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Impacts of Biofumigation and Anaerobic Soil Disinfestation on Strawberry Production

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To the Graduate Council:

I am submitting herewith a thesis written by Jennifer Renee' Wheeler entitled "Impacts of Biofumigation and Anaerobic Soil Disinfestation on Strawberry Production." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

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Impacts of Biofumigation and Anaerobic Soil Disinfestation on Strawberry Production

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Abstract

Due to the phase-out of methyl bromide, there is a need for alternative, non-chemical fumigation treatments in strawberry production. Anaerobic soil disinfestation and biofumigation are two non-chemical methods for controlling soilborne plant pathogens of strawberry. This study was designed to observe strawberry fruit nutrition and soil volatiles of a strawberry field being treated with biofumigation treatments, anaerobic soil disinfestation treatments, and a combination of the two alternative methods. A trial was conducted with 11 pre-plant soil-incorporated treatments arranged in a randomized complete block design with 6 rows (blocks). Biofumigation treatments consisted of deactivated mustard meal, deoiled mustard meal, mustard pellets, and Biofence mustard seed meal. Other treatments included dried molasses as a carbon source for an anaerobic treatment and a Basamid® chemical treatment. Additional combination treatments of deactivated mustard meal combined with molasses, deoiled mustard meal combined with molasses, and molasses combined with soybean meal (to lower amendment Carbon:Nitrogen ratio) were also applied, as well as an untreated control. Soil samples were taken at designated times post irrigation application in order to measure sinigrin and allyl isothiocyanate simultaneously. Harvested fruit were counted, weighed, and graded into marketable and non-marketable categories, and were then analyzed for sugars (fructose, glucose, and sucrose), organic acids (malic and citric), and mineral content (B, Na, Mg, P, S, K, Ca, Fe, Cu, Mn, and Zn). The combination of deoiled mustard meal and molasses can provide a comparable marketable yield as the chemical treatment Basamid®. Fruit sugar and organic acid content did not consistently differ among most treatments. Likewise, there were no consistent patterns of differences among treatments in mineral

content of either fruit or leaf tissues. In general, the alternative methods of biofumigation and soil anaerobic disinfestation produced fruit of equal quality to that produced using the Basamid® chemical treatment. Future work will evaluate pathogen and soil nutrient dynamics affecting productivity in these alternative soil disinfestation treatments.

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Chapter 1: Introduction

Introduction

The U.S. Clean Air Act of 2005 banned the use of methyl bromide due to its ozone depleting properties and U.S. obligations under the Montreal Protocol. Before this time, methyl bromide was one of the most common fumigants that agricultural producers used in order to reduce populations of pathogens. Most of the alternative methods have negative aspects similar to those of methyl bromide. Therefore, vegetable producers are looking for safe and sustainable ways to grow profitable and healthy plants. *Brassicas* have been targeted as rotational crops, due to their chemical composition containing glucosinolates. Glucosinolates are chemicals that have shown pesticidal activity. This can primarily be attributed to one of the secondary products of glucosinolates, allyl isothiocyanates (AITCs). Indian mustard (*Brassica juncea*) has been found to contain high levels of AITCs. Mustard meal, a seed residual remaining after oil extraction, can contain high concentrations of AITC. Mustard meal is an ideal form of biofumigation due to these high AITC concentrations and the natural components from which the meal is derived. Anaerobic soil disinfestation is the process of incorporating a labile carbon source into top soil, covering with a plastic tarp, and then irrigating to field capacity. This process results in an anaerobic environment where anaerobic decomposers promote the buildup of anaerobic by-products that can be toxic to soil pests.

Soilborne pathogens have been known to greatly affect strawberry yields, especially in the Southeast Region of the United States. The USDA reported that over 94% of U.S. households consume strawberries. Therefore, producers have a high consumer demand to meet and cannot afford to lose crops to disease. Combining a biofumigation treatment of mustard meal and an anaerobic soil disinfestation treatment of molasses to soil before transplanting strawberry plants can greatly aid in the avoidance of such diseases. Individual and combination treatments will be monitored in order to examine the possible positive and negative effects on strawberry production.

Glucosinolates

Glucosinolates (GSs) are secondary plant products derived from amino acids (Halkier and Du, 1997). Formerly known as mustard oil glucosides, GSs are known to contain a sulphate and a thioglucose moiety (Halkier and Du, 1997). GSs were first isolated by Toubiquet and Boutron in 1830, when the enzymatic formation of mustard oils was being studied in order to explain the pungent flavor of mustard plants (McDanell et al., 1988; Underhill, 1980; Fahey et al., 2001). Bussy also isolated GSs from a mustard species in 1840 (McDanell et al., 1988).

GSs have only been found in dicotyledonous plants and were determined to mainly occur in the *Capparales* order and *Dapparidaceae*, *Caricaceae, Cruciferae*, *Euphorbiacea , Resedaceae*, *Tovariaceae*, *Moringaceae*, *Limnanthaceae*, *Salvadoraceae*, *Tropaeolaceae,* and *Gyrostemonaceae* families (Kjaer, 1973; Larsen, 1981). GSs located in the *Brassicaceae* family are of special interest due to the number of important vegetables, herbs, and agricultural crops that belong in this family (Larsen, 1981). Some nutritionally significant

species within the *Brassicaceae* family are *B. oleracea*, *B. rapa*, *Raphanus sativus*, *Armoracia rusticana*, *Eutrema japanicum*, *Sinapis alba*, and *Nasturtium officinale*. The predominant GS of *B. juncea* is sinigrin; however, gluconapin and glucobrassicanapin were also detected in some varieties (Kirkegaard and Sarwar, 1998).

Qualitative and quantitative differences have been found among the GSs located within the roots, leaves, and seeds (Underhill, 1980). The highest GS concentrations are found in reproductive organs (Grubb and Abel, 2006). Roots were discovered to contain a greater diversity of GSs than shoots (van Dam et al., 2009). Seeds were found to have approximately tenfold the amount of GSs than shoots (Kirkegaard et al., 1996). Brown et al. (2003) determined GS concentrations to be 63-μmol/g dry weight in seeds and 0.7-μmol/g dry weight in senescent rosette leaves. GSs have been observed to accumulate within the endosperm of seeds, more specifically located within the cytoplasm and vacuoles of cells (Larsen, 1981; Halkier and Du, 1997; Omirou et al., 2009). Up to 15 GSs have been identified within one species (Larsen, 1981).

GSs are sugar anionic thioesters containing a β-D-thioglucose group as well as a sulfonated oxime moiety and a side chain derived from methionine, phenylalanine, typtophane, or aliphatic and aromatic amino acids (Figure A-1) (Palmieri, 1999; Chen et al., 2003; Finley, 2005). GSs are nonvolatile, hydrophilic compounds due to their ionic forms and glucose moiety (Larsen, 1981). GSs are strongly acidic and can only be stored as salts (Sorensen, 1988). Biosynthesis of GSs occurs in a three stage sequence: i) side-chain elongation of amino acids, ii) development of the core structure, and iii) secondary side-chain modifications (Figure A-2) (Grubb and Abel, 2006).

Allylglucosinolate, better known as sinigrin, was first recovered by Bussy as a potassium salt of acid myronique from black mustard seeds in 1840 (Underhill, 1980). In 1897, Gadamer proposed that that the enzymatic hydrolysis of sinigrin to allyl isothiocyanate is produced from a side chain linkage to nitrogen, which resulted in the first proposed structure of the GS sinigrin (Gadamer, 1897; Underhill et al., 1973). Ettlinger and Lundeen (1956) discovered that the Gadamer structure was inaccurate when they found degradation products of nitriles and carboxylic acids rather than the amines that Gadamer had depicted. They corrected the formula for sinigrin in 1956, and this formula is still used today (Ettlinger and Lundeen, 1956; Ettlinger and Lundeen, 1957; Mcdanell et al., 1988; Underhill, 1980). After discovering the correct structure of GSs, Ettlinger and Lundeen (1957) were able to perform the first chemical synthesis of a GS, glucotropaeolate, which was identical to the naturally occurring ion from *Tropaeolum majus* seed.

Most of the research behind GS concentration in plants has been fueled by recent anticarcinogenic effects associated with *Brassica* consumption. An extensive literature review by Verhoeven et al. (1996) revealed that there is a consistent inverse relationship between *Brassica* vegetable consumption and lung, stomach, colon, and rectal cancer. Ambrosone et al. (2004) reported that the consumption of cruciferous vegetables, specifically broccoli, is associated with a reduced risk of premenopausal breast cancer. Zhao et al. (2007) found that consumption of *Brassica* vegetables with high isothiocyanate content resulted in 29% decrease in bladder cancer risk.

Glucosinolate Hydrolysis

GSs are hydrolyzed upon tissue disruption by myrosinase enzymes (Agerbirk et al., 2008). The β-thioglucoside-type bond of GSs can be easily hydrolyzed by a myrosinase-catalyzed reaction to give way to D-glucose, a hydrogen sulfate ion which generates a series of diverse aglucons such as isothiocyanates, nitriles, thiocyanates, and thiones (Figure A-3) (Chew, 1988; Poulton and Moller, 1993; Palmieri, 1999; Agerbirk et al., 2008). The composition of the hydrolysis products is dependent on pH, metal ions, and other protein elements (Bones and Rossiter, 2006). GSs alone have little to no bioactivity; however, hydrolysis products released by myrosinase can be highly bioactive (Borek et al., 1994; Charron et al., 2001). Isothiocyanates (ITCs) are yielded through a proton-dependent Lossen rearrangement with a concerted loss of sulfate (Figure A-4).

Myrosinase is present in *Brassicaceae* species and is particularly abundant in seeds, where they are synthesized in the endoplasmic reticulum by ascorbate (Palmieri, 1999; Bones and Rossiter, 2006). Temperature and photosynthetic photon flux can affect GS content or myrosinase activity due to impact on plant growth and development (Charron and Sams, 2004). Myrosinase activity can also be affected by genotype and season (Charron et al., 2005). ITCs are generally formed at pH 5 to 7, and nitriles are formed under acidic conditions (Borek et al., 1994; Charron et al., 2005). Borek et al. (1994) determined that allyl isothiocyanate is the major reaction product in solutions with pH values above 4.0.

Isothiocyanates

ITCs are volatile substances that were first discovered within mustard plants; thus, early concentrates were named 'mustard oils' (Underhill et al., 1973). Around 1830, studies found that the 'mustard oils' did not reside in the plant but were derived from parent substances called mustard oil glucosides, later termed glucosinolates, upon disruption of the plant tissue (Underhill et al., 1973). In 1968, Hofmann first discovered that mustard oils were ITCs (Underhill, 1980).

GSs are hydrolyzed by the enzyme myrosinase to yield glucose and a labile aglucone which rearranges with the loss of sulfate to form ITCs (Underhill, 1980). ITCs are responsible for the distinctive, pungent flavors and odors that are associated with common mustards used as condiments (Underhill, 1980). Unlike their predecessors, ITCs are hydrophobic, which makes them more likely to adsorb to organic compounds in their environment (Gimsing and Kirkegaard, 2009). Kawakishi and Muramatsu (1966) demonstrated the volatility of ITCs in white mustard after finding no ITC concentration 15 hours post pairing with water.

Allyl isothiocyanate (AITC) has been regarded as the most toxic ITC when compared to methyl, phenyl, and ethyl ITCs (Walker et al., 1937). Gmelin and Virtanen (1959) reported an unpleasant smelling substance that was derived from the enzymatic process that they explained to be the hydrolysis of sinigrin to AITC from *Thlaspi arvense*. Lüthy and Benn (1977) were also able to demonstrate the formation of AITC from sinigrin in *Thlaspi arvense.*

Biofumigation via *Brassica* application

Methyl bromide was once used as a pre-plant soil fumigant that was effective at controlling soilborne diseases, nematodes, insects, and weeds in economically important crops (Ploeg, 2007). At the fourth meeting of the Montreal Protocol in Copenhagen in 1992, methyl bromide was listed as the primary source of stratospheric bromine, which has been reported to be responsible for 20-25% of the austral spring's Antarctic 'ozone hole' (Butler, 1995; Ploeg, 2007). Due to this information, methyl bromide was banned as a soil fumigant in several nations, including in the U.S. in accordance with the U.S. Clean Air Act (Ploeg, 2007). Approximately 20,000 metric tons of methyl bromide were applied to U.S. soils on an annual basis prior to the U.S. Clean Air Act announcement that the phase out plan of methyl bromide was to be completed in 2005, (Ajwa et al., 2003). One cause for concern of methyl bromide use is its persistence in soil for up to two years (Butler, 1995). Methyl bromide can persist in soils and has been found to contaminate ground, surface, and drinking water (Ploeg, 2007). Certain methyl bromide alternatives that are still in use, such as Dazomet and Basamid®, have an active ingredient of methyl isothiocyanate, which has been found to lead to plant toxicity if applied too close to planting date (Brown and Morra, 1997; Ajwa et al., 2003). Collins et al. (2006) reported that soil fumigation with metam sodium has a significant effect on soil microbial populations, including non-plant parasitic, free-living nematodes, and can reduce important soil processes such as carbon and nitrogen mineralization.

Biofumigation was described by Kirkegaard and Sarwar (1998) to be the suppression of soilborne pests and pathogens by biocidal compounds released in soil when GSs in *Brassica* green manure, rotation crops, or seed meal amendments are hydrolyzed.

Gimsing and Kirkegaard (2009) later concluded that the overall aim of biofumigation is to maximize the hydrolysis of GSs and the concentration of ITCs available for pest suppression in soil.

