Anaerobic Soil Disinfestation (ASD) Demonstration

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Anaerobic soil disinfestation or ASD is a method to reduce plant pathogens, weeds and nematodes in soil. It has been tested across a wide range of soil conditions on both fungal and bacterial plant pathogens. Research on ASD was initiated followed the observation that disease was suppressed following periods of flooding. Additional research indicated the disease suppression was enhanced by the addition of organic amendments. The process produces anaerobic conditions that results in volatile compounds such as toxic organic acids and enhances beneficial anaerobic soil microorganisms. ASD is a three stage process: 1) incorporation of organic matter into soil i.e. wheat bran or molasses, 2) irrigate to soil saturation, 3) coverage of soil with plastic film. In addition the process has been effective in both field and high-tunnel situations. Because of the intensity of inputs, it is most often conducted in high tunnels.

Ben Beale, Sarah Hirsh, Habtamu Demissie and Kate Everts trialed this method last fall in two high tunnels (houses) in St. Mary's county, MD. In House 1, the growing area was split in half with a treated side and non treated side. In House 2, the entire area was treated, and compared to two non-treated houses directly adjacent to it. All high tunnel houses had a history of Timber Rot caused by *Sclerotinia sclerotiorum* and Fusarium Crown Rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Losses to Fusarium Crown Rot were 80% in House 1 and 60% in House 2 and adjacent houses the previous year. In past years, houses were planted to non-grafted fusarium crown rot susceptible varieties including Big Beef, Red Deuce, Mountain Fresh Plus, and Cherokeee Purple. House 1 was planted to continuous tomatoes for six years and House 2 and adjacent houses were planted to continuous tomatoes for over 20 years.

ASD Procedure: Wheat middlings and liquid molasses were sourced from a local feed mill. The area was first tilled to a depth of 6-8 inches using a rototiller. Wheat middlings were spread by hand over the treated area at a rate of .58 lbs. per square foot. The wheat middlings were then incorporated to a depth of 6 inches utilizing a rototiller or harrow. Next, molasses was diluted with water at a rate of 1 part molasses to 2 parts water and the solution sprayed onto the treated areas utilizing a 5 hp irrigation pump and 1.5 inch hose at a rate of .175 lbs. of actual molasses per square foot. Finally, the area was irrigated using a series of overhead irrigation sprinklers for approximately 3 hours until the soil was completely saturated to the point of runoff. The treated area was then covered with 3 mil plastic sheeting for a period of 3 months.

Prior to treatment, soil fertility and nematode sampling was conducted to determine baseline levels. Nematode and soil fertility samples were taken from similar locations 3 months after treatment. Soil and nematode cores were taken at a depth of 6-8 inches. Soil temperature was recorded in one location manually and in the second location uti-

