Evaluation of anaerobic soil disinfestation using brewers spent grain and yeast inoculation on weed control in annual hill plasticulture strawberry production

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ABSTRACT

Anaerobic soil disinfestation (ASD) is a promising alternative to chemical fumigation to control soil-borne plant pathogens and weeds. This research focused on evaluating several locally available carbon sources for ASD on weed control, evaluating the performance of brewers' spent grain (a promising carbon source) under field conditions, and evaluating whether yeast addition enhanced the effectiveness of ASD treatments. A series of greenhouse trials were conducted at the Southern Piedmont AREC (Agricultural Research and Extension Center). The greenhouse trials were conducted in PVC tubes, 20 cm tall and 15 cm in diameter. The first set of trials evaluated ASD conducted over 21-day periods of ASD using locally available carbon sources. The carbon sources included brewer`s spent grain, buckwheat (*Fagopyrum esculentum*), cowpea (*Vigna unguiculata*), paper mulch, peanut *(Arachis hypogaea*) shells, rice bran, sorghum-sudangrass (*Sorghum drummondii*), and waste coffee grounds applied at 4 mg of C/g of soil. The targeted weed species included common chickweed (*Stellaria media* (L.) Vill.), redroot pigweed (*Amaranthus retroflexus* L.), white clover (*Trifolium repens* L.), and yellow nutsedge (Cyperus esculentus L.). All ASD treatments significantly reduced weed viability compared to the non-treated control. The yeast amendments enhanced weed control over ASD without yeast. The second set of greenhouse trials was focused on ASD using brewer`s spent grain, and on evaluating ASD at the half and one-third carbon dose rates. The target pests were the same weed species in the first set of trials, and *Pythium irregulare* was added as an additional target pest. This set of trials indicated yeast enhanced addition the effect of BSG in ASD on both weeds and *P. irregulare*, indicating the potential to reduce carbon input necessary for effective ASD. A follow-up, two seasons, open-field trial conducted over two growing seasons at the Hampton Roads AREC focused on understanding the effects of ASD on weed density and strawberry fruit yield and fruit quality in annual hill strawberry production. The treatments included ASD at standard or half carbon dose rates, with or without yeast. Fumigation (80% chloropicrin + 20% 1,3-dichloropropene) and non-treated plots were used as control groups. Weed suppression with ASD was consistent for most of the broadleaf weed species, and total weed counts were significantly reduced compared to non-treated controls. Yield from ASD

with yeast was higher than ASD without yeast and non-treated control in one growing season, while the increase in yield did not occur in another growing season. Yeast may have potentially enhanced the yield effects of ASD but lacked consistency. Yeast may have the potential to enhance ASD effectiveness.

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GENERAL AUDIENCE ABSTRACT

Strawberry is a high-value crop known for its brightly colored, sweet tasting, juicy and fleshy fruit that possesses a unique aroma. The southern region is the second large region of strawberry production in the United States. Strawberry is susceptible to soil-borne pests, including weeds and diseases. Preplant control of soil-borne diseases and weeds is important for strawberry production. Early season weeds can compete with newly transplanted strawberry plugs for nutrients, light, and other resources. However, currently, the limited options of pre-plant chemical fumigants and herbicides available in strawberry plasticulture make weed control a challenge in strawberry production. Anaerobic soil disinfestation (ASD) may be an effective alternative to preplant chemical fumigation. Anaerobic soil disinfestation involves three steps- applying carbon sources to the soil, covering the bed with black tarp, and watering the soil to maintain certain soil moisture to field capacity generally for 21 days.

However, there are only a few studies on weed control using ASD in the southern region; locally available carbon sources also need to be evaluated. Thus, this study focused on evaluating several locally available carbon sources (cover crops, brewer`s spent grain, used coffee ground, paper mulch, peanut shell) for ASD to control troublesome weeds (common chickweed, redroot pigweed, white clover, yellow nutsedge). This study also explored a new method that involves mixing distiller's yeast with solid carbon sources in order to enhance the ASD weed control effect. Additionally, this study evaluated the effect of ASD using reduced carbon inputs, potentially reducing the total cost of ASD by reducing the carbon input. A series of greenhouse studies were conducted at the Southern Piedmont Agricultural Research and Extension Center (AREC), Blackstone, VA, with a follow-up field study done at the Hampton Roads AREC. The greenhouse trials evaluated carbon sources including brewer`s spent grain, buckwheat, cowpea, paper mulch, peanut shells, rice bran, sorghum-sudangrass, and waste coffee grounds. These greenhouse experiments were conducted in containers made from PVC tubes, and strawberry plants were not involved. The main objective of the greenhouse trial was to test the suppression of four troublesome weeds, including common chickweed, redroot pigweed, yellow nutsedge, and white clover. The most effective treatments in the greenhouse studies were further investigated in the field trial. The

brewer`s spent grain was again used in the field trial, and treatments included ASD using a full or half dose of brewer's spent grain, with or without yeast. We evaluated the effects of these treatments on weed control, plant crop growth, and crop yields. Fruit quality factors, including fruit firmness, sweetness, and size, were also evaluated.

In summary, all of the carbon sources evaluated provide similar weed control. Adding yeast showed potential to enhance the effect of ASD using brewer`s spent grain. Adding yeast also increased the effectiveness of the half-rate of the carbon source, showing the potential for effective pre-plant pest control for strawberry using ASD treatments with significantly reduced C dose rates.

DEDICATION

I dedicate this work to my family, friends and colleagues whose continuous support helped me to
achieve my dream.

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CHAPTER I: INTRODUCTION AND LITERATURE REVIEW

1. INTRODUCTION

Crop rotation is useful to suppress soil-borne pests, buffer the soil fertility and have other benefits. However, crop rotation is always limited or dismissed in many specialty crop production systems. Thus, many growers must use other forms of pre-plant treatment methods to suppress weeds, soil-borne pathogens, and nematodes. For the past 40 years, many developed countries have widely used pre-plant soil fumigation. Without fumigation, the soil-borne pathogens, weeds and nematodes often reduce yield. However, fumigation increases environmental and human health risks. Methyl bromide (MeBr) was widely used to control soil-borne pathogens in the past, but it contributed to ozone layer depletion. When the 1993 Montreal Protocol went into effect, the production and importation of MeBr was phased out by 2005 (EPA, 2020). During negotiations, the United States agreed to finally phase out MeBr by 2015. During the long phaseout period, several government exemptions, such as critical use exemptions (EPA, 2020), provided options for the producer who claimed that there was no viable alternative to use MeBr. By the end of 2016, MeBr use was finally phased out for use in strawberry production (Guthman, 2017). The total ban forced growers to use alternative methods such as registered soil fumigants to control soil-borne pests. Registered soil fumigants include 1,3-dichloropropene (1,3-D), chloropicrin (Pic), dimethyl disulfide (DMDS), allyl isothiocyanate along with methyl isothiocyanate generators such as metam potassium or sodium. Those fumigants have shortcomings such as restrictions on use, greater cost, or lower efficacy on certain pests (Eure and Culpepper, 2017; Hanson and Shrestha, 2006; Yu et al., 2019; Zasada et al., 2010). For example, Pic provided effective control on soil-borne pathogens, but it alone could not control weeds and nematodes as effectively as MeBr. The fumigant 1,3-D alone controls nematodes but lacks efficacy on soil-borne pathogens and weeds (Noiling and Beacker, 1994). When Pic was combined with other soil fumigants such as 1,3-D (i.e., as Pic-Clor 60, Pic-Clor 80, Telone C-35, Tri-form 80) or DMDS, the mixture had achieved acceptable weed control efficacy (Boyd et al., 2017; Chase et al., 2006; Ji et al., 2013). However, 1,3-D and chloropicrin have potential to cause acute toxicity in humans (EPA,2007; EPA, 2009). There are additional costs and regulations due to health concerns. For example, use of chloropicrin requires a mandatory buffer zone and notification of neighbors before application; use of respirator masks and other personal protective requirements are required for the applicators; and the site must be closed for five

days before anyone may return (EPA,2012). The health risk and additional costs make soil disinfestation using fumigants not the most ideal option.

Non-synthetic approaches to combat soil-borne pests include, but are not limited to, steam, soil solarization, bio-amendments such as mustard seed meal, cover crops, and anaerobic soil disinfestation. Steam sterilization kills soil-borne pathogens through injection into the soil in a covered field. Steam could effectively control fungal pathogens, bacterial, weeds and a few viruses and nematodes (Baker et al., 1960; Tanaka et al., 2003; Afek and Orenstein, 2002). The disadvantage of this method is the high cost due to the high fuel consumption, labor and time consumed and equipment cost (Dabbene and Tortia, 2003; Luvis et al., 2015). The high cost may even cause a reduction of net return (Samtani et al., 2011; Samtani et al., 2012). The steam sterilization would be well fitted in small-scale environments such as greenhouses, high tunnels and container production, while it is hard to operate in the open field. For now, steam sterilization has not been used in large-scale field production. However, steam still has potential in the future with the research that revealed the use of a mobile steam applicator (Fennimore et al., 2014). Solarization methods use solar energy to heat the soil in order to reduce populations of soil-borne pests and pathogens through high soil temperature (Baysal-Gurel et al., 2019). However, this method requires a hot summer and periods of abundant sunshine during fallow periods, so it is not effective in regions having moderate summer temperatures. Moreover, solarization typically requires a four to six weeks duration for effective suppression of soil-borne pests and weeds. Although this method has the benefit of very low cost, it is not effective in controlling all weed species, resulting in extra weeding costs and lower crop yields compared to chemical fumigation (Samtani et al., 2012). However, soil solarization could be combined with biofumigation or soil amendments to increase the efficacy of soil-borne pests and weeds control (Kewerepe and Labuschagne, 2003; Simmons et al., 2013). Biofumigation via Brassica green manure, Brassica cover cropping and mustard seed meal (MSM) could reduce the population of several soil-borne pathogens by biological compounds released in the soil such as glucosinolates (GSs) and isothiocyanates (ITCs) (Brown and Morra, 1995; Gimsing and Kirkegaard, 2009; Kirkegaard and Sarwar, 1998; Lazzeri et al., 2009). However, the phytotoxicity of ITCs has potential risk of causing lower yields compared to chemical fumigation and other alternatives (Lazzeri et al., 2003; Muramoto et al., 2016). Biofumigant cover crops such as broccoli, cabbage, radish and rapeseed can suppress parasitic nematodes and soil-borne diseases, including, *Fusarium wilt, Verticillium* and other fungal diseases, but

it is a challenge to make cover crops match current crop rotations or to develop new rotations for current crop schedules (Baysal-Gurel et al., 2018; Larkin et al., 2006; Larkin et al., 2007). In summary, an ideal alternative method should: have a relatively low cost; lack adverse health and ecological effects; widely control soil-borne pathogens, weeds and nematodes, and provide crop yields crop comparable to other soil disinfestation methods. Anaerobic soil disinfestation could be a potential method to achieve the above benefits.

Anaerobic soil disinfestation (ASD) was developed separately in Japan (Shinmura, 2000) and in The Netherlands (Blok et al., 2000) as alternatives to chemical soil disinfestations. In the U.S and the Netherlands, this method is called "anaerobic" soil disinfestation (Butler et al., 2012), while in Japan, it is commonly named as "biological" soil disinfestation (BSD) or "reductive" soil disinfestation (RSD), since biological agents play an important role in disinfestation. The key to the method is using a large volume of decomposable organic materials, applying irrigation, and using an impermeable film to prevent air exchange into the soil, and creating a strong soil reduction over a short time period in order to kill soil-borne pathogens and pests.

Strawberries are very sensitive to soil pathogens (*Rhizoctonia*, *Pythium, Fusarium, Macrophomina*), and traditionally growers have depended in the past on soil fumigant methyl bromide to control soil-borne pathogens. After several years' development, ASD has been evaluated on multiple farms in California and Florida, but no research has been done for recommendations (carbon source types and rates, applied protocol and cost) for strawberry growers in Virginia. This study's short-term goal is to optimize the ASD treatment for strawberries growing in Virginia, which will be more economically and environmentally sustainable.

1.1. Research problem

a. The relatively high cost of ASD treatments, especially the cost of carbon sources, is a concern. Cover crops as potential alternative carbon sources showed inconsistent efficacy of pathogen suppression in several pots and field studies (Butlter et al., 2012).
b. ASD treatments have not been evaluated for Virginia strawberry production.
c. Total yields of crops with ASD treatments are not always comparable to chemical fumigants.

d. Several active research programs worldwide continue to develop and evaluate ASD to control plant pathogens, nematodes, and weeds. Investigations on mechanisms of ASD are still ongoing (Shennan et al., 2014). Although the cost of ASD may be relatively low when using locally available carbon at no direct cost as carbon sources, the use of ASD in the USA has only been adopted by a few organic crop producers and some conventional growers. The possible reasons include: the ASD cost is too close to that of conventional soil fumigants, use of composted waste as carbon sources may introduce food safety issues, or the required duration of ASD treatments may be too long to fit into the current production schedule. ASD, therefore, requires more investigation in comparison to other options to chemical fumigants (Butler et al., 2012; Shennan et al., 2014).

1.2. Research question

a. Could we use local carbon sources, such as locally adapted cover crop or agricultural waste, to reduce the cost of ASD ?
b. Could we characterize the interactions among application factors (such as carbon sources type and rates), environment factors (such as soil type, pH, temperature), measurements (such as pH, Eh), and the ASD effectiveness (such as yields and fruit quality) ?
c. Could we improve the ASD protocol to benefit local growers ?

1.3. Research aim

a. Evaluate the effects of local carbon sources (research about cover crops, brewer`s spent grain, paper mulch)
b. Test the effect of combinations of solid carbon source and low concentration (<1 v/v) ethanol applied during ASD treatment.
c. Determine the possibility that the application of yeast or enzyme could enhance the ASD effect.
d. Combine all the above results to optimize the ASD method.

1.4. Research objectives

a. Evaluate the effect of local carbon sources in ASD treatments on weeds control using a completely randomized design under controlled greenhouse environments.

b. Evaluate the effect of reduced rate carbon sources and carbon sources mixed with yeast in ASD under controlled greenhouse environment.
c. Evaluate optimized ASD methods in field condition using tested carbon sources, and to compare the performance of these ASD protocols with fumigated plots and a nontreated control.

2. LITERATURE REVIEW

Anaerobic soil disinfestation (ASD) is a pre-plant, non-chemical soil disinfestation practice, regarded as an alternative to chemical soil fumigation. ASD has been demonstrated to control several soil-borne diseases (*Phytophthora*, *Pythium*, *Sclerotinia*, *Fusarium*, *F. oxysporum*, *Verticillium*, *Rhizoctonia*, and *Sclerotium*), nematodes (*Meloidogyne* spp., *Pratylenchus* spp.), and weeds such as creeping yellow field cress (*Rorippa sylvestris*), common chickweed (*Stellaria media*) and yellow nutsedge (*Cyperus esculentus* L.) in different vegetables and fruit crops (Blok and Lambers, 2001; Meijer and Lamers, 2004; Shennan et al., 2014; Shrestha et al., 2018; Rosskopf et al., 2015). The strategy of ASD is to create a temporary anaerobic soil condition that is appropriate for the growth of facultative and obligate anaerobic microorganisms. Under anaerobic conditions, the carbon source introduced as part of ASD is decomposed by these microorganisms. This decomposition produces multiple phytotoxic compounds, such as volatile fatty acids, alcohols, ammonia, and metal ions. These compounds could suppress specific soil-borne pests and diseases (Huang et al., 2015). The ASD treatment is initiated in three steps: 1) mixing the soil with a decomposable carbon source in order to initiate rapid soil microbial growth and respiration, 2) covering the bed with polyethylene film to obstruct the diffusion of oxygen from the soil surface, and 3) irrigating the soil to field capacity and further reduce the presence of oxygen (Butler et al., 2014; Shennan et al., 2014).

2.1. Mechanisms of ASD

In general, the lethal effect of ASD on soil-borne pathogens is not the result of a single mechanism but the result of a combination of multiple mechanisms. The mechanism may not be the same for different soil-borne pathogens, insect pests, nematodes or weeds. During ASD, soil oxygen is consumed by microorganisms, leading to anaerobic conditions. Soil-borne pathogens are mostly aerobic microorganisms, and they are likely less adapted to the anaerobic condition created by ASD. Under sufficiently anaerobic conditions, the

fermentative decomposition of carbon sources contributes to reductions in soil pH and redox potential (Eh) and production of volatile organic compounds (VOCs) or volatile fatty acids (VFAs) (Hewavitharana et al., 2014). The decrease in soil pH is mainly caused by the production of organic acids (Momma et al., 2006; Momma et al., 2008). If organic acids are not generated, the soil pH may increase due to the basic cations released from the decomposition of organic amendments (Marschner and Noble, 2000; Xu et al., 2006). The changes in soil pH may have indirect effects on disease suppression via changes in the quantity of VOCs or VFAs or manganese and iron ions. For example, acetic acid, a primary VFA, is in the non-ionized toxic form at pH 3, while it is primarily in its non-active form at pH 6 (Conn et al., 2005). In ASD pot trials with wheat bran, the accumulation of acetic and butyric acids was related to decreased soil pH (Momma, 2008). The VOCs or VFAs could be directly related to disease suppression. When added directly to inoculated soils, acetic and butyric acids significantly reduced populations of *Ralstonia solanacearum* (Momma et al., 2006) and *Meloidogyne incognita* (Katase et al., 2009). Researchers reported that the development of organic acids such as acetic and butyric acid in ASD (rice bran, 1.1t ha^{-1}) contributed to the suppression of *Ralstonia solanacearum* populations (Momma et al., 2006), and *Pythium ultimum, Fusarium oxysporum* and *Rhizoctonia solani* AG-5 populations (Hewavitharana et al. 2014). Moreover, the production of organic acids may contribute to manganese (Mn^{2+}) and iron (Fe^{2+}) increase (Momma et al., 2011), and these changes likely also increase ASD effectiveness. For example, *Fusarium oxysporum* could be effectively suppressed in Mn^{2+} and Fe^{2+} solution, which indicated Mn^{2+} and Fe^{2+} might be one of the agents that suppressed pathogens during ASD (Momma et al., 2011).

Another contributing factor to pathogen suppression in ASD is by changing soil microbial communities. As the soil becomes anaerobic during ASD, the anaerobic condition causes a series of changes in redox. For example, compounds with high reduction potential begin to be reduced, such as NO_3^-, Mn^{4+} and Fe^{3+} (Kogel-Knabner et al., 2010). Those reductions could change the solubility of several minerals and affect many small organic molecules (Momma et al., 2011). Those changes, as well as lower Eh, remove the competitive advantages from the bacteria that are primarily competitive in aerobic conditions (Kogel-Knabner et al., 2010). Thus, the aerobic microorganisms, including aerobic soil-borne pathogens, become less competitive, while the anaerobic microorganisms have the potential to thrive during ASD period. In a greenhouse ASD study using wheat bran and *Brassica juncea* as a carbon source, the population of *Fusarium oxysporum* decreased during ASD,

while the population of culturable anaerobic bacteria increased. The increasing anaerobic bacterial belonged to the class *Clostridia*. Those *Clostridia* species likely produced high rates of acetic and butyric acids, which were related to the suppression of pathogens (Mowlick et al., 2012). Significantly different bacterial communities were also observed between ASD and a non-treated control in a trial using rice bran as a carbon source. (Mowlick et al., 2013, 2014). Increased populations of some fungi after ASD were also reported, such as yeasts (Mazzola et al., 2012), *Trichoderma* spp. (Shrestha et al., 2013) and total fungi (Streminska et al., 2014). Correlations among shifts in microbial communities and changes in metabolite abundance were also reported (Hewavitharana et al., 2019).

Aerobic condition during ASD also has suppression effect on some soil insect such as the pupal of *Delia radicum* L. (Neito et al., 2019). The possible explanations for the mortality caused by ASD include compromising protein synthesis (Price, 1963) and contributing to the breakdown of ATP (Heslop et al., 1963). During the tail end of ASD period or just after ASD period, the hypoxic conditions are created when the anaerobic microbe declines. Such hypoxia can lead to soil dipteran pupae suppression or even mortality (Greenberg and Ar, 1996; Neito et al., 2019).

2.2. Factors affecting ASD effectiveness

There are two main types of factors which influence the effect of ASD: application factors such as carbon source rates and types and environmental factors such as soil type, soil pH, and soil temperature. The carbon source application should be adjusted based on environmental factors in order to optimize ASD treatments for local production.

