

A RAPID SEEDLING ASSAY FOR EVALUATING SUGAR BEET FUSARIUM TOLERANCE.

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1. INTRODUCTION: Reductions in yield and quality associated with diseases caused by *Fusarium* spp. impart significant economic losses for sugar beet growers (Hanson and Hill, 2004, Hanson, 2009, Harveson, 2009). *Fusarium* yellows and *Fusarium* yellowing decline, caused by *Fusarium oxysporum* f. sp. *Betae* and *Fusarium secorum*, are responsible for sugar beet root and foliar diseases (Hanson & Jacobsen 2009; Secor et al. 2014). These diseases were recently reported in North Dakota and Minnesota and has since become a problem for growers in the region (Burlakoti et al. 2012; Windels et al. 2005, Secor et al. 2014).

American Crystal Sugar Company (ACSC) initiated a *Fusarium* testing nursery concept in 2005. The field chosen by ACSC for future work had a strip trial that identified significantly susceptible genetics at the Nelson farm near Moorhead, MN. ACSC allocated that field to conduct Official Variety Trial (OVT) *Fusarium* ratings and offered sugarbeet seed companies to screen germplasm for genetic tolerance.



2. OBJECTIVE. The overall objective was to develop a screening procedure to identify genetic tolerance to *Fusarium* species using a pleated paper assay, and compare the results to field nursery trials.

3. METHODS AND MATERIALS. *F. oxysporum* and *F. secorum* collected from sugar beets in the ND and MN were isolated and used as inoculum for the pleated paper bioassay. *Fusarium* tolerant and susceptible varieties identified in the ACSC OVT field nurseries. Both species were grown on PDA medium and inoculum of 1,000, 5,000, 10,000, 15,000 and 20,000 sp/ml were prepared. A susceptible and tolerant variety were planted at each inoculum level and the germination boxes were oriented vertically to promote normal radicles and hypocotyls to develop normally.



Each crease of the pleated paper had a seed planted at two cm from the top and the inoculum (25 microliters) was applied four cm below the seed on moistened pleated paper (30 ml of de-ionized water per pleat). Radicle emergence began within 48-72 hours progressing into the inoculation zone.



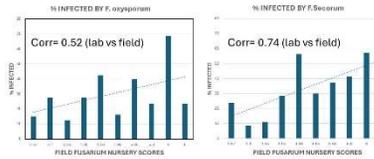
The various inoculum species and concentrations were prepared and tested at germination temperatures of 20C, 25C and alternating 20-30C to assess optimum conditions for seedling infection

Seed samples of eight different varieties with significant differences in *Fusarium* field ratings in Official Variety Trials (OVT) conducted on the Nelson farm near Moorhead MN by ACSC were used for bioassay testing. Seeds of each variety were de-pelleted, randomized and planted in 10 seed replicates at three treatments: 1- None inoculated control, 2- Inoculated with *F. oxysporum* at 10,000 spores/ml and 3- Inoculated with *F. secorum* at 10,000 spores/ml.

Two planting dates with 4 replicates were conducted. Germination boxes were placed in germinator at 25°C with eight hours of light per day. Developed seedlings between the pleats were observed at day seven and each seedling was rated on 1-5 scale where 1 was healthy and 5 was dead.

4. RESULTS: Assay results indicated that significant differences existed between tolerant and susceptible genetics for both species, all inoculum levels and temperatures evaluated. The protocol providing the most

consistent discrimination of seedling infection between the tolerant and susceptible variety was identified at 10,000 spores per ml at 25C.



The least tolerant genetics from the field nursery had the highest percentage of *Fusarium* infected seedlings in the bioassay. The *F. secorum* species of *Fusarium* provided a better correlation to the field scores determined at the ACSC field nursery. Correlation between sick plants in bioassay test and field were 0.52 for *F. oxysporum* and 0.74 for *F. secorum*.

+SEEDLING INFECTION PHENOTYPE: Significant differences existed in the type of infection that occurred. *F. oxysporum* inoculum caused cotyledon infection on approx. 38% of all seedlings evaluated, whereas the *F. secorum* only resulted in cotyledon infection in approx. 14% of the seedlings evaluated. Radicle infection was commonly observed and the restricted hypocotyl elongation was common in the most susceptible germplasm but was observed less frequently in the known tolerant germplasm.



5. DISCUSSION AND FUTURE DIRECTION: Seed testing and rating germination tests are often influenced by diseases typically found in the pericarp and embryo of the germinating seedling. The best temperature for this bioassay was determined to be 25C, however, that temperature is beneficial for many types of seedling pathogens that may interfere with accurate *Fusarium* infection ratings. AOSA rules for germinating seed is 20c which probably serves to minimize pathogen influence on germinating seedlings. It may be beneficial to start test at 20C to minimize early contamination then transfer to 25C at day 3 to allow the *Fusarium* to infect and minimize other external pathogens.

Differences between *F. oxy* and *F. sec* were observed. This would indicate that it is important to understand local inoculum in field trials. This also means that *Fusarium* tolerance may need to be further subset to indicate which species the tolerance has been tested against.

This seven-day test to screen USDA germplasm accessions can be very helpful for quickly identifying genetic tolerance to *Fusarium*. One future objective is to screen wild germplasm (*Beta maritima*) for possible new sources of *Fusarium* tolerance which can be helpful in future variety development.

6. ACKNOWLEDGEMENTS: Thanks to ND State Seed department for germinators and lab space, the seed companies: American Crystal Sugar Company, Hillehog, and SESVanderHave for permitting their varieties to be utilized to compare lab bioassay results with field nurseries impacted by *Fusarium*.

7. REFERENCES:

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Cytiva's Whatman™ 10345576 Grade 3236 Seed Germination Test Paper Pleated Strips. Test Paper Size: 110 x 20mm, Paper Color: Grey, Thickness: 0.22mm, Weight: 10(g/m2)

FUSARIUM MEAN SEEDLING SCORE @25C