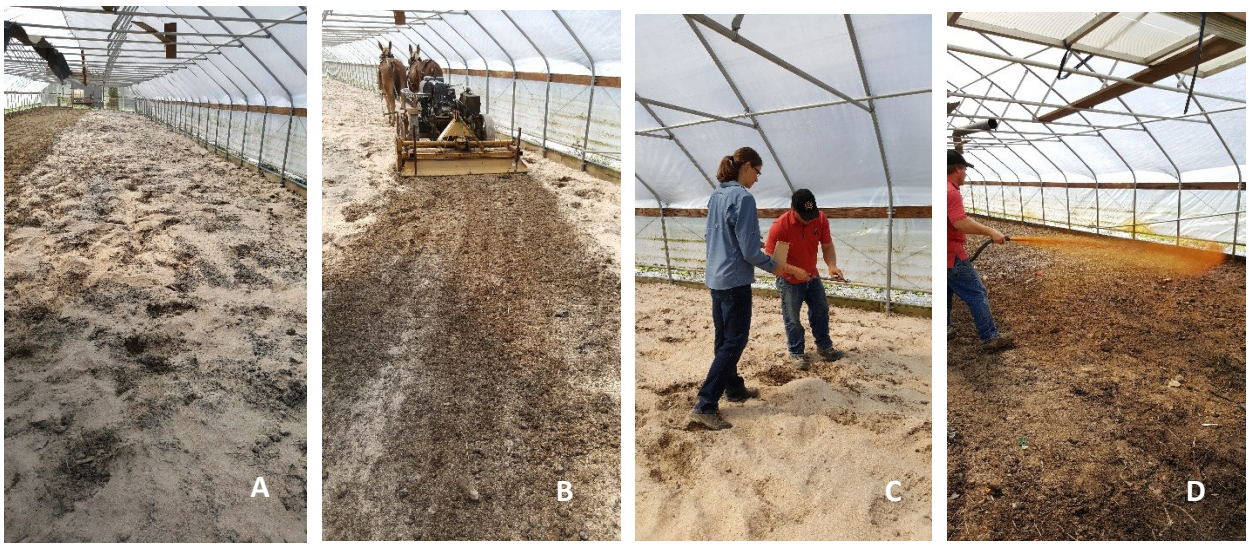

Figure 1) ASD treatement in high tunnel in St. Mary's County A) spreading the wheat middlings, B) incorporating the middlings, C) taking soil samples, D) applying the molasses to the soil.

On the day of treatment, sclerotia, which are the overwintering stage of the pathogen *Sclerotinia sclerotiorum* were placed in fiberglass mesh bags on the surface of the soil or buried at a depth of two or six inches. Following exposure to the ASD treatment for approx. 3 months, the bags were recovered and individual sclerotia were plated on media to determine if they were still viable. In addition, fiberglass mesh bags containing 10 morning glory seeds were buried at a depth of 3 inches and examined for viability by splitting and evaluating the condition of the endosperm. Seed germination tests were not performed. Both sites were visited every month prior to planting and every two weeks during the growing season. Crop observation data was recorded and the percentage of diseased plants noted.

Growing Summary: Tomatoes plants were seeded in late December of 2018 in seedling flats. Small plants were then transplanted to 72 cell trays for one month and then 6 inch pots for approximately one month. Grafting was conducted when plants were 1/8 inch in diameter at the base. House 1 was planted to non-grafted cultivars Red Deuce, Purple Cherokeee and Big Beef. House 2 was planted to cultivars Red Deuce and Big Beef grafted to Rootstock Cultivar RST -04-105-T obtained from Harris Moran Seed. One non-treated house directly adjacent to House 2 was planted to non-grafted plants of the some cultivars and the other non-treated house was planted to grafted plants of the same cultivars and rootstock. Each house was prepared for planting in February by rototilling to a depth of 6 inches. 4 inch raised beds were formed and the entire growing area covered with 3 mil black plastic and two drip tapes installed for each row. Tomatoes were transplanted from 6 inch pots into the houses on March 9. Houses received supplemental wood heat as needed. Plant nutrients were applied through the drip tape. Plants were staked and trellised using the string and weave method. Tomatoes in all houses were suckered by removing the bottom two suckers below the first flower cluster at planting. In house 2, and adjacent houses, lower leaves and branches were removed prior to harvest.

Figure 2) House #2. A. Plants 3 weeks after transplanting from 6 inch pots. B. Plants and fruit 2 weeks prior to harvest. Grower reported the highest yield in twenty years from the ASD treated house.

Figure 3) House # 1. A. Plants 1 week after transplanting from 6 inch pots. B. Plants six weeks later. No evidence of wilting or Fusarium Crown Rot is present yet.

Findings:

Nematode Severity: Nematode results showed a marked decrease in populations of Root Knot and Spiral nematodes following treatment with ASD. We did not observe galling on non-grafted or grafted plants in ASD treated areas. Galling was noted in the non-grafted plants in a non-treated house adjacent to House #2.

Nematode Results	House 1		House 2	
Nematodes in 500 cc of soil	ASD Pre	ASD Post	ASD Pre	ASD Post
Are nematodes a problem?	YES	NO	YES	NO
Lesion, Soybean Cyst, Tobacco Cyst, Stubby Root, Dagger, Stunt, Lance, Ring, Sting, Pin and Sheath	0	0	0	0
Root Knot	240	0	540	0
Spiral	820	60	80	0
Pin	40	0	0	0

Figure 4) A. Summary of nematode counts showing reduction in numbers following treatment. B. Example of root galling caused by Root Knot nematode in tomato.

