OPTIMIZATION OF AMENDMENT C:N RATIO IN ANAEROBIC SOIL DISINFESTATION FOR CONTROL OF *SCLEROTIUM ROLFSII*

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Chemical alternatives to methyl bromide (MeBr) have been registered to aid commercial farmers in controlling soil borne diseases and weeds; however, these alternatives lag behind MeBr regarding their effectiveness to control pests and suitability for diverse production systems. In addition, these alternatives also face regulatory restrictions due to geographic constraints, safety concerns, and accumulation of phytotoxic materials. In this scenario, anaerobic soil disinfestation (ASD; synonymous with biological soil disinfestation) appears to be a potential non-chemical alternative to soil fumigation. ASD treatment uses labile carbon (C) sources from organic amendments (OAs), to create an anaerobic condition through increased microbial activity in moist, polyethylene-covered soils. The antagonistic property is developed in soil by indigenous anaerobic microorganisms with the production of volatile fatty acids or other compounds which act against plant diseases and pests. Various OAs as a C source have been examined for use in ASD including grasses, broccoli residues, wheat bran, rice bran, molasses, potato haulms, ethanol, and cover crops. In addition to determining a suitable OA and rate, identifying a suitable C:N ratio of the OA could play a critical role in determining microbial population structure, decomposition rates, plant growth, and collectively, the effectiveness of ASD. In the present study, molasses and wheat bran were selected as two OAs and the effectiveness of different C:N ratios of these two primary OAs to control *Sclerotium rolfsii* during ASD treatment was evaluated.

A pot study was carried out in a growth chamber (15 to 25°C) in spring 2013. Two agricultural by-products 1) dried molasses (DM) and 2) wheat bran (WB) as C amendments were combined with either soybean meal or corn starch to create four C:N ratios (10:1, 20:1, 30:1 and 40:1) in a completely randomized factorial design with four replications. An unamended, untreated control was included for comparison. Pots were amended with C sources at a rate of 4 mg C g^{-1} soil, watered to saturation, allowed to drain, and covered tightly with black polyethylene mulch. Soil redox potential was recorded during ASD treatment to calculate a measure of cumulative anaerobic soil conditions. Likewise, a field study was established in summer 2013 to evaluate the effectiveness of molasses C amendment for ASD treatment in Crossville, TN. Treatments included four C:N ratios (10:1, 20:1, 30:1 and 40:1) at a C rate of 4 mg C g^{-1} soil, a C:N ratio of 30:1 at a C rate of 2 mg C g^{-1} soil, an untreated control, and a MeBr-fumigated control. Treatments were arranged in a randomized, complete block design with four replications. Amendments were incorporated into soil, raised beds formed and mulched with polyethylene, then drip irrigated to fill pore space. Iron oxihydroxide-coated tubes were installed at the time of treatment to monitor anaerobic soil conditions (Castenson and Rabenhorst, 2006). Four mg C g^{-1} soil from C source mixtures was maintained for both the pot and field studies as suggested by Butler et al., 2012. For both studies, two nylon mesh bags containing 10 sclerotia of *S. rolfsii* each were buried in each pot (15- and 5-cm depths) and field plot (15-cm depth). At the end of the experiment, bags were retrieved and sclerotia assessed for survival. Data recorded included germination of sclerotia, and parasitism by *Trichoderma* spp. and other fungi. Due to logistical constraints, sclerotia of *S. rolfsii* were not introduced into the MeBr-treated plots, which were used for additional pathogen and crop yield data evaluations not presented here. Mixed model analysis of variance was conducted with SAS, and least squares means compared with Fisher's PLSD at the 5% significance level.

In the pot study, there were no significant relationships between C amendments (DM or WB; $p > 0.05$) for germination of introduced sclerotia. However, there was a significant difference in sclerotial germination between C-amended and untreated control pots ($p < 0.001$). While there were no differences in sclerotial germination among different C:N ratio treatments, sclerotial germination from all C-amended treatments (4.5 to 8.3%) were significantly lower that the control (21.7%; Fig. 1). Likewise, C-amendment had a significant positive impact on *Trichoderma* spp. colonization of sclerotia, with higher parasitism in all C:N ratio treatments (93 to 94%) compared to the control (80.8%; Fig. 1). Germination of sclerotia was negatively correlated ($p < 0.01$, r=0.43) with accumulation of anaerobic soil conditions. Cumulative soil anaerobic conditions differed among C:N ratio treatments ($p < 0.001$), with the highest cumulative soil anaerobic condition observed in C:N ratio of 10:1 and the lowest observed in the untreated control (Fig. 2A). In the field study, iron oxihydroxide tubes indicated enhanced anaerobic conditions in C-amended plots at 4 mg C g^{-1} soil (regardless of C:N ratio) compared to the untreated control (Fig. 2B). However, no significant relationship between germination of sclerotia and iron reduction (an indicator of anaerobic conditions) was observed. The germination of sclerotia was relatively low among C amended treatments (1.3 to 11.8%) compared to the untreated control (27.2%), but this difference was not significant ($p = 0.1$). Although results showed higher numbers of the sclerotia parasitized by *Trichoderma* spp., *Fusarium* spp. and other fungi, the result was not significant ($p = 0.1$).