Biological activity of GSs and ITCs is determined by the nature of the side R chain, compound concentration, and type of pest being targeted (Rosa et al., 1997). GSs alone (sinigrin, gluconapin, glucoraphenin, glucotropeolin, dehydroerucin, and sinigrin) were not able to cause mortality of the second-stage juveniles of the population of *Heterodera schachtii* (cyst nematode) (Lazzeri et al., 1993).

Brassica Treatment Effects on Nematodes and Microbes

Biofumigation via *Brassica* incorporation has been found to affect soil nematode and microbe populations. Collins et al. (2006) found that soils treated with mustard had higher microbial biomass carbon (average of 160mg/kg soil) while fallow treatment had less (130 mg/kg soil), and soil treated with metam sodium had the least (118 mg C/kg soil). Henderson et al. (2009) observed the effects of mustard seed meal on *Meloidogyne chitwoodi* in a field setting, and found that mustard meal was able to lower population densities. Lazzeri et al. (2009) also found that in a field setting, defatted mustard meal was better at decreasing populations of *M. chitwoodi* than a treatment of Oxamyl, a pesticide toxic to humans. Henderson et al. (2009) found that the combination of Biofence, a commercial *B. carinata* seed meal, with *Steinernema* spp., a biological control of nematodes, did not decrease instance of *M. chitwoodi.* However, Biofence application significantly reduced *M. chitwoodi* populations and the potato tuber damage caused by such populations (Henderson et al, 2009). Ellenby (1945) used seedlings of cress and black and white

mustards to show the decrease in eelworm larvae emergence in potato soil. The mustard treatments of the potato soil resulted in no permanent injury for the larvae emergence (Ellenby, 1945). Williams et al. (1993) were able to lethally and sub-lethally suppress *Limonius californicus* wireworms and reduce their feeding from 32 to 137 days after amending soil with AITC, which provides protection during the seedling establishment period of crops. Serra et al. (2002) used gluconasturtiin hydrolysis derivatives at concentrations as low as 0.05 mg/ml to adequately control *Globodera rostochiensis* juveniles. Noble et al. (2002) were able to achieve 100% mortality of masked chafer beetle larvae (*Cyclocephala* spp.) when appling *B. juncea* tissue at 8% of soil mass.

Brassica Treatment Effects on Crop Yield

Crop yield is the predominant concern for producers of major cash crops. Several fumigation treatments have been shown to decrease germination or cause phytoxicity of certain plants and decrease yield. It is important to provide methods and alternative treatments that will minimize the possibility of crop damage. Lazzeri et al. (2009) found that when comparing defatted mustard meal to Oxamyl, a carbamate insecticide, neither treatment resulted in phytoxicity of transplanted zucchini plants. For the first 45 days of harvest, yield did not differ between the two treatments (Lazzeri et al, 2009). In the last month of cultivation, plants in the Oxamyl treatment had 40% less yield than plants in the mustard treatment (Lazzeri et al., 2009). The plants treated with the defatted mustard meal treatment had a 14% higher overall yield than the Oxamyl treatment, which was mostly due to the mustard treatment having a longer harvest period by a week (Lazzeri et al., 2009). Henderson et al. (2009) tried to improve potato yield by combining Biofence

with *Steinernema* spp., a biological control of nematodes, but discovered that this did not improve yield beyond each of these treatments used alone.

Persistence and Mobility of GSs and ITCs in Soil Environments

Soil persistence is a major concern of volatile use for fumigation. GSs that have been released from their parent material are mobile in the soil environment, causing them to have high bioavailability (Gimsing and Kirkegaard, 2009). Their fate in soil will be determined by water availability and their functional groups that are able to interact with the soil surface (Figure A-5) (Gimsing and Kirkegaard, 2009). Borek et al. (1995) determined the half-life of AITC to be approximately two days in soil when temperature is 20°C. This demonstrates how AITCs will not be harmful for subsequent plantings; however, a one-time amendment may not be sufficient to fully inhibit or kill the damaging pest for successful control of the growing environment (Borek et al., 1995). Gimsing and Kirkegaard (2006) observed the lives of GSs and ITCs in soil after mustard green manure was incorporated. They found that both concentrations decreased significantly during the first four days but were detected up to eight days after incorporation (Gimsing and Kirkegaard, 2006). Trace concentrations of ITCs were discovered 12 days after incorporation (Gimsing and Kirkegaard, 2006). Maximum concentrations of ITCs (90.6 and 21.6 ITC nmol/g) were measured immediately (30 minutes) following incorporation (Gimsing and Kirkegaard, 2006). These values were reported to be the highest concentration of ITCs in a field setting following *Brassica* incorporation and the highest release efficiency (56%) of a high GS mustard species (Gimsing and Kirkegaard, 2006). The prior high efficiency release rate (26%) was measured by Morra and Kirkegaard in 2002. Both Morra and Kirkegaard (2002) and Gimsing and Kirkegaard (2006) measured the

release efficiency by dividing the maximum soil ITC concentration (nmol/g soil) by the total ITC-liberating GS in incorporated plant material (nmol/g of soil) and then multiplied this by 100. Certain soil environments, such as high clay or peat content, can reduce the efficacy of ITCs (Brown and Morra, 1997; Matthiessen and Shackleton, 2005). Price et al. (2005) determined the times at which AITCs were at their highest concentrations in soil and found that sampling time was significant. The quarter hour and four hour sampling times did not differ statistically and yielded 19% more AITC than the 8 hour sampling time and 95% more AITC than the 24 hour sampling time.

Increased water content has been reported to increase ITC longevity in soil, especially under colder temperatures, and could increase potential for pest inhibition due to longer exposure times (Brown and Morra, 1997). Despite several reports of increased water content increasing ITC concentrations in soil, Gimsing and Kirkegaard (2006) found that irrigation volumes did not have a significant effect on ITC and GS concentrations in soils at any sampling time over 8 days. This is contradictive to their previous findings where they reported that significantly less GSs were recovered from moist soils than from dry soils, and they suggested that this may be due to microbial degradation occurring in moist soils (Gimsing and Kirkegaard, 2005).

Other soil factors affect the persistence of GSs and their ITCs in soil. Price et al. (2005) found that increased soil temperature increased the volatilization of ITCs, therefore causing them to dissipate more quickly. Soil texture also affects soil GS and ITC concentrations, as AITC has been measured in greater concentrations in sandy loams in comparison to clay loams due to the reaction to the organic carbon content of the clay loam

soil (Price et al., 2005). Using ground cover, such as plasticulture or cover crops, has also been linked to an increase in AITC concentration (Price et al., 2005).

Mustard Meal Application

Mustard meal is an alternative to green *Brassica* manure or *Brassica* cover cropping. Brown and Morra (1995) reported that the low moisture content of defatted mustard meal allows the GSs within the meal to remain stable. Lazzeri et al. (2009) found that defatted mustard meal contained a GS concentration of 151 μmol/g, which was over 98% sinigrin. More specifically, Oliveira et al. (2011) measured concentrations of sinigrin at 21.9 mg/g dry weight in the defatted seed meal and 12.2 mg/g dry weight in whole seed meal. They also measured AITC as the major hydrolytic product released by moistened whole seed meal, with concentrations of 5.40 μg/g dry soil detected at 2 hours after water addition, an average concentration of 4.12 ug/g at 6-12 hours, and 0.940 ug/g at 48 hours. For the defatted seed meal, AITC was detected after 2 hours at a concentration of 6.58 ug/g dry soil, then increased to 9.76 ug/g dry soil after 6-12 hours and decreased to 2.66 ug/g dry soil after 48 hours. They concluded that both the whole and defatted seed meals of the wild mustard are applicable for biofumigation due to their high sinigrin content and high AITC release.

The mustard meals used in the field experiments were Wisconsin Spice, Inc. brand Deheated Mustard Meal and Deoiled Overs (478 S. Industrial Park Rd, Berlin, WI), Triumph Italia brand Biofence (Agrium Italia Spa, Livorno, Italy), and Mustard Products and Technologies (MPT) brand Mustard Pellets (Saskatoon, SK, Canada). According to the Wisconsin Spice, Inc. website, the deheated and deoiled mustards are both from oriental

mustard seed (*B. juncea*). This company officially supplies these products for consumptive purposes; however, the available ground form allows for easy application in a field setting. They also offer a variety of purchase quantities, ranging from 2 ounces to 75 pounds. According to the Triumph Italia website, Biofence is 100% vegetable formulation. This product is produced for plant protection and also aims at increasing plant nutrition by increasing chemical and biological fertility of the soil. The listed benefits of Biofence, according to the website, are: 1) the ability to free ground from diseases of plants, creating an unfavorable environment for the development of numerous pathogens of specialty crops (pathogenic fungi, nematodes, wireworms, etc.), 2) rebalancing the microbial flora contributing to high levels of soil organic matter which in turn promotes the development of beneficial organisms, and 3) nourishes the crop by providing nitrogen, available phosphorus, potassium, and trace elements. Biofence is safe for the handler and the applied environment and does not hinder the activity of pollinators when applied as suggested. Their recommended dose is 200-300 grams per square meter. According to the MPT website, MPT mustard treatments provided higher yield than methyl bromide treatments in two separate studies (2010 and 2011/2012). MPT reports that their product line is all organic, biodegradable, and environmentally safe; provides comparable protection against pathogens and nematodes as synthetic alternatives; is a nutritional asset to plants; enriches soil environment; and is a safe economical investment.

Brassica **Treatment for Strawberry Production**

Strawberries are susceptible to pests and diseases. Prior to 2005, methyl bromide was the predominant and most effective fumigant for protection against strawberry pests.

Much of the biofumigation research is aimed toward discovering a fumigant solution that is comparable to methyl bromide's ability to protect strawberry production and harvest.

Lazzeri et al. (2003) found that *B. juncea* manure provided a moderate effect when analyzing strawberry yield comparatively to methyl bromide and general green manure. Strawberry yield in the mustard plots was significantly lower than plots treated with methyl bromide and significantly higher than general green manure treatment plots. Porras et al. (2009) combined biofumigation via *Brassica carinata* incorporation with solarization techniques and was able to increase strawberry fruit weight and significantly increase foliar surface and total yield comparatively to solarization techniques alone.

Mattner et al. (2008) tested *B. rapa* and *B. napus* treatments on well-known strawberry pathogens and found that they were lethal to *Rhizoctonia fragariae*, *Alternaria alternate*, *Colletotrichum dematium*, *Cylindrocarpon destructans*, *Pythium ultimum*, and *Pythium cactorum*, but not lethal to *Fusarium oxysporum*. In a field trial, Koron (2009) demonstrated that *B. juncea* was able to provide a lower frequency (43.4%) of fungal infestation of roots than a Dazomet treatment (50.3%); however, this difference was not statistically significant. The Dazomet treatment increased plant growth compared to the *B. juncea* treatment; however, there was no significant difference in yield between the two treatments. The greenhouse trial of these treatments showed the opposite of the field trial. The *B. juncea* treatment had a higher fungal infestation frequency (57.9%) and a higher yield than the Dazomet treatment, which had only 2.9% fungal frequency. When comparing different *Brassica* varieties on suppression of *Phytophthora cactorum* and *Verticillium dahlia,* Zurera et al. (2009) found that *B. juncea*, *B. carinata*, and *B. nigra* were

more suppressive than *B. rapa*, *B. oleracea*, and *B. sativus.* More specifically, *B. juncea* had the greatest suppression of *Phytophthora cactorum* and *Verticillium dahliae* at pH level 4, and *B. carinata* had the greatest suppression at pH level 10 (Zurera et al., 2009).

Anaerobic Soil Disinfestation

The development of anaerobic soil disinfestation (ASD), also described as biological soil disinfestation, originated in both Japan (Shinmura) and the Netherlands (Blok) (Blok et al., 2000; Momma et al., 2013; Shennan et al., 2014). In Japan, ASD began as an extension of paddy-upland field rotation due to the Montreal protocol phasing out of methyl bromide (Momma et al., 2013; Shennan et al., 2014). Shinmura's method of ASD consisted of incorporating organic matter into a plot, irrigating the plot until saturation, and covering the surface of the soil with plastic film (Momma et al., 2013). Van Bruggen et al. (2014) found ASD to be comparable to methyl bromide regarding reduction of plant pathogenic fungi, nematodes, and bacteria for various crops (asparagus, potato, strawberry, tulip, Norway maple, and Southern catalpa).

Impacts of Anaerobic Soil Disinfestation in a Field Setting

The ASD treatment of wheat bran has been found to decrease soil pH by releasing acetic and butyric acids (Momma, 2008). McCarty et al. (2014) reported no differences occurred among control, ASD with molasses, and biofumigation with mustard meal when measuring soil pH. Momma et al. (2006) found that ASD treatments can keep soil at a lower pH for 15 days after the start of treatment. Butler et al. (2012) reported that none of the cover crop amendments (cowpea, sunn hemp, peal millet, sorghum+sudangrass, cowpea+pearl millet, Cowpea+sorghum-sudan grass, molasses control) which did not have

a combined application of composted poultry litter had a significant effect on pH when compared to the control with no carbon source amendment. However, with the addition of composted poultry litter, the molasses control treatment caused a significantly lower pH reading than all other treatments.

McCarty et al. (2014) found a significant difference of total soil inorganic N between untreated control plots and ASD with molasses as well as between untreated control and biofumigation control. However, there was not a significant difference between ASD with molasses and biofumigation control. The untreated control had significantly more soil inorganic N than the other two treatments.

Hewavitharana et al. (2014) found that anaerobic soil disinfestation applications of rice bran, *B. juncea* seed meal, or orchard grass residue in their apple orchard soil with 10% ethanol as carbon input caused the majority of their apple orchard soil locations to become more acidic. The 10% ethanol, orchard grass residue, and *B. juncea* seed meal provided the greatest control of *P. penetrans* and yielded the most active spectrum of nematicidal volatiles, suggesting an important role for these chemistries in determining the efficacy of anaerobic soil disinfestation for control of lesion nematode in orchard systems. ASD applications have also been successful at significantly decreasing populations of free-living Trichodorids (Korthals et al., 2005). Butler et al. (2012) concluded that combining soil solarization, organic amendments, and a minimal amount of water is sufficient for nematode management.

