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Assessment of Agro-Industrial Wastes as a Carbon Source in Anaerobic Disinfestation of Soil Contaminated with Weed Seeds and Phytopathogenic Bacterium (*Ralstonia solanacearum*) in Tomato (*Solanum lycopersicum*)

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ABSTRACT: Managing weeds and soil-borne pathogens is one of the most challenging aspects of organic crop production. Anaerobic soil disinfestation (ASD) has been identified as a microbial-driven approach capable of suppressing weed species and soil-borne pathogens. The carbon substrate is a critical component of ASD that can be optimized to enhance pest control effectiveness. To assess this potential, a microcosm greenhouse study was conducted to determine the differential impact of agro-industrial waste streams as carbon sources, using molasses + mustard meal (MMM), molasses + chicken manure (MCM), molasses + corn gluten (MCG), and molasses + sweet potatoes (MSP) in anerobic or aerobic soil conditions (covered and not covered with plastic film) on bacterial wilt (caused by *Ralstonia solanacearum*), weed suppression, soil anaerobic conditions, and tomato (*Solanum lycopersicum* L.) crop health. Under anaerobic conditions, the carbon sources effectively controlled weeds by 75−96% compared with no carbon source (NCS) and aerobic treatment and greatly reduced or eliminated *Ralstonia solanacearum* populations from initial 5.6 to final 0 Log_{10} (CFU + 1) g^{-1} dry soil. No phytotoxic effects were observed in tomato plants transplanted 14 days after ASD treatments. These findings encourage further investigations on the interactions of ASD with soil chemistry and microbial biomass in the context of pest management.

KEYWORDS: *agricultural waste management, organic farming, Ralstonia solanacearum, mustard seed meal, molasses, bacterial wilt, Solanum lycopersicum*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the world's most produced vegetable crop.[1,2] The United States is one of the world's leaders in fresh tomato production. In 2020, US growers planted 113,322 hectares and harvested 110,438 hectares of tomatoes worth 1.66 billion US dollars farmgate value.[3] Organic vegetable cultivation has been expanding in the United States to meet consumer taste preference and demands for vegetables produced under chemical-free agricultural practices. An additional 103,319 hectares currently are transitioning to organic production with a 27% increase in overall organic vegetable production since 2017.[4] Organic tomato production increased by 89% from 2011 to 2017; however, according to a recent 2019 survey, production has decreased by 24% since 2017 and more than 65% of organic farms reported facing production and management challenges.[4] Pest management continues to be the biggest challenge for organic growers.

Weeds and soil-borne disease are the major pest issues that affect the US growers' market share and profitability. The use of plastic mulch in vegetables is a common production practice accepted widely in the United States.[5] However, plastic mulch does not adequately control all weeds.[6] Weed control options are limited in conventional tomato production, and even fewer options are available for organic production. Typically, organic growers rely on hand-weeding, which is labor-intensive and costly.[7] Additionally, large acreage growers are not able to hire enough workers to weed due to labor shortages. With high weed pressure, management costs for tomatoes in the southeast can range between $1000 and $1500 per acre.[7] In addition to weeds, soil-borne diseases are another limiting factor for organic crop production. *Ralstonia solanacearum*, a bacterium that causes bacterial wilt of solanaceous plants, is ubiquitous in southern soils and can remain viable in infected soil for more than 10 years.[8] This pathogen can be introduced into fields through infected transplants, water runoff from adjacent contaminated fields or the movement of human, instruments and equipment containing infested soil.[9] With the upsurge in consumer desire for organic and reduced pesticide grown vegetables, methods to combat bacterial wilt in tomatoes is extremely important.

Previously the use of methyl bromide and other soil fumigants had been commonly used for controlling pests in plasticulture production systems.[10,11] The phaseout of methyl bromide and other soil fumigants due to health and environmental concerns, has resulted in various studies seeking more successful techniques to help the domestic tomato industry, both

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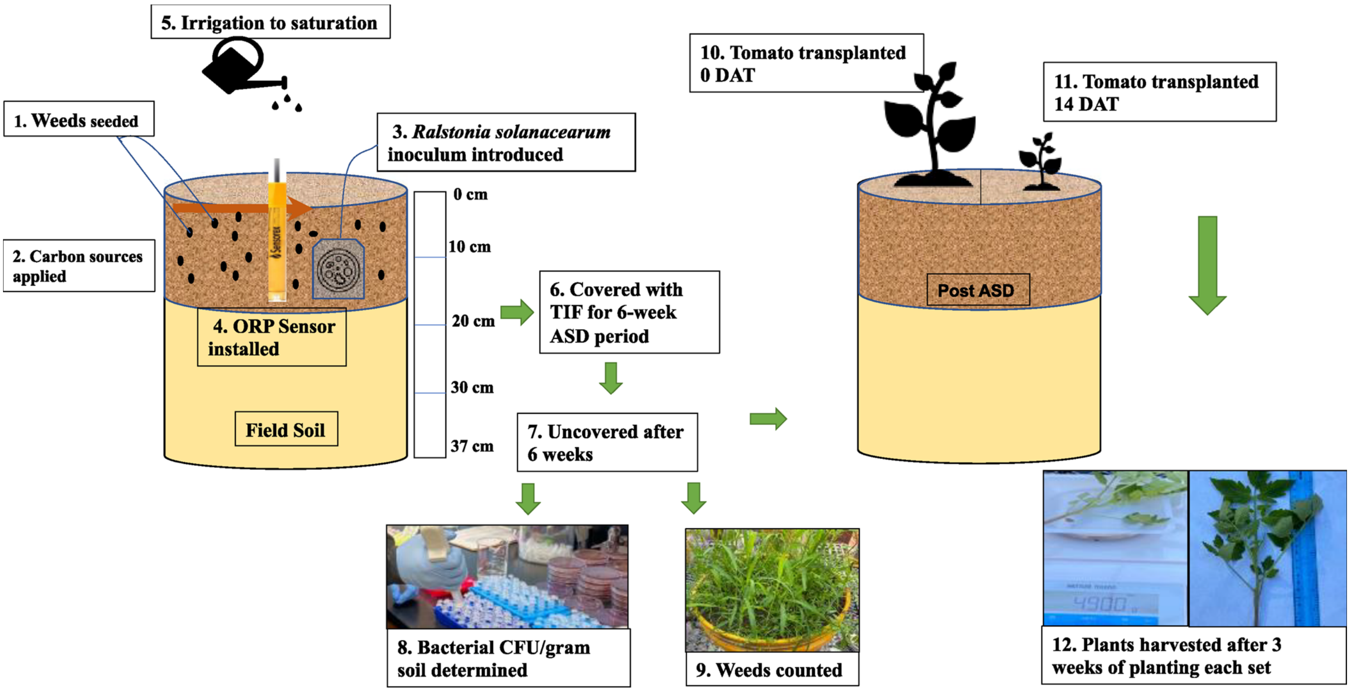

Figure 1. Schematic diagram of the experimental design and research methodology.

