

Anaerobic Soil Disinfestation:

Evaluation of Anaerobic Soil Disinfestation (ASD) for Warm-Season
Vegetable Production in Tennessee

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David Grant McCarty
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ABSTRACT

Anaerobic soil disinfestation (ASD) is a non-chemical, pre-plant soil treatment recently developed for control of pests such as soilborne plant pathogens, plant-parasitic nematodes, and weeds in specialty crop systems. Soil treatment by ASD includes incorporating a labile carbon (C) source, tarping with plastic, and irrigation of the topsoil to saturation to facilitate the development of strongly anaerobic soil conditions driven by soil microbes. Processes occurring during the anaerobic decomposition of the added C source have been reported control plant pests. The goal of this project was to evaluate and adapt the ASD procedure to environmental conditions and production systems in Tennessee and examine the potential for on-farm implementation. To meet this goal, study objectives were to 1) evaluate both cover crop and off-farm C inputs for ASD treatment for production of bell pepper and tomato in a research station field experiment, 2) evaluate cool-season cover crop and off-farm inputs as C sources for ASD treatment in a growth chamber pot experiment with introduced sclerotia of *Sclerotium rolfsii* and propagules of key weed species, and 3) demonstrate and evaluate on-farm implementation of ASD on a commercial tomato farm. Data collection included soil properties, pest assessment, and crop performance. Results indicated no differences in total marketable fruit yields between treatments in any of the studies, likely due to low disease pressure, plant-parasitic nematode populations, and weed populations apparent in all studies. Accumulation of anaerobic soil conditions generally did not differ among treatments, possibly due to the generally low C amendment rates and the soil properties in study locations. Treatment by ASD did impact a number of soil properties, including levels of inorganic soil nitrogen, which will need to be addressed when developing best management practices for ASD implementation. Additional studies are needed on sites with high existing pest pressure to further evaluate the feasibility of ASD for commercial production systems.

TABLE OF CONTENTS

CHAPTER I Literature Review	1
Introduction	1
Methyl Bromide	1
Chemical alternatives to MeBr	2
Non-chemical/Biological alternatives	4
Conclusion	15
CHAPTER II Field evaluation of anaerobic soil disinfestation (ASD) carbon sources for tomato and bell pepper production in Tennessee	17
Abstract	17
Introduction	18
Materials and methods	22
Results	29
Discussion	55
CHAPTER III Evaluation of soil properties and mortality of introduced weeds and sclerotia of <i>Sclerotium rolfsii</i> following anaerobic soil disinfestation using various cool-season cover crops and carbon source amendments	62
Abstract	62
Introduction	63
Materials and methods	66
Results	71
Discussion	96
CHAPTER IV Evaluation of anaerobic soil disinfestation (ASD) for tomato production systems in a Tennessee on-farm trial	103
Abstract	103
Introduction	104
Materials and methods	106
Results	110
Discussion	121
LIST OF REFERENCES	129
APPENDIX	136
VITA	155

LIST OF TABLES

Table 1. Total biomass, carbon and nitrogen content, and C:N ratio of soil amendments and aboveground cover crop biomass from field study, spring 2011.....	30
Table 2. Total biomass, carbon and nitrogen content, and C:N ratio of soil amendments and aboveground cover crop biomass from field study, spring 2012.....	31
Table 3. Mean volumetric soil moisture during treatment and gravimetric soil moisture post treatment from field study.....	33
Table 4. Mean soil temperature during soil treatment from field study, 2011 and 2012...	34
Table 5. Mean soil pH in water (pH_{water}) and CaCl_2 ($\text{pH}_{\text{CaCl}_2}$) from field study, 2011.....	35
Table 6. Mean soil pH in water (pH_{water}) and CaCl_2 ($\text{pH}_{\text{CaCl}_2}$) from field study, 2012.....	36
Table 7. Soil carbon to nitrogen ratio (C:N) from field study, 2011 and 2012.....	39
Table 8. Mean soil ammonium-N ($\text{NH}_4\text{-N}$), nitrate + nitrite-N ($\text{NO}_3 + \text{NO}_2\text{-N}$), and total soil inorganic N (TIN) for composite soil samples from tomato and bell pepper plots from field study, 2011.....	41
Table 9. Mean soil ammonium-N ($\text{NH}_4\text{-N}$), nitrate + nitrite-N ($\text{NO}_3 + \text{NO}_2\text{-N}$), and total soil inorganic N (TIN) for composite soil samples from tomato and bell pepper plots from field study, 2012.....	42
Table 10. Population of <i>Rhizoctonia solani</i> colonizing birch toothpicks immediately following soil treatment from field study.....	47
Table 11. Total biomass, carbon and nitrogen content, and C:N ratio of soil amendments and aboveground cover crop biomass in growth chamber study, Trial 1.....	72
Table 12. Total biomass, carbon and nitrogen content, and C:N ratio of soil amendments and aboveground cover crop biomass in growth chamber study, Trial 2.....	73
Table 13. Mean volumetric soil moisture during treatment and gravimetric soil moisture post treatment from growth chamber study.....	76
Table 14. Mean soil ammonium-N ($\text{NH}_4\text{-N}$), nitrate + nitrite-N ($\text{NO}_3 + \text{NO}_2\text{-N}$), and total soil inorganic N (TIN) from growth chamber study, Trial 1.....	78
Table 15. Mean soil ammonium-N ($\text{NH}_4\text{-N}$), nitrate + nitrite-N ($\text{NO}_3 + \text{NO}_2\text{-N}$), and total soil inorganic N (TIN) from growth chamber study, Trial 2.....	79
Table 16. Mean pre and post treatment soil pH in water (pH_{water}) and CaCl_2 ($\text{pH}_{\text{CaCl}_2}$) from growth chamber study.....	81
Table 17. Soil pH and accumulation of mV h during ASD treatment assay with molasses amendment.....	83
Table 18. Soil carbon to nitrogen ratio (C:N) from growth chamber study, Trial 1 and 2.....	85
Table 19. Water extractable carbon (WEC) and hot water extractable carbon (HWE) from growth chamber study, Trial 1.....	86
Table 20. Nutsedge germination post-soil treatment from growth chamber study, Trial 1 and 2.....	87
Table 21. Crabgrass and pigweed germination post- soil treatment from growth chamber study, Trial 1 and 2.....	89
Table 22. Effect of treatment on germination and parasitism of <i>S. rolfsii</i> sclerotia	

recovered from soil from growth chamber study, Trial 1.	91
Table 23. Effect of treatment on germination and parasitism of <i>S. rolfsii</i> sclerotia recovered from soil from growth chamber study, Trial 2.....	93
Table 24. Early (week 3) and end of trial (week 7) tomato height and stem diameter from growth chamber study, Trial 1 and 2.....	94
Table 25. Mean tomato yield from growth chamber study, Trial 1 and 2.....	95
Table 26. Nitrogen concentration in tomato leaf tissue at harvest from growth chamber study, Trial 1 and 2.....	97
Table 27. Total biomass, carbon and nitrogen content, and C:N ratio of soil amendments from on-farm demonstration study, summer 2012.....	111
Table 28. Mean percentage of ferrihydrite reduction on IRIS tubes from on-farm demonstration study, 2012.....	112
Table 29. Mean post treatment soil temperature at 5 cm and 15 cm depth from on-farm demonstration study, 2012.....	113
Table 30. Mean soil pH in water (pH _{water}) and CaCl ₂ (pH _{CaCl2}) from on-farm demonstration study, 2012.....	115
Table 31. Total nitrogen (TN), total carbon (TC), and carbon:nitrogen ratio (C:N) of soils sampled from on-farm demonstration study, 2012.....	116
Table 32. Soil ammonium (NH ₄ -N), nitrate + nitrite (NO ₃ + NO ₂ -N), and total inorganic nitrogen (TIN) from on-farm demonstration study, 2012.....	118
Table 33. Extractable soil phosphorus from on-farm demonstration study, 2012.....	119
Table 34. Mid-season tomato height and stem diameter of ‘Smarty’ tomatoes from on-farm demonstration study, 2012.....	121
Table 35. Nitrogen concentration in tomato leaf tissue of ‘Smarty’ tomatoes at first harvest from on-farm demonstration study, 2012.....	123
Table 36. Populations of lesion, spiral, root-knot, and stunt nematode second-stage juveniles extracted from soil at harvest, 2012.....	124

LIST OF FIGURES

Figure 1. Total accumulation of anaerobic conditions (mVh) from field study, 2011 and 2012.	38
Figure 2. Cumulative soil respiration (mg CO ₂ g ⁻¹ soil) at 3, 10 , 17, and 30 days during incubation at 25° C for 2012 field study soil treatments.....	43
Figure 3. Area under disease progress curve for early blight of tomato (<i>Alternaria</i> spp.), 2011 and 2012.....	46
Figure 4. Mean monocot weed populations in field study, 2011 and 2012.....	49
Figure 5. Mean dicot weed populations in field study, 2011 and 2012.....	50
Figure 6. Mean total tomato yield (2011 and 2012), total marketable yields, large tomato yield, and culled tomato yield.	51
Figure 7. Mean total pepper yield (2011 and 2012), total marketable yields, and culled pepper yield.....	53
Figure 8. Accumulation of anaerobic conditions (mVh) during soil treatment in growth chamber study, Trial 1.	75
Figure 9. AUDPC of early blight from growth chamber study, Trial 1.....	90
Figure 10. Total, marketable, and green fruit yields (kg ha ⁻¹) of grape tomatoes from on-farm demonstration study, 2012.....	123

CHAPTER I

LITERATURE REVIEW

Introduction

In many specialty crop production systems with limited crop rotation, it is necessary for growers to utilize some form of soil disinfestation method in order to suppress pests that include weeds, soilborne plant pathogens, and plant parasitic nematodes. Without the use of soil disinfestation growers may have difficulty controlling these pests and as a result would yield declines. Many producers utilize chemical soil fumigants applied prior to planting to suppress these pests. These methods have resulted in good control of pests, but have led to reliance on these chemicals, which have many limitations in terms of environmental, economic, and social sustainability. Alternative methods of soil disinfestation have been developed, including solarization, steam disinfestation, biofumigation, anaerobic soil disinfestation, and the use of organic soil amendments. Previous research has shown the potential of several of these methods for controlling weeds, soilborne plant pathogens, and plant-parasitic nematodes. This review will examine the published research related to the use of methyl bromide and non-chemical/biological alternatives in specialty crop production systems for control of plant pests.

Methyl Bromide

Methyl bromide (MeBr) is a preplant soil fumigant historically used in vegetable production systems that has broad spectrum activity for controlling plant-parasitic nematodes, weeds, and soilborne plant pathogens. For most growers, MeBr is applied in combination with chloropicrin to raised beds that are immediately covered with plastic mulch. Crops are typically transplanted from several days to a few weeks after soil treatment. Many agricultural industries are dependent on MeBr for use as a pre-plant soil fumigant, including 88% of strawberry production in

California, numerous southeastern vegetable industries, and winter vegetable production in Florida (Byrd et al. 2006a; Carter et al. 2000; Haar et al. 2003; Noling and Becker 1994). Since 2005, a full mandate has been in place following U.S. ratification of the Montreal Protocol on Ozone Depleting Substances in 1988. This ban has been projected to have a detrimental impact on many of the vegetable and small fruit industries in the U.S. Some studies have projected substantial losses, such as 1 billion dollars in the winter vegetable industry in Florida; a 10 to 15% decline in yields and a 10% decline in acreage in the California strawberry industry; and a large reduction of cucurbitaceous and solanaceous crops in Florida (Byrd et al. 2006b; Carter et al. 2000; Deepak et al. 1996). At the same time, since Mexico does not implement the ban until 2015, industries could seize larger market share in the U.S. for certain crops (Carter et al. 2000). In fact, it is projected that Mexico would take a larger share of winter vegetable production from Florida, and with that, change production of vegetables would cease during certain months and completely end in certain regions in Florida (Deepak et al. 1996). While this mandate has reduced the use of MeBr, many industries have been allowed Critical Use Exemptions (CUE) by the U.S. Environmental Protection Agency, where they can continue to use the chemical and purchase it based on economic need if no viable alternatives are available (Martin 2003). For instance, with a CUE, strawberry production in California continued to use 1,800 Mg of MeBr in 2005, even with a shift toward other chemical alternatives (Trout 2006). With the CUE status leading to a waning supply of this chemical, growers are experiencing increased production costs and pressure to no longer rely on this chemical (Noling et al. 2009). This current situation presents a need for a suitable alternative that can replace the use of MeBr as a soil fumigant.

Chemical alternatives to MeBr

Chemical Alternatives. In place of MeBr, many growers have shifted to other chemicals. One alternative is chloropicrin, which has been traditionally combined with MeBr at low concentrations as a warning agent but added in higher concentrations in recent years, chloropicrin has activity against certain soilborne pathogens, nematodes, and weeds while being cost-effective (Byrd et al. 2006a; Deepak et al. 1996). A major issue is that many alternative fumigants lack the broad-spectrum efficacy of MeBr. According to Noling et al. (2009), chloropicrin, applied without MeBr, can control some fungal diseases but has limited efficacy against weeds or nematodes, while 1,3-dichloropropene (1, 3-D; e.g., Telone®, InLine®) can control nematodes and some fungal pathogens but not weeds or many bacterial pathogens. Metam sodium (e.g., Vapam®) and metam potassium (e.g., K-Pam®) can control weeds only when well-incorporated into the soil. Methyl iodide has shown potential for broad-spectrum efficacy (Noling et al. 2009), but is no longer commercially available in the U.S. due largely to human health concerns (Chawkins and Marcum 2012). Dimethyl disulfide (Paladin®), has been used when combined with chloropicrin as a broad spectrum control and achieved the same yields as MeBr in Florida and Spain studies of ‘Camarosa’ strawberry (*Fragaria x anassa* Duch.; Santos et al. 2006). These chemical alternatives to MeBr have been promoted by the industries with Florida tomato production promoting 1,3-D + chloropicrin and metam sodium, Georgia bell pepper and other vegetables moving toward the Georgia 3-way combination (1,3-D, chloropicrin, and metam sodium), and methyl iodide in California strawberry (Byrd et al. 2006a; Culpepper et al. 2008; Gilreath and Santos 2008; Martin 2003). Given that alternative chemical fumigants have yet to reach the level of consistent, broad-spectrum control provided by MeBr, especially weed control, additional options are needed.

Non-chemical/Biological Alternatives

Anaerobic/Biological Soil Disinfestation. Anaerobic soil disinfestation (ASD; sometimes referred to as biological soil disinfestation) is a pre-plant method developed to control pests in specialty crop production systems. It involves adding an easily-decomposable carbon source such as a cover crop or an organic amendment that is incorporated into the soil, irrigation, and tarping with plastic. Amendments used can vary, but must contain an adequate supply of labile carbon to support soil microbial growth (Momma 2008). During ASD treatment, organic acids, particularly acetic and n-butyric acids, are released into the soil solution as the carbon source decomposes anaerobically (Momma 2008). The application of soil amendments (e.g., molasses, wheat bran, cover crops, crop residues) have led to reductions in soil pH and redox potential (Eh) implying that oxygen is being consumed and organic acids are being added (Momma 2008). At the same time, the efficacy of the ASD method is connected with the presence of organic acids (Kubo et al. 2005; Momma et al. 2005). In a pot study examining ASD using wheat bran as the carbon source, the production of acetic and butyric acid was detected (Momma et al. 2006). Researchers reported that development of organic acids (acetic, n-butyric) in ASD contributed to control of *Ralstonia solanacearum* populations (Momma et al. 2006). Separately, when these acids were added that were not derived from wheat bran, they suppressed *R. solanacearum* to lower concentrations than those under ASD treatment (Momma et al. 2006).

Ethanol (200 kg ha⁻¹ of 1% ethanol) as a carbon source has shown potential in effectively controlling *Fusarium oxysporum* f. sp. *lycopersici* while wheat bran (10.3 Mg ha⁻¹) as a carbon source could not produce the same results (Momma et al. 2010). Alternatively, in a pot study, treatment with wheat bran (40 g per 2.5 kg of soil) was able to control bacterial wilt (*R.*

solanacearum) to below detectable rates but only when combined with irrigation and a plastic mulch, indicating the importance of anaerobic decomposition of the added carbon source (Momma 2008). In addition, wheat bran (10 Mg ha^{-1}) has been effective in reducing soilborne plant pathogens such as *Fusarium oxysporum* (Yossen et al. 2008).

Molasses has also been shown to be an effective carbon source for ASD and the use of liquid molasses has been promoted due to its potential to be easily incorporated into the soil via irrigation (Momma 2008). In an field experiment in the Netherlands, ASD treatment of 30 Mg ha^{-1} of potato (*Solanum tuberosum*) haulms and grass (species not given) residue resulted in a 92.5% decrease in *R. solanacearum* compared to the control but did not alter the bacterial species richness; however, researchers stressed the importance of a organic matter that is easy to decompose and the use of plastic mulch (Messiha et al. 2007). Without these two components, plant pathogen populations stabilized. In Japan, following ASD treatment using wheat bran (20 Mg ha^{-1}) as a carbon source, the severity of bacterial wilt (*R. solanacearum*) in tomato production decreased and also delayed disease severity when compared to the control (Momma 2008). In the Netherlands, a study examining *Fusarium redolens* f.sp. *asparagi*, *Rhizoctonia tuliparum*, and *Verticillium dahliae* in asparagus, reported that all three were reduced by the carbon source of broccoli (3.8 kg m^{-2} ; *Brassica oleracea*) and grass residue (4.0 kg m^{-2} ; species not given; Blok et al. 2000). In an experiment in the Netherlands examining the treatment of ASD on *V. dahliae* there was an 85% reduction in soil inoculum in all treatments (40 to 54 Mg ha^{-1} of Italian ryegrass amended or not amended then tarped or not tarped) compared to the control and also led to decreases in *Verticillium* wilt occurrence (Goud et al. 2004). In an experiment in Florida, *Phytophthora capsici* inoculum buried prior to ASD treatment, did not survive in any ASD or solarization treatments and was equivalent to the MeBr control and

greater than an untreated control while a treatment of molasses as the carbon source significantly affected *Fusarium oxysporum* inoculum mortality (Butler et al. 2012b). Following ASD treatment with various cover crops, introduced *F. oxysporum* inoculum was reduced by 97% when a carbon source was added compared to the control (no carbon source) while *Sclerotium rolfsii* was inconsistently affected by carbon source and ASD treatment (Butler et al. 2012a). A laboratory study in Rhode Island examined the effect of butyric acid in the controlling soilborne pathogens; *V. dahliae*, *Rhizoctonia solani*, and *R. fragariae*, which were killed following the addition of butyric acid (0.88 and 8.8 mg g⁻¹ soil) and 2-day incubation and (Browning et al. 2006).

For ASD to be considered a reliable alternative to MeBr it would ideally include some control of weed species and some research has examined the effects of ASD on weed control. For instance, in a greenhouse pot experiment examining different carbon sources in ASD treatment, yellow nutsedge (*Cyperus esculentus*) planted when cover crops were incorporated for ASD treatment showed that germination was low among carbon source treatments with liquid molasses having the lowest nutsedge germination and the control without an added carbon source having the highest germination (Butler et al. 2012a). This demonstrates that many different carbon sources, including the amendment of molasses, could be effective in ASD treatment. Under ASD conditions in another pot experiment, yellow nutsedge germinated at shallow depths but not at deeper depths when tubers in mesh bags were planted and different carbon sources were added, a possible indication that resupply of oxygen at the soil surface during treatment acted as a limiting factor (Muramoto et al. 2008).

Plant-parasitic nematodes have also been shown to be affected by ASD treatment. Of the organic acids that may be created in the soil environment with ASD treatment, plant-parasitic

nematodes are likely most affected by butyric acid (Browning et al. 2004). This was examined in a laboratory study in Rhode Island examining 12 nematode species with varying concentrations of butyric acid. Reductions of plant-parasitic nematode genera *Tylenchorhynchus*, *Helicotylenchus*, and *Pratylenchus* occurred after a 2-day incubation in sand with an application of 0.88 mg butyric acid g⁻¹ soil (Browning et al. 2004). In one study, populations of root lesion nematode (*Pratylenchus* spp.) were lower under a plastic-covered treatment with 4.6 Mg ha⁻¹ of Italian ryegrass compared to an uncovered control (Goud et al. 2004). A further study in Rhode Island examined juveniles of *Meloidogyne hapla* and *M. incognita* submerged into various butyric acid solutions to determine survivability. In this study, a 4-h immersion in 0.1 M and 1 M butyric acid resulted in 100% mortality of juveniles. This study also examined the formation of root galls following the immersion of egg masses of both *M. hapla* and *M. incognita* before being placed on the roots of tomato plants. A reduction of 73 to 100% was reported when using the 0.1 and 1 M solution of butyric acid (Browning et al. 2006). A pot study in Florida examining warm-season legumes and grasses in ASD for control of *M. incognita* found a reduction of galling by *M. incognita* for all treatments with an added carbon source compared to the unamended control (Butler et al. 2012a).

Biofumigation. The general term biofumigation is used to describe a soil disinfestation method that can control soilborne pests and pathogens through the decomposition of brassicaceous plants or seed meals incorporated in the soil (Kirkegaard and Sarwar 1998). Common in most plant species of the Brassicaceae family, a class of sulfur compounds known as glucosinolates are particularly important in biofumigation (Bellostas et al. 2007). Evolutionarily, glucosinolates are generally used by brassicaceous plants to combat pests such as insects, fungal pathogens, and other diseases (Buskov et al. 2002; Kirkegaard et al. 1996; McGuire 2003). When physically

disrupted and added to the soil in the presence of adequate moisture, the co-occurring myrosinase enzyme triggers hydrolysis reactions that release isothiocyanates (ITCs; Kirkegaard and Sarwar 1998; Matthiessen and Kirkegaard 2006). As this occurs, ITCs are released along with nitriles, cyanides, and ionic thiocyanates that suppress soil-borne pests and pathogens (Kirkegaard and Sarwar 1998). The potential for ITCs to impact pest levels depends on the type of proteins and amino acids present, which then form bonds to react with sulphhydryl groups (Brown and Morra 1997). Differing glucosinolates at varying concentrations are found among different species within different tissues and parts of the plants and as these degrade, ITCs are produced that will target pests (Brown and Morra 1997). Knowing what part of the plant will provide the highest concentration of glucosinolates and the glucosinolates present will allow for the greatest effect to be achieved for pest management in any soil disinfestation treatment.

While glucosinolate properties and concentration are a major consideration in biofumigation, additional consideration must also be given to crop management and treatment methods. These include maceration, tillage, soil temperature, and plant maturity (Mattner et al. 2008; Ploeg 2007). Much of the impact of maceration for biofumigation is tied to the production of glucosinolates. Depending on how finely the crop is macerated, the level of ITCs released increases. This has been evident in research by Mattner et al. (2008) in which the incorporation of a *Brassica rapa* and *B. napus* crop by a rotary tractor did not release ITCs while Matthiessen and Kirkegaard (2006) found that a finely ground cover crop led to high levels of ITCs in the soil. Additionally, flail mowing of broccoli residues has been shown to be an effective biofumigation treatment (Martin and Bull 2002).

Control of plant disease using brassicas in biofumigation shows promise. In a crop rotation experiment in Maine where cover crops were grown in the summer with potatoes

planted in the spring, both canola (*B. napus* cv. Hyola 401) and rapeseed (*B. napus* cv. Dwarf Essex) crops reduced incidence of black scurf (*R. solani*) 70 to 80%, compared to reductions between 25 and 75% for a rotation with oat (*Avena sativa*; Larkin and Griffin 2007). In addition, all brassica species in this study inhibited fungal growth to a degree and reduced incidence of *R. solani*. Of the brassica species, Indian mustard (*Brassica juncea*) was the most effective in controlling fungal diseases possibly based on the high level of glucosinolates present (Larkin and Griffin 2007). Similar results were found by Charron and Sams (1999) in that Indian mustard controlled *Pythium ultimum* and could be used in the tomato industry to control *R. solani*. In fact, flail mowed broccoli residues and rotations have been incorporated by farmers in order to reduce the impact of Verticillium wilt (Martin 2003). In a study looking at various brassica species in which tomatoes were planted following flail mowing of cover crops, the cover crops ‘Caliente 119’ mustard (*B. juncea* and *Sinapis alba*), ‘Caliente 61’ mustard (*B. juncea*), and ‘Nemat’ arugula (*Eruca sativa*) showed no difference compared to the unseeded control plots when examining marketable yield, growth of plants, and blossom end rot (Baysal and Miller 2009).

Control of plant-parasitic nematodes has also been demonstrated by brassicas in biofumigation. The seed meal, dry leaf meal, and defatted seed meal of Indian mustard has shown potential in decreasing levels of the southern root-knot nematode (*M. incognita*), specifically gall number, eggs, and egg masses in tomato production in Brazil (Oliveira et al. 2011). Arugula, specifically the ‘Nemat’ cultivar, has been shown to control *M. hapla*, *M. chitwoodi*, and *M. incognita* by acting as a trap crop (Ploeg 2007). An important key in using brassicas may be not only the decline in nematode species through eradication, but the potential to restrict the nematode communities to tolerable levels for the cash crop. Mustard seed meal was

capable of reducing *M. chitwoodi* levels, but not at the same level attainable by an organophosphate nematicide (ethoprop; Henderson et al. 2009).

Cover Crops. Cover crops are crops that are grown to enhance nutrient cycling, increase soil organic matter, and prevent erosion prior to planting cash crops; they may also enhance an agroecosystem through pest management. Other benefits include increases in yield and fruit size, suppression of soilborne diseases and weeds, conservation and improvement of soil organic matter and improved soil water holding capacity (Abawi and Widmer 2000; Chellemi 2002). Cover crops can also be utilized in a soil disinfestation treatment and could thus perform added benefits of improving pest control. The choice of a cover crop for use in soil disinfestation will need to be examined in order to achieve goals specific to pest management needed in a given farming system. Because of the wide range of cover crops such as grasses, legumes, brassicas, and other broadleaf plants, each can deliver different benefits to the soil environment and the agroecosystem.

The addition of cover crops to the soil environment can alter pathogen levels as they decompose, altering the soil microclimate and potentially limiting contact between pathogens and the cash crop. Through the decomposition of the biomass and root exudates following flail mowing in Maryland, both hairy vetch (*Vicia villosa*) and cereal rye (*Secale cereale*) led to an increase in the soil microbial biomass of the tomato rhizosphere (Buyer et al. 2010). Hairy vetch has further shown increases of polyunsaturated fatty acids in the soil when combined with compost that could lead to altering populations of fungi and protozoa (Carrera et al. 2007). In a study in Idaho, *V. dahliae* in potato production was best controlled by the green manures of corn (*Zea mays*) and sudangrass (*Sorghum bicolor* var. *sudanense*) compared to the untreated control,

Austrian winter pea (*Pisum sativum*), a two cultivar mix of rape (*Brassica napus* cvs. ‘Dwarf Essex’ and ‘Bridger’), oat (*Avena sativa* ‘Monida’), and cereal rye (Davis et al. 1996).

Cover crops also can control weeds through alteration of the soil environment, competition for resources, production of allelopathic compounds, and maintenance of surface residues (Snapp et al. 2005). Between cash crop growing seasons, a cover crop can also modify the weed seed bank by preventing a fallow period to persist that may allow for increased weed seed production, which could increase weed pressure in spring and summer. In a field experiment in California, sorghum-sudangrass (*Sorghum bicolor* x *S. bicolor* var. *sudanense*), when shredded and left on the surface as a mulch, led to a decline in weed biomass between 35 to 100% when vegetable transplants were grown (Stapleton et al. 2010). Sunn hemp (*Crotalaria juncea*) and cowpea (*Vigna unguiculata*), have also been suggested for weed suppression based on the amount of biomass produced by these summer cover crops (Wang et al. 2002).

Cover crops can also impact nematode populations through the incorporation and decomposition of the crop residue (Nyczepir and Thomas 2009). Summer cover crops such as cowpea, sunn hemp, sorghum-sudangrass, velvet bean (*Mucuna pruriens*), and hairy indigo (*Indigofera hirsuta*) have commonly been used in Florida systems to manage nematode populations (Gill and McSorley 2011). In research in North Carolina by McBride et al. (2000), the visual rating of root galling was significantly reduced following the addition of cereal rye that suppressed nematodes and assisted tomato growth (McBride et al. 2000). In a study looking at the effect of grass (family Poaceae) residues, the combination of poaceous amendments and temperatures of 38 C°/27 C° (day/night) triggered a substantial change in galling by the nematode *M. incognita* on tomato plants (cv. Cherry Belle), with reductions of 98 to 100% (Stapleton et al. 2010). Certain cover crops also act as non-hosts for nematodes, meaning that the

use of the cover crop could have the added benefit of managing parasitic nematode populations by limiting growth and reproduction during the off-season (Gill and McSorley 2011).

Organic amendments. Organic soil amendments are typically defined as organic residues that are either plant or animal in origin. This can include manures, composts, seed meals, crop residues, and animal byproducts applied to benefit cash crops or the agroecosystem in general through nutrient release or the beneficial effects of increased soil organic matter. Amendments can be incorporated into soil to breakdown and release nutrients gradually, or left on the surface. These soil amendments may also be used in conjunction with solarization or other soil disinfestation methods to improve efficacy of treatment (Lazarovits et al. 1991). An example of this would be the addition of an organic amendment that releases a large amount of ammonia that can be maintained at a high concentration in the soil to provide for improved pest control. Factors that influence pest control include amendments that contain nitrogen and secondary metabolites from plant material as well as the influence of soil C content and pH, as well as other environmental factors (Gamliel et al. 2000).

Plant-parasitic nematode control by the addition of organic soil amendments is well-established (Akhtar and Malik 2000; Oka 2010). As the organic matter decomposes, a greater population of soil organisms is present that can compete with the plant parasitic nematodes. At the same time, high levels of ammonia are released and ammonia has been shown to be an effective mechanism for controlling nematodes (Rodriguez-Kabana 1986). In addition, other decomposition byproducts such as nitriles, hydrogen sulfide, organic acids, and other chemicals that come from the degradation of soil amendments have the potential to impact nematode eggs and juveniles (Elmilligy and Norton 1973; Hollis and Rodriguez-Kabana 1967; Sayre et al. 1964; 1965). Some studies have shown that a narrow C:N ratio (higher nitrogen with lower carbon),

like those found in animal manures, legume green manures, and oilcakes or seed meals, are much better at controlling nematodes than those with a wider ratio (Akhtar and Malik 2000). If this is the case, then an optimum ratio along with the added benefits provided by the degradation of the soil amendment will need to be considered.

