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Project Report

Carbon Sources for Anaerobic Soil Disinfestation in Southern California Strawberry

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Abstract: Anaerobic soil disinfestation (ASD) has been adopted in over 900 ha in California strawberry production as an alternative to chemical fumigation. Rice bran, the predominant carbon source for ASD, has become increasingly expensive. In 2021–22 and the 2022–23 field studies, we evaluated 20–30% lower-priced wheat middlings (Midds) and dried distillers' grain (DDG) at 21,800 kg ha^{-1} (in 2021) and 17,000 kg ha^{-1} (in 2022) as alternative carbon sources to rice bran. The study was placed at Santa Paula, California in September of each season in preparation for strawberry planting in October. Soil and air temperatures were 18–26 °C during that time. After the incorporation of carbon sources into the top 30 cm of bed soil, beds were reshaped, and irrigation drip lines were installed and covered with totally impermeable film (TIF) to prevent gas exchange. Beds were irrigated to saturate the bed soil within 48 h after TIF installation. Anaerobic conditions were measured with soil redox potential (Eh) sensors placed at 15 cm depth in all plots. Both DDG and Midds plots maintained Eh at −180 to 0 mV during the two ASD weeks, while untreated soil was aerobic at 200 to 400 mV. Permeable bags with inocula of *Macrophomina phaseolina*, a lethal soil-borne pathogen of strawberry, and tubers of a perennial weed *Cyperus esculentus* were placed 15 cm deep in the soil at ASD initiation and retrieved two weeks later for analyses. Two weeks after that, holes were cut to aerate beds and 'Victor' or 'Fronteras' bare-root strawberries were transplanted into them. ASD with DDG reduced viable microsclerotia of *M. phaseolina* by 49% in the first season and 75 to 85% with both carbon sources in the second season. Both ASD treatments reduced tuber germination of *C. esculentus* 86–90% compared to untreated soil in one of two years. Additionally, Midds and DDG provided greater sufficiency of plant-available nitrogen and phosphorus compared to untreated soil with synthetic pre-plant fertilizer and improved fruit yields by 11–29%. ASD with these carbon sources can suppress soil pathogens and weeds and help sustain organic strawberry production in California.

Keywords: yellow nutsedge; soil-borne pathogens; organic strawberry production

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1. Introduction

California produces approximately 706,000 tons of strawberry fruit annually, nearly 80% of all fresh market strawberries produced in the United States [1]. Similar to other production regions in the US, California experiences challenges with increasing costs and labor, land, and pest management exacerbated by climate change-induced weather variability and regulatory policies [2,3]. Due to increasing costs and restrictions on fumigant use, research has focused on developing non-fumigant alternatives to chemical applications to the soil. These alternative technologies place greater emphasis on an integrated approach to soil pests and plant-soil environment management compared to standard chemical fumigation [4,5]. Anaerobic soil disinfestation (ASD) has been optimized and adopted primarily by organic strawberry growers in California as a method of soil treatment that utilizes a labile carbon source and moisture to produce anaerobic conditions in soil for two to four weeks [6]. As a result of chemical, physical, and microbiological changes in soil, ASD may suppress important strawberry pathogens, such as charcoal rot (*Macrophomina phaseolina*), and Verticillium wilt (*Verticillium dahliae*), and troublesome weeds, such as yellow nutsedge (*Cyperus esculentus* L.) [6]. Successful ASD can improve strawberry productivity compared with untreated soil to levels similar to that of fumigated soil [6,7]. ASD was also effective in reducing Fusarium wilt (*Fusarium oxysporum* f. sp. *Fragariae*) at soil temperatures above 30 °C [8]. Continued ASD adoption in California is challenged by the increasing cost of rice bran, the most commonly used carbon source. Therefore, recent research has focused on alternative locally-sourced carbon products that can be more affordable than rice bran [8,9]. The selection of such carbon sources for evaluation in ASD has been based on their carbon-to-nitrogen ratio, costs of delivery, and ease of application [10].

Based on these characteristics and analyses (Table 1), wheat middlings (Midds) and dried distillers' grain (DDG) were chosen as carbon sources for the study and sourced locally (Western Milling, McFarland, CA, USA).

Table 1. Analyses of wheat middlings (ASD-Midds) and dried distiller's grain (ASD-DDG) evaluated for anaerobic soil disinfestation trial at Santa Paula, California (as is basis).