For the application factors, the species and volumes of different carbon sources can have a profound influence on the effectiveness of ASD. The carbon source used should contain an adequate supply of labile carbon to support or stimulate the growth of soil microbes (Momma 2008). Various labile carbon sources have been studied, such as molasses (Butler et al., 2012), rice bran (Shennan et al., 2009), wheat bran (Momma, 2008), ethanol (Uematsu et al., 2007), other forms of plant biomass (Mehissa et al., 2007) or agricultural byproducts (Serrano-Pérez et al., 2016). A meta-analysis of 533 ASD studies showed most carbon sources used significantly reduced pathogens, and the combination of different types of carbon sources had a similar effect to the use of a single carbon source (Shrestha et al., 2016). Recommended carbon-to-nitrogen ratio (C: N ratio) ranges from 10:1 to 35:1 (Shennan et al., 2014). The low C: N ratio caused the loss of organic nitrogen, while the high

C: N ratio caused N deficiency in the soil. However, the volume of organic materials applied can be adjusted according to the actual conditions, such as the species and population levels of soil pathogens, the sensitivity of the pathogens to ASD treatment, soil temperature, and the treatment length (Momma et al., 2006; Butler et al., 2012). Increasing the application rates of organic materials applied may be appropriate when the soil-borne pathogen density is high.

Soil temperature is also a crucial factor influencing ASD performance. Under anaerobic conditions, the survival period of pathogens could be shortened by increasing the temperature, although effects differed among different species (Ebihara and Uematsu, 2014). Similarly, the effect of ASD on pathogen suppression increased when soil temperature was greater than 35 °C (Shrestha et al., 2016). The effectiveness of ASD against *Fusarium oxysporum* was reduced when soil temperature was below 25°C compared to soil temperature above 25°C (Shrestha et al., 2021). In tropical and subtropical regions, it is advisable to take advantage of high temperatures during the summer season.

2.3. Cover crop as carbon source

Cost and operability are important factors that constrain the application of ASD methods. The application of ASD needs a large volume of organic materials and irrigation, which result in additional labor costs compared to field with chemical fumigant. The extra inputs indicate that ASD should only be used for high-value crops such as strawberries. The use of cover crops or local agricultural organic waste may reduce the cost of ASD. The anaerobicity and suppression of *Pythium* spp. were similar in ASD trials using grass residues versus ethanol as carbon sources (Hewavitharana et al., 2014). That study indicates that the locally available carbon source may enable reducing the cost of ASD without reducing its effectiveness. Moreover, the ecological interactions between cover crops and plant-parasitic nematodes and soil-borne plant pathogens could result in pathogen suppression. For example, arugula *(Eruca sativa*), cowpea (*Vigna unguiculate*), and sorghum-sudangrass (*Sorghum drummondii*), consistently suppressed *Meloidogyne* associated with roots and are able to control root-knot nematode in ASD (Kokalis-Burelle et al., 2013).

Several cover crops were studied as potential carbon sources by Butler et al. (2012) in ASD trials in 5.2-L volume pots in the greenhouse. The cover crops included cowpea (*Vigna unguiculate*), sunn hemp *(Crotalaria juncea),* pearl millet *(Pennisetum glaucum),* sorghum-sudangrass (*Sorghum bicolor (L.) Moench x S. bicolor var. sudanense),* cowpea+pearl millet,

cowpea+sorghum-sudan grass, molasses control. There were similar anaerobicity levels in cover crops- treated pots to molasses-amended pots. Suppressions of *Fusarium oxysporum* were observed in all carbon source treatments, while suppression of *Sclerotium rolfsii* was inconsistent across cover crop treatments. This might be attributed to fluctuation in quantity and quality of cover crops. In addition, ASD trials using cover crops as carbon sources were not as effective under field conditions as ASD trials under pot conditions in terms of creating anaerobic conditions. This indicates that ASD using the cover crop as carbon sources may had different effectiveness under field conditions compared to pot conditions. The species of cover crops, or the biochemical composition of cover crops may affect the ASD effectiveness.

2.4. Ethanol and fermentation

Liquid carbon sources such as ethanol, organic acids, and liquid molasses can be applied through drip irrigation, and multiple applications during the ASD period can enhance the consistency of the anaerobic condition. Liquid carbon sources are easily decomposed in soil and rapidly translocate to the soil profile, which suggests that they could be more effective in ASD than solid carbon sources. In Japan, ethanol has been used as a carbon source for ASD on a relatively large scale (Momma et al., 2013). In Florida, liquid molasses has been studied, and researchers commonly used that as a liquid carbon source for ASD studies (Butler et al., 2012; Rosskopf et al., 2014).

Ethanol was reported as an efficient carbon source for ASD against root-knot nematodes, fungi, and bacteria (Kobara et al., 2007; Momma et al., 2010; Uematsu et al., 2007). Ethanol was a potential carbon source in effectively controlling *Fusarium oxysporum* f. sp. *lycopersici*, while wheat bran (10.3 Mg ha^{-1}) could not produce the same results when used as a carbon source (Momma et al., 2010). Kobara et al. (2007) and Uematsu et al. (2007) also tested the possibility of using ethanol as a carbon source for ASD. Kobara discovered that the redox potential dropped significantly in soil saturated with 1 % ethanol. Additionally, Uematsu et al. (2007) reported that ASD using a low-concentration ethanol (0.5–1.0 %, v/v) successfully suppressed root-knot nematodes (*Meloidogyne incognita*), *F. oxysporum* f. sp. *cucumerinum*, and *Ralstonia solanacearum*.

Moreover, ethanol requires a relatively short incubation period for ASD. For example, ASD with wheat bran needed at least nine days to suppress chlamydospores of *F. oxysporum*

f. sp. lycopersici buried in soil, while with ethanol, only three days were needed with 2 % (v/v) ethanol, six days for 1 % ethanol, and nine days for 0.5 % ethanol (Momma et al.2010).

Although researchers indicate that ethanol is more effective than solid form carbon sources, the high cost of ethanol makes it unsuitable for large-scale ASD use in the US. Bioethanol produced from agricultural waste is a potential way to solve this problem. Honda et al. (2008) and Kitamoto et al. (2011) reported a novel bioethanol fermentation method. The new method used silage and forage crops to produce ethanol under field conditions. Unlike the original process of bioethanol production, bioethanol from ASD can be produced without preliminary distillation or purification. Moreover, fermentation can occur in the field, where disinfestation is needed. Greenhouse and field experiments were conducted by Horita and Kitamoto (2015). Ethanol fermentation was conducted in the field using forage rice plants during fermentation, and the final product [14% (v/v) ethanol] was collected. Another bioethanol solution was generated from sweet sorghum juice [brix ca. 13%(w/v)] mixed with yeast. The ethanol concentrations of those solutions were adjusted, and then those solutions were applied as the carbon source in ASD trials. The ASD tests in the field with 0.5 or 1.0 % (v/v) bioethanol solution consistently suppressed Fusarium *oxysporum,* while the treatment with wheat bran or 1.0 % (v/v) ethanol did not always suppress *Fusarium oxysporum.* This study indicated organic residue from the bioethanol fermentation had the potential to enhance the effect of the ethanol-based ASD treatment.

Another locally available agricultural waste that could be used for ethanol fermentation is brewer`s spent grain (BSG). BSG is a solid residue from the beer-brewing process. BSG mainly consists of grain husks. Several researchers showed the potential to produce ethanol from BSG mixed with yeast or enzymes (Caetanoa et al., 2013; Hassan et al., 2020; Olugbenga and Ibiyemi, 2011). For example, when BSG was pretreated with enzymes and supplemented with yeast (*S. cerevisiae* NRRL YB 2293), the yeast cells produced 12.79 g/L ethanol within 24 h (Liguori et al., 2015). However, for BSG without any other nutrient, all the glucose was consumed by the yeast cells, and the same ethanol concentration of 12.0 g/L was achieved (Liguori et al., 2015). Although few studies have been conducted on bioethanol fermentation using BSG under field conditions, this strategy is a possible method to generate low-cost ethanol or even enhance the effect of ASD treatment, especially if yeast or enzyme are added during ASD.

2.5. ASD and strawberry weeds control

In Virginia, strawberries have primarily been grown in the annual plasticulture production system over the past few decades. In general, the strawberry cultivars are transplanted early fall. High-value crops such as strawberries are often planted continuously, with just enough time to disinfest the soil before the next crop. Pre-plant weed control is necessary for strawberry because strawberry is sensitive to weed competition (Guerena and Born, 2007). Weeds in strawberry fields may be annuals (summer or winter), biennials, or perennials. Some of the summer weeds are as followed: common lamb's quarters (*Chenopodium album),* redroot pigweed *(Amaranthus retroflexus*), hemp nettle *(Galeopsis tetrahit*), and corn spurry *(Spergula arvensis)*. Winter weeds include shepherd's purse (*Capsella bursa-pastoris*), European field pansy *(Viola arvensis Murr)*, common groundsel (*Senecio vulgaris*), and common chickweed *(Stellaria media*). Biennials include wild carrot (*Daucus carota*), evening primrose (*Oenothera spp.*), and perennials include dandelion *(Taraxacum officinale*), Canada thistle *(Cirsium arvense*), quackgrass *(Elymus repens*), creeping buttercup (*Ranunculus repens*), and yellow nutsedge (*Cyperus esculentus*). New weed control methods are needed to optimize costs and minimize adverse effects on strawberry plants Melanson et al., 2021).

According to a meta-analysis of 533 ASD experiments, including 88 ASD studies on weed suppression (Shrestha et al., 2016), populations of *Chenopodium album* and *Cyperus esculentus* have been reduced with ASD, while suppression was not observed for *Amaranthus retroflexus.* The weed suppression effects of ASD ranged from 32% to 81% (Shrestha et al., 2016). Weed suppression only occurred with high soil temperature and only when the carbon source rate was greater than 1 kg m^{-2}. Moreover, the liquid carbon source such as ethanol reduced weeds more than the solid carbon source such as rice bran. It was also reported that ASD strongly reduced survival rates of sow thistle (*Sonchus arvensis*) under both laboratory and field conditions (Bleeker, 2008; Zeeland and Weide, 2006). The effectiveness of ASD on weed suppression varied based on weed species, carbon source types, and soil types (Muramoto et al., 2008; Di Gioia et al., 2016; McCarty et al., 2014; Strauss and Kluepfel, 2015; Butler et al., 2012; Shrestha et al., 2016; Lamers et al., 2010).

The mode of action of ASD on soil-borne pathogens suppression has not been fully investigated, and there were little research studies on the mechanism of ASD on weed suppression. Muramoto et al. (2008) indicated the lack of oxygen could be the possible

reason for weed suppression during ASD. Shrestha et al. (2018) evaluated the effect of ASD with different C: N ratio on the suppression of yellow nutsedge (*Cyperus esculentus*). This research found ASD had effective suppression on tuber sprouting at a15 cm depth, while ASD had much less effect at a 5 cm depth. This finding was similar to another previous study (Muramoto et al., 2008). The different depths may cause different anaerobic periods (lower redox potential for a longer time period). However, studies at the same depth (15 cm) showed the cumulative anaerobic conditions were not the main factor that affected yellow nutsedge suppression (Muramoto et al., 2008; Shrestha et al., 2018; Paudel et al., 2020). The lowest soil pH was observed for ASD treatments that had the lowest tuber sprouting rates (Shrestha et al., 2018). The low soil pH during ASD causes by the production of organic acids (Momma et al., 2006), suggested that the pH might be linked with weed suppression. The decomposed or rotted yellow nutsedge tubers observed in ASD treatments suggest microbe activities have a potential effect on the mortality of tubers (Shrestha et al., 2018). The studies on ASD for weed suppression are still few, and most of the studies were conducted in pots. Thus, more studies focusing on the mechanisms of ASD on weed suppression on various weed species are needed, particularly on a field scale.

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CHAPTER II: WEED CONTROL ASSESSMENT OF VARIOUS CARBON SOURCES FOR ANAEROBIC SOIL DISINFESTATION

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ABSTRACT

Greenhouse trials were conducted to evaluate the effect of several locally available carbon (C) sources on weed suppression using anaerobic soil disinfestation (ASD). carbon sources included rice bran, sorghum-sudangrass, cowpea, buckwheat, paper mulch, brewer`s spent grain, waste coffee grounds and peanut shells applied at 4 mg of C/g of soil. All trials were 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 was reduced similarly with all carbon sources used for ASD. The addition of distiller's yeast at 10 kg/ha to carbon sources at 4 mg of C/g of soil provided similar or better weed control than ASD treatments with carbon sources alone. ASD treatments in all trials reduced weed viability from 38 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.
Keywords. Brewer's spent grain, cover crops, distiller's yeast, ethanol, paper mulch

1. INTRODUCTION

In many high-value specialty crop production systems where crop rotation is not practical, growers must utilize pre-plant treatments to suppress soil-borne pests, including weeds, plant pathogens, and plant parasitic nematodes (Shennan et al., 2009). For the past 40 years, pre-plant soil chemical fumigation has been widely used in many developed countries to achieve good pest control efficacy and higher crop yields relative to non-treated soils. However, fumigation increases the environmental and human health risk, along with the increasing production costs from barrier films and expanding buffer zones (Gao et al., 2011; Fennimore et al., 2013). The chemical fumigants 1,3-dichloropropene (1,3-D) and chloropicrin are now applied as alternatives to MeBr for pest control in strawberry (Rosskopf et.al, 2005). However, 1,3-D and chloropicrin are toxic to humans, and chloropicrin is not as effective on weeds as methyl bromide (MeBr) fumigation (Noling and Becker, 1994). Due to the health risks and relatively high cost associated with chemical fumigants, it is necessary to evaluate alternative strategies to disinfest soils.

Anaerobic soil disinfestation (ASD) was independently developed in Japan (Shinmura, 2000) and in the Netherlands (Blok et al., 2000) as alternatives to chemical soil disinfestation. In the U.S and the Netherlands, this method is called “anaerobic” soil disinfestation (Butler et al., 2012). The method involves using a large number of decomposable organic materials, applying irrigation to field capacity, and use of polyethylene mulch film to limit gas and create anaerobic condition. Under anaerobic conditions, the carbon source is also decomposed by other microorganisms, which produce compounds that could suppress specific soil-borne pests and diseases (Huang et al., 2015).

In general, the types and amounts of carbon sources have profound influence on the ASD effect. The carbon sources used should contain an adequate supply of labile carbon to support soil microbial growth (Momma, 2008), and various labile carbon sources have been studied, such as molasses (Butler et al., 2012), rice bran (Shennan et al., 2009), wheat bran (Momma, 2008), ethanol (Uematsu et al., 2007), other forms of plant biomass (Messiha et al., 2007) or other agricultural byproducts (Serrano-Pérez et al., 2016). Other carbon sources such as ethanol have shown promise in controlling soil-borne pests (Momma et al., 2010; Uematsu et al., 2007). However, the high cost of ethanol makes it unsuitable for large-scale usage in the US, and the application of ethanol to soil is regulated in US. Bioethanol produced from agricultural waste is a potential way to solve this problem. Honda et al. (2008)

and Kitamoto et al. (2011) reported a method to process bioethanol production under field conditions using local forage crops, and Horita and Kitamoto (2015) evaluated the effect of the products and byproducts from such bioethanol fermentation as carbon sources for ASD. This study indicated that residual organic substances in the bioethanol fermentation products had the potential to enhance the effect of ASD treatment. Locally available waste materials, such as brewer`s spent grain (BSG), have been shown to produce bioethanol when mixed with yeast (*Saccharomyces cerevisiae*) (Liguori et al., 2015). Although few field studies on bioethanol fermentation used wastes such as BSG, BSG could provide a possible way to generate low-cost ethanol or even to enhance the effect of ASD treatment by applying yeast during ASD.

In Virginia, strawberries are mostly grown using an annual plasticulture production system with strawberry cultivars transplanted in early fall (Christman and Samtani, 2019). Strawberries are planted continuously with limited time to prepare land post-harvest and disinfest the soil before the next transplant season. Pre-plant weed control is necessary because strawberry is sensitive to weed competition. Strawberry yields are depressed due to competition with annual weeds such redroot pigweed *(Amaranthus retroflexus),* shepherd's purse (*Capsella bursa-pastoris*) and common chickweed *(Stellaria media*); biennials including wild carrot (*Daucus carota*); and perennials include dandelion *(Taraxacum officinale*), quackgrass *(Elymus repens*), white clover (*Trifolium repens*) and yellow nutsedge (*Cyperus esculentus*) (Melanson et al., 2019).

According to a meta-analysis of 533 ASD experiments, including 88 ASD studies on weed suppression (Shrestha et al, 2016), density of common lamb's quarters (*Chenopodium album*) and yellow nutsedge were significantly reduced by ASD, while suppression of redroot pigweed was not achieved. Moreover, weed suppression was primarily at high soil temperature during ASD treatment, and only when the carbon source rate was greater than 1 kg m^{-2}. In addition, liquid carbon sources showed better effects compared to dry solid carbon sources in terms of weed suppression.

Strawberries are also very susceptible to soil-borne plant pathogens *(e.g., Phytophthora*, *Cylindrocarpon, Colletotrichum, Macrophomina* and *Rhizoctonia*). Traditionally growers depended on soil fumigation with mixtures of MeBr and chloropicrin to control soil-borne pathogens along with weed propagules. Although ASD has been evaluated for strawberry in some geographic sites in the U.S (Shennan et al., 2019, Mazzola

et al., 2018), there are no recommendations (carbon source types and rates, application protocol, and treatment duration) for strawberry growers in Virginia and the mid-Atlantic region. The objective of this study was to evaluate weed control efficacy of local carbon sources used in ASD treatments, and consisted of three trials, each with separate objectives. The objective of trial 1 was to evaluate the effect of several local carbon sources on weed control in ASD. The objective of trial 2 was to evaluate the effect of local carbon sources mixed with ethanol in ASD. The objective of trial 3 was to evaluate if carbon sources mixed with distiller`s yeast could stimulate bioethanol fermentation during the ASD process, and whether this carbon source and distiller's yeast combination could enhance ASD weed control efficacy. Local carbon sources tested included brewer's spent grain, paper mulch, waste ground coffee, and cover crops.

2. MATERIALS AND METHOD

2.1. Trial setup.

Greenhouse ASD pot trials were initiated at the Southern Piedmont Agricultural Research and Extension Center (AREC), Blackstone, VA beginning in May 2017. In the greenhouse, custom-made "bioreactors" (182 cm^2 soil surface area) were constructed of PVC tubing 20 cm tall with a 15 cm diameter, and a piece of bone voile fabric mesh was attached to the bottom of each tube (Fig 2.1). The bioreactor was filled with 6.8 kg of field topsoil (sandy loam, pH = 6.5) from the Southern Piedmont AREC that was premixed in a tub with treatment appropriate carbon sources, and 2-liter tap water added to bring soil to 20% moisture content, which was the field capacity for sandy loam soil (Blok et al., 2000). The ORP sensors and temperature sensors were buried near the bottom of the bioreactor at 15 cm depth, and inoculum bags were placed close to the sensors. The black 1.25 mil virtually impermeable plastic film (VIF, Raven Industries Engineered Films Division, Sioux Falls, SD, USA) was secured on top of bioreactor with duct tape for all treatments except the non-treated control.

All the carbon source values were calculated based on the recommendations of Butler et al. (2012), with a C rate was 4 mg C/g soil, or 16 t/ha in these trials. These calculations were based on 15 cm soil depth and soil density of 1.08 g/cm^3 carbon sources were chosen considering the availability and costs in our region during the time period of strawberry bed preparation and dry rice bran was included as a positive control. The carbon sources used in these trials and the amount used (gram/ container) were: dry rice bran (64 g), sorghum-

sundangrass (*Sorghum* × *drummondii*) residue (67 g), cowpea residue (74 g), buckwheat residue (80 g), velvet bean residue (*Mucuna pruriens*, 56g), pelleted paper mulch (32 g; Lebanon Seaboard Corporation), brewer`s spent grain (BSG, 64 g, Commonwealth Brewering Company, Virginia Beach, VA, USA), waste coffee grounds (local Starbucks, 112 g), and peanut shell (63 g, Wakefield Peanut Co LP, Wakefield, VA, USA). The cover crops were planted in the field in April 2017. Eight weeks following cover crop planting, above-ground cover crop tissue was harvested on June 2017, dried (35 °C) and chopped to use as carbon sources for ASD trials. In trial 3, to simulate ethanol fermentation in soil, distiller`s yeast (Distiller's Active Dry Yeast, Red Star Yeast Co., Milwaukee, WI, USA) was mixed with carbon sources in appropriate treatments.