conventional and organic, overcome pest management problems.[12−16] Several biological control agents including *Acinetobacter* sp., *Bacillus thuringiensis*, *Bacillus subtilis*, *Bacillus* sp., *Enterobacter* sp., *Burkholderia nodosa*, *B. sacchari*, *B. tericola*, *B. pyrrocinia* and bacteriophages are found effective to delay the appearance of the bacterial wilt symptoms and reduced the disease incidence in solanaceous crops.[17] However, frequent soil inoculation with microbial cultures may not be feasible for commercial growers with each crop rotation. Growing bacterial wilt-resistant cultivars is the most effective and environmentally friendly method of disease control.[17] However, public acceptance is required before such genetically modified crops can be used commercially. Furthermore, bacterial wilt reduction in many plants has generally been inversely proportional to crop yield and quality and Ralstonia strains have led to the development of resistant defenses that are effective in some growing regions but ineffective in others.[18]

Nonchemical techniques such as solarization, flooding, and steam sterilization are some available options for pest management in organic production. However, these environmentally friendly approaches have limitations that hinders the commercial adoption, including long treatment process (>2 months) and high-temperature requirements for solarization (36−60 °C), long treatment period with high water utilization for flooding (4−6 weeks), and high application costs for steam sterilization (>$12,000/ha).[19−21] Biosolarization has been effective in controlling pests in organic production.[22] This method is modified from solarization, which uses organic amendments and irrigation in addition to tarping with clear mulch. Another promising nonchemical option available is anaerobic soil disinfestation (ASD), which slightly differs from biosolarization, which is not dependent on solar heat supply.

ASD method has been studied as a preplant control measure for a wide range of soil-borne pathogens, nematodes, and weeds.[16,23] ASD is facilitated by adding carbon-rich amendments or macerated debris to the soil, tarping with a vapor impermeable plastic film and saturating the soil under the film with water, which rapidly creates an anaerobic environment that kills many of the obligate aerobic plant pathogens.[12−15,23] Several studies report that the changes in microbial composition, release of volatile organic compounds (VOCs), decreased soil pH, and reduced soil conditions all aid in pest suppression during ASD.[23−25] Carbon input, temperature, incorporation technique, soil type, and weed species were all identified as critical variables affecting pest management via ASD.[23,26−31] The ASD method is not reliant on the sun's rays and appears to destroy pathogens deeper in the soil profile than solarization due to both the anaerobic conditions and the release of various VOC.[32,33] From shallow growing plants such as strawberries to deep-rooted nut trees, ASD has proved effective for control of several plant pathogens across multiple plant species.[23]

In addition to pest control, ASD can reduce crop production costs and environmental pollution, by utilizing agro-industrial wastes as carbon sources. ASD can utilize large amounts of agro-industrial waste that may otherwise end up in landfills or incinerated, leading to excessive greenhouse gas (GHG) and pollutant emissions. On the other hand, the aerobic composting process often results in unwanted byproducts, such as odors, gases (CH_4, NO_2, and NH_3), and leachate production, which may cause secondary pollution. ASD has been shown to sustain crop yield while having no substantial negative effect on tomato quality in terms of pH, firmness, dry matter content, and total soluble solids when compared to chemical soil fumigation.[14,15,27] The application of ASD in commercial tomato production systems has not yet gained widespread acceptance due to a lack of a standardized cost-effective carbon source capable of providing multipest control.[23] For ASD to become widely used by organic tomato producers, it must be as effective and result in equal or more crop profitability than the conventional methods of pest and weed control. Research is still lacking on several key factors for different geological regions, such as locally available soil amendments as carbon sources, cost-effectiveness, and ultimate potential as a pest management strategy.

In this study, we evaluated the effects of different combinations of carbon sources (molasses + sweet potatoes, molasses + liquid corn gluten, molasses + chicken manure, and molasses + mustard meal) in creating anaerobic conditions, weed suppression, *Ralstonia solanacearum* reduction and tomato plant growth response after ASD in microcosm-based greenhouse trials. This simulated ASD in microcosms can serve as a decision-making tool for determining the most effective carbon source from agro-industrial wastes that warrants further investigation in more resource-intensive field experiments.

■ MATERIALS AND METHODS

Greenhouse experiments were conducted at Clemson University Coastal Research and Education Center (CREC), Charleston, SC, USA (32.794091, −80.068349). The experiments were conducted twice, with trial 1 initiated on October 15, 2019, and trial 2 initiated on September 23, 2020. Average daily maximum/minimum or day/night temperatures of greenhouses were set to 26/22 ± 2 °C.

Experimental Setup. The soil for experiments was collected from the surface horizon (0−15 cm) at the USDA Organic Crops Unit in Charleston and passed through a 4 mm sieve. Soil is characterized as Charleston Loamy fine sand (thermic *Aquultic* Hapludalfs) with pH 6.4 and 0.8% soil organic matter. Soil was filled in 19,000 cm^3 plastic containers (microcosms) with 37 cm height and 30 cm diameter (The Home Depot, Atlanta, GA, USA), which were used as the experimental units. Carbon source used in this study was molasses (Unsulfured Blackstrap Molasses, North Georgia Still Co., Dahlonega, GA, USA), sweet potatoes (Valpredo Farms Bakersfield, CA, USA), corn gluten (ICT Organics, Baltimore, MD, USA), chicken manure (Pearl Valley Organix, Pearl City, IL, USA), and mustard meal (PESCADERO GOLD Mustard meal, Farm Fuel Inc., Watsonville, CA, USA) at a rate of 13.5 m^3/ha, 2.24 t/ha, 1.01 m^3/ha, 20.34 t/ha, and 2.17 t/ha, respectively. Rates of carbon sources were based on the literature and preliminary studies conducted at USDA-ARS, United States Vegetable Laboratory, Charleston, SC, USA, and detailed information is provided (Table S1). The experiments were designed as a randomized complete block design (RCBD) with four replications. The treatments were structured as a factorial with five carbon source combinations, (i) molasses + sweet potatoes (MSP), (ii) molasses + liquid corn gluten (MCG), (iii) molasses + chicken manure (MCM), (iv) molasses + mustard meal (MMM), and (v) no carbon source (NCS), each either covered with plastic (ASD treatment) or not covered (non-ASD treatment). NCS, noncovered and no carbon source, and plastic-covered treatments served as controls. The research methodology and experimental design are presented in the form of a schematic diagram (Figure 1).

Chemical analyses of carbon amendments used in this study were conducted through Waters Agricultural Laboratories in Camilla, GA, and key parameters are listed in Table 1. All the experimental units were seeded with 100 seeds (1408 seeds/m^2) of three economically significant weed species Palmer amaranth (*Amaranthus palmeri* S. Wats.), Barnyardgrass (*Echinochloa cruss-galli* (L.) *P. Beauv*), and large crabgrass (*Digitaria sanguinalis* L. Scop) at 15 cm depth in each microcosm. Carbon amendments were mixed in the upper 0−20 cm soil in the microcosms. Before application, liquid molasses was diluted with water (1:1 on v/v) to ease application and poured onto soils. Carbon sources were chosen as relatively widely available agricultural byproducts that could be used as organic amendments for ASD treatment.

