Title: Disease and weed control efficacy of anaerobic soil disinfestation using brewer's spent grain and yeast inoculation

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Public Abstract:

Greenhouse trials were conducted to evaluate the effect of brewer`s spent grain (BSG) carbon source ± distiller`s dry yeast on weed suppression using anaerobic soil disinfestation (ASD). The trial was conducted in containers of 0.2-m height and 0.15-m diameter. The germination of common chickweed, redroot pigweed, white clover and yellow nutsedge, and the count of *Pythium* was significantly reduced with BSG ± yeast, and the suppression was comparable to ASD using rice bran. The addition of distiller's yeast at 10 kg/ha to C sources at 4 mg of C/g of soil provided similar or better weed and *Pythium* control than ASD treatments with C sources alone. ASD treatments reduced weed viability and *Pythium* viability from 65% to 100% compared to the non-treated control. Redox potential in all ASD treatments during the 3-week treatment was lower (more anaerobic) than the non-treated control.

Introduction:

Strawberry production in the southern region of the U.S. is threatened by disease pressure. Many strawberry growers in the region hold small acreages of land and grow berries each year without crop rotation or cover crop adoption. This practice, in the long run, could impact soil health and build disease pressure. 'Sweet Charlie' and 'Chandler' are two popular cultivars in the southern region and are very susceptible to *Phytophthora cactorum* (**crown rot**). Infection by *P. cactorum* is common on poorly drained, wet soils, or during long periods of rain (Louws and Ridge, 2014). Black root rot, a disease complex primarily caused by *Pythium* and *Rhizoctonia* spp., is also especially problematic and is perhaps the primary motivating factor for soil fumigation in the region (Louws, 2014). More recently, *Fusarium oxysporum* f. sp. *fragariae,* has been reported as an increasing threat to strawberry in multiple regions throughout the world, including the south-Atlantic area (Koike and Gordon, 2015). Strawberries in this region are also very susceptible to *Colletotrichum* spp. (anthracnose), particularly under warm (25-30°C) and moist conditions (Louws et al., 2014). This pathogen can survive for up to 9 months in the soil without a host plant (Bolda et al., 2016). Weeds such as *Stellaria* and *Cerastium* spp. (chickweed) and *Vicia* spp. (vetch) commonly observed in strawberry production, are known hosts for this pathogen (Bolda et al., 2016).

Weeds and limited herbicides. Weed control is important, particularly during the early stages of strawberry production, when transplants are being established in the field. Herbicides registered for use in strawberry plasticulture production are few and make weed control a challenge nationwide. With the lack of interest from chemical companies to register herbicides for many specialty crops, herbicide options will likely stay limited. Many herbicides currently registered have a 30 day –preplant application period (oxyfluorfen and flumioxazin), while others have continued to cause phytotoxicity (flumioxazin and napropamide) to strawberry plants (Southeast Regional Strawberry IPM Guide, 2019).

Anaerobic soil disinfestation (ASD) has demonstrated efficacy in suppression of many soil-borne pests across a diversity of cropping systems and environments (Shennan et al., 2014; Shennan et al., 2018; Shrestha et al., 2018). The basic method involves the addition of a relatively labile carbon (C) source to stimulate microbial growth and respiration, complete coverage with polyethylene mulch to limit gas exchange, and irrigation to fill soil pore space with water. Anaerobic conditions are created due to an initial rapid growth of aerobic microorganisms, which depletes remaining soil oxygen, and subsequently the microbial community shifts to facultative and obligate anaerobes. Production of volatile fatty acids (e.g., acetic, n-butyric acids) via anaerobic decomposition of the added C, the release of volatile compounds, accumulation of Fe^{2+}, and biocontrol by microorganisms that flourish during ASD and the crop production cycle are all mechanisms responsible for pest suppression with ASD (Momma, 2008). Anaerobic conditions are maintained for a period of two to six weeks. The period varies with soil temperature and C-sources used (Shennan et al., 2014). The tarp is then either

removed or planting holes are punched through the tarp to allow oxygen back into the soil and to stimulate the degradation of remaining by-products of anaerobic decomposition. Higher temperatures during ASD allow for a shorter treatment period and/or smaller quantities of C inputs (Butler et al., 2014). For the widespread adoption of ASD, a range of C sources ideally derived from locally-available waste products need to be identified in each region (Shennan et al., 2018).