Our preliminary results suggest that the application of C amendments for ASD at 4 mg C g^{-1} soil induces anaerobic soil conditions facilitating suppression of introduced sclerotia of *S. rolfsii*, partly due to parasitism by beneficial fungi including *Trichoderma* spp. However, further research on soil incorporation of sclerotia (without mesh bags) will help to enhance understanding of C:N ratio of ASD carbon amendments on plant disease.

Butler, D.M., S.E. Eichler Inwood, D.G. McCarty, C.E. Sams, A.L. Wszelaki, M.E. Dee, and B.H. Ownley. 2012. Optimizing Anaerobic Soil Disinfestation for Tennessee. *In* 2012 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions, San Diego, California, USA. p. 13.11-13.14.

Castenson, K.L., and M.C. Rabenhorst. 2006. Indicator of reduction in soil (IRIS): Evaluation of a new approach for assessing reduced conditions in soil. *Soil Sci. Soc. Am. J.* 70:1222–1226.

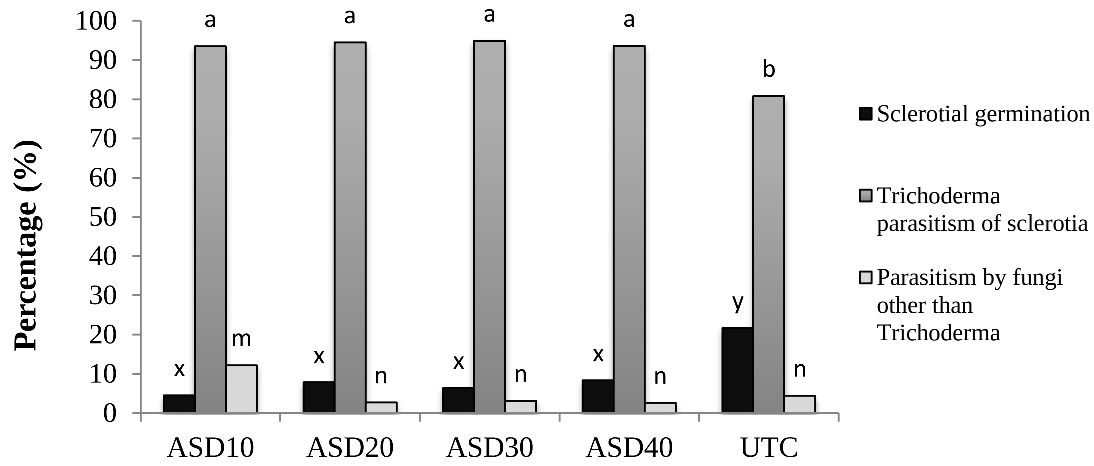

Figure 1. Effect of amendment C:N ratio on mean germination of sclerotia of *S. rolfsii*, percentage sclerotia parasitized by *Trichoderma* spp. and other fungi during ASD treatment in pots. Within category, bars indicated by the same letters are not significantly different ($p > 0.05$). ASD10= C:N ratio 10:1, ASD20= C:N ratio 20:1, ASD30= C:N ratio 30:1, and ASD40= C:N ratio 40:1, UTC=untreated control.

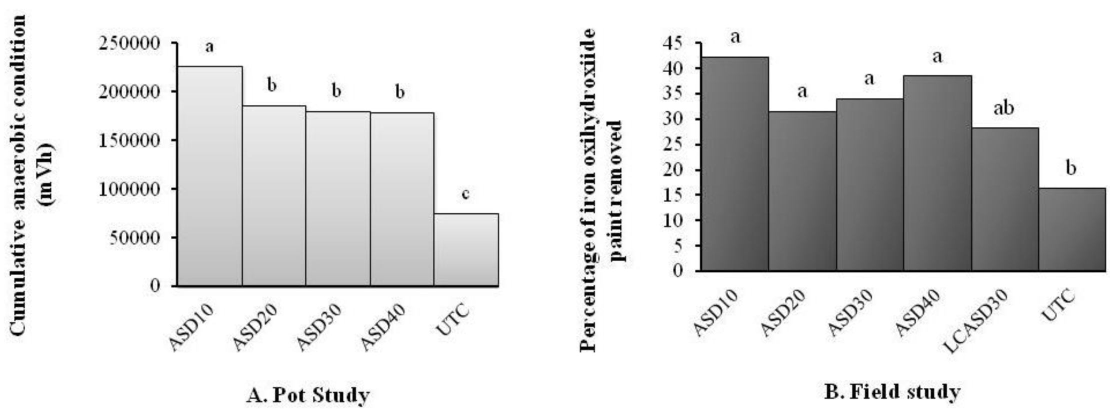

Figure 2. A. Effect of amendment C:N ratio on mean cumulative soil anaerobic conditions (mVh) in the pot study. B. Percentage of iron oxihydroxide removed during ASD treatment in the field study. Bars indicated by the same letters are not significantly different ($p > 0.05$). ASD10= C:N ratio 10:1, ASD20= C:N ratio 20:1, ASD30= C:N ratio 30:1, and ASD40= C:N ratio 40:1, LCASD30=Low carbon 2mg C/g at 30:1 C:N ratio, UTC= untreated control.