Biological Approaches to Verticillium wilt Management

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Verticillium wilt is a vascular disease caused by Verticillium dahliae, a highly prolific soil-borne fungal pathogen that negatively affects mint. Some chemical fumigants can control this disease but can negatively impact beneficial soil organisms. Biological fumigation using anaerobic soil disinfestation (ASD) is an alternative approach that is successfully used to control V. dahliae in other crops like strawberries. During the ASD process, a labile carbon amendment is applied to the field, and then the soil is saturated with water and covered with a fumigation tarp for about four weeks. This study aims to determine whether ASD can control Verticillium wilt in mint using local carbon substrates to make it economically feasible. The carbon substrates chosen for the study included: chicken litter, dried distiller's grain from corn, soybean meal, and a Brassica cover crop. The study was initiated during the summer of 2020. Soil samples were collected pre and post-ASD treatment, and quantification of changes in V. dahliae populations was initiated. Other soil health metrics, including pH, active soil carbon, mineralizable nitrogen, and microbial activity, are also being quantified to determine whether the ASD process could provide other benefits. Finally, the incidence and severity of Verticillium wilt were monitored in the experimental plots, and changes in biomass and amount of essential oil were quantified at harvest.

Mint Production

Field trial details

All treatments were applied, and tarps were laid down on August 12 and 13, 2020
ASD/soil solarization (tarp) treatments were applied for 4 weeks
Mint planted on April 2, 2021
Harvested on August 3, 2021
Distilled on August 5, 2021
Oil samples analyzed by Labbeemint – September 7, 2021

Application rate

C	Untreated Control	n/a
CLT	Chicken Litter with Tarp	9.6 tons/A (100 lb/plot)
DDGT	Dried Distiller's Grain with Tarp	9.6 tons/A (100 lb/plot)
MCCNT	Mustard Cover Crop, No Tarp	Var. Caliente 199 at 22 lb/A
MCCT	Mustard Cover Crop with Tarp	Var. Caliente 199 at 22 lb/A
SBMT	Soybean Meal with Tarp	9.6 tons/A (100 lb/plot)
ST	Solarization with Tarp	n/a

Cover crops received 625 lb/A Sustain 8-2-4 fertilizer in 2020 (50 N, 12.5 P_2O_5, 25 K_2O)

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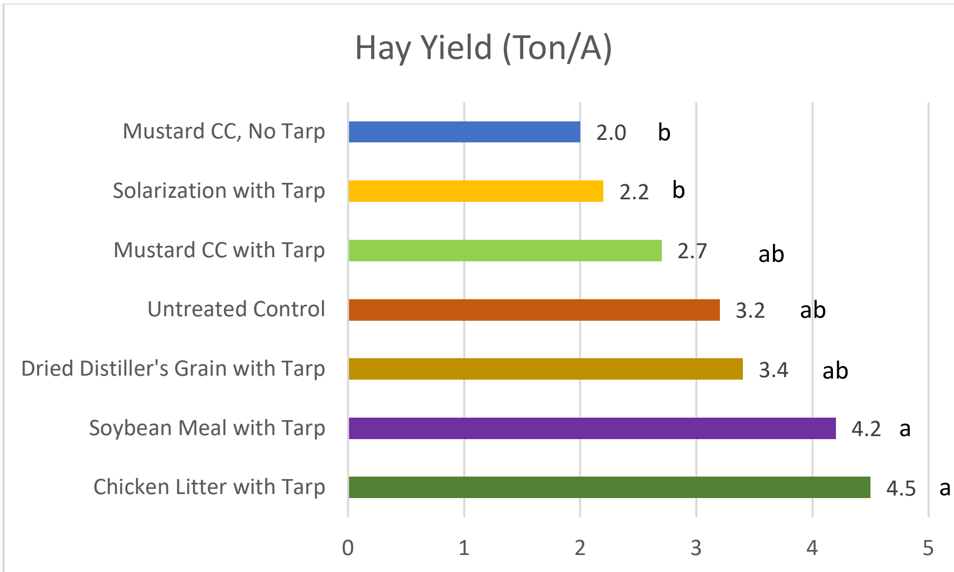
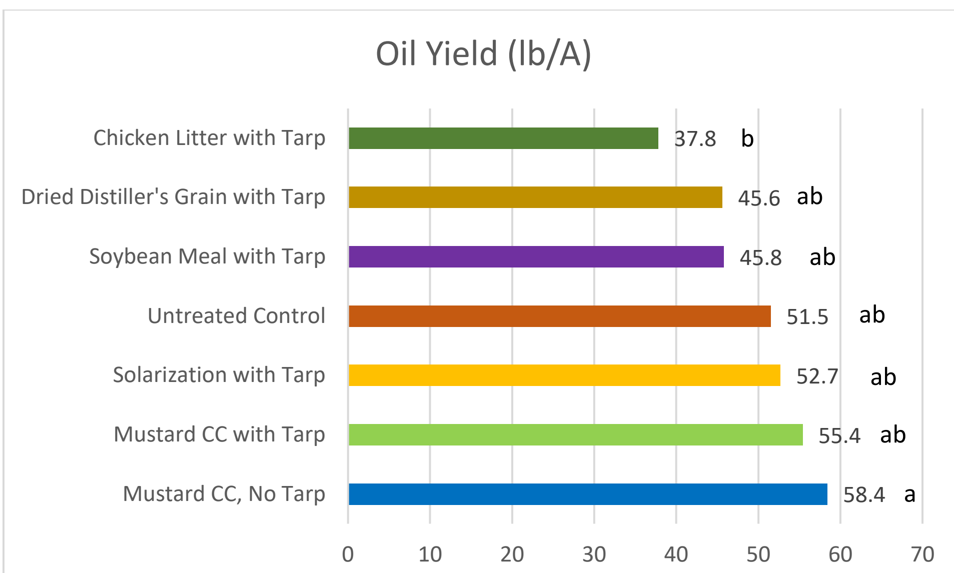
Hay and Oil Yield