Various soil amendments have been effective in managing various plant parasitic nematodes. In a pot study in Israel using tomato plants, galling by *M. javanica* was reduced by using soybean oilcake, broiler litter, cottonseed meal, and feather meal, all at a rate of 4.0 to 8.0 g kg⁻¹ soil, but were not as effective in controlling root-knot nematode populations (*M. incognita* and *M. javanica*) alone, when not combined with soil solarization (moistened soil covered by polyethylene plastic) methods (Oka et al. 2007). *M. incognita* was further controlled by the application of chicken manure (Lopez-Perez et al. 2005). The use of poultry litter at a rate of 2.8 or 8.2 Mg ha⁻¹ in crop rotation in a Maryland study was likewise effective in controlling populations of *M. incognita* and *P. penetrans* (Everts et al. 2006).

Considering that soilborne plant pathogens infect crop plants from the soil, a soil amendment affects a plant pathogen's impact on a host plant (Islam and Toyota 2004). Animal manures such as swine and poultry, animal by-products, and seed meals such as soybean meal all contain high N levels, which have been shown to have a significant effect in reducing pathogen populations of *V. dahliae* (Lazarovits et al. 2001). Additional studies have demonstrated the importance of nitrogen in reducing populations of many soil-borne pathogens and that level of control is based on factors such as nitrification rate, organic matter content, pH, and soil buffering capacity (Lazarovits et al. 2001). Further, the use of poultry manure and other farm yard manures that have lower C:N ratios could be triggering higher microbial activity. If this is the case, a lower C:N ratio may be necessary for control of pathogens in order to shift microbial

communities to manage the disease. In a study in North Carolina examining the treatment of tillage and surface mulches, southern blight (*S. rolfsii*) incidence was lower in a cotton gin trash treatment mulched at the soil than other amendments: poultry manure, rye-vetch green manure, or synthetic fertilizer (Liu et al. 2007).

A concern for solanaceous crops in many regions is bacterial wilt, since this pathogen can survive in soils at deeper layers and has a broad host range (Islam and Toyota 2004). If bacterial wilt can be further controlled through the use of a soil amendment along with the additional benefits provided to the plant, this could prove to be a useful management tool. In a field experiment in Ethiopia comparing different organic matter additives in tomato production, complete suppression of *R. solanacearum* was seen with soils that had 5 and 10% farm yard manure, which consisted of cow manure and teff (*Eragrostis tef*) straw, and 10% green compost when compared to a non-amended control (Yadessa et al. 2010). A study in Japan examining different soil amendments reported that bacterial wilt incidence was reduced by adding a mixture of cow manure/rice straw and poultry manure but not by adding tree bark (species not given), coffee residues, or swine manure (Islam and Toyota 2004). A study in the Netherlands combining compost amendment with a simulated solarization environment in a lab setting showed the decline in population of *R. solanacearum* when compared to the control (Schönfeld et al. 2003).

A factor that must be considered for effective control of *R. solanacearum* is soil type. Considering that soil type will affect the soil microbial community and *R. solanacearum* populations, the amendment added to the soil may or may not be effective in suppression. In a study by Michel and Mew (1998), examining soil properties and application of an amendment containing urea and CaO, alkaline soil decreased the *R. solanacearum* population while an acidic

soil had no decline in populations. The properties of both the soils used influenced the available nitrate accumulation that occurred with the added urea (Michel and Mew 1998).

Soil amendments have also proven somewhat successful in improving weed control in specialty crop production systems. One strategy is the use of soil amendments in combination with solarization. In this case, the breakdown of an amendment under a plastic tarp in combination with increased soil temperatures can inhibit weed germination and promote decomposition of weed propagules. This was seen in a study in Lebanon where the addition of chicken manure combined with solarization inhibited germination of the weed seed of bean broomrape (*Orobanche crenata*), while separately neither method could attain the same level of control (Haidar and Sidahmed 2000). Allelopathy may also be another component of using soil amendments for weed control. The production of acids, nutrients, and other products could keep weeds from actively germinating. Composts and manures can control weeds through heat generated during decomposition, release of decomposition by-products (ammonia, acetic acid, humic and fulvic acids, and phenols) as well as increased microbial activity (Liebman and Davis 2000). Factors that may pertain to the effectiveness of compost in weed management includes age and application (incorporated vs. surface-applied).

Conclusion

The use of non-chemical soil disinfestation has shown potential in controlling plant pests. At the same time, many of the non-chemical methods have limitations that have prevented their wide-scale adoption. These include lack of broad-spectrum pest control, lack of cost effectiveness/increased treatment costs, and need for specialized equipment needs. One of the most promising alternative treatments is ASD, which has shown high potential in previous

research in other regions, further evaluation and optimization of ASD in Tennessee will serve to provide solutions for Tennessee growers interested in production without the use of soil fumigants.

CHAPTER II

FIELD EVALUATION OF ANAEROBIC SOIL DISINFESTATION (ASD) CARBON SOURCES FOR TOMATO AND BELL PEPPER PRODUCTION IN TENNESSEE

Abstract. Anaerobic soil disinfestation (ASD) is a non-chemical, pre-plant soil treatment developed to control soilborne plant pathogens, plant-parasitic nematodes, and weed populations in specialty crop production systems. Soil treatment by ASD involves the incorporation of a labile carbon (C) source, tarping with plastic, and irrigation of the topsoil to saturation (~ 5 cm irrigation) in order to create conditions conducive to anaerobic decomposition of the added C source. A field study was implemented beginning in fall 2010 and repeated in fall 2011 in Knoxville, TN to evaluate potential C sources for ASD prior to production of fresh-market tomato (*Solanum lycopersicum* cv. Red Defender) and red bell pepper (*Capsicum annum* cv. Red Knight X3R). Carbon sources included: 1) dried molasses, 2) cereal rye (*Secale cereale*) cover crop residue, 3) cereal rye residue supplemented with dried molasses, 4) mixture of mustards (*Brassica juncea* and *Sinapsis alba*) and arugula (*Eruca sativa*) cover crop residue, 5) mixture of mustard/arugula cover crop residue supplemented with dried molasses, 6) mustard seed meal (biofumigant control), and 7) an untreated control. Following incorporation of amendments and cover crops, beds were formed in the center of each plot, covered with black polyethylene mulch, and irrigated. Following ASD treatment, tomato and bell pepper were transplanted on half of each plot and treatment impacts on soil properties, weed control, plant pathogens, and crop performance evaluated. Accumulation of anaerobic conditions did not differ among treatments in spring/summer 2011 or 2012, but were generally greater in 2012 than 2011. There were also few differences in weed populations, disease pressure, and pepper and tomato yields among treatments. While this study establishes a potential management system for ASD in Tennessee,

further research is needed on sites with high pathogen, nematode and/or weed pressure to adequately evaluate the effectiveness of ASD treatments for warm-season vegetable production.

Introduction

Many vegetable, small fruit and ornamental producers are transitioning away from the use of methyl bromide (MeBr) as a pre-plant soil fumigant in their production systems. This is largely due to increased regulation due to the global phase-out of this ozone-depleting chemical as part of the Montreal Protocol and increased costs for a reduced supply of available MeBr. Given the limitations of alternative chemical soil fumigants in controlling plant-parasitic nematodes and weeds, many growers would likely be amenable to implementing a non-chemical approach to soil disinfestation if comparable crop yields and control of soilborne plant pathogens, plant-parasitic nematodes, and weeds could be achieved. The use of anaerobic soil disinfestation (ASD) is one non-chemical approach that has shown promise in Japan, the Netherlands, Florida, and California (Butler et al. 2012a; 2012b; 2012c; Goud et al. 2004; Momma et al. 2006; Shennan et al. 2010). Soil treatment by ASD involves incorporation of an easily-decomposable carbon (C) source, covering the soil with plastic, and then irrigating the topsoil to field capacity. Amendments used can vary, but must contain an adequate supply of labile C to support soil microbial growth during a treatment period that can range from 2 to 6 weeks in length (Momma 2008). During ASD treatment, organic acids, particularly acetic and n-butyric acids, are released into the soil solution as the C source decomposes anaerobically (Momma 2008).

In a field study in the Netherlands, declines in *Ralstonia solanacearum* survival rates were observed when anaerobic soil conditions were created by adding a mown grass amendment

(species not given), irrigating to field capacity, and mulching with a polyethylene silage tarp (0.135mm; Messiha et al. 2007). In addition, the researchers concluded that reductions in inoculum of *R. solanacearum* were dependent on incorporation of an easily-decomposable C source and covering with a plastic tarp as neither amendment nor tarping alone had the same impact on pathogen survival rates. In a lab experiment in California, the addition of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) residues to moist soil covered with clear polyethylene (0.031 mm) and maintained at a temperature of 23°C in a laboratory bioreactor reduced populations of Southern root-knot nematode (*Meloidogyne incognita*) by 97 and 56% compared to the non-amended control, respectively (Stapleton et al. 2010). In a study by Blok and colleagues (2000) in the Netherlands, researchers examined control of soilborne plant pathogens when soils were amended with perennial ryegrass (*Lolium perenne* L.) or broccoli (*Brassica oleracea* L. convar. *botrytis* (L.) Alef. var. *cymosa* Duch.) residues or left unamended, and then irrigated following amendment incorporation. The researchers concluded that both incorporated amendments (i.e., labile C source) and mulching with the polyethylene silage tarp (0.135 mm) were necessary for control of *Verticillium dahliae* and *Rhizoctonia solani* (Blok et al. 2000). In an experiment examining the impact of ASD treatment on inoculum densities of endemic *V. dahliae* in the Netherlands, an 85% reduction in inoculum was reported for all treatments where a grass amendment with a C:N ratio of either 13:1 or 22:1 was incorporated and covered with plastic (0.115 mm), compared to the unamended, uncovered control (Goud et al. 2004).

In a field study in Japan, the use of wheat bran as a C source in ASD demonstrated potential for reducing disease severity of bacterial wilt (*R. solanacearum*) on tomato (Momma 2008). In a separate field study in Japan, which utilized a 1% ethanol solution as the C source for

ASD, mortality of *F. oxysporum* inoculum was greatest when ethanol was used as the C source as compared to wheat bran or an untreated control (Momma et al. 2010). In a previous study, wheat bran as a C source for ASD treatment decreased *F. oxysporum* conidia and chlamydospores (Momma et al. 2005). In addition, many researchers have stressed the importance of organic acid generation in the effectiveness of ASD treatment. The presence of high levels of both acetic and butyric acids through the degradation of wheat bran during ASD treatment suppressed *R. solanacearum* at low concentration levels of these acids while the maximum concentration did not suppress *F. oxysporum* chlamydospores (Momma et al. 2006). A recent examination of the organic acids produced by ethanol during ASD treatment noted the presence of the metal ions of Fe^{2+} and Mn^{2+} when soil reduction occurred and hypothesized that this may play a role in suppression of *F. oxysporum* (Momma et al. 2011).

Studies of ASD in Florida have also shown promising results in the ability of ASD to control pests. Liquid molasses as a C source for ASD treatment combined with solarization significantly increased *F. oxysporum* inoculum mortality compared to an untreated control to levels comparable with the MeBr control (Butler et al. 2012b). Populations of *M. incognita* and ratings of root gall on eggplant at the end of the study were generally equivalent to the MeBr control and less than solarization alone when molasses was used as an amendment in ASD treatment (Butler et al. 2012b). When molasses and poultry litter were applied in ASD treatments combined with solarization, control of yellow nutsedge (*Cyperus esculentus* L.) was generally equivalent to the MeBr control (Butler et al. 2009). In a greenhouse pot study of ASD treatment with various warm-season legume and grass cover crops, introduced *F. oxysporum* inoculum was reduced by 97% when a C source was added compared to the control (no C source) while *Sclerotium rolfsii* was inconsistently affected by C source and ASD treatment (Butler et al.

2012a). In the same study, mortality of yellow nutsedge tubers was greatest when molasses was used as a C source (31% germination compared to 76% germination in the control) and numbers of *M. incognita* were low in all treatments with an added C source (Butler et al. 2012a). In a California pot study, wheat bran was used as a C source with simulated coastal California environmental conditions that included treatments of different tarps and burial depths of mesh packets containing weed seeds of annual bluegrass (*Poa annua*) and common purslane (*Portulaca oleracea*). While weed seed germination was inhibited by all tarps and soil types used, anaerobic activity was not related to inhibition of germination (Muramoto et al. 2008). A separate study evaluated yellow nutsedge (*Cyperus esculentus*) tuber germination at two different burial depths (2 and 15 cm). Results indicated that there was limited survival of yellow nutsedge tubers buried at the 15-cm depth where anaerobic conditions existed while 66% of tubers survived at the 2-cm depth indicating a relationship between anaerobic soil conditions and tuber survival (Muramoto et al. 2008). Additional pot and field studies in California demonstrated the potential of ASD in controlling *V. dahliae* when using C sources of ethanol, wheat bran, mustard cake, or rice bran for ASD treatment with the recommendation that strawberry producers could adopt rice bran cost-effectively for ASD treatment (Shennan et al. 2010).

There exists a need to evaluate ASD in environmental conditions and production systems representative to Tennessee in order to evaluate and optimize the procedure for use by commercial producers in the state and other states in the Southeast. Research conducted in other geographic locations has applicability to Tennessee, but environmental factors (e.g. climate, soil types, pathogens, weeds) and production systems can differ markedly in Tennessee as compared to locations where ASD has been successfully implemented (Japan, Netherlands, Florida,

California). This experiment was initiated to adapt the ASD system for warm-season vegetable production in Tennessee using bell pepper and tomato as model production systems by evaluating locally-available C sources and seasonally-adapted cover crop C sources for ASD treatment and examining impacts on soil properties, soilborne pathogens, weed control, and crop productivity.

The objectives of this field study were to (1) evaluate redox potential (as a measure of reduced (anaerobic) soil conditions) and soil properties for a range of C sources used in ASD treatment, (2) evaluate weed control, incidence of galling by root-knot nematodes, and incidence of disease caused by soilborne plant pathogens among C source treatments and (3) compare growth, yields, and fruit quality of both tomato and bell pepper between ASD treatments.

Materials and Methods

A randomized complete block design including six C source treatments and a fallow, untreated control with four replicates (blocks) was established at the East Tennessee Ag Research and Education Center in Knoxville, TN beginning in fall 2010 and repeated on the same plots beginning in fall 2011. The experimental area had been cropped with a diverse mix of vegetables during the previous growing seasons. The soil type is a Dewey clay loam (fine, kaolinitic, thermic, typic Paleudult). Soil samples were taken from the experimental site in fall 2010 and stockpiled poultry litter (34 kg N ha⁻¹, 53 kg P ha⁻¹, 67 kg K ha⁻¹) was applied to all plots at a rate of 4.5 Mg ha⁻¹ and incorporated to increase soil phosphorus (P) and potassium (K) levels and support cover crop growth. In 2011 poultry litter was again applied at a rate of 4.5 Mg ha⁻¹ (125.1 kg N ha⁻¹, 55.8 kg P ha⁻¹, 51.3 kg K ha⁻¹). Each treatment was established in a plot 2.4 m by 9.1 m. Treatments included: (1) a fallow, unamended control (hereafter referred to as the

‘untreated control’), (2) fallow with mustard seed meal (Wisconsin Spice, Berlin, WI; 2.24 Mg ha⁻¹) added prior to treatment (hereafter referred to as the ‘biofumigant control’), (3) fallow with dried molasses (Westway Feed, Tomball, TX; 5.6 Mg ha⁻¹) added prior to ASD treatment, (4) a commercial mixture (‘Caliente 61’, High Performance Seed, Moses Lake, WA) of Indian mustard (*Brassica juncea*) and white mustard (*Sinapsis alba*) seeded in a 1:1 mixture with arugula (*Eruca sativa* cv. Nemat) at 7.8 kg ha⁻¹ mixture with a reduced rate of dry molasses (1.12 Mg ha⁻¹) added prior to ASD treatment, (5) mustard and arugula cover crop, (6) a cereal rye (*Secale cereale*, variety not stated) cover crop (seeded at 134 kg ha⁻¹ Albert Lea Seed House, Albert Lea, MN) with a reduced rate of dry molasses (1.12 Mg ha⁻¹) added prior to ASD treatment, and (7) cereal rye cover crop. All amendment rates given are based on an application area of 16.7 m² (9.15 m by 1.83 m). Cover crops for treatments 1, 2, 5 and 6 were drilled (Light Duty Grain Drill, ALMACO, Nevada, IA) on September 27, 2010 and again on October 26, 2011 while all other plots remained fallow during the fall and winter of both seasons.

All treatments containing a cover crop were flail mowed (SH74 Flail Mower, Alamo Industrial, Seguin, TX) on April 7, 2011 and on March 28, 2012. Molasses and mustard seed meal were applied by hand to a 1.8-m by 9.1-m strip along the center of the cereal rye + molasses, mustard/arugula + molasses, molasses, and the biofumigant control. All plots except the biofumigant control also had feather meal applied to balance N added in other amendments. Plots were rotovated (RTC240, Bush Hog, Inc., Selma, AL) immediately after amendments were applied, and beds formed (0.9-m width) and covered with black polyethylene (0.032-mm embossed, Pliant-Berry Plastics, Evansville, IN). A drip irrigation line (T-Tape, John Deere/T-Systems, San Diego, CA) was installed in the center of each bed. Five cm of water was applied

to all beds based on the calculated amount of water needed to approximately fill soil pore space to a depth of 20 cm.

Soil temperature, redox potential, and soil moisture were continually monitored, averaged, and recorded hourly during treatment using soil sensors (combination ORP electrode, Sensorex Corp., Garden Grove, CA and 5TM soil moisture and temperature sensor, Decagon Devices, Pullman, WA) and an automatic datalogging system (CR1000 with AM 16/32 multiplexers, Campbell Scientific, Logan, UT). Each sensor was placed to monitor at a 15-cm soil depth. Raw soil redox potential values were corrected to relate to the redox potential of a standard hydrogen electrode (Fiedler et al. 2007). Anaerobic conditions were indicated based on the soil redox potential, when measured on an average hourly reading, to be below a critical redox potential (CEh). Critical redox potential was calculated using the formula: $CEh = 595 \text{ mV} - (60 \text{ mV} \times \text{soil pH})$, with soil pH determined at the termination of ASD treatment used to calculate CEh. Over a three-week treatment period, for values below CEh, the absolute value of the difference between a given value and CEh were summed to give a measure of accumulation of anaerobic soil conditions (Butler et al. 2012a). In 2012, an IRIS (indicator of reduction in soil) tube, a section of polyvinyl-chloride pipe painted with a ferrihydrite solution, was placed into each plot at a depth of 0 to 15 cm at treatment and removed following the treatment period. This method has been reported to correlate with anaerobic soil conditions based on the solubilization of ferrihydrite paint on the tube surface (Castenson and Rabenhorst 2006). Reduction of iron (Fe) was assessed by using a plastic grid (15 cm) with each square (0.25 cm^2 , 390 total) marked when 50% or more of the paint was solubilized (Rabenhorst 2012).

Soil and cover crop samples were collected in April 2011 and March 2012, prior to the initiation of the ASD treatments. Soil cores (0 to 15-cm depth, 1.75-cm internal diameter) were

collected from random locations within each plot and composited. A subsample was oven-dried (105° C) to determine gravimetric moisture content and the remaining sample was air-dried and sieved (< 2 mm). Soil pH was determined on air-dried samples in deionized water (1:1; pH_{water}) and in 0.01-M CaCl₂ (1:2; pH_{CaCl2}) using a pH electrode (Orion 3-Star Plus pH Benchtop Meter, Thermo Scientific, Waltham, MA). Soil samples were also analyzed for inorganic and total nitrogen (TN) and total C (TC). Briefly, 5 g of air-dried soil was extracted with 1-M KCl for 30 min on a reciprocating shaker, centrifuged, and filtered (Whatman 42, Whatman Ltd., Kent, UK) prior to colorimetric analysis for NH₄-N and NO₃-N + NO₂-N using a microplate spectrophotometer (Powerwave XS, Biotek, Winooski, VT) as described by Sims et al. (1995). Air-dried and ground soil samples were analyzed by flash combustion for TN and TC (Flash EA 1112 NC Soil Analyzer, Thermo Scientific, Waltham, MA, USA). Extractable soil phosphorus was determined using a malachite green microplate method (D'Angelo et al. 2001) following extraction of soil as described by Mehlich (1953). In plots with a cover crop, aboveground biomass was sampled by randomly placing two square frames (0.25 m²) in each plot and cutting all vegetation within the square at the soil surface. Cover crop samples were weighed, and then oven dried (65°C) to determine moisture content and TN/TC content by combustion as described for soil samples.

In 2012, C and N mineralization from soil samples with incorporated amendments was evaluated utilizing methods based on Drinkwater et al. (1999) and Franzluebbers (1999). For each field plot, pre-treatment soil samples were mixed at field rates with soil amendments and biomass (cereal rye and mustard/arugula) collected one day prior to field treatments. For each field plot, one jar was assembled with four 20-mL scintillation vials containing 10 g of soil to which respective amendments were added at field rates and water added to 50% soil porosity.

Each jar also contained a scintillation vial containing 2 mL of 2-M NaOH to capture respired CO₂ and jars were then placed in an incubator at 30°C. At 3, 10, 17 and 30 days, the scintillation vial from each jar was removed with 2 mL of 1.5 M BaCl₂ and 30 µL of phenolphthalein solution (1%) added. This solution was then titrated using 0.25 M HCl to determine soil respiration rates, as a measure of labile C in each treatment. After removal of the vial of NaOH, 2 mL of 2-M NaOH was added to a clean scintillation vial and placed into the jar for the next time period. At each sampling interval, one of the four initial vials containing soil and amendments was removed from each jar and extracted to determine inorganic soil N as previously described.

At the termination of soil treatments (May 3, 2011 and April 24, 2012), soil cores were collected from each bed and analyzed as described previously. Because of hail damage to plastic mulch on April 27, 2011, a second layer of plastic was placed over all beds on May 6, 2011. On May 10, 2011 and April 25, 2012, tomato transplants (cv. Red Defender, Reimer Seeds, Mount Holly, NC) were planted in one randomly selected half of each block, with bell pepper transplants (cv. Red Knight X3R, Johnny's Selected Seeds, Winslow, ME) planted in the remaining half (May 10, 2011 and May 2, 2012). Tomatoes were planted at 45.7-cm spacing in one row (10 plants per bed) while peppers were planted 30.5 cm between and within a double row per bed (32 plants per bed). No herbicides were applied for weed control. Insect pests were controlled with applications of pyrethrum (PyGanic® Crop Protection EC 1.4, Arbico Organics, Oro Valley, AZ) and extract of neem oil (70% Neem oil, Monterey Lawn and Garden Products, Fresno, CA) as necessary. Weed populations (to species) and incidence of disease (visual rating) were monitored throughout the growing season on a weekly basis. Plant height and stem diameter at crown were measured at flowering and at first harvest. At first harvest, recently

matured leaves were collected from each treatment, dried at 65°C, and total N and C determined by combustion as described previously.

Populations of *Rhizoctonia solani* were assessed at planting by placing 20 birch toothpicks in the soil at the base of plants (10 pepper and 10 tomato plants) of each plot using the methods described by Paulitz et al. (2005). Toothpicks were removed after 48 h and plated onto semi-selective media with propagules per g of soil were derived based on the toothpick surface area and soil bulk density. Disease pressure by *Alternaria* spp. (*A. solani*, *A. porri*; McCarty et al. 2012b) was measured based on a weekly visual rating of foliar disease symptoms beginning in mid-June of each year. Incidence of southern stem blight (*S. rolfsii*) was recorded weekly throughout the growing season. Ratings of root galling by root-knot nematode were based on Bridge and Page (1980); following rinsing with water the extent of root galling present on both tomato and bell pepper roots rated as follows: 0 = no galling, 1 to 4 = galling of secondary roots only, 5 to 10 = galling of primary laterals and tap root. Each root system was also scored for physical root condition on a scale of 1 to 5 (1 = brown with signs of decay, 5 = white and healthy). Three root systems from each plot were evaluated for both bell pepper and tomato on August 19, 2011. In 2012, tomato roots systems were removed and rated on August 20 and bell pepper root systems removed and rated on October 2. Soil samples for nematode population assessment were taken at harvest at the end of the experiment in 2012 from both tomato and bell pepper plots and sent to the Plant Diagnostic Lab at Auburn University, Auburn, AL. Nematode populations (second-stage juveniles) in 100-cm³ soil samples were assessed by a sugar flotation technique to identify plant-parasitic populations at the genus level (Mullen et al. 2005).

Tomatoes and peppers were harvested and graded according to USDA Standards (USDA-AMS, 2005a; USDA-AMS, 2005b). In 2011, harvest for both bell pepper and tomatoes occurred

once a week during mid to late July. In 2012, harvest began in mid-July for tomatoes and in mid-August for peppers. Tomatoes were harvested based on first sign of red fruit color while peppers were harvested based on predominate (>75%) red color. At the end of the season, all green fruit were harvested and included in total yield estimates. Fruit were harvested from the center 6 plants of tomatoes and all pepper plants with the exception of the plants at the end of each row. Yields were converted to kg ha^{-1} based on an estimate of 5,467 m linear bed ha^{-1} (1.83-m between bed spacing, on center). At each harvest with adequate numbers of fruit, measurements of bell pepper pericarp thickness (two replicate measures per fruit), diameter, and length were taken from three fancy peppers (or No.1 peppers if needed) from each plot. Soil samples were also taken at first harvest and analyzed as described previously.

Measures of soluble solids, titratable acidity, and fruit pH were determined for tomato and pepper (two harvests for tomatoes and one harvest for peppers in each year). Fruit was selected to obtain five fruits from each plot at peak ripeness and either No.1 or fancy grade when applicable. Fruit were processed into a slurry with peppers requiring the addition of deionized water (1:1 ratio; Jen and Robinson 1984) using a blender (Oster Pro Blender, Sunbeam-Jarden Corporation, Providence, RI) for 1 minute. Forty mL of the solution was added to a 50-mL centrifuge tube, centrifuged (3500 RPM for 15 min), then 15 mL pipetted into beaker for measurement of soluble solids and fruit pH. For soluble solid determination, 40 μL of the slurry of each treatment was placed on a refractometer (REF103, General Tools, New York, NY). Fruit pH was determined on the slurry using a pH electrode (Orion 3-Star Plus pH Benchtop Meter, Thermo Scientific, Waltham, MA). Titratable acidity was measured following pH determination by titrating to pH 8.1 using 0.1 M NaOH in order to reach the citric acid standard (Tandon et al. 2003).

Results were analyzed using the GLM procedure in SAS 9.2 (SAS Institute Inc, Cary, NC). Main effects and differences between means were considered significant at $p < 0.05$, unless otherwise noted. Protected means were separated using Fisher's least significant difference procedure.

Results

Amendment biomass, carbon, and nitrogen. In 2011, total biomass incorporated for soil treatments ranged from 1,120 kg ha⁻¹ for the untreated control with added feather meal to 11,839 kg ha⁻¹ for cereal rye + molasses (Table 1). In 2012, total biomass ranged from 1,120 kg ha⁻¹ for the untreated control with added feather meal to 21,855 kg ha⁻¹ for mustard/arugula + molasses (Table 2). The primary difference between seasons was the lower biomass contribution of the mustard cover crop during 2011, due to winterkill likely influenced by the earlier planting date and colder winter conditions compared to 2012. Total C incorporated for the mustard/arugula (2,953 kg C ha⁻¹) and mustard/arugula + molasses (3,389 kg C ha⁻¹) treatments in 2011 was slightly higher than the amount of C added by molasses (2,335 kg C ha⁻¹). In 2011, total C added by cereal rye and cereal rye + molasses treatments were the highest (4,230 and 4,494 kg C ha⁻¹, respectively). For treatments with mustard/arugula, arugula total carbon was similar to 2011 (2,000 to 2,007 kg C ha⁻¹ in 2011 and 2,807 to 3,047 kg ha⁻¹ in 2012), while mustard biomass was greater with 3,688 and 3,460 kg C ha⁻¹ in 2012 compared to 306 and 329 kg C ha⁻¹ in 2011. The contribution of C from cereal rye biomass was similar in both 2011 and 2012. The ratio of C in amendments incorporated to the estimated soil volume to a 15-cm depth ranged from 0.29 mg C g⁻¹ soil for the untreated control to 2.3 and 2.5 mg C g⁻¹ soil in the cereal rye + molasses and cereal rye treatments in 2011, respectively (Table 1).

Table 1. Total biomass, carbon and nitrogen content, and C:N ratio of soil amendments and aboveground cover crop biomass from field study, spring 2011.

	-----2011-----				
	Biomass	TC	TN	TC	C:N
	-----kg ha ⁻¹ -----			mg C g ⁻¹ soil	ratio
Untreated control[†]	1,120	560	179	0.29	3.1
Biof. control	2,240	937	114	0.50	4.2
Molasses	6,238	2,335	164	1.18	14.4
Molasses	5,600	2,016	62	1.03	32.5
Feather meal	638	319	102	0.16	3.1
Must./aru. + mol.	8,460	3,389	316	1.70	10.7
Arugula	5,005	2,007	110	1.03	18.2
Mustard	865	329	20	0.17	19.5
Molasses	1,120	406	12	0.57	33.8
Feather meal	1,030	515	165	0.26	3.1
Weeds	440	132	9	0.07	15.2
Mustard/arugula	6,375	2,953	334	1.49	8.8
Arugula	5,000	2,000	125	1.02	16.0
Mustard	806	306	23	0.16	13.1
Feather meal	1,120	560	179	0.29	3.1
Weeds	255	87	6	0.04	14.0
Cereal rye + mol.	11,839	4,494	371	2.30	12.1
Cereal rye	9,636	3,565	193	1.79	18.9
Molasses	1,120	403	12	0.21	32.8
Feather meal	1,030	515	165	0.26	3.1
Weeds	53	11	1	0.00	8.7
Cereal rye	10,536	4,230	368	2.20	11.5
Cereal rye	9,290	3,623	186	1.84	19.5
Feather meal	1,120	560	179	0.29	3.1
Weeds	126	47	3	0.02	16.0

[†]Represents contribution of applied feather meal.

Table 2. Total biomass, carbon and nitrogen content, and C:N ratio of soil amendments and aboveground cover crop biomass from field study, spring 2012.

