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, 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).

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.* (in progress)

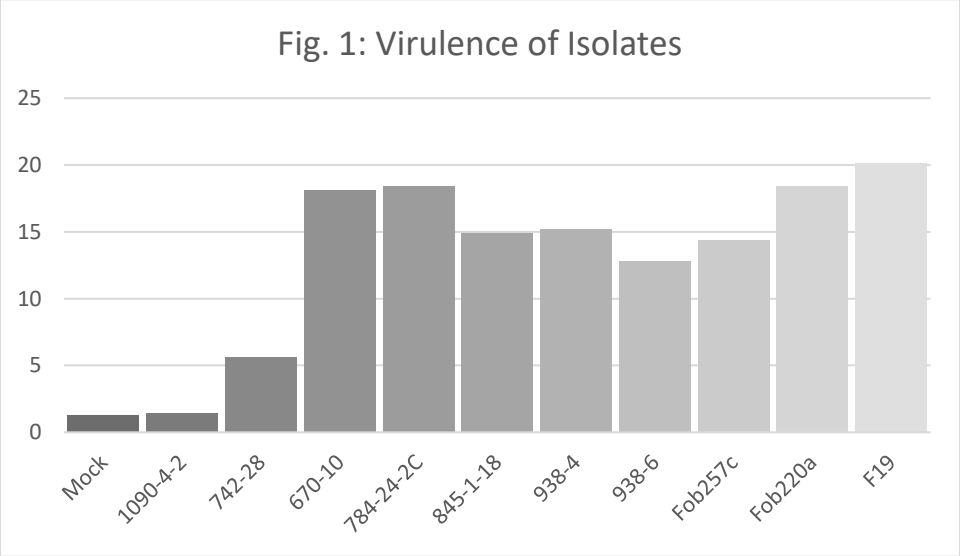
Materials and Methods

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 supplemented with Osmocote 14-14-14 slow release fertilizer (Scotts, Marysville, OH). Plants will be 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. Ten 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 two inoculation dates (experiments). At each inoculation date, two replicates will be performed where each isolate is inoculated to a block of five sugar beet plants per germplasm and replicate two times. 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.

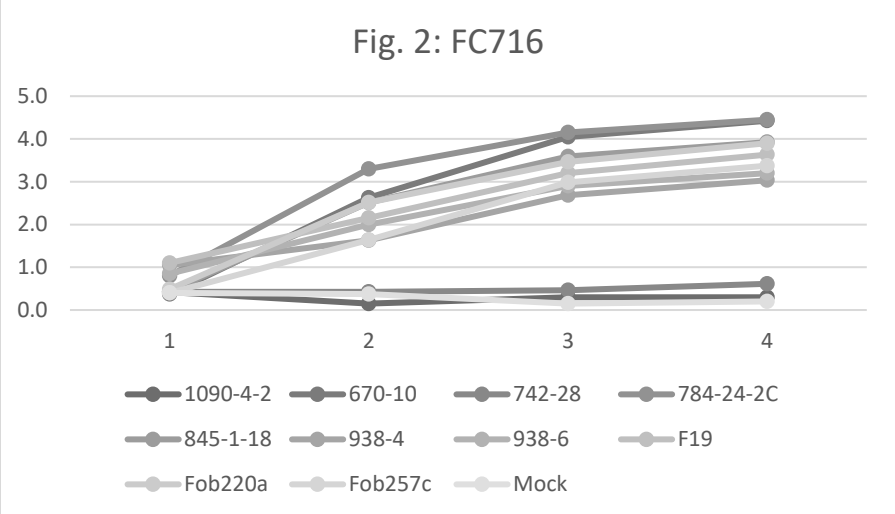
***Fusarium secorum* inoculations.** At inoculation, sugar beet plants that are at 4weeks after planting will be inoculated by dipping the root into a spore suspension of $1x10^5$ conidia ml^{-1} for 2-8 min without agitation (Burlakoti et al. 2012;Secor et al. 2014). Plants will be inoculated with multiple 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

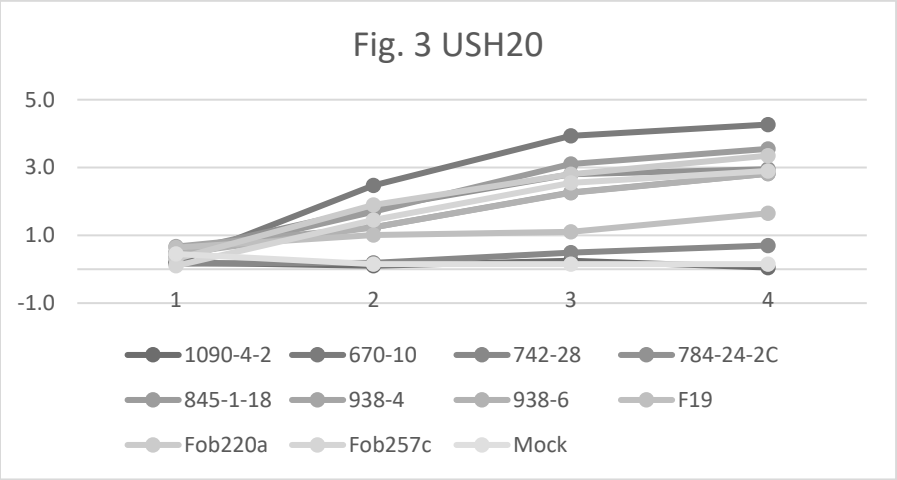
yellowing decline symptoms will be evaluated using a modified 0-5 Fusarium yellows disease severity rating (Hanson et al. 2009).

Results and Discussion. We were interested in two primary questions 1) Is there a variation in virulence of the *F. secorum* population and 2) Do sugar beet lines and/or cultivars differ in severity of Fusarium yellowing decline. In preliminary experiments, we determined that the *F. secorum* varies in virulence to sugar beet and that this is influenced by variety (Fig. 1). *F. oxysporum* isolates F19 and Fob220a and *F. secorum* isolates 670-10 and 784-24-2C are highly virulent, and mostly end up completely killing the sugar beet plants after 4weeks. *F. secorum* isolates 845-1-18, 938-4, and 938-6 and *F. oxysporum* Fob257c are moderately virulent. One *F. secorum* isolate 742-28, is weakly virulent only causing minor symptoms and is dependent on the cultivar in question. One isolate, 1090-4-2 was non-pathogenic in our studies and will not be included in future screening of potential resistant varieties.



Preliminary results have also indicated that sugar beet cultivars did react differently to the *F. secorum* isolates with some lines having more severe disease symptoms than others. In general, the most susceptible cultivar in these tests was VDH46177 and the least susceptible variety was USH20. Symptoms associated with *F. secorum* such as the half leaf yellowing also appeared to be associated with the cultivar being tested rather than based on isolate however, this trait was not specifically recorded. In future experiments we will record these observations for each isolate by genotype interaction. In general, each cultivar reacted differently to the isolates inoculated. For example, on sugar beet line FC716 all isolates tested caused generally the same amount of disease (Fig. 2) whereas on other lines such as USH20, a clear difference in the susceptibility to some isolates was observed (Fig. 3).





In conclusion, it appears that the *F. secorum* population varies in virulence to sugar beet but that this is similar to the variation that we see for Fusarium yellows caused by *F. oxysporum*. Likewise, there are differences in susceptibility of sugar beet and therefore it is important to screen for both *F. secorum* and *F. oxysporum* populations for each sugar beet production region. Testing for resistance to *F. secorum* was proposed for FY18-19 and findings will be reported in the future.