Analytical Parameters	Wheat Middlings (Midds)	Dried Distiller Grain (DDG)
Total N, %	2.4	3.9
Total C, %	30.3	43.7
C:N ratio	12.7	11.4
Total P_2O_5, %	2	1.9
Total K_2O, %	1.2	1.2
EC, dS/m	4.2	25.2
Total chloride, %	0.1	0.2
pH	4.4	4.5
Boron, ppm	4.5	2.9
Zinc, ppm	66.5	51.8
Manganese, ppm	120	13
Iron, ppm	96	90
Copper, ppm	9.1	4.6
Moisture, %	8.6	8.2

The objective of these field trials was to evaluate Midds and DDG for their potential to generate anaerobic conditions and to assess the resulting effects on charcoal rot pathogen and yellow nutsedge in ASD-treated soil. Strawberry fruit production after these treatments was also evaluated.

2. Materials and Methods

2.1. Carbon Sources Application for ASD

Field experiments arranged in randomized complete block design with four replications were conducted during the 2021–2022 and 2022–2023 strawberry seasons at Santa Paula, California. Soil treatments were applied in fall and fruit production was in winter and spring.

The soil at the experimental site was 75% clay and 25% silt, with a pH of 7.6 and 1.1% organic matter [11]. Beds 1.3 m wide and 0.3 m tall were shaped, and 3 m long treatment plots were marked in beds. The soil at the site was fumigated three years prior to the study initiation with chloropicrin at 360 kg ha^{-1} and remained fallow until 2021.

The ASD process was accomplished by spreading carbon sources on bed tops at 21,800 kg ha^{-1} (in 2021) and 17,000 kg ha^{-1} (in 2022), incorporating them into the top 20–30 cm of soil with a rototiller, placing two irrigation drip lines (John Deere, Moline, IL, USA or Aqua-Traxx® Toro Ag., Rome, Italy), covering beds with totally impermeable plastic mulch (AGRO-TIF, no pigment/natural clear, 1-mil-thick; USA Extruded Plastics, Anaheim, CA, USA) and irrigating within 48 h until soil saturation (about 0.01 ha m^{-1}), according to previously developed protocols [9]. These drip lines were delivering about 4 L min^{-1} per 100 m. In untreated check plots, 310 kg ha^{-1} of ammonium sulfate (21-0-0-24S) was incorporated as pre-plant fertilizer instead of carbon sources.

After initiation of the ASD process, oxidation/reduction potential (ORP mV) was automatically monitored every 30 s in all treatments with oxidation/reduction potential sensors (S500CD-ORP, Sensorex. Garden Grove, CA, USA) installed at 15 cm soil depth and connected to a data logger (CR1000, Campbell Scientific, Logan, UT, USA). The ORP reading in mV was converted to Eh mV by adding 199 mV. To compare the intensity of anaerobic conditions, the cumulative Eh mV hours under 200 mV were calculated for each plot using hourly averages of soil Eh [6]. The value of 200 mV has been previously selected as the threshold below which soil was considered anaerobic at a soil pH of 6.58 [12].

In addition to the ORP sensors, TEROS 12 sensors (Meter Group Inc., Pullman, WA, USA) were installed at 15 cm depth and connected to a ZL6 datalogger (Meter Group Inc., Pullman, WA, USA) to measure soil temperature during the ASD process.

2.2. *M. phaseolina and C. esculentus Survivorship Evaluation during ASD*

In addition to sensors, permeable nylon bags filled with 50 g of soil inoculated with *M. phaseolina* at about 500 microsclerotia g^{-1} soil (in 2021) and 1000 microsclerotia g^{-1} soil (in 2022) were buried in all plots at 15 cm depth. A sterile cornmeal/sand mixture was prepared and infested with *M. phaseolina* by the method described in Koike et al. (2016) [13]. After preparation, the density of microsclerotia in the inoculum was quantified by the soil plating method described below.

Separate bags containing ten locally collected *C. esculentus* tubers in each were filled with 50 g of soil and buried in all plots at 15 cm depth. In 2021, soil in bags was not augmented with carbon sources, while in 2022, Midds and DDG were mixed into the bags at rates corresponding to the plot application rates.