Strawberry Production

In 2012, the U.S. consumed almost 8 pounds of strawberries per person (Wright, 2014). Strawberries can be produced in different systems, with the most common being perennial matted row system and the annual plasticulture system. The latter is the most common field system in the U. S. Plasticulture provides an opportunity for an earlier growing season, which can attract consumers for a longer portion of the year (Wright, 2014). Consistent extended seasons can present an opportunity for retaining customers (Wright, 2014). While investment for plasticulture is high (upwards of $15,000 per acre), there is an increased potential for higher yield and a better quality crop (Wright, 2014).

Strawberry fields should be located near a water source (Pritts). Ideally, soil should be well drained with moderate to high organic matter (Pritts). Sandy loams and clay loams are best for building and shaping the raised bed of plasticulture (Poling; Wright, 2014). These raised beds will aid in water drainage, which is crucial for decreasing the chance for soil pathogens (Poling; Pritts). Fields with gentle slopes can also aid in draining excess surface water (Poling). Beds should be built in a north/south orientation to encourage more uniform plant development and ripening on both sides of the rows (Poling). Raised beds are often built 8-10 inches high, 28-30 inches wide, and slightly crowned on top (Poling; Pritts). Drip irrigation is typically used in the plasticulture system for less water waste, the availability for use during harvest, and to decrease supply water to the areas between rows where weeds can prevail (Pritts). Drip tubing is installed before plastic is laid or during the laying of plastic, with help from specific machinery (Pritts; Wright, 2014).

One of the major functions of the use of black plastic on production beds in plasticulture is to hinder weed growth (Pritts). Weeds cause a greater economic loss in berry crops than disease and insects combined (Pritts). Implementing a summer cover crop will also reduce weed stress while preventing soil erosion (Poling). Hand weeding is also encouraged during times of harvest in order to increase the life of the strawberry plants (Pritts). A preemergent herbicide is recommended for use prior to transplanting plug plants (Pritts). In order to decrease the potential threat of *Verticillium* wilt, strawberries should not be planted after potatoes, tobacco, peppers, eggplants, or tomatoes (Wright, 2014).

Many strawberry cultivars are available for production. One common cultivar is 'Chandler'. 'Chandler' strawberries were introduced in 1983 (Chandler and Legard). They are economically important for the Southeastern U.S. due to their high yields of attractive, exceptionally flavored fruit (Chandler and Legard). Plasticulture producers prefer 'Chandler' strawberries because they survive well as a plug plant for transport and are fairly cold hardy (Poling). Transplanting plug plants should generally occur between September 10 and September 20 (Wright, 2014). Double-row hills are preferred for 'Chandler' strawberries, where there are 12-14 inches between plant rows and 12-16 inches between plants within the rows (Poling; Pritts).

Fertilizer applications vary based upon soil testing and cover cropping. A standard nitrogen application consists of 30 pounds per acre four weeks after planting (Bushway, 2004). Sufficient potassium, phosphorus, magnesium, and calcium should be added prior to planting in order to assist in plant growth and development (Pritts). Fertigation should

begin in the spring, once row covers are removed, and continue once weekly until harvest is complete (Wright, 2014). Row covers can be used throughout harsh winter months to protect the crowns from desiccation (Pritts). Floating covers should be used to provide a smooth transition in the spring months, when frost can coincide with warm weather (Pritts; Wright, 2014). Harvest will begin in early to mid-May and can last up to 5 weeks. Strawberries should be full color at harvest due to no increase in post-harvest quality (Wright, 2014).

Objectives

Biofumigation and ASD treatment methods can be used in a strawberry production environment in order to remediate soil pests. We will observe strawberry fruit yield and nematode counts in order to determine the impacts of these treatments on strawberry production. Also, we will measure the concentrations of sinigrin and AITC to show the break-down of sinigrin to AITC within strawberry soil. This is crucial for the fumigation of soil pests and can also have an impact on strawberry production. Other quality tests, such as fruit and leaf mineral content, fruit sugar and organic acid content, soil nitrogen content, and soil pH will be completed to help determine the positive and negative impacts of using biofumigation and/or ASD treatments for strawberry production.

References:

Agerbirk, N., Warwick, A. I., Hansen, P. R., Olsen, C. E. (2008). Sinapis phylogeny and evolution of glucosinolates and specific nitrile degrading enzymes. *Phytochemistry* 69(17): 2937-2949.

Ajwa, H.A., Klose, S., Nelson, S. D., Minuto, A. M., Gullino, M. L., Lamberti, F., Lopez-Aranda, J. M. (2003). Alternatives to methyl bromide in strawberry production in the United States of America and the Mediterranean region. *Phytopathol. Mediterr.* 42(3): 220-244.

Ambrosone, C. B., McCann, S. E., Freudenheim, J. L., Marshall, J. R., Zhang, Y., Shields, P. G. (2004). Breast Cancer Risk in Premenopausal Women Is Inversely Associated with Consumption of Broccoli, a Source of Isothiocyanates, but Is Not Modified by GST Genotype. *The Journal of Nutrition* 134:1134-1138.

Blok, W. J., Lamers, J. G., Termorshuizen, A. J., Bollen, G. J. (2000). Control of Soilborne Plant Pathogens by Incorporating Fresh Organic Amendments Followed by Tarping. *Disease Control and Pest Management* 90 (3): 253-259.

Bones, A. M., Rossiter, J. T. (2006). The enzymic and chemically induced decomposition of glucosinolates. *Phytochemistry* 67(11): 1053-1067.

Borek, V., Morra, M. J., Brown, P. D., McCaffrey, J. P. (1994). Allelochemicals Produced during Sinigrin Decomposition in Soil. *Journal of Agricultural and Food Chemistry* 42(4): 1030-1034.

Borek, V., Morra, M. J., Brown, P. D., McCaffrey, J. P. (1995). Transformation of the Glucosinolate-Derived Allelochemicals Allyl Isothiocyanate and Allylnitrile in Soil. *Journal of Agricultural and Food Chemistry* 43(7): 1935-1940.

Brown, Paul D, Morra, M. J. (1995). Glucosinolate-Containing Plant Tissues as Bioherbicides. *J. Agric. Food Chem* 43(12): 3070-3074.

Brown, P. D., Morra, M.J. (1997). Control of soil-borne plant pests using glucosinolate-containing plants. *Advances in Agronomy*. D. L. Sparks. New York, Academic Press: 167-231.

Brown, Paul D., Tokuhisa, J. G., Reichelt, M., Gershenzon, J. (2003). Variation of glucosinolate accumulation among different organs and developmental stages of Arabidopsis thaliana. *Phytochemistry* 62:471-481.

Bushway, L. (2004). Nutrient Management in Strawberries. *New York Berry News* 3(5):4.

Butler, J. H. (1995). Methyl bromide under scrutiny. *Nature* 376: 469-470.

Butler, D. M., Kokalis-Burelle, N., Muramoto, J., Shennan, C., McCollum, T. G., Rosskopf, E. N. (2012). Impact of anaerobic soil disinfestation combined with soil solarization on plant-parasitic nematodes and introduced inoculum of soilborne plant pathogens in raised-bed vegetable production. *Crop Protection* 39: 33-40.

Butler, D. M., Rosskopf, E. N., Kokalis-Burelle, N., Albano, J. P., Muramoto, J., Shennan, C. (2012). Exploring warm-season cover crops as carbon sources for anaerobic soil disinfestation (ASD). *Plant Soil* 355:149-165.

Chandler, C.K., Legard, D. E. Strawberry Cultivars for Annual Production Systems. University of Florida. <http://strawberry.ifas.ufl.edu/Strawberrycultivars.htm>.

Charron, C. S., Kopsell, D. A., Randle, W. M., Sams, C. E. (2001). Sodium selenate fertilisation increases selenium accumulation and decreases glucosinolate concentration in rapid-cycling *Brassica oleracea*. *Journal of the Science of Food and Agriculture* 81(9): 962-966.

Charron, C. S., Sams, C. E. (2004). Glucosinolate content and myrosinase activity in rapid-cycling *Brassica oleracea* grown in a controlled environment. *Journal of the American Society for Horticultural Science* 129(3): 321-330.

Charron, C. S., Saxton, A. M., Sams, C. E. (2005). Relationship of climate and genotype to seasonal variation in the glucosinolate-myrosinase system. I. Glucosinolate content in ten cultivars of *Brassica oleracea* grown in fall and spring seasons. *Journal of the Science of Food and Agriculture* 85(4): 671-681.

Chen, S. X., Glawischnig, E., Jorgensen, K., Naur, P., Jorgensen, B., Olsen, C. E., Hansen, C. H., Rasmussen, H., Pickett, J. A., Halkier, B. A. (2003). CYP79F1 and CYP79F2 have distinct functions in the biosynthesis of aliphatic glucosinolates in Arabidopsis. *Plant Journal*, 33: 923-937.

Chew, F. S. (1988). Biological Effects of Glucosinolates. *Am. Chem. Soc*. 380:155-181.

Collins, H. P., Alva, A., Boydston, R. A., Cochran, R. L., Hamm, P. B., McGuire, A., Riga, E. (2006). Soil microbial, fungal, and nematode responses to soil fumigation and cover crops under potato production. *Biology and Fertility of Soils* 42(3): 247-257.

Ellenby, C. (1945). The influence of crucifers and mustard oil on the emergence of larvae of the potato-root eelworm, Heterodera rostochiensis Wollenweber. *Annals of Applied Biology* 32(1):67-70.

Ettlinger, M. G., Lundeen, A. J. (1956). The Structures of Sinigrin and Sinalbin; an Enzymatic Rearrangement. *J. Am. Chem. Soc.* 78:4172-4173.

Ettlinger, M. G., Lundeen, A. J. (1957). First Synthesis of a Mustard Oil Glucoside; the Enzymatic Lossen Rearrangement. *J. Am. Chem. Soc.* 79:1764-1765.

Fahey, J. W., Zalcmann, A. T., Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 56: 5-51.

Finley, J. W., Robbins, R. J., Keck, A. S., Banuelos, G. (2005). Cultivation Conditions and Selenium Fertilization Alter the Phenolic Profile, Glucosinolate, and Sulforaphane Content of Broccol. *Journal of Medicinal Food* 8(2):204-214.

Gadamer, J. (1897). Uber das Sinigrin. *Berichte der deutschen chemischen Gesellschaft* 30: 2322-2327.

Grubb, C. D., Abel, S. (2006). Glucosinolate metabolism and its control. *Trends in Plant Science* 11(2): 89-100.

Gimsing, A. L., Kirkegaard, J. A. (2005). Extraction and determination of Glucosinolates from soil. *Journal of Agricultural and Food Chemistry* 53(25): 9663-9667.

Gimsing, A. L., Kirkegaard, J. A. (2006). Glucosinolate and isothiocyanate concentration in soil following incorporation of *Brassica* biofumigants. *Soil Biology & Biochemistry* 38(8): 2255-2264.

Gimsing, A. L., Kirkegaard, J. A. (2009). Glucosinolates and biofumigation: fate of glucosinolates and their hydrolysis products in soil. *Phytochemistry Reviews* 8(1): 299-310.

Halkier, B. A., Du, L. C. (1997). The biosynthesis of glucosinolates. *Trends in Plant Science* 2(11): 425-431.

Henderson, D. R., Riga, E., Ramirez, R. A., Wilson, J., Snyder, W. E. (2009). Mustard biofumigation disrupts biological control by Steinernema spp. nematodes in the soil. *Biological Control* 48(3): 316-322.

Hewavitharana, S. S., Ruddell, D., Mazzola, M. (2014). Carbon source-dependent antifungal and nematicidal volatiles derived during anaerobic soil disinfestation. *European Journal of Plant Pathology* 140(1):39-52.

Kawakishi, S., Muramatsu, K. (1966). Studies on the Decomposition of Sinalbin Part I. The Decomposition Products of Sinalbin. *Agr. Biol. Chem.* 30(7): 688-692.

Kirkegaard, J. A., Wong, P. T. W., Desmarchelier, J. M. (1996). In vitro suppression of fungal root pathogens of cereals by *Brassica* tissues. *Plant Pathology* 45(3): 593-603.

Kirkegaard, J. A., Sarwar, M (1998). Biofumigation potential of *Brassicas* - I. Variation in glucosinolate profiles of diverse field-grown *Brassicas*. *Plant and Soil* 201(1): 71-89.

Kjaer, A. (1973). The Natural Distribution of Glucosinolates: a Uniform Group of Sulfur-Containing Glucosides. *Chemistry in Botanical Classification: Medicine and Natural Sciences*: 229 -234.

Koron, D. (2009). The Influence of Biofumigation on Mycorrhizal Fungi and Growth of Strawberries. *Proc. VIth Internat. Strawberry Symposium*: 381-384.

Korthals, G.W., Visser, J. H. M., Molendijk, L. P. G. (2005). Improvement and Monitoring Soil Health. *Acta Horticulturae (ISHS)* 698:279-284.

Larsen, P. O. (1981). Glucosinolates. The Biochemistry of Plants. New York: Academic Press, Inc.

Lazzeri, L., Tacconi, R., Palmieri, S. (1993). In Vitro Activity of Some Glucosinolates and Their Reaction Products toward a Population of the Nematode Heterodera schachtii. *J. Agric. Food Chem* 41(5): 825-829.

Lazzeri, L., Malaguti, B. G., Antoniacci, L. (2003). Replacing methyl bromide in annual strawberry production with glucosinolate-containing green manure crops. *Pest Manag. Sci.* 59:983-990.