The trial 1 had four replicates repeated twice, but in trials 2 and 3, due to limited number of sensors and larger set of treatments, trials had two replications of each treatment in each run and these trials were repeated twice. All of the containers were arranged in trays in a completely random design. The treatments of each trial were as follows:

Trial 1: rice bran (64 g) as positive control, sorghum-sundangrass (67 g), cowpea (74 g), buckwheat (80 g), velvet bean (56 g), paper mulch (32 g), and a non-treated control. This trial was repeated twice from 7 Sep. 2017 to 27 Sep. 2017 and from 13 Oct. 2017 to 2 Nov. 2017.

Trial 2: BSG (64 g), BSG (32 g) + ethanol, paper mulch (32 g), paper mulch (16 g) + ethanol, rice bran (64 g) and rice bran (32 g) + ethanol. A 70% ethanol solution was applied at 50 ml per container, which contained 2 mg C /g soil. To keep the anaerobic condition in bioreactors, the ethanol was sprayed evenly across the soil surface using syringe through 6 spots on the top and was applied 1 week after ASD was initiated. This trial was repeated, and the trial runs were from 16 Nov 2017 to 7 Dec. 2017 and from 25 Jan. 2018 to 15 Feb. 2018.

Trial 3. waste coffee grounds (112 g) +/- yeast, paper mulch (32 g) +/-yeast, BSG (64 g) +/- yeast, BSG (32 g) +/- yeast, rice bran (64g) +/- yeast, peanut shell (63g) +/- yeast and non-treated control. Treatments with yeast had 0.06 g of yeast/bioreactor. This trial was repeated twice, once from 1 Mar. 2018 to 22 Mar. 2018 and the second time from 29 Mar. 2018 to 19 Apr. 2018. Results from trial 1 and 2 were considered when making carbon source choices for trial 3.

2.2. Inoculum preparation and sensor installation

In trial 1 and 2, 100 common chickweed (*S. media*), 100 redroot pigweed (*A. retroflexus*) and 10 yellow nutsedge (*C. esculentus*) were used. All weeds were put in one inoculum bag, and the bag was buried in the soil at 2.5 cm above the container bottom. For trial 3, two inoculum bags were used. One inoculum bag contained 10 yellow nutsedge tubers and 100 common chickweed seeds, and another inoculum had 100 white clover (*T. repens)* seeds and 100 redroot pigweed seeds. Common chickweed, redroot pigweed and white clover were procured from Herbiseed, Twyford, England. Yellow nutsedge tubers were harvested from a local farm in Virginia Beach, VA, USA.

Redox potential (Eh) sensors (ORP2000 Extended Life ORP Sensor, Sensorex, Garden Grove, CA, USA) were installed at a 15 cm depth to evaluate soil anaerobic conditions during the ASD process, and the sensors were connected to an automatic data logging system (CR-1000, Campbell Scientific, Logan, UT, USA). Soil temperature sensors (U12 Deep Ocean Temperature Data Logger, Onset, Bourne, MA, USA) were installed at a 15 cm depth in the containers. Due to the limited sensors available, redox potential was monitored only in half of the replicates per treatment in all trials and soil temperature was measured in three of the four replicates per treatment in trial 1 and two replicates in trials 2 and 3, with the sensor recording readings every 10 minutes.

2.3. ASD treatment initiation and post assessments

At the time of treatment initiation, the top surface of the containers (except the non-treated control) was covered by a piece of black VIF and sealed with duct tape. All of the containers were arranged in trays in a completely randomized design. The trays were filled with tap water and the water level was kept above the bottom of the bioreactors to maintain soil moisture content. Water levels were maintained for the duration of each trial. The non-treated controls were not saturated. Each ASD trial ran 25 days. After ASD, survival of weed seed was determined by a Tetrazolium chloride (TZ) assay (Peters, 2000), and survival rate of yellow nutsedge tubers was determined by sprouting the tubers in a growth chamber (23 °C, 16 h day length).

2.4. Statistical analyses

The data were analyzed by JMP v. 14 (SAS Institute Inc., Cary, NC, USA). The temperature data were averaged for the time of treatment duration, and cumulative redox potential was calculated based on the hourly average redox potential, and 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). Weed species data were subject to Johnson Su transformation to satisfy assumptions of normality. Thus, the original least-square means are presented in table, but the separation letters are based on transformed mean values. In trial 1, the carbon source by run interaction was not significant and data were pooled over runs. Survival of inoculum was analyzed by one-way analysis of variance (ANOVA), comparing the means of the treatments by Fisher's least significant difference (LSD) at alpha = 0.05. Factor analysis was conducted for trials 2 and 3. Due to lack of a non-treated control with ethanol or yeast, the non-treated control was not involved in factorial analysis. In trial 2, factor analysis among carbon source types, ethanol and runs were conducted. The three-way or two-way interactions showed that the run had no significant effect ($p>0.5$), and only the main effect of ethanol and carbon source type was significant. Hence, data from the two runs were pooled (including non-treated control data) to run a one-way ANOVA, and to run LSD at alpha=0.05 to compare means of the treatments. In trial 3, similar to trial 2, factor analysis was conducted only for all treatments that had carbon sources among carbon source type, yeast and runs. The two-way or three-way interactions showed the effect of runs was not significant. Hence, data from two runs, including non-treated control was pooled and a one-way ANOVA was conducted and mean separation was done using LSD test at alpha=0.05.

3. RESULTS

3.1. Weed germination

In trial 1, all ASD treatments suppressed germination of common chickweed, redroot pigweed, and sprouting of yellow nutsedge compared to the non-treated control by at least 50% (Table 2.1). There were no significant differences among carbon sources, which indicated that for further trials, the consideration of carbon sources could focus primarily on local availability and cost.

In trial 2, interaction analysis, among carbon source types, ethanol, and runs were conducted (data not presented). For redroot pigweed, only the effect of ethanol was significant on germination ($p<0.05$) when the three-way ANOVA was run, and there was no

effect of different trial runs. However, for yellow nutsedge when the three-way ANOVA was run, only the main effect of carbon source affected weed seed viability ($p=0.02$). For common chickweed, neither carbon source nor ethanol affected germination rates. Anaerobic soil disinfestation treatments reduced weed germination compared to the non-treated control. Reduced rates of BSG +ethanol application decreased the germination rate of redroot pigweed and common chickweed compared to BSG application at full rate (Table 2.2). Nutsedge emergence was not any different in BSG at full rate and BSG at half rate with ethanol. These results suggest that a mix of liquid carbon source i.e., ethanol and solid carbon source can be as effective in weed suppression as solid carbon source at full dose rate.

For trial 3, the influence of yeast on weed suppression varied with carbon sources (Table 2.3). Interaction analysis, among carbon source, yeast and runs were conducted for all four weed species. There was no effect of different runs ($p>0.6$), but the main effects of carbon source and yeast application were significant ($p<0.01$). Moreover, for yellow nutsedge, yeast x carbon source had a significant effect on the sprouting rate ($p<0.0001$). These results support the hypothesis that yeast application could enhance weed control when certain carbon sources are used for ASD. Yeast application significantly suppressed the emergence of white clover and sprouting of yellow nutsedge when the brewer`s spent grain was the carbon source. For waste coffee grounds, yeast application suppressed the emergence of all weed species except white clover. The addition of yeast improved the effect of rice bran on weed suppression for common chickweed, redroot pigweed, and white clover. All carbon sources reduced yellow nutsedge emergence over the non-treated compared to the nontreated control.

3.2. Cumulative soil anaerobic conditions

There were significant effects of carbon source on cumulative soil anaerobic conditions for all three trials (Tables 2.1,2.2 and 2.3). Overall, the non-treated control had the least anaerobic conditions. Cumulative anaerobic conditions in trial 1 were similar for all carbon sources, highest for BSG in trial 2 and, in trial 3, cumulative soil anaerobic conditions were the lowest in the non-treated control and not significantly different from paper mulch + yeast and peanut shell treatments.

3.3. Temperature

Overall, there were no significant difference in average temperature within the bioreactors/ achieved among all treatments during the ASD period. Soil temperatures at a 15 cm depth generally ranged from 9 °C to 33°C in trial 1, 9°C to 31°C in trial 2, and 15°C to 39°C in trail 3. There were no significant differences in average temperature among treatments in trial 1. In trial 2, paper mulch with ethanol had the highest average temperature. In trial 3, there was no significant differences in average temperatures among treatments.

4. DISCUSSION

In this study, all tested carbon sources had a significant effect on suppressing of chickweed, redroot pigweed, yellow nutsedge and white clover compared to the non-treated control, and some combinations, such as brewer`s spent grain with yeast inoculation, had comparable effects as rice bran. Trial 2 was conducted to test the hypothesis that ethanol supplementation to solid carbon sources could improve the weed suppression of ASD, and the addition of ethanol to reduced dosage of solid carbon sources provided similar weed efficacy as full dose rate of solid C. Ethanol application is not an economically practical application. The current price of ethanol in US is around $0.4/l (U.S. grains council, 2019), which indicates the cost of ethanol application for ASD is ~ $1.15/$m^2$, or $11,500/ha making ethanol unaffordable and impractical for growers. However, there is economic potential to develop the bioethanol fermentation in open-field conditions as an alternative to ethanol application. Trial 3 evaluated the potential of stimulation bioethanol fermentation during the ASD process, using a simple bioethanol production method, which is the distiller`s yeast that are used in craft-brewery to produce alcohol. Although the distiller's strain of yeast may not be suitable for all carbon sources used in ASD, it has been reported that BSG can generate ethanol following inoculation with yeast (Liguori et al., 2015). In trial 3, yeast application only improved weed suppression for certain carbon sources and weed species and additional research is warranted. Compared to the fumigant cost of $2700/ha (Sydorovych et al., 2006), the material cost of BSG is relatively low, which is free (Table 2.4) for the amendment and the cost of yeast is approximately $145/ha. However, the cost of labor associated with carbon sources application may be higher than using fumigant. More economically analysis is needed for local growers to make decision. The suppression of all four weed species was achieved in the present study, while previous studies indicate that ASD had an effect on suppression of several weed species except redroot pigweed (*Amaranthus retroflexus*)

(Shrestha et al., 2016). However, there is no prior study done that uses distiller`s dry yeast in the ASD process for pest management.

Although the meta-analysis study by Shrestha et al. (2016) showed that the effect of ASD on weed suppression required high temperatures (>35 °C), Shrestha et al., (2018) showed that under moderate temperatures (mean 25°C) ASD treatment did significant suppress yellow nutsedge. Moreover, there are several possible reasons why weed control was achieved in our study at low temperatures. Many studies with a higher mean temperature recorded temperature data at 10 cm depth while in this study the temperature was recorded at greater depth of 15 cm. This study maintained the anaerobic conditions by keeping a relatively high water level at around 7-10 cm, outside the container which meant that temperatures may have been moderated by the immediate environment outside the container. The high soil moisture in the soil may retard the temperature from increasing. Meanwhile, the much higher anaerobicity may promote the weed suppression under low temperature. For example, in a 3-week ASD study on suppression of yellow nutsedge (Butler et al., 2012), using molasses as carbon source, yellow nutsedge was significantly reduced under mean temperature of 25°C. However, cumulative soil anaerobicity in that study was relatively lower as compared to this study (116935 mVh vs 135000 mVh, the mean anaerobicity for all carbon sources in trial 3). Moreover, compared to some studies that achieved high temperature (>35 °C) (Blok et al., 2000, Mowlick et al., 2013, Katase et al., 2009), this study showed relatively higher anaerobicity. In addition, all of the untreated control groups were not irrigated to saturation. Thus, there is a need to study the interaction among soil moisture, soil temperature and weed suppression in ASD, especially using C mixed with yeast for ASD as well as a need to standardize the protocol at which temperature and anaerobicity data are recorded during ASD treatment. The results of this study suggest several locally carbon sources could be used in ASD to control weeds as a pre-plant treatment and indicate the potential to mix certain carbon sources with brewery yeasts. In order to optimize the ASD method for local strawberry production, the cost of ASD needs to be reduced, and the price of the carbon source is a major factor in overall cost. Thus, practices such as yeast inoculation or supplementation with reduced rates of liquid carbon sources should be considered to reduce overall costs, if efficacy can be maintained under field conditions.

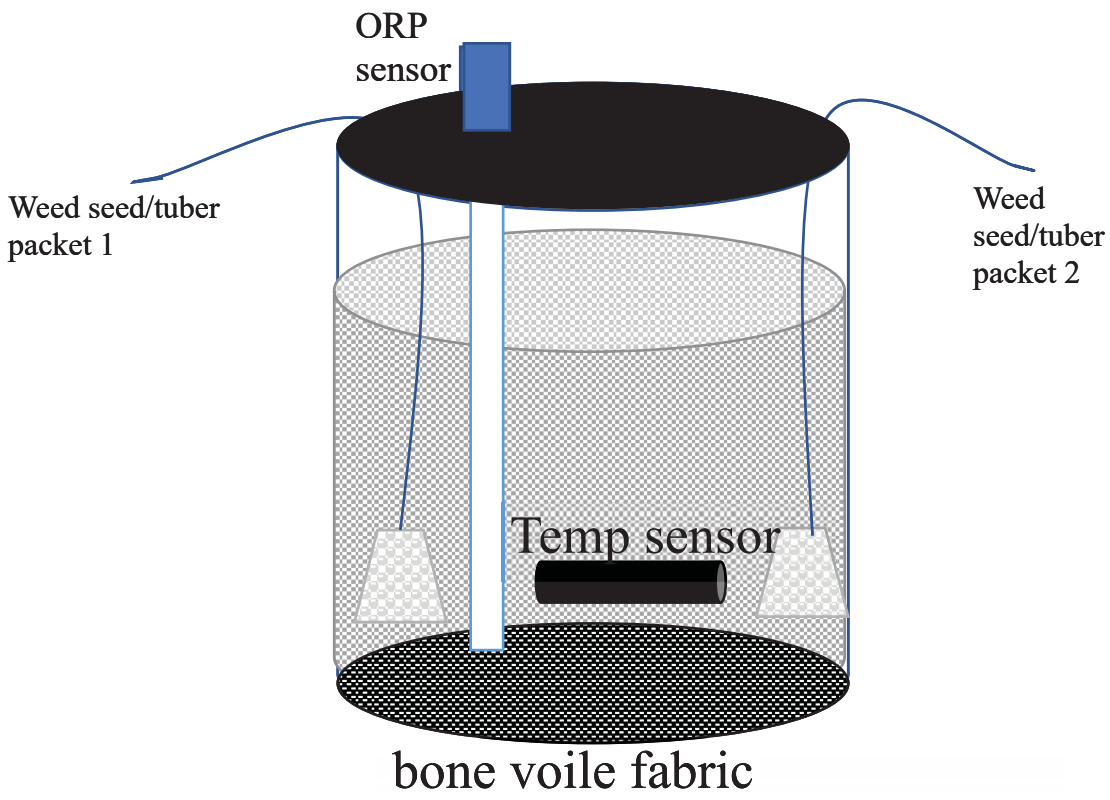

Figure 2.1. Bioreactor used in anaerobic soil disinfestation trials.

Table 2.1. Weed germination rates and cumulative soil anaerobic conditions after anaerobic soil disinfestation (ASD) process with several different carbon sources.

Treatments [a]	Weed germination rate (%) [a]			Cumulative soil anaerobic conditions (V hr)	Mean temperature(°C)
	Common chickweed	Redroot pigweed	Yellow nutsedge		
Buckwheat 80 g	23.0 b	24.0 b	5.0 bc	159 ab	20.0
Cowpea 74 g	34.0 b	33.2 b	20.0 b	261 a	20.4
Velvet bean 56 g	32.2 b	26.7 b	10.0 bc	100 ab	20.4
Paper mulch 32 g	22.5 b	22.6 b	5.0 bc	179 a	20.1
Rice bran 63 g	21.1 b	20.0 b	5.0 bc	226 a	20.5
Non-treated control	68.6 a	66.7 a	65.0 a	0 c	20.2
P value	0.010	0.007	<0.0001	<0.0001	0.29

[a] Means followed by different letters within a column are statistically different using least significance difference at P≤0.05.

Table 2.2. Weed germination rates and cumulative soil anaerobic conditions after anaerobic soil disinfestation (ASD) process with several different carbon sources and ethanol application.

Treatments [a]	Weed germination rate (%) [b]						Cumulative soil anaerobic condition (V hr)		Mean temperature (°C)	
	Common chickweed		Redroot pigweed		Yellow nutsedge					
	w/o ethanol	w/ ethanol	w/o ethanol	w/ ethanol	w/o ethanol	w/ ethanol	w/o ethanol	w/ ethanol	w/o ethanol	w/ ethanol
Brewer's spent grain	22.2 b	14.0 bc	24.5 b	11.9 d	25.0 b	28.8 b	309 a	163 d	17.4 b	17.5 b
Paper mulch	19.5 bc	17.5 bc	22.3 bc	18.9 bcd	10.0 b	5.0 b	257 c	279 b	17.3 b	23.5 a
Rice bran	15.5 bc	11.5 c	15.2 cd	11.8 d	0 c	11.3 b	150 e	91 f	17.3 b	17.3 b
Non-treated control	83.8 a	N/A	85.2 a	N/A	74.4 a	N/A	4.8 g	N/A	17.5 b	N/A
P value	<0.0001		<0.0001		<0.0001		0.01		0.01	

[a] The rates of different carbon sources were as followed: BSG 64 g without ethanol, BSG 32g with 50 ml70% ethanol, paper mulch 32g without ethanol, paper mulch 16 g with 50 ml 70% ethanol, rice bran 63g without ethanol, rice bran 31g with 50ml 70% ethanol, non-treated control without ethanol.

[b] Means followed by different letters within a column are statistically different using least significance difference at $P \leq 0.05$.

Table 2.3. Weed germination rates and cumulative soil anaerobic conditions after anaerobic soil disinfestation (ASD) process with several different carbon sources and yeast amendment.

Treatments [a]	Weed germination rate (%) [b]								Cumulative soil anaerobic conditions (V hr)		Mean temperature(° C)	
	Common chickweed		Redroot pigweed		White clover		Yellow nutsedge					
	w/o ethanol	w/ ethanol	w/o ethanol	w/ ethanol	w/o ethanol	w/ ethanol	w/o ethanol	w/ ethanol	w/o ethanol	w/ ethanol	w/o ethanol	w/ ethanol
Brewer's spent grain	22 c	17 d	25 cd	19 de	21 bc	10 ef	7 c	0 d	195 a	19 a	22	21
Coffee grounds	34 b	23 c	33 b	20 de	28 bc	18 bcd	22 b	0 d	165 ab	116 ab	20	22
Paper mulch	31 b	29 b	26 bc	25 bc	15 cdef	10 def	0 d	0 d	91 ab	51 bc	22	22
Peanut shell	23 c	14 d	20 de	17 e	16 bcde	8 f	0 d	0 d	80 bc	206 a	22	22
Rice bran	28 b	21 c	26 bc	19 de	26 bc	11 ef	0 d	0 d	154 ab	103 ab	22	22
Non-treated control	83 a	N/A	86 a	N/A	72 a	N/A	80 a	N/A	3 c	N/A	22	N/A
P value	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		0.178	

[a] The rates of different carbon sources were as followed: BSG 64 g, coffee ground 112g, paper mulch 32g, peanut shell 63g, rice bran 63g, and yeast 0.06g per treatment.
[b] Means followed by different letters within a column are statistically different using least significance difference at $P \leq 0.05$.

Table 2.4. Carbon rate, carbon to nitrogen ratio and approximate cost of evaluated carbon sources

Carbon source	C (%)	C/N ratio	Cost	References
Sorghum-Sudangrass	0.42	53	$5/kg, $100-200/ha	https://content.ces.ncsu.edu/summer-cover-crops
Cowpea	0.37	21	$5/kg, $170-250/ha	
Buckwheat	0.35	34	$3/kg, $240-300/ha	
Paper mulch	0.87	100	$1.2/kg, ~$10,000/ha	https://www.lebanonturf.com/products?cat=0
Brewer`s spent grain	0.44	14	free as byproduct	Paula Serrano-Pérez et al., 2017
Rice bran	0.45	21	~4400/ha	Shennan et al., 2018
Coffee grounds	0.25	20	free as byproduct	http://compost.css.cornell.edu/chemistry.html
Peanut shell	0.45	17	free as byproduct	Nalluri, N. and Karri, V. 2018.