In both seasons, after two weeks after the ASD process initiation, bags with the pathogen and weed inoculum were excavated from plots. *Macrophomina phaseolina* viability was analyzed by soil plating and counting morphologically identifiable colonies. This method was based on Cloud and Rupe (1991) [14]. Briefly, the soil was air dried and ground in a Wiley mill soil grinder, then passed through a #40 mesh sieve (425 µm square mesh pore size). Then, 5 g subsamples were mixed with 100 mL 1% sodium hypochlorite in a Waring commercial blender for 30 s, and the mixture was poured over a 38 µm square mesh sieve. Particles collected in the sieve were rinsed with deionized water, then dislodged with 30–50 mL of sterile deionized water into a flask. Liquid potato dextrose agar (PDA; 90 mL) was added to the flask, mixed by agitation, and then poured into seven 100 mm petri dishes. Plates were incubated at 30 °C for 7–14 days before *M. phaseolina* colonies were counted. Each sample was assayed twice by this method, and the average of the two technical replicates was used for statistical analysis. *C. esculentus* germination was assessed by counting shoot number at excavation time and during the three following weeks, after placing tubers in water-saturated filter paper at 25 °C.

2.3. *Strawberry Fruit Production Assessment after ASD*

During the second week of October 2021 and 2022, after four weeks since ASD initiation, 15 cm long slits were punched through plastic in four rows per bed (and 35 cm between holes) in preparation for planting, which also provided bed aeration. Within 48 h after cutting holes, bare-root strawberry transplants of 'Victor' (in 2021) and 'Fronteras' (in 2022) from a high-elevation nursery were planted, and trial sites were managed according to standard strawberry production practices in California [15]. During the strawberry growth cycle in November, December, and January, four soil sub-samples were taken at

0–30 cm depth in each plot, combined into 500 g samples, and analyzed for nutrient content. No fertilization was completed during the strawberry growing season.

Strawberry fruits were harvested twice a week at maturity from all 16 plants per plot January-June in 2022 and 2023. Fruits were separated into marketable and unmarketable and weighed in each category.

Analyses of variance and differences between treatment means were determined using Fisher's least significant difference test ($p > 0.05$). All statistical computations were performed using SAS (version 9.3; SAS Institute, Cary, NC, USA).

3. Results

3.1. Carbon Sources Application for ASD

In both seasons, Midds and DDG were effective in creating anaerobic conditions (Table 2). The cumulative value reflecting the strength of anaerobiosis after 14 d approached 50,000 in 2021 and doubled that in 2022. The weaker anaerobic conditions in 2021, in spite of higher carbon sources application rate, were likely due to temporary damage to plastic by animals after ASD initiation. Although birds commonly puncture the plastic, in this study, a dog was observed on the bed top during the ASD process in 2021 (Stephanie Gomez, personal communication). The plastic integrity was restored, and anaerobic conditions developed again within 24 h after the incident. A cumulative value of 50,000 was previously used as a threshold for successful control of *Verticillium dahliae* in soil [6]. In this study, anaerobic conditions persisted after 14 d (data not shown), but the inoculum bags were retrieved at 14 d after ASD initiation.

Table 2. Anaerobic conditions, *Macrophomina phaseolina* survivorship and marketable strawberry fruit yields after anaerobic soil disinfestation (ASD) with wheat middlings (ASD-Midds) and dried distiller's grain (ASD-DDG) at Santa Paula, California.

Treatments	Cumulative Anaerobiosis after 14 d, Eh mV below 200 mv (mV hrs)		*Macrophomina phaseolina* (Microsclerotia g soil^{-1})		Marketable Fruit Yield, (g plant^{-1}) January–June	
	2021	2022	2021	2022	2022	2023
ASD-Midds	46,000	90,000	547 a [c]	230 b	360 a	680 a [f]
ASD-DDG	48,000	110,000	278 b	132 b	390 a	651 ab
Untreated [d]	564 [e]	14,000	548 a	912 a	280 b	606 b

[c] Values in a column followed by different letters are significantly different according to Fisher's LSD test ($p > 0.05$). [d] Untreated beds received 330 kg ha^{-1} of 21-0-0-24S pre-plant fertilizer. [e] A single electrode (one in four replicated plots) was used to determine Eh mV in the untreated check. [f] Yield data were collected between January and 1 June 2023.