Table 1: Peppermint hay production after two days of infield drying



Pr > F
0.0033

Table 2: Peppermint oil production



Pr > F
0.0442

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Oil Quality

Table 3: Significant treatment effects on the main components of peppermint oil

	Menthone[z]	IsoMenthone[z]	Esters[z]	Menthol[z]	Carvone[z]	TMenthone[z]	TMenthol[z]
Treatment				**Percent**			
Untreated Control	20.2 abc	2.7 ab	2.6 ab	33.0 ab	0.8 ab	23.0 abc	41.5 abc
Chicken Litter with Tarp	22.0 ab	2.8 ab	2.1 b	31.2 b	1.4 a	24.8 ab	38.8 bc
Dried Distiller's Grain with Tarp	22.6 ab	2.8 ab	2.2 b	31.0 b	1.2 ab	25.4 ab	38.6 bc
Mustard CC, No Tarp	17.4 c	2.5 b	3.8 a	34.8 a	0.7 b	20.0 c	45.1 a
Mustard CC with Tarp	19.1 bc	2.6 ab	2.7 ab	32.5 ab	0.7 b	21.7 bc	40.9 abc
Soybean Meal with Tarp	23.6 a	2.9 a	1.9 b	30.8 b	0.7 b	26.5 a	38.1 c
Solarization with Tarp	19.3 bc	2.6 ab	3.3 ab	33.7 ab	0.8 ab	21.9 bc	43.2 ab
Pr > F	***0.0003***	***0.0169***	***0.0039***	***0.0023***	***0.0112***	***0.0004***	***0.0007***

[z] Means followed by the same letter are NOT significantly different at P = 0.05, Tukey-Kramer.

Table 4:

	Limonene	Cineol	Sab Hydrate	Furan	Pulegone
			Percent		
Untreated Control	1.93	4.86	2.30	10.52	2.55
Chicken Litter with Tarp	1.91	4.32	2.15	11.69	2.95
Dried Distiller's Grain with Tarp	1.80	4.31	2.28	11.63	2.91
Mustard CC, No Tarp	1.97	4.67	2.25	10.00	2.35
Mustard CC with Tarp	1.83	4.61	2.40	9.98	2.50
Soybean Meal with Tarp	1.85	4.29	2.25	11.47	2.87
Solarization with Tarp	1.86	4.36	2.13	10.65	2.39
Pr > F	**NS**	**NS**	**NS**	**NS**	**NS**

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Differences in Microbial Communities among Treatments

Bacterial communities were significantly different with respect to the control for chicken litter and soybean meal treatments. Fungal communities were significantly different with respect to the control for mustard cc with tarp and soybean meal treatments (Figure 1).

Figure 1 – Results of pairwise PERMANOVA tests comparing bacterial and fungal communities in all treatments

Bacteria	C	CLT	DDGT	MCCNT	MCCT	SBMT	ST
C							
CLT							
DDGT							
MCCNT							
MCCT							
SBMT							
ST							

Fungi	C	CLT	DDGT	MCCNT	MCCT	SBMT	ST
C							
CLT							
DDGT							
MCCNT							
MCCT							
SBMT							
ST							

Green color denotes a significant correlation with $p<0.05$.