Brewer`s spent grain (BSG) is a solid residue from breweries consisting of exhausted grain husks obtained after mashing and lautering. BSG could be a potential C source of ASD for three reasons: **(i)** BSG could be provided for free, as BSG is a waste from beer production. There is an increasing trend of draft-breweries in Virginia and neighboring states. **(ii)** BSG can produce ethanol when mixed with distiller's yeast (Liguori et al., 2015), and ethanol is an effective C source for ASD (Momma et al., 2013). Due to the high cost of ethanol, using low-cost BSG to produce bioethanol under field conditions could be feasible. **(iii)** Fresh BSG has an optimal C: N ratio of ~14:1, which could reduce the nitrogen inputs from fertilizer. The recommended C: N ratio is 10:1 to 35:1 (Shennan et al., 2014).

Materials and Methods:

Greenhouse trials were initiated at the Southern Piedmont Agricultural Research and Extension Center (AREC), Blackstone, VA in late spring/summer 2019. We made ASD "bioreactors" with a surface area of 182 cm^2 by attaching a 20 x 20 cm piece of bone voile to the bottom of each 20 cm x 15 cm PVC tube (Fig. 1). We added 6.8 kg of topsoil from a field at the Southern Piedmont AREC research farm to each container. Carbon source treatments and application rates (4 mg C/kg soil) were as follows:

i) brewer's spent grain (fresh weight 64 g/container),

ii) brewer's spent grain + distiller's yeast (fresh weight 64 g/container + 4.1kg/acre of yeast)

iii) rice bran as a positive control (fresh weight 80 g/container)

iv) rice bran + distiller's yeast

v) nontreated control

vi) nontreated control + distiller's yeast

Two inoculum bags were buried in the soil at a depth of 2.5 cm above the container bottom. One inoculum bag contained 10 yellow nutsedge (*Cyperus esculentus*) tubers and 100 seeds each of common chickweed (*Stellaria media*), redroot pigweed (*Amaranthus retroflexus*) and white clover (*Trifolium repens)*. A second inoculum bag contained a colonized substrate of *Pythium irregulare*, which were inoculated into sterile V8 juice-vermiculite-oat medium and mixed with the same soil as in the container. In each container, redox potential sensors (ORP2000 Extended Life ORP Sensor; a measure of soil anaerobic conditions) and temperature sensors (U12- 015, Onset Hobo Data Loggers)

were positioned next to inoculum bags (Fig. 1). The sensors collected data every 10 min. during the treatment period. The container was covered with a polyethylene mulch film and sealed. Each treatment had four replicates. The containers were arranged in a completely randomized design in black bins, with each bin holding four containers. The bins were partially filled with water such that the container bases of the anaerobic treatment containers maintained high soil moisture content. The non-treated containers were elevated so the container base is not sitting in water. The experiment set up was maintained for 3 weeks and the study was repeated twice (two runs). First run period in the greenhouse was from 17 April, 2019 through 8 May, 2019. The second run period was from 6 June, 2019 through 26 June, 2019.

Data collection. We collected data on soil temperature, redox potential, soil volatile fatty acid concentrations, and efficacy of these treatments on weed and pathogen propagules. Weed and Pythium inoculum bags were retrieved post-treatment and weed seeds were subject to a tetrazolium viability assay. Nutsedge sprouting was evaluated under growth chamber conditions at 23 °C using 16 h day length. For the Pythium assay, bag soil was air-dried and sieved by mesh. Ground soil samples (0.5-g) were added to 20 mL water, and 1 mL of the soil solution was sieved by a syringe with a filter membrane. The filtered solution was spread to a modified cornmeal agar medium with pimaricin, ampicillin, rifampicin, Benomyl and Rose Bengal (Moorman and May, 2018). Over the next 12 to 48 h, the number of colonies on each plate was determined and expressed as CFU per gram of dry soil. The presence of volatile fatty acids in soils during treatment was determined by KCl extraction of soil samples at day 3 of ASD treatment (Lawongsa et al., 1987), followed by analysis by HPLC (Angeles et al., 2006). The study was repeated in early fall 2019.

