APPLICATION SEQUENCE AND SOIL BIOLOGY INFLUENCE ANAEROBIC SOIL DISINFESTATION INDUCED DISEASE SUPPRESSION

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Anaerobic soil disinfestation (ASD) and mustard seed meal (MSM) soil amendments can yield significant control of a diversity of soil-borne pests and pathogens. The mechanisms functional in disease suppression are diverse and with regard to MSM amendment, soil biology has been shown to have a significant or even primary role in the resulting disease control (Cohen et al., 2005; Mazzola et al., 2007). Soil biology is thought to contribute to disease control obtained in response to ASD treatments through the generation of anaerobic by-products (e.g. acetic acid, butyric acid, etc.) that are toxic to pathogens (Katase et al., 2009). As such, when applied collectively in an integrated approach these individual treatments have capacity to enhance or diminish the functional disease control mechanisms that operate in response to the independent methods. Studies were undertaken to assess the effect of application sequence on growth performance of strawberry in response to integration of ASD with MSM amendment and to determine the influence of soil biology on long-term ASD induced suppression of root infection by the oomycete pathogen *Pythium ultimum*.

Studies were conducted in the greenhouse using a natural strawberry field soil from Santa Maria, CA. The MSM amendment consisted of *Brassica juncea* seed meal applied at 1.25 ton per acre and rice bran for ASD treatments was applied at a rate of 9 tons per acre. ASD and MSM amendments were applied individually or consecutively using the sequence of ASD/MSM or MSM/ASD, with a two or a three week interval between treatment applications. Additional treatments consisted of a no treatment control and application of MM+ASD concurrently. Soils were dispensed into 1 gallon pots. MSM only treatments were sealed in air tight Bitran bags for 24 h to simulate tarping and concentrate the activity of the biologically active volatile allyl isothiocyanate. All pots containing ASD as a component of the treatment were irrigated with 300 mls of sterile distilled water and then pots were sealed in a double layer bitran bag, and were incubated for one week at 24°C prior to subsequent application of MSM or planting. Soil pH was determined immediately prior to planting. Soils were planted to strawberry (cv Camarosa) one week after application of the final treatment and controls were fertilized with 125 ml Hoagland's solution. Plants were grown using a 16 hour photoperiod and a day/night temperature regime of 24/16°C. Plants were harvested after 15 weeks and biomass determined.

A greenhouse bioassay was conducted to determine whether soil biology contributed to long-term suppression of root infection by *P. ultimum*. ASD was applied to an apple orchard soil that was or was not pasteurized prior to treatment application. At completion of the one week incubation period soils were inoculated with *P. ultimum* to attain an initial population density of 2500 cfu g^{-1}. Apple seedlings were used as the test plant in the bioassay. *P. ultimum* soil density and root infection frequency were determined at harvest four weeks after planting.

Sequence of application significantly influenced the effect of MSM and ASD treatments on soil pH and growth of strawberry in Santa Maria, CA strawberry field soil. Initial soil pH was 7.9 in the un-treated control and ranged from 7.7 to 8.1 in soils that received MSM alone or in soils where MSM followed ASD in treatment sequence. In contrast, pH ranged from 6.2 to 6.4 in soils that received ASD alone or those in which ASD succeeded MSM in the application sequence. Plant growth was significantly improved in response to all ASD or MSM treatments relative to the control. However, when MSM was applied to soil two or three weeks after ASD treatment, strawberry growth was significantly improved relative to the individual treatments or when ASD followed MSM application (Fig. 1A). ASD alone, concurrently with MSM, or when applied in the treatment sequence MSM/ASD produced a bacterial community that was distinct from that realized in response to MSM alone or the treatment sequence ASD/MSM (Fig. 1B).

ASD treated soil was suppressive to root infection by the introduced isolate of *P. ultimum*, but suppressiveness did not develop in pasteurized soil that were treated with ASD prior to pathogen infestation (Fig. 2A). Interestingly, suppression of root infection was realized even though the ASD treatment in a natural orchard soil resulted in elevated soil density of the pathogen (Fig. 2B).

