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). 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 for Fusarium yellows (Hanson *et al.* 2009). However, it is unknown if the resistance to Fusarium yellows found in sugar beet will provide any protection against the emerging Fusarium yellowing decline. Therefore, this project proposed 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; published Webb et al. 2019. Plant Pathology. 68:1654-1662)**

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. **(Completed; manuscript submitted)**

Objective 2: Characterize *F. secorum* population and evaluate phylogenetic relationship with current *F. oxysporum* f. sp. *betae* regional populations. **(Completed; published Webb et al. 2019. Plant Pathology. 68:1654-1662)**

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 (Table 1). 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).

Plant treatment(s). Six susceptible and 26 resistant or tolerant sugar beet lines/germplasm were provided by the breeding program of Dr. Leonard Panella, USDA-ARS, Fort Collins, CO, SESVanderhave and Betaseed for screening (Table 2). Two sets of experiments were completed with the screening the susceptible lines being performed first, followed by a second experiment to screen putative resistant lines. For all experiments, sugar beet seed were planted into 6.5cm black plastic "conetainers" filled with pasteurized potting soil. Plants were grown in a greenhouse with an average daytime temperature of 24°C and average nighttime temperature of 18°C with a 16h photoperiod for 4 weeks. For all inoculations, approximately two weeks prior to inoculation, spore suspensions were started by plating each isolate to 10 plates of half strength PDA (Becton, Dickinson, and Co., Sparks, MD) and incubating at 25°C with a 12 hr light/dark cycle. After incubation for two weeks, 5 mL sterile nanopure water was added to each plate and the surface of the agar scraped with a sterile "hockey stick" to loosen fungal hyphae and spores. The contents of all 10 plates were then poured through autoclaved double layered cheesecloth and the resulting spore suspension collected into a sterile beaker. The spore concentration was determined with a

hemocytometer and then adjusted to a final concentration of approximately 1 x 10^4 conidia per mL by adding nanopure water (100 mL total volume) (Hanson et al. 2009).

Sugar beet varieties were screened by randomly assigning each variety to one of seven "inoculation sets", most of which contained 4-5 varieties. For screening of resistant mterials, each set also always contained two varieties that were used as susceptible controls and checks for effectiveness of inoculations (Monohikari and 902735) (Tanabe et al. 1991; Webb et al. 2019). Each set was inoculated on different experimental dates with each of the eight *Fusarium* isolates (plus one mock negative control; nanopure water) at each inoculation date. Each "set" of varieties were inoculated a total of two times over two experimental dates (replicates). Therefore, up to a total of 10 plants (n=10) were inoculated for each variety by isolate combination, with some combinations having fewer plants due to differences in germination of plants and/or sporulation of the isolates at each experimental date. After inoculation disease severity was rated on a 0-5 Fusarium yellows rating scale (Hanson & Hill, 2004).

Data were analyzed using JMP Pro (SAS Institute Inc., Cary, NC). Due to the complexity of the resistant materials data set (26 varieties, 9 isolate/treatments), data were analyzed using an unsupervised hierarchical clustering using the Ward method to group varieties based on their mean score for the isolate in the panel (Ward, 1963). Each plant was classified as susceptible (score 4-5), moderate resistant/susceptible (score 2-3) and resistant (score 0-1). Isolates were considered highly virulent (score 4-5), moderately virulent (3), lowly virulent (1-2) or non-pathogenic (0) (Table 1). The average score for each variety from each inoculated plant (n=10 plants) was then calculated and this information was used to group varieties with similar patterns of response to the entire *Fusarium* isolate panel; four phenotypical "clusters" were subjectively identified with 3-12 varieties per cluster. One-way ANOVA was used to compare clusters for each isolate to identify significant pathogen by variety interactions using JMP Pro. Significant differences were identified at $p<0.05$.

