefly, sugar beet seed will be planted into 6.5cm black plastic

"conetainers" using pasteurized potting soil. Plants will be grown in a greenhouse with an average daytime temperature of 24°C and nighttime temperature of 18°C and a 16h photoperiod for 4 weeks. Five plants will be used for each treatment and will be performed using an augmented split block experimental design (Federer 2005). Briefly, germplasm will be randomly assigned to one of multiple "sets" of inoculations which will be based on the final number of sugar beet germplasm and *F. secorum* isolates. "Sets" will then represent the blocking for the statistical analysis for this experiment. Each inoculation "set" will then be used for four inoculation dates (experiments).

***Fusarium secorum* inoculations.** At inoculation, sugar beet plants that are at 4-5weeks after planting will be inoculated by dipping the root into a spore suspension of $1x10^5$ conidia ml^{-1} for 2-8 min without agitation (Burlakoti et al. 2012;Secor et al. 2014). Plants will be inoculated with multiple isolates of *F. secorum* including the wild type *F. secorum* (670-10; Secor et al. 2014) and which represent the diversity of the pathogen population throughout the Red River Valley. *F. oxysporum* f. sp. *betae* isolate "F19" will be used as a known positive control for Fusarium yellows and distilled water as the negative control. Treated plants will be maintained in the greenhouse and evaluated for Fusarium yellowing decline symptoms on a weekly basis for 4 weeks after inoculation. Fusarium yellowing decline symptoms will be evaluated using a modified 0-5 Fusarium yellows disease severity rating (Hanson et al. 2009). Differences in disease severity will be evaluated using an area under the disease progress curve. Statistical analyses will be conducted using SAS Proc Mixed (SAS Institute, version 9.2, Cary, NC, USA).

Phylogenetic analysis of *F. secorum/F. oxysporum* f. sp. *betae* populations. All *F. secorum* isolates will be cultured in liquid media and DNA extractions performed as previously described by Webb et al. (2012). Gene sequences for *TEF1-α* and ITS will be amplified from each isolate also as previously described (Hill et al. 2011, Webb et al. 2012). All genes will then be manually edited and phylogenetic analysis performed using Geneious software (Newark, NJ) and as described by others (Hill et al. 2011, Webb et al. 2012, and Covey et al. 2014). An individual data set will be generated for each gene (*TEF1-α* and ITS) using the sequence data from all isolates (*F. secorum* and previous *F. oxysporum* isolates).

Time Line of Anticipated Accomplishments:

It is estimated that this project will be two year project. The first year of the project has been completed and was used to evaluate 6 susceptible lines and germplasm against 11 different *F. secorum* and *F. oxysporum* f. sp. *betae* isolates. The second year of the project will entail screening 28 selected USDA-ARS germplasm and donated lines for resistant screening utilizing only virulent *F. secorum* and *F. oxysporum* f. sp. *betae* isolates. Based on findings from year 1 of the study, we would like to include a phylogenetic analysis of current *F. secorum* isolates in comparison to what we currently know about the *F. oxysporum* f. sp. *betae* population.

Progress Toward Objectives On-going Projects:

All greenhouse studies have been completed on six previously identified susceptible sugar beet germplasm and/or commercial lines. For these studies we obtained 7 suspected *F. secorum* isolates from Dr. G. Secor and included 3 *F. oxysporum* f. sp. *betae* isolates representing the diversity of this population throughout the US production region. We also included a negative control (water) for all comparisons. These studies were necessary to

determine 1) if there were differences in virulence of the *F. secorum* population and 2) if differences in host susceptibility were present and to determine which susceptible lines should be used in future studies as control(s). We found that there were differences in virulence of the different isolates tested. Generally, the *F. oxysporum* f. sp. *betae* isolates (F19 and Fob 220a) were the most virulent. There was a second group of pathogens that were moderately virulent and contained mostly *F. secorum* isolates however one isolate "Fob257c" was among this group. We also found that one *F. secorum* isolate was "weakly virulent" and would only cause disease symptoms on only a few lines. Finally one isolate was non-pathogenic in our studies and will be discarded in future resistance screening. There were differences in susceptibility of sugar beet lines, and the line determined which isolates were the most virulent on that line. We also observed that traditional symptoms of Fusarium yellowing decline (in particular presence of half leaf symptoms) appeared to be contingent on the variety tested rather than on the isolate, as many of our *F. oxysporum* f. sp. *betae* isolates also caused half leaf yellowing on certain susceptible lines. However, this is one component of the study that we would like to more fully document in future experiments.

Budget:

	USDA	SBREB
Labor (100%; Part-time 180-day employee)	$0	$11,210.00
Equipment (over $250.00)	$0	$0
Supplies	$5,000.00	$0
Travel	$1,500.00	$1,500.00
TOTAL	$6500.00	$12,710.00

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