Soil Fertility: Results from pre and post treatment soil test are presented in Figure 5. Data are presented in UMD Fertility Index Value scale unless otherwise noted. In both houses, soil pH increased by approximately 0.5 points, which was not expected . One result of the anaerobic fermentation process is the production of acids, which we assumed would lower the pH. Also of note is an increase in potassium levels across both houses and a decrease in calcium levels. We also did not observe any appreciable increase in soil organic matter in the three month period. This study did not examine the availability of Nitrogen following treatment or soil biological activity. Based upon plant vigor and growth, we assume that some nitrogen is mineralized and available for plant uptake from the ASD process. Future work to quantify nitrogen availability is needed.

House 2	Pre	Post	Diff		House 1	Pre	Post	Diff
1:1 Soil pH	7.1	7.6	0.5		1:1 Soil pH	5.6	6.2	0.6
Organic Matter %	2.1	2.3	0.2		Organic Matter %	3.2	3.2	0
Potassium FIV	75	201	126		Potassium FIV	304	368	64
Calcium FIV	301	239	-62		Calcium FIV	162	125	-37
Magnesium FIV	313	250	-63		Magnesium FIV	136	159	23
Sulfate-S ppm S	24	50	26		Sulfate-S ppm S	109	102	-7
Zinc ppm Zn	32.7	25.1	-7.6		Zinc ppm Zn	17.2	12.7	-4.5
Manganese ppm Mn	48.5	60	11.5		Manganese ppm Mn	82.7	58	-24.7
Boron ppm B	1.2	0.85	-0.35		Boron ppm B	1.2	0.8	-0.4
Phosphorus (FIV)	> 400	> 400	n/a		Phosphorus (FIV)	338	321	-17

Figure 5) Soil test results from treated houses.

Soil Temperature: Soil temperatures peaked at 115° F approximately 14 days after treatment. Temperatures gradually decreased following ambient air temperatures in the house. It doesn't appear that the ASD treatment resulted in the soil temperatures increasing more than what would be expected for soil that is covered with clear plastic in a closed house. Approximately 10 days after treatment, a distinct musky odor could be noticed inside and up to 100 feet away from the treated areas. The odor dissipated after 3 weeks. The houses remained closed during the three month treatment period and then reopened during the later half of December and all of January so soil would freeze.