Lazzeri, L., Curto, G., Dallavalle, E., D'Avino, L., Malaguti, L., Santi, R., Patalano, G. (2009). Nematicidal Efficacy of Biofumigation by Defatted *Brassicaceae* Meal for Control of *Meloidogyne incognita* (Kofoid et White) Chitw. On a Full Field Zucchini Crop. *Journal of Sustainable Agriculture* 33(3): 349-358.

Matthiessen, J. N., Shackleton, M. A. (2005). Biofumigation: environmental impacts on the biological activity of diverse pure and plant-derived isothiocyanates. *Pest Management Science* 61(11): 1043-1051.

Mattner, S.W., Porter, I. J., Gounder, R. K., Shanks, A. L., Wren, D. J., Allen, D. (2008). Factors that impact on the ability of biofumigants to suppress fungal pathogens and weeds of strawberry. *Crop Protection* 27: 1165-1173.

McCarty, D.G., Ownley, B.H., Wszelaki, A.L., Eichler Inwood, S.E., Sams, C.E., Butler, D.M. (2014). Field evaluation of anaerobic soil disinfestation (ASD) carbon sources for tomato and bell pepper production in Tennessee. *HortScience* 49(3):272-280.

Mcdanell, R., McLean, A. E. M., Hanley, A. B., Heaney, R. K., Fenwick, G. R. (1988). Chemical and Biological Properties of Indole Glucosinolates (Glucobrassicins) - a Review. *Food and Chemical Toxicology* 26(1): 59-70.

Momma, N., Yamamoto, K., Simandi,P., Shishido, M. (2006). Role of organic acids in the mechanisms of biological soil disinfestation (BSD). *Journal of General Plant Pathology* 72: 247-252.

Momma, N. (2008). Biological Soil Disinfestation (BSD) of Soilborne Pathogens and Its Possible Mechanisms. *Japan Agricultural Research Quarterly* 42 (1): 7-12.

Momma, N., Kobara, Y., Uematsu, S., Kita, N., Shinmura, A. (2013). Development of biological soil disinfestations in Japan. *Applied Microbiology and Biotechnology* 97(9): 3801-3809.

Morra, M. J., Kirkegaard, J. A. (2002). Isothiocyanate release from soil-incorporated *Brassica* tissues. *Soil Biology and Biochemistry* 34:1683-1690.

Mustard Products and Technologies. 111 Research Drive, Suite 101, Saskatoon, SK S7N 3R2, Canada. Website: <http://www.mptmustardproducts.com/index.html>.

Noble, R. R., Harvey, S. G., Sams, C.E. (2002). Toxicity of Indian Mustard and Allyl Isothiocyanate to Masked Chafer Beetle Larvae. *Plant Health Progress.* (doi:10.1094/PHP-2002-0610-01-RS).

Oliveira, R. D. L., Dhingra, O. D., Lima, A. O., Jham, G. N., Berhow, M. A., Holloway, R. K., Vaughn, S. F. (2011). Glucosinolate content and nematicidal activity of Brazilian wild mustard tissues against Meloidogyne incognita in tomato. *Plant and Soil* 341(1-2): 155-164.

Omirou, M. D., Papadopoulou, K. K., Papastylianou, I., Constantinou, M., Karpouzas, D. G., Asimakopoulos, I., Ehaliotis, C. (2009). Impact of Nitrogen and Sulfur Fertilization on the Composition of Glucosinolates in Relation to Sulfur Assimilation in Different Plant Organs of Broccoli. *Journal of Agricultural and Food Chemistry* 57(20): 9408-9417.

Palmieri, S. (1999). Glucosinolates nutraceutical products. *Agro Food Industry Hi-Tech* 10(1): 28-29.

Ploeg, A. (2007). Biofumigation To Manage Plant-Parasitic Nematodes. *Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes* 2: 239-248.

Poling, E. B. (2001). An Introductory Guide to Strawberry Plasticulture. North Carolina State University. 12 December 2014 <http://hortintl.cals.ncsu.edu/articles/introductory-guide-strawberry-plasticulture>.

Porras, M., Barrau, C. Romero, E., Zurera, C., Romero, F. (2009). Effect of Biofumigation with *Brassica carinata* and Soil Solarization on *Phytophthora* spp. and Strawberry Yield. *Proc. VIth Internat. Strawberry Symposium:* 969-972.

Poulton, J. E., Moller, B. L. (1993). Glucosinolates. *Methods in Plant Biochemistry* 9: 209-37.

Price, A. J., Charron, C. S., Saxton, A. M., Sams, C. E. (2005). Allyl isothiocyanate and carbon dioxide produced during degradation of *Brassica juncea* tissue in different soil conditions. *Hortscience* 40(6): 1734-1739.

Pritts, M. Perennial Strawberry Production. Cornell University. <http://www.fruit.cornell.edu/berry/production/pdfs/strperennprod.pdf>

Rosa, E.A.S., Heaney, R. K., Fenwick, G. R., Portas, C. A. M. (1997). Glucosinolates in Crop Plants. *In Horticultural reviews* 19:99-215.

Serra, B., Rosa, E., Iori, R., Barillari, J., Cardoso, A., Abreu, C., Rollin, P. (2002). In vitro activity of 2-phenylethyl glucosinolate, and its hydrolysis derivatives on the root-knot nematode Globodera rostochiensis (Woll.). *Scientia Horticulturae* 92: 75-81.

Shennan, C., Muramoto, J., Lamers, J., Mazzola, M., Rosskopf, E. N., Kokalis-Burelle, N., Momma, N., Butler, D. M., Kobara, Y. (2014). Anaerobic Soil Disinfestation for Soil

Borne Disease Control in Strawberry and Vegetable Systems: Current Knowledge and Future Directions. *Acta Horticulturae* 1044: 165-176.

Sorensen, H. (1988). Glucosinolates - Structure-Properties-Function. *Abstracts of Papers of the American Chemical Society* 195: 79-AGFD.

Triumph Italia, Agrium Italia Spa, Via delle Cateratte, 68, Livorno LI, Italy. Website: <http://www.triumphitalia.com/it/2009/01/biofence/>.

Underhill, E. W., Wetter, L. R., Chisholm, M. D. (1973). Biosynthesis of glucosinolates. *Biochem Soc Symp.* (38):303–326.

Underhill, E. W. (1980). Glucosinolates. *Secondary Plant Products* 8: 493-511.

Van Bruggen, A., Blok, W., Gu, G. (2014). Soil Disinfestation and Food Safety Issues. *Acta Horticulturae (ISHS)* 1044: 437-446.

Van Dam, N. M., Tytgat, T. O., Kirkegaard, J. A. (2009). Root and shoot glucosinolates: a comparison of their diversity, function and interactions in natural and managed ecosystems. *Phytochemistry Reviews* 8(1):171-186.

Verhoeven, D. T., Goldbohm, R. A., van Poppel, G., Verhagen, H., van den Brandt, P. A. (1996). Epidemiological Studies on *Brassica* Vegetables and Cancer Risk. *Cancer Epidemiology, Biomarkers & Prevention* 5: 733-748.

Walker, J. C., Morell, S., Foster, H. H. (1937). Toxicity of Mustard Oils and Related Sulfur Compounds to Certain Fungi. *American Journal of Botany* 24(8):536-541.

Williams, L III, Morra, M. J., Brown, P. D., McCaffrey, J. P. (1993), Toxicity of Allyl Isothiocyanate-Amended Soil to Limonius californicus (Mann.) (Coleoptera: Elateridae) Wireworms. *Journal of Chemical Ecology* 19(6): 1033-1046.

Wisconsin Spice, Inc., 478 S. Industrial Park Rd, Berlin, Wi. Website: <http://www.wisconsinspice.com/index.php>.

Wright, S. (2014). Annual Plasticulture Strawberry Production. University of Kentucky Cooperative Extension Service. <http://www.uky.edu/Ag/CCD/introsheets/plasticulturestrawberry.pdf>.

Zhao, H., Lin, J., Grossman, H. B., Hernandez, L. M., Dinney, C. P., Wu, X. (2007). Dietary isothiocyanates, GSTM1, GSTT1, NAT2 polymorphisms and bladder cancer risk. *Int. J. Cancer* 120:2208-2213.

Zurera, C., Romero, E., Poras, M., Barrau, C., Romero, F., (2009). In Vitro Suppression of *Phytophthora cactorum* and *Verticillium dahlia* Potential Strawberry Pathogens by *Brassica* Tissues. *Proc. VIth Internat. Strawberry Symposium*: 267-270.

Appendix A:

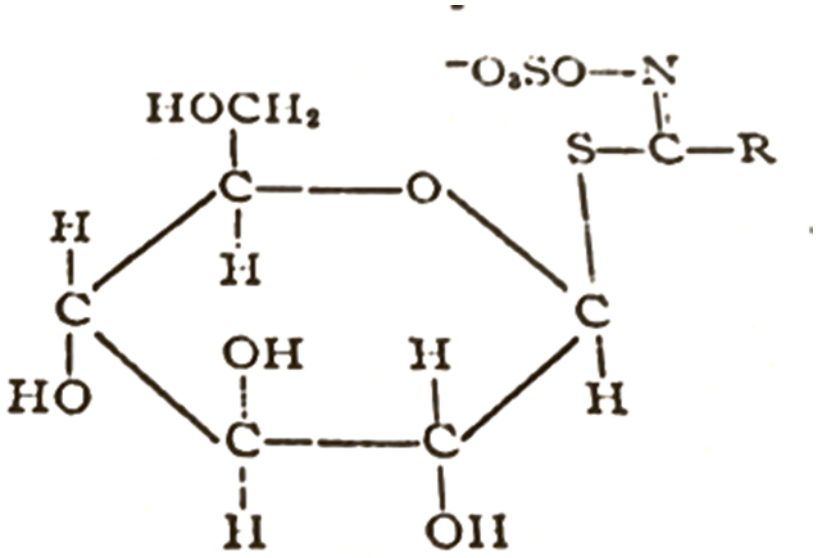

Figure A-1: General Structure of Glucosinolates (Ettlinger and Lundeen, 1957)

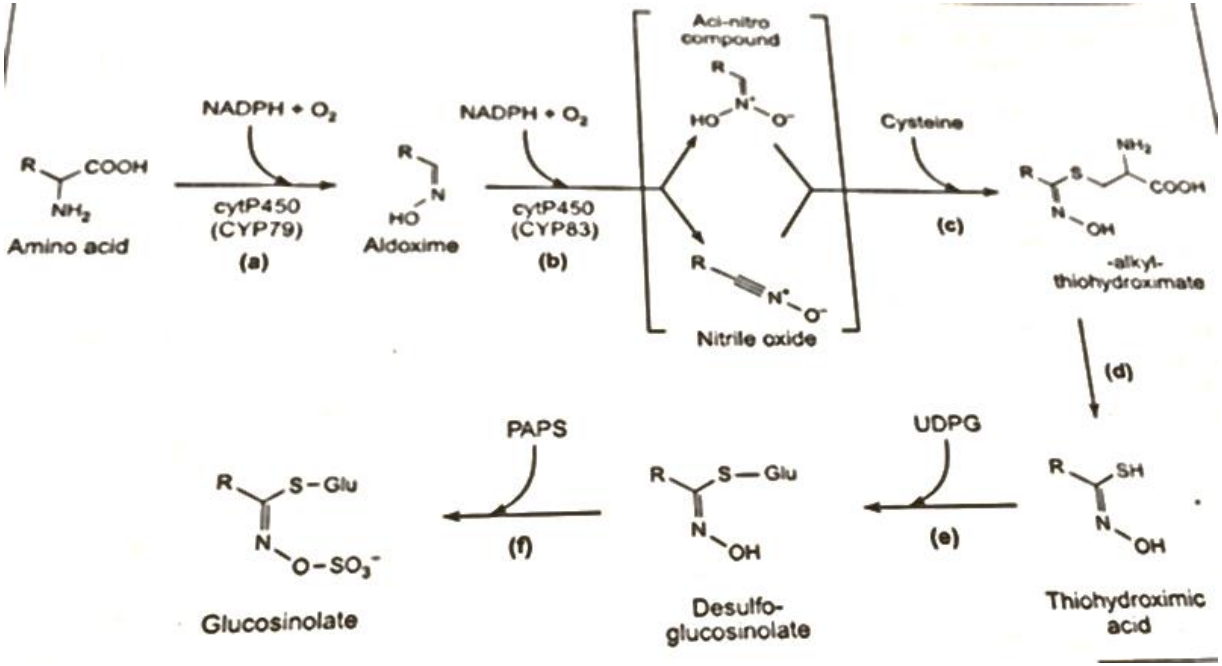

Figure A-2: Desulfoglucosinolate production (Quinsac and Ribaillier, 1991)