Bacterial Cultured Soil Inoculum Preparation and Detection of Colony-Forming Units (CFU) after ASD. A local isolate of *Ralstonia solanacearum* was obtained from naturally infected tomato plants at the USDA-ARS Vegetable Laboratory in Charleston, South Carolina, and verified by PCR[34] and biochemically[35,36] to be *R. solanacearum* race1 biovar 1. The isolate was grown in a 1 L culture of CPG (casamino acids, peptone, and glucose) broth[37] amended with 10 mL of 10 mg/mL RIF at 24 °C for 48 h in the dark at 200 rpm. The bacterial suspension was pelleted at 8000 RPM for 10 min, rinsed and diluted in sterile distilled water and then mixed with 10 kg of autoclaved soil for a final colony density of approximately 10^{10} cells per g dry soil. Mesh bags (PouchMart, Oxnard, CA, USA) with 0.5 mm sieve size containing 20 g of this inoculated soil were buried 15−20 cm deep in each I. Inoculum bags were removed at the end of the 6-week ASD period, when the microcosms were uncovered. From each mesh bag 10 g of soil were subsampled from the 20 g subsample and suspended in 20 mL of sterile distilled H_20. The soil solution was vortexed for 60 s, and serial diluted by taking 100 μL of soil solution into 900 μL of H_2O for 4 times and repeated twice. Dilutions were plated onto SMSA medium[38] amended with cycloheximide 100 mg/L and rifampicin 100 μg/L, as well as TTC medium[37] amended with rifampicin 100 mg/L and cycloheximide 100 μg/L. Plates were then incubated for 3 days at 28 °C. Small (1−2 mm) disk-shaped colonies, typical for *R. solanacearum* biovar 1, were counted and CFU densities g/dry soil were calculated and converted into log values.

Table 1. Chemical Composition of Carbon Amendments Used in a Study that Evaluated the Impacts of Anaerobic Soil Disinfestation (ASD) on Redox Potential, Weeds Control, Bacterial Wilt Control and Tomato Response[a]

parameters	chicken manure content (%)[b]	mustard meal	sweet potato	molasses	corn gluten meal
nitrogen-total	2.12	5.98	0.29	0.32	1.80
organic nitrogen	1.69	5.60	0.25	0.26	0.69
P2O5-total	3.04	2.33	0.10	0.05	0.19
K2O-total	3.18	1.24	0.62	1.33	0.09
sulfur	0.67	1.41	0.04	0.08	0.13
boron	0.01	0.001	0.01	0.001	0.001
zinc	0.03	0.01	0.02	0.001	0.003
manganese	0.05	0.003	0.01	0.001	0.001
iron	0.15	0.01	0.01	0.003	0.001
copper	0.02	0.001	0.01	0.001	0.001
calcium	7.41	0.54	0.07	0.40	0.02
magnesium	0.66	0.44	0.03	0.07	0.008
sodium	0.75	0.01	0.01	0.05	0.28
aluminum	0.18	0.01	0.01	0.004	0.001
total carbon	14.90	47.52	6.36	30.82	7.44
total organic carbon	10.64	38.17	6.17	29.80	7.13
carbon: nitrogen	7:1	8:1	22:1	96:1	4:1
pH	8.97	5.0	4.66	5.0	5.0

[a] Experiment was conducted in the microcosms at the greenhouse research facility, Clemson University Coastal Research and Education Center (CREC), Charleston, SC, USA. [b] Wet basis %.

Sensor Installation. Oxidation−reduction potential sensors (Pt combination electrodes, Ag/AgCl reference; Sensorex, Garden Grove, CA, USA) were installed in the center of each microcosm at a 15-cm depth to monitor soil conditions. A data logging system (CR-1000× with AM 16/32 multiplexers, Campbell Scientific, Logan, UT, USA) was used to record the output from the sensors every 30 s and averaged on an hourly basis. All microcosms were irrigated with tap water to saturation at the initiation of the trial. For ASD treatments, the microcosms were covered with 15-μm-thick transparent totally impermeable film (TIF) polyethylene/plastic mulch (TriEst Ag Group, Greenville, NC) and secured using reinforced rubber bands (Global industries, Buford, GA, USA), whereas in non-ASD treatments, the microcosms were left uncovered. Microcosms were set on greenhouse benches in a completely randomized block design and kept stationary for the 6-week treatment phase. The experiment ended on November 30, 2019, and November 7, 2020, in Trials 1 and 2, respectively.

Weed Assays and Crop Performance. After 6 weeks, ASD was terminated by uncovering plastic mulch covers from microcosms. Weed ratings were conducted immediately, which consisted of percent weed control ratings and individual weed counts. Percent weed control was estimated by visual observations on a scale of 0 to 100%, by comparing weed infestation between control (NCS, noncovered) and all other treated experimental units in each replication, where 0% weed control refers to control treatment and 100% refers to complete weed mortality in a microcosm.

Following weed species assessments, tomato plants were transplanted twice in each microcosm by dividing the base area of microcosm into two halves. The cultivar transplanted was mountain magic (Johnny's Selected Seeds, Winslow, ME, USA). The first set of tomatoes were transplanted immediately post-trial and the second set 14-days post-trial to determine the impact of treatments on the growth and biomass response of plants at two-time intervals.

After 3 weeks of each planting set, aboveground height and biomass of plants were recorded, because in this period phytotoxicity symptoms, such as chlorosis and leaf necrosis, became apparent. For aboveground biomass, plants from each treatment were clipped and oven dried in a general protocol oven (Heratherm, Thermo Scientific, MA, USA) at 70 °C for 72 h and weighed.

Data Analysis. The experiment followed a two-factor (carbon source type and polythene cover) factorial design with four replications of each treatment and the experiment was repeated. All data was subjected to analysis of variance using mixed model methodology (JMP v. 16). Carbon source, plastic cover, trial run, and all interactions between these effects were considered fixed while replication was considered random. Percent weed control, weed counts, shoot height, dry weight, bacterial counts, and redox potential data were pooled for both trials, because there was no treatment by trial interaction. All data sets were examined for normal distribution with the Shapiro−Wilk and Anderson−Darling tests. When necessary, either square root, log or arcsine-square-root transformation was used to normalize the data. Weed Counts data were normalized by square root transformation. The transformed data were used for statistical interpretation, but the back-transformed data were presented. CFU are converted into Log$_{10}$ (CFU + 1) g^{-1} of dry soil. Means were separated using Tukey−Kramers HSD test.