	-----2012-----				
	Biomass	TC	TN	TC	C:N
	-----kg ha ⁻¹ -----			mg C g ⁻¹ soil	ratio
Untreated control[†]	1,120	560	179	0.29	3.1
Biof. control	2,240	937	114	0.50	4.2
Molasses	6,238	2,335	164	1.18	14.4
Molasses	5,600	2,016	62	1.03	32.5
Feather meal	638	319	102	0.16	3.1
Must./aru. + mol.	21,855	8,197	534	4.20	15.4
Arugula	7,928	2,807	159	1.44	17.7
Mustard	9,173	3,688	147	1.89	25.1
Molasses	1,120	406	12	0.57	33.8
Feather meal	1,030	515	165	0.26	3.1
Weeds‡	2,604	781	52	0.40	15.2
Mustard/arugula	20,597	7,683	588	3.94	13.1
Arugula	8,606	3,047	172	1.57	17.7
Mustard	9,060	3,460	192	1.77	18.0
Feather meal	1,120	560	179	0.29	3.1
Weeds	1,811	616	45	0.32	14.0
Cereal rye + mol.	12,590	4,823	422	2.47	11.4
Cereal rye	8,967	3,596	215	1.84	16.7
Molasses	1,120	403	12	0.21	32.8
Feather meal	1,030	515	165	0.26	3.1
Weeds	1,473	309	29	0.16	10.5
Cereal rye	13,464	5,288	466	2.71	11.3
Cereal rye	10,079	3,890	242	1.94	16.1
Feather meal	1,120	560	179	0.29	3.1
Weeds	2,265	838	45	0.43	18.5

[†]Represents contribution of applied feather meal. [‡] Values of C and N for weed species in 2012 are estimated based on 2011 TC and TN concentrations of weed biomass.

In 2012, this range was between 0.29 mg C g⁻¹ soil in the untreated control to 4.2 mg C g⁻¹ soil in the mustard/arugula + molasses treatment (Table 2).

Total N in soil amendments in 2011 ranged between 114 kg N ha⁻¹ for the biofumigant control treatment to 371 kg N ha⁻¹ for the cereal rye + molasses treatment while total N in 2012 ranged between 114 kg N ha⁻¹ for the biofumigant treatment to 588 kg N ha⁻¹ for the mustard/arugula treatment. In 2011, the greatest C:N ratio of soil amendments was from the molasses treatment (14.4) followed by the cereal rye + molasses treatment (12.1). In 2012, amendments for the mustard/arugula + molasses and molasses treatments had the greatest C:N ratios (15.4 and 14.4, respectively). In both years, the biofumigant control (4.2) and the untreated control with feather meal (3.1) had the lowest C:N ratio for soil amendments.

Soil properties. In 2011, no differences were observed in volumetric soil moisture content during soil treatments or in gravimetric soil moisture content determined from soil samples taken at treatment termination (planting; Table 3). In 2012, volumetric soil moisture content during soil treatments was generally higher than in 2011, averaging from 0.29 to 0.34 cm³ cm⁻³ for each treatment in 2012 as compared to 0.18 to 0.24 cm³ cm⁻³ in 2011. Gravimetric soil moisture content at treatment termination in 2012 differed slightly among treatments, with the highest soil moisture content observed from the cereal rye treatment (0.27 g g⁻¹), which was significantly greater than all other treatments (0.21 to 0.24 g g⁻¹). In 2011, mean soil temperature during treatment did not differ among treatments, ranging from 20.7 to 21.0°C (Table 4). In 2012, there also were no differences among treatments with mean soil temperatures ranging between 20.4°C in the biofumigant control to 21.0°C in the molasses treatment. In 2011, there were no differences between treatments in harvest soil pH_{water} or pH_{CaCl2} (Table 5). Post treatment, the highest pH_{water} and pH_{CaCl2} values were observed from the cereal rye + molasses treatment (6.8

Table 3. Mean volumetric soil moisture during treatment and gravimetric soil moisture post treatment from field study.

	During Treatment ^a	Post Treatment ^b	During Treatment	Post Treatment
	-----2011-----		-----2012-----	
	-----cm ³ cm ⁻³ -----	-----g g ⁻¹ -----	-----cm ³ cm ⁻³ -----	-----g g ⁻¹ -----
Untreated control	0.18	0.23	0.34	0.21c [†]
Biofumigant control	0.24	0.20	0.34	0.22bc
Molasses	0.18	0.23	0.30	0.24b
Mustard/arugula + molasses	0.20	0.22	0.29	0.22bc
Mustard/arugula	0.20	0.22	0.33	0.22bc
Cereal rye + molasses	0.18	0.23	0.32	0.21c
Cereal rye	0.18	0.24	0.31	0.27a

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.^a Volumetric moisture determined using 5TM sensor during treatment. ^bGravimetric moisture determined using soil sample collected from raised beds post treatment.

Table 4. Mean soil temperature during soil treatment from field study, 2011 and 2012.

	2011	2012
	-----Soil temperature, °C-----	
Untreated control	20.7	20.5
Biofumigant control	21.0	20.4
Molasses	20.8	21.0
Mustard/arugula + molasses	20.7	20.8
Mustard/arugula	20.9	20.7
Cereal rye + molasses	21.0	20.5
Cereal rye	20.8	20.8

[†]Temperature recorded at 15-cm depth during soil treatments April 6 to May 3, 2011 and March 28 to April 24, 2012. [†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table 5. Mean soil pH in water (pH_{water}) and CaCl₂ (pH_{CaCl2}) from field study, 2011.

	Pre-treatment		Post-treatment		Harvest	
	pH _{water}	pH _{CaCl2}	pH _{water}	pH _{CaCl2}	pH _{water}	pH _{CaCl2}
Untreated control	6.23c [†]	5.99bc	6.42cd	6.30c	6.78	6.75
Biofumigant control	6.38bc	5.97c	6.24d	6.19c	6.82	6.65
Molasses	6.32bc	5.93c	6.36cd	6.20c	6.68	6.72
Mustard/arugula + molasses	6.52ab	6.19abc	6.58abc	6.46abc	6.89	6.86
Mustard/arugula	5.52bc	6.12abc	6.52bc	6.39bc	6.66	6.76
Cereal rye + molasses	6.55ab	6.32a	6.76a	6.74a	7.04	6.96
Cereal rye	6.68a	6.29ab	6.68ab	6.65ab	7.27	6.96

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

and 6.7, respectively), which was greater than mustard/arugula, molasses, biofumigant control, and untreated control treatments (less than 6.6). In 2012, pre-treatment pH_{water} did not differ significantly among treatments (Table 6). Pre-treatment $\text{pH}_{\text{CaCl}_2}$ was highest for cereal rye (6.3) and cereal rye + molasses (6.3), which were greater than the untreated control, biofumigant control, and molasses treatment (less than 6.0). Post-treatment pH_{water} was highest in the cereal rye + molasses treatment (6.4) which differed from all treatments except for cereal rye and molasses. The lowest pH_{water} values were observed from treatments that did not include cereal rye amendment (6.0 to 6.1).

There were no significant differences in accumulation of anaerobic conditions among treatments for both 2011 and 2012 (Fig 1). In general, there was a greater accumulation of anaerobic conditions for all treatments during the second season of the study. Similarly, IRIS tubes introduced prior to treatment in 2012 indicated no differences among treatments in anaerobic conditions with percentage reductions ranging from 14 to 32% (data not shown). The soil C:N ratio prior to treatment in 2011 indicated slight differences among treatments, with the biofumigant control having the greatest ratio (10.1), which was higher than the cereal rye + molasses treatment (9.3; Table 7). Post-treatment, the molasses treatment soil had the greatest C:N ratio (10.2) and differed from mustard/arugula, cereal rye + molasses, biofumigant control, and the untreated control (9.4 to 9.6). In 2012, there were no differences among treatments in any of the soil C:N ratios, ranging between 7.8 to 9.9 prior to amendment incorporation to 9.6 to 9.9 at planting. Prior to incorporation of soil amendments in 2011, the mustard/arugula treatment plots had the highest level of soil $\text{NO}_3 + \text{NO}_2\text{-N}$ ($42.2 \text{ mg N kg}^{-1}$ soil) and total soil inorganic N ($49.0 \text{ mg N kg}^{-1}$ soil), which was higher than that observed from the cereal rye treatment ($10.5 \text{ mg N kg}^{-1}$ and $16.8 \text{ mg N kg}^{-1}$ soil, respectively; Table 8). Soil $\text{NH}_4\text{-N}$

Table 6. Mean soil pH in water (pH_{water}) and CaCl₂ (pH_{CaCl2}) from field study, 2012.

	Pre-treatment		Post-treatment		Harvest tomato		Harvest pepper	
	pH _{water}	pH _{CaCl2}	pH _{water}	pH _{CaCl2}	pH _{water}	pH _{CaCl2}	pH _{water}	pH _{CaCl2}
Untreated control	6.76	5.96b [†]	6.04b	5.73bc	6.17	5.62b	6.14ab	5.64ab
Biofumigant control	6.74	5.94b	6.05b	5.68c	6.40	5.66ab	6.26a	5.55b
Molasses	6.73	5.97b	6.13ab	5.77bc	6.37	5.60b	6.28a	5.60ab
Mustard/arugula + molasses	6.96	6.12ab	6.06b	5.86abc	6.38	5.82a	6.32a	5.71ab
Mustard/arugula	6.90	6.07ab	6.05b	5.85abc	6.23	5.60b	6.00b	5.58ab
Cereal rye + molasses	7.02	6.29a	6.40a	6.01ab	6.42	5.75ab	6.16ab	5.73a
Cereal rye	7.12	6.31a	6.28ab	6.07a	6.31	5.80a	6.23a	5.68ab

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

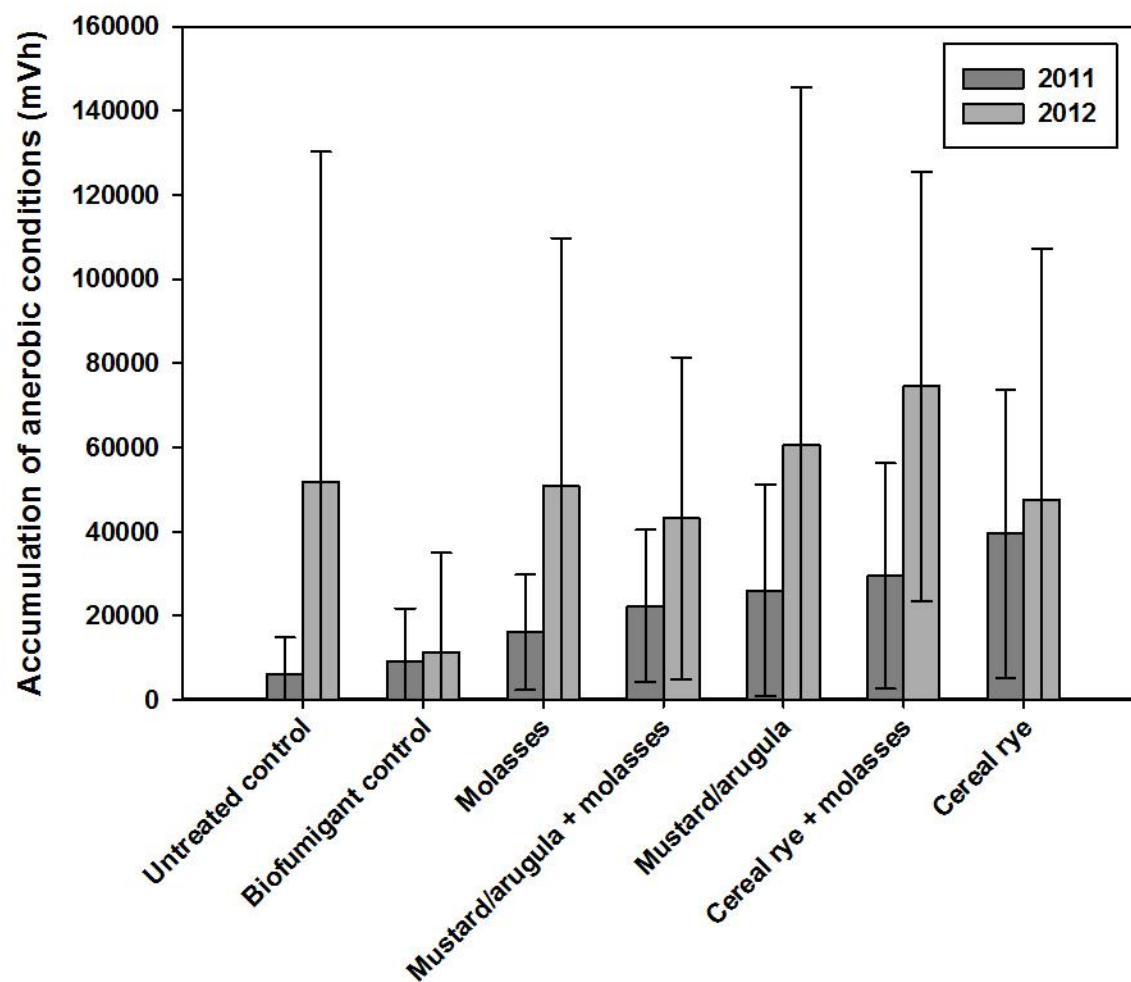


Figure 1. Total accumulation of anaerobic conditions (mVh) from field study, 2011 and 2012.. Error bars represent 95% confidence intervals.

Table 7. Soil carbon to nitrogen ratio (C:N) from field study, 2011 and 2012.

	Pre treatment	Post treatment	Harvest	Pre treatment	Post treatment	Harvest tomato	Harvest pepper
	-----2011-----			-----2012-----			
	-----TC/TN-----						
Untreated control	9.4ab [†]	9.4b	9.3bc	9.8	9.6	8.2	9.3
Biofumigant control	10.1a	9.6b	9.4bc	9.9	9.9	8.1	9.0
Molasses	9.7ab	10.2a	9.8ab	9.8	9.6	8.3	9.4
Mustard/arugula + molasses	9.6ab	9.8ab	9.5bc	9.9	9.7	8.4	8.9
Mustard/arugula	9.5ab	9.5b	9.1c	7.8	9.7	8.4	9.0
Cereal rye + molasses	9.3b	9.6b	10.2a	9.6	9.8	8.7	9.4
Cereal rye	9.7ab	9.7ab	9.6b	9.9	9.7	8.5	9.2

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

was generally low ($< 7 \text{ mg N kg}^{-1}$) and did not differ among treatments prior to amendment incorporation. At the termination of soil treatments in 2011 (at planting), the highest total inorganic N ($114.3 \text{ mg N kg}^{-1} \text{ soil}$) and $\text{NO}_3+\text{NO}_2\text{-N}$ ($109.1 \text{ mg N kg}^{-1} \text{ soil}$) was observed from the untreated control treatment, which was greater than both the biofumigant control and cereal rye treatments (Table 8). $\text{NH}_4\text{-N}$ (mean $5.8 \text{ mg N kg}^{-1} \text{ soil}$) did not differ among treatments at planting. Similarly, there were no differences in total inorganic N, $\text{NO}_3+\text{NO}_2\text{-N}$, or $\text{NH}_4\text{-N}$ at harvest among treatments. In 2012, there were no differences in $\text{NH}_4\text{-N}$, $\text{NO}_3+\text{NO}_2\text{-N}$, or total inorganic N prior to incorporation of soil amendments (Table 9). Post treatment (at planting), the mustard/arugula and mustard/arugula + molasses treatments had the highest total soil inorganic N levels (27.3 and $27.9 \text{ mg N kg}^{-1}$, respectively). The untreated control, biofumigant control, and molasses treatments all had the lowest total inorganic N at planting (12.4 to $14.4 \text{ mg N kg}^{-1}$). Total soil N content (TN) prior to amendment incorporation in 2011 ranged from 1.07 g N kg^{-1} for the molasses and 1.08 g N kg^{-1} mustard/arugula + molasses treatments to 1.23 g N kg^{-1} for the cereal rye + molasses treatment (data not shown). At planting, there were no differences in soil TN (mean 1.16 g N kg^{-1}). In 2012, there were no differences in pre-treatment soil TN (mean 1.04 g N kg^{-1}) while cereal rye + molasses and mustard/arugula + molasses had the greatest TN post treatment with 1.06 g N kg^{-1} and 1.01 g N kg^{-1} , respectively.

Soil respiration from 2012 soil samples and amendments indicated treatment differences, although this varied depending on incubation length (Fig 2). For day 3, cereal rye + molasses, cereal rye, mustard/arugula, and mustard/arugula + molasses all had greater cumulative soil respiration compared to the untreated control, biofumigant control, and molasses treatments. At day 3, all treatments had greater cumulative soil respiration compared to the untreated control

Table 8 Mean soil ammonium-N ($\text{NH}_4\text{-N}$), nitrate + nitrite-N ($\text{NO}_3 + \text{NO}_2\text{-N}$), and total soil inorganic N (TIN) for composite soil samples from tomato and bell pepper plots from field study, 2011.

	NH ₄ N	NO ₃ + NO ₂ -N	TIN	NH ₄ N	NO ₃ + NO ₂ -N	TIN	TIN
	-----Pre treatment-----			-----Post treatment-----			---Harvest---
	----- mg N kg ⁻¹ soil-----						
Untreated control	5.3	32.3ab †	38.8ab	5.2	109.1a	114.3a	13.8
Biofumigant control	4.5	20.9ab	25.4ab	5.5	70.0b	75.5b	15.1
Molasses	4.8	17.8ab	22.6ab	5.6	78.2b	84.1ab	11.8
Mustard/arugula + molasses	4.6	21.5ab	26.4ab	6.2	93.0ab	99.2ab	13.2
Mustard/arugula	6.8	42.2a	49.0a	5.3	86.1ab	91.4ab	16.1
Cereal rye + molasses	5.9	23.8ab	29.7ab	7.3	81.1ab	88.4ab	23.4
Cereal rye	6.3	10.5b	16.8b	5.5	66.3b	71.8b	14.3

†Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table 9. Mean soil ammonium (NH₄-N), nitrate + nitrite (NO₃ + NO₂-N), and total inorganic nitrogen (TIN) from field study, 2012.

	NH ₄ N	NO ₃ + NO ₂ -N	TIN	NH ₄ N	NO ₃ + NO ₂ -N	TIN	TIN Tomato	TIN Pepper
	-----Pre treatment-----			-----Post treatment-----		-----Harvest-----		
	----- mg N kg ⁻¹ soil-----							
Untreated control	2.1	5.4	7.5	1.8	12.7cd [†]	14.4c	15.0ab	17.0
Biofumigant control	1.8	5.8	7.6	2.8	10.1d	12.9c	12.7ab	18.6
Molasses	2.1	4.5	6.7	3.0	9.4d	12.4c	8.9b	16.5
Mustard/arugula + molasses	1.8	5.2	7.0	1.9	26.0a	27.9a	37.3ab	39.4
Mustard/arugula	0.8	4.7	5.5	2.8	24.5ab	27.3a	44.1a	35.0
Cereal rye + molasses	1.8	4.5	6.2	3.2	19.9abc	23.1ab	28.7ab	48.0
Cereal rye	2.2	4.1	6.4	1.7	17.1bcd	18.8bc	26.2ab	36.7

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

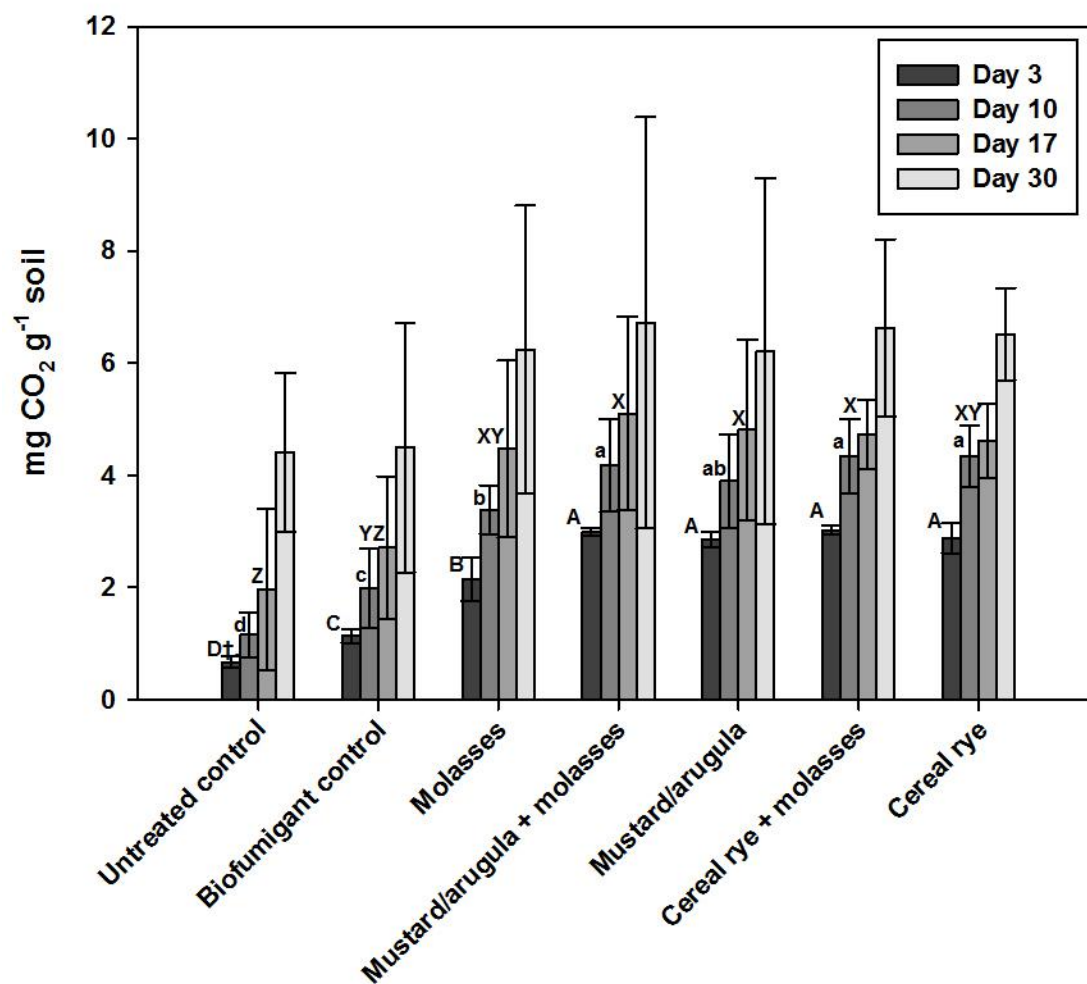


Figure 2. Cumulative soil respiration ($\text{mg CO}_2 \text{ g}^{-1} \text{ soil}$) at 3, 10, 17, and 30 days during incubation at 25°C for 2012 field study soil treatments. †Within day, means indicated by different letters are significantly different, $p < 0.05$. Error bars represent 95% confidence intervals.

(0.70 mg CO₂ g⁻¹), with the greatest soil respiration observed from the cereal rye + molasses treatment (3.02 mg CO₂ g⁻¹). This trend was also apparent at day 10 and day 17 except for the biofumigant control. At day 30, no significant differences in cumulative soil respiration were observed among treatments, possibly due to the loss of a few treatment replicate vials which increased treatment variability.

Nitrogen mineralized during soil incubation of 2012 soil samples (taken prior to amendment incorporation) and amendments indicated treatment differences during the 32-day incubation (data not shown). On day 3, there were no differences in soil NH₄-N, but this was not the case for soil NO₃+NO₂-N. In this case, the untreated control had the largest amount of NO₃+NO₂-N which was significantly greater than all other treatments. The total inorganic N mineralized during the first 3 days of incubation was greatest from the mustard/arugula, mustard/arugula + molasses, and untreated control treatments (18.2, 19.1, and 15.7 mg N kg⁻¹ respectively). Following 10 days of incubation, there were no significant differences in total soil inorganic N among treatments, which ranged from 11.5 mg N kg⁻¹ soil for the untreated control to 29.4 mg N kg⁻¹ soil for the mustard/arugula treatment. Following 17 days, total inorganic N was greatest in the mustard/arugula + molasses treatment (56.3 mg N kg⁻¹ soil), which was greater than the molasses, biofumigant control, and untreated control treatments (21.0 to 34.1 mg N kg⁻¹ soil). By day 30, mustard/arugula + molasses also had the highest NO₃+NO₂-N and total inorganic N with 94.4 and 94.9 mg N kg⁻¹ soil, respectively, which was greater than all treatments except for the cereal rye treatment.

In 2011, few differences were observed in extractable soil P (data not shown). Soil P ranged from 21.0 to 38.3 mg P kg⁻¹ prior to amendment incorporation, 22.2 to 28.3 mg P kg⁻¹ at planting, and 12.6 to 26.8 mg P kg⁻¹ at harvest. Similarly, there were no significant differences in

soil P in 2012. Soil P ranged from 25.3 to 33.1 mg P kg⁻¹ prior to amendment incorporation and 24.9 to 34.0 mg P kg⁻¹ soil at post- treatment. At tomato harvest in 2012, soil P ranged from 13.3 to 16.9 mg P kg⁻¹. At pepper harvest, extractable soil P was greatest from the mustard/arugula + molasses treatment (32.0 mg P kg⁻¹), which was higher than that observed from the molasses treatment (20.4 mg P kg⁻¹).

Disease incidence and weed populations. The area under disease progress curve (AUDPC) for early blight (*Alternaria* spp.; McCarty et al. 2012b) on tomato indicated no differences among treatments in 2011 or 2012 (Fig 3). Limited southern stem blight (*S. rolfsii*) occurrence was noted in the field during both seasons with 3 to 5 incidences in each year in blocks 2 and 4, with no observable relationship to treatment. No visible galling by root-knot nematode was observed on any of the root systems examined from the field in 2011 or 2012 (data not shown).

Assessment of nematodes extracted from soil in 2012 indicated no root-knot nematodes present in any plots of the study (data not shown). Root condition ratings were in general consistent among treatments with limited discoloration and ratings consistently near 1 indicating healthy root systems (data not shown).

Differences among treatments were observed for populations of *R. solani* colonizing toothpicks placed in the soil at planting (Table 10). In 2011, cereal rye + molasses, molasses, and mustard/arugula + molasses treatments had a greater number of propagules g⁻¹ soil than the cereal rye and biofumigant control treatments. In 2012, cereal rye + molasses also had the highest propagules g⁻¹ soil, which was greater than cereal rye, mustard/arugula, mustard/arugula + molasses, molasses, and the biofumigant control. Between 2011 and 2012 seasons, a decline in *R. solani* populations was observed in all treatments except for cereal rye, cereal rye + molasses, and the untreated control.

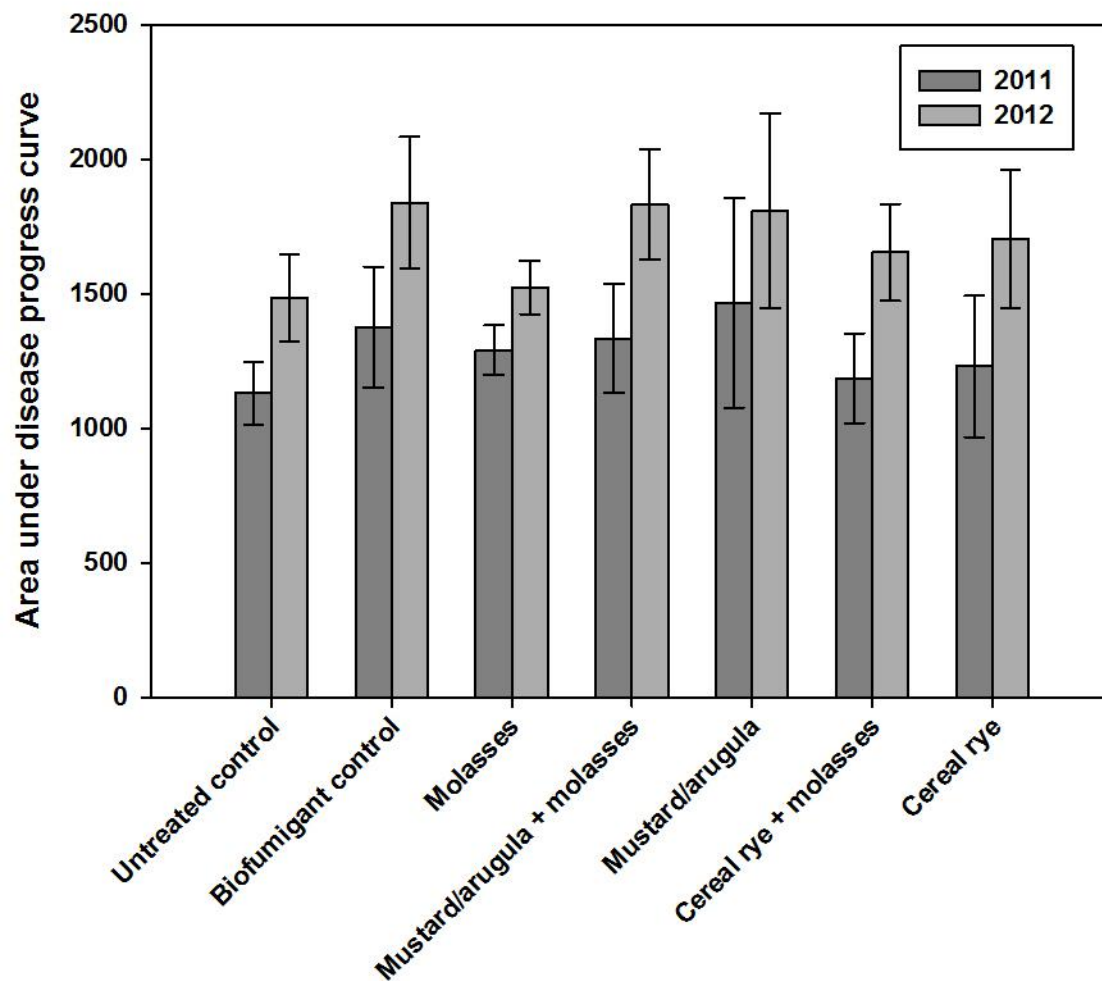


Figure 3. Area under disease progress curve for early blight of tomato (*Alternaria* spp.) from field study, summer 2011 and 2012. Error bars represent 95% confidence intervals.

Table 10. Population of *Rhizoctonia solani* colonizing birch toothpicks immediately following soil treatment from field study.

	<i>R. solani</i> (propagules g ⁻¹ soil)		Change (%) in <i>R. solani</i> populations in soil from 2011 to 2012
	-----2011-----	-----2012-----	
Untreated control	38.2ab [†]	50.3ab	+ 31.7
Biofumigant control	15.4b	13.8c	– 10.4
Molasses	60.8a	36.9bc	– 39.3
Mustard/arugula + molasses	55.4a	29.3bc	– 47.1
Mustard/arugula	34.0ab	20.1c	– 40.9
Cereal rye + molasses	52.6a	66.7a	+ 26.8
Cereal rye	15.5b	35.4bc	+ 128.4

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Numbers of monocot weeds emerging from planting holes in 2011 were greatest in the molasses treatment and the untreated control, which were higher than that observed from the mustard/arugula, mustard/arugula + molasses, and cereal rye + molasses treatments (Fig 4). Monocot weed populations were in general low (< 1.5 weeds m^{-1} bed length). Dicot weed populations did not differ among treatments in 2011 (Fig 5). In 2012, neither monocot nor dicot weed populations differed among treatments.