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 conclusions

Little was known about the range of virulence within *F. secorum* nor how this related to the overall *Fusarium* population previously described from sugar beet. To further characterize the *F. secorum* pathogen population, we obtained *Tef1-α* sequence from seven isolates of *F. secorum* and added this data to a phylogenetic tree that included *F. oxysporum* f. sp. *betae* (Hill et al. 2011, Webb et al. 2012, Covey et al. 2014 : **Objective 2**). Unexpectedly, the *F. secorum* strains nested into a distinct clade (Clade B) that had included several isolates previously designated as *F. oxysporum* f. sp. *betae*, suggesting that those previous isolates were actually *F. secorum* and had been identified in the broader sugar beet production region prior to discovery of the pathogen (data not shown; Webb et al. 2019). These results prompted an expanded analysis of the *Tef1-α* sequence from genome sequences of publicly available *Fusarium* spp. which indicated that other isolates previously reported as *F. oxysporum* f. sp. *betae* from Clade A were actually *F. commune*, a species that was not previously known to be a sugar beet pathogen. However, isolates previously reported within Clade C could continue to be considered as part of the *Fusarium oxysporum* species complex (data not shown, Webb et al. 2019). Inoculation on susceptible sugar

beet with differing genetic backgrounds demonstrated that *F. secorum* strains ranged in virulence from low to highly virulent depending on cultivar (**Objective 1**). This work was published in the journal Plant Pathology (Webb et al. 2019).

Screening of resistant lines (experiment 2, **Objective** 1) was completed in 2020 and a manuscript reporting results has been submitted for publication. Twenty six sugar beet germplasm and commercial hybrids were screened for resistance against the same panel of *F. secorum* isolates from the first experiment. Based on their disease response, these 26 sugar beet varieties could be grouped into four general susceptibility/resistant "clusters" ranging from highly susceptible to highly resistant. Four varieties were resistant to all *F. secorum* isolates, likewise three varieties were susceptible to all isolates (Table 3). However, the other lines appeared to have variable tolerance levels depending on the isolate with some lines being moderately susceptible and other lines moderately resistant. Results from these experiments have been submitted for publication in the Journal of Sugar Beet Research (Webb et al. *submitted*)

Table 1. Panel of *Fusarium* isolates used for screening of sugar beet germplasm and lines.

Isolate name	Original Identified Species[†]	Current Species Designation[‡]	Virulence[‡]	Donor[§]	Year collected	Location collected
F19	*F. oxysporum*	*F. commune*	HV	L. Hanson	2001	Salem, OR
670-10	*F. secorum*	*F. secorum*	HV	G. Secor	2005	Sabin, MN
845-1-18	*F. secorum*	*F. secorum*	MV	G. Secor	2010	Foxhome, MN
784-24-2C	*F. secorum*	*F. secorum*	HV	G. Secor	2007	Sabin, MN
Fob220a	*F. oxysporum*	*F. secorum*	HV	H. Schwartz	1998	Iliff, CO
Fob257c	*F. oxysporum*	*F. secorum*	MV	H. Schwartz	1998	Brush, CO
938-4	*F. secorum*	*F. secorum*	MV	G. Secor	2010	Moorhead, MN
742-28	*F. secorum*	*F. secorum*	LV	G. Secor	2006	Sabin, MN

[†]Original identified *Fusarium* species as provided by donor of isolates.
[‡]Current *Fusarium* species designation and virulence to sugar beet as reported by Webb et al. 2019. *Plant Pathology.* 68: 1654-1162. HV=Highly virulent, MV=Moderately virulent, LV=Lowly virulent.
[§]Institution of each donor: G. Secor, Dept. Plant Pathology, North Dakota State University, Fargo, ND; L. Hanson, USDA-ARS, Sugarbeet and Bean Research Unit, East Lansing, MI; H. Schwartz, formerly with Dept. of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO.

Table 2. List of sugar beet germplasm and/or commercial hybrids received for resistance screening to *Fusarium secorum.*