Average soil temperatures during the ASD process were 29.1 °C in ASD-Midds, 29.5 °C in ASD-DDG, and 27.9 °C in untreated plots in the first season, whereas these were 24.6 °C in ASD-Midds, 24.8 °C in ASD-DDG, and 24.0 °C in untreated plots in the second season. Cumulative hours of soil temperatures above 30 °C during the ASD process were 195 h, 189 h, and 122 h in ASD-Midds, ASD-DDG, and untreated plots, respectively, in the first season. In contrast, these were 112 h, 87 h, and 7 h in ASD-Midds, ASD-DDG, and untreated plots, respectively, in the second season. The increases in soil temperature at ASD plots are common and appear to be caused by microbial heat generation during the fermentation process at these plots [16]. These are generally associated with effective ASD, a process that benefits from warm soil [8].

3.2. *M. phaseolina* and *C. esculentus* Survivorship Evaluation during ASD

In 2021, there was no reduction in the pathogen microsclerotia levels in ASD-Midds, but in ASD-DDG treatment, there was 49% fewer viable microsclerotia than in untreated soil (Table 2). In 2022, ASD-Midds and ASD-DDG treatments reduced *M. phaseolina* microsclerotia levels by 75% and 85%, respectively, compared to untreated soil. Uninterrupted anaerobic conditions in 2022 and the addition of carbon sources to the soil in inoculum bags were likely responsible for greater ASD efficacy, compared with 2021.

Similar to results with *M. phaseolina* microsclerotia, ASD efficacy in suppressing *C. esculentus* germination was greater in 2022 than in 2021 (Table 3). In 2021, there were no significant differences in germination between treatments, even though tubers in ASD treatments tended to have fewer shoots than in untreated soil. In 2022, on the day of excavation, ASD-Midds and ASD-DDG treatments had tubers with 86 and 90% fewer shoots, respectively, than tubers from untreated soil (Table 3). However, once removed from the soil after 14 d burial in 2022, the tubers from all treatments continued to germinate in the lab, and by 21 d after excavation, only ASD-Midds treatment had 68% fewer shoots than from untreated soil (Table 3).

Table 3. *Cyperus esculentus* germination after anaerobic soil disinfestation (ASD) with wheat middlings (ASD-Midds) and dried distiller's grain (ASD-DDG) at Santa Paula, California.

Treatments	Cumulative Average Shoot Number per 10 Tubers							
	2021				2022			
	Days after Excavation from Soil							
	0	7	14	21	0	7	14	21
ASD-Midds	1.4 a [c]	1.5 a	1.5 a	1.5 a	1.7 b	3 b	4.3 b	5 b
ASD-DDG	2.3 a	2.5 a	2.5 a	2.5 a	1.3 b	3.5 b	9.0 ab	11 ab
Untreated	4 a	4.3 a	4 a	4 a	12.5 a	12.5 b	13.8 a	15.5 a

[c] Values in a column followed by different letters are significantly different according to Fisher's LSD test ($p > 0.05$).

3.3. Strawberry Fruit Production Assessment after ASD

In 2022, total marketable yields were 28% greater in ASD-Midds and 39% greater in ASD-DDG compared to untreated plots (Table 2). Even though untreated plots were fertilized and all treatments had similar nitrate levels in the 0–30 cm soil profile in November (around 40 ppm), mineralization of organic nitrogen from the two carbon sources and conversation to oxidized forms resulted in 140 ppm nitrate-nitrogen in ASD treatments compared to 15 ppm in untreated soil in December (data not shown).

Additionally, Midds and DDG provided phosphorus to the soil and Olsen P_2O_5 levels in the root zone were 100–250 ppm in ASD plots compared to 80–100 in untreated soil.

Increased supply of plant-available nitrogen and phosphorus from the two carbon sources likely improved plant vigor (data not shown) and fruit production (Table 2).

In 2023, marketable fruit yield for ASD-Midds was 11% greater than in untreated control and similar to yields in ASD-DDG. There were no significant differences in fruit yields between ASD-DDG and the untreated control (Table 2).