RESULTS

Chemical Composition of Tested Carbon Sources. Total carbon inputs from tested agro-industrial byproducts ranged from 6.36 to 47.52% (63.6 to 475 g kg^{-1}), and C/N ratios ranged from 4:1 to 96:1 (Table 1). Major differences between the chemical composition of tested agro-industrial byproducts were their total nitrogen (N), total carbon (C), and hence C/N ratios (Table 1). The mustard seed meal used in this microcosm study contained 47.52% (475 g kg^{-1}) C and 5.98% (58 g kg^{-1}) N with a C/N ratio of 8:1. Chicken manure had 14.90% (149 g kg^{-1}) C and 2.12% (21.2 g kg^{-1}) N with a C/N ratio of 7:1. Sweet potatoes had a lower N concentration of 0.29% (2.9 g kg^{-1}) and a higher C/N ratio of 22:1. Molasses also had a lower N concentration of 0.32% (3.2 g kg^{-1}) and a much higher C/N ratio of 96:1. Corn gluten meal had a lower N concentration of 1.80% (18 g kg^{-1}) and a lower C/N ratio of 4:1. Mustard seed meal contained about 2.5 times as much sulfur as chicken manure and more than 10 times that of sweet potatoes, molasses, and corn gluten meal. Mustard seed meal and chicken manure had much higher concentrations of phosphorus pentoxide (P_2O_5). Chicken manure had a higher concentration of potassium oxide (K_2O), followed by mustard seed meal and molasses, while sweet potato and corn gluten had the lowest K_2O concentration. Other elements, such as Na, Zn, and Mg, were comparable in all five agro-industrial byproducts amendments. The pH of chicken manure was 8.97, indicating a tendency toward alkalinity, whereas the pH of all other carbon sources was near to 5, indicating tendency toward acidity.

Redox Potential Measurements (Anaerobic Conditions). The soil redox potential in each microcosm was measured every 30 s and averaged on an hourly basis. To calculate average anaerobic conditions throughout the experiment, redox potential (Eh) readings were averaged, and 200 mV was selected as the threshold below which the soil is considered in the anaerobic phase.[14] The reduction in the Eh value (<200 mV) implied the consumption of oxygen and formation of anaerobic conditions in the soil. The effects of carbon source, plastic cover and their interaction had a significant influence on accumulated soil anaerobic conditions ($P < 0.001$). Overall, the NCS noncovered treatment had the least anaerobic conditions. Higher anaerobic conditions (<−100 mV) were achieved in plastic-covered treatment amended with MMM, followed by MCM (<−50 mV) (Figure 1). NCS plastic-covered treatment was not able to attain anaerobic conditions, however it was less aerobic in comparison to all noncovered carbon source treatments or control (Figure 2).

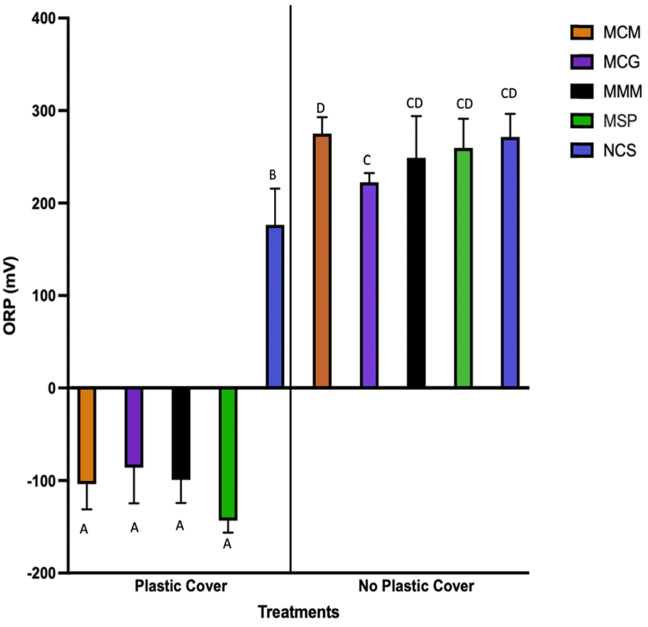

Figure 2. Mean and standard error ($n = 8$) of the soil redox potential (mV) measured during 6 weeks of Anaerobic Soil Disinfestation (ASD) in soil carbon source treatments covered and not covered with plastic film in microcosms, amended with molasses + chicken manure (MCM), molasses + corn gluten meal (MCG), molasses + mustard meal (MMM), and molasses + sweet potato (MSP). Data are also given for no carbon source (NCS) control soil treatments. Data were pooled for both trials, because there was no treatment by trial interaction Bars indicated by different letters are significantly different at $p < 0.05$ according to Tukey's HSD test. Soil conditions typically considered aerobic when redox potential is >200 mV and anaerobic when <200 mV. Experiment was conducted at the greenhouse research facility, Clemson University Coastal Research and Education Center (CREC), Charleston, SC, USA.

Percent Weed Control and Weed Counts. Barnyardgrass and large crabgrass were the two most prevalent grass weeds infesting our experimental microcosms. The average heights of crabgrass, Palmer amaranth, and barnyardgrass plants were 22 ± 5 cm, 15 ± 5 cm, and 20 ± 5 cm, respectively, in the noncovered treatments. The other broadleaf weeds, whose seeds were naturally present in soil identified, were carpet weed (*Mullugo verticillate* L.), corn spurry (*Spergula arvensis* L.), cutleaf evening-primrose (*Oenothera laciniata* Hill), and swinecress (*Cornopus didymus* L.). The carbon sources, plastic cover, and their