Variety	Provider[†]	Citation (if available)
Monohikari	L. Panella	Tanabe et al. 1991. Sugarbeet [*Beta vulgaris*] cultivar "Monohikari", its development and characteristics. Research Bulletin of the Hokkaido National Agricultural Experiment Station 155:1-47.
902735	SesVanderhave	
FC708	L. Panella	Hecker and Ruppel. 1981. Registration of FC 708 and FC 708 CMS sugarbeet germplasm. Crop Sci. 21:802.
20101008	L. Panella	Panella et al. 2013. Registration of FC1028, FC1037, FC1038, and FC1036 multigerm sugarbeet germplasm with multiple disease resistances. J. Plant Reg. 7:1-9.
20111031	L. Panella	Panella et al. 2013. Registration of FC1028, FC1037, FC1038, and FC1036 multigerm sugarbeet germplasm with multiple disease resistances. J. Plant Reg. 7:1-9.
20131011	L. Panella	
FC221	L. Panella	Panella et al. 2008. Breeding for multiple disease resistance in sugarbeet: registration of FC220 and FC221. J. Plant Reg. 2:146-155.
FC1740	L. Panella	Panella et al. 2018. Registration of FC1740 and FC1741 multigerm, Rhizomania-resistant sugar beet germplasm with resistance to multiple diseases. J. Plant Reg. 12:257-263.
20131010 H14	L. Panella	
20131010 H15	L. Panella	
FC201	L. Panella	Panella and Lewellen. 2005. Registration of FC201, a heterogeneous, disease-resistant, monogerm, O-type sugarbeet population. Crop Sci. 45:1169-1170.
20141022 PF	L. Panella	
20151038 PF	L. Panella	
7927-4-309	L. Panella	

5927-4-308	L. Panella
SV-Hybrid FR1+2	SesVanderhave
SV-Hybrid CR3	SesVanderhave
SV-Hybrid B-R1	SesVanderhave
SV-Hybrid A-S	SesVanderhave
TOL 1	KWS Seeds, LLC
TOL 2	KWS Seeds, LLC
TOL 3	KWS Seeds, LLC
MOD 1	KWS Seeds, LLC
MOD 2	KWS Seeds, LLC
MOD 3	KWS Seeds, LLC
SUSC 1	KWS Seeds, LLC
SUSC 2	KWS Seeds, LLC
SUSC 3	KWS Seeds, LLC

[†]Institution of each seed donor: L. Panella, formerly with USDA-ARS, 1701 Centre Ave. Fort Collins, CO; SesVanderhave, 5908 52nd Ave. South, Fargo, ND; KWS Seeds, LLC,5705 W. Old Shakopee Road, Suite 110, Bloomington, MN.

Table 3. Percentage of sugar beet plants that displayed each respective resistance phenotype against the panel of *Fusarium* isolates (one *F. commune* and seven *F. secorum*).

Variety	Susceptible†	Moderate Resistant†	Resistant†	Cluster assignment‡
Susc 2	89.86%	0.00%	10.14%	1
SV Hybrid A-S	88.16%	0.00%	11.84%	1
Susc 3	89.61%	0.00%	10.39%	1
5927-4-308	39.39%	36.36%	24.24%	2
20131011	30.65%	54.84%	14.52%	2
FC221	62.50%	26.56%	10.94%	2
Mod 1	60.32%	26.98%	12.70%	2
FC708	38.89%	48.15%	12.96%	2
Tol 2	66.67%	22.22%	11.11%	2
20131010 H15	35.09%	45.61%	19.30%	2
FC201	52.31%	21.54%	26.15%	2
20101008	8.70%	73.91%	17.39%	2
20151038 PF	22.06%	52.94%	25.00%	2
FC1740	19.18%	43.84%	36.99%	2
Susc 1	35.62%	52.05%	12.33%	3
20111031	0.00%	80.95%	19.05%	3
20141022 PF	13.33%	64.44%	22.22%	3
Mod 2	5.00%	66.67%	28.33%	3
SV Hybrid B-R1	9.09%	43.94%	46.97%	3
SV Hybrid CR3	29.41%	0.00%	70.59%	3
7927-4-309	0.00%	30.14%	69.86%	4
Tol 1	0.00%	0.00%	100.00%	4
20131010 H14	0.00%	50.00%	50.00%	4
Mod 3	11.90%	33.33%	54.76%	4
SV Hybrid FR1+2	10.98%	0.00%	89.02%	4
Tol 3	11.11%	0.00%	88.89%	4

†Percentage of plants (out of 90 plants total; all isolates tested) that had a susceptible (score 4-5), moderate resistant (score 2-3) or resistant (score 0-1) phenotype.

‡Phenotype cluster assignment was based on a multivariate analysis using Jmp Pro which assigned each germplasm/line with a similar response into phenotypic clusters.