Crop performance. In 2011, height of tomato and bell pepper plants at flowering and first-harvest did not differ among treatments (data not shown). First-harvest bell pepper stem diameter was generally similar among treatments, although the cereal rye treatment had a lower stem diameter than all other treatments except for the untreated control. In 2012, there were no differences in height at flowering or at first-harvest for tomato plants. Likewise, no differences in tomato stem diameter were observed among treatments at first-harvest. At flowering, heights of bell peppers were greatest for the mustard/arugula (18.1 cm) and the untreated control (17.9 cm) treatments, which were greater than the biofumigant control (13.8 cm) and cereal rye treatments (13.4 cm). Bell pepper stem diameter at flowering did not differ among treatments in 2012. At first-harvest, there were no differences among treatments for height or stem diameter of bell peppers. In 2011, total tomato yields (marketable + cull yields) ranged from 83,214 kg ha^{-1} for cereal rye to 98,944 kg ha^{-1} for the molasses treatment but did not differ significantly among treatments (Fig. 6). Total marketable fruit (USDA No.1, 2, and 3) also did not differ among treatments, ranging from 40,864 kg ha^{-1} for cereal rye to 52,665 kg ha^{-1} for mustard/arugula + molasses. When US No.1 and 2 were further analyzed by the largest size classes (large, extra-large, and jumbo), mustard/arugula + molasses, molasses, mustard/arugula, and the untreated

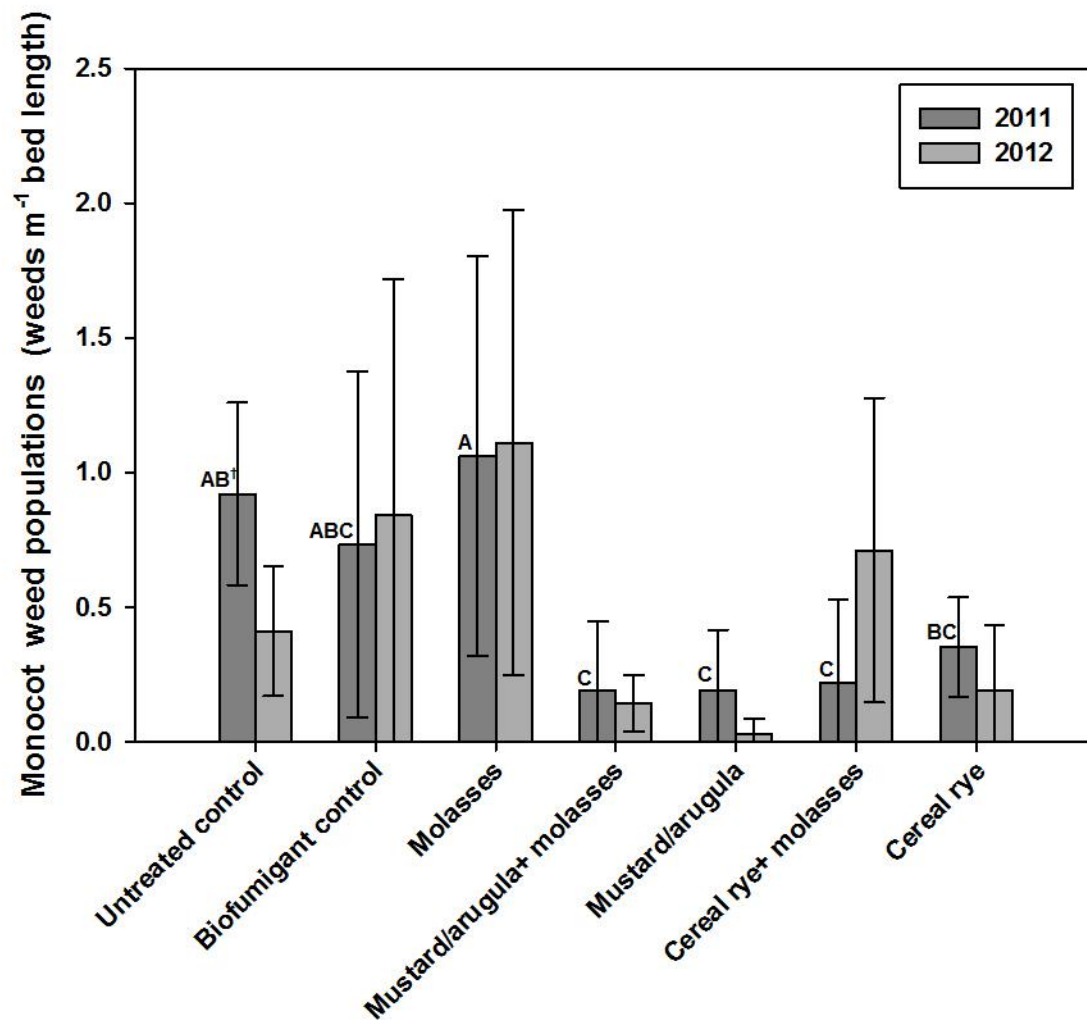


Figure 4. Mean monocot weed populations, 2011 and 2012. [†]Within year, means indicated by different letters are significantly different, $p < 0.05$. Error bars represent 95% confidence intervals.

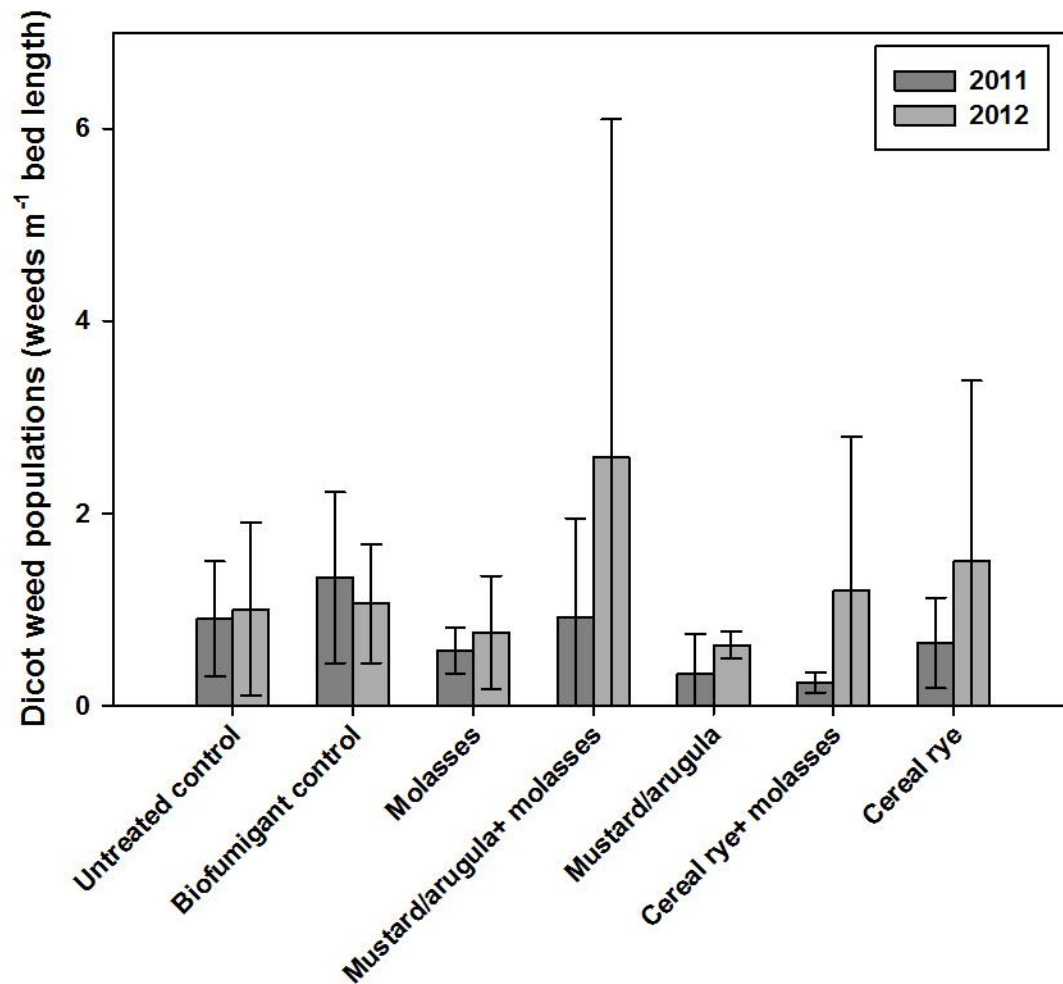


Figure 5. Mean dicot weed populations, 2011 and 2012. Error bars represent 95% confidence intervals.

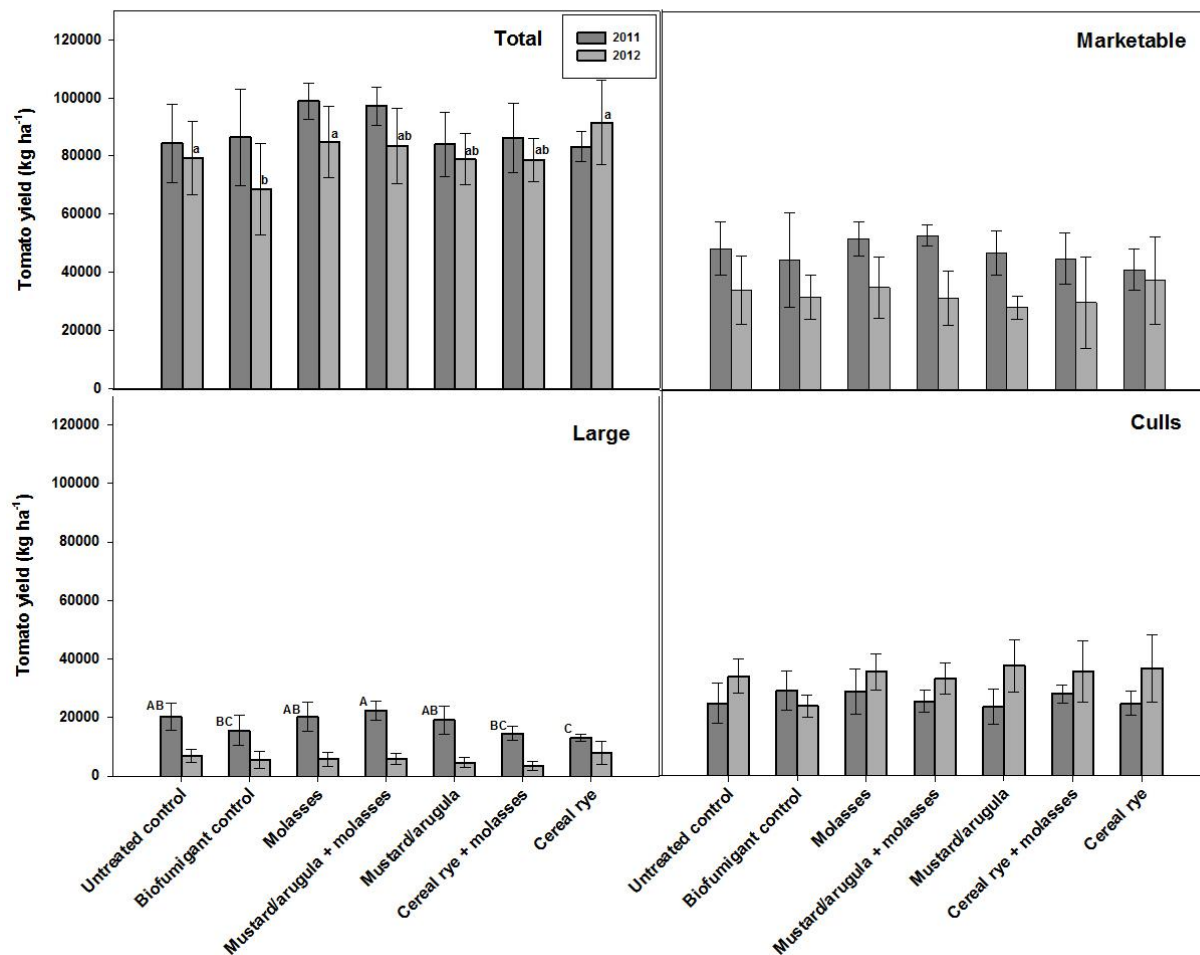


Figure 6. Mean total tomato yield (2011 and 2012), total marketable yields, large tomato yield, and culled tomato yield. † Within yield class and year, means indicated by different letters are significantly different, $p < 0.05$. Error bars represent 95% confidence intervals.

control treatments all had the greatest yields of fruit in these larger classes, which differed from the cereal rye treatment. There were no differences in culled tomato fruit weights among treatments. The percentage of culls ranged between 32% for mustard/arugula + molasses to 39% for the untreated control.

In 2012, total tomato yield was greatest from the untreated control, molasses, and cereal rye treatments, which were significantly greater than the biofumigant control, which had the lowest yield (Fig. 6). Total marketable yields did not differ among treatments, which ranged between 29,731 kg ha⁻¹ for cereal rye + molasses treatment to 37,336 kg ha⁻¹ for the cereal rye treatment. There were also no differences in large fruit yields among treatment, which ranged between 5,612 kg ha⁻¹ for the biofumigant control to 7,815 kg ha⁻¹ for the cereal rye treatment. Culled tomato fruit weights were higher in 2012 compared to 2011 ranging between 23,552 kg ha⁻¹ (43% of total yields) for the biofumigant control to 37,197 kg ha⁻¹ (57% of total yields) for the mustard/arugula treatment. Culls for both 2011 and 2012 were typically due to blossom end rot, insect damage, cracking, and zippering.

There were no differences observed in tomato fruit pH or titratable acidity in 2011 (data not shown). There was a difference among treatments in soluble solids content with all treatments (ranging from 3.99 to 4.10%), which was greater greater than the untreated control (3.77%). In 2012, there were no differences among treatments for soluble solids content, fruit pH, or titratable acidity (data not shown). In 2011, there were no differences between treatments for total bell pepper yields, which ranged from 29,815 kg ha⁻¹ for cereal rye to 41,483 kg ha⁻¹ for mustard/arugula (Fig. 7). Similarly, there were no differences among treatments in total marketable yields or culled fruit weights. Total marketable (USDA No. 1, No. 2, and fancy)

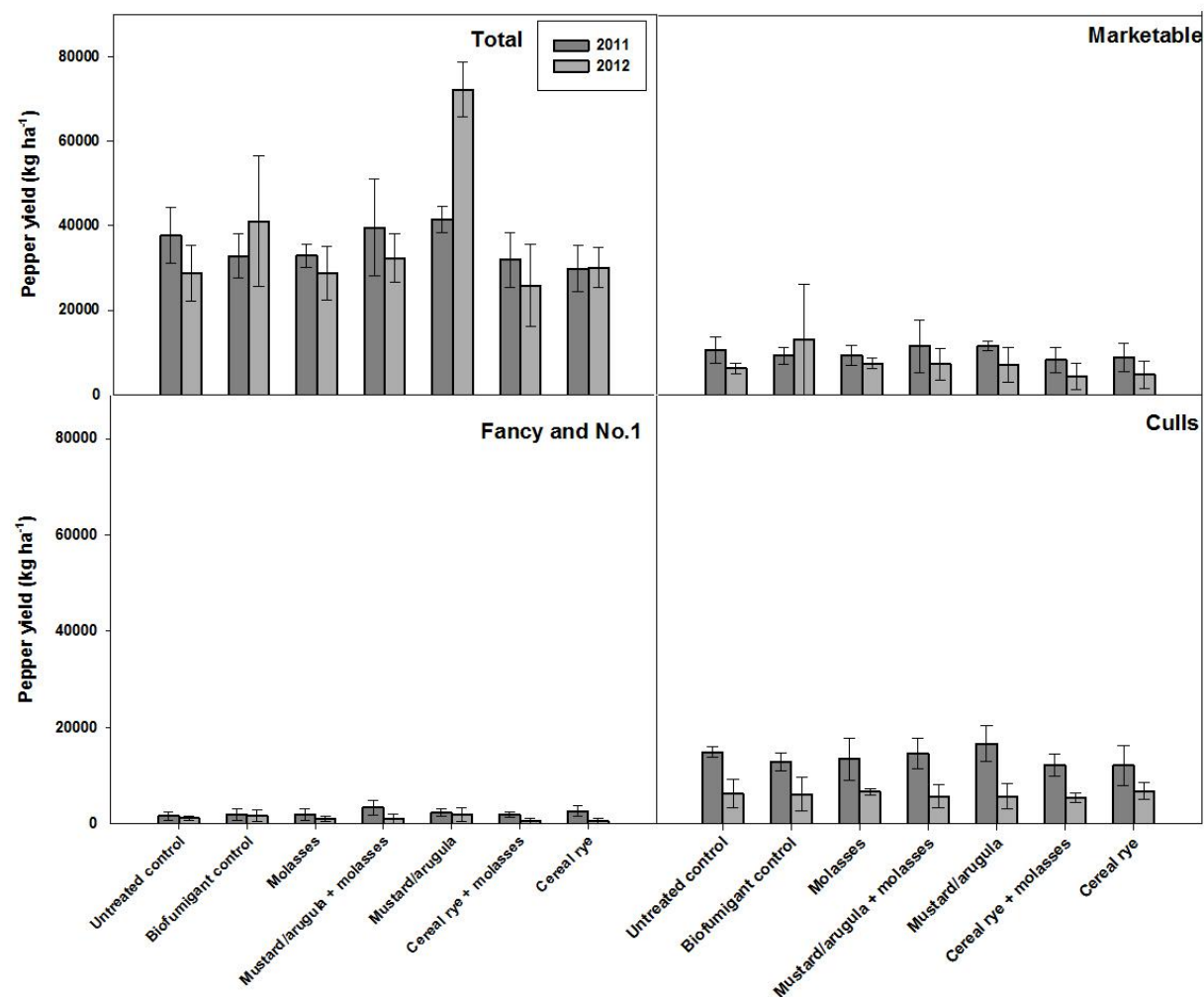


Figure 7. Mean total bell pepper yield (2011 and 2012), total marketable yields, and culled bell pepper yields. Error bars represent 95% confidence intervals.

yields ranged from 8,060 kg ha⁻¹ for cereal rye to 11,367 kg ha⁻¹ for the mustard/arugula treatment. In 2012, there were also no significant differences among treatments in total yields, marketable yields, or culled fruit weights, although a great deal of within treatment variability was observed. Total bell pepper yields ranged from 25,787 kg ha⁻¹ for cereal rye + molasses to 41,036 kg ha⁻¹ for the biofumigant control. Marketable fruit yields ranged between 4,202 kg ha⁻¹ for cereal rye + molasses to 12,869 kg ha⁻¹ for the biofumigant control. Generally, 2011 yields were greater than 2012 for total, marketable, and culled fruit. Culled fruit were generally a result of blossom end rot, sunscald, or insect damage.

In 2011, bell pepper fruit pH did not differ among treatments, ranging between 5.33 for the molasses treatment to 5.46 for the mustard/arugula treatment (data not shown). Soluble solids content also did not differ significantly among treatments ranging between 4.40% in the untreated control and cereal rye + molasses to 5.03% in the molasses treatment. In addition, titratable acidity did not differ among treatments ranging between 0.050 g citric acid 100 mL⁻¹ in mustard/arugula + molasses and mustard/arugula treatments to 0.066 g citric acid 100 mL⁻¹ in the molasses treatment. There were no differences observed in pericarp thickness (6.0 to 6.7 mm), pepper fruit length (81.1 to 90.7 mm), or pepper fruit diameter (84.2 to 88.2 mm). In 2012, fruit pH values had a lower range, between 4.96 in the cereal rye + molasses treatment to 5.07 in the biofumigant control and mustard/arugula treatments. Soluble solid contents were generally lower in 2012 than 2011. While no differences were observed among treatments, soluble solids content ranged between 3.11% in the cereal rye treatment to 3.41% in the mustard/arugula treatment. Titratable acidity was generally higher in 2012 than 2011. While no differences were observed among treatments, titratable acidity ranged between 0.09 g citric acid 100 mL⁻¹ in the untreated control to 0.14 g citric acid 100 mL⁻¹ in the molasses treatment. There were no differences in

pericarp thickness (6.1 to 7.4 mm), pepper fruit length (69.2 to 81.1 mm), or pepper fruit diameter (47.8 to 67.3 mm).

Leaf tissue N content of tomato and bell pepper did not differ among treatments in 2011 and 2012 (data not shown). In 2011, tomato leaf N concentration ranged from 4.5 to 5.0 cg N g^{-1} for the biofumigant control and mustard/arugula treatments, respectively. In 2012, tomato leaf N concentration was between 6.1 cg N g^{-1} and 6.4 cg N g^{-1} for all treatments except the untreated control at 8.1 cg N g^{-1} . In 2011, pepper leaf N ranged between 3.62 cg N g^{-1} in the molasses treatment to 4.59 cg N g^{-1} in the mustard/arugula treatment. In 2012, bell pepper leaf N concentration ranged between 4.2 cg N g^{-1} for the untreated control to 4.7 cg N g^{-1} for the mustard/arugula + molasses treatment.

Discussion

The anaerobic soil conditions that were accumulated during the ASD treatments in this study were similar to those reported in previous ASD studies. Butler et al. (2012a) reported accumulation of anaerobic conditions (mVh) values for a molasses ASD treatment below 5,000 mVh and above 30,000 mVh in years 1 and 2 of a Florida field study. Compared to our molasses treatment, this was similar for 2011 with 16,079 mVh but different for the second year of our study where 50,753 mVh were observed on average. The increase in anaerobic activity may have been influenced by adaption of the soil microbial community to large inputs of labile C as well as to the temporally-variable reducing conditions in the soil. In California, Shennan et al. (2010) reported that a threshold of 50,000 mVh at 25°C soil temperature is necessary for control of *V. dahliae*. While this threshold is linked to the suppression only of *V. dahliae* at this time, it is an important starting point to gauge the effectiveness of ASD treatments for pathogen control. In

2011, treatments in our study did not reach this threshold, while the untreated control, mustard/arugula, cereal rye + molasses, and molasses treatments in 2012 were all above this threshold. However, mean soil temperatures during ASD treatment in 2011 and 2012 were slightly below 25°C in all treatments.

In a Netherlands study, anaerobic soil conditions were reported as mV only (without cumulative values) from a study in which treatments of broccoli residue amendment led to a soil redox potential of -193 mV by day 48 whereas the lowest redox potential of a nonamended treatment was 110 mV (Blok et al. 2000). In 2011, the lowest mean redox potential value in our study was -148 mV in the cereal rye + molasses treatment while in 2012, the lowest mean redox value was -134 mV in the cereal rye + molasses treatment. An additional study by Blok et al. (2008) reported that ASD treatments consistently had redox potential values near -200 mV following the addition of fresh grass amendment at 38, 56, or 93 Mg fresh biomass ha⁻¹.

Examining biomass produced by treatments containing a cover crop during 2012 compared to 2011, it could be expected that greater accumulation of anaerobic conditions would occur due to increased biomass available to support soil microbial growth and respiration. This is especially notable when we compare the mustard/arugula + molasses treatment where a biomass increase from 6,310 kg ha⁻¹ in 2011 to 19,705 kg ha⁻¹ in 2012 was observed. The influence of the increasing biomass of the treatments in 2011 and 2012 did not closely correlate to differences in cumulative mV h values. While increases were observed in cumulative mV h from 2011 to 2012, this was also the case for treatments without increased amendment rates in 2012 (i.e., untreated and biofumigant controls, molasses treatment). These results are in contrast to Butler and colleagues (2012a) who reported that all C sources used in a greenhouse pot study (molasses, various warm-season cover crop residues) contributed to a cumulative mVh that was greater than

the untreated control under ASD treatment. The difference may be attributable to the sandy soil type utilized in the Butler et al. study, which likely had less labile C available to support soil microbial activity without additional C amendment.

In an ASD pot study in Florida, a molasses treatment was reported to accumulate nearly 120,000 mVh while all other C source treatments were above a no C source control (fallow without added C source) of 80,000 mVh (Butler et al. 2012a). Only the cereal rye + molasses treatment in 2012 came close to reaching the molasses treatment from this pot study while all treatments in 2011 were below the untreated control and molasses treatment. Differences in soil moisture, soil properties, temperature, and C source rates and properties (i.e. C:N ratio) all likely affected the accumulation of anaerobic conditions. Carbon sources added in the study by Butler and colleagues ranged between 0.42 mg C g soil⁻¹ for the sunn hemp residue amended treatment to 2.65 mg C g soil⁻¹ in the molasses treatment (2012a). This compares to our treatments, which ranged between 0.29 mg C g soil⁻¹ in the untreated control to 2.3 mg C g soil⁻¹ for the cereal rye + molasses treatment in 2011 and 0.29 mg C g soil⁻¹ in the untreated control to 4.2 mg C g soil⁻¹ in the mustard/arugula + molasses treatment in 2012. The C:N ratio for our treatments ranged between 3.1 in the untreated control to 14.4 in molasses treatment in 2011 while 3.1 in the untreated control to 15.4 in the mustard/arugula + molasses treatment in 2012, which was the only treatment near the range of 14.7 to 57.9 reported by Butler and colleagues for sunn hemp and sorghum-sudangrass amended treatments, respectively. Our treatment amendments in both years generally were at the lower end or below the C:N ratios reported for the Florida study. Gravimetric soil moisture was similar in the Florida pot study post treatment (0.19 to 0.20 g g⁻¹) to that observed in both years of our study.

A decline in soil pH following ASD soil treatment has been reported to be an indicator of whether organic acids are being added to the soil solution through the anaerobic decomposition of the added C amendments (Momma 2008). In 2011, there were generally few differences in soil pH comparing soil samples taken prior to treatment and immediately following soil treatments. This is counter to 2012 where declines in soil pH_{water} values were observed post treatment for most treatments. Given that greater biomass was added in 2012 than in 2011, this may indicate a relationship between an increase in biomass and a decline in pH resulting from the greater contribution of organic acids. Of the treatments without composted poultry litter in the Florida pot study, low soil pH values were reported for all treatments compared to the control without added C amendment (Butler et al. 2012a).

Total soil inorganic nitrogen was higher in 2011 at post-treatment compared to 2012 while the nitrogen concentration in tomato and bell pepper leaf tissue was higher in 2012 than 2011. This would not necessarily be expected since more readily available soil N would be expected to result in greater plant uptake in 2011 compared to 2012. Foliar disease pressure in 2012 could have inhibited crop biomass production and growth, with less N uptake leading to greater availability of soil N. The differences among treatments for total inorganic N post-treatment 2011 and 2012 did not seem to be closely related to yield of either tomato or bell pepper. Visual symptoms of N deficiency were not observed among field treatments, with the exception of bell pepper late in the 2012 season in the molasses treatment. This is mirrored in the low soil N values at harvest for this treatment in 2012, suggesting that soil N limitation may be an issue that should be addressed when molasses is used for ASD treatments.

The mustard/arugula, mustard/arugula + molasses, and biofumigant control all could have influenced disease severity based on the biofumigant (i.e. isothiocyanates produced from the

degradation of glucosinolates) potential in each of these treatments. Declines in populations of *R. solani* between 2011 and 2012 were greatest in the mustard/arugula + molasses and mustard/arugula treatments, which both had a large increase in C inputs in the second year of our study. Statistically, in both 2011 and 2012 cumulative mV h did not explain the differences in *R. solani* populations, which is emphasized by the observation that the untreated control, cereal rye, and cereal rye + molasses treatments had large increases in cumulative eH but also increases in *R. solani* populations. Several previous studies have indicated the potential of Indian mustard to control *R. solani* and other fungal diseases (Charron and Sams 1999; Friberg et al. 2009; Larkin and Griffin 2007).

Previous ASD studies have indicated potential in controlling *R. solanacearum* with wheat bran (10.3 mg C g soil⁻¹), potato haulms (*Solanum tuberosum*; 15.4 mg C g soil⁻¹) and grass (species not given) while populations of *Fusarium redolens* f.sp. *asparagi*, *Rhizoctonia tuliparum*, and *V. dahliae* have all been reported to be negatively impacted by ASD treatment with C sources of broccoli (1.9 mg C g soil⁻¹) or grass residue (2.0 mg C g soil⁻¹; species not given; Blok et al. 2000; Messiha et al. 2007; Momma 2008). *Verticillium dahliae* was further reduced and occurrence decreased by Italian ryegrass amendment (estimated 0.88 to 3.00 mg C g soil⁻¹; Goud et al. 2004). A similar amount of grass residues (estimated 3.21 and 5.92 mg C g soil⁻¹) led to reported declines in buried inoculum of *Fusarium redolens* f.sp. *asparagi*, *Rhizoctonia tuliparum*, and *V. dahliae* at the soil depth of 80 cm (Blok et al. 2008). *R. solani* has also been reported to be controlled by the incorporation of *B. juncea* (2.33 to 3.49 Mg biomass ha⁻¹) in sugar beet production and mustard seed meal (3,050 kg ha⁻¹) in soybean production (Fayzalla et al. 2009; Motisi et al. 2009). Given that weed populations, disease incidence, and plant-parasitic nematode pressure were low in our study, evaluation of our treatments on a site

with greater pest populations could have indicated more differences among treatments than were observed.

Currently, yield data from previous ASD studies have only been reported preliminarily (Butler et al. 2012b; Butler et al. 2009; Shennan et al. 2010). While there were no significant differences in marketable yields or culls among treatments in our study, culled fruit percentages were generally much greater in 2012 for tomatoes, which might be related to the presence of *Alternaria* spp. and weather conditions. The percentages of cull weights were consistent in 2011 and 2012. Bell pepper yield trends from 2011 to 2012 were similar to the tomato yield trend as lower yields were observed in 2012 compared to 2011, likely due to extremely high temperatures (> 38°C) occurring for several days at tomato and bell pepper flowering in 2012. Total bell pepper yields for cereal rye + molasses, molasses, and the untreated control all were greater than the biofumigant control in 2012. These results were offset somewhat by a greater percentage of culls compared to marketable fruit compared to the biofumigant control.

