SCREENING OF SUGAR BEET GERMPLASM FOR RESISTANCE TO FUSARIUM YELLOWING DECLINE.

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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 & Niehaus, 2011; Hanson & Hill, 2004; Hanson & Jacobsen, 2009; Stewart, 1931) including Fusarium yellows (Stewart, 1931) and Fusarium tip root (Harveson & 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).

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 & Hansen, 1940) but can be caused by other *Fusarium* spp. including *F. acuminatum*, *F. avenaceaum*, *F. solani,* and *F. moniliforme* (Hanson & 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 & 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; manuscript submitted)**

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. **(2 of 4 replications completed)**

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

Materials and Methods

***Fusarium* isolates**

Fusarium isolates used for these studies were obtained from the long-term culture collections located at either the USDA-ARS Soil Management and Sugar Beet Research Unit (SMSBRU) in Fort Collins, CO or from Dr. Gary Secor. Six *F. secorum* isolates (670-10; 742-28; 784-24-2C; 845-1-18; 938-4; 938-6; and 1090-4-2) and three *F. oxysporum* f. sp. *betae* isolates (F19; Fob220a; and Fob257c) were used for all inoculations. Working cultures of all isolates were maintained on potato dextrose agar plates (PDA; Becton, Dickinson, and Co., Sparks, MD) at room temperature until used, and transferred using established protocols (Leslie & Summerell, 2006). To validate identification of each isolate as either *F. secorum* or *F. oxysporum* f. sp. *betae*, each isolate was grown on ½ PDA and carnation leaf agar (CLA) at 25°C with continual lights for 3-4 weeks. Morphological characteristics were recorded according to the descriptions of *Fusarium* species (Leslie & Summerell, 2006).

Plant treatment(s)

Six susceptible and 32 resistant or tolerant sugar beet lines/germplasm were provided by the breeding program of Dr. Leonard Panella, USDA-ARS, Fort Collins, CO, SESVanderhave, Betaseeds, and Syngenta-Hilleshog for screening (data not shown). Two sets of experiments are being completed with the screening of a set of 6 susceptible lines being performed first, followed by screening of Fusarium yellows resistant lines and other lines provided by seed companies. For the first set of experiments, six susceptible lines (USH20; FC716; Monohikori; VDH46177; 902735; and SYN07064964) were inoculated with all *Fusarium* isolates as described below. Disease severity was rated on a 0-5 Fusarium yellows rating scale (Hanson & Hill, 2004) and an area under the disease progress (AUDPC) was used to detect significant differences in pathogen aggressiveness using SAS as previously described (Webb, Brenner & Jacobsen, 2015).

Screening of the resistant sugar beet lines is being performed using an augmented split block experimental design (Federer, 2005). Briefly, germplasm are randomly assigned to one of six "sets" of inoculations. "Sets" will then represent the blocking for the statistical analysis for this experiment. Each inoculation "set" is then being used for two-three inoculation dates (experiments or replicates). Experiments are being performed as previously described by Secor et al. (2014). Briefly, sugar beet seed are planted into 6.5cm black plastic "conetainers" using pasteurized potting soil supplemented with Osmocote 14-14-14 slow release fertilizer (Scotts, Marysville, OH). Plants are grown in a greenhouse with an average daytime temperature of 24°C and average nighttime temperature of 18°C and a 16h photoperiod for 4 weeks.

***Fusarium secorum* inoculations.** Plants are inoculated at the 2-3 leaf stage by dipping the root into a spore suspension of $1x10^4$ conidia ml^{-1} for 5 min with gentle agitation (Hanson & Hill, 2004; Hanson *et al.* 2009; Burlakoti *et al.* 2012; Secor *et al.* 2014) with 5 plants being inoculated for each isolate per variety. 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). Statistical analyses will be conducted using SAS Proc Glimmix (SAS Institute, version 9.2, Cary, NC, USA) and the best linear unbiased estimates (Blups) compared to the respective negative and positive controls.

DNA extractions and translation elongation factor PCR amplification

Fusarium isolates were grown in 50 mL potato dextrose broth (PDB; Becton, Dickinson and Co.) by inoculating with a 7 mm diameter mycelium plug taken from a fresh culture of each isolate. Liquid cultures were grown in the dark for 5-7 days at 25°C on a rotary shaker at 100 RPM. Mycelia masses were collected by pouring the filtrate through a double layer of sterile cheese cloth, rinsed with de-ionized water, and then lyophilized at -50°C for 48 h. Lyophilized tissue was ground into a fine powder using a spatula, and DNA extracted using the Invitrogen Easy-DNA extraction kit (Carlsbad, CA) utilizing the manufacturer's protocol for small amounts of plant tissues. Each isolate had 2 biological replicates for PCR amplification and DNA sequencing.

Tef1-α primers were used for PCR amplification (O'Donnell *et al.* 1998) using Thermo Scientific *Taq* polymerase (Waltham, MA) and the following PCR conditions; one cycle of 94ºC for 5 min followed by 33 cycles of 94ºC for 1 min, 55°C for 1 min, and an extension cycle of 72°C for 2 min, followed by final extension cycle of 72°C for 5 min using a Mastercyler gradient thermocycler (Eppendorf, Hamburg, Germany). PCR products were held at 4°C until they could be removed from the thermocycler. PCR amplicons were visualized on a 1.5% agarose gel and purified using the Epoch GenCatch PCR extraction kit (Missouri City, TX). Products were sequenced by Eurofins, MWG/Operon (Huntsville, AL) using primers used for *Tef1-α* amplification. *Tef1-α* gene sequences were manually edited and consensus sequences built using a pair-wise sequence alignment in Genious 6.1.8 (Newark, NJ) for each isolate. Novel gene sequences from *F. secorum* isolates amplified in this study can be obtained from GenBank under accession numbers MH926020-MH926026.

Results and Discussion

Little is known about the range of virulence within *F. secorum* nor how this relates to the overall *Fusarium* population previously described. We obtained *Tef1-α* sequence from seven isolates of *F. secorum* and added this data to a phylogenetic tree that includes *F. oxysporum* f. sp. *betae* (**Objective 2**). Unexpectedly, the *F. secorum* strains nested into a distinct clade (Clade B) that included several isolates previously designated as *F. oxysporum* f. sp. *betae*, suggesting those species designations are outdated. These results prompted an expanded phylogenetic analysis of the *Tef1-α* sequence from genome sequences of publicly-available *Fusarium* spp. This analysis further designated isolates previously reported as *F. oxysporum* f. sp. *betae* from Clade A as *F. commune*, a species that is not known to be a sugar beet pathogen. Sugar beet isolates within Clade C nested within the *Fusarium oxysporum* species complex, confirming those isolates as *F. oxysporum*. Whole genome analysis was performed on representative isolates from Clade B (670-10 and Fob257c) and Clade C (F19 and non-pathogenic isolate F29). Comparative genomics supports the identification of isolate Fob257c as *F. secorum* and the identification of Clade C isolates (F19/F29) with *F. commune*. Inoculation on susceptible sugar beet with differing genetic backgrounds demonstrate that *F. secorum* strains range in virulence from low to highly virulent depending on cultivar (**Objective 1**). This work has been submitted for publication and is currently under review (Webb et al. *submitted*).

Screening resistant lines is currently in progress. 32 lines have been provided by multiple seed companies and breeding programs and are being inoculated with all of the pathogenic isolates identified from the preliminary experiments above. Two of four replicates of screening has been completed with the additional replications currently in progress throughout 2019.

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