Acronyms

C Untreated Control

CLT Chicken Litter with Tarp

DDGT Dried Distiller's Grain with Tarp

MCCNT Mustard Cover Crop, No Tarp

MCCT Mustard Cover Crop with Tarp

SBMT Soybean Meal with Tarp

ST Solarization with Tarp

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Dominant phylotypes

There was a high evenness in bacterial communities. Dominant phylotypes were mostly shared among all soils, but some were characteristic of different treatments, such as *Alicyclobacillus* and *Deltaproteobacteria* representative 0319-6G20 for soybean meal treatment (Figure 2). Fungal evenness was lower than for *Bacteria*. Several dominant phylotypes were characteristic of the control treatment (Figure 3). Taxonomy of *Fungi* was more difficult to discern than for *Bacteria*.

Figure 2 – Heat map of dominant phylotypes of *Bacteria* domain

Phylotype	Taxonomy	Characteristic	C	CLT	DDGT	MCCNT	MCCT	SBMT	ST
Otu00001	Aquicella	DDGT+MCCT+SBMT							
Otu00002	Candidatus_Udaeobacter								
Otu00003	Nocardioides								
Otu00004	Xanthomonadaceae_unclassified								
Otu00005	Actinobacteria_unclassified	DDGT+SBMT							
Otu00006	OM27_clade	C+DDGT+MCCNT+MCCT+ST							
Otu00007	Rhodoplanes								
Otu00008	Adhaeribacter								
Otu00009	Oxalobacteraceae_unclassified								
Otu00010	Myxococcaceae_unclassified								
Otu00011	uncultured_ge								
Otu00012	Burkholderia-Caballeronia-Paraburkholderia								
Otu00013	Pedosphaeraceae_unclassified								
Otu00014	Alicyclobacillus	SBMT							
Otu00015	Chthoniobacteraceae_unclassified								
Otu00016	Vicinamibacteraceae_ge	C+DDGT+MCCNT+MCCT+SBMT							
Otu00017	0319-6G20_ge	SBMT							
Otu00018	Roseiflexaceae_unclassified	C+DDGT+MCCNT+MCCT+SBMT							
Otu00019	RB41								
Otu00020	Gaiellales_unclassified								
Otu00021	Gemmatimonadaceae_unclassified								
Otu00022	Gaiellales_unclassified								
Otu00023	RB41	C+DDGT+MCCNT							
Otu00025	67-14_ge								
Otu00026	Aquicella								

Code
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0.1%
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9.0%
≥10%

Treatment Acronyms: C: untreated control**; CLT:** chicken litter with tarp; **DDGT:** dried distiller's grain with tarp; **MCCNT**: mustard cc no tarp; **MCCT**: mustard cc with tarp; **SBMT**: soybean meal with tarp; **ST**: solarization with tarp

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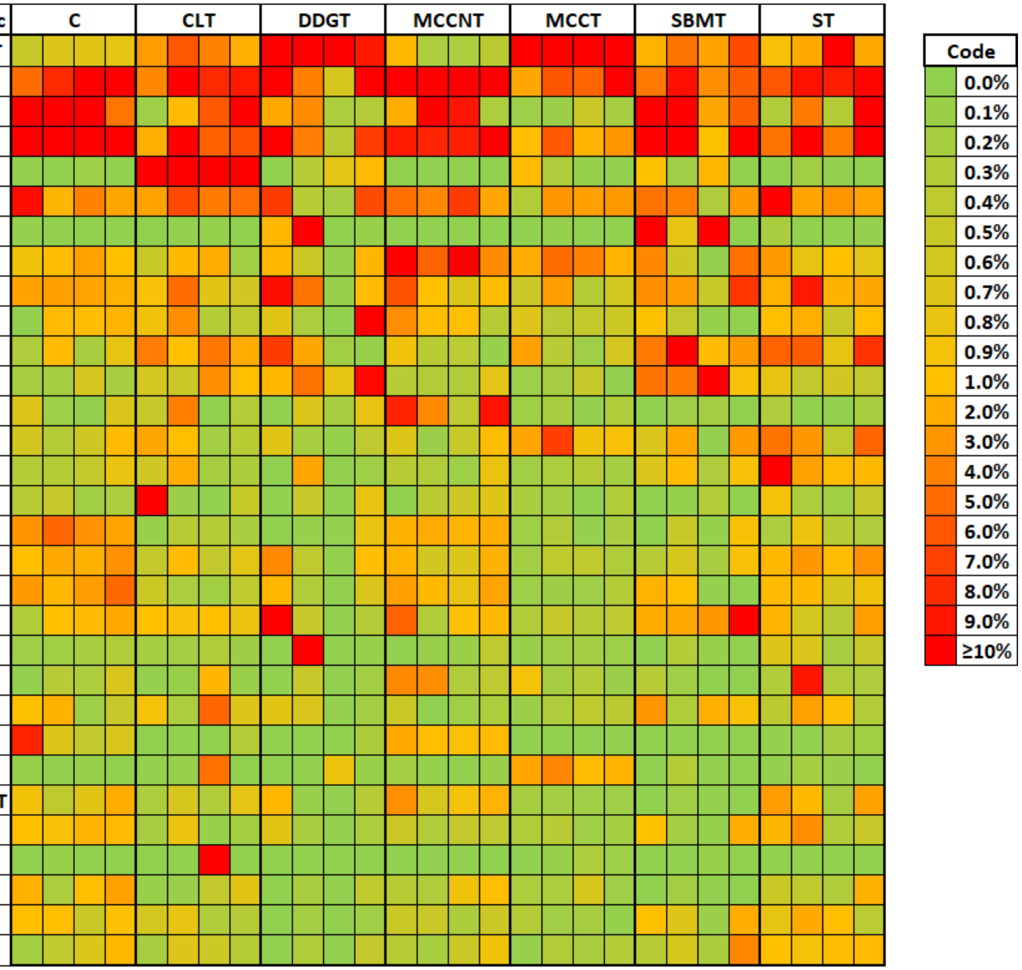
Figure 3 – Heat map of dominant phylotypes of *Fungi* domain