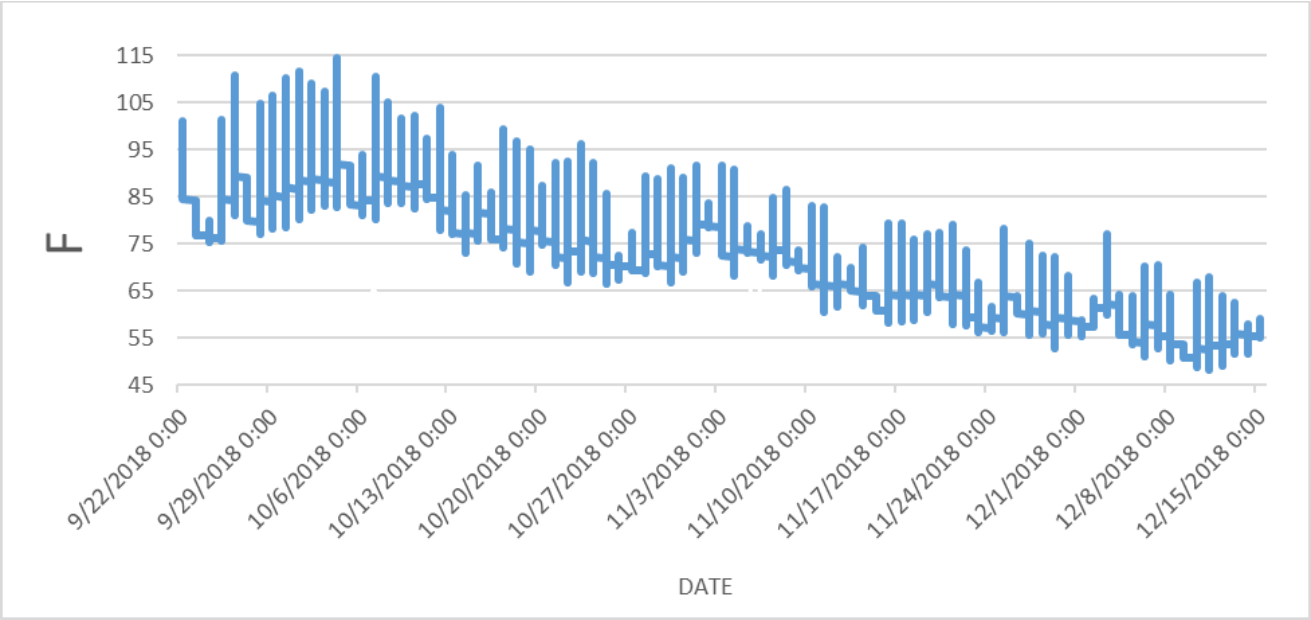

Figure 6) Soil temperature recorded at 3 inch depth during the treatment period.

Sclerotia: Significantly more sclerotia survived in the areas of the high tunnels that did not receive the ASD treatment. Only 21% of sclerotia survived the ASD treatment and more than 95% survived where no ASD was applied (P=0.0217). Sclerotia survival did not differ by soil depth (P=0.0587) and varied from 11 to 30% in the ASD treated sections and from 79 to 100% in the untreated sections. This data indicates that overwintering fruiting structures and initial disease inoculum levels in the house can be effectively reduced with ASD treatment. However, Timber Rot and sclerotia fruiting bodies were observed in both treated houses at low levels (6 symptomatic plants per house). Spores from timber rot can enter the house through air currents and infect plants, thus reduction of fruiting bodies inside houses will reduce but not eliminate Timber Rot occurrence.

Figure 7) Mesh bags with sclerotia prior to treatment (A), burying the mesh bags (B), and processing the sclerotia following treatment to determine viability (C).

Morning Glory Seed Survival: Morning glory seeds in the ASD treated houses had decomposed or decayed, with no viable seeds present after treatment. Morning glory seeds in the non-ASD treated areas also showed some decomposition, however 60% remained firm and the endosperm appeared viable. After removal of the cover in December there was some germinating grasses which indicates the treatment may not be effective for all plants. It is possible that the germinating grass seed was on top of the soil surface and thus not affected by the ASD process. In both houses, the entire growing area was covered with black or white on black plastic mulch which prevent d weed growth after planting, so season long weed control could not be evaluated.

Fusarium Crown Rot:

Although the treatment seemed successful for reducing *Sclerotinia sclerotiorum*, House 1 reported that the crop planted in the spring was not thriving. Some plants were wilting during the daytime and fruit production was limited. Samples from wilting plants were submitted to the UMD Plant Diagnostic lab and confirmed for the presence of Fusarium Crown Rot. We obtained soil samples from the high tunnels and conducted a small bioassay to determine how much Fusarium inoculum remained. We found no difference (P=0.8580) between the treated and untreated sections of the high tunnels. It should be noted that our sample was taken several months after the treatment was completed, and the fungus may have reinvaded treated areas. In addition, we could not differentiate between pathogenic or saprophytic Fusarium species. However, our results may indicate that our treatment was less successful in reducing Fusarium than Sclerotinia.

Example of wilting plant infected with Fusarium Crown Wilt from House 1.

In House 2, plants thrived. However we have no way to compare the efficacy of the ASD treatment versus the efficacy of resistant rootstock on grafted plants. Overall yields from House 2 were outstanding and exceeded yields from the non-treated ASD house with grafted plants by 30%. The adjacent non-treated house planted to non-grafted susceptible plants performed poorly with losses of approximately 75% due to Fusarium Crown rot. and Root Knot nematode. The author's recommend the use of grafted plants with resistant a rootstock in combination with the ASD treatment if Fusarium Crown rot or Root Knot nematode are present. Follow-up studies are being conducted during the 2019-2020 season comparing the ASD treatment, mustard seed meal treatment and combination of grafted versus non-grafted plants.