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CHAPTER III: YEAST AMENDMENT WITH BREWER`S SPENT GRAIN SHOWS POTENTIAL FOR ANAEROBIC SOIL DISINFESTATION

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ABSTRACT

BACKGROUND: Anaerobic soil disinfestation (ASD) is a promising alternative to chemical fumigation to control soilborne plant pathogens and weeds. Two greenhouse studies were conducted to evaluate the effect of a locally available carbon (C) source, brewer's spent grain (BSG), for ASD on certain weeds and *Pythium irregulare*. Inoculum in each pot included common chickweed (*Stellaria media*), redroot pigweed (*Amaranthus retroflexus*), white clover (*Trifolium repens*), yellow nutsedge (*Cyperus esculentus*), and *Pythium irregulare*. The first study evaluated BSG at a standard rate (4 mg of C/g of soil) and with distiller's dry yeast. Rice bran with and without yeast was used as a comparison. The non-treated control with and without yeast was used as control treatments. The second study assessed BSG at a half and one-third dose, with or without yeast, and at a full dose without yeast. Studies were conducted in PVC tubes, 20 cm tall and 15 cm in diameter.

RESULTS: Both studies showed ASD treatments with and without yeast significantly reduced ($P \leq 0.05$) the viability of all weed species and *P. irregulare* compared to the non-treated control. Combining yeast with BSG significantly reduced seed viability of all weed species but did not reduce viability of yellow nutsedge tubers. Adding yeast to reduced rates of BSG provided similar weed control and *Pythium* suppression to that from the full BSG rate, except for common chickweed. Adding yeast with reduced rates of BSG increased suppression of all weed species and *P. irregulare* compared to BSG alone at the same rates.

CONCLUSION: Yeast enhanced the effect of BSG in ASD on both weed species and *P. irregulare* in this research, reducing the C input necessary for effective ASD. The economic cost of C input is one of the major hindrances for the widespread adoption of ASD to control soilborne pathogens. The results of this work may be useful in expanding the implementation of ASD in production agriculture.

Keywords. Carbon rate, ethanol, fumigant alternatives, weed, *Pythium*.

1. INTRODUCTION

For many specialty crop producers that cultivate perennial crops or annuals without crop rotation, the density of specific weeds, plant pathogen, and plant-parasitic nematode species can increase over time due to no crop rotation (Shennan et al., 2009). Pre-plant soil disinfestation strategies are useful and recommended for effective pest management at these farms. For the past 40 years, pre-plant soil chemical fumigation has been widely adopted in many developed countries to achieve efficient pest control and relatively higher economic return compared to non-treated soils (Muramoto et al., 2008). However, chemical fumigation could increase the environmental and human health risk, along with the increasing production costs from barrier films and expanding buffer zones (Fennimore et al., 2013; Gao et al., 2011). The chemical fumigants such as1, 3-dichloropropene (1,3-D) and chloropicrin have been used as alternatives to methyl bromide (MeBr) for pest control in strawberry and several other crops (Rosskopf et al., 2005). However, 1,3-D and chloropicrin are toxic to humans, and chloropicrin does not provide similar weed suppression as MeBr fumigation (Noling and Becker, 2005). There is a need to develop and evaluate alternative strategies for chemical fumigation.

Anaerobic soil disinfestation (ASD) was separately developed in Japan (Shinmura et al., 2000) and the Netherlands (Blok et al., 2000) and has been studied as an alternative to chemical soil disinfestation. In the U.S and the Netherlands, this method is called “anaerobic” soil disinfestation (Butler et al., 2012). The method involves using multiple tons of decomposable organic materials per hectare, irrigating to field capacity, and covering with a polyethylene tarp to limit gas and to create anaerobic conditions. Under anaerobic conditions, the carbon source is decomposed by facultative anaerobic microorganisms. Those microorganisms could produce toxic compounds that could suppress or are toxic to several soil-borne pests and diseases (Huang et al., 2015).

In general, the types and rate of carbon sources are important components of ASD. The carbon sources should contain sufficient labile carbon and have a moderate carbon, nitrogen ratio to support soil microbial growth (Momma, 2008). Currently, various labile carbon sources have been studied, such as molasses (Butler et al., 2012), rice bran (Shennan et al., 2009), wheat bran (Momma, 2008), ethanol (Uematsu et al., 2007), grass (Messiha et al., 2017), or other agricultural byproducts (Serrano-Pérez et al., 2017). Other carbon sources such as ethanol have

shown a promising effect in controlling soilborne pests (Momma, 2008; Serrano-Pérez et al., 2017). However, the high cost of ethanol makes it unrealistic for large-scale usage in the USA, and there is a regulation of ethanol applied for agricultural use (EPA, 2006). Unlike ethanol, low-cost bioethanol produced from agricultural waste, or even the byproduct from bioethanol fermentation could potentially be used in ASD. Honda et al. (2008) and Kitamoto et al. (2011) demonstrated a method to produce bioethanol under field conditions using local forage crops, and Horita and Kitamoto (2015) showed the effect of the products and residue from such bioethanol fermentation as carbon sources for ASD. Moreover, this study indicated that residue from the bioethanol fermentation productions had the potential to enhance the effect of ASD treatment. Although the carbon sources used in that study are not available or cost-effective in the US, locally available alternatives, such as brewer`s spent grain (BSG), could be used in ASD, given they have been shown to produce bioethanol when mixed with yeast (*Saccharomyces cerevisiae*) (Liguori et al., 2015).

BSG is a solid byproduct of the beer-brewing process, representing around 85% of the generated byproducts. The main composition of BSG is exhausted barley malt grain husks in a mixture with part of the pericarp and seed coat layers. Although the composition of BSG may change based on the operating conditions, BSG is generally rich in polysaccharides (cellulose and hemicellulose), proteins, and minerals (Mussatto, 2014). The fermentable polysaccharides in BSG make it a potential resource for yeast fermentation (Liguori et al., 2015). Besides, the anaerobic condition created by ASD is also aided by yeast, especially for the facultative anaerobic *S. cerevisiae* (Feldman, 2012). Liu et al. (2020) evaluated the effects of several carbon sources mixed with ethanol and yeast on weed control and found a yeast amendment enhanced the suppressing effect of ASD. However, just like applying other carbon sources in ASD, applying several tons of BSG to a field may release excess nitrogen to the environment. The excess nitrogen from ASD may also cause salt damage to crop (Muramoto et al., 2008). Moreover, excess nitrogen may cause cropping systems issues such as excessive vegetative growth, increased lodging, delayed fruit maturity, attracting insects and diseases, and enhancing weed growth (Scarsbrook, 2015). Reducing C input from ASD could not only mitigate the environmental impacts but also reduces the material cost as well as the labor cost for applying C. However, research on determining optimal C rates for consistent ASD effects is ongoing, and no results are available to suggest whether or not yeast could enhance ASD at a low C rate.

In Virginia and the mid-Atlantic region of the USA, strawberries are mostly grown using an annual hill plasticulture production system with strawberry cultivars transplanted in early fall (Christmas and Samtani, 2019). Strawberries are often planted consecutively on the same piece of land with limited time in fallow summer months to prepare the land after -harvest and disinfest the soil before the next planting. Pre-plant weed control is necessary because strawberry is adversely strongly affected by weed competition. A strawberry crop may yield less as a result of competition with annual weeds such redroot pigweed *(Amaranthus retroflexus),* shepherd's purse (*Capsella bursa-pastoris*), and common chickweed *(Stellaria media*); biennials including wild carrot (*Daucus carota*); and perennials include dandelion *(Taraxacum officinale*), quackgrass *(Elymus repens*), white clover (*Trifolium repens*) and yellow nutsedge (*Cyperus esculentus*) (Melanson et al., 2021).

Previous studies, such as a meta-analysis of 533 ASD experiments, including 88 ASD studies on weed suppression (Shrestha et al., 2016), showed the density of common lambsquarters (*Chenopodium album*) and yellow nutsedge were significantly reduced by ASD, while suppression of redroot pigweed was not achieved. Moreover, weed suppression was primarily at high soil temperature (>35°C) during ASD treatment, and only when the carbon source rate was greater than 1 kg biomass m^{-2}.

Strawberries are very susceptible to soilborne plant pathogens *(e.g., Pythium spp., Phytophthora*, *Cylindrocarpon, Colletotrichum, Macrophomina, Fusarium,* and *Rhizoctonia*). *Pythium irregulare* is the most prevalent species of *Pythium* causing black root rot, and also causes damping-off, in both greenhouse and field production (Louws and Cline, 2019). There are a few studies on *Pythium* control using ASD (Browne et al., 2018), but we found no study evaluating the effect of ASD on *P. irregulare* control. Moreover, strawberry relies on preplant soil fumigation, and the potential of using ASD on strawberry has been reported (Mazzola et al., 2018; Shennan et al., 2018). Thus, the study on *P. irregulare* control could increase the potential of ASD for strawberry soil-borne disease control in general and would extend the potential spectrum of use for ASD. In recent research, ASD has been evaluated for strawberry and several other crops in some geographic sites in the USA (Mazzola et al., 2018; Shennan et al., 2018). The research (Shennan et al., 2018) indicated that ASD with rice bran provided control of several pathogens such as *V. dahilae*, *Fusarium oxysporum,* and *Pythium* spp, and also provided

marketable strawberry yields which were equivalent to chemical fumigation. Those studies showed that ASD could be a potential and viable alternative method for strawberry growers. However, no ASD protocol has been developed for the conditions that strawberry growers in Virginia and the mid-Atlantic region face. The objective of this study was to evaluate the efficacy of BSG with yeast in ASD treatments for weed control and *P. irregulare* suppression and to determine if yeast could enhance ASD effectiveness using reduced C dose rates.

2. MATERIAL AND METHODS

2.1. Trial setup

Two greenhouse trials were initiated at the Southern Piedmont Agricultural Research and Extension Center (AREC), Blackstone, VA, starting in April 2019. In the greenhouse, 20 cm tall and 15 cm diameter hand-made bioreactors (Liu et al., 2020) were used. The bioreactor was filled with 6.8 kg of topsoil (sandy loam, pH = 6.5) from the Southern Piedmont AREC that was premixed in a tub with treatment appropriate carbon sources, and 2 L of tap water added to bring the soil to a 20% moisture content, which was the field capacity for the sandy loam soil (Blok et al., 2000). Oxidation-reduction-potential (ORP) probes and temperature sensors were buried near the bottom of the bioreactor at a 15 cm depth, and inoculum bags were placed close to the sensors. The black 1.25 mil virtually impermeable plastic film (VIF, Raven Industries Engineered Films Division, Sioux Falls, SD, USA) was secured on top of each bioreactor with duct tape for all treatments except the non-treated control. The black sheer voile fabric (Joann Fabric, Virginia Beach, VA, USA) was secured on the bottom to create a permeable layer to allow for drainage in the bioreactor.

All the carbon source values were calculated based on the recommendations of Butler et al. (2012), with a carbon rate of 4 mg carbon/g soil, or 16 t/ha in these trials. These calculations were based on a 15 cm soil depth and soil density of 1.08 g/cm^3. Dry rice bran was included as a positive control. The full dose rate of (gram/ container) carbon sources used was dry rice bran (64 g) and brewer`s spent grain (BSG, 64 g, Commonwealth Brewing Company, Virginia Beach, VA, USA). To simulate ethanol fermentation in soil, distiller`s yeast (Distiller's Active Dry Yeast, Red Star Yeast Co., Milwaukee, WI, USA) was mixed with carbon sources in appropriate treatments. The full dose rate of distiller`s dry yeast was 0.06 g/container.

Both greenhouse trials had four replicates and were conducted twice. All of the containers were arranged in trays in a completely randomized design. The treatments for each trial were as follows: Trial 1: BSG (64g), BSG (64g) + yeast (0.06g), rice bran (63g), rice bran (63g) + yeast (0.06g), non-treated control and non-treated control + yeast (0.06g). This trial was conducted from 17 April 2019 to 8 May 2019 and repeated from 6 June 2019 to 26 June 2019. Trial 2: BSG at full rate (64 g), BSG at half rate (32 g), BSG at half rate (32g) + yeast (0.03g), BSG at one-third rate (21g), BSG at one-third rate (21g) + yeast (0.02g), non-treated control and non-treated control + yeast (0.06g). The trial runs were from 18 July 2019 to 8 August 2019 and from 22 October 2019 to 13 November 2019.

2.2. Inoculum preparation and sensor installation

In both trial 1 and 2, 100 common chickweed (*S. media*) seeds, 100 redroot pigweed (*A. retroflexus*) seeds, 100 white clover (*T. repens)* seeds, 10 yellow nutsedge tubers (*C. esculentus*), and 15 millet (*Urochloa ramosa* L.) seeds infected by *Pythium irregulare* were used in each container. All weeds were put in one inoculum bag, and the millet seeds were put in another inoculum bag. Both the bags were buried in the soil at 2.5 cm above the container bottom. Common chickweed, redroot pigweed, and white clover were procured from Herbiseed, Twyford, England. Yellow nutsedge tubers in trial 1 were harvested from a local farm in Virginia Beach, VA, USA, and tubers in trial 2 were harvested from the Hampton Roads Agricultural Research and Extension Center (AREC). The tubers used in both trials were around 1cm in diameter and without abiotic and biotic damage.

The *P. irregulare* isolates were provided by Ms. Xuemei Zhang (Missi), a doctoral candidate at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, VA. *P. irregulare* was cultured on potato dextrose agar (PDA). Millet seeds were prepared by first soaking the seeds in deionized water (40 g of millet seeds were soaked in 100 ml of water to make 150 ml of millet seed-water mixture in a 250-ml flask) for an hour, draining the excess water, and autoclaving the millet seeds at 121°C for 45 min, once a day for three consecutive days. Inoculum was prepared by removing 3-5, 5 by 5 mm plugs from a PDA culture of the pathogen and mixing these with the sterilized millet seeds. The millet seeds and *P. irregular*-colonized agar pieces (8-10 pieces) were then incubated at 25°C in the dark for 2 weeks. Flasks

were shaken by hand once a day the first two days to ensure that PDA plugs were evenly distributed among the seeds. Approximately 15 colonized millet seeds were also plated onto a modified PARP medium (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) (Browne et al., 2018) at the same time that bioreactors were inoculated to confirm that *P. irregulare* remained present in the inoculated seed. The inoculum bag was prepared with nylon mesh fabric (2mm sieve) and each bag contained 15 infested millet seeds.

Redox potential (Eh) sensors (ORP2000 Extended Life ORP Sensor, Sensorex, Garden Grove, CA, USA) were installed at a 15 cm depth to evaluate soil anaerobic conditions during the ASD process, and the sensors were connected to an automatic data logging system (CR-1000, Campbell Scientific, Logan, UT, USA). Soil temperature sensors (U12 Deep Ocean Temperature Data Logger, Onset, Bourne, MA, USA) were installed at a 15 cm depth in the containers. Due to the limited sensors available, redox potential was monitored only in half of the replicates per treatment in both trials and soil temperature was measured in three of the four replicates per treatment in both trials with the sensor recording readings every 10 minutes.

2.3. ASD treatment initiation and post-assessments

At the time of treatment initiation, the top surface of the bioreactors (except the non-treated control) was covered by black VIF and sealed with duct tape. All of the containers were arranged in several trays (76 cm x 40 cm) in a completely randomized design. The trays were filled with tap water and the water level was kept above the bottom of the bioreactors to maintain field capacity. Water levels were maintained for the duration of each trial, except the non-treated controls. The non-treated controls were not covered, and the pots for the non-treated controls were raised by wooden blocks and kept away from water in the trays. The non-treated controls were watered from the top once the soil was dried. Each trial lasted for 21 days. At the end of the treatment period, the viability of weed seeds was determined by a Tetrazolium chloride (TZ) assay (Peters, 2000). The seeds of chickweed, pigweed and white clover were cut in half using a scalpel blade, and the TZ solution was dropped on the incision of the seeds. The viability of yellow nutsedge tubers was determined by sprouting the tubers in plastic nursery pots (10 cm diameter and 9 cm height, one treatment per pot, five tubers per pot) in a growth chamber (23 °C, 16 h day length). The viability of *P. irregulare* was determined by a modified PARP medium

(Browne et al., 2018). After ASD, the substances from *P. irregulare* inoculum bags were air-dried and ground using a sterile mortar and pestle. Then, ground samples (4g) were added to 20ml deionized water. The well-mixed sample solution (1ml) was transferred to a modified PARP medium using an inoculating loop. The modified PARP medium was made by combining cornmeal agar (17g/L), Tween 20 (1ml/L), Rose Bengal (25 µg/ml, a pink stain), rifampicin (10 µg/ml), ampicillin (250 µg/ml), pimaricin (5 µg/ml) and benomyl (40 µg/ml). The inoculated plates were stored in the dark at 25°C for 48h. Macroscopically visible colonies of *P. irregulare* were counted 24h and 48h after plating and reported as CFU g^{-1} dry millet seed.

The soil samples (50g/container) were collected at a 15 cm depth in trial 1 on 8 May 2019, and trial 2 on 18 August 2019. The samples were stored in 50 mL centrifuge tubes and then sent on ice bags to the University of Tennessee for determination of volatile fatty acids (VFAs) in soil. Briefly, 30-g of water saturated soil was extracted with 20-mL1M KCl (Pacharee et al., 1987) for 30-min, centrifuged, filtered (0.2µm), and analyzed for concentrations of acetic, propionic, *n*-butyric, isobutyric, valeric, and isovaleric acids by HPLC as described by Shrestha et al. (2020).

2.4. Statistical analyses

The data were analyzed by JMP v. 14 (SAS Institute Inc., Cary, NC, USA). The temperature data were averaged for the 21-day treatment duration, and cumulative redox potential (CuEh) (Butler et al., 2012) was calculated based on the hourly average redox potential, and the absolute value of the difference between each hourly average redox potential and the 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.

Data were analyzed for the treatment effect and run effect. In both trials, the treatment by run interaction was not significant ($P>0.1$) and the data were pooled over runs. Data for weed propagule viability, *P. irregulare* viability, and redox potential were subject to the Wilcoxon test given that the data did not meet the assumptions of normality. The multiple comparison method was used for nonparametric comparison for each pair using the Wilcoxon method. The

temperature data were analyzed by one-way analysis of variance (ANOVA), comparing treatment means by Fisher's least significant difference (LSD) at alpha = 0.05.

3. RESULTS

3.1. Weed and P. irregulare viability

In trial 1 (Table 3.1), all ASD treatments significantly reduced ($P < 0.05$) viability of common chickweed, redroot pigweed, white clover, and yellow nutsedge, and the survival of *P. irregulare* compared to the non-treated control with or without yeast. Compared to BSG without yeast, BSG with yeast significantly reduced the viability of all weeds except yellow nutsedge, as well as the survival of *P. irregulare*. All ASD treatments had significantly reduced yellow nutsedge viability compared to non-treated control with/without yeast. Weed viability and *P. irregulare* survival were similar for BSG without yeast to that for rice bran without yeast. *P. irregulare* survival was greater for BSG with yeast than for rice bran with/without yeast.

Reduced rates of BSG (half and one-third) with yeast provided similar weeds suppression efficacy to that from the full BSG rate without yeast in trial 2 (Table 3.2). The BSG at the full rate provided the greatest efficacy on *P. irregulare* compared to all the other treatments. The addition of yeast enhanced the efficacy of BSG at the half rate on common chickweed, redroot pigweed, white clover, and *P. irregulare*, while it did not affect yellow nutsedge sprouting. For BSG at a one-third rate, the amendment of yeast significantly improved the control of all four weed species, although it did not provide greater efficacy on *P. irregulare*.

3.2. Cumulative soil anaerobic conditions

The redox potential during the 21 days treatment period was significantly ($P<0.0001$) lower (i.e., more anaerobic) for all ASD treatments compared to the nontreated control with/without yeast in both trials (Table 3.1 and 3.2). The amendment of yeast did not increase cumulative soil anaerobic conditions for any treatments statistically. The BSG with/without yeast at the full rate had numerically higher cumulative soil anaerobic conditions than rice bran with/without yeast.

3.3. Temperature

Overall, there was no significant difference in average soil temperature within the bioreactors among all treatments during the ASD period in both trials (Table 3.3 and 3.4). Soil temperatures at a 15 cm depth generally ranged from 17 °C to 43 °C in trial 1, and 20 °C to 43 °C in trial 2. The mean temperature for all treatments in trial 1 was around 26 °C, and 29 °C in trial 2.

3.4. Volatile fatty acids

The results (Table 3.5 and 3.6) showed that acetic acid (AA), n-butyric acid (BA), propionic acid (PA), isobutyric acid (IBA), and isovaleric acid (IVA) were found in relatively high concentrations in the soil during ASD compared to the non-treated control. In trial 1, the BSG treatments without yeast had the highest concentrations for AA, BA, PA, and IBA. The results indicated that BSG with yeast had lower (significantly in AA, PA, IBA, IV, and numerically in BA) VFAs concentrations than BSG without yeast. Conversely, the yeast amendment enhanced the IVA concentrations for rice bran treatments, and numerically increased AA and BA concentrations for both rice bran treatments and non-treated control. In trial 2, BSG at the full rate was the only treatment that generated all five kinds of VFAs. Meanwhile, BSG at the full rate had the numerically highest concentrations for AA, BA, PA and IBA, and significantly higher concentration for IV.