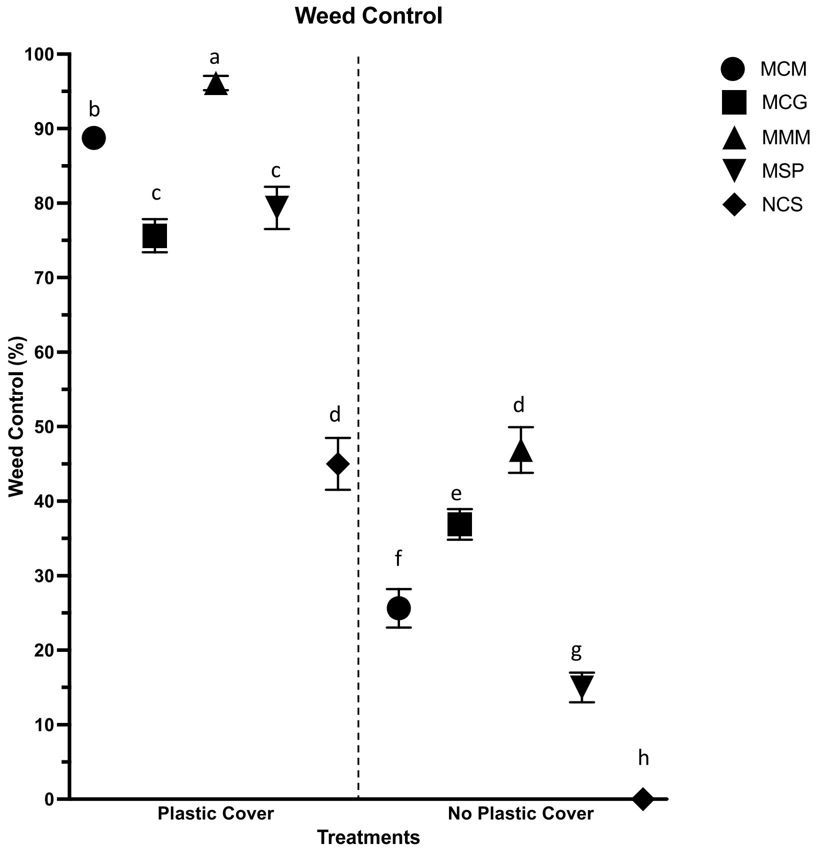

Figure 3. Mean and standard error ($n = 8$) of weed control ratings after 6 weeks of Anaerobic Soil Disinfestation (ASD) in soil carbon treatments covered and not covered with plastic film in microcosms, amended with molasses + chicken manure (MCM), molasses + corn gluten meal (MCG), molasses + mustard meal (MMM), or molasses + sweet potato (MSP), compared to no carbon source (NCS) in microcosms. Data were pooled for both trials, because there was no treatment by trial interaction Control based on visual scale of 0 to 100 by comparing weed infestation between untreated and treated microcosms in each replication, where 0% control refers NCS and 100% refers to complete weed mortality. Bars indicated by different letters are significantly different at $P < 0.05$ according to Tukey HSD test. Experiment was conducted at the greenhouse research facility, Clemson University Coastal Research and Education Center (CREC), Charleston, SC, USA.

interaction had a significant inhibitory effect on weed control ($P < 0.001$). MMM plastic-covered treatment reduced weeds by 96% as compared to the control (NCS noncovered) (Figure 3). MCM plastic-covered treatment exhibited 89% weed control and was second most effective in reducing weed infestation, followed by MSP and MCG plastic-covered treatments, which reported weed control by 75% and 79%, respectively (Figure 3). NCS plastic-covered treatment was effective in preventing weed growth by 45%. Interestingly, noncovered MMM treatment reduced weeds by 47% (Figure 3).

The effects of carbon source, plastic cover and their interactions on grass weed seedlings counts were significant ($P < 0.001$). For both grass weeds (barnyardgrass and large crabgrass), shoot counts were lowest in microcosms treated with MMM plastic cover treatment (Figure 4A,B). Other carbon sources (MSM, MCM, or MCG) plastic-covered treatments having similar reduced grass weed counts in relation to nontreated or carbon sources noncovered treatments (Figure 4A,B). Palmer amaranth weed seedlings counts were significantly reduced with plastic-covered relative to the noncovered treatments ($P < 0.001$). In all plastic-covered treatments, regardless of carbon sources, complete control of Palmer amaranth was observed (Figure 4C). Additionally, other weeds were counted that were present naturally in the soil, such as carpet weed, corn spurry, cutleaf evening-primrose, and swinecress. These weeds were significantly suppressed by the carbon source, plastic cover, and their interaction ($P < 0.001$, data not shown in detail because these weeds were not seeded).

Effect of Treatments on *Ralstonia solanacearum* Survival. The effects of carbon source, plastic cover (covered or noncovered), and their interaction on bacterial CFU were significant ($P < 0.001$). The CFUs were observed to be significantly reduced in all carbon source plastic-covered treatments in comparison to NCS plastic-covered or all uncovered treatments (Figure 5). The initial population of *R. solanacearum* at the starting point of the experiment was 5.6 Log_{10} (CFU + 1) gm^{-1}. After 6 weeks of ASD treatment, the observed populations recovered from the inoculated soil ranged from 0 to 6 Log_{10} (CFU + 1) g^{-1} of dry soil. At the end of each trial, *R. solanacearum* was not detected in soil treated with MMM and MCM plastic-covered treatments, indicating 100% mortality of *R. solanacearum* (Figure 5). MCG and MSP plastic-covered treatments were observed as second and third in reducing the pathogen population with 0.33 and 1.71 Log_{10} (CFU + 1) g^{-1}, respectively. A minor reduction in CFU in *R. solanacearum* cells was also observed in unsealed carbon sources in which MMM and MCG were used as carbon sources. In this experiment, 4 weeks after transplanting, no wilting symptoms were observed on tomato seedlings grown in any treatment.

Tomato Plants Response to ASD. Plants transplanted immediately after ASD in MSP plastic cover treated microcosms showed some phytotoxic effects in terms of yellowing of leaves,