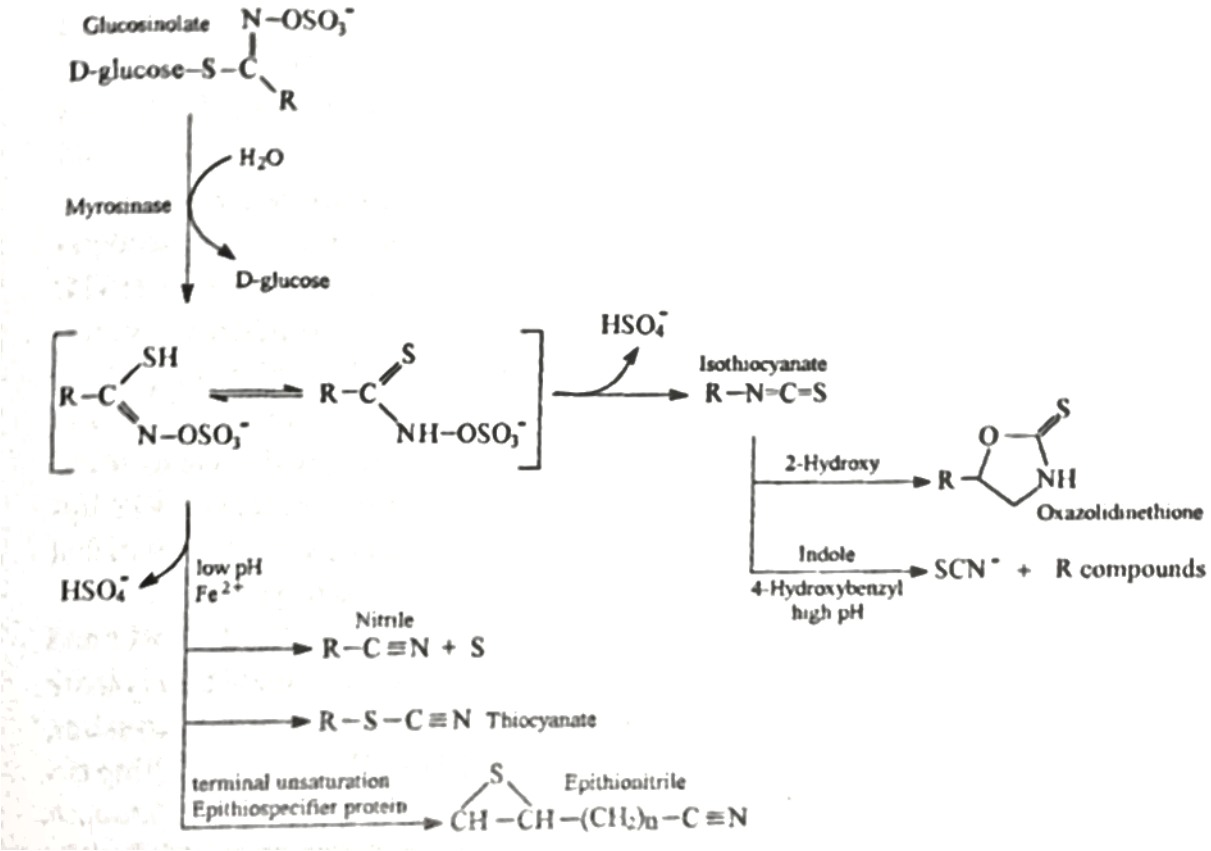

Figure A-3: Glucosinolate hydrolysis and subsequent products (Brown and Morra, 1997)

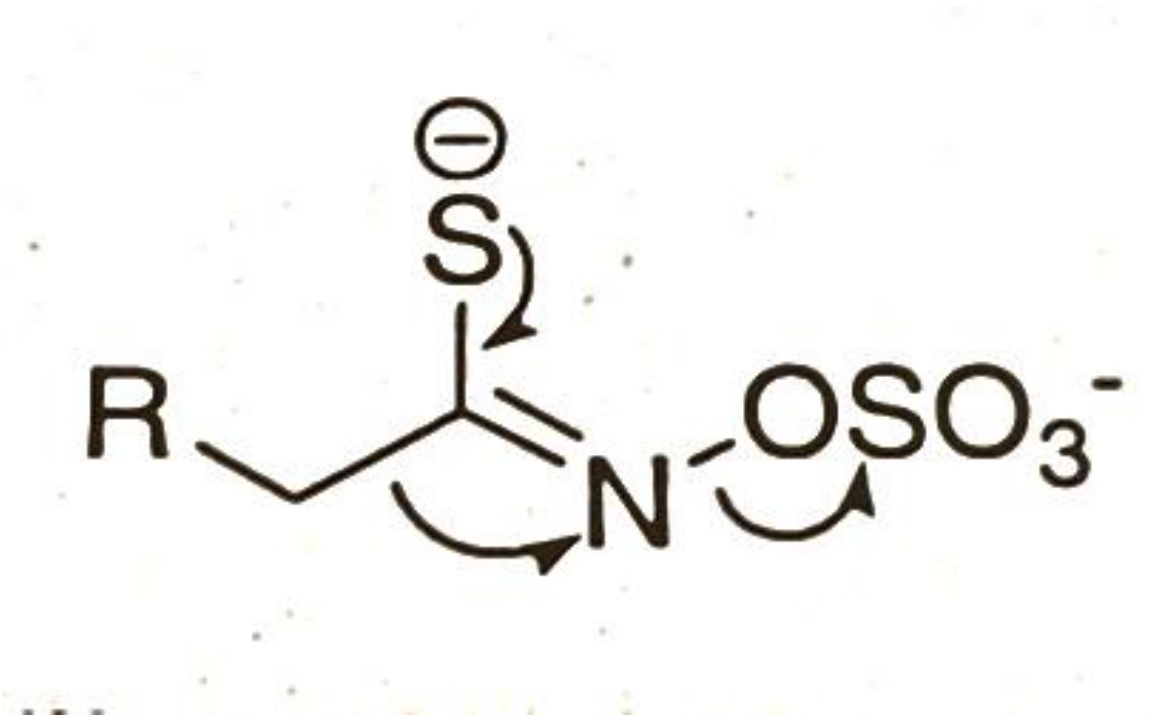

Figure A-4: Lossen rearrangement of Glucosinolates that results in isothiocyanates (Bones and Rossiter, 2006)

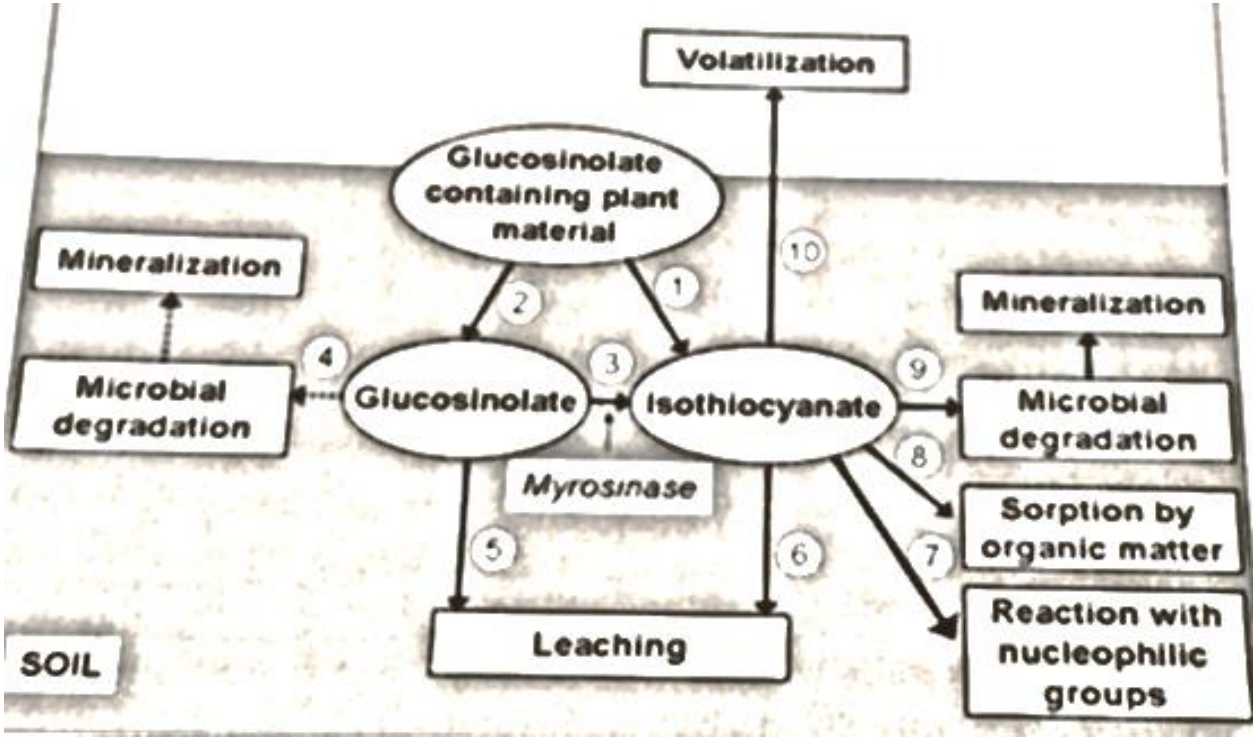

Figure A-5: Glucosinolate and isothiocyanate activity in soil environment (Gimsing and Kirkegaard, 2009)

Chapter 2: Impacts of Biofumigation and Anaerobic Soil Disinfestation on Overall Strawberry Plant and Soil Nutrition

Abstract

Anaerobic soil disinfestation and biofumigation are two non-chemical methods for controlling soilborne plant pathogens of strawberry. Due to their high mineral contents, both treatments could potentially increase mineral content in strawberry plants and thus impact fruit quality, but research in this area is limited. A trial was conducted with 11 pre-plant soil-incorporated treatments arranged in a randomized complete block design with 6 rows (blocks). Biofumigation treatments consisted of deactivated mustard meal, deoiled mustard meal, mustard pellets, and Biofence mustard seed meal. Other treatments included dried molasses as a carbon source for an anaerobic treatment and a Basamid® chemical treatment. Additional combination treatments of deactivated mustard meal combined with molasses, deoiled mustard meal combined with molasses, and molasses combined with soybean meal (to lower amendment C:N ratio) were also applied, as well as an untreated control. Harvested fruit were counted, weighed, and graded into marketable and non-marketable categories, and were then analyzed for sugars (fructose, glucose, sucrose), organic acids (malic and citric), and mineral content (B, Na, Mg, P, S, K, Ca, Fe, Cu, Mn, and Zn). Harvested leaves were analyzed for mineral content. Plots treated with Basamid® had an average yield of 228 g/plant but were not different statistically from those treated with the combination of the biofumigation treatment with deoiled mustard meal and anaerobic soil disinfestation with molasses (184 g/plant; $P>0.05$). Plants in the untreated plots produced the lowest overall yield (134 g/plant). Plots treated with Basamid® had the overall largest yield of non-marketable fruit (85.0 g/plant). Plots treated with Basamid® (143 g/plant) and those in the combination treatment of deoiled meal and molasses (110 g/plant) were not different statistically in marketable yield

(P>0.05). The plots treated with the biofumigation treatment of mustard pellets provided the largest overall marketable yield among the alternative methods (113 g/plant). However, the Basamid® treated plots had a larger marketable yield by contrast than all other biofumigation treatment plots combined (P<0.05). There were no differences among treatments for glucose and fructose. However, fruit from the plots treated with the combination treatment of molasses with deoiled mustard meal did have significantly more sucrose than the control (P<0.05). Fruit citric and malic acid contents were greater in the anaerobic disinfestation plots treated with the combination of molasses and soybean meal than in the plot treated with Basamid® (P<0.05). However, fruit sugar and organic acid content did not consistently differ among most treatments. Likewise, there were no consistent patterns of differences among treatments in mineral content of either fruit or leaf tissues (P<0.05). However, plants in Basamid® treated plots had a greater concentration of Ca than those treated with molasses and soybean meal (P<0.05). The combination of the biofumigation treatment of deoiled mustard meal and the anaerobic soil disinfestation treatment with molasses can provide a comparable marketable yield as the chemical treatment Basamid®. In general, the alternative methods of biofumigation and soil anaerobic disinfestation produced fruit of equal quality to that produced using the Basamid® chemical treatment. Future work will evaluate pathogen and soil nutrient dynamics affecting productivity in these alternative soil disinfestation treatments.

Introduction

As a result of the Montreal Protocol, methyl bromide was listed as the primary source of stratospheric bromine (Butler, 1995; Ploeg, 2007). Subsequently, methyl bromide was banned in several nations as a soil fumigant, including in the U.S. in

accordance with the U.S. Clean Air Act (Ploeg, 2007). Methyl bromide was once used as a pre-plant soil fumigant that was effective in controlling soilborne diseases, nematodes, insects, and weeds in economically important crops, such as strawberries (Ploeg, 2007). Certain methyl bromide alternatives that are still in use, such as Dazomet and Basamid, have an active ingredient of methyl isothiocyanate, which has been found to lead to plant toxicity if applied too close to planting date (Brown and Morra, 1997; Ajwa et al., 2003). Strawberry producers are interested in finding alternative, sustainable methods for soilborne pest control.

Biofumigation is one alternative method that has been found to be effective at controlling strawberry pathogen populations (Matter et al., 2008; Koron, 2009; Zurera et al., 2009). Kirkegaard and Sarwar (1998) described biofumigation as the suppression of soilborne pests and pathogens by biocidal compounds released in soil when glucosinolates (GSs) in *Brassica* green manure, rotation crops, or seed meal amendments are hydrolyzed. Isothiocyanates (ITCs) are hydrolysis products of GSs that are effective at fumigating soil (Lazzeri et al., 1993; Charron and Sams, 1999).

Biofumigation via *Brassica* incorporation is effective for controlling plant parasitic nematode and soilborne plant pathogen populations. Henderson et al. (2009) measured the effects of mustard seed meal on *Meloidogyne chitwoodi* in a field setting, and found that mustard meal is able to lower population densities. Lazzeri et al. (2009) also reported that in a field setting, defatted mustard meal was better at decreasing populations of *M. chitwoodi* than a treatment of Oxamyl. Henderson et al. (2009) reported that the combination of Biofence, a commercial *B. carinata* seed meal, with *Steinernema* spp., a biological control of nematodes, did not decrease instance of *M. chitwoodi*. However,

Biofence application significantly reduced *M. chitwoodi* populations and the tuber damage caused by such populations. Williams et al. (1993) were able to lethally and sub-lethally suppress *Limonius californicus* wireworms and postpone the onset of their feeding from 32 to 137 days after amending soil with AITC, allowing for the establishment of seedlings.