4. DISCUSSION AND CONCLUSIONS

In this study, brewer`s spent grain at three different dose rates significantly reduced viability of common chickweed, redroot pigweed, white clover, yellow nutsedge, and the survival of *P. irregulare* compared to the non-treated control. Moreover, brewer`s spent grain with yeast inoculation had effects comparable to rice bran. Weed suppression similar to rice bran was also reported by other researchers for common chickweed seed (Zeeland et al., 2004) and yellow nutsedge tubers (Muramoto et al., 2008; Shrestha et al., 2018). However, the reduction of redroot pigweed seen was not consistent with results from another research (McCarty, 2012). The main difference between our studies and the cited literature is carbon sources used, which may affect weed control efficacy. For example, our studies use BSG as a carbon source, while

the cited studies (Muramoto et al., 2008; Zeeland et al., 2004; McCarty, 2012; Shrestha et al., 2018) used carbon sources such as cover crops (i.e., *Brassica juncea, Sinasis alba, Eruca sativa,* and *Secale cereale*), molasses, or a green manure crop (*Festuca perennis* Lam.). The different moisture, texture, mixture, and rates of carbon sources may cause differences in weed control. There is no other research on the effect of ASD on *P. irregulare,* while there are several research reports on other *Pythium* species and *Phytophthora* such as *P.intermedium* (Van Os et al., 2015), *P. ultimum* (Browne et al., 2018; Strauss et al., 2017) and *Phytophthora nicotianae* (Schonbeck, 2019). The results of van Os et al., 2015 also indicated that microbial volatiles played an important role in suppressing pathogens, such as *Pythium*. That finding may support the significant P. *irregulare* suppression in our studies, and also indicates that more research to investigate the mechanisms of ASD suppresses pathogens by microorganism activities or by their metabolites such as VFAs.

Although the precise mechanism of ASD is not fully understood, VFAs have been found in various ASD studies, and have been reported as an important component of control for some soil-borne pathogens during ASD (Blok et al., 2000; Huang et al., 2015, Momma, 2008). The VFAs could become lethal to many soilborne pathogens, which could be due to their ability to readily move across cell membranes, acidifying the cell cytoplasm (Browing et al., 2006). Our study showed multiple VFAs were generated during ASD, similar to previous literature (Huang et al., 2015; Shrestha et al., 2018). However, amending BSG with yeast did not increase VFAs concentrations. One possible explanation could be that other organisms compete with carbon for labile carbon, and those organisms produce fewer VFAs due to metabolizing less carbon. As ASD is initiated, organisms such as *Clostridium, Enterobacter,* and *Acetobacter* rapidly reproduce and break down carbon sources into VFAs, alcohols, and CO_2 (Momma, 2008). The competition between yeast and these other microorganisms may occur at this stage. After that, those products such as VFAs could be utilized by facultative anaerobic organisms such as *Bacillus* species, which have been reported as biocontrol organisms (Huang et al.,2015; Nakano and Zuber, 1998). Yeast may enhance alcohol production and provide more labile carbon for such biocontrol organism growth. However, while the yeast amendment enhanced weed control in our research, it did not enhance VFAs concentrations, indicating that the VFAs concentrations may not be the predominant mechanism for weed suppression. The biological effects may directly affect weed seeds and tubers, but more research is needed to verify such conclusions.

Previous research (Shrestha et al., 2018) showed that yellow nutsedge viability changes might differ based on the carbon-nitrogen ratio or soil depth. Compared to the higher carbon-nitrogen ratio treatments (40:1), lower carbon-nitrogen ratio treatments (10:1) had lower yellow nutsedge sprouting and reproduction rates after ASD. Previous research (Shrestha et al., 2018) also showed that the emergence of yellow nutsedge tubers was greater at a 5 cm depth compared to at 15 cm depth with ASD. Other research (Muramoto et al., 2008) also showed yellow nutsedge tubers at a 2 cm depth where the soil is slightly drier, had higher emergence rates with ASD compared to tubers at a 15 cm depth. It is possible that the soil moisture and anaerobicity are different at different soil depths, which may lead to various organic acids or another ASD produces concentrations at different soil depths. Such variable concentrations and anaerobicity may differ the weeds or pathogens suppression effect. Combining those research trials and our studies, we could hypothesize that the lack of oxygen, along with increased anaerobic by-products, and anaerobic microbial activity at greater depths, may increase tuber mortality, even though yellow nutsedge tubers have a high flooding tolerance (Schonbeck, 2019). The research on the distribution of ASD products and related soil physical and chemical parameters at different soil depths is needed to better understand the ASD behavior in soil.

Our studies showed ASD treatments resulted in a moderate mean soil temperature (25°C and 30°C) compared to a higher temperature (>35 °C) observed in other studies (Butler et al., 2012). However, a meta-analysis by Shrestha et al. (2016) showed that the effect of ASD for weed control required higher temperatures (>35 °C). There are several hypotheses for effective weed control at low or moderate temperatures. Unlike many studies that recorded soil temperature data at a 10 cm depth, the temperature data in this study was recorded at a greater depth of 15 cm. Moreover, in many reported studies, the containers were flooded only at the beginning of the ASD period to create the anaerobic condition; in our study, the containers were immersed in water and maintained at a relatively high water level at around 7-10 cm from the bottom of the container for 21 days to keep the anaerobic condition in the container. The water could enter containers through the fabric-covered bottom opening, which meant that soil temperatures might have been moderated in our studies by the immediate environment surrounding the container. The high soil moisture level may mitigate the soil temperature changes. Another explanation could be that the much greater anaerobic conditions promoted weed suppression under moderate temperature. Compared to some studies that had effective

weed control at higher temperatures (>35 °C) (Katase et al., 2009; Mowlick et al., 2013), this study showed relatively higher anaerobic conditions (100-300 Vh versus 40-50 VH). While a low oxygen condition was generated in our studies, similar to other research using BSG (Strauss et al., 2017), there was no strong correlation between weed control and CuEh, as with other studies (Butler et al., 2012; McCarty, 2012). This lack of correlation indicates that strong anaerobicity may not be a direct factor for weed control. There is a need to study the interaction among soil moisture, soil temperature, and weed suppression in ASD under field conditions.

Suppression of all four weed species and *P. irregulare* was achieved in the present study, and the effect of distiller`s dry yeast enhancing weed suppression in ASD was observed. Compared to the fumigant material cost of $1,000-1,800/ha (Sydoroyych et al., 2006), the material cost for BSG applied with yeast is relatively inexpensive (BSG currently free, and $145/ha for yeast). However, the material cost for BSG would likely increase as soon as demand increases significantly. The labor costs associated with the transportation and application of large volumes of carbon sources could make ASD less economical, but the strategy of reducing carbon dose rates using yeast could make ASD more affordable. Brewer's spent grain contains organic nitrogen which could substitute for the application of synthetic fertilizer. For example, the full rate BSG at 5t/ha could provide approximately 350 kg/ha nitrogen. The general recommended preplant nitrogen application rate for strawberry is 68 kg/ha, which is far less than the nitrogen from BSG. The half-rate of BSG could also provide nearly 125 kg/ha nitrogen. Although the nitrogen from BSG would not be fully decomposed, the preplant nitrogen fertilizer might not be necessary if only 20% of the nitrogen supplied by a full rate of BSG were available during the first five months, offsetting the cost of ASD by approximately $ 100/ha. Additionally, BSG also contains several other nutrients such as silicon, phosphorus, and calcium (Mussatto, 2014). Thus, the use of BSG may potentially reduce fertilizer cost or increase soil fertility. The research on the short-term and long-term impacts of ASD on soil nutrient availability are needed. Future research is also needed, which would focus on whether the nutrients from carbon sources in ASD have potential impacts on the growth of strawberry crop, such as root and crown growth, canopy development, and timing and dynamics of blooming, flowering or fruit set.

The application of yeast in ASD could reduce the dosage of carbon sources needed for effective ASD, subsequently reducing the cost. Future research is needed on developing

techniques of yeast application, such as adjusting the rate of yeast, priming yeast before application, or pre-treating carbon sources to increase their nutritional suitability for yeast. For example, BSG has a high content of hemicellulose and cellulose fractions, while yeast primarily uses glucose resulting from cellulose breakdown. Acid or enzymatic pre-treatment could enhance the conversion of cellulose to glucose (Mussatto, 2014). Such treatment may also be efficacious for other carbon sources, such as cover crops or crop residues. Thus, further research on the behavior of BSG at reduced rates in field conditions is necessary. Meanwhile, the economic analysis of ASD for large scale field production is also essential.

In conclusion, amending BSG with yeast could enhance suppression of weeds and *P. irregulare* with reduced rates of BSG, making it possible to reduce the carbon rates necessary for ASD. This could have important implications in reducing the carbon dosage needed for ASD in field conditions, potentially improving the economic viability of this non-fumigant system.

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Table 3.1. Weed viability, *P. irregulare* viability and cumulative soil anaerobic conditions after treatment completion with different carbon sources and yeast in trial 1.

Treatment [a]	Weed viable rate (%) [b]				*P. irregulare* viability (CFU g^{-1})	Cumulative soil anaerobic conditions (V hr)
	Common chickweed	Redroot pigweed	White clover	Yellow nutsedge		
Brewer`s spent grain full						
Without yeast	21.0 b	27.0 b	21.0 b	2.5 b	51.0 b	183.7 a
With yeast	14.0 c	15.0 c	11.0 c	0.0 b	28.0 c	175.9 a
Rice bran						
Without yeast	24.0 b	23.0 b	13.0 c	0.0 b	53.0 b	144.8 a
With yeast	18.0 c	20.0 c	15.0 c	0.0 b	52.0 b	96.5 a
Non-treated control						
Without yeast	73.0 a	74.0 a	82.0 a	75.0 a	164.0 a	5.0 b
With yeast	65.0 a	68.0 a	78.0 a	70.0 a	172.0 a	4.2 b
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a] The rates of carbon sources and yeast/container were as follows: brewer spent grain 64g, rice bran 63g, yeast 0.06g.
[b] Means followed by different letters within a column are statistically different using the Wilcoxon test at $P \leq 0.05$.

Table 3.2. Weed viability and cumulative soil anaerobic conditions after anaerobic soil disinfestation (ASD) process with several different C dose rates and yeast amendment in trial 2.

Treatment [a]	Weed viability rate (%) [b]				*P. irregulare* viability (CFU g^{-1})	Cumulative soil anaerobic conditions (V hr)
	Common chickweed	Redroot pigweed	White clover	Yellow nutsedge		
Brewer`s spent grain full [c]						
Without yeast	17.0 c [d]	19.0 c	25.0 c	3.0 c	46.3 e	315.7 a
Brewer's spent grain half						
Without yeast	31.0 b	48.0 b	47.0 b	9.0 c	87.5 c	273.7 a
With yeast	21.0 c	19.0 c	25.0 c	9.0 c	70.0 d	223.3 a
Brewer`s spent grain one-third						
Without yeast	33.0 b	44.0 b	44.0 b	20.0 b	68.8 d	142.9 a
With yeast	24.0 c	22.0 c	25.0 c	6.0 c	61.9 d	321.9 a
Non-treated control						
Without yeast	77.0 a	73.0 a	77.0 a	73.0 a	186.9 a	22.2 b
With yeast	75.0 a	75.0 a	74.0 a	66.0 a	147.5 b	5.9 b
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a] The rates of carbon sources and yeast/container were as follows: brewer`s spent grain full rate 64g, brewer`s spent grain half rate 32g, half rate yeast 0.03g, brewer`s spent grain one-third rate 21g, one-third rate yeast 0.02g, non-treated control with yeast 0.06g.
[b] Means followed by different letters within a column are statistically different using the Wilcoxon test at $P \leq 0.05$.
[c] There was no full rate BSG with yeast treatment for this variable.
[d] Means are statistically different from the mean for brewer`s spent grain and rice bran with/without yeast using the Wilcoxon test at $P \leq 0.05$.

Table 3.3. Mean, minimum and maximum temperatures pooled over the two runs for 3-week periods involved in the anaerobic soil disinfestation (ASD) process with several different carbon sources and yeast amendment in trial 1.

Treatment [a]	Temperature (°C)					
	Mean for three weeks	Mean for week 1	Mean for week 2	Mean for week 3	Minimum for three weeks	Maximum for three weeks
Brewer`s spent grain full						
Without yeast	25.9 [b]	25.3	25.5	27.1	17.4	42.2
With yeast	26.6	25.8	26.0	28.0	18.1	43.3
Rice bran						
Without yeast	26.3	25.7	25.7	27.7	17.8	43.1
With yeast	26.6	25.9	26.1	28.0	17.5	41.2
Non-treated control						
Without yeast	25.7	24.9	25.8	26.4	18.2	41.7
With yeast	26.1	25.2	25.7	27.4	17.3	40.2
P-value	0.45	0.26	0.96	0.06	0.56	0.32

[a] The rates of carbon sources and yeast were as followed: brewer spent grain 64g, rice bran 63g, yeast 0.06g.
[b] There were no significant differences amongst mean values within a column.

Table 3.4. Mean, min and max temperature of two runs for 3-weeks period anaerobic soil disinfestation (ASD) process with several different C dose rates and yeast amendment in trial 2.

Treatment [a]	Temperature (°C)					
	Mean for three weeks	Mean for week 1	Mean for week 2	Mean for week 3	Minimum for three weeks	Maximum for three weeks
Brewer`s spent grain full						
Without yeast	28.7 [b]	29.2	29.5	27.1	19.5	41.5
With yeast	*Nt* [c]	*Nt*	*Nt*	*Nt*	*Nt*	*Nt*
Brewer's spent grain half						
Without yeast	29.5	30.0	30.5	27.9	19.3	43.9
With yeast	30.0	30.6	30.6	28.7	18.9	44.5
Brewer`s spent grain one-third						
Without yeast	29.4	30.0	30.2	28.0	18.8	43.1
With yeast	29.4	30.1	30.1	28.0	19.1	42.5
Non-treated control						
Without yeast	28.7	29.2	29.5	27.1	19.5	41.5
With yeast	28.5	28.9	29.2	27.0	20.9	40.4
P-value	0.38	0.34	0.29	0.39	0.85	0.17

[a] The rates of carbon sources and yeast per container were as follows: brewer`s spent grain full rate 64 g, brewer`s spent grain half rate 32 g , half rate yeast 0.03g, brewer`s spent grain one-third rate 21g, one-third rate yeast 0.02 g, non-treated control with yeast 0.06 g.
[b] There were no significant differences amongst mean values within a column.
[c] There was no full rate BSG with yeast treatment for this variable; *Nt* indicates that the treatment was not included in the experiments.

Table 3.5. Volatile fatty acids concentration for different carbon sources and yeast amendment in trial1.

Treatment [a]	Acetic acid (mmol kg^{-1} of soil)	n-butyric acid (mmol kg^{-1} of soil)	Propionic acid (mmol kg^{-1} of soil)	Isobutyric acid (mmol kg^{-1} of soil)	Isovaleric acid (mmol kg^{-1} of soil)
Brewer`s spent grain full					
Without yeast	2.80 a [b]	0.92 a	0.15 a	0.29 a	0.17 b
With yeast	1.87 b	0.60 a	0.10 b	0.12 bc	0.08 bc
Rice bran					
Without yeast	0.30 c	0.05 b	0.03 c	0.01 d	0.07 bc
With yeast	0.86 c	0.21 b	0.03 c	0.06 cd	0.33 a
Non-treated control					
Without yeast	0.06 c	0.00 c	0.01 c	0.00 d	0.00 c
With yeast	0.38 c	0.12 b	0.02 c	0.00 d	0.06 bc
P-value	<0.0001	<0.0001	<0.0001	<0.0001	0.008

[a] The rates of carbon sources and yeast per container were as follows: brewer spent grain 64 g, rice bran 63 g, yeast 0.06 g.
[b] Means followed by different letters within a column are statistically different using Wilcoxon test at $P \leq 0.05$.

Table 3.6. Volatile fatty acids concentration for different carbon sources dose rates and yeast amendment in trial 2.

Treatment [a]	Acetic acid (mmol kg^{-1} of soil)	n-butyric acid (mmol kg^{-1} of soil)	Propionic acid (mmol kg^{-1} of soil)	Isobutyric acid (mmol kg^{-1} of soil)	Isovaleric acid (mmol kg^{-1} of soil)
Brewer`s spent grain full					
Without yeast	0.80 a [b]	0.23 a	0.20 a	0.24 a	3.90 a
With yeast	*Nt* [c]	*Nt*	*Nt*	*Nt*	*Nt*
Brewer's spent grain half					
Without yeast	0.07 a	0.00 a	0.00 a	0.00 a	0.50 b
With yeast	0.24 a	0.00 a	0.00 a	0.00 a	0.50 b
Brewer`s spent grain one-third					
Without yeast	0.19 a	0.07 a	0.00 a	0.09 a	1.50 b
With yeast	0.11 a	0.00 a	0.00 a	0.04 a	1.63 b
Non-treated control					
Without yeast	0.00 a	0.00 a	0.00 a	0.00 a	0.00 b
With yeast	0.00 a	0.00 a	0.00 a	0.00 a	0.00 b
P-value	0.25	0.47	0.08	0.27	0.0024

[a] The rates of carbon sources and yeast per container were as follows: brewer`s spent grain full rate 64 g, brewer`s spent grain half rate 32 g, half rate yeast 0.03 g, brewer`s spent grain one-third rate 21g, one-third rate yeast 0.02 g, non-treated control with yeast 0.06 g.
[b] Means followed by different letters within a column are statistically different using Wilcoxon test at $P \leq 0.05$.
[c] There was no full rate BSG with yeast treatment for this variable; *Nt* indicates that the treatment was not included in the experiments.

CHAPTER IV: EFFECT OF ANAEROBIC SOIL DISINFESTATION WITH YEAST AMENDMENT ON WEED CONTROL AND STRAWBERRY YIELD

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ABSTRACT

Anaerobic soil disinfestation (ASD) is a promising alternative to chemical fumigation to control soil-borne plant pathogens and weeds. Field trials were conducted over two growing seasons to evaluate ASD using brewer's spent grain and yeast inoculation in open-field annual hill strawberry production. Each replicate was comprised of a 10.7 m long bed , 0.7 m wide at the top. Strawberry plugs were transplanted into the center 4.6 m (2018/19 growing season) or 3.6 m (2019/20 growing seasons) of each replicate. There were seven treatments including ASD at full (4 mg carbon g^{-1} of soil) and half carbon (2 mg carbon g^{-1} of soil) amendment rates with and without yeast, fumigation with chloropicrin (80%) + 1,3-dichloropropene (20%) at 196 kg/ha, and non-treated controls with and without yeast. The duration of the ASD was 21 days. The soil temperature and anaerobic conditions were measured during the ASD period at 15 cm depth. The weed density was evaluated from January to March in each growing season. The strawberry yield data were collected over the harvest season (April to June), and strawberry fruit size, firmness, and sugar content were also evaluated. Over both seasons, plots treated with all ASD treatments consistently and significantly had lower density of most of the broadleaf weed species and total weed density compared to non-treated plots. Strawberry marketable and total yield in 2018/19 growing season was not significantly different among all ASD treated plots and those non-treated plots, while all ASD treated plots had a higher marketable and total yield than non-treated control in 2019/20. In 2019/20, yeast addition enhanced the crop yield of the plot treated with ASD treatments compared to those without yeast. Therefore, the yeast amendment had the potential to enhance ASD using brewer's spent grain and paper mulch as carbon sources for weed control and improvement of strawberry yield. Half carbon rate of ASD with yeast could be a potential strategy to reduce the carbon input.

Keywords: broadleaf weeds, fumigant alternatives, fruit firmness, total soluble solids, weed density,

1. INTRODUCTION

In Virginia and the USA's mid-Atlantic region, strawberries are mostly grown using an annual hill plasticulture production system with strawberry cultivars plants transplanted in early fall (Christmas and Samtani, 2019). Many small acreage farms grow strawberries without annual crop rotation or cover crop adoption. The long-term lack of rotation with cover crops negatively impacts soil health and increases disease occurrence. Black root rot is a common disease for strawberry production in many states. Black root rot is a disease complex involving infection by multiple fungi and oomycetes and root-infecting nematodes. Fungi include oomycetes include various *Pythium* spp. [including *Pythium irregulare* Buisman, (1927)], *Rhizoctonia* spp. and *Fusarium* spp. *Pratylenchus* spp. are also often involved. Black root rot is estimated to reduce strawberry yields by as 20% to 40%, and for this reason pre-plant soil fumigation is routinely practiced in the region (Louws and Cline, 2019).