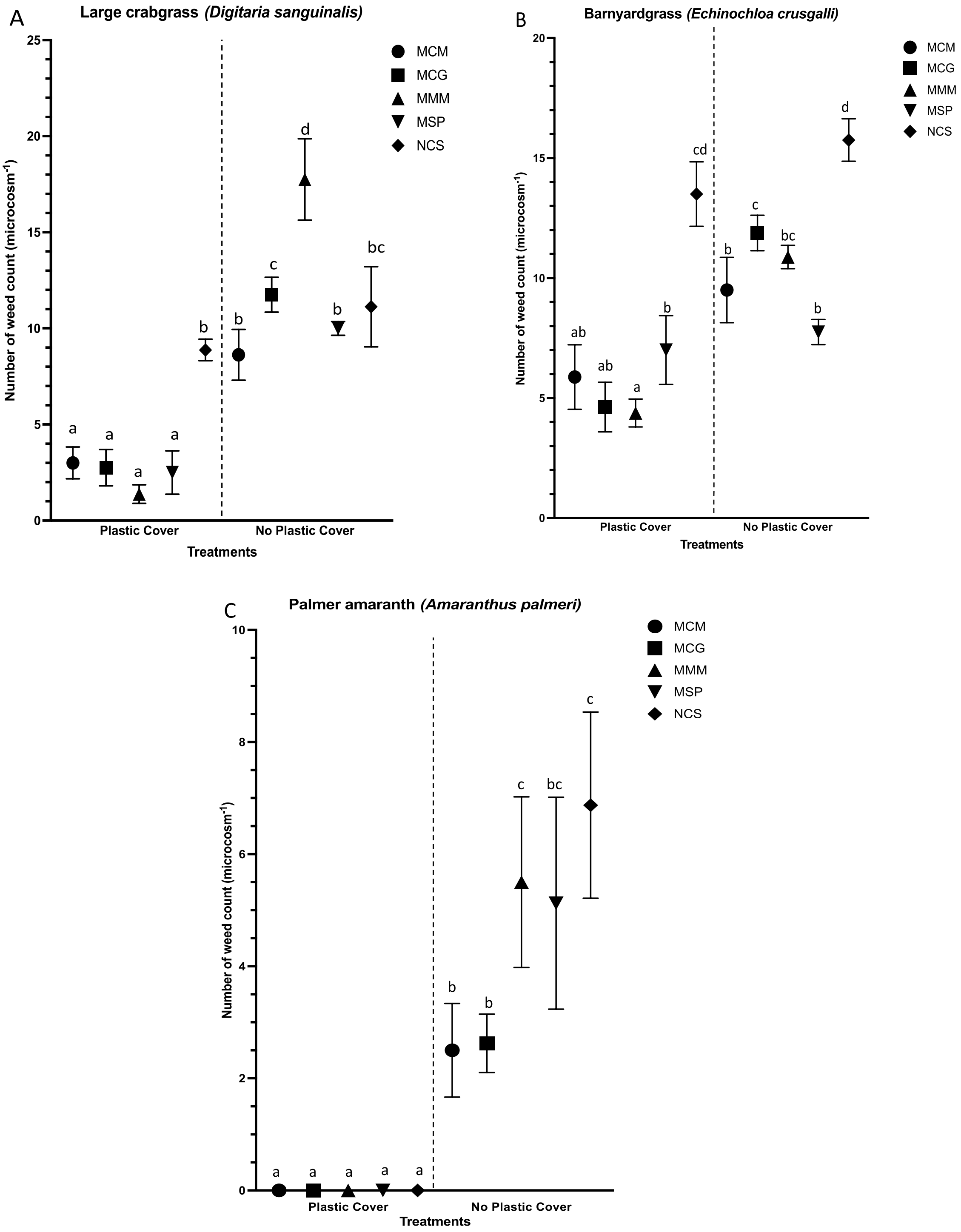

Figure 4. Mean and standard error ($n = 8$) of weed shoot counts of (A) *Digitaria sanguinalis*, (B) *Echinochloa crusgalli* (C) *Amaranthus palmeri* taken after 6 weeks of Anaerobic Soil Disinfestation (ASD) in soil carbon treatments covered and not covered with plastic film in microcosms, amended with molasses + chicken manure (MCM), molasses + corn gluten meal (MCG), molasses + mustard meal (MMM), molasses + sweet potato (MSP). Data are also given for no carbon source (NCS) control treatments. Data were pooled for both trials, because there was no treatment by trial interaction Bars indicated by different letters are significantly different at $P < 0.05$ based on Tukey's HSD test. Experiment was conducted at the greenhouse research facility, Clemson University Coastal Research and Education Center (CREC), Charleston, SC, USA.

stunted growth, and reduced biomass in comparison to other treatments including control. This may be due to the allelopathic effects of decomposition of sweet potatoes in the anaerobic environment. Significant differences were detected in the shoot mass and height of tomato plants grown in MSP plastic cover/ASD treatment transplanted immediately after ASD (Table 2). However, plants transplanted 14 days after ASD were apparently unaffected in all treatments.

■ DISCUSSION

The Lynchburg soils, which formed from sandy and loamy marine sediments, are the most common type of soil found in

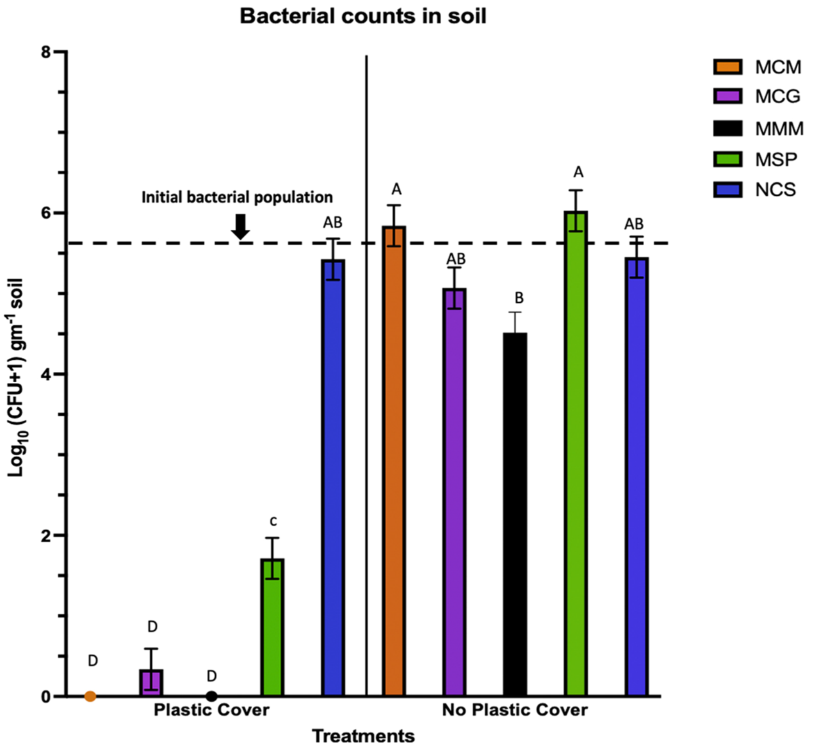

Figure 5. Mean and standard error ($n = 8$) of *Ralstonia solanacearum* population in soil after 6 weeks of Anaerobic Soil Disinfestation (ASD) in soil carbon treatments covered and not covered with plastic film in microcosms, amended with molasses + chicken manure (MCM), molasses + corn gluten meal (MCG), molasses + mustard meal (MMM) and molasses + sweet potato (MSP). Data are also given for no carbon source (NCS) control treatments. Data were pooled for both trials, because there was no treatment by trial interaction Colony-forming units are converted into Log$_{10}$ (CFU + 1) gm^{-1} of dry soil. Bars indicated by different letters are significantly different at $p < 0.05$ according to Tukey HSD test. Experiment was conducted at the greenhouse research facility, Clemson University Coastal Research and Education Center (CREC), Charleston, SC, USA.

Table 2. Effects of Carbon Sources, Plastic Cover and Their Interactions on Tomato Plants Growth Transplanted 0 and 14 DAT or Anaerobic Soil Disinfestation (ASD)[a]

		tomato transplanted 0 DAT[c]		tomato transplanted 14 DAT[c]	
plastic cover	carbon source[b]	shoot length (cm)	dry weight (gm)	shoot length (cm)	dry weight (gm)
covered	MCM	21.75 ± 1.07 B[d]	1.42 ± 0.17 AB	24.25 ± 0.7 A	1.59 ± 0.12 A
	MCG	23.25 ± 1.29 AB	1.13 ± 0.19 BC	25.75 ± 1.19 A	1.86 ± 0.24 A
	MMM	23 ± 1.25 AB	1.39 ± 0.18 AB	25.51 ± 1.18 A	2.01 ± 0.21 A
	MSP	18.12 ± 0.92 C	0.83 ± 0.08 C	24.12 ± 0.93 A	1.61 ± 0.16 A
	NCS	20.75 ± 1.38 B	1.08 ± 0.15 BC	23.75 0.83 A	1.58 ± 0.18 A
not covered	MCM	27.75 ± 0.76 A	1.97 ± 0.12 A	26 ± 1.0 A	1.96 ± 0.13 A
	MCG	24.5 ± 1.48 AB	1.63 ± 0.20 AB	25 ± 0.88 A	1.81 ± 0.18 A
	MMM	27.25 ± 0.93 A	2.03 ± 0.22 A	28.25 ± 1.09 A	2.29 ± 0.25 A
	MSP	20.5 ± 0.7 B	1.01 ± 0.06 BC	25.5 ± 1.54 A	2.03 ± 0.41 A
	NCS	24 ± 1.08 AB	1.41 ± 0.14 AB	24.5 ± 1.80 A	1.77 ± 0.28 A
		p-value			
carbon source		<0.01	<0.001	NS	NS
plastic cover		<0.001	<0.001	NS	NS
carbon source x plastic cover		NS	NS	NS	NS