Nutrient losses to leaching in 2023 were high due to abnormally high rainfall during the winter (720 mm, compared to the 30-year average of 380 mm during the same period) [17]. Soil Nitrate nitrogen (0–30 cm) in November was 59 ppm in ASD-Midds, 62 ppm in ASD-DDG, and 32 ppm in untreated fertilized soil. In March, nitrate levels dropped to 2.6, 1.9, and 1.7 ppm, respectively, in the three treatments.

4. Discussion

These trials showed that Midds and DGG were suitable carbon sources for effective ASD in Southern California. Currently, they cost 25–30% less than commonly used rice bran (Robert Brzezinski, personal communication). The incorporation of Midds and DDG at rates commonly used for rice bran provided strong anaerobic conditions at this test site. The ASD treatment with both materials was effective in suppressing key strawberry pathogen *M. phaseolina* when its survival structures were exposed to soil augmented with Midds or DDG even though the cumulative soil temperatures above 30 °C did not exceed the threshold reported to control *Fusarium oxysporum* f. sp. *fragariae* by ASD [8]. This suggests the soil temperature threshold for controlling *M. phaseolina* is likely to be lower than the one for *Fusarium oxysporum* f. sp. *fragariae.* In the absence of soil fumigation in organic fields and lack of *M. phaseolina*-resistant strawberry cultivars in commercial production, such reduction in pathogen inoculum in the root zone may slow disease development and plant collapse [4]. In previous ASD trials with rice bran as the carbon source, end of the season mortality due to *M. phaseolina* in an infested field was reduced by 50–65% compared to the previous season (Kris Gean, personal communication). Similarly, in a demonstration trial on an organic strawberry farm in southern California, end of the season mortality mainly caused by *M. phaseolina* was reduced from 26.4% at the grower standard plot to 16.2% at the ASD rice bran 20 tons/ha plot [7]. These studies show that DDG and Midds may be even more efficacious in suppressing this pathogen.

Anaerobic conditions with ASD-Midds provided excellent control of *C. esculentus* shoots germinating from tubers. If tubers remained in the soil beyond the two weeks (when they were excavated in the trial), the continued soil anaerobiosis could have provided additional control. *C. esculents* tubers remain viable for three years in Southern California soils and germinate most actively in summer and fall [18]. Reducing fall-germinated cohorts in fall-planted strawberries is important in minimizing competition and weeding costs during plant establishment. The gradual germination pattern of *C. esculentus* tubers over time was documented previously [18] and shoots germinated after ASD completion would require additional control. In this study, anaerobic conditions persisted after inoculum bags were removed 14 d after burial for analyses. Continued exposure to anaerobic conditions could have provided additional suppression of germination of the next cohorts of *C. esculentus*, further enhancing ASD efficacy.

Improvements in strawberry fruit production after ASD compared to untreated soil have been widely documented [6–8]. In our study, the field soil did not contain common strawberry pathogens, and weed interference was eliminated by hand weeding. However, fruit yields after ASD-Midds and ASD-DDG significantly exceeded those in untreated soil in 2022. This may have been due to nitrogen and phosphorus that were mineralized gradually from these carbon sources and became available later in the fruiting season compared to pre-plant applied ammonium sulfate. The C:N ratio of Midds (12.7) and DDG (11.4) is lower than the one for rice bran (typically ~20 [6]). Therefore, the nitrogen mineralization rate of these materials is likely to be greater than the one of rice bran [19]. Although this may favor strawberry plants in the southern California region, where a high early yield is extremely important economically, the wheat bran application rate may need to be reduced according to its nitrogen mineralization rate, which should be determined in the future.

Even though we did not measure changes in soil pH in this study, ASD typically results in a reduction in soil pH [7] and improved micronutrient availability in calcareous soils of Southern California. In 2023, these differences were not significant as leaching reduced the levels of nitrate dramatically in all treatments. However, fruit production in ASD plots was not lower than in fertilized soil that contained no known pathogens or weeds.

This study showed that ASD with Midds or DDG can be an effective tool for soil-borne pathogen and weed management and serve as an economical alternative to ASD with rice bran. These carbon sources are being certified for use in organic production, thus, facilitating adoption among California organic strawberry growers.

While the evaluation of carbon sources for ASD continues, it has been essential to integrate this technology with other management practices, such as the use of resistant cultivars, establishing adequate crop rotations, and implementing basic sanitation and prevention, to sustain fruit production in non-fumigated fields of coastal California [4].

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