Strawberry is also strongly affected by weed competition. The competition between strawberries and annual weeds can result in loss of yield and reduced net return (Pritts and Kelly, 2004). Troublesome annual weeds include common chickweed (*Stellaria media* L. Vill.), redroot pigweed (*Amaranthus retroflexus* L.) and shepherd's purse (*Capsella bursa-pastoris* L. Medik.), and biennials include wild carrot (*Daucus carota* L.); and perennials include dandelion (*Taraxacum officinale* L. Weber ex F.H.Wigg.), quackgrass (*Elymus repens* L. Gould), white clover (*Trifolium repens* L.) and yellow nutsedge (*Cyperus esculentus* L.) (Melanson et al., 2021).

Although weed control is essential, the few herbicides registered for strawberry plasticulture production make weed control a nationwide challenge. The few options of herbicides are possibly caused by the lack of interest from chemical companies to register herbicides for many specialty crops. Options for strawberry weed control will likely remain limited (Fennimore and Doohan, 2008). Currently, registered herbicides have some limitations. For example, many herbicides have a 30 day preplant application period (oxyfluorfen and flumioxazin), while others have continued to cause phytotoxicity (flumioxazin and napropamide) to strawberry plants (Melanson et al., 2021). Therefore, there is an increasing need to develop reliable alternative weed control practices.

Anaerobic soil disinfestation (ASD) has demonstrated efficacy in suppressing many soilborne pests across a diversity of cropping systems and environments (Shennan et al.,

2014; Shennan et al., 2018; Shrestha et al., 2018a). The method involves using several tons of decomposable organic materials per hectare, irrigating to field capacity, and covering with polyethylene film to block gas diffusion and create an anaerobic condition. Under anaerobic conditions, the carbon source is decomposed by facultative and obligate anaerobic microorganisms. Those microorganisms could produce toxic or suppressive compounds such as organic acids, aldehydes, alcohols, metal ions, and volatile organic acids (Huang et al., 2015).

In general, the types and rate of carbon sources are essential components of ASD. The carbon sources should contain sufficient labile carbon and have a moderate carbon to nitrogen (N) ratio to support soil microbial growth (Momma, 2008). Brewer's spent grain (BSG) is a solid byproduct generated from the beer-brewing process. The BSG represents around 85% of the total byproducts of brewing. The main composition of BSG includes exhausted grain husks obtained after mashing and lautering. BSG could be a potential carbon source of ASD for three reasons: (1) BSG is currently available free of material costs, as BSG is a waste from beer production. There is an increasing number of draft-breweries in Virginia and neighboring states. (2) BSG can produce ethanol when mixed with distiller's yeast (*Saccharomyces cerevisiae* Meyen ex E.C. Hansen) (Liguori et al., 2015), and ethanol is a useful carbon source for ASD (Momma et al., 2013). Due to ethanol's high cost, using low-cost BSG to produce bioethanol under field conditions could be feasible. Moreover, Horita and Kitamoto (2015) showed that organic residue from bioethanol fermentation had the potential to enhance ASD treatment. (3) Fresh BSG has an optimal carbon-to-nitrogen ratio (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., 2018). Liu et al. (2020) evaluated the effect of several carbon sources mixed with ethanol and yeast on weed control under controlled environment conditions, which indicated the potential of a yeast amendment in ASD to enhance the suppression effect of ASD. However, there is no research on the performance of ASD with BSG and yeast under field conditions. There is no reported research on results from ASD at a low carbon rate under field conditions. Thus, this study's objective was to evaluate weed density, crop nitrogen status, and strawberry yield following anaerobic soil disinfestation using BSG and yeast inoculation in open-field annual hill production and to determine if yeast could enhance ASD effectiveness when ASD is applied at a half carbon rate.

2. MATERIALS AND METHODS

2.1, Experimental Design

Field trials were conducted at the Hampton Road Agricultural Research and Extension Center, Virginia Beach, Virginia (36°9′N, 76°2′W) during the 2018/19 and 2019/20 growing seasons. Both trials were arranged in a randomized complete block design with four replicates. There were seven treatments in both growing seasons, with each replicate comprising a bed 10.7 m long, 0.7 wide on the bed tops, and 0.15 m high. The beds were oriented north-south. All seven treatments (Table 4.1) were randomized in each block. During the 2018/19 growing season, the center 4.6 m length of each bed was used for strawberry plug transplanting and data collection. During the 2019/20 growing season, the center 5.1 m length of each bed was used for strawberry plug transplanting. The center 5.1 m length section was divided into a 3.6 m length section and a 1.5 m length section. The 3.6 m length section was used for fruit harvest and data collection, while the 1.5 m length section was only used for nitrogen estimation due to the destructive sampling of crop plants involved in estimating nitrogen. In the 2018/19 growing season, three blocks had strawberry cultivation history, and one block was primarily grassy vegetation that was maintained by mowing. Preplant soil tests were conducted in both trials by sending soil samples to the Virginia Tech. Soil Testing laboratory in late July. The soil test report provided recommendations for pH adjustment and preplant fertilizers. The soil type at the site was Tetotum loam (sandy loam, moderately well-drained, deep, parent material: loamy, fluvial, and marine sediments) (USDA-NRCS,2004). The soil pH was adjusted to 6.2 by broadcasting limestone at 898 kg/ha in early August 2018. Total carbon and nitrogen in BSG (Commonwealth Brewing Company, Virginia Beach, VA, USA) samples were sent to the University of Tennessee (NC Soil Analyzer, CE Elantech, Lakewood, NJ, USA) for C: N ratio determination in late July 2018. The recommended rate of carbon source in ASD is 4 mg C g^{-1} soil (Shennan et al., 2018). The BSG had a C: N ratio of 14:1, which indicated that BSG would provide 271 kg ha^{-1} organic N to the soil at the 4 mg C g^{-1} soil rate. The total N from BSG was much higher than the soil test recommendation and recommendation from the regional production guide (Poling et al., 2005). Thus, to reduce the N added to the soil, the BSG was mixed with pelleted paper mulch (C: N ratio 57:1, Lebanon Seaboard Corporation, Lebanon, PA). However, there was three times more paper mulch than BSG in the mixture when the N rate was 78 kg ha^{-1}, which was the N rate from the soil test recommendation. This study primarily focused on the effect of BSG, so that there was a need to increase the BSG

volume at least equal to the paper mulch volume. The C: N ratio of BSG (14:1) is much lower than paper mulch (57:1). Thus, when the carbon source mixture was at a constant carbon rate, the proportion of BSG increased compared to paper mulch along with the increase of the total N rate. To reach a balance between the BSG volume and total N rate, the N rate used in this study for all plots was 117 kg N ha^{-1}. There was 60% (w/w) fresh BSG in the mixture at the full carbon rate at that N rate, and 97% (w/w) fresh BSG in the mixture at half carbon rate. Synthetic preplant N fertilizers (urea, 46-0-0, PCS Sales, Inc, Northbrook, IL, USA) at the above rate were only applied to non-treated control plots and plots treated with 1,3-D + Pic (80% chloropicrin + 20% 1,3-dichloropropene) at 196 kg ha^{-1}. No phosphorus or potassium was needed as determined by the soil test. The amendments (carbon sources, yeast, or fertilizers, Table 4.1) were applied broadcast manually before final bedding on 29-30 August 2018 and 30 August 2019. The amendments were incorporated to a 15 cm depth by a machine that cultivated, shaped beds, installed drip tapes and laid plastic film in one pass. All beds, including non-treated controls, were covered with a 0.03 mm virtually impermeable film (TriEst Ag Group, Inc, Greenville, NC, 27835, US). A 0.38 mm single drip line with a 30.5 cm emitter spacing (Chapin; Jain Irrigation, Inc., Watertown, NY, USA) was used to saturate the soil for ASD and irrigate and fertigate the beds during the growing season. The drip line was approximately 5 cm depth under the bed surface.

On 5 September 2018 and 5 September 2019, the redox potential sensors (ORP sensor, ORP2000 Extended Life ORP Sensor, Sensorex, Garden Grove, CA, USA) were installed at the center of the bed at a 15 cm depth to evaluate soil anaerobic conditions during the ASD period, and the sensors were connected to an automatic data logging system (CR-1000, Campbell Scientific, Logan, UT, USA). Soil temperature sensors (U12 Deep Ocean Temperature Data Logger, Onset, Bourne, MA, USA) were installed simultaneously and in the same depth as the ORP sensors. Due to the limited sensors available, ORP sensors and temperature sensors were installed only in half of the four blocks for both trials. Each of the treatments except 1,3-D + Pic had two plots containing sensors. The 1,3-D + Pic plots had no sensors set up. Both sensors recorded readings every 60 minutes for the duration of the treatments.

On 5 September 2018 and 5 September 2019, the ASD treatments were initiated by irrigating the beds to maintain the soil moisture content at field capacity (23%) (Elmore et al., 1997). The soil moisture prior to ASD initiation was approximately 13% for both trials. The

soil moisture was measured by the Field Scout TDR 100 soil moisture meter (Spectrum Technologies, Inc., Aurora, IL). The TDR meter was used periodically over the 21-days ASD period to determine the irrigation times and durations. Due to the landing of Hurricane Florence in September 2018, all the sensors and data loggers were temporarily removed from beds from 12 September 2018 to 15 September 2018. For the following 2019/20 growing season, some hurricane precautions such as waterproof sealant for dataloggers and portable batteries were applied before hurricane Dorian landed in September 2019. Thus, the recordings in the 2019/20 growing season were not interrupted. The 1,3-D + Pic was applied at 196 kg ha-1 on 7 September in both growing seasons. On 26 September 2018 and 27 September 2019, the 21-days ASD period ended, and the sensors were removed. Meanwhile, the planting holes were made. Prior to punching holes for transplanting strawberries, Italian ryegrass [*Lolium perenne* ssp. *multiflorum* (Lam.) Husnot] was seeded at 280 kg ha^{-1} as a cover crop in furrows between strawberry beds on 21 September 2018 and 26 September 2019. Italian ryegrass was mowed as needed throughout the strawberry growing season.

After completing preplant treatments, 'Ruby June' strawberry plugs (Aarons Creek Farms, Buffalo Junction, VA, USA) were transplanted in all beds on 9 October in both growing seasons, in two rows at a 36 cm in-row spacing. There were 16 plugs per bed in the 2018/19 growing season and 18 plugs per bed (12 plugs used for harvest and fruit quality data collection, six plugs used for N estimation only) in the 2019/20 growing season. For both growing seasons, typical post-planting fertilization, pest, and irrigation management practices for conventional strawberries in the region (Poling et al., 2005) were conducted unless otherwise stated.

2.2. Weed Density

The 1.5 m long by 0.7 m wide viewing window at the top of each bed was established on 2 October 2018 and 4 October 2019. The weed viewing window was created by replacing the black 0.03 mm VIF tarp (TriEst Ag Group, Inc, Greenville, NC, 27835, USA) with a 0.025 mm clear tarp (Robert Marvel Plastic Mulch, LLC, Annville, PA, 17003, USA). This was done to separate the treatment effect from the suppressive effects of the black plastic tarp. The dates for weed evaluation were chosen when the emerged weeds in most window areas appeared to be competing with the crops or when the weed canopy covered approximately 50% of the window area. On each evaluation date, the emerged weeds in the window area were identified and counted by species. Both the weed counts by species for each treatment

from each evaluation date and the total counts for each treatment from each evaluation date were summed separately. The summed total counts were recorded as cumulative weed density. After each evaluation, all counted weeds in the window area were carefully removed by hand to keep the entire weed plant, including shoot and root. Replicates were recorded, and the fresh and dry biomass of the harvested weeds was recorded. In the 2018/2019 growing season, weeds were counted 13 weeks after transplanting (WAT) on 10 January 2018, 18 WAT on 14 February 2019, and 24 WAT on 28 March 2019. In the 2019/20 growing season, weeds were counted 13 WAT on 6 January 2020, 19 WAT on 19 February 2020, and 25 WAT on 31 March 2020. After each counting, emerged weeds in strawberry planting holes and from the bed shoulders were not included in the weed count but were hand-weeded at each weed counting date.

2.3. Crop Stand, and Health Index

In both growing seasons, crop stand and health were noted monthly from November to June. All plants within the 3.6 m length section were evaluated for vigor and health. Crop vigor and health were categorized using a scale of 0 (all plants are dying) to 10 (all plants are vigorous and no disease).

2.4. Nitrogen Estimation

To estimate nitrogen content in leaves in the early harvest season to determine plant nutrients status, leaf tissue and petiole samples were collected on 12 April 2019 and 6 April 2020. The samples collected were most recently mature trifoliate leaves with the associated petiole, 25 leaves and petioles for each treatment (6-7 leaves per plot). The samples were sent to the North Carolina Department of Agriculture and Consumer Services (Raleigh, NC, USA) for nutrients analysis.

2.5.Yield, Fruit Size, Fruit Firmness, and Total Soluble Solids Content

Strawberry fruits were harvested starting 16 April 2019 and 12 April 2020, up through 14 June 2019 and 18 June 2020. The fruit was harvested by hand twice a week, and the harvested fruits were sorted into marketable and nonmarketable categories. The nonmarketable fruits were fruits that were less than 10 g, diseased, rotten, deformed, overripe, or misshapen. Diseased berries included those infested with Anthracnose or Botrytis. Post-harvest data parameters included measuring fruit diameter, fruit firmness, fruit

total soluble solids content, and pH of the fruit juice. Fruit diameter was collected on five marketable fruits collected randomly from each plot once a week, and fruit diameter was recorded using a Vernier caliper scale. We checked the firmness of the fruit using a tabletop fruit texture analyzer (GS-15 Fruit Texture Analyzer, QA Supplies, Norfolk, VA, USA). At every other harvest date, five marketable fruits from each replicate were randomly tested for firmness. The same five fruits were tested for total soluble solids using a refractometer (MA871 Refractometer, Milwaukee, Rocky Mount, NC) and were tested for pH using pH tester electrode (combo pH/conductivity/TDS tester, HANNA Instruments, Smithfield, RI, USA). In the 2019/20 growing season, six whole plants per plot were collected for biomass analysis on 23 June 2020 and 24 June 2020. Each plant was divided into six parts, which included leaves, petioles, crown, root, flower, and berries. The soil that stuck to the roots were was removed carefully by hand hands and then gently rinsed by tape water gently. Both the fresh weight and dry weight of all six parts components were recorded.

2.6. Statistical Analysis

Data were analyzed using JMP v. 14 (SAS Institute Inc., Cary, NC, USA). Prior to the ANOVA, data were checked for normality and homogeneity of variance assumptions. The temperature data were averaged for the 21-day ASD treatment duration, and maximum and minimum temperatures achieved during the treatment period were recorded. The cumulative Eh was calculated basing on the hourly average redox potential and the absolute value of the difference between each hourly average redox potential and the calculated critical redox potential (CEh; redox potential value below, which is considered anaerobic) and was summed over the whole 21-day ASD period. The critical redox potential was calculated by the formula CEh=595mV - 60mV * soil pH (Rabenhorst and Castenson, 2005; USDA-NRCS, 2010). The soil temperature and cumulative Eh data were analyzed by two-way ANOVA (treatments x growing seasons). The data were analyzed separately by growing season if either the treatment by growing season interaction or growing season effect was significant ($P<0.05$). The data were pooled over growing seasons if the treatment main effect was significant ($P<0.05$) and treatments by growing seasons interaction was not significant ($P>0.05$). The multiple comparisons were conducted by using protected Fisher's least significant difference (LSD) ($P<0.05$).

The weed density data did not meet the assumptions of normality, and the rank transformation was used. Then the transformed data were analyzed by two-way ANOVA.

Non-transformed means were presented. The strawberry health index, fruit yield, fruit firmness, fruit size and fruit total soluble solids data were analyzed similarly to temperature and Eh data by two-way ANOVA. The fruit firmness and fruit size data were averaged for each replicate by each evaluation date for each growing season. The percentage of marketable yield (marketable yield rate) data were transformed by log(x+1) for analysis, and only the non-transformed data are presented.

3. RESULTS

3.1. Temperature and Cumulative Redox Potential

The temperature and redox potential data were not collected for plots treated with Pic-Clor 80. There was no significant treatment main effect for mean and high temperature at a 15 cm depth over the two growing seasons (Table 4.2 and 4.3). The overall mean soil temperature in the 2018/29 growing season was significantly higher than that in the 2019/20 growing season. Many soil organisms, including weeds, are negatively affected at over 40 °C (Stapleton et al., 1995). Thus, the cumulative hours that the temperature was higher than 40 °C were calculated. In the 2018/19 growing season, the plot treated with ASD at full rate with yeast had the greatest number of hours that temperature above 40 °C, which was significantly longer than plots treated with ASD at the half rate with/without yeast or non-treated control plots with or without yeast. In the 2019/20 growing season, plots treated with ASD at a full rate with yeast had a significantly greater number of hours that temperature above 40 °C than the plots treated with the rest other five treatments.

The cumulative Eh data had no significant ($P>0.05$) treatment by growing season interaction. Thus, the cumulative Eh data were pooled over two growing seasons. Plots treated with all four ASD treatments had significantly higher cumulative Eh than two non-treated control plots (Table 4.3). There was no significant difference among plots treated with ASD treatments for cumulative Eh.

3.2. Weed Density

In both growing seasons, the dominant broadleaf weeds species were carpetweed (*Mollugo verticillate* L.), Carolina geranium (*Geranium carolinianum* L.), cudweed (*Gnaphalium* spp.), henbit (*Lamium amplexicaule* L.), and white clover (*Trifolium repens* L.). The dominant grass weed species were bermuda grass (*Cynodon dactylon* L. Pers.) and

crabgrass (*Digitaria sanguinalis*). Shepherd's purse (*Capsella bursa-pastoris* L. Medik.) and yellow nutsedge (*Cyperus esculentus* L.) were the dominant weed species only in the 2018/19 growing season. Shepherd`s purse and yellow nutsedge were rarely observed in the 2019/20 growing season.

In the 2018/19 growing season, ASD treated plots had a significantly lower density of shepherd`s purse (Table 4.4) and cumulative weed total density than the non-treated with and without yeast (Table 4.7). For large crabgrass, only plots treated with ASD at a full rate without yeast and 1,3-D + Pic had significantly lower weed density than the two non-treated plots (Table 4.6). For yellow nutsedge, only plots treated with ASD at a half rate with yeast and 1,3-D + Pic significantly reduced weed density.

In the 2019/20 growing season, plots treated with all ASD treatments and 1,3-D + Pic had a significantly lower density of cumulative weed density, fresh and dry weed biomass compared to plots with non-treated control without yeast (Table 4.7). For cumulative density, plots treated with ASD with yeast at both rates had a significantly lower density than plots treated with, 1,3-D + Pic, as well as ASD without yeast.

For carpetweed, plots treated with all ASD treatments had similar weed density, which was significantly lower than the two non-treated plots but was significantly higher than plots treated with 1,3-D + Pic (Table 4.4). For Carolina geranium (Table 4.4), plots treated with all ASD treatments had significantly lower Carolina geranium density than the two non-treated plots, as well as lower density than plots with 1,3-D + Pic. The yeast amendment significantly reduced the Carolina geranium density for plots treated with ASD at the full rate compared to that without yeast. For cudweed (Table 4.4), the plot treated with all ASD treatments and 1,3-D + Pic had a significantly lower density than the two non-treated plots. For henbit and white clover (Table 4.4), plots treated with all ASD treatments and 1,3-D + Pic had similar weed density, which was significantly lower than plots with non-treated control without yeast. The plots with non-treated control with yeast had no significantly different henbit density compared to plots treated with all ASD treatments and had similar white clover density compared to plots with ASD at a full rate without yeast. The overall average cudweed, henbit and white clover density for all treatments in 2018/19 growing seasons were significantly higher than those in the 2019/20 growing season (Table 4.5). For bermuda grass (Table 4.6), plots treated with ASD without yeast at both rates and 1,3-D + Pic had a significantly lower density than the two non-treated plots, while there was no

significant difference among plots with all ASD treatments. The yeast amendment did not affect weed density in plots with non-treated control.

3.3. Crop Stand Counts and Crop Health

The strawberry stand counts had no two-way or main effect significance ($P>0.05$) (data not shown). For the plant health index (Table 4.8), treatment main effect and growing season effect were significant ($P<0.05$). There was no significant difference among plots treated with all ASD treatments and 1,3-D + Pic. The plots with non-treated control without yeast had a significantly lower health index than plots treated with all ASD treatments and 1,3-D + Pic, while plots with non-treated control with yeast had similar health index compared to plots treated with ASD at a full rate without yeast and with 1,3-D + Pic. The overall average health index for all treatments in the 2018/19 season was significantly lower than that in the 2019/20 growing season (Table 4.9). The main reason that led to a lower index for the non-treated plots was that crops were less vigorous or small in size.