As phytotoxicity can be a problem with soil fumigation, crop yield is a focus of concern when comparing treatment methods. Lazzeri et al. (2003) found that *B. juncea* green manure provided a moderate treatment when analyzing strawberry yield comparatively to methyl bromide and general green manure. Strawberry yield in the mustard plots was significantly lower than plots treated with methyl bromide and significantly higher than general green manure treatment plots. Porras et al. (2009) combined biofumigation via *B. carinata* incorporation with solarization techniques and were able to increase strawberry fruit weight and significantly increase foliar surface and total yield comparatively to solarization techniques alone. In a field trial, Koron (2009) demonstrated that a Dazomet treatment provided larger plant growth than a *B. juncea* treatment; however, there was no significant difference in yield between the two treatments. The greenhouse trial of these treatments concluded with the *B. juncea* treatment having a higher yield than the Dazomet treatment.

Mustard meal is a current innovation that certain companies have begun to produce as an alternative to green *Brassica* manure or *Brassica* cover cropping. Brown and Morra (1995) reported that the low moisture content of defatted mustard meal allows the GSs within the meal to be more stable. Oliveira et al. (2011) measured AITC as the major hydrolytic product released by moistened whole seed meal, with concentrations of 5.4-μg/g dry soil detected at two hours after water addition. For the defatted seed meal, AITC

was detected in the greatest amount after six to 12 hours at a concentration of 9.76-ug/g dry soil. They concluded that both the whole and defatted seed meal of the wild mustard are applicable for biofumigation due to their high sinigrin content and high AITC release.

Anaerobic soil disinfestation (ASD) is another common methyl bromide alternative that has been found to be successful at controlling pathogens (Blok et al., 2000; Momma et al., 2010; McCarthy et al., 2014; Van Bruggen et al., 2014). ASD originated in both Japan and the Netherlands (Momma et al., 2013). In the Netherlands, Blok et al. (2000) recorded that combining organic amendments with air tight plastic coverings in field production significantly decreased soil pathogens.

Momma et al. (2010) speculated that the addition of organic amendments could cause nutrient overloading to the soil. Van Bruggen et al. (2014) conducted field and laboratory experiments that resulted in the use of an ASD treatment causing a depletion of NO_3-N and an increase in NH_4-N, Fe2+, and Mn3+ in the soil solution. Butler et al. (2012) did not find any negative impacts on soil fertility or plant nutrition following the implementation of ASD.

McCarty et al. (2014) determined that total, culled, and marketable yields of tomatoes and peppers were not affected by treatment when comparing a range of carbon sources for ASD. Shennan et al. (2011-2012) reported that there was no significant difference in strawberry yields when comparing ASD with Pic-Clor 60 (a common, soil fumigant).

The majority of the research surrounding biofumigation and ASD are concerned with their treatment effects on plant and soil pathogens, nematodes, and soil health. Research is lacking on the impacts of these treatment alternatives on over-all plant health.

The objectives of this research are to determine the effects of biofumigation, ASD, and a combination of the two on strawberry fruit and plant health, marketable and total yield, nematode count, and soil health for strawberry field production.

Materials and Methods

Mustard Meal Sources

The mustard meal used in the field experiments are Wisconsin Spice, Inc. brand deactivated mustard meal and deoiled overs (Berlin, WI), Triumph Italia brand Biofence (Agrium Italia Spa, Livorno, Italy), and Mustard Products and Technologies (MPT) brand mustard pellets (Saskatoon, SK, Canada). The dried molasses was OMALASS from Westway Feed Products LLC (New Orleans, LA). The soybean meal was Hi-Pro brand (Friona, TX).

2013-2014 Plant Science Farm Strawberry Field Test

A trial was conducted with 11 pre-plant, soil-incorporated treatments arranged in a randomized complete block design with 6 rows (blocks) at the East Tennessee Agricultural Research and Education Center in Knoxville, TN. The soil was a Shady-Whitwell complex originating from loamy alluvium derived from limestone, sandstone, and shale. The biofumigation treatments consisted of deactivated mustard meal, deoiled mustard meal, mustard pellets, and Biofence mustard seed meal. Other treatments included dried molasses as a carbon source for an ASD treatment and a Basamid® chemical treatment. Additional combination treatments of deactivated mustard meal and molasses, deoiled mustard meal and molasses, molasses and soybean meal (to lower amendment C:N ratio) were also applied, as well as a control with no treatment. In August of 2013, the intended field was pretreated with Round-Up® and Basagram twice each in order to eliminate

weeds. A perimeter was marked for six plant rows that were 67.1 m long. Beds were then formed at a width of 1.52 m, and the lengths of the rows were divided into 11 plots of 6.10 m length each with a buffer region of 1.22 m at the end of each plot.

On September 18 of 2013, the 11 treatments were incorporated into designated plots, with one treatment for each row (Tables B-1). The treatments were tilled into approximately 0.150 m of the soil depth. The beds were completed with an addition of two John Deere 16.0 mm, 30.0 cm spacing between emitters drip-tapes per row with a delivery rate of 15.0 psi and covered immediately with black plastic. The field was then drip irrigated for 32 hours.

At 21 days post-treatment, 'Chandler' strawberries purchased from Cottle Strawberry Nursery Inc. (Faison, NC) were transplanted into the plots with 0.300 m between plants in row and 0.360 m between double plant rows. A total of 32 plants were planted per plot. Cereal rye grass was grown as a ground cover crop in between rows in order to help aid in decrease of weed pressure.

The plants were irrigated once a week over a six hour time period. A once a week injection of a 20-20-20 fertilizer at 1.4 kg/wk/acre was applied until November 1, 2013. On November 11, 2013, soil samples were taken to measure pH and nitrogen levels. The plants were covered with a floating row covers at the beginning of December of 2013, in order to shield from colder temperatures. In early February of 2014, the covers were taken off, and dead leaves removed. Covers remained in field in the event the plants might need freeze protection. Fertigation began within a week of new leaf growth with an alternation between an injection of 20-20-20 fertilizer at 1.10 kg/wk/acre and an injection of calcium

nitrate at 1.4 kg/wk/acre. In March, April, and early May of 2014, overhead irrigation began at a rate of 0.500 cm/hr until sun-up.

Harvest began April 28, 2014. At harvest, fertigation alternated between an injection of calcium nitrate at 1.40 kg/wk/acre and an injection of potassium nitrate at 1.36 kg/wk/acre. Harvest of fruit and plant runners was done twice a week of only the center 28 plants of each plot (excluding two plants at the ends of plots). Weight and fruit number were recorded. The fruit was also graded into marketable and non-marketable fruit. Non-marketable fruit was subdivided into categories pertaining to physical appearance and size: deformed, rotten, and small marketable. Deformed fruit were the berries that had a physical appearance that are not marketable, such as 'nubbins' or 'button berries. Rotten strawberries are the fruit that contained blemishes. Small marketable strawberries were the fruit that did not contain a deformity or blemishes, but were less than 10 grams in weight. On May 14, 2014, fruit was sampled for mineral, sugar, and organic acid analysis and soil was sampled for soil pH, inorganic nitrogen, and nematode analysis. Leaves were also sampled for nutrient analysis.

Mineral Extraction of Fruit and Leaf Tissue

Mineral extraction of the strawberry fruit and leaf tissues was performed according to a method described by Barickman et al. (2013). In short, the strawberry fruit tissue sampled from the field experiment was weighed, freeze-dried, and then ground with liquid nitrogen in a mortar and pestle for analysis. The strawberry leaf tissue was weighed, air-dried in an oven at 45°C, reweighed, and then ground with a Magic Bullet. Dried tissue (0.5 g of leaf and fruit) was weighed into a 15.0 mL centrifuge tube. Each sample was added to a separate Teflon vessel and topped with 10.0 mL of nitric acid. These vessels were placed

upon a rotor apparatus that was added to an Ethos 1112 microwave digestion unit, where tissue was adequately digested for mineral analysis. Once the digestion program was completed, each sample was removed separately from each vessel. The sample (0.100 mL) was placed into a new 15.0 mL centrifuge tube that was filled with 9.9 mL of ICP matrix. The ICP matrix consists of 20% nitric acid and 5% hydrochloric acid. The samples were processed on an Agilent 7500 Series ICP-MS.

Sugar Analysis

Sugar analysis of fruit tissue was performed following Barickman et al. (in press) with minor edits. In short, the strawberry fruit tissue sampled from the field experiment was weighed, freeze-dried, and then ground with liquid nitrogen in a mortar and pestle for analysis. Dried and ground fruit tissue (0.100 g) was weighed into a 16x100-mm glass culture tube. Reverse osmosis water (1.00 mL) was added to the tube. The tubes were vortexed and then shaken horizontally for 15 minutes at 200 RPM. The samples were centrifuged at 14000 RPM for 10 minutes. The supernatant (500 µL) was transferred to new 16x100-mm glass culture tubes. Acetonitrile (0.700 mL) was added to the supernatant; the tubes were mixed by inversion, and then kept at room temperature for 30 minutes. This mixture was centrifuged at 14000 RPM for 10 minutes. The supernatant (500 µL) was placed into new 16x100-mm glass culture tubes. This sample was dried via evaporation under the fume hood. Once dry, the sample was rehydrated with 500 µL 75% acetonitrile. This was filtered with a 13.0 mm syringe filter into 12x32 mm clear standard crimp top vials and then capped and stored in the freezer until HPLC analysis.

HPLC analysis was performed on an Agilent 1100 series HPLC equipped with a refractive index detector (RID). Samples were injected at 10.0 µL, and the flow rate was set

at 1.00 mL/min for 16 minutes. The mobile phase consisted of 75% Acetonitrile in 25% reverse osmosis water which was kept isocratic for the entire 16 minute run. There was a two minute equilibration period prior to each injection. Separations were achieved using a 150 x 4.6 mm i.d., 5 μm analytical scale Zorbax Carbohydrate column (Agilent Technologies), which was equipped with a Zorbax NH2 4.6 x 12.5 mm i.d. guard cartridge (Agilent Technologies). The column temperature was kept at the standard 40°C. Samples were measured on the RID at a 254 nm wavelength. Data were collected, recorded, and integrated using Chemstation Software (Agilent Technologies). Sample composition was based on standard curves of malic and citric acids.

Organic Acid Analysis

Organic acid analysis of the fruit tissue was conducted following Barickman et al. (in press) with minor edits. Briefly, 1.00 g of the fresh strawberry fruit tissue harvested in the field was weighed into a 15.0 mL plastic tube. 2.00 mL of 80% ethanol was added to the tube, and then the tube was placed into an ultrasonic bath for five minutes. The sample tube was then centrifuged for five minutes at 1090 xg. The supernatant was decanted, and 2.00 mL of 80% ethanol was added once more to the tube. The sample tube was placed in an ultrasonic bath for five minutes and then centrifuged five minutes at 1090 xg. The supernatant was added to the prior supernatant and then evaporated to dryness with nitrogen gas. The sample was then dissolved in 5.00 mL reverse osmosis water and filtered with a 13.0 mm syringe filter into 12x32 mm clear standard crimp top vials that were capped and stored in the freezer until HPLC analysis.

HPLC analysis was performed on an Agilent 1100 series HPLC equipped with a refractive index detector (RID). Samples were injected at 10.0 μL, and the flow rate was set

at 1.00 mL/minute for 16 minutes. The mobile phase consisted of 100% 0.1 M H2SO4 which was kept isocratic for the entire 15 minute run. There was a two minute equilibration period prior to each injection. Separations were achieved using a 300 x 7.7-mm i.d., 8 µm analytical scale Hi-Plex H column (Agilent Technologies), which was equipped with a Zorbax NH2 4.6 x 12.5 mm i.d. guard cartridge (Agilent Technologies). The column temperature was kept at 50°C. Samples were measured on the RID at a 254 nm wavelength. Data were collected, recorded, and integrated using Chemstation Software (Agilent Technologies). Sample composition was based on standard curves of malic and citric acids.

Soil pH Analysis

Soil pH was conducted according to Kissel et al. (2009; 2012). In summary, soil samples taken from the field were air dried, sieved, and weighed out to approximately 5.00 g into 50.0 mL plastic tubes. 10.0 mL of 0.01 M CaCl2 was added to each of the tubes, and samples were mixed well. This solution was allowed to settle for approximately 10 minutes. Soil pH was then determined by placing a pH electrode (Orion 3-Star Plus pH Benchtop Meter; Thermo Scientific) into the solution. Soil pH values were recorded as pH $CaCl_2$ with an addition of 0.6 to standardize values to soil pH in water.

Soil Inorganic Nitrogen Analysis

Soil inorganic nitrogen analysis was performed on the dried and sieved soils used for pH. Samples were weighed out to 5.00 g (+/- 0.100 g) in a 50.0 mL plastic tube. 40.0 mL of 1 M KCl was added to each sample tube, and the tubes were placed on the shaker at 180 rpm for one hour. Sample tubes were then centrifuged at 3500 rpm for five minutes.

Supernatant was filtered through Whatman #42 filter paper into 20.0 mL scintillation vials to store in the freezer until analysis. Samples were analyzed colorimetrically for NO_3-N + NO_2-N and NH_4-N using a microplate reader based on Sims et al. (1995).

Nematode Analysis

Approximately 100 grams of soil was taken from each plots through zig-zag sampling six times 15.2 cm into the soil. Samples were placed in whirl-pack bags and shipped to Dr. Kathy Lawrence's entomology and plant pathology lab (Plant Diagnostics, Auburn, AL) in a cooler with ice packs. The soil was then analyzed for nematodes, which were identified by class: bacterial feeders, root-knot, reniform, spiral, tylenchulus, stunt, lesion, SCN, lance, sheath, and pin.