The use of IRIS tubes in our study in 2012 proved a useful tool in examining anaerobic soil conditions over a greater soil volume than redox electrodes. While none of the treatments differed significantly, the percentage of Fe solubilized was highest for the cereal rye treatment, which was also the highest for accumulation of anaerobic conditions determined by redox potential electrodes. This trend however was not consistent for all treatments. Additional studies and the use of a greater number of IRIS tubes in each plot would be useful to determine if this technology can be successfully calibrated to relate to effectiveness of ASD treatment. Overall, accumulated anaerobic conditions tended to not influence monocot or dicot weed populations, tomato or bell pepper yields, or *R. solani* populations.

This study establishes the potential of the adoption of ASD in Tennessee. While few differences were observed among treatments, this study establishes management of ASD treatments in Tennessee that can contribute to high accumulations of anaerobic conditions, which do not result in yield reductions compared to an untreated control. However, more studies are needed to further evaluate the effectiveness of ASD treatments, particularly on sites with high soilborne plant pathogen or plant parasitic nematode pressure. Further, evaluation of ASD treatments in a controlled environment with introduced inoculum of soilborne plant pathogens and weed species would also be useful for determining the most promising ASD methods for use in field trials with commercial soil fumigants and grower demonstrations to allow growers to adapt the technology to their production systems.

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CHAPTER III

EVALUATION OF SOIL PROPERTIES AND MORTALITY OF INTRODUCED WEEDS AND SCLEROTIA OF *SCLEROTIUM ROLFSSII* DURING ANAEROBIC SOIL DISINFESTATION USING VARIOUS CARBON SOURCE AMENDMENTS

Abstract. Used as a non-chemical, pre-plant soil treatment, anaerobic soil disinfestation (ASD) has been developed to control soilborne plant pathogens, plant-parasitic nematodes, and weeds in specialty crop production systems. Control of these pests is achieved by incorporating a labile carbon (C) source, tarping with plastic, and irrigating the topsoil to saturation (~ 5 cm irrigation) which leads to anaerobic decomposition of the added C source. To assess the potential of cool-season cover crops and C source amendments in ASD, a greenhouse/growth chamber study was implemented in fall 2011 and repeated beginning in spring 2012. Treatments for this study were (1) a cereal rye (*Secale cereale*) cover crop (seeded at 134 kg ha⁻¹), (2) a commercial mixture of Indian mustard (*Brassica juncea*) and white mustard (*Sinapsis alba*) seeded in a 1:1 mixture with arugula (*Eruca sativa* cv. Nemat; seeded at 7.8 kg ha⁻¹), (3) fallow with dried molasses (5.6 Mg ha⁻¹), (4) fallow with mustard seed meal (2.24 Mg ha⁻¹), (5) cereal rye cover crop with a reduced rate of dry molasses (1.12 Mg ha⁻¹), (6) mustard and arugula cover crop mixture with a reduced rate of dry molasses (1.12 Mg ha⁻¹), (7) crimson clover (*Trifolium incarnatum*, seeding rate: 16.8 kg ha⁻¹), (8) hairy vetch (*Vicia villosa*, seeding rate: 24.6 kg ha⁻¹), (9) wheat (*Triticum aestivum* seeding rate: 134 kg ha⁻¹) and (10) a fallow, untreated control were established with four replicates. After 16 weeks of growth, cover crops were cut and incorporated into the soil and pots were reconstructed to include yellow nutsedge tubers (*Cyperus esculentus*; 200 tubers m⁻²), redroot pigweed seeds (*Amaranthus retroflexus*; 2000 seeds m⁻²), large crabgrass seeds (*Digitaria sanguinalis*; 2000 seeds m⁻²), and packets containing sclerotia of *Sclerotium rolfsii*. At the end of treatment, dwarf tomatoes (cv. Florida lanai) were planted to assess crop performance,

and weed germination was continually monitored. No differences were observed among treatments in the level of anaerobic conditions, weed germination, tomato plant health, or mortality of sclerotia of *S. rolfsii*, while the molasses as a C source tended to have similar results to other cover crop treatments. Rates of C added in amendments may have been too low for effective treatment. Additional trials are needed to assess the viability of these treatments in accumulating anaerobic conditions and managing pest pressure.

Introduction

Methyl bromide (MeBr) is a preplant soil fumigant that was historically used extensively in specialty crop systems to control weeds, plant-parasitic nematodes, and soilborne plant pathogens. Because of this broad-spectrum activity, many industries have come to depend on the use of this chemical including 88% of strawberry production in California, several warm-season vegetable production industries in the southeast, and winter vegetable production in Florida (Byrd et al. 2006; Carter et al. 2000; Haar et al. 2003; Noling and Becker 1994). However, this chemical is being phased out in the United States as part of the Montreal Protocol on Ozone Depleting Substances, ratified by the United States in 1988. Therefore, there exists a need for a suitable alternative to MeBr. While a great deal of effort and funding has been expended to develop and evaluate chemical alternatives to MeBr fumigation, there has not been a comparable effort to develop non-chemical alternatives. Non-chemical alternatives may be especially attractive to producers due to the level of regulatory oversight of chemical soil fumigants necessary to protect human health and environmental quality.

Anaerobic soil disinfestation (ASD) is considered a possible non-chemical alternative. Under ASD treatment, an easily decomposable carbon (C) source is incorporated into the soil prior to covering with plastic mulch and irrigating the soil to fill soil porosity. During a 2 to 5

week treatment period, a shift occurs from aerobic to anaerobic conditions leading to organic acid creation and production of volatile compounds during the decomposition of the added C source. The use of a cover crop as a C source for ASD treatment may provide additional beneficial pest control effects.

Previously, ASD treatment has shown potential for controlling several pest issues in specialty crop production, including Japan, the Netherlands, Florida, and California (Butler et al. 2012a; Butler et al. 2012b; Goud et al. 2004; Momma et al. 2006; Shennan et al. 2010). ASD treatment has also been used in greenhouse pot studies to determine the feasibility of pest management. For instance, in a greenhouse pot experiment examining different carbon sources in ASD treatment, yellow nutsedge (*Cyperus esculentus*) planted when cover crops were incorporated for ASD treatment showed that germination was low among carbon source treatments with liquid molasses having the lowest nutsedge germination and the control without an added carbon source having the highest germination (Butler et al. 2012a). Under ASD conditions in another pot experiment, yellow nutsedge germinated at shallow depths but not at deeper depths when tubers in mesh bags were planted and different carbon sources were added, a possible indication that resupply of oxygen at the soil surface during treatment acted as a limiting factor (Muramoto et al. 2008).

In general, cover crops impact (positively or negatively) pest problems through ecological interactions with plant-parasitic nematodes and soilborne plant pathogens (Snapp et al. 2005; Stivers-Young 1998). For ASD treatment, cover crops can also act as a labile C source to increase microbially-driven anaerobic soil conditions when chopped and incorporated into the soil. In addition, the differences among cover crops will likely influence the effectiveness of the ASD treatment. Important differences between legume, brassica, and grass species cover crops

include C:N ratio, lignin content, hemicelluloses, biomass, and other biochemical constituents (Creamer and Baldwin 2000; Snapp et al. 2005). The impact of the biomass and relative availability of C compounds is likely particularly important in ASD given that anaerobic conditions are largely driven by microbial growth and respiration, a process that is typically limited by availability of C to soil microbes. The importance of the C:N ratio of the added amendment has been examined in ASD treatments, but no effect was reported in a narrow range of C:N ratio from 13:1 to 22:1. In this case, endemic *V. dahliae* in the Netherlands was reduced by 85% in all treatments where a grass amendment with a C:N of either 13:1 or 22:1 was incorporated, compared to the unamended, uncovered control (Goud et al. 2004).

The use of cover crops in ASD for weed control has been examined previously. In a greenhouse pot experiment examining different C sources in ASD treatment, yellow nutsedge (*Cyperus esculentus*) planted when cover crops were incorporated for ASD treatment showed that germination was low among C source treatments with liquid molasses having the lowest nutsedge germination and the control without an added C source having the highest germination (Butler et al. 2012a). The results from this study demonstrate that many different C sources including the amendment of molasses and warm-season cover crop residues could be used effectively in ASD treatment. Under ASD conditions in another pot experiment, yellow nutsedge germinated at shallow depths but not at deeper depths when tubers in mesh bags were planted and different C sources were added, a possible indication that resupply of oxygen at the soil surface during treatment acted as a limiting factor (Muramoto et al. 2008).

While greenhouse studies of ASD have shown supporting data to recognize the importance of cover crops in ASD, further research is needed. Under controlled conditions, ASD treatments from the field research project described by McCarty et al. (2012a) were examined to

evaluate the control of weeds and soilborne plant pathogens that were either not present or were unevenly distributed in the location of the field study. This experiment also allowed for examination of relationships between soil properties, C input quality and quantity, soil microbial respiration, weed control, pathogen control, and crop performance in ASD-treated soils. Further, a better evaluation of the potential of ASD will offer needed information for optimizing ASD procedures for field implementation.

Our objectives were to (1) evaluate redox potential and soil properties for regionally-adapted cool-season cover crops and C source amendments for ASD treatment, (2) determine the potential for weed control of yellow nutsedge, redroot pigweed (*Amaranthus retroflexus*), and large crabgrass (*Digitaria sanguinalis*) during ASD treatment, and (3) determine mortality of introduced sclerotia of *Sclerotium rolfsii* during ASD treatment.

Materials and methods

The seven treatments from the field study reported by McCarty et al. (2012a) and three additional cover crop treatments were established in a completely randomized pot study on the campus of the University of Tennessee, Knoxville, TN. The soil type was a Dewey silt clay loam (fine, kaolinitic, thermic, typic Paleudult) collected from the East Tennessee Ag Research and Education Center in Knoxville, TN in summer 2011. For each pot, a soil mixture of a 1:1 ratio of field soil and fine sand was placed in pots 12.7 cm in height with a 124-cm² volume. Treatments included: (1) a cereal rye (*Secale cereale*) cover crop (Albert Lea Seed House, Albert Lea, MN, seeded at 134 kg ha⁻¹), (2) a commercial mixture ('Caliente 61', High Performance Seed, Moses Lake, WA) of Indian mustard (*Brassica juncea*) and white mustard (*Sinapsis alba*) seeded in a 1:1 mixture with arugula (*Eruca sativa* cv. Nemat, seeded at 7.8 kg ha⁻¹), (3) fallow with dried

molasses (Westway Feed, Tomball, TX; 5.6 Mg ha⁻¹) added prior to ASD treatment, (4) fallow with mustard seed meal (Wisconsin Spice, Berlin, WI; 2.24 Mg ha⁻¹) added prior to ASD treatment, (5) cereal rye cover crop with a reduced rate of dry molasses (1.12 Mg ha⁻¹) added prior to ASD treatment, (6) mustard and arugula cover crop mixture with a reduced rate of dry molasses (1.12 Mg ha⁻¹) added prior to ASD treatment, (7) crimson clover (*Trifolium incarnatum*, cv. Dixie, seeded at 16.8 kg ha⁻¹), (8) hairy vetch (*Vicia villosa*, cultivar unstated, seeded at 24.6 kg ha⁻¹), (9) wheat (*Triticum aestivum*, cultivar unstated, seeded at 134 kg ha⁻¹) and (10) a fallow, untreated control were established with four replicates and repeated. The first trial began in September 2011 and the second in January 2012. Blood meal (752 kg ha⁻¹; Miracle-Gro® Organic Choice®, The Scotts Company, Marysville, Ohio) was applied to all treatments to provide N for cover crop biomass production.

Following one month of growth in a greenhouse with temperatures maintained at 20 to 28 °C, pots were moved to a walk-in growth chamber (EGC Corporation, Chagrine Falls, Ohio). Temperature was maintained at 8°C at night and 12°C during the day with an 8-h day length for approximately one month. Afterwards, temperatures were increased to 24°C during the day and 15°C at night with a 12-h daylength for approximately 2 months to allow for biomass growth and cover crop maturity prior to incorporation. At incorporation, vegetation was chopped (< 5 cm) and sampled (~50 g), and soil samples collected (~50 cm³). Vegetation and additional soil amendments including feather meal to provide N as specified by treatment (Tables 1, 2) were incorporated into the soil and a sample of the soil and vegetation mixture collected (~50 cm³). The soil sample without biomass was oven-dried (105°C) to determine gravimetric moisture content and the remaining sample was air-dried and sieved (< 2 mm). Soil pH was determined on air-dried samples in deionized water (1:1; pH_{water}) and in 0.01-M CaCl₂ (1:2; pH_{CaCl2}) using a pH

electrode (Orion 3-Star Plus pH Benchtop Meter, Thermo Scientific, Waltham, MA). Soil samples were also analyzed for inorganic and total nitrogen (TN) and total C (TC). Briefly, 5 g of air-dried soil was extracted with 1-M KCl for 30 min on a reciprocating shaker, centrifuged, and filtered (Whatman 42, Whatman Ltd., Kent, UK) prior to colorimetric analysis for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N} + \text{NO}_2\text{-N}$ using a microplate spectrophotometer (Powerwave XS, Biotek, Winooski, VT) as described by Sims et al. (1995). Extractable soil phosphorus was determined using a malachite green microplate method (D'Angelo et al. 2001) following extraction of soil as described by Mehlich (1953). Both air-dried and ground soil samples and vegetation subsamples were analyzed by flash combustion for TN and TC (Flash EA 1112 NC Soil Analyzer, Thermo Scientific, Waltham, MA, USA).

Soil samples that contained added biomass were analyzed for water soluble C (WSC) and hot water extractable C (HWEC) as measures of readily-available C (Curtin et al. 2006; Ghani et al. 2003). Both methods extracted 3 g of moist soil containing the incorporated amendments with 30 mL of distilled water. Samples of WSC were shaken for 30 minutes and then centrifuged (3500 rpm for 15 min) and filtered (Whatman 42). Samples of HWEC were extracted for 16 h at 80°C in a hot water bath, then filtered (Whatman 42). Total organic C in extracts was determined using a total organic carbon analyzer (Shimadzu, Kyoto, Japan).

As pots were re-filled, pigweed (25 seeds pot^{-1} /2000 seeds m^{-2}) and large crabgrass seeds (25 seeds pot^{-1} /2000 seeds m^{-2}) were placed in the surface 1-cm while yellow nutsedge tubers (5 tubers pot^{-1} /200 tubers m^{-2}) were placed in the surface 2-cm of soil. Two mesh bags containing 10 sclerotia each of *Sclerotium rolfsii* were buried at 5-cm and at 15-cm depths to assess survival during treatment. The isolate of *S. rolfsii* was collected from a 2005 hybrid field tomato project at East Tennessee Ag Research Education Center, Knoxville, TN. *S. rolfsii* were grown on petri

dishes (100 mm x 15 mm) using potato dextrose agar (PDA, Fisher Scientific, Pittsburgh, PA). Following 7 days, plugs were transferred to the center of new PDA plates. Using these new PDA plates, cultures were grown for an additional 7 days at room temperature to allow for mycelium growth then transferred into a cold room at 3.3°C for 4 hours. Plates were returned to room temperature and kept in the dark to form mature sclerotia over a 4 to 6 week period. Sclerotia were then harvested and dried overnight under a laminar flow hood, then stored in a sterile glass vial until use in the mesh bags.

One oxidation-reduction electrode per pot and one soil temperature/volumetric moisture probe per treatment were inserted to a 15-cm soil depth (Combination ORP Electrode, Sensorex Corp., Garden Grove, CA and 5TM Soil Moisture Probe, Decagon Devices, Pullman, WA). Soil temperature, redox potential, and volumetric soil moisture were continually monitored, averaged, and recorded hourly during treatment using an automatic datalogging system (CR1000 with AM 16/32 multiplexers, Campbell Scientific, Logan, UT). Irrigation (600 mL) was added to each pot to saturate the soil and begin the ASD treatment period. Immediately after irrigation, black plastic mulch (32-µm embossed, Pliant-Berry Plastics, Evansville, IN) was placed onto each pot and secured with a heavy duty rubber band (January 26, 2012; May 15, 2012).

At the end of treatment, mesh bags containing sclerotia of *S. rolfsii* were removed and assessed according to the methods described by Rodriguez-Kabana et al. (1980). Soil samples were taken from each pot and analyzed as previously described. Post-treatment emergence of weeds was also noted and germinated weeds were removed from the pots. Dwarf tomato plants (cv. Florida lanai) were grown in each pot in order to monitor plant growth and incidence of disease. Weed emergence was recorded on a weekly basis. Height and stem diameter (mm) of tomato plants was measured at three and six weeks following planting. Foliar disease pressure

was evaluated weekly and ratings used to calculate an area under disease progress curve for each pot (AUDPC). Tomatoes were harvested weekly when ripe and weighed and number of fruit recorded. At the end of an eight-week tomato growth period, aboveground biomass from each tomato plant was removed, oven-dried at 65°C for 48 hours, and weighed. Total N of tomato biomass was analyzed by combustion as previously described. Wet root biomass was removed, weighed, and root condition evaluated. A final soil sample was collected and analyzed as previously described. Pots were also deconstructed and ungerminated nutsedge tubers were retrieved and visually assessed for viability based on state of decomposition.

A separate repeated assay was conducted in fall 2012 to examine soil pH changes over the course of ASD treatment. The molasses treatment was used as the model ASD treatment system for evaluation to determine changes in soil pH occurring over the course of ASD treatment in relation to soil redox potential. In a growth chamber maintained at 15 to 24° C, 20 pots for each trial were established as described previously and amended with molasses and feather meal as described for the molasses treatment previously (Table 1). At days 0, 1, 3, 10, 17, and 30, four random pots were removed and 40 to 45 g of soil sampled in a centrifuge tube. Tubes were centrifuged for 30 minutes at 5,000 rpm, and pH determined by inserting a pH electrode in the supernatant. Measures of accumulation of anaerobic conditions up to the time of pot destruction were used to assess the relationship between soil pH, time, and anaerobic conditions.

Results were analyzed using the GLM procedure in SAS 9.2 (SAS Institute Inc, Cary, NC). Main effects and differences between means were considered significant at $p < 0.05$, unless otherwise noted. Protected means were separated using Fisher's least significant difference procedure.

Results.

Amendment biomass, carbon, and nitrogen. Amendment biomass added in trial 1 ranged between 1,120 kg ha⁻¹ in the untreated control to 6,238 kg ha⁻¹ in the molasses treatment (Table 11). Among cover crop treatments, biomass inputs ranged from 5,620 kg ha⁻¹ in the hairy vetch treatment to 7,250 kg ha⁻¹ in the mustard/arugula + molasses treatment. In trial 2, cover crop treatment biomass ranged between 4,420 kg ha⁻¹ in the cereal rye treatment to 13,120 kg ha⁻¹ in the hairy vetch treatment (Table 12). In trial 2, hairy vetch, crimson clover, and mustard/arugula all produced a greater amount of biomass than trial 1. Total C added by amendments in trial 1 ranged between 560 kg C ha⁻¹ (0.29 mg C g⁻¹ soil) in the untreated control to 2,938 kg C ha⁻¹ (1.21 mg C g⁻¹ soil) in the cereal rye treatment. In trial 2, C inputs ranged between 1,910 kg C ha⁻¹ (0.98 mg C g⁻¹ soil) in the cereal rye treatment to 5,300 kg C ha⁻¹ (2.72 mg C g⁻¹ soil) in the hairy vetch treatment.

In trial 1, total N in cover crop amendments ranged between 250 kg N ha⁻¹ cereal rye + molasses treatment to 404 kg N ha⁻¹ in the hairy vetch treatment. In trial 2, total N in cover crop amendments ranged from 241 kg N ha⁻¹ in the wheat treatment to 755 kg N ha⁻¹ in the hairy vetch treatment. Total N content was lowest in the biofumigant control, molasses, and the untreated control treatments. The C:N ratio of amendments was lowest for the untreated control and biofumigant control (3.1 and 4.2, respectively). Cover crop amendment C:N ratios in trial 1

Table 11. Total biomass, carbon and nitrogen content, and C:N ratio of soil amendments and aboveground cover crop biomass in growth chamber study, Trial 1.

-----Trial 1-----					
	Biomass	TC	TN	TC	C:N
	-----kg ha ⁻¹ -----			mg C g ⁻¹ soil	ratio
Untreated control[†]	1,120	560	179	0.29	3.1
Biof. control	2,240	937	114	0.50	4.2
Molasses	6,238	2,335	164	1.18	14.4
Molasses	5,600	2,016	62	1.03	32.5
Feather meal	638	319	102	0.16	3.1
Must./aru. + mol.	7,250	2,757	304.3	1.4	9.1
Must/aru	5,100	1,836	127.5	0.94	14.4
Molasses	1,120	406	12	0.21	33.8
Feather meal	1,030	515	164.8	0.26	3.1
Mustard/arugula	7,620	3,429	374	1.49	7.8
Mustard/arugula	6,500	2,340	195	1.2	12
Feather meal	1,120	560	179	0.29	3.1
Cereal rye + mol.	6,750	2,807	250.4	1.44	11
Cereal rye	4,600	1,886	73.6	0.97	25.6
Molasses	1,120	406	12	0.21	33.8
Feather meal	1,030	515	164.8	0.26	3.1
Cereal rye	6,920	2,938	289	1.51	10.2
Cereal rye	5,800	2,378	110	1.21	21.6
Feather meal	1,120	560	179	0.29	3.1
Crimson Clover	4,620	1,925	319	0.99	6.0
Crimson Clover	3,500	1,365	140	0.7	9.8
Feather meal	1,120	560	179	0.29	3.1
Hairy vetch	5,620	2,450	404	1.26	6.0
Hairy Vetch	4,500	1,890	225	0.97	8.4
Feather meal	1,120	560	179	0.29	3.1
Wheat	5,720	2,400	289.4	1.23	8.3
Wheat	4,600	1,840	110.4	0.94	16.7
Feather meal	1,120	560	179	0.29	3.1

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$..

Table 12. Total biomass, carbon and nitrogen content, and C:N ratio of soil amendments and aboveground cover crop biomass in growth chamber study, Trial 2.

-----Trial 2-----					
	Biomass	TC	TN	TC	C:N
	-----kg ha ⁻¹ -----			mg C g ⁻¹ soil	ratio
Untreated control[†]	1,120	560	179	0.29	3.1
Biof. control	2,240	937	114	0.50	4.2
Molasses	6,238	2,335	164	1.18	14.4
Molasses	5,600	2,016	62	1.03	32.5
Feather meal	638	319	102	0.16	3.1
Must./aru. + mol.	6,650	2,561	319	1.3	8.0
Must/aru	4,500	1,640	142.2	0.84	11.5
Molasses	1,120	406	12	0.21	33.8
Feather meal	1,030	515	164.8	0.26	3.1
Mustard/arugula	7,820	3,072	373	1.58	8.2
Mustard/arugula	6,700	2,512	194	1.29	12.9
Feather meal	1,120	560	179	0.29	3.1
Cereal rye + mol.	6,250	2,598	258.8	1.33	10
Cereal rye	4,100	1,677	82.0	0.86	20.5
Molasses	1,120	406	12	0.21	33.8
Feather meal	1,030	515	164.8	0.26	3.1
Cereal rye	4,420	1,910	245	0.98	7.8
Cereal rye	3,300	1,350	66	0.69	12.3
Feather meal	1,120	560	179	0.29	3.1
Crimson Clover	6,920	2,723	399.4	1.40	6.8
Crimson Clover	5,800	2,163	220.4	1.11	9.8
Feather meal	1,120	560	179	0.29	3.1
Hairy vetch	13,120	5,300	755	2.72	7.0
Hairy Vetch	12,000	4,740	576	2.43	8.2
Feather meal	1,120	560	179	0.29	3.1
Wheat	5,020	2,150	241	1.11	8.9
Wheat	3,900	1,590	62	0.82	14.5
Feather meal	1,120	560	179	0.29	3.1

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$..

ranged between 6.0 in the hairy vetch and crimson clover treatments to 11.0 in the cereal rye + molasses treatment. In trial 2, the C:N ratio of amendments ranged between 6.8 in the crimson clover treatment to 10.0 in the cereal rye + molasses treatment.

Data indicated a generally positive response of soil redox potential and accumulation of anaerobic conditions to the addition of C sources (Fig 8). The cereal rye + molasses treatment with 116,947 mV h and crimson clover treatment with 84,290 mV h were greater than the untreated control, biofumigant control, and molasses treatments. Accumulation of anaerobic conditions was not recorded in trial 2 due to a datalogger error.

Soil properties. Mean soil temperatures did not differ among treatments in either trial (data not shown). Trial 1 had a range of mean soil temperatures between 20.7°C for the biofumigant control to 21.3°C for the hairy vetch treatment. In trial 2, slightly lower mean soil temperatures were observed than trial 1, with a range between 18.3°C for the mustard/arugula + molasses to 19.1°C for the cereal rye + molasses treatment. During treatment soil volumetric moisture ranged between 0.14 cm³ cm⁻³ (Table 13) in the biofumigant control (fallow, no cover crop) to 0.24 cm³ cm⁻³ in the molasses treatment in trial 1. In trial 1, post treatment gravimetric soil moisture did not differ among treatment ranging between 0.12 g g⁻¹ in the mustard/arugula treatment to 0.17 g g⁻¹ in the cereal rye + molasses treatments. In general, gravimetric soil moisture during trial 2 was slightly higher than trial 1, ranging between 0.17 g g⁻¹ in the untreated control to 0.28 g g⁻¹ in the cereal rye treatment. Gravimetric soil moisture post treatment in trial 2 showed cereal rye having the greatest soil moisture content at 0.17 g g⁻¹, which was greater than that observed from the untreated control, biofumigant control, hairy vetch, molasses, and cereal rye + molasses treatments.

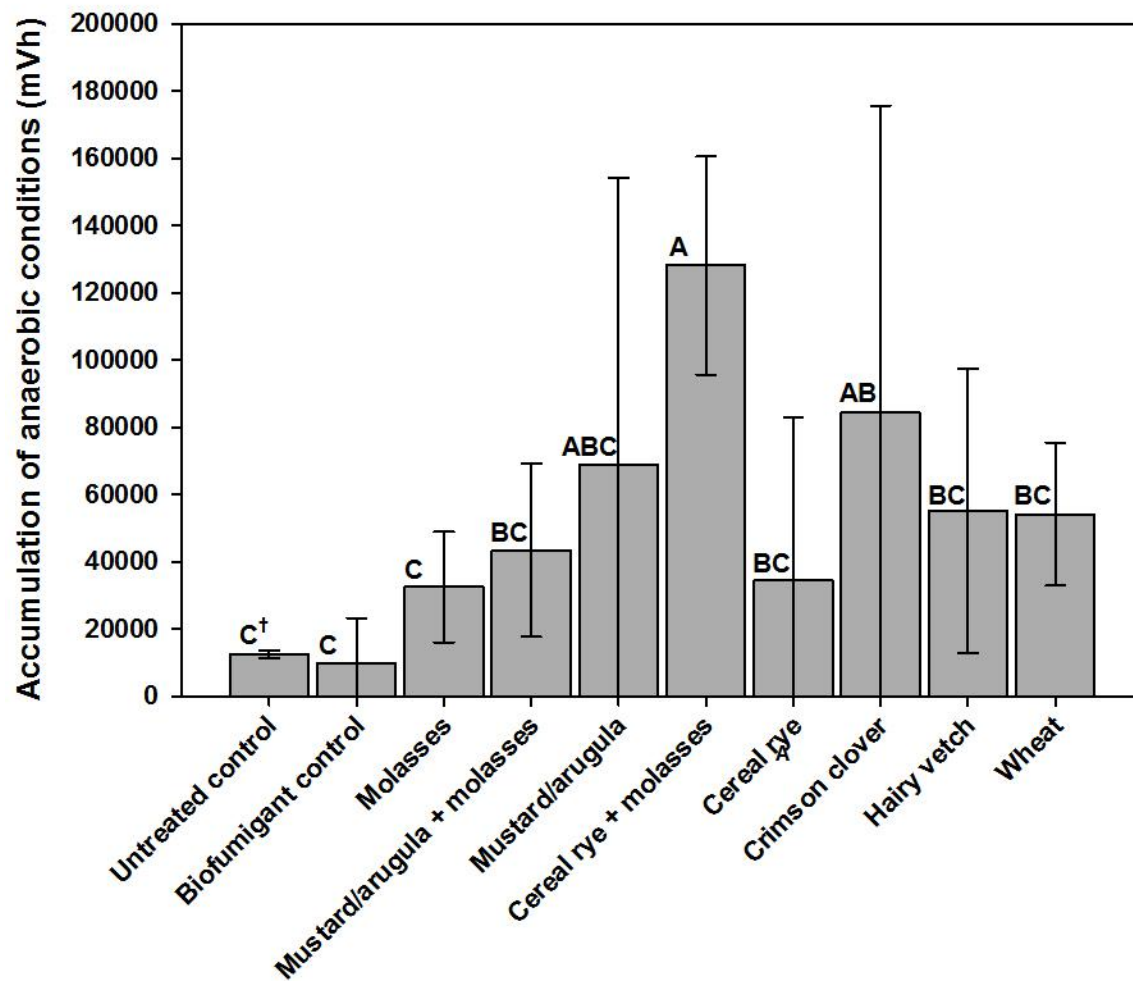


Figure 8. Accumulation of anaerobic conditions (mVh) during soil treatment of growth chamber study, Trial 1. [†] Means indicated by different letters are significantly different, $p < 0.05$. Error bars represent 95% confidence intervals.

Table 13. Mean volumetric soil moisture during treatment and gravimetric soil moisture post treatment from growth chamber study.

	During Treatment ^a	Post Treatment ^b	During Treatment	Post Treatment
	-----Trial 1-----		-----Trial 2-----	
	-----cm ³ cm ⁻³ -----	-----g g ⁻¹ -----	-----cm ³ cm ⁻³ -----	-----g g ⁻¹ -----
Untreated control	0.20 [†]	0.14	0.17	0.13c
Biofumigant control	0.14	0.13	0.19	0.13c
Molasses	0.24	0.16	0.27	0.15bc
Mustard/arugula + molasses	0.19	0.16	0.26	0.16ab
Mustard/arugula	0.20	0.12	0.26	0.15ab
Cereal rye + molasses	0.22	0.17	0.21	0.15b
Cereal rye	0.22	0.15	0.28	0.17a
Crimson clover	0.18	0.13	0.24	0.16ab
Hairy vetch	0.19	0.15	0.23	0.15bc
Wheat	0.18	0.14	0.26	0.16ab

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$..^a Volumetric moisture determined using 5TM sensor during treatment. ^bGravimetric moisture determined using soil samples collected from pots.