Data analysis. We analyzed the data using SAS v. 9.4 (SAS Institute Inc.). We have checked the data for normality and homogeneity of variance assumptions. The cumulative redox potential was calculated basing on the hourly average redox potential. The absolute value of the difference between each hourly average redox potential and calculated critical redox potential (CEh; redox potential value below which is considered anaerobic) was summed up over the whole three-week ASD period. The critical redox potential was calculated by the formula CEh=595mV - 60mV * soil pH (Rabenhorst and Castenson 2005; USDA-NRCS 2010). We analyzed the interaction between experimental runs (time) and treatment as fixed effect factors, and there was no effect of runs. Hence, data from two runs were polled to run the Wilcoxon test. We used the Wilcoxon for each pair test to compare means (α=0.05).

Results and Discussion:

Weed and *Pythium* viability

The weed data (Table 1) showed all ASD treatments reduced weed seed viability by around 60% to 100%. The addition of yeast increased the weed efficacy for pigweed, chickweed and white clover when C source was BSG. Yeast only improved the efficacy

of rice bran in the case of pigweed species. All ASD treatments showed outstanding control on yellow nutsedge.

Pythium viability was the lowest in BSG+ yeast, even lower than rice bran treatments. However, all ASD treatments significantly reduced the viability of *Pythium* over nontreated control. Our findings indicate that yeast could have the potential to improve the effect of ASD treatments using BSG as C source.

Temperature and Cumulative redox potential

Overall, there was no significant difference in mean temperature among all treatments. All ASD treatments had significantly higher cumulative redox potential than non-treated control, and there was no statistical effect of yeast application.

Volatile fatty acids

There were five organic acids detected in the soil which were acetic acid (AA), propionic acid (PA), isobutyric acid (IBA), n-butyric acid (BA) and isovaleric acid (IVA). Overall, ASD treatments with BSG had more organic acids (AA, BA, PA, IBA) produced than non-treated control and rice bran treatments. In summary, the ASD treatments using BSG as C source with or without yeast had good suppression on four tested weed species and *Pythium irregulare*, but further research in the open-field condition is needed.