[a] Experiment was conducted at the greenhouse research facility, Clemson University Coastal Research and Education Center (CREC), Charleston, SC, USA. [b] MCM (mustard meal + chicken manure), MCG (molasses + corn gluten), MMM (molasses + mustard meal), MSP (molasses + sweet potato), NCS (no carbon source). [c] Tomato plants were transplanted twice in each microcosm by dividing the base area of microcosm into two halves. The first set was transplanted immediately after treatment or ASD (0 DAT), when the microcosms were uncovered and the second set transplanted 14 days after treatment or ASD (14 DAT). [d] Means within the column followed by the same letter are not significantly different based on Tukey's honestly significant difference (HSD) test ($P < 0.05$). Data were pooled for both trials because there was no treatment by trial interaction. *P*-values of the analysis of variance (ANOVA) of the effect of plastic cover, carbon source type, and their interaction are also provided.

the southeast coastal area. ASD is promising for sandy soils with high-temperature regions, possibly because of pathogen and weed suppression caused by elevated soil temperatures, as well as significant beneficial effects of organic matter additions on the chemical, biological, and physical characteristics of sandy soils.[14,31] In this experiment, all carbon sources in anaerobic conditions suppressed tested weed species significantly more than the nontreated control and effective in reducing or eliminating *R. solanacearum*.

Chemical Composition of Tested Carbon Sources. This simulated-microcosm ASD study aimed to assess the potential of various agro-industrial wastes as carbon sources for pest management in ASD conditions. The redox potential (Eh) values in all tested carbon sources in plastic-covered microcosms were similar (Figure 2). Therefore, the chemical analysis report of tested carbon sources (Table 1) and average soil redox potential (Eh) during ASD indicates no observable relationship between the amount of C added or the C:N ratio and average soil Eh. One of the major limitations of this study is that we did not conduct in-depth chemical analyses of the mixed treatments and treated soil post-ASD. More research is required to understand better the effects of agro-industrial byproducts as carbon sources and their relationship with different nutrient concentrations and pest management. However, the treatments containing mustard meal (MMM) and chicken manure (MCM) in addition to molasses had a higher concentration of N, P, K, Ca, and Mg contents, which may have related to their greater effectiveness in controlling weeds and *Ralstonia solanacearum*. Similarly, these chemicals in various carbon sources have been linked to the control of soil-borne diseases in tomato in previous ASD studies.[39]

Redox Potential Measurements (Anaerobic Conditions). Accumulated anaerobic conditions are reported as a key indicator for successful pest control.[15] In this experiment, soil anaerobic conditions were significantly greater in carbon-treated soils covered with plastic mulch as compared to the NCS plastic-covered (control) and all other treatments without plastic cover. Redox reactions in higher anaerobic settings results in the release of poorly oxidized substances such as methane and ethylene gases, alcohol, and organic acids, all of which are lethal to plant pathogenic bacteria.[15,31] Plant pathogenic bacteria, such as *R. solanacearum* are aerobic microbes that require oxygen to survive and proliferate. As a result, anoxic conditions may inhibit the development and multiplication of *R. solanacearum*, resulting in a reduction or elimination of bacterial populations in the soil environment. Additionally, ASD influences the composition of the soil microbial population, resulting in the dominance of anaerobic microorganisms in the soil.[25,40] The lower *R. solanacearum* populations seen in this study might be a result of the species' low compatibility and competitiveness with anaerobic microbes during the ASD process.

Weed Control. The phaseout of the methyl bromide due to health and environmental concerns has resulted in numerous studies to find other effective strategies for controlling weeds.[22,23,28] Currently available weed control options in organic specialty crops are limited. Moreover, in conventional polyethylene mulched vegetable production, the application of herbicides, both as premulching application or through the under-mulch drip irrigation system, has been a unique tool for weed control.[41,42] However, with the lack of effective herbicide options in specialty crops and an increase in documented cases of herbicide resistance, the future of herbicide-based weed management programs is uncertain.[43] Mixed results have been documented in terms of ASD effects on weed control.[28] For example, mustard seed meal (3.3 Mg ha^{-1}) and rice bran (20 Mg ha^{-1}) mixed carbon treatment moderately reduced weed populations in California in one of 2 years of field studies, but rice bran alone provided unacceptable level of weed control as compared to steam and chemical fumigant treatments.[44] In another ASD study conducted in Florida, carbon sources such as composted poultry litter and molasses demonstrated an average weed control efficacy of 85% in one location but an unacceptable level of weed control in another location.[27] Different carbon sources (wheat bran, molasses, ethanol, and chicken litter) completely inhibited yellow nutsedge tubers when buried 15 cm deep in pots, according to another Florida study.[45] In a greenhouse pot study conducted in Tennessee, wheat bran-based treated pots contained 92% less weed propagule than untreated control pots.[16] Overall, variations in carbon sources, temperature, integration process, soil composition, and weed species have all been studied as key factors influencing weed control in previous ASD studies.[16,23,27,28]

In this microcosm study, weeds were suppressed in ASD treatments with mixed carbon amendments and significant effects were observed within carbon source plastic-covered treatments (Figure 3). The MMM treatment combination was significantly more effective for overall weed control and for reducing each weed seedling counts than the other mixed carbon sources treatments. It is possible that differences in carbon source efficacy in weed control could have been related to the specific microbial communities associated with degradation of organic carbon sources, soil pH, or generated anaerobic conditions in the treated soil.

Previous studies reported that crabgrass is not affected by ASD.[20] In contrast, this study's findings indicate ASD with mixed carbon treatment significantly reduces crabgrass infestation (Figure 4A). Palmer amaranth is recognized as a major problematic weed in vegetable production in North and South Carolina. Establishment of glyphosate-resistant genotypes of Palmer amaranth on farms in the southern United States has resulted in the adoption of physical weed control methods such as hand-weeding and tillage, resulting in greatly increased production costs.[46] In this study, Palmer amaranth emergence was reduced to zero counts in all plastic-covered treatments regardless of carbon source, which may be attributed to seeding depth, or light requirements in all covered mesocosms.[47] While contrasting the large crabgrass emergence, barnyardgrass was more resistant to the effects of ASD treatments in our study. Overall, the most effective mixed treatment for weed control in this study was MMM (Molasses and Mustard meal). Molasses is a common carbon source used to facilitate ASD in southeastern states such as Florida,[48] and mustard seed meal, a byproduct of the oil extraction process of Brassica crops, contains a class of secondary plant metabolites called glucosinolates. Allelopathic compounds such as isothiocyanates (ITC) form by degradation of glucosinolates, which suppress certain weed species.[49] The findings of this study indicate that when molasses is combined with allelopathic organic amendments such as mustard meal, it is possible to target the soil weed seed bank. Molasses acts as a chelating agent or organic stimulant when combined with other organic amendments, providing a readily available source of carbon energy and carbohydrates to feed and accelerate the growth of beneficial microbes. As demonstrated in this study, ASD with mixed carbon treatments can be an advantageous strategy for weed control in field plasticulture settings.