Soil P did not differ among treatments (data not shown). At post treatment, soil P in trial 1 ranged between 12.8 mg P kg⁻¹ in the hairy vetch treatment to 22.2 mg P kg⁻¹ in the untreated control while at harvest, this range was 19.4 mg P kg⁻¹ in the crimson clover treatment to 28.6 mg P kg⁻¹ in the cereal rye treatment. In trial 2, post treatment soil P ranged between 7.8 mg P kg⁻¹ in the cereal rye treatment to 19.1 mg P kg⁻¹ in the hairy vetch treatment. At harvest, the range was between 23.1 mg P kg⁻¹ in the cereal rye + molasses treatment to 29.3 mg P kg⁻¹ in the biofumigant control.

In trial 1, pre-treatment soil NH₄-N ranged between 2.2 mg N kg⁻¹ soil in the crimson clover treatment to 9.3 mg N kg⁻¹ in the molasses treatment (Table 14). Nitrite and nitrate-N ranged between 0.7 mg N kg⁻¹ in the wheat treatment to 48.9 mg N kg⁻¹ in the untreated control. Total soil inorganic N ranged between 4.1 mg N kg⁻¹ in the crimson clover treatment to 55.8 mg N kg⁻¹ biofumigant control treatment. In trial 1 at post-treatment, some differences were observed in soil NH₄-N content with 12.4 g N kg⁻¹ observed from the untreated control, which was greater than all other treatments. The hairy vetch and wheat treatments had the lowest soil NH₄-N with 3.5 mg N kg⁻¹ and 4.5 mg N kg⁻¹, respectively. There were no differences observed in NO₃+NO₂ -N, which ranged from 54.0 mg N kg⁻¹ for the wheat treatment to 89.1 mg N kg⁻¹ in the untreated control. Total inorganic N did not differ among treatments, ranging between 53.6 mg N kg⁻¹ in the biofumigant control to 101.4 mg N kg⁻¹ in the untreated control. At harvest in trial 1, differences were observed in soil NO₃+NO₂-N and total inorganic N, but all values were rather low at less than 5 mg N kg⁻¹. In trial 2, pre-treatment soil NH₄-N ranged between 1.7 mg N kg⁻¹ in the wheat treatment to 4.4 mg N kg⁻¹ for the biofumigant control (Table 15). Soil NO₃+NO₂-N concentrations ranged between 0.4 mg N kg⁻¹ in the wheat treatment to 66.8 mg N kg⁻¹ in the untreated control (fallow, no cover crop).

Table 14. Mean soil ammonium-N (NH₄-N), nitrate + nitrite-N (NO₃ + NO₂-N), and total soil inorganic N (TIN), from growth chamber study, Trial 1.

	NH ₄ -N	NO ₃ + NO ₂ -N	TIN	NH ₄ -N	NO ₃ + NO ₂ -N	TIN	NH ₄ -N	NO ₃ + NO ₂ -N	TIN
	----Pre treatment----			----Post treatment----			-----Harvest-----		
	----- mg N kg ⁻¹ soil-----								
Untreated control	4.6 [†]	38.8	43.5	12.4a	89.1	101.4	1.6	2.4a	4.1a
Biofumigant control	6.9	48.9	55.8	4.8cd	48.8	53.6	0.4	1.4bc	1.8b
Molasses	9.3	21.5	40.8	5.9bcd	58.7	64.5	0.3	1.1bc	1.4b
Mustard/arugula + molasses	3.1	1.7	4.8	8.0bc	60.6	68.6	0.6	0.8c	1.4b
Mustard/arugula	6.2	2.1	8.3	8.8b	79.5	88.3	0.6	1.2bc	1.8b
Cereal rye + molasses	3.5	2.2	5.6	4.8cd	60.9	65.7	0.7	1.0bc	1.6b
Cereal rye	3.6	1.0	4.7	5.0cd	72.9	77.9	1.0	1.2bc	2.2b
Crimson clover	2.2	2.0	4.1	5.7bcd	68.7	74.4	0.5	1.7ab	2.1b
Hairy vetch	4.8	1.9	6.7	3.5d	64.0	67.5	0.6	1.3bc	1.8b
Wheat	3.6	0.7	4.3	4.5d	54.0	58.4	0.7	0.8c	1.5b

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table 15. Mean soil ammonium-N (NH₄-N), nitrate + nitrite-N (NO₃ + NO₂-N), and total soil inorganic N (TIN) for tomato from growth chamber study, Trial 2.

	NH ₄ -N	NO ₃ + NO ₂ -N	TIN	NH ₄ -N	NO ₃ + NO ₂ -N	TIN	NH ₄ -N	NO ₃ + NO ₂ -N	TIN
	----Pre treatment----			----Post treatment----			-----Harvest-----		
	----- mg N kg ⁻¹ soil-----								
Untreated control	2.7 [†]	66.8	69.4	4.2b	75.7b	80.1b	2.5bc	3.1	5.6
Biofumigant control	4.4	21.5	25.9	3.3b	34.6c	38.0c	1.8c	2.2	4.0
Molasses	4.2	8.4	12.6	3.1b	29.4c	32.7c	2.0bc	2.6	4.6
Mustard/arugula + molasses	3.2	1.8	5.0	3.4b	35.4c	38.9c	2.3bc	2.9	5.3
Mustard/arugula	3.5	1.2	4.7	3.5b	29.4c	33.0c	1.9bc	2.5	4.4
Cereal rye + molasses	3.0	0.7	3.8	3.4b	46.4bc	49.9bc	1.9bc	2.8	4.7
Cereal rye	2.4	1.0	3.4	81.2a	169.9a	253.9a	3.4a	3.0	6.4
Crimson clover	3.0	1.2	4.2	4.4b	71.2b	75.8b	2.6b	6.5	9.1
Hairy vetch	7.3	12.5	19.8	4.3b	52.8bc	57.2b	2.1bc	7.9	10.0
Wheat	1.7	0.4	2.1	2.7b	26.4c	29.2c	2.3bc	2.0	4.4

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Total inorganic N ranged between 2.1 mg N kg⁻¹ in the wheat treatment to 69.4 mg N kg⁻¹ in the untreated control. At post treatment, the cereal rye treatment had the highest NH₄-N level with 81.2 mg N kg⁻¹. In addition, soil NO₃+NO₂ -N was highest in the cereal rye treatment of 169.9 mg N kg⁻¹, which was higher than that observed from all other treatments. The lowest levels of soil NO₃+NO₂ -N were observed from the molasses, biofumigant control, mustard/arugula, wheat, and mustard/arugula + molasses treatments. Total inorganic soil N was greatest in the cereal rye treatment (253.0 mg N kg⁻¹) and lowest in the wheat, mustard/arugula, mustard/arugula + molasses, biofumigant control, and molasses treatments. At harvest in trial 2, soil inorganic N was generally low. Total soil inorganic N did not differ among treatments, ranging between 4.4 mg N kg⁻¹ in the wheat treatment to 10.0 mg N kg⁻¹ in the hairy vetch treatment.

All treatments in trial 1 and 2 showed declines in soil pH over the course of soil treatment (Table 16). The pre-treatment soil pH_{water} in trial 1 ranged between 6.1 in the untreated control to 7.0 in the wheat treatment while the pH_{CaCl2} ranged between 5.7 in the untreated control to 6.4 in the wheat treatment. Pre-treatment pH_{water} in trial 2 ranged between 6.3 in the untreated control to 7.2 in the mustard/arugula + molasses treatment while pH_{CaCl2} ranged between 5.7 in the molasses treatment to 6.5 in the wheat treatment. In trial 1, the wheat treatment had the highest pH_{water} and pH_{CaCl2}, which differed from all treatments except for pH_{water} in cereal rye + molasses. In trial 2, the wheat treatment had the highest pH_{water} with 6.7 at post treatment, which differed significantly from the cereal rye, crimson clover, and the untreated control treatments. Post treatment pH_{CaCl2} was highest for the cereal rye + molasses treatment (6.3), which was greater than that observed from the untreated control, cereal rye, and molasses treatments.

Table 16. Mean pre and post treatment soil pH in water (pH_{water}) and CaCl₂ (pH_{CaCl2}) from growth chamber study.

	Trial 1				Trial 2			
	Pre treatment		Post treatment		Pre treatment		Post treatment	
	pH _{water}	pH _{CaCl2}	pH _{water}	pH _{CaCl2}	pH _{water}	pH _{CaCl2}	pH _{Water}	pH _{CaCl2}
Untreated control	6.11	5.70	5.54e [†]	5.67e	6.30	5.93	5.87c	5.45d
Biofumigant control	6.35	5.74	5.92d	5.57d	6.89	6.06	6.46ab	6.02ab
Molasses	6.17	5.76	6.00cd	5.69cd	6.55	5.66	6.45ab	5.88bc
Mustard/arugula + molasses	6.77	5.84	6.00cd	5.69cd	7.16	6.12	6.53ab	6.12ab
Mustard/arugula	6.65	5.78	5.98cd	5.71cd	6.97	6.09	6.42ab	6.04ab
Cereal rye + molasses	6.68	6.15	6.22ab	5.95b	6.86	6.14	6.56ab	6.27a
Cereal rye	6.75	6.00	6.04bcd	5.76c	7.10	6.39	5.91c	5.59cd
Crimson clover	6.61	6.06	5.92d	5.69cd	6.95	6.29	6.30b	6.01ab
Hairy vetch	6.88	6.31	6.17bc	5.98b	6.90	6.44	6.42ab	6.11ab
Wheat	7.04	6.44	6.39a	6.17a	7.15	6.51	6.70a	6.04ab

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

During the ASD assay for the molasses treatment, day 1 had the highest soil pH with 7.19 (Table 17), which was greater than all days except for day 17 with 6.96. The pH measurements did not decline as days increased. Cumulative mV h did increase with day 30 reaching 286,140 mV h, which was greater than all other days. Day 17 (185,289 mV h) was also greater than day 1, 3, and 10, which were the lowest and did not show any difference.

Total soil nitrogen (TN) did not differ among treatments at pre-treatment, post-treatment, or at harvest in Trial 1 (data not shown). At pre-treatment mean soil TN was 0.5 g N kg⁻¹ soil, post-treatment mean soil TN was 0.8 g N kg⁻¹ soil, and at harvest mean soil TN was 0.6 g N kg⁻¹. At pre-treatment in trial 2, TN ranged between 0.4 g N kg⁻¹ in the molasses treatment to 0.8 g N kg⁻¹ in mustard/arugula, wheat, and cereal rye treatments. Post-treatment cereal rye had the highest TN with 1.0 g N kg⁻¹ soil which was greater than all treatments except for hairy vetch (0.9 g N kg⁻¹ soil). At harvest, there were no differences among treatments (mean 0.8 g N kg⁻¹ soil).

The range of C in trial 1 prior to treatments was between 4.1 g C kg⁻¹ soil in the molasses treatment to 7.0 g C kg⁻¹ in the mustard/arugula treatment (data not shown). Post-treatment, the cereal rye + molasses had the greatest amount of soil C with 9.0 g C kg⁻¹, which was greater than that observed from the mustard/arugula + molasses, wheat, hairy vetch, crimson clover, and molasses treatments. At harvest, there were no differences observed among treatments. In trial 2, pre-treatment soil C was lower than that observed in pre-treatment soil in trial 1. In this trial, the range of soil C was between 1.7 g C kg⁻¹ soil in the molasses treatment to 5.1 g C kg⁻¹ in the wheat treatment. At post treatment, the hairy vetch treatment had the highest soil C with 7.7 g C kg⁻¹ which was greater than that observed from the biofumigant control, molasses, mustard/arugula + molasses, wheat, and mustard/arugula treatments.

Table 17. Soil pH and accumulation of mV h during ASD treatment assay with molasses amendment. Means represent averages of both trials.

Day	pH _{water}	Cumulative mVh
0	6.73bc [†]	N/A
1	7.19a	5,167d
3	6.59c	18,930cd
10	6.54c	95,337c
17	6.96ab	185,289b
30	6.70bc	286,140a

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

At harvest, there were no differences observed among treatments, ranging between 5.3 g C kg⁻¹ in the crimson clover treatment to 7.2 g C kg⁻¹ in the cereal rye treatment.

Soil C:N ratio prior to treatment in trial 1 ranged between 10.1 in the biofumigant control to 11.6 in the mustard/arugula + molasses treatment (Table 18). Post-treatment, the cereal rye + molasses treatment had the highest C:N ratio (10.2), which was greater than that observed from the untreated control, biofumigant control, crimson clover, hairy vetch, and wheat treatments. At harvest, there were no differences observed among treatments, ranging between 11.3 in the hairy vetch, crimson clover, and untreated control treatments to 12.3 in the mustard/arugula + molasses treatment. In trial 2, soil C:N ratio at pre-treatment ranged between 3.9 in the molasses treatment to 6.2 in the untreated control. At post treatment, the wheat and mustard/arugula treatments had the highest C:N ratio with 9.9, which was greater than the hairy vetch, cereal rye, and mustard/arugula + molasses treatments. At harvest, there were no differences among treatments, which ranged between 7.8 in the crimson clover treatment to 8.7 in the untreated control. Trial 2 C:N ratios tended to be smaller than C:N ratios observed in trial 1.

For trial 1, WEC ranged from 0.02 mg L⁻¹ in the hairy vetch treatment (Table 19) to 0.10 mg L⁻¹ in the mustard/arugula + molasses treatment. HWECC ranged between 0.12 mg L⁻¹ in the molasses treatment to 0.30 mg L⁻¹ in the mustard/arugula + molasses treatment.

Disease incidence and weed populations. In trial 1, yellow nutsedge emergence was highest (35%) from the crimson clover treatment (Table 20). However, this did not differ significantly from most of the other treatments where nutsedge germination immediately post treatment was in the range of 15 to 25%. In trial 1, the untreated control treatment had no nutsedge emergence post treatment, which was lower than all other treatments. Cumulative nutsedge germination did not differ among treatments, ranging between 10% for the untreated control to 70%

Table 18. Soil carbon to nitrogen ratio (C:N) from growth chamber study, Trial 1 and 2.

	Pre-treatment	Post-treatment	Harvest	Pre-treatment	Post-treatment	Harvest
	-----Trial 1-----			-----Trial 2-----		
	-----TC/TN-----					
Untreated control	10.8 [†]	7.9bc	11.3	6.2	9.2abcd	8.7
Biofumigant control	10.1	7.8bc	12.1	5.5	9.8ab	8.3
Molasses	10.7	9.2ab	11.8	3.9	9.6abc	7.9
Mustard/arugula + molasses	11.6	8.7ab	11.5	5.2	9.1bcd	8.5
Mustard/arugula	11.0	8.1bc	12.3	5.6	9.9a	8.4
Cereal rye + molasses	11.5	10.2a	12.1	5.0	9.3abcd	8.1
Cereal rye	10.8	9.2ab	12.0	5.2	6.7e	7.9
Crimson clover	11.2	8.2bc	11.3	5.0	9.0cd	7.8
Hairy vetch	10.4	6.9c	11.3	5.0	8.7d	7.9
Wheat	10.9	8.4bc	10.9	6.0	9.9a	8.2

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table 19. Water extractable carbon (WEC) and hot water extractable carbon (HWEC) from growth chamber study, Trial 1.

	WEC	HWEC
	-----mg C g ⁻¹ soil-----	
Untreated control	0.02	0.14
Biofumigant control	0.04	0.12
Molasses	0.06	0.12
Mustard/arugula + molasses	0.10	0.28
Mustard/arugula	0.04	0.15
Cereal rye + molasses	0.05	0.17
Cereal rye	0.05	0.23
Crimson clover	0.05	0.19
Hairy vetch	0.02	0.17
Wheat	0.02	0.13

Table 20. Nutsedge germination post-soil treatment from growth chamber study, Trial 1 and 2.

	-----Trial 1-----		-----Trial 2-----	
	Post-treatment	Cumulative	Post-treatment	Cumulative
	-----germination (% of introduced tubers)---			
Untreated control	0b [†]	10	25	30
Biofumigant control	15ab	50	55	80
Molasses	10ab	65	25	30
Mustard/arugula + molasses	25ab	40	30	50
Mustard/arugula	25ab	70	30	65
Cereal rye + molasses	25ab	60	35	55
Cereal rye	25ab	60	30	35
Crimson clover	35a	65	40	65
Hairy vetch	20ab	40	45	70
Wheat	15ab	55	35	55

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

in the mustard/arugula treatment. While crimson clover had the highest nutsedge germination post treatment of (35%), by the final weed count cumulative nutsedge germination was only 65% of planted tubers. Crabgrass and pigweed germination was minimal (Table 21). There was no statistical relationship among weed germination, post treatment to soil moisture or cumulative mV h.

During trial 2, nutsedge germination during treatment did not differ among treatments, but a greater percentage of planted tubers germinated as compared to trial 1. The highest germination (55%) occurred with the biofumigant control and the lowest germination was observed from the untreated control and molasses treatments (25%), although these did not differ significantly. Cumulative nutsedge germination during the tomato growth period was 80% in the biofumigant control and 70% in the hairy vetch treatment. While not significantly different than trial 1, germination was observed to be higher for both crabgrass and pigweed in trial 2. There were no differences observed among any of the treatments with crabgrass germination ranging between 2% for the mustard/arugula treatment to 8% for the wheat, untreated control, and crimson clover treatments. For pigweed, there were no differences among treatments ranging between 7% germination in hairy vetch and mustard/arugula + molasses treatments to 19% in mustard/arugula + molasses. *Disease incidence and Sclerotium rolfsii*. Early blight (*Alternaria solani*) was observed in Trial 1, however no differences were observed between treatments (Fig 2). Foliar disease pressure was not observed in Trial 2 and thus weekly ratings were not conducted.

Following trial 1 treatment, total sclerotial germination, while not differing among treatments, ranged between 2.5% in the hairy vetch treatment to 22.5% in the cereal rye +

Table 21. Crabgrass and pigweed germination post- soil treatment from growth chamber study, Trial 1 and 2.

	-----Trial 1-----		-----Trial 2-----	
	Crabgrass	Pigweed	Crabgrass	Pigweed
	-----germination (% of introduced seeds)-----			
Untreated control	0	0	8	12
Biofumigant control	1	3	6	17
Molasses	0	0	6	16
Mustard/arugula + molasses	0	2	6	19
Mustard/arugula	0	3	2	10
Cereal rye + molasses	0	2	4	7
Cereal rye	0	8	7	12
Crimson clover	0	0	8	12
Hairy vetch	0	6	6	7
Wheat	0	0	8	18

*Within columns, means indicated by different letters are significantly different, $p < 0.05$.

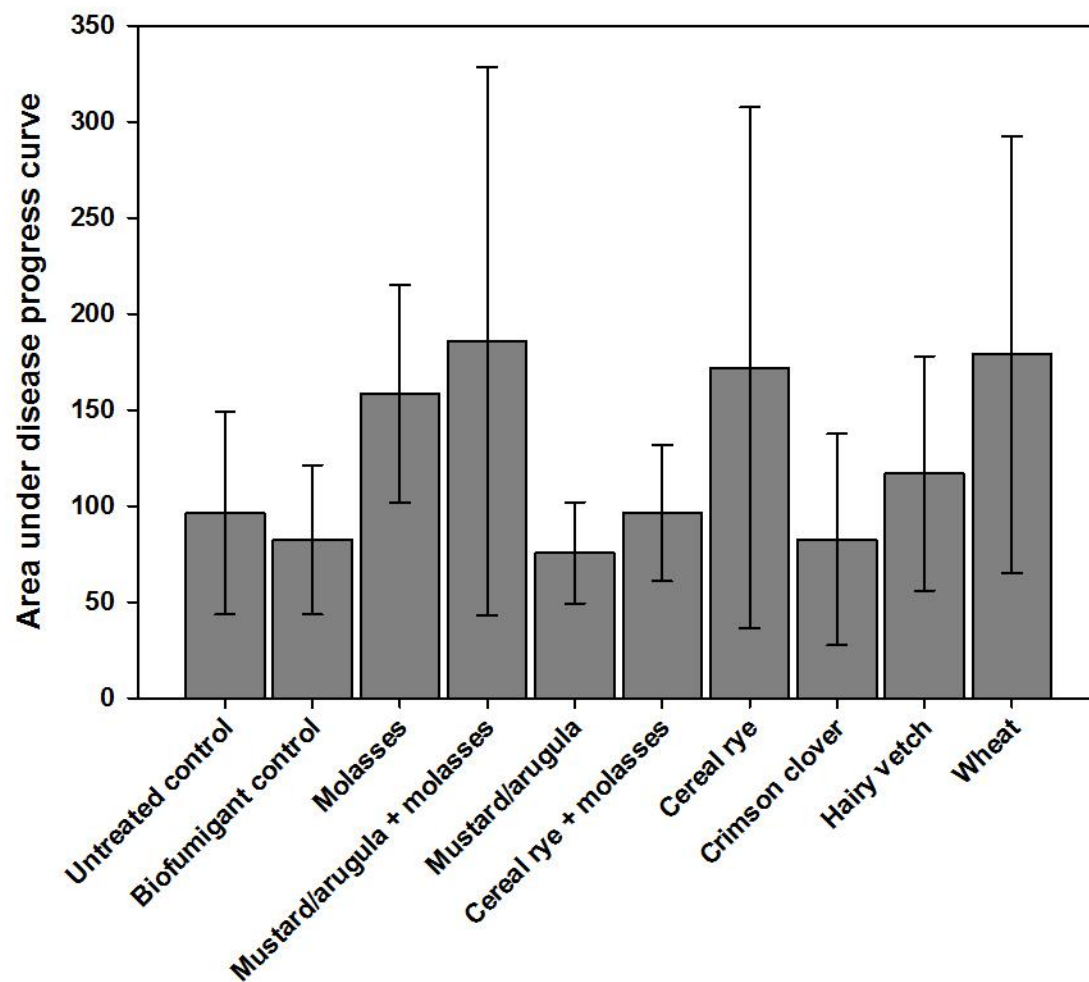


Figure 9. AUDPC of Early Blight in growth chamber study, Trial 1..Error bars represent 95% confidence intervals.

Table 22. Effect of treatment on germination and parasitism of *S. rolf sii* sclerotia recovered from soil in growth chamber study, Trial 1.

	Total sclerotial germination	Sclerotia parasitized by <i>Trichoderma</i> spp.	Sclerotia parasitized by <i>Fusarium</i> spp.	Sclerotia parasitized by other fungi	Disintegrated sclerotia	Intact sclerotia that did not germinate
	-----%					
Untreated control	5.0	58.8	0	2.5	60.0	0
Biofumigant control	13.8	76.3	2.5	3.8	3.8	0
Molasses	7.5	37.5	17.5	3.8	41.3	0
Mustard/Arugula + Molasses	11.3	51.3	8.8	12.5	16.3	0
Mustard/Arugula	7.5	42.5	2.5	15.0	38.8	1.3
Cereal rye + Molasses	22.5	56.3	3.8	11.3	6.3	0
Cereal rye	3.3	63.8	0	5.0	22.5	5.0
Crimson clover	10.0	63.8	0	3.8	22.5	0
Hairy vetch	2.5	38.8	0	0	58.8	0
Wheat	5.0	66.3	1.3	10.0	17.5	0

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

molasses treatment (Table 22). There were no differences among treatments for sclerotia parasitized by *Trichoderma* spp., *Fusarium* spp., or other fungi. The percentage of disintegrated sclerotia also did not differ among treatments, with a range between 3.8% for the biofumigant control to 60.0% in the untreated control. Intact sclerotia that did not germinate occurred only in the mustard/arugula and cereal rye treatments. In trial 2, total sclerotial germination was greatest for the biofumigant control (33.8% germination), which differed from all other treatments (Table 23). Sclerotia parasitized by *Trichoderma* spp. were low in the untreated control (21%) which was significantly lower than all treatments except hairy vetch. Similar to that observed in trial 1, there were no differences among treatments for sclerotia colonized by *Fusarium* spp. or other fungi in trial 1. Disintegrated sclerotia were highest for the untreated control (53.8%), which differed from all other treatments.

Crop performance. During trial 1, plant height did not differ among treatments at week 3, with a range between 16.4 cm for the untreated control to 11.3 cm for the cereal rye + molasses treatment (Table 24). Stem diameter was greatest from the wheat treatment (10.5 mm) and least from the cereal rye + molasses treatment (6.3 mm). At week 7, tomatoes in the hairy vetch treatment had the greatest height (20.2 cm) but did not differ from the untreated control (19.8 cm) or other treatments. Tomato plants in the cereal rye + molasses and mustard/arugula + molasses treatments were the shortest (15.0 and 16.1 cm, respectively). For stem diameter, tomatoes in the hairy vetch treatment were again the largest (13.3 mm) but did not differ from any of the other treatments. For trial 2, there were no differences among treatments at either week 3 or week 6 measurements of stem diameter or plant height. No differences in total yield were observed among treatments in trial 1 (Table 25). Yields ranged between 0.07 kg plant⁻¹ to

Table 23. Effect of treatment on germination and parasitism of *S. rolfsii* sclerotia recovered from soil from growth chamber study, Trial 2.

	Total sclerotial germination	Sclerotia parasitized by <i>Trichoderma</i> spp.	Sclerotia parasitized by <i>Fusarium</i> spp.	Sclerotia parasitized by other fungi	Disintegrated sclerotia	Intact sclerotia that did not germinate
	-----%-----					
Untreated control	11.5b [†]	25.0c	1.3	5.0	53.8a	1.3
Biofumigant control	33.8a	60.0ab	3.8	2.5	2.5d	0
Molasses	5.0b	73.8ab	3.8	15.0	3.8cd	0
Mustard/Arugula + Molasses	11.3b	78.8a	1.3	2.5	6.3bcd	0
Mustard/Arugula	13.8b	72.5ab	2.5	8.8	0d	2.5
Cereal rye + Molasses	11.3b	63.8ab	1.3	8.8	13.8bcd	1.3
Cereal rye	1.3b	60.0ab	0	5.0	23.8bc	10.0
Crimson clover	11.3b	66.3ab	2.5	10.0	7.5bcd	2.5
Hairy vetch	17.5ab	45.0bc	0	8.8	25.0b	3.8
Wheat	10.0b	77.5a	1.3	5.0	7.5bcd	0

[†]Within columns, means indicated by different letters are significantly different, $p > 0.05$.

Table 24. Early (week 3) and end of trial (week 7) tomato height and stem diameter from growth chamber study, Trial 1 and 2.

	-----Trial 1-----				-----Trial 2-----			
	Height ¹	Stem diameter	Height ²	Stem diameter	Height ³	Stem diameter	Height ⁴	Stem diameter
	Week 3		Week 7		Week 3		Week 7	
	--cm--	--mm--	--cm--	--mm--	--cm--	--mm--	--cm--	--mm--
Untreated control	16.4 [†]	9.6ab	19.8	11.5	9.8	5.8	20.0	8.6
Biofumigant control	14.0	8.5abcd	16.2	11.6	12.7	5.6	20.8	7.7
Molasses	14.2	8.9abc	16.7	11.5	12.4	6.3	23.9	8.9
Mustard/arugula + molasses	14.1	9.1abc	16.1	12.6	11.3	5.9	22.4	8.1
Mustard/arugula	13.3	7.8bcde	17.0	11.5	10.0	5.9	23.3	8.2
Cereal rye + molasses	11.3	6.3e	14.9	11.7	11.2	5.0	23.8	8.7
Cereal rye	14.0	7.4cde	16.7	11.2	10.9	5.6	23.0	11.3
Crimson clover	11.9	6.7de	17.3	9.7	9.6	4.8	20.1	9.1
Hairy vetch	14.3	8.8abcd	20.2	13.3	9.5	5.0	23.7	9.4
Wheat	13.8	10.5a	16.7	12.6	13.6	6.2	22.3	6.6

¹March 13, 2012; ²April 10, 2012; ³July 2, 2012; ⁴July 20, 2012; [†]Within columns, means indicated by different

letters are significantly different, $p < 0.05$.

Table 25. Mean tomato yield from growth chamber study, Trial 1 and 2.

	Trial 1	Trial 2
	-----kg plant ⁻¹ -----	
Untreated control	0.08	0.005e [†]
Biofumigant control	0.07	0.04cde
Molasses	0.09	0.07abc
Mustard/arugula + molasses	0.07	0.08a
Mustard/arugula	0.09	0.05bcd
Cereal rye + molasses	0.07	0.08a
Cereal rye	0.09	0.08a
Crimson clover	0.08	0.02de
Hairy vetch	0.12	0.03cde
Wheat	0.09	0.09a

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

0.12 kg plant⁻¹ in trial 1. In trial 2, yields were generally lower than in trial 1 with the highest yields observed from the mustard/arugula + molasses, cereal rye + molasses, cereal rye, and wheat treatments and the lowest yields observed from the untreated control treatment.

No differences were observed among treatments in the N content of leaf tissue (Table 26) in trial 1. The range among treatments was between 1.4 cg N g⁻¹ for mustard/arugula + molasses to 4.1 cg N g⁻¹ content of the hairy vetch treatment. While many of the treatments in trial 1 were below 2.0 cg N g⁻¹, all treatments in trial 2 were greater than 2.0 cg N g⁻¹. In trial 2, the highest N concentration in leaf tissue was in the crimson clover treatment (4.0 cg N g⁻¹), which was greater than that observed in the wheat, molasses, mustard/arugula + molasses, biofumigant control, and cereal rye + molasses treatments.

Discussion

Cover crop biomass added in this study was much lower in trial 1 (3.5 to 6.5 Mg ha⁻¹) compared to trial 2 (3.3 to 12 Mg ha⁻¹). This level of cover crop biomass addition is also lower than that reported in a Tennessee ASD field trial of many of the same cover crops by McCarty and colleagues (2012; 4.6 to 7.2 Mg ha⁻¹ in trial 1 and 4.4 to 13 Mg ha⁻¹ in trial 2). This is likely due to differences in growing conditions from the field to the small pots in the greenhouse and growth chamber. Likewise, the rate of molasses application in this study (5.6 Mg ha⁻¹) was lower than that applied in a Florida ASD pot study (14.6 Mg dry matter ha⁻¹; Butler et al. 2012a). At the same time, the C:N ratio of the liquid molasses was 35.2 compared to a ratio of 14.4 in the dry molasses used in this study, likely due to the grain by-products used in production of dry molasses, which due to higher N content can lower the C:N ratio of the bulk product.

Table 26. Nitrogen concentration in tomato leaf tissue at harvest from growth chamber study, Trial 1 and 2

	Trial 1	Trial 2
	-----cg N g ⁻¹ -----	
Untreated control	2.7	3.4abc [†]
Biofumigant control	1.7	2.7bcd
Molasses	1.7	2.4d
Mustard/arugula + molasses	1.4	2.6bcd
Mustard/arugula	1.7	3.1abcd
Cereal rye + molasses	1.8	2.5cd
Cereal rye	1.7	3.1abcd
Crimson clover	2.0	4.0a
Hairy vetch	4.1	3.5ab
Wheat	1.6	2.8bcd

^{††} Within columns, means indicated by different letters are significantly different, $p < 0.10$.