Preliminary studies demonstrate that ASD alters soil biology and results in development of a microbial community that is suppressive to disease incited by *Pythium* spp. Reports also suggest that different organic substrates applied in the conduct of ASD results in production of different volatile profiles which differ in their capacity to suppress fungal pathogens including *F. oxysporum* and *P. ultimum* (Mazzola, unpublished). However, due to the temporal nature of the ASD application relative to the introduction of the pathogen, these volatiles would not have had a role in the observed soil suppressiveness towards reintroduced *P. ultimum*. These findings suggest that a multiplicity of mechanisms may contribute to ASD-induced disease control. Application sequence significantly influenced growth of strawberry in ASD/MSM treated soils. Effective use of ASD or its integration with other methods such as MSM requires an understanding of mechanisms of action to avoid the diminution or elimination of disease control activity, as was observed in pot trials when MSM application preceded ASD.

References:

Cohen et al., 2005. Soil Biology & Biochemistry 37:1215–1227.

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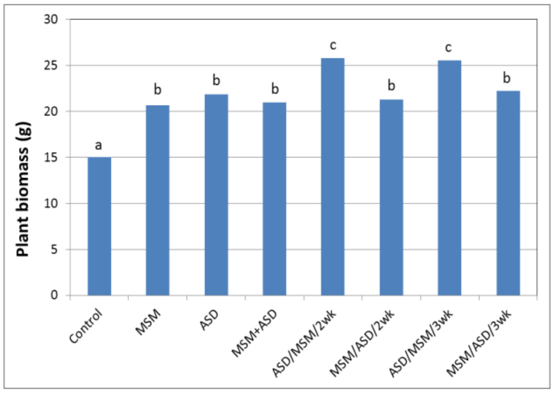
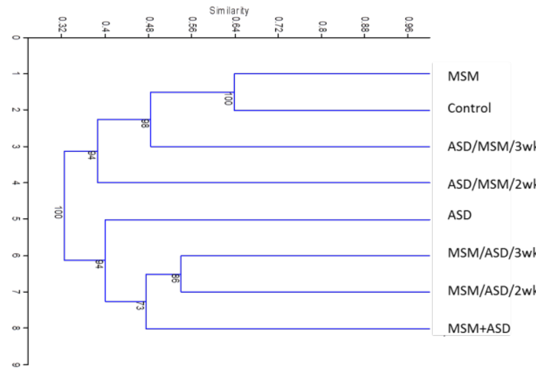
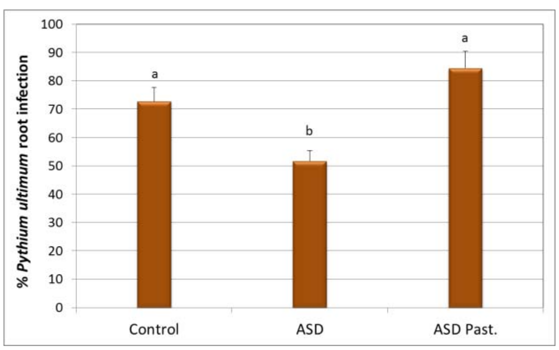


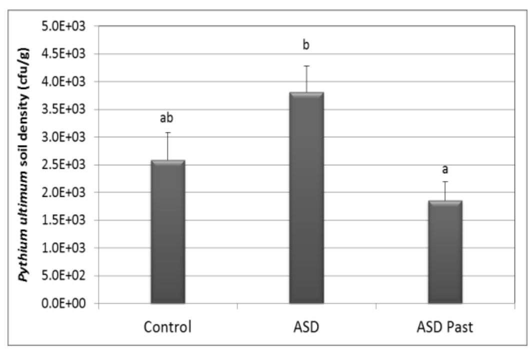


A: Growth of strawberries

B: Bacterial communities

Figure 1. Effect of ASD, mustard seed meal (MSM), sequence of application and period between treatment applications on A: strawberry plant biomass after 15 weeks and B: relative similarity of bacterial communities as determined by T-RFLP analysis, in a Santa Maria, CA strawberry field soil.





A: Root infection

B: Soil density

Figure 2. Effect of ASD treatment of a natural and pasteurized apple orchard soil on A: subsequent apple root infection by an introduced isolate of *Pythium ultimum*, and B: density of the pathogen in soil after four weeks.