Effect of Treatments on *Ralstonia solanacearum* Survival. ASD has been shown to be an effective method for reducing or eliminating several soil-borne phytopathogens. Bacterial wilt caused by *R. solanacearum* is the most crop limiting factor of tomatoes in the southern United States. Currently, there is no effective method available for organic growers for controlling this pathogen in South Carolina. Our experiment findings indicate that ASD is capable of greatly reducing and even eliminating *R. solanacearum*. In this study, combination of liquid molasses and easily decomposable organic amendments, was found to strongly reduce the inoculated soil population densities of *R. solanacearum*. Petri plates demonstrating the *Ralstonia solanacearum* colonies plated after 6 weeks of ASD (Figure 6).

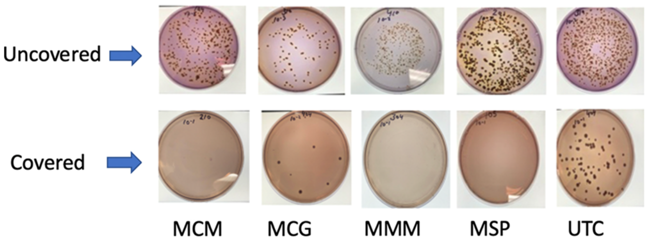

Figure 6. Petri plates demonstrating the *Ralstonia solanacearum* colonies plated after 6 weeks of Anaerobic Soil Disinfestation (ASD). Samples taken from soil carbon treatments covered and not covered with plastic film in microcosms, amended with molasses + chicken manure (MCM), molasses + corn gluten meal (MCG), molasses + mustard meal (MMM), molasses + sweet potato (MSP) and no carbon source (NCS). Experiment was conducted at the greenhouse research facility, Clemson University Coastal Research and Education Center (CREC), Charleston, SC, USA.

Our findings are consistent with previous ASD work in which wheat bran was used as a carbon source in a simulated ASD study and the population of *R. solanacearum* decreased below the detection limit after 14 days of treatment.[50] In this study, 250 g of infested soil was treated with ASD using 4 g of wheat bran. The effect of ASD was observed in this study as the experimental microcosms with only plastic covering and no carbon amendment, which were exposed to the same conditions as the ASD ones, did not exhibit a decline in *R. solanacearum* populations. Similar effects were observed in a previous study amended with fresh grass as a carbon source.[51] The soil environment created by ASD is inhospitable for plant pathogens as a result of the generation of toxic volatile and nonvolatile fatty acids by the decomposition of carbon sources by soil microbial populations.[33]

The greater control of *R. solanacearum* observed in MMM amended ASD-treated soil may be due to biocidal effects of isothiocyanates produced by mustard meal. Our results indicate mixed carbon sources and plastic-covered treatments have the potential to suppress *R. solanacearum* populations in soil and reduce bacterial wilt incidence in greenhouse microcosm experiments. The choice of carbon source plays a critical role in the efficacy of ASD. For instance, MCG and MSP amended, covered soil was comparatively less effective in suppressing *R. solanacearum* than MMM and MCM (Figure 6). Since these type of carbon sources, or their components under anaerobic conditions have been reported to have fungicidal, nematocidal, and antibacterial activities they could be used in integrated management of soil-borne disease in tomato. According to a previous study, different carbon sources increase the production of antagonistic compounds, including Fe^{2+} and Mn^{2+}, citric acid, succinic acid, and ammonium, as well as improve the soil chemical (lower Eh, NO_3^-, SO_4^{2-}, and higher pH) and biological (increase dehydrogenase and urease), activities that have been associated with soil-borne disease control in tomato.[39] Soil treated with these types of compounds can be an alternative to the use of methyl bromide. However, further research is needed to assess the efficacy of carbon sources in managing tomato bacterial wilt in field conditions with high population densities. Additionally, the mechanism of action of the ASD utilized by different carbon sources against *R. solanacearum* needs to be investigated.

Tomato Plant Response to ASD. ASD's ability to suppress weed growth appears to be driven by phytotoxic volatiles produced by microbial activity.[28] The phytotoxic effect of ASD on crop plant growth is a matter of concern among growers.[48] In this simulated ASD microcosm study, we observed no negative impact of ASD on tomato plants transplanted 14 days after ASD, although significant negative effects were observed in tomato plants transplanted immediately after ASD, as evidenced by significant shoot growth stunting and decreased biomass in MSP plastic cover treatment. Our results are consistent with a previous study, which indicated that a minimum of 14 days of aerobic soil remediation is required to eradicate phytotoxins from treated soil.[22] Another study suggested that the time of tomato plant transplantation after ASD should be longer than 7 days.[52] These findings suggest that plants may not be transplanted immediately following ASD; however, a gap period between the end of ASD and crop transplantation is essential to enable the soil that has been anaerobically treated to fully recover aerobic conditions needed for plant root growth. According to the results, 14 days may be an appropriate time for crop transplantation following ASD; however, this may vary depending on the carbon source treatment and crop plant cultivars.

■ CONCLUSIONS

With the increasing demand for organically produced vegetables, several southern states witnessed expanding the number of growers transitioning from conventional to certified organic production in the United States. In parallel, weeds and soil-borne pathogens control continue to be the biggest challenges for organic growers. ASD is a biological process that eliminates the need for chemical preplant pesticides. ASD may be a viable option for organic growers looking to treat soil-borne pathogens and weeds without using chemical fumigants, and this process can be easily integrated into the traditional field preparations completed prior to planting vegetable crops. Today, sustainable agriculture is essential for meeting long-term agricultural demands via the use of farm-based, environmentally friendly resources which do not degrade the environment. Relative to field conditions, this simulated ASD in microcosms using field soil permits screening of carbon sources from various agro-industrial waste streams ahead of resource-intensive field trials. This microcosm study found that ASD with mixed carbon treatments provided strong anaerobic conditions and an acceptable level of weed control and was effective in killing *R. solanacearum*, a devasting pathogen of solanaceous crops. In addition to reducing agro-industrial wastes and pollution, the use of agricultural byproducts in pest management may also benefit small farm-based industries and growers who produce and sell these carbon sources. The local availability of these byproducts at low cost would increase the likelihood that ASD may be adopted widely as a management practice for weed and pathogen control by growers. The various ASD carbon substrates may also stimulate the growth of particular bacterial groups due to differences in the degradability of their carbon components. Further research is needed to explore specific microbial communities associated with the degradation of particular organic carbon sources and their role in sustainable

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agricultural production due to their ability to promote plant growth, enhance biotic and abiotic stress resistance, remediate contaminated soils, recycle nutrients, manage soil fertility, and reduce the use of fertilizers or pesticides in agriculture.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsagscitech.2c00071.

Carbon sources rates and manufacturing information (PDF)

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