This C:N ratio was larger than all other C:N ratios of treatment amendments in both trial 1 and 2. Gravimetric soil moisture content was higher for the Florida pot study post treatment (0.19 to 0.20 g g⁻¹) compared to our moisture level range of 0.12 to 0.17 g g⁻¹ in trial 1 and 0.13 to 0.17 g g⁻¹ in trial 2. As mentioned in Butler et al. (2012a), much of the accumulation of mV h could be tied to maintenance of moisture content in soil. While there was no difference in post soil moisture content in trial 1, the highest level of soil moisture (0.17 g g⁻¹) was in the cereal rye + molasses treatment. Unfortunately, while differences were observed in post treatment soil moisture among treatments in trial 2, the accumulated mV h is not available (due to a datalogger error) to determine if this trend influenced the accumulation of anaerobic conditions.

Accumulation of anaerobic soil conditions during the ASD treatments in this study were similar to those reported in previous ASD studies. The mV h observed in the Tennessee ASD field study reported by McCarty et al. (2012a) had a range between 6,246 mV h to 39,470 mV h in the first year and 11,258 to 74,505 mV h in the second year. In general, mV h values in our study excluding the untreated control were higher than the first year of the field study. In the second year of the field trial, our cereal rye + molasses and crimson clover treatments exceeded all treatments. The biofumigant control treatment in the Tennessee field study reached 55,561 mV h, which was lower than our treatments of crimson clover, cereal rye + molasses, and mustard/arugula treatments. In the Florida pot study, a molasses treatment was reported to accumulate nearly 120,000 mV h while all other C source treatments were above an untreated control (fallow without added C source) of 80,000 mV h (Butler et al. 2012a). Compared to our values in trial 1, only the cereal rye + molasses treatment exceeded 80,000 mV h while our molasses treatment accumulated 11,571 mV h. In a field study using liquid molasses, Butler et al. (2012b) reported accumulated mV h below 5,000 mV h and above 30,000 mV h in year 1 and

2, respectively while treatments with the application of composted poultry litter were below 10,000 mV h and above 20,000 mV h in years 1 and 2 of the study, respectively. This is a similar range compared to the accumulated mV h in the molasses treatment in our study.

A study of ASD for control of *V. dahliae* in California strawberry production by Shennan et al. (2010) reported that a threshold of 50,000 mV h at 25 °C soil temperature was necessary for control of *V. dahliae*. While this threshold is linked to the suppression of this particular pathogen, it is an important gauge of the mV h we may expect to observe in our pots and the potential for controlling other pathogens. In a Netherlands study, anaerobic soil conditions were reported as mV in which treatments of broccoli residue amendment reached a soil redox potential of -193 mV by day 48 whereas a non-amended plot did not fall below 110 mV (Blok et al. 2000). In trial 1, our lowest soil redox potential was -159 mV averaged from the wheat treatment. A further study by Blok et al. (2008) reported ASD conditions induced redox potentials near -200 mV following the addition of grass at 38, 56, and 93 Mg ha⁻¹ (fresh biomass weight), which was similar to the values we observed in both pot trials. Cover crop amendment biomass was generally lower (average 4.3 Mg ha⁻¹ in trial 1) than the amendments added by Blok and colleagues but reaching a greater average mV, likely due to the nature of soil water relations in pots versus in situ. In the Tennessee ASD field study (2012), McCarty and colleagues observed an average biomass of 6.67 Mg ha⁻¹ in trial 1 and 11.2 Mg ha⁻¹ in trial 2. In that study, the lowest redox potential value in trial 1 was -323 mV in the cereal rye + molasses treatment while in trial 2, the lowest redox value was -159 mV in the cereal rye + molasses treatment. Our average biomass (5.2 Mg ha⁻¹) and redox potentials in trial 1 were similar to that reported by McCarty and colleagues.

A decline in soil pH following ASD soil treatment has been reported to be an indicator of whether organic acids are being added to the soil solution through the anaerobic decomposition of the added C amendments (Momma 2008). In both trials, there were declines in soil pH_{water} and $\text{pH}_{\text{CaCl}_2}$ during treatment.. The greatest decline in soil pH during treatment occurred in the mustard/arugula + molasses treatment (-0.77) in pH_{water} while crimson clover had the greatest decline in $\text{pH}_{\text{CaCl}_2}$ (-0.37). In trial 2, the greatest decline in pH_{water} (-1.19) and $\text{pH}_{\text{CaCl}_2}$ (-0.80) occurred in the cereal rye treatment. Of the treatments without composted poultry litter in the Florida pot study, low soil pH values were reported for all treatments compared to the control without added C amendment (Butler et al. 2012a). In the field study reported by McCarty and colleagues (2012), there were declines in pH_{water} values at post treatment (mean of -0.79) for most treatments in 2012, possibly based on the contribution of greater biomass added in 2012 than in 2011.

Yellow nutsedge germination was lowest from or equal to other treatments for the untreated control for both trials 1 and 2. Much of this could be due to observed compaction in the untreated control pots due to lack of organic matter additions to the pots. The molasses control, while not adding a large amount of biomass, did not differ from the untreated control but still had a higher rate of germination (30%). This differed from that reported by Butler et al. (2012a) who reported that an untreated control had the highest rate of cumulative nutsedge germination compared to a molasses treatment and a number of warm-season cover crop residue amendments. However, the range of nutsedge germination for cover crop treatments of 41 to 60% in the Butler et al. study was similar to our range for both trials of 35 to 70%. The rate of total C of the molasses treatment ($2.64 \text{ mg C g}^{-1} \text{ soil}$) in the Butler et al. study was much greater than the total C in our molasses treatment ($1.18 \text{ mg C g}^{-1} \text{ soil}$) while cover crops in the Butler et

al. study ranged between 0.42 to 1.15 mg C g⁻¹ soil, which was comparable to our range of 0.99 to 1.49 mg C g⁻¹ in trial 1 and 0.98 to 2.52 mg C g⁻¹ in trial 2. No correlation was observed between weed germination and that of cumulative mV h. Under ASD conditions in another pot experiment, yellow nutsedge germinated at shallow depths but not at deeper depths when tubers in mesh bags were planted and different C sources were added, a possible indication that resupply of oxygen at the soil surface during treatment acted as a limiting factor (Muramoto et al. 2008). Within our study, nutsedge was planted at the soil surface after incorporation of treatment amendments. Perhaps at a deeper depth we would have observed greater nutsedge control, however, this would be an unrealistic representation of nutsedge pressure in field situations. At the same time, control of nutsedge tubers buried more deeply in the soil may be possible with ASD treatment.

Butler and colleagues (2012a) reported the highest survival of sclerotia of *S. rolfsii* in a control treatment without added C source and noted a relationship between survival and added carbon source. Control was greatest (increased mortality) in a molasses treatment, sorghum-sudangrass, cowpea/pearl millet, and pearl millet treatments in one trial, while sunn hemp, sorghum-sudangrass, and pearl millet treatments induced the highest levels of control in a second study. However, in our study few differences were observed in survival of *S. rolfsii* during ASD treatment. While hairy vetch had the greatest total C added (2.72 mg C g⁻¹) in trial 2, total sclerotial germination was 17.5%, which did not differ from the untreated control (11.5%). The total C added by many of our treatments was within the range or above the level of total C added by the cover crop treatments in the Florida study.

Yields in this trial were similar to that reported by Butler et al. study (2012). In our study, yields in trial 1 ranged between 0.07 kg plant⁻¹ in the mustard/arugula + molasses treatment to

0.120 kg plant⁻¹ in the hairy vetch treatment. While there were no differences in yields for the treatments in the Butler et al. study, our results for trial 2 show greater yields in the wheat (0.09 kg plant⁻¹), cereal rye + molasses (0.09 kg plant⁻¹), cereal rye (0.08 kg plant⁻¹), and the molasses (0.07 kg plant⁻¹) treatment than the untreated control.

The molasses treatment tended to not differ in weed germination, yields, C:N ratio, and cumulative mV h compared to the other cover crop treatments. Therefore, it seems that molasses would be an acceptable choice for ASD adoption in place of a cover crop treatment. While other cover crops could show similar results as the molasses treatment, these would require additional inputs of growing cover crops before incorporation, which while providing numerous agronomic and environmental benefits, would not easily fit into all specialty crop production systems. At the same time, for growers wishing to limit off-farm inputs, it seems that cover crops and crop residues can be used to effectively increase accumulation of mV h in ASD treated soils. Given that cumulative mV h could not be reported for trial 2, although differences were observed in soil properties, weed germination, and yields, additional trials could help to better assess relationships between cumulative mV h and these variables. Additional studies are needed to determine the potential for the other cover crops used in this study.

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CHAPTER IV

ON-FARM DEMONSTRATION AND EVALUATION OF ANAEROBIC SOIL DISINFESTATION (ASD) FOR TOMATO PRODUCTION SYSTEMS IN TENNESSEE

Abstract. Anaerobic soil disinfestation (ASD) is a non-chemical, pre-plant soil treatment that involves the incorporation of a labile carbon (C) source, tarping with plastic, and irrigation of the topsoil to saturation (~ 5 cm irrigation). The conditions created allow for the anaerobic decomposition of the added C source which may control soilborne plant pathogens, plant-parasitic nematodes, and weeds in specialty crop production systems. To assess the feasibility of ASD in commercial production systems, a demonstration trial was conducted at a tomato farm in Rutledge, TN beginning in July 2012. Soil treatments included: (1) methyl bromide (MeBr) + chloropicrin (50:50; 224 kg ha⁻¹), (2) metam sodium (Vapam®; 374.8 L ha⁻¹), (3) ASD with dry molasses (11,000 kg ha⁻¹), (4) ASD with dry molasses + soybean meal (9,041 and 2,211 kg ha⁻¹, respectively), and (5) an untreated control. The molasses and molasses + soybean meal treatments were applied to the tops of pre-formed raised-beds, covered with polyethylene mulch, and irrigated to fill soil porosity to begin ASD treatment. At end of treatment, tomato was planted. Data collection included assessment of anaerobic soil conditions, crop performance, and soil properties during the growing season. Post treatment (at planting), molasses and molasses + soybean meal ASD treatments had higher levels of total soil nitrogen (N) compared to the other treatments. Furthermore, at post treatment the highest levels of soil phosphorus, C:N ratio, and soil NH₄-N were observed from the molasses treatment. Total soil inorganic N was highest from the molasses + soybean meal ASD treatment. Overall, there were no differences in yield and plant-parasitic nematode populations among treatments. Additional on-farm

demonstration trials are needed to continue to refine ASD treatment and to collect data from diverse production systems and field sites over a number of production years.

Introduction.

Methyl bromide (MeBr) is a preplant soil fumigant used in vegetable production systems that has broad spectrum activity for controlling plant-parasitic nematodes, weeds, and soilborne plant pathogens. For most growers, MeBr is applied in combination with chloropicrin to raised beds which are immediately covered with plastic mulch. Crops are typically transplanted several days to a few weeks following soil treatment. Many industries are dependent on MeBr for use as a pre-plant soil fumigant. These industries include California strawberry production, several southeastern vegetable production industries, and winter vegetable production in Florida (Byrd et al. 2006a; Carter et al. 2000; Haar et al. 2003; Noling et al. 2009). Since 2005, a full mandate has been in place following U.S. ratification of the Montreal Protocol on Ozone Depleting Substances in 1988. This ban has been projected to have a large detrimental impact on many of the vegetable industries in the U.S. Many studies have projected substantial losses such as \$1 billion on the winter vegetable industry in Florida, a 10 to 15% decline in yields and a 10% decline in acreage in the California strawberry industry, and a reduction in Florida industries of cucurbitaceous and solanaceous crops (Byrd et al. 2006b; Carter et al. 2000; Deepak et al. 1996). While this mandate has reduced the use of MeBr, many industries have been allowed critical use exemptions (CUE) where they can continue to use the chemical and purchase it based on the economic need if no viable alternatives are available (Martin 2003). With the CUE status delaying the expected and waning supply of this chemical, growers are seeing increased MeBr

prices and pressure to no longer rely on MeBr (Noling et al. 2009). This current situation presents a need for a suitable alternative that can replace MeBr as a soil fumigant. While several chemical alternatives have been adopted by growers, none of these have the broad-spectrum pest control provided by MeBr. According to Noling et al. (2009), chloropicrin can control some plant pathogens but has limited efficacy against weeds or plant-parasitic nematodes, while 1, 3-dichloropropene (e.g., Telone®, PicChlor60) can control nematodes but not weeds or certain plant pathogens. Metam Sodium (Vapam®) and Metam Potassium (K-Pam®) control weeds only when appropriately incorporated in the soil. Methyl iodide has shown potential for broad-spectrum efficacy (Noling et al. 2009), but is no longer commercially available in the U.S. due largely to human health concerns (Chawkins and Marcum 2012).

There exists a need for a suitable alternative, particularly a nonchemical method, that is easily-adaptable in systems currently using MeBr. One method that has demonstrated promise is anaerobic soil disinfestation (ASD; sometimes referred to as biological soil disinfestation), which is a pre-plant method developed to control pests in specialty crop production systems. It involves adding an easily decomposable carbon source, such as a cover crop or organic amendment to the soil, which is then tarped with plastic mulch and irrigated. Amendments used can vary, but must contain an adequate supply of labile C to support soil microbial growth (Momma 2008). During ASD treatment, organic acids, particularly acetic and n-butyric acids, are released into the soil as the C source decomposes anaerobically (Momma 2008). The application of soil amendments (e.g., molasses, wheat bran, cover crops, crop residues) have led to reductions in soil pH and redox potential (Eh) implying that oxygen is being consumed and organic acids are being added

(Momma 2008). At the same time, the efficacy of the ASD method is connected with a reduction in pH and presence of organic acids (Kubo et al. 2005; Momma et al. 2005). In a pot study examining ASD using wheat bran as the C source, the production of acetic and butyric acid occurred. At the same time, when these acids were added that were not derived from wheat bran, they appeared to have the same effect as ASD treatment (Momma et al. 2006). For plant pathogens, researchers also acknowledge that development of organic acids (acetic, n-butyric) in ASD can contribute to control of *Ralstonia solanacearum* (Momma et al. 2006).

Presently, studies show that the elimination of MeBr could result in substantial economic losses to vegetable and small fruit producers in a number of states (Byrd et al. 2006a; Byrd et al. 2006b; Carter et al. 2000; Deepak et al. 1996). As such, there currently exists a need to evaluate MeBr alternatives for Tennessee crops. Particularly, with a robust tomato industry in Grainger County, the lack of sustainable alternatives in TN could result in economic losses, ecological harm, or impacts on human health. In order to further industry-wide acceptance of ASD as an alternative to MeBr fumigation, an on-farm demonstration trial was established to demonstrate and evaluate ASD under standard grower management practices. Crop yields, weed populations, and disease occurrence between chemical fumigants, two ASD treatments differing in C:N ratio and N content of amendments, and an untreated control were evaluated..

Materials and Methods

A large-scale tomato producer agreed to host a demonstration trial in Rutledge, Grainger County, Tennessee, beginning in July 2012. Treatments consisted of: (1) MeBr + chloropicrin (50:50; 224 kg ha⁻¹), (2) metam sodium (Vapam®; 374.8 L ha⁻¹), (3) ASD using dry molasses

(11,000 kg ha⁻¹; Westway Feed, Tomball, TX), (4) ASD using dry molasses + soybean meal (9,041 kg molasses ha⁻¹; 2,211 kg soybean meal ha⁻¹, Tennessee Farmer's Cooperative, LaVergne, TN), and (5) an untreated control. Six raised beds (~ 90 m in length by ~ 0.9 m width) were used in the study. Each raised bed was divided in half with each block representing three adjacent raised beds ~ 45-m in length. Locations of fumigants were randomly assigned within each section of three adjacent beds and applied by the producer in advance of ASD treatments as part of standard production practices. Fumigant rates were reported by the producer. Due to the nature of the fumigation application and to simplify producer management, fumigant treatments (metam sodium and MeBr + chloropicrin) were applied to the entire ~ 90-m bed length, spanning two adjacent blocks. The remaining untreated bed was divided into three 15-m beds per block, and the untreated control, ASD with molasses and ASD with molasses + soybean meal treatments randomly assigned to one ~ 15-m bed per block. Pre-plant fertilizer (12% N, 24% P₂O₅, 24% K₂O, derived from diammonium phosphate, urea, and potassium chloride; Tennessee Farmer's Cooperative, LaVergne, TN) was applied to all plots by the producer at a reported rate of 560 kg ha⁻¹ prior to initiation of the study. On July 9, 2012, soil temperatures were recorded at 5-cm and 15-cm depths in all non-fumigated plots. Soils in non-fumigated beds were sampled with soil cores (0 to 15-cm depth, 1.75-cm internal diameter) collected from random locations within each plot and composited. A soil subsample was oven-dried (105° C) to determine gravimetric moisture content and the remaining sample was air-dried and sieved (< 2 mm) prior to further analyses.

Molasses and molasses + soybean meal were applied by hand to appropriate plots on pre-formed raised beds only (i.e., not to row middles between bed). Due to heavy rain (~ 2.5 cm) occurring immediately after amendment application, amendments were not further incorporated

into raised beds and polyethylene mulch (0.032-mm, white on black) was applied to raised beds by hand. Two IRIS (indicator of reduction in soil) tubes were inserted in the soil in each plot with the exception of fumigated plots at a depth of 15 cm. These indicator tubes consisted of a polyvinyl-chloride tube painted with a ferrihydrite solution, which have been reported to relate to anaerobic soil conditions based on the solubilization of ferrihydrite paint from the tube surface. A loss of 20% of the paint solution has been reported to translate to an 87% soil reduction (Castenson and Rabenhorst 2006). An additional 2.5 cm of irrigation was applied by the producer on the day following amendment application.

Grape tomatoes (*Solanum lycopersicum* cv. Smarty) were planted on July 23, 2012, effectively ending ASD treatments. Soils were again sampled on July 24, 2012 and soil temperatures recorded as described previously. All plots were fertigated weekly during the growing season by the producer with 56 kg ha⁻¹ of calcium nitrate, 56 kg ha⁻¹ of potassium chloride, and 56 kg ha⁻¹ of a mixed water soluble fertilizer (20% N, 20% P₂O₅, 20% K₂O, derived from ammonium phosphate, potassium nitrate, urea, sodium borate, copper EDTA, iron EDTA, manganese EDTA, and sodium molybdate; Tennessee Farmer's Cooperative, LaVergne, TN). On August 20, 2012 near first flowering, plant height and stem diameter was recorded on 2 plants per plot. Marketable fruit were harvested on October 2, 9, and 15, 2012 from subplots containing four contiguous plants in each plot, and weighed. On October 19, 2012 all green fruit was stripped from plants on the final harvest as an estimation of yield potential. Soils were again sampled at harvest from each plot, as described previously. Soil samples from all sampling dates were analyzed for soil pH, soil inorganic N, extractable P, total N, and total C. Soil pH was determined on air-dried samples in deionized water (pH_{water}; 1:1) and in 0.01-M CaCl₂ (pH_{CaCl2}; 1:2) using a pH electrode (Orion 3-Star Plus pH Benchtop Meter, Thermo Scientific, Waltham,

MA). Soil samples were also analyzed for inorganic and total nitrogen (TN) and total carbon (TC). Briefly, 5 g of air-dried soil was extracted with 1-M KCl for 30 min, centrifuged, and filtered (Whatman 42, Whatman Ltd, Kent, UK) prior to colorimetric analyses for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N} + \text{NO}_2\text{-N}$ using a microplate spectrophotometer (Powerwave XS, Biotek, Winooski, Vermont) as described by Sims et al. (1995). Air-dried and ground soil samples were analyzed by flash combustion for TN and TC (Flash EA 1112 NC Soil Analyzer, Thermo Scientific, Waltham, Massachusetts, USA). Extractable soil phosphorus was determined using a malachite green microplate method (D'Angelo et al. 2001) following extraction of soil as described by Mehlich (1953).

Two root systems were sampled from each sub plot at the end of the season and rated for root galling by root-knot nematodes as described by (Bridge and Page 1980) with the extent of root galling present on tomato plants rated as follows: 0 = no galling, 1 to 4 = galling of secondary roots only, 5 to 10 = galling of primary laterals and tap root. Each root system was also scored for physical root condition on a scale of 1 to 5 (1 = brown with signs of decay, 5 = white and healthy). Soil samples (0 to 15-cm) for nematode population assessment were taken at harvest at the end of the experiment and sent to the Plant Diagnostic Lab at Auburn University, Auburn, AL. Nematode populations (second stage juveniles) in 100-cm³ soil samples were assessed by a sugar flotation technique to identify plant-parasitic nematode populations to the genus-level (Mullen et al. 2005). In addition, weed population and disease pressure were noted during the growing season. Leaf tissue (10 leaves from each plot) was also collected at harvest and analyzed by flash combustion as previously described.

Results were analyzed using the GLM procedure in SAS 9.2 (SAS Institute Inc, Cary, NC). Main effects and differences between means were considered significant at $p < 0.05$, unless

otherwise noted. Protected means were separated using Fisher's least significant difference procedure.

Results

Amendment rates. Biomass added from molasses and molasses + soybean treatments was similar (11,000 and 11,252 kg ha⁻¹, respectively; Table 27). These treatments were designed to have a similar contribution of total C, but a large difference in C:N ratio and N content. The contribution of total C was similar between these two treatments, with 3,885 kg C ha⁻¹ (1.95 mg C g⁻¹ soil) in the molasses treatment and 3,939 kg C ha⁻¹ (2.00 mg C g⁻¹ soil) in the molasses + soybean meal treatment. As expected, there were large differences in total N and C:N ratio of amendments between these two treatments. Molasses + soybean meal amendment contributed a greater rate of N (267 kg N ha⁻¹) compared to the molasses-amended ASD treatment (111 kg N ha⁻¹). The C:N ratio of molasses amendment was 35 while the C:N ratio of molasses + soybean meal treatment was less than half of that at 14.

Soil properties. No significant differences among ASD treatments or the untreated control were observed in the amount of ferrihydrite paint solubilized from IRIS tubes during soil treatment (Table 28). However, there was a distinct trend of slightly higher removal from both ASD treatments (6.0% and 3.7% reduction) compared to the untreated control (0.67%). Soil temperature did not differ among treatments at treatment initiation or at planting, ranging from 28.1 to 30.2°C at the 5-cm depth and 28.2 to 29.3°C at the 15-cm depth prior to soil treatment (Table 29). Prior to alternative soil treatments, gravimetric soil moisture did not differ among treatments, averaging 0.053 g g⁻¹ (data not shown).

Table 27. Total biomass, carbon and nitrogen content, and C:N ratio of soil amendments from on-farm demonstration study, 2012.

	Biomass		Total carbon	Total	C:N Ratio
				nitrogen	
	-----kg ha ⁻¹ -----		---mg C g ⁻¹ soil-	---kg ha ⁻¹ ---	----C:N---
ASD: molasses	11,000	3,885	1.95	111	35
ASD: molasses + soybean meal	11,252	3,939	2.00	267	14
Molasses	9,041	3,165	1.60	90	35
Soybean meal	2,211	774	0.40	177	4
Untreated control	N/A	N/A	N/A	N/A	N/A

Table 28. Mean percentage of ferrihydrite paint solubilization on IRIS tubes from on-farm demonstration study, 2012.

	-----%---
MeBr + chloropicrin	N/A
Metam sodium	N/A
ASD: molasses	6.0
ASD: molasses + soybean meal	3.1
Untreated control	0.67

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table 29. Mean post treatment soil temperature at 5-cm and 15-cm depth from on-farm demonstration study, 2012

Treatment	5-cm depth	15-cm depth
	-----Soil temperature, °C----	
MeBr + chloropicrin	30.2	28.5
Metam sodium	29.8	29.3
ASD: molasses	29.0	28.7
ASD: molasses + soybean meal	29.4	29.0
Untreated control	28.1	28.2

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

[§]Sample taken following planting, producer had begun fertigation.

No significant differences were observed among treatments for pre-treatment soil pH_{water} or $\text{pH}_{\text{CaCl}_2}$ (Table 30). Soil pH_{water} averaged 6.55 and soil $\text{pH}_{\text{CaCl}_2}$ averaged 6.21. At planting, soil $\text{pH}_{\text{CaCl}_2}$ was highest in the molasses treatment (6.53), which was higher than all other treatments including the soil fumigant controls. No differences were observed among treatments at planting for soil pH_{water} , which ranged from 6.66 for ASD with molasses + soybean meal to 7.04 for ASD with molasses. At harvest, soil pH_{water} increased from planting in all treatments, although no differences were observed among treatments (range of 7.13 in the ASD with molasses + soybean meal treatment to 7.47 in the MeBr + chloropicrin treatment). Soil $\text{pH}_{\text{CaCl}_2}$ also increased in this time period, ranging from 6.59 in the metam sodium treatment to 6.74 in the molasses treatment.

Total soil nitrogen (TN) did not differ prior to soil treatments (excluding fumigant controls; average of 0.74 g N kg^{-1} ; Table 31). At planting, ASD treatments with molasses or molasses + soybean meal had higher total soil N than all other treatments with 0.88 g N kg^{-1} . Neither of the soil fumigant treatments differed from the untreated control (mean of 0.65 g N kg^{-1}). Total soil C did not differ between treatments prior to treatment application (excluding the soil fumigants) with an average of 7.2 g C kg^{-1} soil. At planting, the ASD with molasses and ASD with molasses + soybean meal treatments had the highest amount of total soil C (7.8 and 6.9 g C kg^{-1} soil, respectively), which were significantly higher than the total soil C observed in the fumigant control treatments (average of 5.05 g C kg^{-1}). The soil C:N ratio prior to treatment did not differ among the ASD treatments with molasses or molasses + soybean meal, and the untreated control (range of 9.7 to 10.2). At planting, the ASD with molasses treatment had the highest soil C:N ratio (8.9), which was greater than all treatments except the untreated control.

Table 30. Mean soil pH in water (pH_{water}) and CaCl_2 ($\text{pH}_{\text{CaCl}_2}$) from on-farm demonstration study, 2012

	-----Pre Treatment-----		-----Post Treatment-----		-----Harvest-----	
	pH_{water}	$\text{pH}_{\text{CaCl}_2}$	pH_{water}	$\text{pH}_{\text{CaCl}_2}$	pH_{water}	$\text{pH}_{\text{CaCl}_2}$
MeBr + chloropicrin	N/A	N/A	6.78	6.31b [†]	7.47	6.61
Metam sodium	N/A	N/A	6.69	6.30b	7.26	6.59
ASD: molasses	6.55	6.21	7.04	6.53a	7.34	6.74
ASD: molasses + soybean meal	6.56	6.23	6.66	6.37b	7.13	6.71
Untreated control	6.54	6.19	6.75	6.40b	7.38	6.71

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table 31. Total nitrogen (TN), total carbon (TC), and carbon:nitrogen ratio (C:N) of soils sampled from on-farm demonstration study, 2012.

	-----Pre- treatment-----			-----Post-treatment§-----			-----Harvest-----		
	TC	TN	C:N	TC	TN	C:N	TC	TN	C:N
	g C kg ⁻¹	g N kg ⁻¹		g C kg ⁻¹	g N kg ⁻¹		g C kg ⁻¹	g N kg ⁻¹	
MeBr + chloropicrin	N/A [†]	N/A	N/A	5.0c	0.65b	7.53b	6.6	0.67	9.8ab
Metam sodium	N/A	N/A	N/A	5.1c	0.65b	7.67b	6.0	0.66	9.2b
ASD: molasses	7.2	0.75	10.2	7.8a	0.88a	8.92a	8.1	0.77	10.4a
ASD: molasses + soybean meal	7.2	0.73	9.84	6.9ab	0.88a	7.64b	7.3	0.71	10.1ab
Untreated control	7.2	0.75	9.70	5.7bc	0.70b	8.24ab	6.7	0.67	10.0ab

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$. §Sample taken following planting, producer had begun fertigation.

At harvest, the C:N ratio was greatest in the molasses treatment (10.4), which was greater than the metam sodium treatment.

Prior to treatment, soil $\text{NH}_4\text{-N}$, $\text{NO}_3\text{+NO}_2\text{-N}$, and total inorganic N did not differ among treatments (Table 32). Soil $\text{NH}_4\text{-N}$ ranged between 4.7 mg N kg^{-1} soil in the ASD with molasses + soybean meal treatment to 9.0 mg N kg^{-1} in the untreated control. Soil $\text{NO}_3\text{NO}_2\text{-N}$ ranged between $40.0 \text{ mg N kg}^{-1}$ in the ASD with molasses + soybean meal treatment to $51.6 \text{ mg N kg}^{-1}$ in the ASD with molasses treatment. Total inorganic N ranged between $44.9 \text{ mg N kg}^{-1}$ soil in the ASD with molasses treatment to $58.5 \text{ mg N kg}^{-1}$ in the untreated control. At planting the ASD with molasses treatment, while not differing from the ASD with molasses + soybean meal treatment, was highest with $1.78 \text{ mg N kg}^{-1}$ in soil $\text{NH}_4\text{-N}$. Soil $\text{NO}_3\text{NO}_2\text{-N}$ was greatest for the ASD with molasses + soybean meal treatment with $94.4 \text{ mg N kg}^{-1}$, which differed from all other treatments. In addition, the ASD with molasses + soybean meal treatment also had the highest total soil inorganic N with $95.7 \text{ mg N kg}^{-1}$, which was significantly higher than all other treatments. At harvest, there were no differences among treatment of TIN with a range between 6.2 mg N kg^{-1} in the MeBr + chloropicrin treatment to $25.2 \text{ mg N kg}^{-1}$ in the molasses + soybean meal treatment.

Extractable soil P did not differ among non-fumigant treatments prior to treatment application (Table 33), ranging between $60.1 \text{ mg P kg}^{-1}$ soil to $68.5 \text{ mg P kg}^{-1}$. At planting, the ASD with molasses treatment had a higher level of extractable soil P ($62.4 \text{ mg P kg}^{-1}$), which was greater than that observed from the fumigant and untreated controls. At harvest, there was difference among treatments, ranging between 55.9 to $58.8 \text{ mg P kg}^{-1}$.

Table 32. Soil ammonium (NH₄-N), nitrate + nitrite (NO₃ + NO₂-N), and total inorganic nitrogen (TIN) from on-farm demonstration study, 2012.