3.4. Crop Yield, Fruit Size, Fruit Firmness, and Total Soluble Solids

The total yields were calculated by marketable yield added to non-marketable yield. The non-marketable yield was mainly caused by fruit rot diseases (anthracnose and botrytis) and rot due to excessive moisture on the beds. Most non-marketable fruits were heavier than 10 g. The growing season by treatment interaction was significant for marketable yield, total yield, monthly total yield and marketable yield rate (Table 4.10 and 4.11). In the 2018/19 growing season, there were no significant differences among plots treated with ASD treatments and non-treated plots for both marketable and total yield (Table 4.10). Plots treated with 1,3-D + Pic had the highest marketable yield. In the 2019/20 growing season, plots treated with ASD treatments and 1,3-D + Pic provided significantly higher marketable and total yield for the whole harvest season than plots with both non-treated controls. The treatment main effect was no significant for the marketable yield rate (marketable rate = marketable yield/ total yield x 100%). Plots treated with ASD treatments at both rates with yeast had a comparable marketable and total yield to plots with 1,3-D + Pic, while plots treated with ASD without yeast had significantly lower yield than plots treated with 1,3-D + Pic. Thus, the yeast amendments significantly enhanced the marketable and total yield for plots treated with ASD treatments. These trends were also found for monthly total yield in May and June during the 2019/20 growing season (Table 4.11). The weekly total yields were variable for each week in

both growing season (Figure 4.1 and 4.2), which indicated the distribution of strawberry yield was not uniform over the harvest season. In both growing seasons, the fruit's fruit size, fruit firmness and total soluble solids were not significantly different among treatments (data not shown).

4. DISCUSSION

The mean soil temperature during the ASD period in the 2018/19 growing season was significantly higher than in the 2019/20 growing season. That difference was consistent with the air temperature data retrieved from the U.S. Department of Agriculture Natural Resources Conservation Service, National Water and Climate Center (USDA-NRCS) for the nearest station, which was at Norfolk International Airport. The average air temperature in September 2018 was 26.1 °C while it was 24.5 °C in September 2019. The highest soil temperature from ASD treatments was around 10°C higher than the highest air temperature in September 2018 and September 2019. This difference in maximum soil temperatures between the soil and the air was observed in our previous greenhouse trials (Liu et al., 2020). The difference in the cumulative numbers of hours that the soil temperature was above 40 °C may have caused the significant growing season by treatment interaction, especially in the non-treated control plots (Table 4.3). Even though cumulative number of hours in both growing seasons that the mean soil temperature was above 40 °C may not have been enough to eliminate some soilborne pests on its own, the black tarp may have enhanced weed suppression by blocking the necessary light for weed germination and growth (Johnson and Fennimore, 2005). The cumulative number of hours that mean soil temperature was between 30 and 35 °C in the 2019/20 growing season were greater than that in the 2018/19 growing season (data are not shown). The temperature ranged from 30 to 35 °C, and was likely an ideal temperature range for yeast metabolism and fermentation (Feldman, 2012). The greater number of hours in 2019/20 when mean soil temperature ranged from 30 to 35 °C may have led to more metabolites produced from yeast fermentation. That difference in yeast activities could be an explanation for the better performance of yeast in the 2019/20 growing season than in the 2018/19 growing season. For example, plots treated with ASD with yeast had significantly lower total weed density than ASD without yeast in the 2019/20 growing season, but there was no significant difference in total weed density for plots treated with ASD either with or without yeast in the 2018/19 growing season.

Inconsistent weed control by ASD has been reported (Shrestha et al., 2016; Guo et al., 2017), but in this study, although the weed control effect from ASD varied for different weed species, ASD consistently suppressed several weeds including carpetweed, Carolina geranium, cudweed, henbit and white clover. Compared to the research that had inconsistent weed control, this study had several different factors that may lead to the difference. The different factors included different locations and soil types, different carbon sources and C:N ratio and different soil temperature. For example, Guo et al. reported inconsistent yellow nutsedge, grass and broadleaf weed control, which had sandy soil type and molasses as carbon sources. In this study, we used sandy loam soil and BSG mixed with paper mulch as carbon sources. The interaction between carbon source C:N ratio and yellow nutsedge tuber suppression were also reported (Shrestha et al., 2018). There was a trend that relative lower amendment C:N ratio (10:1 or 20:1) led to lower soil pH, which may indicate higher organic acids content (Shrestha et al., 2018). It possible that the organic acids have potential impacts on weed suppression. The weed density in this trial was relatively low, especially in the 2019/20 growing seasons. Other studies indicated ASD could effectively control weeds when their density is low (Di Gioia et al., 2016; Guo et al., 2017). Thus, the efficacy of ASD with yeast on weed control needs to be evaluated under high weed density field conditions. For the weed species that ASD did not consistently suppress, there was a trend that all ASD treatments numerically reduced all weed counts when weed density was higher than ten counts m^{-2}. The weed density data in this study were collected from the areas covered by clear tarp. However, the annual plasticulture strawberry production system typically uses black tarps, which suppress weeds more than clear tarp (Johnson and Fennimore, 2005). However, clear tarp warms soil more than black tarp; conventional strawberry plasticulture with clear tarp could provide higher yield than with black tarp (Johnson and Fennimore, 2005). The combination of ASD and solarization using clear tarp might have the potential to provide adequate weed control as well as higher yield compared to ASD with black tarp (Butler et al., 2014 a). The anaerobic condition is an essential factor in ASD, and the volatile organic compounds produced during the ASD period were related to soil-borne pest control (Hewavitharana et al., 2014; Mowlick et al., 2012). The plastic film that highly reduced gas emission from soil may also lead to better ASD performance. Song et al. (2020) evaluated ASD with virtually impermeable film (VIF), polyethylene (PE), and totally impermeable film (TIF) and observed TIF had the highest suppression against *Fusarium* spp. and *Phytophthora* spp.

Although the environmental factors and plastic film could influence the soil anaerobic conditions for ASD (Butler et al., 2014 b), cumulative Eh (the indicator of anaerobic conditions) may not consistently affect weed control effectiveness from ASD. In our studies, there was no significant difference in weed density of several weed species among plots treated with all ASD treatments. The cumulative Eh was also not significantly different among plots treated with ASD treatments. The cumulative Eh in this study was significantly higher for plots treated with ASD treatments than non-treated plots, while that difference did not lead to significant suppression on large crabgrass and yellow nutsedge in the 2018/19 growing season. Yellow nutsedge is a troublesome weed species but was at a really low weed density in this study. Some studies which had relatively high yellow nutsedge density and significant yellow nutsedge suppression showed there was no strong correlation between cumulative Eh and yellow nutsedge suppression (Muramoto et al., 2008; Shrestha et al., 2018; Paudel et al., 2020). Some other factors involved in ASD may have greater influence, such as volatile fatty acid accumulation, carbon source, and microbial communities' decomposition of weed propagules (Guo et al., 2018; Shennan et al., 2018; Shrestha et al., 2018). The microbial community may also enhance the nutrient availability in the soil by increasing the rate of carbon source decomposition (Guo et al., 2018). That may be the reason that ASD with yeast had a higher yield than without yeast.

A significant crop yield increase resulting from ASD treatments was obtained in this study, although only in the 2019/20 growing season. The promotion of ASD on yield was also reported by other researchers, such as ASD on strawberry (Song et al., 2020), eggplant and bell pepper (Butler et al., 2014 a), and tomato (Di Gioia et al., 2016). However, ASD does not consistently enhance the yield. The marketable and total yield in this study were not increased in the 2018/29 growing season. McCarty et al. (2014) showed ASD did not increase yield for either tomato or bell pepper. There are many possible reasons responded for the yield increase. The application rate and the type of carbon used in ASD could affect the crop yield (Shrestha et al., 2016). The suppression of disease and weeds could affect crop yield. The application of carbon sources could also increase the soil fertility level and then influence the crop yield (Guo et al., 2017). For example, ASD could greatly increase ammonium nitrogen while reducing nitrate nitrogen in the soil (Liu et al., 2016). The leaf tissue report in this study also showed a similar trend than leaves from ASD treatments had less nitrate nitrogen than non-treated control and 1,3-D + Pic treated plots (Table 4.9). The

increase in the utilization rate of nitrogen, phosphorus, and potassium by ASD was also reported (Song et al., 2020).

In both growing seasons, the leaf tissue analysis was conducted on April 2018 and April 2019, and several nutrients were analyzed, such as nitrogen (N), phosphorus (P), and potassium (K). No synthetic fertilizer was applied to any ASD treatments in either growing season. Although the available N from the ASD carbon sources was the same as the available N from the synthetic fertilizer applied to the non-treated control and 1,3-D + Pic groups (Table 4.1), the available N from the organic ASD carbon sources would likely nor be fully decomposed or mineralized during the growing season. The result of leaf tissue analysis supported this assumption (Table 4.12). The nitrogen index value for ASD with synthetic fertilizer was 50 (S, or satisfactory). This nitrogen index value indicated that the full rate of BSG mixed with paper mulch did not provide excessive nitrogen to the crop. Therefore, specific carbon sources possessing more nitrogen than recommended will be unlikely to cause excess nitrogen issues to the crop, as long as the nitrogen rate of the carbon sources used for ASD is within a specified dose range. Excess nitrogen issues such as excessive vegetative growth, decreased or delayed flowing, or fruit set would negatively impact plant health and yield. The dynamics of additional soil nitrogen should be monitored in order to verify the influence of BSG on soil fertility.

In this study, ASD did not significantly affect non-marketable yield (Table 4.10), because the non-marketable fruits were mostly due to disease (anthracnose and botrytis), and environmental factors, which were unlikely to have been influenced by ASD. Thus, it indicated that the total yield (marketable yield + non-marketable yield) increase caused by ASD was not the main result of ASD reducing non-marketable yield. The yield increase may be related to the increased average fruit weight. There was a significant difference in average fruit weight (Table 4.13), and plots treated with ASD with yeast had significantly higher fruit weight than plots treated with ASD without yeast or with non-treated controls. However, the differences in fruit size, firmness, and soluble solid were not significant (data are not shown). Thus, higher fruit weight may result from higher fruit water content or fruit dry matter content such as higher proportion of cell wall content. The dry matter content is likely to be higher using organic fertilization compared to inorganic fertilization (Reganold et al., 2010). In this study, the yeast activities in soil may change the soil fertility and provide similar conditions to organic fertilization, which may contribute to higher dry matter content. a

Therefore, additional research on whether yeast impact soil fertility, bud and fruit numbers, and fruit nutrient concentrations are needed, in order to verify yield effects and explore the mechanisms of ASD for yield increase. An economic analysis of ASD for strawberry field production is also necessary.

5. CONCLUSIONS

ASD using BSG as a source of carbon, as well as yeast amendment, demonstrated potential to control certain weed species and to enhance cumulative strawberry yield compared to a non-treated control, similar to a fumigant standard treatment. However, the effect was not consistent across the two growing seasons. The variability in effects across growing seasons suggests that more replicated trials are needed to verify the effect of BSG and yeast, and that additional work should be conducted in order to elucidate the mechanisms affecting ASD system performance.

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Table 4.1. Treatment list including the rates of carbon sources in the bedded area and nitrogen rate from both carbon sources and fertilizer.

Treatments [a]	Yeast	BSG	Paper mulch	N from carbon sources	N from synthetic fertilizer	Total carbon	Total N
	(kg dry matter ha^{-1})			(kg ha^{-1})			
Carbon full							
(1) Without yeast	0	4,082	12,330	117	0	6,700	117
(2) With yeast	10	4,082	12,330	117	0	6,700	117
Carbon half							
(3) Without yeast	0	6,080	1,570	117	0	3,350	117
(4) With yeast	5	6.080	1,570	117	0	3,350	117
Non-treated control							
(5) Without yeast	0	0	0	0	117	0	117
(6) With yeast	10	0	0	0	117	0	117
(7) 1,3-D + Pic	0	0	0	0	117	0	117

[a] The rates of carbon sources and yeast were as follows: carbon sources full rate 4 mg carbon g^{-1} soil, 6.7 t carbon ha^{-1}, yeast full rate 10 kg ha^{-1}, carbon sources half rate 2 mg carbon g^{-1} soil, 3.35 t carbon ha^{-1}, yeast half rate 5 kg ha^{-1}, non-treated control with yeast 10 kg ha^{-1}. The rate of 1,3-D + Pic was 196 kg ha^{-1}. The BSG is brewer`s spent grain, which has 44% carbon and 3.7% nitrogen on dry-matter basis. The BSG we applied was fresh matter with 69% moisture content (w/w). The weight of fresh BSG for carbon full rate was 13,169 kg^{-1}, and for carbon half rate was 19,614 kg ha^{-1}. The paper mulch applied was dry matter, which has 40% carbon rate and 0.7 % nitrogen rate.

Table 4.2. Overall mean soil temperature for all treatments at 15-cm depth for 21-day period during the 2018-2019 and 2019-2020 growing season.

Growing season	Mean temperature (°C)
2018-2019	30 a [a]
2019-2020	28.6 b
P treatment effect [b]	N.S.[c]
P season effect	<0.0001
P treatments x seasons	N.S.

[a] Means in the same column with the same letters are not significantly different based on Fisher`s Least Significant Difference at alpha=0.05
[b] Only the growing season effect was significant for mean temperature (P<0.005). Thus, only the overall mean temperature for all treatments was present.
[c] N.S.=not significant (P>0.05).

Table 4.3. High soil temperature at 15-cm depth for 21-day period of anaerobic soil disinfestation (ASD) with different C dose rates and yeast amendment.

Treatment [a]	High temperature(°C)		> 40 °C Hours		Cumulative Eh (Vhr)
	2018/19	2019/20	2018/19	2018/19	2019/20
C sources full					
Without yeast	45.9 [c]	45.0	14 ab	15 b	80 a
With yeast	46.1	45.3	21.5 a	34 a	57 a
C sources half					
Without yeast	41.0	41.9	3.5 b	6 b	81 a
With yeast	42.0	44.0	7 b	14 b	74 a
Non-treated control					
Without yeast	34.3	43.9	0 b	11 b	15 b
With yeast	33.4	44.0	0 b	16 b	13 b
P treatment effect [b]	N.S.[d]		0.0014		0.0024
P season effect	0.0047		0.0011		N.S.
P treatments x seasons	0.018		0.018		N.S.

[a] The rates of carbon sources and yeast were as follows: carbon sources full rate 4 mg carbon g^{-1} soil, 6.7 t carbon ha^{-1}, yeast full rate 10 kg ha^{-1}, carbon sources half rate 2 mg carbon g^{-1} soil, 3.35 t carbon ha^{-1}, yeast half rate 5 kg ha^{-1}, non-treated control with yeast 10 kg ha^{-1}.

[b] Growing season by treatment interaction was significant for high temperature and hours > 40 °C. For cumulative Eh, only the treatment main effect was significant, and the data were pooled over the two growing seasons.

[c] A column without any letter means there is no treatment effect (P>0.05) based on Fisher`s Least Significant Difference at alpha=0.05; Means in the same column with the same letters are not significantly different based on Fisher`s Least Significant Difference at alpha=0.05.

[d] N.S.=not significant (P>0.05).

Table 4.4. Weed density for broadleaf in 1.5 m lengths of windows for the 2018/2019 and 2019/2020 growing seasons after preplant treatments.

Treatment [a]	Carpetweed	Carolina geranium	Cudweed	Henbit	White Clover	Shepherd's Purse	
						2018/19	2019/20
	(plants m^{-2})						
Carbon full							
Without yeast	22 b [c]	13 cde	21 b	3 bc	44 bc	2 cd	0
With yeast	16 b	8 f	16 b	2 bc	33 c	4 bc	0
Carbon half							
Without yeast	13 b	17 bcd	17 b	3 bc	37 c	2 c	0
With yeast	10 b	12 def	22 b	3 bc	38 c	32 ab	0
Non-treated control							
Without yeast	29 a	33 a	52 a	18 a	67 a	50 a	0
With yeast	33 a	23 abc	47 a	17 ab	59 ab	41 a	0
1,3-D + Pic	0 c	25 ab	14 b	0 c	32 c	0 d	0
P treatment effect [b]	<0.0001	<0.0001	<0.0001	0.0129	0.0003	0.0079	
P season effect	N.S.[d]	N.S.	<0.0001	0.0075	0.01	0.0042	
P treatments x seasons	N.S.	N.S.	N.S.	N.S.	N.S.	0.0028	

[a] The rates of carbon sources and yeast were as follows: carbon sources full rate 4 mg carbon g^{-1} soil, 6.7 t carbon ha^{-1}, yeast full rate 10 kg ha^{-1}, carbon sources half rate 2 mg carbon g^{-1} soil, 3.35 t carbon ha^{-1}, yeast half rate 5 kg ha^{-1}, non-treated control with yeast 10 kg ha^{-1}.

[b] Growing season by treatment interaction was significant for Shepherd's purse. For carpet weed and Carolina geranium, only the treatment main effect was significant, and data are pooled over the two growing seasons. For cudweed, henbit and white clover, the treatment effect and growing season effect were significant, thus the only the treatment main effect was presented in this table, and the season effect was presented in the table 4.5.

[c] Means in the same column with the same letters are not significantly different based on Fisher`s Least Significant Difference at alpha=0.05.

[d] N.S.=not significant (P>0.05).

Table 4.5. Overall weed density for cudweed, henbit and white clover average for all treatments in 1.5 m lengths of windows for the 2018/2019 and 2019/2020 growing seasons.

Growing season	Cudweed	Henbit	White clover
	(plants m^{-2})		
2018-2019	44 a [a]	8 a	50 a
2019-2020	9 b	4.8 b	38 b
P treatment effect [b]	<0.0001	0.0129	0.0003
P season effect	<0.0001	0.0075	0.01
P treatments x seasons	N.S [c]	N.S.	N.S.

[a] Means in the same column with the same letters are not significantly different based on Fisher`s Least Significant Difference at alpha=0.05

[b] The growing season effect and treatment main effect were significant for cudweed, henbit and white clover (P<0.005). Thus, the overall weed density for all treatments was present. The treatment main effect was presented in Table 4.4.

[c] N.S.=not significant (P>0.05).

Table 4.6. Weed density for grass and yellow nutsedge in 1.5 m lengths of windows for the 2018/2019 and 2019/2020 growing seasons after preplant treatments.

Treatment [a]	Bermuda grass	Large crabgrass		Yellow nutsedge	
		2018/19	2019/20	2018/19	2019/20
	(plants m^{-2})				
Carbon full					
Without yeast	2 bc [c]	6 cd	5	5 ab	0
With yeast	3 ab	8 bc	2	2 ab	0
Carbon half					
Without yeast	2 bc	12 ab	4	3 ab	0
With yeast	2.5 abc	10 abc	6	2 bc	0
Non-treated control					
Without yeast	16 a	17 ab	2	9 a	0
With yeast	13 ab	18 a	7	8 a	1
1,3-D + Pic	0 c	0 d	2	0 c	0
P treatment effect [b]	0.04	<0.0001		0.0006	
P season effect	N.S. [d]	<0.0001		<0.0001	
P treatments x seasons	N.S.	0.002		0.0015	

[a] The rates of carbon sources and yeast were as follows: carbon sources full rate 4 mg carbon g^{-1} soil, 6.7 t carbon ha^{-1}, yeast full rate 10 kg ha^{-1}, carbon sources half rate 2 mg carbon g^{-1} soil, 3.35 t carbon ha^{-1}, yeast half rate 5 kg ha^{-1}, non-treated control with yeast 10 kg ha^{-1}.
[b] Growing season by treatment interaction was significant for large crabgrass and yellow nutsedge. For bermuda grass, only the treatment effect was significant, thus the data were pooled over the two growing seasons.
[c] A column without any letter means there is no treatment effect (P>0.05) basing on Fisher`s Least Significant Difference at alpha=0.05; Means in the same column with the same letters are not significantly different based on Fisher`s Least Significant Difference at alpha=0.05.
[d] N.S. = not significant (P>0.05).

Table 4.7. Cumulative weed density and cumulative fresh and dry biomass in 1.5 m lengths of windows for the 2018/2019 and 2019/2020 growing seasons after preplant treatments.

