OPTIMIZING AMENDMENT C:N RATIO FOR *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* SUPPRESSION UNDER ANAEROBIC SOIL DISINFESTATION

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Summary
Anaerobic soil disinfestation (ASD) is a pre-plant soil treatment developed for control of soilborne pathogens in high-value specialty crop production. Treatment by ASD relies on the incorporation of organic amendments to provide labile carbon (C) to stimulate microbial activity in saturated soil mulched with polyethylene. Pathogen control is due to changes in chemical, physical and biological soil properties occurring due to temporal anaerobic conditions in ASD-treated soils. To date, ASD has been evaluated in diverse cropping systems with a range of C-source amendments and amendment rates, to evaluate impacts on various pathogens and nematodes, but results have been variable. More work is needed to evaluate factors which influence the effectiveness of ASD treatment in order to adapt the procedure for diverse cropping systems and environments.

Amendment C:N ratio is widely reported to influence soil microbial activity, suggesting that the microbially-driven ASD process is likely influenced by this amendment property. Amendment C:N ratio also influences post-treatment nutrient availability for crops, ultimately impacting yield of crops following ASD treatments. To evaluate amendment C:N ratio for ASD treatment, we established a randomized complete block field study with four replications at the Plateau Research and Education Center in Crossville, TN in May 2014. Dry molasses was used as the primary C-source amendment at four C:N ratios (10:1, 20:1, 30:1 and 40:1) at a total C rate of 4 mg C g^{-1} soil. The C:N ratio was adjusted using soybean meal or corn starch. In addition, a C:N ratio of 30:1 at a lower C rate of 2 mg C g^{-1} soil, an untreated control, and a MeBr-fumigated control were included. The soil type is a Lily series (Fine-loamy, siliceous, semiactive, mesic Typic Hapludult). Amendments were applied in each plot using a drop fertilizer spreader and were thoroughly incorporated with a rotovator. Raised beds (~5 cm) were formed, mulched with standard black polyethylene and drip irrigated to fill pore space. Anaerobic soil conditions were monitored using iron oxihydroxide-coated tubes, which were installed after applying plastic mulch and before irrigation (Castenson and Rabenhorst, 2006).

Four inoculum packets containing propagules of *Fusarium oxysporum* (*Fo*) were buried at a 10 to 15 cm depth in each bed. *Fo* packets (5 cm × 5 cm) were made with apertured Delnet® polyolefin fabric (DelStar Technologies, Austin, TX) and contained 2 g of dehydrated rice grain inoculum (*F. oxysporum* f. sp. *lyscopersici* isolated from tomato

plants at the UT Organic Crops Unit, Knoxville, TN). Due to logistical constraints, *Fo* inoculum packets were not introduced into the MeBr-fumigated plots. At the end of the ASD treatment (3 wk), inoculum packets were retrieved and *Fo* propagule survival was assessed by dilution plating of recovered inoculum on Nash-Snyder agar medium (Nash and Snyder, 1962) using the spread plate method. After 14 days, *Fo* colony identification was confirmed under a compound microscope at 100× magnification by examining morphological features, and then counted and log transformed [$\log_{10}$[CFU+1] g^{-1} soil] prior to statistical analyses. Soil samples (10 composite cores to 15-cm depth) from each plot were collected 1) before applying amendments (preASD), 2) at ASD termination (endASD), and 3) 3 wk following ASD treatment termination (postASD). Soil samples were analyzed similarly to *Fo* packet inoculum. Mixed model analysis of variance was conducted with SAS (9.3 SAS Institute, Cary, NC), data were checked for normality and homogeneity of variances, and transformed as needed. Least squares means were compared with Fisher's P-LSD at the 5% significance level and untransformed (except for *Fo* populations) means are reported.

There was a significant treatment effect on percentage of oxihydroxide paint removal, indicating enhanced anaerobic conditions driven by soil microbial activity in amended plots compared to the unamended (untreated control) treatment ($p < 0.001$). At 4 mg C g^{-1} of soil, all four amendment C:N ratios created similar anaerobic conditions, but with the reduction in C rate (to 2 mg C g^{-1} soil) anaerobic conditions were reduced (Fig. 1). Across all treatments, there was no interaction and no significant differences for *Fusarium* counts from soil samples. Endemic *Fusarium* populations from soil include a mixture of pathogens, saprophytes, and beneficial endophytes, and Nash-Snyder agar is not specifically selective for *F. oxysporum*. From inoculum packets, the lowest *Fo* survival was observed in amended plots ($p < 0.001$) with the highest suppression of *Fo* inoculum at C:N ratios of 20:1 and 30:1(3.2 CFU; Fig. 2).