Results

Strawberry Fruit Yield

Total strawberry yield (marketable and non-marketable) was not significantly affected by treatment ($P=0.31$; Figure B-1). Plots treated with Basamid® provided an overall yield of 231 g/plant, and the plantst in the untreated plots yielded 137 g/plant. The plots treated with the combination of the biofumigation treatment with deoiled mustard meal and anaerobic soil disinfestation with molasses did not differ statistically (187 g/plant) from the Basamid® plots ($P>0.05$). As a whole, total yields from plots treated with biofumigation treatments combined with anaerobic disinfestation treatments did not differ statistically from the chemical treatment of Basamid® ($P>0.05$); however, total yield from plots treated with only biofumigation treatments or ASD treatments were statistically less than plants in Basamid® plots ($P<0.05$).

Marketable strawberry yield was not significantly affected by treatment (P=0.24; Figure B-2). Plots treated with Basamid® provided a marketable yield (143 g/plant) that did not differ statistically from plots treated with the combination treatment of deoiled meal and molasses (110 g/plant; P>0.05). The plots treated with the biofumigation treatment of mustard pellets provided the largest overall marketable yield for the alternative methods (113 g/plant). However, all other biofumigation treatments plots yielded statistically less than the Basamid® plots when compared as a whole via contrast (P<0.05).

The non-marketable strawberry fruit was subdivided into cat-faced, rotten, and small marketable (less than 10.0 grams in weight). Non-marketable strawberry fruit yield was not significantly affected by treatment (P=0.40; Figure B-3). For deformed (Figure B-4) and rotten fruit (Figure B-5), plots treated with Basamid® had significantly more yield than the control plots (P<0.05). Plots treated with Basamid® yielded significantly more small marketable fruit (32.6 g/plant) than plots treated with soybean meal (20.8 g/plant; Figure B-6).

Strawberry Fruit and Leaf Mineral Content

Treatments did not affect leaf tissue B, Mg, P, S, K, Fe, or Cu concentrations (P<0.05; Table B-2). However, both plants in the Basamid® and the molasses and soybean combination plots had greater Na concentrations than the plants in the Biofence and control plots (P<0.05). Basamid® treated plots had greater Cu concentrations in leaves than plants in the combination plot of molasses and deoiled mustard meal (P<0.05). Basamid® plots had a greater concentration of Mn that the control, soybean, molasses, molasses and deactivated mustard meal, and molasses and deoiled mustard meal

treatment plots ($P<0.05$). Basamid® plots also had a greater concentration of Zn than the control and the molasses and soybean plots ($P<0.05$).

With regard to fruit minerals, treatments did not differ for B, Na, P, S, K, and Cu ($P<0.05$; Table B-3). However, plots treated with deactivated mustard meal had a significantly higher Mg concentration than those treated with the combination of molasses with soybean meal ($P<0.05$). Basamid® plots had a greater concentration of Ca than those treated with molasses and soybean meal ($P<0.05$). Plots treated with deoiled overs had a greater concentration of Fe than the control plots ($P<0.05$). Plots treated with Basamid® had a greater concentration of Mn than the control, deoiled mustard meal, molasses, Biofence, molasses and soybean meal, molasses and deoiled mustard meal, and molasses and deactivated mustard meal ($P<0.05$). Plots treated with the combination treatment of deactivated mustard meal and molasses had a greater concentration of Zn than those treated with molasses and soybean meal ($P<0.05$).

Strawberry Fruit Sugar Content

The overall analysis of sugar content showed treatments had no effect on fructose, glucose, and sucrose in the strawberry fruit tissue ($P>0.05$). However, when comparing treatments as contrasts, fruit from the plots treated with the combination treatment of molasses with deoiled mustard meal had significantly more sucrose (272 mg/g) than the fruit from the control (173mg/g), deactivated mustard meal (201 mg/g), and deoiled mustard meal (198 mg/g) plots ($P<0.05$; Figure B-7).

Strawberry Fruit Organic Acid Content

Citric and malic acid contents had similar responses to soil treatment (Figure B-8). Plots treated with molasses produced fruit with significantly more malic and citric acid concentrations than the fruit harvested from control plots and plots treated with deoiled meal, soybean meal, mustard pellets, Biofence, molasses combined with soybean meal, and molasses combined with deoiled meal. Strawberries harvested from the plots treated with molasses had mean concentrations of 44.3 mg of malic acid per g of soil and 10.4 mg of citric acid per g of soil, while strawberries harvested from the plots treated with soybean meal had mean concentrations of 20.5 mg of malic acid per g of soil and 5.21 mg of citric acid per g of soil. Strawberries harvested from plots treated with molasses combined with soybean meal had concentrations of 28.8 mg of malic acid per g of soil and 6.88 mg of citric acid per g of soil. This was significantly different than the molasses only treatment ($P<0.05$).

Soil pH

The pH of soil sampled on November 11, 2013 significantly differed among treatments ($P<0.001$). As expected, the control plot with no treatment had the highest mean soil pH of 6.09 (Figure B-9). This is significantly different than all other treatments ($P<0.05$). The ASD treatment of molasses alone had the lowest mean pH of 5.37. This is significantly different than plots treated with soybean meal, mustard pellets, Basamid®, and the combination of treatment of molasses and deactivated meal ($P<0.05$). Soil pH of soil sampled during harvest on May 14, 2014 did not differ significantly among treatments ($P>0.05$; Figure B-11).

Soil Inorganic Nitrogen

Total inorganic N (NH_4, NO_3, and NO_2) of the soil sampled on November 11, 2013 was not significantly affected by treatment ($P>0.05$). The soil treated with the combination treatment of molasses and soybean meal (60.1 mg/kg) had significantly more total inorganic nitrogen than the soil from the control plot (21.4 mg/kg) and the Biofence plot (27.4 mg/kg; $P<0.05$; Figure B-12). Total inorganic N of the soil sampled on May 14, 2014 was also not significantly affected by treatment ($P>0.05$). The soil treated with the ASD treatment of molasses had significantly more total inorganicN (21.4 mg/kg) than the combination treatment of molasses with soybean meal (8.21 mg/kg; $P<0.05$; Figure B-10).

Nematodes

For the soil cores taken a month after treatment incorporation, the untreated plots had statistically similar counts of bacterial feeders (beneficial nematodes) (76/500cm^3) as the plots treated with soybean meal (136/500cm^3; $P>0.05$); however, soils collected from the untreated plots had significantly less beneficial nematodes than all other plots ($P<0.05$). *Rotylenchulus reniformis* (reniform nematode) was only found in one plot treated with soybean meal (87/500cm^3). No differences occurred among alternative treatment methods with regard to beneficial nematode counts ($P>0.05$). *Helicotylenchus* spp. (spiral nematodes) were found in statistically similar numbers in all treatment plots ($P=0.83$). *Tylenchulus* spp. (Citrus nematode) was found in significantly greater numbers in plots treated with mustard pellets (8/500cm^3) than in plots treated with deoiled meal (0/500cm^3) and the combination treatment of deactivated meal with molasses (0/500cm^3; $P<0.05$). Plots treated with deoiled meal had a significantly larger *Tylenchorhynchus* spp. (stunt nematode) count (19/500cm^3) than plots treated with deactivated meal

(0/500cm^3), molasses (0/500cm^3), Basamid® (1/500cm^3), and the combination treatment of deoiled meal with molasses (0/500cm^3; $P<0.05$). *Hoplolaimus galeatus* (lance nematode) was only found in one plot treated with soybean meal (87/500cm^3) and one control plot (87/500cm^3). Overall, plots treated with soybean meal had significantly more parasitic nematodes (70/500cm^3) than plots treated with the combination treatment of deoiled meal with molasses (2/500cm^3; $P<0.05$).

For the soil cores taken during harvest, the biofumigation treatment of deactivated mustard meal had the highest number of beneficial nematodes (605/500cm^3 of soil) when compared to the control plot (142/500cm^3), soybean meal (283/500cm^3), Biofence (180/500cm^3), Basamid® (296/500cm^3), and the combination of molasses with soybean meal (270/500cm^3). The other treatment plots (deoiled meal, mustard pellets, molasses, molasses combined with deoiled meal, and molasses combined with deactivated meal) did not differ statistically from the deactivated mustard meal plots ($P>0.05$; Figure B-11). *Helicotylenchus* spp. (spiral nematode) was only found in one plot treated with deactivated meal (77/500cm^3) and one plot treated with Biofence (77/500cm^3). The occurrence of *Tylenchulus* spp. (Citrus nematode) was not significantly different among treatment plots ($P=0.72$). The presence of *Tylenchorhynchus* spp. (stunt nematode) was noted more significantly in plots treated with deactivated overs (3/500cm^3) than plots treated with the combination treatments (none were found; $P<0.05$). *Pratylenchus* spp. (lesion nematode) was only observed in one plot treated with the combination treatment of deactivated meal with molasses (77/500cm^3). Overall, more parasitic nematodes were found in plots treated with soybean meal (109/500cm^3) than plots treated with deoiled meal (5/500cm^3), molasses (5/500cm3), Basamid® (4/500cm^3), soybean meal combined with molasses

(5/500cm3), and the control plots (4/500cm3; $P<0.05$). All treatment plots had statistically similar amounts of parasitic nematodes as Basamid® ($P>0.05$).

Discussion

The combination of ASD with biofumigation treatments did not significantly differ from the chemical treatment of Basamid® in regards to total yield of strawberry fruit ($P=0.10$). However, the plots treated with Basamid® yielded significantly more fruit than the plots treated with biofumigation treatments ($P=0.015$) and ASD treatments ($P=0.014$) when applied separately. This is contrary to Koron (2009), who reported that the total strawberry fruit yield of the biofumigation treatment of *B. juncea* did not differ significantly from the total yield of Dazomet (Basamid®). The greenhouse trial conducted in conjunction with that field experiment found that the pots treated with the *B. juncea* treatment yielded significantly more strawberry fruit than the pots treated with Basamid® (Koron, 2009). The difference in yield between the plots treated with a chemical treatment and the plots treated with the ASD treatment of molasses also conflicts with the 2011-2012 field study of Shennan et al. (2012), who found that the total yield of plots treated with the chemical treatment of Pic-Clor60 did not differ from the total yield of the plots treated with ASD treatment. In our field study, the total yield from the combination treatment plots did not differ significantly from the total yield of the control plots ($P=0.14$). The marketable yield of strawberries (strawberries that were at least 10 g and did not have any visible blemishes) behaved the same as total yield. However, individually, more biofumigation treatment plots did not significantly differ from the plots treated with Basamid®. The only biofumigation treatment that was significantly different from Basamid® when compared on its own was Biofence ($P<0.05$). The marketable yield harvested from plots treated with

the combination of molasses with soybean meal also did not statistically differ from the total marketable yield harvested from plots treated with Basamid® ($P>0.05$).

There are not many reports regarding how biofumigation treatments or ASD treatments affect overall plant nutrition. We chose to report this data to show the effects of alternative treatments on a strawberry production system beyond pest suppression. Overall, there were no treatment differences for leaf mineral content (B, Mg, P, S, K, Fe, and Cu). The sodium concentration for leaves from the Basamid® plots and combination treatment plots of molasses and soybean meal significantly differed from the concentrations in the leaves harvested from the control and the Biofence plots. The leaves harvested from the plots treated with Basamid® had significantly more Ca than the leaves harvested from the plots treated with the combination treatment of molasses and deoiled meal ($P>0.05$). However, there were no other treatment differences as a whole or individually from all other treatments and Basamid® regarding leaf Ca concentration ($P>0.05$). There was a significant difference between the Mn content of the leaves harvested from the Basamid plots (268 mg/g) as opposed to the leaves harvested from the control (132 mg/g), soybean meal (153 mg/g), Biofence (151 mg/g), molasses and deoiled meal combination (124 mg/g), and molasses and deactivated meal combination plots (122 mg/g; $P<0.05$). Finally, there was a significant difference in the Zn content of the leaves harvested from the plots treated with Basamid® (27.20 mg/g) and the plots treated with the combination of molasses and soybean meal (20.34 mg/g) and the control plot (20.4 mg/g; $P<0.05$). There were more significant differences for the fruit minerals than there were for the leaf minerals. The only mineral for which there were no treatment differences (whether combined or individually) was B ($P>0.05$). Overall, the fruit harvested from the

plots treated with soybean meal had significantly less Na concentration (66.5 mg/g) than the fruit harvested from the plots treated with molasses (97.8 mg/g), Basamid® (98.9 mg/g), and the combination of molasses and deactivated meal (96.4 mg/g; $P<0.05$). The fruit harvested from the plots treated with soybean meal also had significantly less Mg and P contents than the fruit harvested from plots treated with deactivated meal and deoiled meal; had significantly less K content than the plots treated with deactivated meal, deoiled meal, and molasses; and had significantly less Cu content than the fruits harvested from plots treated with the combination of molasses and soybean meal ($P<0.05$). The Ca content of the fruit harvested from the plots treated with soybean meal was significantly different (1210 mg/g) than the fruit harvested from the plots treated with Basamid® (1960 mg/g), mustard pellets (1730 mg/g), deoiled meal (1720 mg/g), and the control plot with no treatment (1860 mg/g; $P<0.05$). The fruit harvested from the plots treated with the combination treatment of molasses and soybean meal had significantly less Ca content (1450 mg/g) than the plots treated with Basamid® ($P<0.05$). The fruit harvested from the plots treated Basamid® had significantly more Mn content (90.3 mg/g) than the fruit harvested from the plots treated with deoiled meal (59.2 mg/g), molasses (50.1 mg/g), Biofence (62.1 mg/g), the combination of molasses and soybean meal (57.7 mg/g), the combination of molasses and deoiled meal (55.0 mg/g), and the combination of molasses and deactivated meal (53.8 mg/g; $P<0.05$). The fruit harvested from the plots treated with deoiled meal had a significantly larger Fe content (27.4 mg/g) than the fruit harvested from the plots treated with soybean meal (6.87 mg/g) and the control plots (5.66 mg/g; $P<0.05$). Finally, the fruit harvested from the plots treated with the combination treatment of molasses and deactivated meal had significantly more Zn content (17.7 mg/g) than the

plots treated with soybean meal (13.5 mg/g) and the combination of molasses and soybean meal (13.1 mg/g; $P<0.05$). Overall, the plots treated with soybean meal yielded fruit with reduced nutrient content when compared to many of the other treatments. From a nutritional standpoint, this would not be a viable treatment for strawberry production. The biofumigation, ASD, and combination treatments were, for the most part, significantly similar to the chemical treatment of Basamid®. Nutrient analysis of each of these treatments should be conducted to explain for the differences in mineral content that occurred between and among treatments.