Treatment [a]	Cumulative weed density (plants m^{-2}) [b]		Cumulative fresh biomass (g m^{-2})		Cumulative dry biomass (g m^{-2})	
	2018/19	2019/20	2018/19	2019/20	2018/19	2019/20
Carbon full						
Without yeast	113 c [c]	68 bc	3,045 ab	919 bc	2,553 ab	604 b
With yeast	133 bc	44 d	3,381 abc	761 c	2,851 ab	449 b
Carbon half						
Without yeast	119 c	76 b	2,821 abc	1,021 b	2,396 abc	662 b
With yeast	139 bc	57 cd	2,630 bc	955 bc	2,185 bc	615 b
Non-treated control						
Without yeast	283 a	148 a	4,336 a	2,006 a	3,605 a	1,667 a
With yeast	223 ab	135 a	5,294 a	1,642 a	4,298 a	1,318 a
1,3-D + Pic	60 d	67 bc	1,874 c	1,008 b	1,453 c	657 b
P treatment effect	<0.0001		<0.0001		<0.0001	
P season effect	<0.0001		<0.0001		<0.0001	
P treatments x seasons	0.0001		0.02		0.02	

[a] The rates of carbon sources and yeast were as follows: carbon sources full rate 4 mg carbon g^{-1} soil, 6.7 t carbon ha^{-1}, yeast full rate 10 kg ha^{-1}, carbon sources half rate 2 mg carbon g^{-1} soil, 3.35 t carbon ha^{-1}, yeast half rate 5 kg ha^{-1}, non-treated control with yeast 10 kg ha^{-1}.
[b] Growing season by treatment interaction was significant for cumulative weed counts and cumulative fresh and dry biomass.
[c] Means in the same column with the same letters are not significantly different based on Fisher`s Least Significant Difference at alpha=0.05.

Table 4.8. Strawberry plant health index for the whole growing seasons.

Treatment [a]	Health index [b]
Carbon full	
Without yeast	8.4 ab
With yeast	8.6 a
Carbon half	
Without yeast	8.6 a
With yeast	8.5 a
Non-treated control	
Without yeast	7.9 c
With yeast	8.0 bc
1,3-D +chloropicrin	8.3 abc
P treatment effect	0.0086
P season effect	<0.0001
P treatments x seasons	N.S. [d]

[a] The rates of carbon sources and yeast were as follows: carbon sources full rate 4 mg carbon g^{-1} soil, 6.7 t carbon ha^{-1}, yeast full rate 10 kg ha^{-1}, carbon sources half rate 2 mg carbon g^{-1} soil, 3.35 t carbon ha^{-1}, yeast half rate 5 kg ha^{-1}, non-treated control with yeast 10 kg ha^{-1}.
[b] The crop health indicates by index from 0 (all plants are dead) to 10 (all plants are vigorous and no disease). The evaluation was based on all strawberry plants in the 3.6m length section. The growing season effect and treatment main effect was significant for health index ($P<0.005$). Thus, the treatment main effect was present in this table. The overall health index for all treatments for health index was presented in table 4.9.
[c] Means in the same column with the same letters are not significantly different based on Fisher`s Least Significant Difference at alpha=0.05.
[d] N.S.=not significant ($P>0.05$).

Table 4.9. Strawberry plant health index for 2018/19 and 2019/20 growing seasons.

Growing season	Health index (°C)
2018-2019	7.8 b [a]
2019-2020	8.8 a
P treatment effect [b]	0.0086
P season effect	<0.0001
P treatments x seasons	N.S. [c]

[a] Means in the same column with the same letters are not significantly different based on Fisher`s Least Significant Difference at alpha=0.05.
[b] The growing season effect and treatment main effect was significant for health index (P<0.005). Thus, the overall health index for all treatments was present in this table. The treatment main effect for health index was presented in table 4.8.
[c] N.S.=not significant (P>0.05).

Table 4.10. Effect of treatments and growing season in marketable, non-marketable and total yield during the harvest period in 2018/19 and 2019/20 growing season in Virginia Beach, VA.

Treatment [a]	Marketable yield		Non-marketable yield	Total yield		Marketable yield rate (%) [c]	
	2018/19	2019/20		2018/19	2019/20	2018/19	2019/20
	(g plant^{-1})						
Carbon full							
Without yeast	397 b [b]	410 c	78 b	488 b	474 c	81	86
With yeast	361 b	684 a	76 b	446 b	752 a	81	91
Carbon half							
Without yeast	419 ab	418 c	84 b	530 ab	475 c	79	88
With yeast	327 b	572 b	80 b	420 b	639b	76	90
Non-treated control							
Without yeast	297 b	226 d	64 b	379 b	270 d	78	84
With yeast	319 b	287 d	66 b	414 b	323 d	77	89
1,3-D + Pic	536 a	559 b	139 a	663 a	710 ab	81	79
P treatment effect	<0.0001		<0.0001	<0.0001		N.S.	
P season effect	0.0127		0.0006	N.S.		<0.0001	
P treatments x seasons	0.0019		N.S. [d]	0.0052		0.04	

[a] The rates of carbon sources and yeast were as follows: carbon sources full rate 4 mg carbon g^{-1} soil, 6.7 t carbon ha^{-1}, yeast full rate 10 kg ha^{-1}, carbon sources half rate 2 mg carbon g^{-1} soil, 3.35 t carbon ha^{-1}, yeast half rate 5 kg ha^{-1}, non-treated control with yeast 10 kg ha^{-1}.

[b] A column without any letter means there is no treatment effect (P>0.05) based on Fisher's LSD at P<0.05; Means in the same column with the same letters are not significantly different based on Fisher`s Least Significant Difference at alpha=0.05.

[c] Marketable yield rate = marketable yield / total yield.

[d] N.S. = not significant (P>0.05).

Table 4.11. Monthly cumulative yield for the 3-month harvest period.

Treatment [a]	April total yield		May total yield		June total yield	
	2018/19	2019/20	2018/19	2019/20	2018/19	2019/20
	(g plant^{-1})					
Carbon full						
Without yeast	119	125 b [b]	337 b	228 c	32	120 b
With yeast	115	187 a	309 b	372 a	22	192 a
Carbon half						
Without yeast	115	125 b	381 ab	238 c	28	112 b
With yeast	79	154 ab	292 b	313 b	23	170 a
Non-treated control						
Without yeast	86	51 c	264 b	160 d	30	58 c
With yeast	106	67 c	290 b	188 cd	18	67 c
1,3-D +chloropicrin	120	148 ab	514 a	379 a	30	182 a
P-value treatments x seasons	0.045		0.0031		<0.0001	

[a] The rates of carbon sources and yeast were as follows: carbon sources full rate 4 mg carbon g^{-1} soil, 6.7 t carbon ha^{-1}, yeast full rate 10 kg ha^{-1}, carbon sources half rate 2 mg carbon g^{-1} soil, 3.35 t carbon ha^{-1}, yeast half rate 5 kg ha^{-1}, non-treated control with yeast 10 kg ha^{-1}.
[b] A column without any letter means there is no treatment effect (P>0.05) based on Fisher`s Least Significant Difference at alpha=0.05; Means in the same column with the same letters are not significantly different basing on Fisher`s Least Significant Difference at alpha=0.05.

Table 4.12. Nitrogen content in leaves of 30-week old plants, expressed by nitrogen index.

Treatment [a]	Nitrogen Index value [b & c]		Ammonium nitrogen (mg L^{-1})	
	2018/19	2019/20	2018/19	2019/20
Carbon full				
Without yeast	42 (L)	51 (S)	1,590	2,990
With yeast	42 (L)	40 (L)	1,100	406
Carbon half				
Without yeast	42 (L)	43 (L)	1,690	642
With yeast	46 (L)	46 (L)	1,970	2,110
Non-treated control				
Without yeast	54 (S)	46 (L)	4,280	2,410
With yeast	51 (S)	55 (S)	4,400	2,750
1,3-D + Pic	69 (S)	62 (S)	6,510	3,810

[a] The rates of carbon sources and yeast were as follows: carbon sources full rate 4 mg carbon g^{-1} soil, 6.7 t carbon ha^{-1}, yeast full rate 10 kg ha^{-1}, carbon sources half rate 2 mg carbon g^{-1} soil, 3.35 t carbon ha^{-1}, yeast half rate 5 kg ha^{-1}, non-treated control with yeast 10 kg ha^{-1}.

[b] The nitrogen index is made by comparing the nitrogen concentration to the established sufficiency range for nitrogen in strawberry and translated to a numerical index between 0 and 124.

[c] Letters in parentheses indicate interpretive groupings of sufficiency indices - L = low, index range from 25-49; S = sufficient, index range from 50-74.

Table 4.13. 2019/20 growing season, average fruit weights.

Treatment [a]	Average fruit weight(g)
Carbon full	
Without yeast	29.99 cd [b]
With yeast	34.67 a
Carbon half	
Without yeast	29.49 cd
With yeast	32.68 ab
Non-treated control	
Without yeast	28.25 d
With yeast	30.81 cd
1,3-D + Pic	30.85 bc
P-value	0.0012

[a] The rates of carbon sources and yeast were as follows: carbon sources full rate 4 mg carbon g^{-1} soil, 6.7 t carbon ha^{-1}, yeast full rate 10 kg ha^{-1}, carbon sources half rate 2 mg carbon g^{-1} soil, 3.35 t carbon ha^{-1}, yeast half rate 5 kg ha^{-1}, non-treated control with yeast 10 kg ha^{-1}.
[b] Means followed by different letters within a column were statistically different using Fisher`s Least Significant Difference at alpha=0.05.

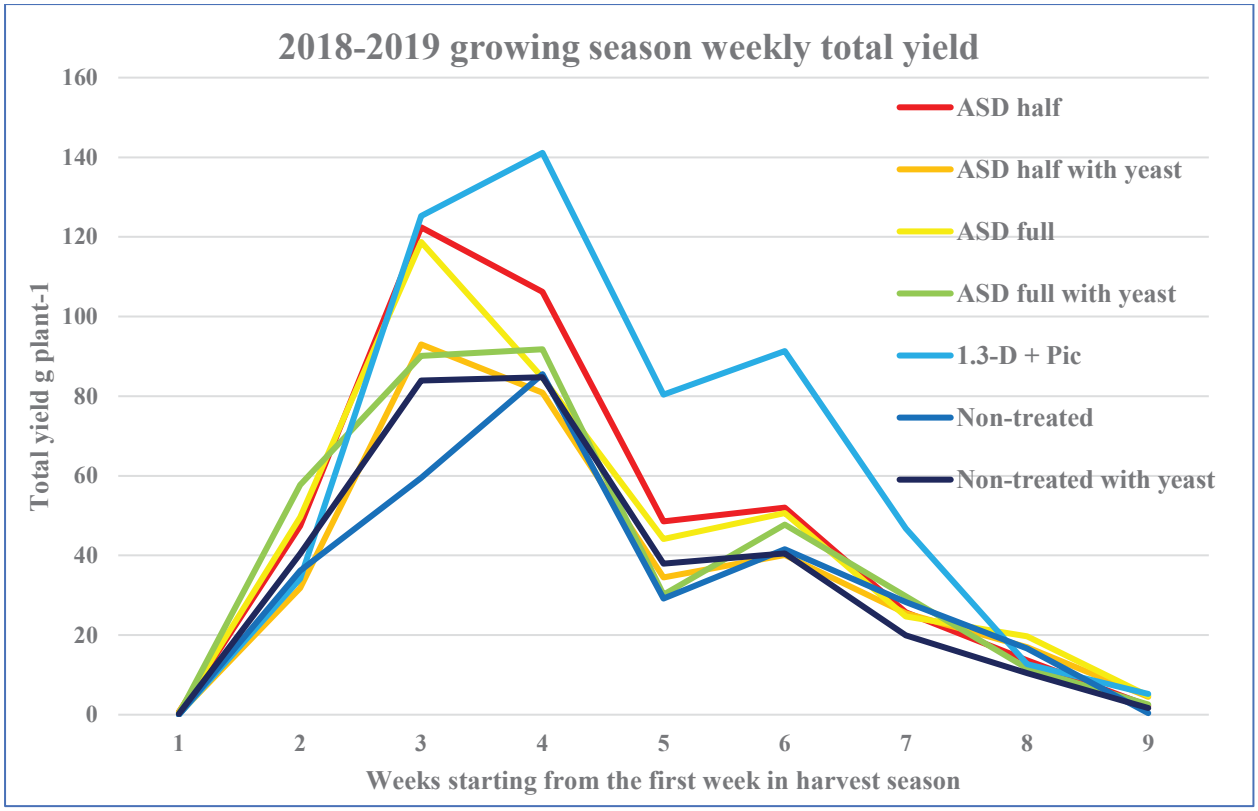

Figure 4.1. The monthly cumulative total yield in 2018/19 growing season. ASD full = ASD at carbon sources full rate, ASD half = ASD at carbon sources half rate, and 1,3-D + Pic = chloropicrin (80%) + 1,3-dichloropropene (20%). The rates of carbon sources and yeast were as follows: carbon sources full rate 4 mg carbon g^{-1} soil, 6.7 t carbon ha^{-1}, yeast full rate 10 kg ha^{-1}, carbon sources half rate 2 mg carbon g^{-1} soil, 3.35 t carbon ha^{-1}, yeast half rate 5 kg ha^{-1}, non-treated control with yeast 10 kg ha^{-1}. The rate of 1,3-D + Pic was 196 kg ha^{-1}. The harvest season in 2018/29 growing season started on 18 April 2019, and the harvest season in 2019/20 started at 10 April 2020.

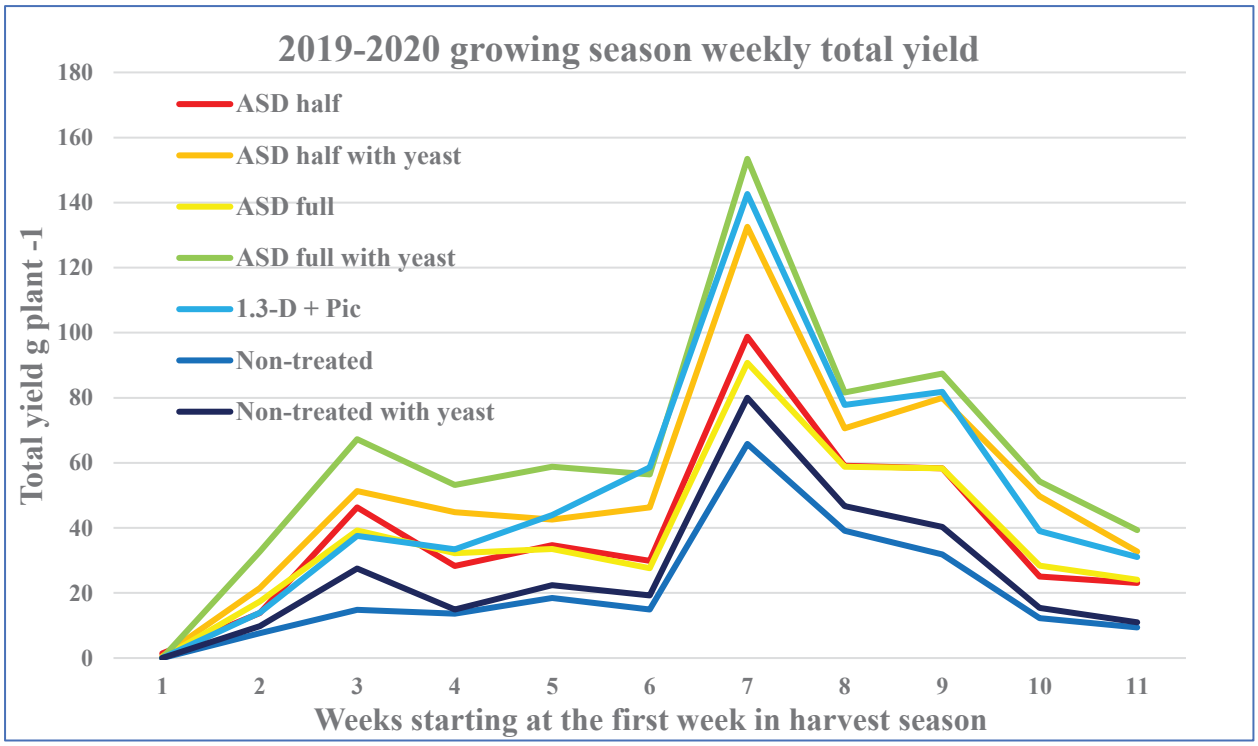

Figure 4.2. The monthly cumulative total yield in 2019/20 growing season. ASD full = ASD at carbon sources full rate, ASD half = ASD at carbon sources half rate, and 1,3-D + Pic = chloropicrin (80%) + 1,3-dichloropropene (20%). The rates of carbon sources and yeast were as follows: carbon sources full rate 4 mg carbon g^{-1} soil, 6.7 t carbon ha^{-1}, yeast full rate 10 kg ha^{-1}, carbon sources half rate 2 mg carbon g^{-1} soil, 3.35 t carbon ha^{-1}, yeast half rate 5 kg ha^{-1}, non-treated control with yeast 10 kg ha^{-1}. The rate of 1,3-D + Pic was 196 kg ha^{-1}. The harvest season in 2018/29 growing season started on 18 April 2019, and the harvest season in 2019/20 started at 10 April 2020.

CHAPTER V: CONCLUSION

SUMMARY

Anaerobic soil disinfestation (ASD) is a promising preplant method that could be a potential alternative to methyl bromide and chemical fumigants. Anaerobic soil disinfestation involves incorporating carbon amendments into soil in beds to be planted, covering the bed with black polyethylene tarp, and irrigating the soil to field capacity. The effectiveness of ASD in pest suppression could be influenced by multiple factors, including environmental conditions, application rates, and types of carbon sources, especially the carbon: nitrogen ratio in the carbon sources. Among those factors, the ASD effect is highly likely correlated with types of carbon sources. Moreover, the cost of ASD is also highly correlated to the rates and types of carbon sources applied, since the recommend carbon source rates would be around 10~20 ton ha^{-1}. More widely studied carbon sources such as rice bran and molasses have relatively low availability and are less economical in Virginia and the mid-Atlantic region. Thus, there is a need to evaluate carbons sources that have higher local availability and also maintain a similar pest control effect. Another strategy is reducing the volume of carbon sources utilized reduce the total cost. Thus, there is a need to develop new methods to enhance the ASD effect at lower carbon rates. To achieve the above objectives, we initiated a series of greenhouse trials and a two-year open-field trial. Although this research indicates the potential of using brewer`s spent grain and yeast in ASD, effectiveness may be affected by several factors, including physicochemical properties of brewer's spent grain, soil properties, and environmental factors, which have not yet been fully described.

In our greenhouse trials, we evaluated several locally available carbon sources, including several cover crops that match the strawberry season, brewer`s spent grain, paper mulch, used coffee grounds, and peanut shells. The target pests focused on troublesome weed species. Fewer studies have been conducted with a focus on weed pests versus soilborne plant pathogens. The targeted weed species included common chickweed, redroot pigweed, yellow nutsedge, and white clover. These greenhouse trials were conducted in pots filled with field soil. Results indicated no significant differences among the different carbon sources tested on the control of certain weeds. To enhance the ASD effect, we hypothesized mixing solid carbon sources with liquid carbon sources, such as ethanol, in order to improve treatment effects. We initiated a second series of greenhouse trials to evaluate the effect of soil carbon sources with ethanol on weed control. The results showed that ethanol has the potential to

enhance weed suppression from ASD on certain weed species, and the effectiveness was correlated with the type of solid carbon source. Although ethanol is not locally available and economical, new methods that produce bioethanol under field conditions might make the use of ethanol more feasible. Literature also indicated byproducts from bioethanol fermentation could enhance the ASD effect. Thus, we developed a new strategy, which was adding yeast to carbon sources to facilitate bioethanol fermentation during the ASD period. We initiated greenhouse and open-field trials to evaluate the effect of a carbon source mixed with yeast. The carbon source evaluated was the brewer`s spent grain (BSG) since this carbon source had the lowest cumulative anaerobicity when mixed with ethanol. These studies also evaluated carbon at reduced dose rates. The target pests were troublesome weed species and *Pythium irregulare*, which is one cause of black root rot of strawberry. The greenhouse trials showed ASD using BSG as the carbon source had significant effect on weed and *P. irregulare* suppression. The BSG at half or one-third rate without yeast did not achieve comparable weed and *P. irregulare* suppression as BSG at the full rate. However, the BSG with yeast at half and one-third rate had similar weed suppression as BSG at a full rate without yeast, while the BSG at the full rate still had greater *P. irregulare* suppression. The field trials not only evaluated the effect of ASD on weed suppression but also strawberry yield and fruit quality. The ASD provided variable weed control on different weed species, but ASD did reduce total weed density to an acceptable degree. The yeast application in ASD has great potential for increasing strawberry yield. The ASD at a half rate with yeast provided comparable weed suppression and yield as ASD at a full rate.