Treatment	-----Pre treatment-----			-----Post treatment [§] -----			-----Harvest-----		
	NH ₄ N	NO ₃ NO ₂ - N	TIN	NH ₄ N	NO ₃ NO ₂ - N	TIN	NH ₄ N	NO ₃ NO ₂ - N	TIN
	----- mg N kg ⁻¹ soil-----								
MeBr + chloropicrin	N/A [†]	N/A	N/A	0.7c	30.4b	31.1b	1.07	5.2	6.2
Metam sodium	N/A	N/A	N/A	0.9bc	20.6bc	21.5bc	1.0	9.7	10.7
ASD: molasses	6.3	51.7	58.1	1.8a	12.8c	14.6c	1.2	19.6	20.8
ASD: molasses+ soybean meal	4.7	40.0	44.9	1.4ab	94.4a	95.7a	1.0	24.2	25.2
Untreated control	9.0	49.2	58.5	1.1bc	28.1b	29.2b	1.4	9.2	10.6

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$. [§]Sample taken following planting, producer had begun fertigation.

Table 33. Extractable soil phosphorus from on-farm demonstration study, 2012.

Treatment	Pre treatment	Post treatment	Harvest
	----- mg P kg ⁻¹ soil-----		
MeBr + chloropicrin	N/A [†]	49.4b	56.9
Metam sodium	N/A	35.5c	55.9
ASD: molasses	68.5	62.4a	58.8
ASD: molasses + soybean meal	60.1	54.9ab	55.9
Untreated control	67.6	45.0bc	57.2

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.§Sample taken following planting, producer had begun fertigation.

Crop performance. Measurements of tomato plant height and stem diameter did not differ among treatments at flowering (Table 34). Plant height ranged between 54.4 cm in the ASD with molasses treatment to 65.7 cm in the untreated control treatment. Tomato stem diameter ranged between 9.1 mm for the untreated control to 10.3 mm in the ASD with molasses + soybean treatment. At harvest, N concentration in tomato leaf tissue did not differ among treatments (Table 35). Leaf N concentration ranged between 5.51 cg g⁻¹ in the ASD with molasses and untreated control treatments to 5.93 cg g⁻¹ in the MeBr + chloropicrin treatment.

Total yields (marketable and green fruit) were similar among treatments (Fig. 8), ranging from 11,766 kg ha⁻¹ in the metam sodium treatment to 13,269 kg ha⁻¹ in the untreated control. For total yields, the untreated control had a 95% confidence interval that was wider than all other treatments (9,082 to 17,456 kg ha⁻¹). Total marketable tomato yields did not differ among treatments ranging between 4,056 kg ha⁻¹ in the metam sodium treatment to 4,747 kg ha⁻¹ in MeBr + chloropicrin treatment. Green tomato yields were also similar among treatments ranging between 7,711 kg ha⁻¹ in the metam sodium to 8,936 kg ha⁻¹ in the untreated control.

Root galling and nematode populations. Ratings of root galling did not differ among treatments, with ratings consistently below 1 for all treatments (data not shown). Similarly, nematode populations extracted from soil samples taken at harvest did not differ among treatments (Table 36). Lesion nematode (*Pratylenchus* spp.) populations ranged between 1.0 juvenile 100 cm⁻³ soil in the ASD with molasses treatment to 9.0 juveniles 100 cm⁻³ soil in the untreated control. Spiral nematodes (*Helicotylenchus* spp.) had the highest of the nematode genera observed with a range between 5.5 juveniles 100 cm⁻³ soil in the untreated control to 24.0 juveniles 100 cm⁻³ soil in the MeBr + chloropicrin treatment. Root-knot nematodes (*Melodogyne* spp.) ranged between 0.5

Table 34. Mid-season tomato height and stem diameter of ‘Smarty’ tomatoes from on-farm demonstration study, 2012.

	Height	Stem diameter
	--cm--	--mm--
MeBr + chloropicrin	57.8 [†]	9.8
Metam sodium	63.2	9.6
ASD: molasses	54.4	9.6
ASD: molasses + soybean meal	56.4	10.3
Untreated control	65.7	9.1

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table 35. Nitrogen concentration in tomato leaf tissue of ‘Smarty’ tomatoes at first harvest from on-farm demonstration study, 2012.

	TN
	--- cg g ⁻¹ ---
MeBr + chloropicrin	5.93 [†]
Metam sodium	5.64
ASD: molasses	5.51
ASD: molasses + soybean meal	5.73
Untreated control	5.51

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

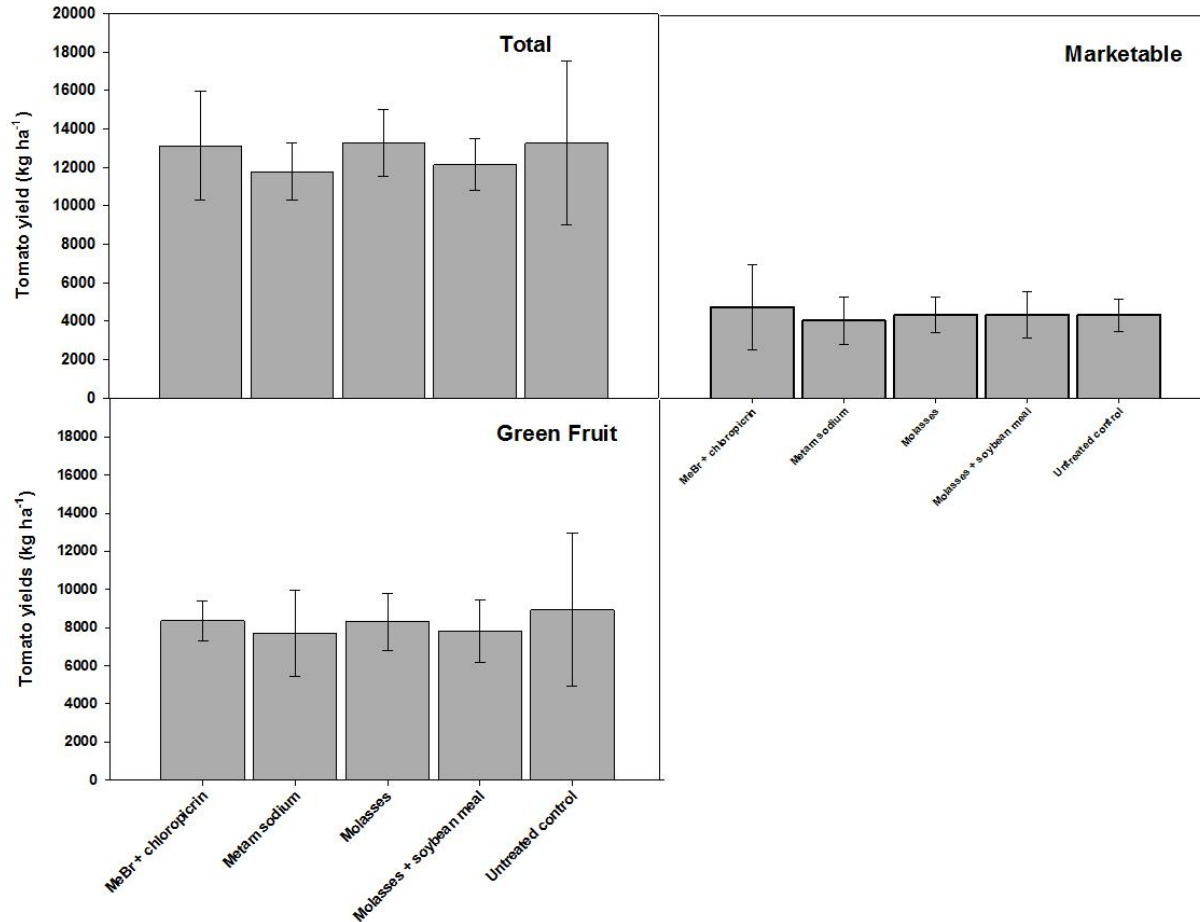


Figure 10. Total, marketable, and green fruit yields (kg ha⁻¹) of grape tomatoes from on-farm demonstration study, 2012. Within yield category, means indicated by different letters are significantly different, $p < 0.05$. Error bars represent 95% confidence intervals.

Table 36. Populations of lesion, spiral, root-knot, and stunt nematode second-stage juveniles extracted from soil at harvest, 2012.

	Lesion	Spiral	Root-knot	Stunt
	-----nematodes 100 cm ⁻³ soil-----			
MeBr + chloropicrin	8.0 [†]	24.0	17.0	0.0
Metam sodium	2.5	14.5	6.5	0.0
ASD: molasses	1.0	22.5	6.5	0.0
ASD: molasses + soybean meal	2.5	6.5	0.5	2.5
Untreated control	9.0	5.5	22.0	0.0

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

juveniles 100 cm⁻³ soil in the ASD with molasses + soybean meal treatment to 22.0 juveniles 100 cm⁻³ soil in the untreated control.

Discussion

At planting, soil properties did differ in many respects in the ASD treatments (molasses or molasses + soybean meal) compared to treatments without organic amendments. For example, soil total N and C were generally higher in these treatments, which would have provided a greater amount of C and N for microbial growth during treatment. Furthermore, at planting the ASD with molasses treatment had the highest soil P, soil C:N ratio, and soil NH₄-N; soil NO₃+NO₂-N, and total inorganic N were highest from the ASD with molasses + soybean meal treatment. However, these differences did not affect total marketable tomato yields. Weekly fertility was uniform for all treatments and any noticeable differences in N were likely outweighed by weekly fertigation at a high rate of fertility.

The ASD treatment with molasses + soybean meal included a greater total N application in the C amendments than the ASD with molasses treatment (267 compared to 111 kg N ha⁻¹). As the total C added in C amendments was similar between treatments, the ASD treatment with molasses + soybean meal had a much lower C:N ratio than the ASD treatment with molasses alone (14 compared to 35). In previous ASD studies, molasses treatments have been applied at a lower rate (5,600 kg ha⁻¹) and combined with feather meal (638 kg ha⁻¹; McCarty et al. 2012a; McCarty et al. 2012b). The C:N ratio of liquid molasses reported by Butler and colleagues (2012a) was 35.2 with a total C amount of 2.64 mg C g⁻¹.

The fumigant control treatments used in our study generally did not differ from the untreated control in regard to soil nutrient properties, likely due to high rates of fertility used by

conventional vegetable growers and the lack of organic matter additions in these treatments. The impact on crop performance and plant-parasitic nematode populations also did not differ significantly from any of the nonchemical and untreated control treatments; however, pest populations were low for all treatments including the untreated control, limiting the interpretation of results.

The IRIS tubes indicated that the ASD treatments were leading to anaerobic conditions in the soil. In a previous study, the percentage of ferrihydrite paint solubilized ranged between 18 and 37%, although no differences were observed among treatments (McCarty et al. 2012a). For our current study, the range was between 6% for the ASD with molasses treatment ($2.0 \text{ mg C g soil}^{-1}$) to 0.7% in the untreated control. McCarty and colleagues (2012a) observed that a molasses treatment ($1.18 \text{ mg C g soil}^{-1}$) resulted in a 14% ferrihydrite paint solubilization. The reason behind the lower reduction in our current study could be based on the surface application of amendments and possibly the differences in soil texture and properties within the site. Given that soil saturation is an important part of Fe reduction (Castenson and Rabenhorst 2006), the ability of soil type to hold saturation for a long period of time will in turn trigger greater reduction. It is possible that the soil type here with the higher sand fraction triggered the lower Fe reduction. At the same time, the soluble components of the molasses should have transported by the irrigation deeper into the soil profile and been easily consumed by soil microbial communities. Additional studies are needed to better assess the relationship of IRIS tube data to soil anaerobic activity triggered by ASD treatments.

Currently, yield data from previous ASD studies has only been reported preliminarily (Butler et al. 2012b; Butler et al. 2009; Shennan et al. 2010). There were no differences in yields among treatments in our study. However, there tended to be increased variability with the

untreated control especially in regard to total tomato yields. A different tomato variety other than a grape-type and grading of subsequent tomato variety could have resulted in observed differences in size, fruit quality, culls, and possibly yields. It could also be compared much more easily to the results from McCarty et al. (2013), which did not find any differences in total yield but did observe some differences in large fruit yields in an ASD with molasses treatment. A pot study by McCarty and colleagues (2013) reported that a dwarf tomato variety, 'Florida Lanai', in a molasses treatment (applied at 5,600 kg ha⁻¹) had greater yields than the untreated control while not differing from other cover crop amended ASD treatments.

Nematodes did not appear to be a limiting factor for crop performance in our study. The nematode populations and incidences of galling that were noted were very low (all gall ratings less than 2; low plant-parasitic nematode populations). If nematode populations were more robust, greater differences among treatments may have been observed, especially given that the range of populations was wide for root-knot nematode (0.05 to 22.0 juveniles to 100 cm⁻³ soil). In a previous ASD study combined with solarization, molasses (8.2 Mg ha⁻¹) with and without poultry litter and with irrigation (5 and 10 cm) at the end two seasons of a double-crop of bell pepper and eggplant indicated that ASD treatments led to lower populations of *M. incognita* compared to the untreated control and solarization alone treatments (Butler et al. 2012b). While ASD treatment does present the possibility of controlling nematode populations, the untreated control in our experiment did not have high populations, which would not be expected to greatly impact yield. Additional studies on sites with high existing root-knot nematode populations would help to clarify the response of root-knot nematode to ASD treatment.

Further studies are needed to assess the viability of ASD treatment in Tennessee warm-season vegetable production. While there is evidence that these ASD alternatives do not

negatively impact yield of grape tomato, the lack of pest pressure in the field study inhibits generalizations and recommendations for tomato growers. This study presents data from one year of an on-farm demonstration which can be used as a starting point for additional on farm-demonstrations of ASD in Tennessee and Southeastern US. On-farm studies over several years from a number of diverse sites with high pest pressure are likely needed to adequately assess ASD treatment versus standard grower practices.

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APPENDIX

Table A1. Mean ammonium (NH₄-N), nitrate + nitrite (NO₃ + NO₂-N), total inorganic nitrogen (TIN) mineralized from soil samples in 2012 during 30-day incubation at 25°C, field study 2012.

	NH ₄ -N	NO ₃ +NO ₂ -N	TIN	NH ₄ -N	NO ₃ +NO ₂ -N	TIN	NH ₄ -N	NO ₃ +NO ₂ -N	TIN	NH ₄ -N	NO ₃ +NO ₂ -N	TIN
	Day 3			Day 10			Day 17			Day 30		
	----- mg N kg ⁻¹ soil-----											
Untreated control	2.5†	13.2a	15.7ab	0.5	20.4	20.8	0.6a	34.0	34.1bcd	0.4	55.0c	55.5c
Biofumigant control	2.5	5.4b	7.9bc	0.5	17.2	17.7	0.4ab	23.3	24.0cd	0.1	48.1c	48.2c
Molasses	1.8	4.0b	5.8c	1.1	10.5	11.5	0.2b	20.0	20.9d	0.5	52.6c	53.1c
Mustard/arugula + molasses	11	8.1ab	19.1a	0.6	23.7	24.3	0.4ab	47.7	56.3a	0.5	94.3a	94.8a
Mustard/arugula	14.4	3.8b	18.2a	0.8	28.6	29.4	0.3ab	42.7	43.4abc	0.6	71.4b	72.0b
Cereal rye + molasses	2.1	4.9b	7.0bc	0.8	20.2	21.1	0.4ab	41.1	41.9abc	0.3	74.9b	75.2b
Cereal rye	5.8	6.3b	5.8c	1.0	23.4	24.4	0.2b	45.0	45.6ab	0.3	84.0ab	84.3ab

[†]Within columns, means indicated by different letters are significantly different, *p* < 0.05.

Table A2. Total soil carbon (TC), field study 2011 and 2012.

	Pre treatment	Post treatment	Harvest	Pre treatment	Post treatment	Harvest tomato	Harvest pepper
	-----2011-----			-----2012-----			
	-----g C kg ⁻¹ soil-----						
Untreated control	1.1ab [†]	1.12	0.89b	1.13	0.89c	1.06bc	1.04b
Biofumigant control	1.19ab	1.10	0.92b	1.01	0.92bc	0.97c	1.00b
Molasses	1.07b	1.10	0.91b	1.05	0.91bc	1.02c	1.04b
Mustard/arugula + molasses	1.08b	1.05	0.94ab	1.06	1.01a	1.31a	1.08b
Mustard/arugula	1.14ab	1.07	0.91b	0.88	1.00ab	1.11abc	1.04b
Cereal rye + molasses	1.23a	1.17	1.07a	1.06	1.06a	1.24ab	1.24a
Cereal rye	1.13ab	1.06	0.99ab	1.09	0.97abc	1.08bc	1.11ab

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A3. Total soil nitrogen (TN), field study 2011 and 2012.

	Pre treatment	Post treatment	Harvest	Pre treatment	Post treatment	Harvest tomato	Harvest pepper
	-----2011-----			-----2012-----			
	-----g N kg ⁻¹ soil-----						
Untreated control	0.12 [†]	0.12	0.10ab	0.12	0.09	0.13bc	0.11
Biofumigant control	0.12	0.12	0.10ab	0.10	0.09	0.11c	0.11
Molasses	0.11	0.11	0.09b	0.11	0.09	0.12bc	0.11
Mustard/arugula + molasses	0.11	0.11	0.10ab	0.11	0.11	0.16a	0.12
Mustard/arugula	0.12	0.11	0.10ab	0.11	0.10	0.13bc	0.12
Cereal rye + molasses	0.13	0.12	0.11a	0.11	0.11	0.14ab	0.13
Cereal rye	0.12	0.11	0.10ab	0.11	0.10	0.13bc	0.12

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A4. Extractable soil phosphorus, field study 2011 and 2012.

	Pre treatment	Post treatment	Harvest	Pre treatment	Post treatment	Harvest Tomato	Harvest Pepper
	-----2011-----			-----2012-----			
	-----mg P kg ⁻¹ soil-----						
Untreated control	28.3ab [†]	28.3	12.6	33.1	24.9	21.0	21.8ab
Biofumigant control	21.0b	25.0	18.3	26.7	28.9	23.7	21.3ab
Molasses	23.5b	22.9	13.4	26.7	27.9	18.1	20.4b
Mustard/arugula + molasses	27.5b	22.2	22.7	29.0	34.0	20.1	32.0a
Mustard/arugula	29.6ab	23.8	16.2	25.3	38.5	22.5	27.8ab
Cereal rye + molasses	38.3a	25.8	26.8	28.6	29.7	24.1	28.6ab
Cereal rye	25.7b	22.2	18.4	25.6	28.5	20.5	20.8ab

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A5. Early and mid-season height and stem diameter, field study bell peppers 2011 and 2012.

	Early Season		Mid Season		Early Season		Mid Season	
	Height	Diameter	Height	Diameter	Height	Diameter	Height	Diameter
	-----2011-----				-----2012-----			
	--cm--	--mm--	--cm--	--mm--	--cm--	--mm--	--cm--	--mm--
Untreated control	33.2 [†]	4.4	55.5	19.3a	17.9ab	7.0	56.5	18.5
Biofumigant control	30.1	4.2	58.4	19.4a	13.8cd	6.5	58.9	18.9
Molasses	30.3	4.4	60.8	19.8a	14.9bcd	6.0	55.3	15.8
Mustard/arugula + molasses	29.4	3.7	56.1	17.5ab	16.2abcd	6.5	53.9	17.8
Mustard/arugula	31.1	4.3	59.0	18.3a	18.1a	6.7	59.1	17.2
Cereal rye + molasses	28.3	3.7	54.4	18.8a	16.5abc	6.3	54.6	18.4
Cereal rye	30.1	3.6	54.1	14.9b	13.4d	5.2	55.2	17.2

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A6. Early and mid-season height and stem diameter of tomato, field study 2011 and 2012.

	Early Season		Mid Season		Early Season		Mid Season	
	Height	Diameter	Height	Diameter	Height	Diameter	Height	Diameter
	-----2011-----				-----2012-----			
	--cm--	--mm--	--cm--	--mm--	--cm--	--mm--	--cm--	--mm--
Untreated control	52.9a [†]	5.9bc	99.4	16.0	42.6	12.7	98.3	18.9
Biofumigant control	55.3	6.0b	98.5	17.2	41.2	12.9	97.5	19.5
Molasses	57.8	6.8a	102.4	20.2	37.9	12.7	98.5	18.3
Mustard/arugula + molasses	56.8	6.3ab	96.6	17.6	37.9	13.5	95.5	20.0
Mustard/arugula	53.0	6.2ab	97.9	16.9	46.8	14.1	102.4	19.8
Cereal rye + molasses	56.6	6.4a	95.3	18.9	45.4	13.7	99.2	18.2
Cereal rye	49.5	5.2c	92.9	17.0	45.4	14.2	102.6	16.8

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A7. Length, diameter, and pericarp thickness from ‘fancy’ peppers, field study 2011.

	Length	Diameter	Pericarp thickness	Length	Diameter	Pericarp thickness
	-----mm-----					
Untreated control	87.9 [†]	87.9	6.3	77.1	67.3	6.6
Biofumigant control	87.2	86.2	6.4	81.1	65.3	6.8
Molasses	84.6	87.5	6.1	69.8	65.5	5.7
Mustard/arugula + molasses	90.7	84.2	6.7	65.7	63.9	6.6
Mustard/arugula	81.1	86.6	6.2	50.5	47.8	6.1
Cereal rye + molasses	88.1	85.1	6.2	69.2	58.4	7.4
Cereal rye	86.4	88.2	6.0	73.5	63.5	6.7

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A8. Fruit pH, soluble solids content (SSC) and titratable acidity, field study 2011.

	Pepper pH	Tomato pH	Pepper SSC %	Tomato SSC %	Pepper titratable acidity g citric acid 100 mL ⁻¹	Tomato titratable acidity g citric acid 100 mL ⁻¹
Untreated control	5.38 [†]	4.67	4.40	3.77b	0.06	0.23a
Biofumigant control	5.36	4.61	4.65	4.01a	0.06	0.24a
Molasses	5.33	4.63	5.03	4.06a	0.07	0.24a
Mustard/arugula + molasses	5.34	4.66	4.95	4.06a	0.05	0.21a
Mustard/arugula	5.46	4.60	4.53	3.98a	0.05	0.23a
Cereal rye + molasses	5.40	4.66	4.40	4.10a	0.06	0.27a
Cereal rye	5.34	4.66	4.90	3.99a	0.05	0.21b

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A9. Fruit pH, soluble solids content (SSC) and titratable acidity, field study 2012.

	Pepper pH	Tomato pH	Pepper SSC %	Tomato SSC %	Pepper titratable acidity g citric acid 100 mL ⁻¹	Tomato titratable acidity g citric acid 100 mL ⁻¹
Untreated control	5.02 [†]	4.68	3.44	3.42	0.09	0.31
Biofumigant control	5.07	4.68	3.19	3.19	0.11	0.34
Molasses	5.01	4.73	3.40	3.30	0.14	0.28
Mustard/arugula + molasses	5.00	4.70	3.33	3.40	0.10	0.29
Mustard/arugula	5.07	4.87	3.41	3.46	0.12	0.26
Cereal rye + molasses	4.96	4.69	3.30	3.41	0.11	0.31
Cereal rye	4.98	4.78	3.11	3.33	0.10	0.22

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A10. Mean percentage of ferrihydrite paint solubilization on IRIS tubes, field study 2012.

	---% reduced---
Untreated control	32 [†]
Biofumigant control	29
Molasses	14
Mustard/arugula + molasses	28
Mustard/arugula	36
Cereal rye + molasses	37
Cereal rye	18

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A11. Nitrogen concentration in tomato and bell pepper leaf tissue at first harvest, field study 2011 and 2012.

	Tomato	Pepper	Tomato	Pepper
	-----2011-----		-----2012-----	
	-----cg N g ⁻¹ -----			
Untreated control	4.69†	4.43	8.05	4.18
Biofumigant control	4.97	4.28	6.07	4.38
Molasses	4.68	3.62	6.23	4.34
Mustard/arugula + molasses	4.45	3.90	6.34	4.66
Mustard/arugula	4.97	4.59	6.36	4.45
Cereal rye + molasses	4.83	4.15	6.31	4.24
Cereal rye	4.77	3.65	6.22	4.34

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A12. Soil moisture, growth chamber study Trial 1.

	Pre ^a treatment	During ^b treatment	Post ^a treatment
	---g g ⁻¹ ---	-cm cm ⁻³ -	---g g ⁻¹ ---
Untreated Control	0.09 [†]	0.2	0.14
Biofumigant Control	0.06	0.14	0.13
Molasses	0.07	0.24	0.16
Mustard/Arugula + Molasses	0.11	0.19	0.16
Mustard/Arugula	0.12	0.20	0.12
Cereal Rye + Molasses	0.12	0.22	0.17
Cereal Rye	0.12	0.22	0.15
Crimson Clover	0.10	0.18	0.13
Hairy Vetch	0.17	0.19	0.15
Wheat	0.10	0.18	0.14

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$. ^a Gravimetric moisture determined using soil sample collected from raised beds post treatment. ^b Volumetric moisture determined using 5TM sensor during treatment.

Table A14. Aboveground tomato biomass and root biomass, growth chamber study Trial 1 and 2.

	-----Trial 1-----		-----Trial 2-----	
	Shoot Biomass, Dry	Root Biomass, Wet	Shoot Biomass, Dry	Root Biomass, Wet
	-----g pot ⁻¹ -----			
Untreated Control	20a [†]	40a	50	30a
Biofumigant Control	20a	30ab	50	30a
Molasses	20a	30ab	40	30a
Mustard/Arugula + Molasses	10ab	20ab	40	20bc
Mustard/Arugula	20a	30ab	30	20bc
Cereal Rye + Molasses	10ab	20ab	40	20bc
Cereal Rye	20a	20ab	40	40a
Crimson Clover	10ab	20ab	40	40a
Hairy Vetch	10ab	30ab	40	20bc
Wheat	20a	30ab	50	20bc

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A15. Total soil nitrogen (TN), growth chamber study trial 1 and 2.

	Pre treatment	Post treatment	Harvest	Pre treatment	Post treatment	Harvest
	-----Trial 1-----			-----Trial 2-----		
	-----g N kg ⁻¹ soil-----					
Untreated control	0.06 [†]	0.09	0.07	0.06	0.08bc	0.08
Biofumigant control	0.05	0.09	0.06	0.07	0.06e	0.08
Molasses	0.04	0.07	0.06	0.04	0.05e	0.07
Mustard/arugula + molasses	0.04	0.08	0.06	0.06	0.06cde	0.08
Mustard/arugula	0.06	0.09	0.06	0.08	0.06de	0.07
Cereal rye + molasses	0.06	0.09	0.06	0.07	0.07cde	0.08
Cereal rye	0.05	0.08	0.06	0.08	0.10a	0.09
Crimson clover	0.04	0.07	0.05	0.06	0.08bcd	0.07
Hairy vetch	0.05	0.08	0.06	0.07	0.09ab	0.08
Wheat	0.03	0.07	0.04	0.08	0.06de	0.08

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A16. Total soil carbon (TC), growth chamber study trial 1 and 2.

	Pre treatment	Post treatment	Harvest	Pre treatment	Post treatment	Harvest
	-----Trial 1-----			-----Trial 2-----		
	----- g C kg ⁻¹ soil -----					
Untreated control	0.60 [†]	0.76ab [†]	0.80	0.40	0.73ab	0.68
Biofumigant control	0.52	0.72ab	0.68	0.43	0.57cd	0.63
Molasses	0.41	0.63bc	0.67	0.17	0.53d	0.52
Mustard/arugula + molasses	0.46	0.69bc	0.85	0.34	0.58cd	0.68
Mustard/arugula	0.70	0.75ab	0.80	0.46	0.61bcd	0.62
Cereal rye + molasses	0.67	0.90a	0.75	0.33	0.65abcd	0.61
Cereal rye	0.51	0.71ab	0.74	0.42	0.66abcd	0.72
Crimson clover	0.48	0.58bc	0.55	0.33	0.69abc	0.53
Hairy vetch	0.55	0.50c	0.68	0.36	0.77a	0.60
Wheat	0.31	0.56bc	0.43	0.51	0.61bcd	0.62

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A17. Extractable soil phosphorus, growth chamber study Trial 1 and 2.

	-----Trial 1-----			-----Trial 2-----		
	Pre treatment	Post treatment	Harvest	Pre treatment	Post	Harvest
	-----mg P kg ⁻¹ -----					
Untreated control	13.6 [†]	22.2	26.4	17.3	15.7	23.9
Biofumigant control	12.9	18.4	26.4	25.6	18.2	29.3
Molasses	16.8	14.8	22.9	13.2	15.5	24.5
Mustard/arugula + molasses	13.4	15.1	23.4	28.7	18.2	26.2
Mustard/arugula	16.9	16.8	26.5	23.8	14.1	25.7
Cereal rye + Molasses	14.3	17.4	24.7	25.6	15.3	23.1
Cereal rye	13.3	16.1	28.6	26.5	7.8	28.1
Crimson clover	10.9	16.1	19.4	25.4	14.6	25.6
Hairy vetch	13.8	12.8	21.9	30.7	19.1	26.8
Wheat	11.0	15.7	25.8	0.9	9.3	28.3

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A18. Mean soil temperatures during soil treatment, growth chamber study Trial 1 and 2.

	Trial 1	Trial 2
	-----Soil temperature, °C-----	
Untreated control	20.9 [†]	19.4
Biofumigant control	20.7	18.9
Molasses	20.9	18.9
Mustard/arugula + molasses	20.9	18.3
Mustard/arugula	21.1	18.7
Cereal rye + molasses	21.1	19.1
Cereal rye	20.9	18.5
Crimson clover	21.0	18.9
Hairy vetch	21.3	18.5
Wheat	20.8	18.7

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A19. Rating of root-galling by root-knot nematode at harvest, on-farm study 2012.

Root galling	
----- Visual rating-----	
MeBr + chloropicrin	0.50 [†]
Metam sodium	0.50
ASD: molasses	0.38
ASD: molasses +	0.00
soybean meal	
Untreated control	0.38

[†]Within columns, means indicated by different letters are significantly different, $p < 0.05$.

Table A20. Mean gravimetric soil moisture, on-farm study 2012.

	Pre	Post
	treatment	treatment
	-----g g ⁻¹ -----	
MeBr + chloropicrin	N/A	0.19
Metam sodium	N/A	0.24
Molasses	0.06	0.21
Molasses + soybean meal	0.05	0.28
Untreated control	0.05	0.19

^aWithin columns, means indicated by different letters are significantly different, $p < 0.05$. ^bGravimetric moisture determined using soil sample collected from raised beds post treatment.

VITA

David Grant McCarty is from Kodak, TN. He received a Bachelor's of Arts in Sociology from Maryville College in 2008. Following this, he worked with UT's Organic and Sustainable Crop Production Unit as the Intern Coordinator in Summer 2010.