Phylotype	Taxonomy	Characteristic
Otu001	f__Bolbitiaceae_unclassified	DDGT+MCCT
Otu002	Fungi_unclassified	
Otu003	o__Hypocreales_unclassified	
Otu004	o__Spizellomycetales_unclassified	C
Otu005	f__Onygenaceae_unclassified	CLT
Otu006	Polyporales	
Otu007	g__Olpidium_unclassified	
Otu008	p__Ascomycota_unclassified	MCCNT
Otu009	c__Sordariomycetes_unclassified	
Otu010	Fungi_unclassified	
Otu011	f__Nectriaceae_unclassified	
Otu012	p__Ascomycota_unclassified	
Otu013	p__Chytridiomycota_unclassified	
Otu014	Fungi_unclassified	MCCT+ST
Otu015	Monoblepharidales	
Otu016	g__Stachybotrys_unclassified	
Otu017	p__Ascomycota_unclassified	C+MCCNT
Otu018	o__Orbiliales_unclassified	
Otu019	Calvatia	C+MCCNT
Otu020	Udeniozyma_ferulica	
Otu021	f__Psathyrellaceae_unclassified	
Otu022	o__Hypocreales_unclassified	
Otu023	g__Trichoderma_unclassified	
Otu024	Fungi_unclassified	
Otu026	g__Sampaiozyma_unclassified	
Otu027	o__Spizellomycetales_unclassified	C+MCCNT+ST
Otu028	f__Mycosphaerellaceae_unclassified	
Otu029	p__Ascomycota_unclassified	
Otu030	o__Spizellomycetales_unclassified	
Otu031	g__Tilletiopsis_unclassified	C+SBMT+ST
Otu032	g__Vermispora_unclassified	



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Relationship of dominant phylotypes with mint weight and oil production

Both fungal and bacterial communities were organized along an axis that negatively correlated weight and oil produced. Thus, dominant phylotypes promote higher mint weight, higher oil yield, or none of them but not both.

Alicyclobacillus was significantly correlated with the weight of mint at harvest. However, it was significantly and negatively correlated with the mint oil yield. Only the *Deltaproteobacteria* 0319-6G20 phylotype related with oil production was found (Figure 4). Most dominant bacterial phylotypes were correlated with weight but not oil.

A phylotype classified as an *Onygenaceae* family member was very correlated with mint weight. An unclassified fungus was correlated with oil yield. *Fungi* dominant phylotypes could be classified as growth or oil yield promoters (Figure 5). Deeper insights into fungal community identification at the taxonomic level should be conducted.

