7 May 2020. Sukhwinder Kaur & Lynn Epstein

***F. oxysporum* f. sp. *apii* soil infestation for screening *Apium* lines**

**Inoculum preparation:**

Pearl millet grains were obtained from Win Co food store. Grains were washed thoroughly 4-5 times in warm tap water and were soaked overnight in tap water. All the water was drained and approx. 1/3 (up to 100.0 ml marking on the 500 ml capacity flask) was filled with grain. Sponge plugs were used to close the flasks. Plugs were covered with aluminum foil and & grain was autoclaved for 30 min. (Cycle #1)

5-7 FOA culture plugs (5 mm diam) growing on PDA (6-7 days old) or FOA cultures stored on dried filter papers (3-5 mm size and 5-7 in number) were transferred to the flask aseptically. The flasks were incubated at RT for 8-10 days under lights. Flasks were shaken every other day to distribute the inoculum uniformly. On the day of inoculation, twice steam-sterilized perlite and GH soil mix (3:1) and inoculum was thoroughly mixed by hand in 1:15 ratio of inoculum to soil mix. Two month (after germination) old Apium lines were planted in FOA infested soil. 3/4 of planting tube was filled with clean soil at the bottom and on top 1/4 of planting tube was filled with infested soil with inoculum mixed as above except in healthy controls in which case only sterilized greenhouse soil (perlite:GH3:1) was used.

**NO millet seeds were added to unincoulated controls.**

**FROM BMC genomics-2020 MS draft**

To produce *F. oxysporum* inoculum for soil infestation, millet seeds were hydrated overnight. One hundred cc of drained seeds per 500 ml flask were autoclaved, and then infested with either plugs from one-week-old cultures grown on potato dextrose agar (PDA) or dried conidia that had been stored on filter paper. Cultures were incubated under approx. 5000 lux cool-white fluorescent lights at 22 °C for 8 to 10 days; cultures were shaken vigorously every other day for more uniform colonization of the substrate.

For uninfested planting media in the greenhouse, we mixed steam-sterilized University of California Davis greenhouse soil (GHS) as a 3:1 (v/v) mix of perlite:GHS. The perlite:GHS mix was placed 6.4 cm diam planting tubes. For celery in infested soil, the bottom ¾ of tube was filled with the perlite:GHS mix and the upper ¼ was filled with a thoroughly mixed preparation of a 1:15 (v/v) ratio of inoculum to the perlite-GHS mix. Uninfested controls had neither inoculum nor millet seeds.

Two-month-old celery was transplanted into the infested soil. All plants were maintained in a greenhouse that was maintained between 27 and 29 °C. Foliar symptoms were recorded weekly.

Harvested plants were washed and scored for typical vascular discoloration on a 0 to 5 severity scale: 0, asymptomatic: 1, some discoloration in the lateral root vasculature; 2, some discoloration in the main root vasculature; 3, some discoloration in the crown vasculature; 4, extensive discoloration of the crown vasculature; and 5, plant dead. Based on the mock-inoculated controls, isolates with a mean of $\leq$1.0 were rated as nonpathogenic.

To confirm that symptoms were caused by pathogens, Koch's postulates were completed on the isolates