Our results suggest that the application of C rates at 4 mg C g^{-1} soil for ASD treatment induces more anaerobic soil conditions and greater mortality of Fo inoculum compared to 2 mg C g^{-1} soil, at a C:N ratio of 30:1. Analysis of marketable yield and impacts on inoculum of *Sclerotium rolfsii* are ongoing.

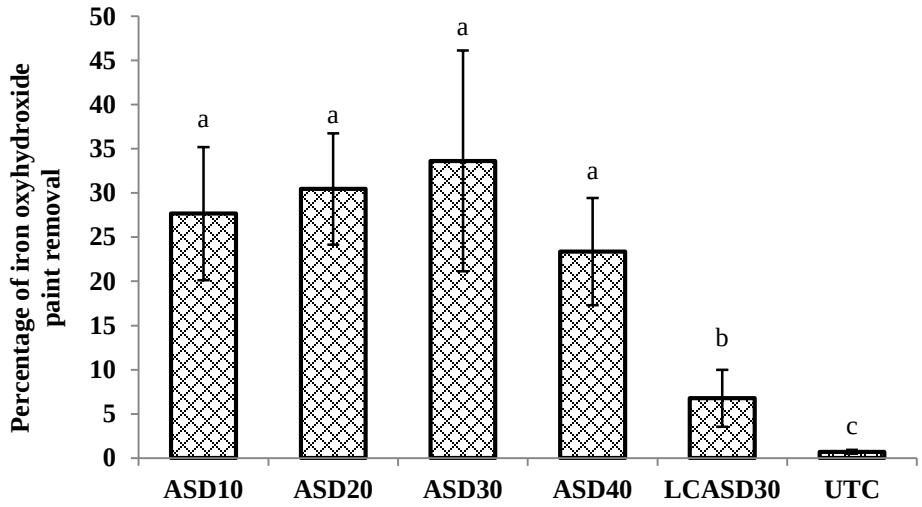

Figure 1. Percentage of iron oxihydroxide removed during ASD treatment. Bars indicated by the same letters are not significantly different ($p > 0.05$). ASD10 = C:N ratio 10:1, ASD20 = C:N ratio 20:1, ASD30 = C:N ratio 30:1, and ASD40 = C:N ratio 40:1, all at C rates of 4 mg C g^{-1} soil; LCASD30 = low carbon rate of 2 mg C g^{-1} soil and 30:1 C:N ratio; UTC = untreated (unamended) control. Error bars represent standard error of the mean.

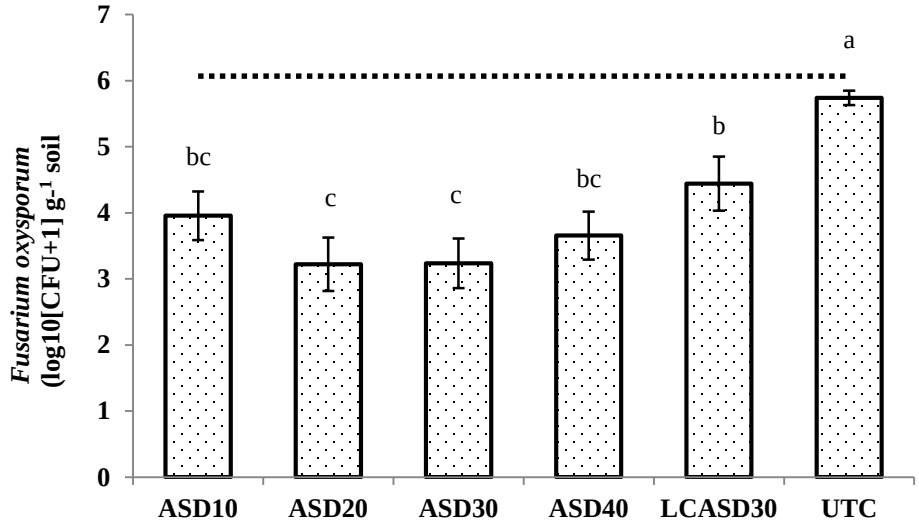

Figure 2. Effect of amendment C:N ratios on mean *Fo* populations during ASD treatment, 2014. Bars indicated by the same letters are not significantly different ($p > 0.05$). Dashed line in the plot represent *Fo* populations (6.07 log10 [CFU+1]g^{-1} soil) from packets not buried in the field. ASD10 = C:N ratio 10:1, ASD20 = C:N ratio 20:1, ASD30 = C:N ratio 30:1, and ASD40 = C:N ratio 40:1, all at C rates of 4 mg C g^{-1} soil; LCASD30 = low carbon rate of 2 mg C g^{-1} soil and 30:1 C:N ratio; UTC = untreated (unamended) control. Error bars represent standard error of the mean.