Figure 4 – Principal Components Analysis linking mint weight and oil produced with dominant bacterial phylotypes (numbers in the figure refer to phylotypes shown in Figure 2 with the corresponding number)

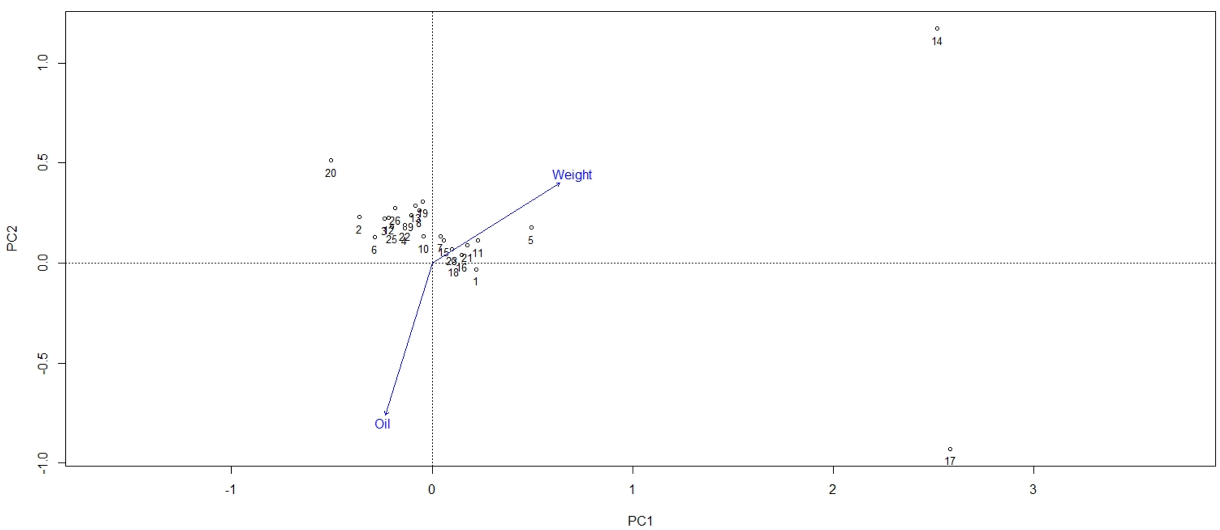

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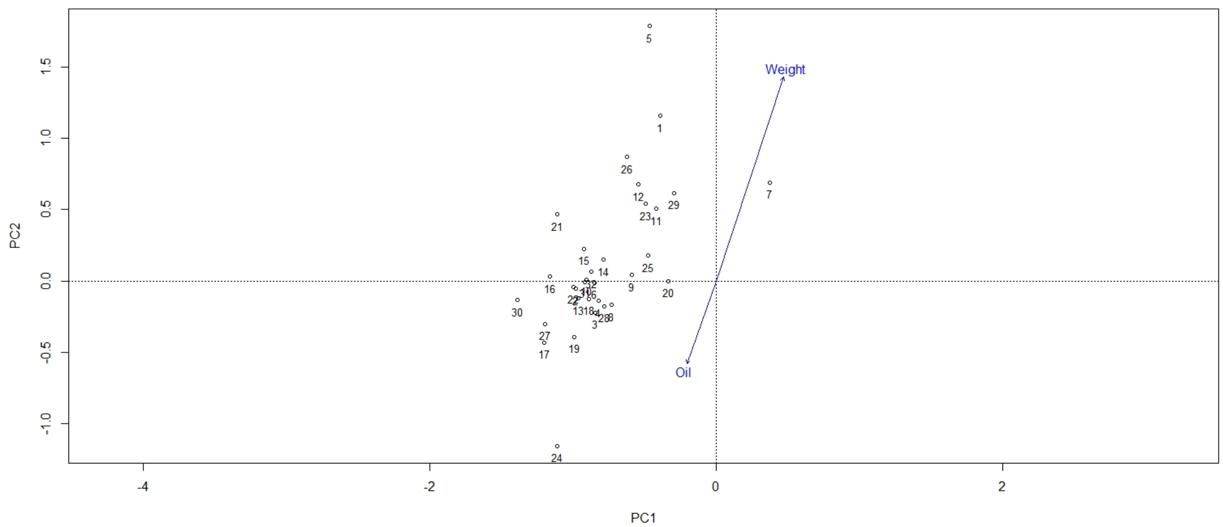
Figure 5 – Principal Components Analysis linking mint weight and oil produced with dominant fungal phylotypes (numbers in the figure refer to phylotypes shown in Figure 2 with the corresponding number)



Main takeaways from this research

- High hay yield does not translate into more oil.
- Mustard CC with no tarp produced a significantly higher oil yield than the chicken litter treatment.
- All treatments produced acceptable oil quality.
- With respect to the control, only soybean meal with tarp treatment significantly changed the bacterial and fungal communities of the soil.
- Soybean meal with tarp treatment promoted the growth of bacteria with the capacity to provide high yields in mint weight and oil produced.
- No treatment could promote fungal phylotypes that could significantly increase yield in mint weight and oil produced at the same time.
- Soybean meal with tarp provided the most suitable microbiome for mint production among the treatment studied.

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