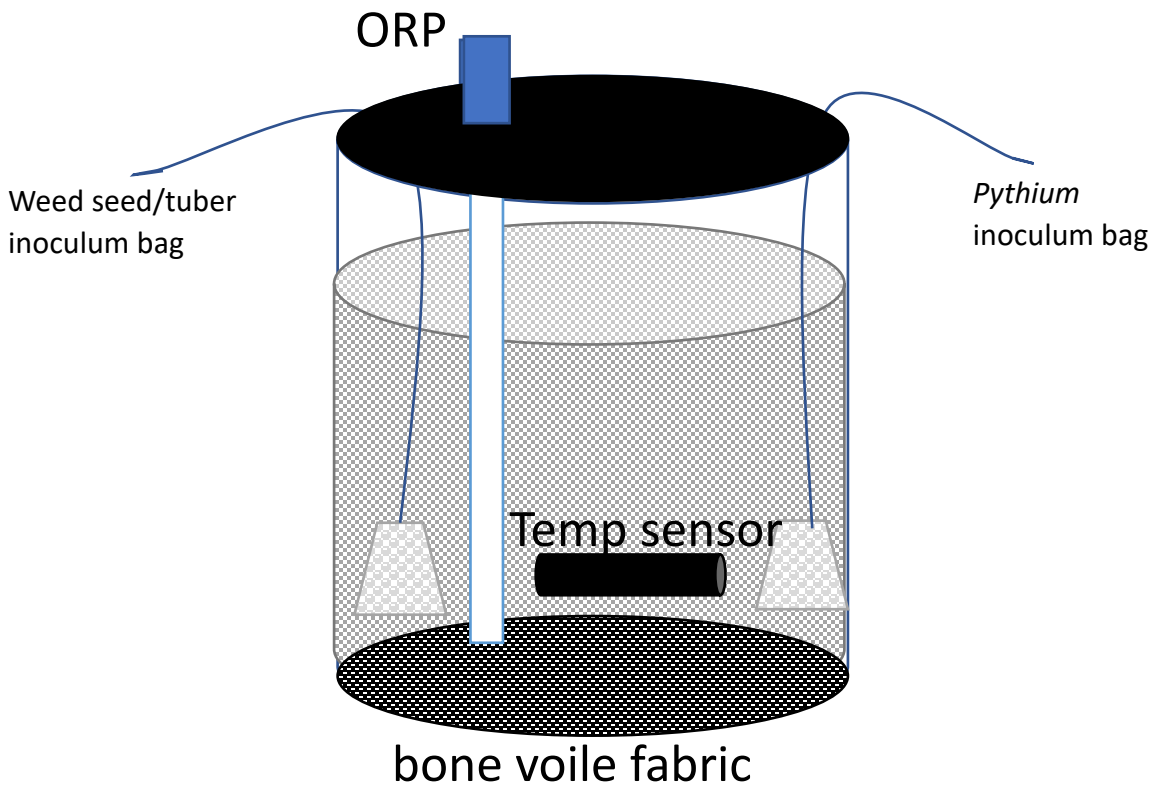

Figure 1. Bioreactor used in anaerobic soil disinfestation trials

Table 1. Weed germination rates, *Pythium* count, mean temperature and cumulative soil anaerobic conditions after anaerobic soil disinfestation (ASD) process.

Treatment	**Pigweed (%)**	**Chickweed (%)**	**Clover (%)**	**Nutsedge (%)**	**Pythium (CFU/g)**	**Mean Temperature(°C)**	**Cumulative Redox potential (mV hr)**
Brewer`s spent grain (4 mg of C / g soil, 15 ton / acre)	27 b	21 c	21 b	2.5 b	51.3 b	25.9	175922 a
Brewer`s spent grain + distiller`s yeast (4mg of C / g soil, 4.1kg / acre yeast)	15 c	14 d	11 c	0 b	28 c	26.5	183707 a
Rice bran (4mg of C / g soil, 15 ton / acre)	23 b	24 c	13 c	0 b	54 b	26.3	96571 a
Rice bran + distiller`s yeast (4mg of C / g soil 4.1kg / acre yeast)	2 c	18 c	15 c	0 b	52 b	26.5	144827 a
Nontreated control	74 a	73 a	82 a	75 a	164a	25.6	5023 b
Nontreated control + distiller`s yeast (4.1kg / acre)	68 a	65 b	78 a	70 a	172a	26.0	4214 b
Wilcoxon/Kruskal-Wallis Test-1-way test, ChiSquare Approximation							
P value	<0.001	<0.001	<0.001	<0.001	<0.001	0.98	0.003

Table 2, Volatile fatty acids amount in soil after ASD process

Treatment	Acetic acid (AA)*	Propionic acid (PA)	Isobutyric acid (IBA)	N-butyric acid (BA)	Isovaleric acid (IVA)
Brewer`s spent grain (4 mg of C / g soil, 15 ton / acre)	2.80 a	0.15 a	0.29 a	0.92 a	0.17 b
Brewer`s spent grain + distiller`s yeast (4mg of C / g soil, 4.1kg / acre yeast)	1.87 b	0.09 b	0.11 bc	0.59 a	0.08 bc
Rice bran (4mg of C / g soil, 15 ton / acre)	0.30 c	0.02 c	0.00d	0.05 b	0.07 bc
Rice bran + distiller`s yeast (4mg of C / g soil, 4.1kg / acre yeast)	0.86 c	0.03 c	0.06 cd	0.21 b	0.33 a
Nontreated control	0.06 c	0.01 c	0.00 d	0.00 c	0.00 c
Nontreated control + distiller`s yeast (4.1kg / acre)	0.38 c	0.02 c	0.00 d	0.12 b	0.06 bc
Wilcoxon/Kruskal-Wallis Test-1-way test, ChiSquare Approximation					
P value	<0.001	<0.001	<0.001	<0.001	<0.001

*The unit of acids was mmole/kg of dry soil