The fruit content of glucose, a major carbohydrate in strawberry fruit tissue, did not differ among the treatment plots ($P>0.05$). However, fruit harvested from the plots treated with the combination treatment of molasses and deoiled meal had significantly more fructose content (305 mg/g) than the fruit harvested from the plots treated with soybean meal (264 mg/g; $P<0.05$). The fruit harvested from the plots treated with the combination of molasses and deoiled meal also had a larger sucrose content (272 mg/g) than the fruit harvested from the plots treated with deactivated meal (201 mg/g), deoiled meal (198 mg/g), and the control plot (173 mg/g; $P<0.05$). Overall, the combination treatment of molasses and deoiled meal resulted in higher carbohydrate content for the strawberry fruit. The higher carbohydrate content provides the sweet flavor of the strawberries, which is an important marketing point for strawberry producers. Schwieterman et al. (2014) conducted a taste panel regarding what drives consumers to favor strawberries and discovered that the sweetness associated with fruit carbohydrates are one of the most important factors.

The plots treated with the ASD treatment of molasses yielded fruit that had significantly higher malic acid concentrations (44.3 mg/g) than the plots treated with deactivated meal (28.7 mg/g), deoiled meal (27.3 mg/g), mustard pellets (27.7 mg/g), Biofence (26.3 mg/g), combination treatment of molasses and deoiled meal (26.0 mg/g), molasses combined with soybean meal (28.8 mg/g), soybean meal (20.5 mg/g), and the control plot (25.6 mg/g; $P<0.05$). The plots treated with Basamid® also had significantly higher malic acid content (40.6 mg/g) than the plots treated with soybean meal and the control plot ($P<0.05$). Again, the fruit harvested from the plots treated with the ASD treatment of molasses had significantly higher citric acid concentrations (10.4 mg/g) than the plots treated with the combination treatment of molasses and deoiled meal (5.96 mg/g), molasses combined with soybean meal (6.88 mg/g), Biofence (6.03 mg/g), mustard pellets (6.81 mg/g), soybean meal (5.21 mg/g), deoiled meal (6.53 mg/g), and the control plots (6.31 mg/g; $P<0.05$). The fruit harvested from the plots treated with Basamid® also had a significantly higher concentration of citric acid (8.70 mg/g) than the fruit harvested from the plots treated with soybean meal ($P<0.05$). It is not surprising that the ASD treatment plots yielded fruit with a higher organic acid content, because ASD treatments are known to cause the soil environment to become more acidic (Momma, 2008; Momma et al., 2006).

The soil pH of the plots taken a month after treatment incorporation had a significant treatment differences ($P=0.0003$). Soil cores taken from the control plots had significantly higher pH (6.09) than the cores taken from plots treated with deactivated meal (5.51), deoiled meal (5.53), soybean meal (5.72), mustard pellets (5.76), molasses (5.37), Biofence (5.45), Basamid® (5.72), molasses combined with soybean meal (5.47),

the combination treatment of molasses and deoiled meal (5.47), and the combination treatment of molasses and deactivated meal (5.66; $P<0.05$). The soil cores taken from the plots treated with the ASD treatment of molasses had a significantly lower pH than the plots treated with soybean meal, mustard pellets, Basamid®, and the combination treatment of molasses and deactivated meal ($P<0.05$). The soil cores taken during fruit harvest did not differ significantly among treatments with regard to soil pH ($P=0.64$). The higher acidity of the plots treated with ASD treatments coincide with the release of organic acid into soils from ASD treatments (Momma, 2008; Momma et al., 2006). McCarty et al. (2014) did not find a significant difference in soil pH between plots treated with biofumigation treatments of mustard meal and plots treated with ASD treatments of molasses.

When measured a month after treatment incorporation, total soil inorganic N was significantly higher in the plots treated with molasses combined with soybean meal (60.1 mg/kg) than in plots treated with Biofence (27.4 mg/kg) and the control plots (21.4 mg/kg; $P<0.05$). The soil cores taken during harvest showed that the plots treated with the ASD treatment of molasses had significantly more soil inorganic N (21.37 mg/kg) than soil treated with molasses combined with soybean meal (8.21 mg/kg; $P<0.05$). McCarty et al. (2014) reported that there was no significant difference in soil inorganic N between the plots treated with the ASD treatment of molasses and the plots treated with the biofumigation treatment of mustard meal. They found that the control plots with no treatment had significantly more soil inorganic N than both ASD and biofumigation treatments.

For both sampling dates, beneficial nematodes were found in statistically similar amounts for the alternative methods (biofumigation, ASD, and the combination of both) as well as the chemical application (Basamid®). The control plots for both sampling dates had significantly less beneficial nematodes than most of the other treatment plots. Also, plots treated with soybean meal contained larger numbers of parasitic nematodes. None of the other treatment methods proved to be a better control for parasitic nematodes. Henderson et al. (2009) were able to control parasitic nematodes with Biofence applications; however, they did not report the effect of Biofence on beneficial nematodes.

Conclusion

In regard to total strawberry fruit yield, biofumigation and ASD treatments on their own were not comparable to the chemical treatment of Basamid® which is contrary to past research (Koron, 2009; Shennan et al., 2012); however, combining biofumigation and ASD treatments provided a yield that was significantly the same as Basamid®. More importantly, marketable yield harvested from plots treated with all biofumigation (minus Biofence), ASD, and combination treatments were statistically equal to those harvested from plots treated with Basamid®. Therefore, any would be a viable alternative to chemical treatments if marketable yield is the main concern. Leaf mineral analysis did not vary enough among treatments for one treatment to be considered superior. While fruit mineral analysis had differences, the differences were not consistent in regard to a better treatment. Fruit quality with respect to carbohydrate analysis differed from the combination treatment of deoiled meal and molasses, providing a carbohydrate content superior to some of the other treatments. ASD treatment of molasses yielded fruit with higher organic acid content, while also decreasing the soil pH at treatment incorporation

but not at harvest. These two observations could be related. Further research on the effects of higher soil pH from ASD treatments on strawberry fruit organic acid concentrations should be performed. For this analysis, the increase in organic acid content of the strawberry fruit could cause a bitter taste that may be detrimental to consumptive sales. The addition of soybean meal to the ASD treatment of molasses was able to increase the concentration of inorganic N in the soil solution after incorporation; however, when measured at harvest, the concentration of total inorganic N was significantly lower than most other treatments. Parasitic nematodes were equally not present in plots treated with the alternative methods; however, beneficial nematodes were found in these plots. Overall, combining ASD with biofumigation is effective at increasing total yield; however, for all other aspects of strawberry production, each treatment is efficient on their own in comparison to the chemical treatment of Basamid®.

References:

Ajwa, H.A., Klose, S., Nelson, S. D., Minuto, A. M., Gullino, M. L., Lamberti, F., Lopez-Aranda, J. M. (2003). Alternatives to methyl bromide in strawberry production in the United States of America and the Mediterranean region. *Phytopathol. Mediterr.* 42(3): 220-244.

Barickman, T. C., Kopsell, D., Sams, C.. In Press. Foliar applications of abscisic acid improve greenhouse tomato fruit quality.

Barickman, T.C., Kopsell, D., Sams, C. (2013). Selenium Influences Glucosinolates and Isothiocyanates and Increases Sulfur Uptake in Arabidopsis thaliana and Rapid-Cycling *Brassica oleracea*. *Journal of Agriculture and Food Chemistry* 61: 202-209.

Blok, W. J., Lamers, J. G., Termorshuizen, A. J., Bollen, G. J. (2000). Control of Soilborne Plant Pathogens by Incorporating Fresh Organic Amendments Followed by Tarping. *Disease Control and Pest Management* 90 (3): 253-259.

Brown, Paul D, Morra, M. J. (1995). Glucosinolate-Containing Plant Tissues as Bioherbicides. *J. Agric. Food Chem* 43(12): 3070-3074.

Brown, P. D., Morra, M.J. (1997). Control of soil-borne plant pests using glucosinolate-containing plants. *Advances in Agronomy*. D. L. Sparks. New York, Academic Press: 167-231.

Butler, J. H. (1995). Methyl bromide under scrutiny. *Nature* 376: 469-470.

Butler, D. M., Kokalis-Burelle, N., Muramoto, J., Shennan, C., McCollum, T. G., Rosskopf, E. N. (2012). Impact of anaerobic soil disinfestation combined with soil solarization on

plant-parasitic nematodes and introduced inoculum of soilborne plant pathogens in raised-bed vegetable production. *Crop Protection* 39: 33-40.

Butler, D. M., Rosskopf, E. N., Kokalis-Burelle, N., Albano, J. P., Muramoto, J., Shennan, C. (2012). Exploring warm-season cover crops as carbon sources for anaerobic soil disinfestation (ASD). *Plant Soil* 355:149-165.

Charron, C. S., Sams, C. E. (1999). Inhibition of *Pythium ultimum* and *Rhizoctonia solani* by shredded leaves of *Brassica* species. *Journal of the American Society for Horticultural Science* 124(5): 462-467.

Henderson, D. R., Riga, E., Ramirez, R. A., Wilson, J., Snyder, W. E. (2009). Mustard biofumigation disrupts biological control by Steinernema spp. nematodes in the soil. *Biological Control* 48(3): 316-322.

Kirkegaard, J. A., Sarwar, M (1998). Biofumigation potential of *Brassicas* - I. Variation in glucosinolate profiles of diverse field-grown *Brassicas*. *Plant and Soil* 201(1): 71-89.

Kissel, D.E., Sonon, L., Vendrell, P F., Isaac, R. A. 2009. Salt concentration and measurement of soil pH. Commun. *Soil Sci. Plant Anal*. 40:179–187.

Kissel, D.E., Vendrell, P. F. 2012. Soil testing: Soil pH and salt concentration. The University of Georgia Cooperative Extension. *Circular 875*. Athens, GA.

Koron, D. (2009). The Influence of Biofumigation on Mycorrhizal Fungi and Growth of Strawberries. *Proc. VIth Internat. Strawberry Symposium*: 381-384.

Lazzeri, L., Tacconi, R., Palmieri, S. (1993). In Vitro Activity of Some Glucosinolates and Their Reaction Products toward a Population of the Nematode Heterodera schachtii. *J. Agric. Food Chem* 41(5): 825-829.

Lazzeri, L., Malaguti, B. G., Antoniacci, L. (2003). Replacing methyl bromide in annual strawberry production with glucosinolate-containing green manure crops. *Pest Manag. Sci.* 59:983-990.

Lazzeri, L., Curto, G., Dallavalle, E., D'Avino, L., Malaguti, L., Santi, R., Patalano, G. (2009). Nematicidal Efficacy of Biofumigation by Defatted *Brassicaceae* Meal for Control of *Meloidogyne incognita* (Kofoid et White) Chitw. On a Full Field Zucchini Crop. *Journal of Sustainable Agriculture* 33(3): 349-358.

Mattner, S.W., Porter, I. J., Gounder, R. K., Shanks, A. L., Wren, D. J., Allen, D. (2008). Factors that impact on the ability of biofumigants to suppress fungal pathogens and weeds of strawberry. *Crop Protection* 27: 1165-1173.

McCarty, D.G., Ownley, B.H., Wszelaki, A.L., Eichler Inwood, S.E., Sams, C.E., Butler, D.M. (2014). Field evaluation of anaerobic soil disinfestation (ASD) carbon sources for tomato and bell pepper production in Tennessee. *HortScience* 49(3):272-280.

Momma, N., Yamamoto, K., Simandi,P., Shishido, M. (2006). Role of organic acids in the mechanisms of biological soil disinfestation (BSD). *Journal of General Plant Pathology* 72: 247-252.

Momma, N. (2008). Biological Soil Disinfestation (BSD) of Soilborne Pathogens and Its Possible Mechanisms. *Japan Agricultural Research Quarterly* 42 (1): 7-12.

Momma, N, Momma, M., Kobara, Y. (2010). Biological soil disinfestation using ethanol: effect on Fusarium oxysporum f. sp. Lycopersici and soil microorganisms. *Journal of General Plant Pathology* 76: 336-344.

Momma, N., Kobara, Y., Uematsu, S., Kita, N., Shinmura, A. (2013). Development of biological soil disinfestations in Japan. *Applied Microbiology and Biotechnology* 97(9): 3801-3809.

Oliveira, R. D. L., Dhingra, O. D., Lima, A. O., Jham, G. N., Berhow, M. A., Holloway, R. K., Vaughn, S. F. (2011). Glucosinolate content and nematicidal activity of Brazilian wild mustard tissues against Meloidogyne incognita in tomato. *Plant and Soil* 341(1-2): 155-164.

Ploeg, A. (2007). Biofumigation To Manage Plant-Parasitic Nematodes. *Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes* 2: 239-248.

Porras, M., Barrau, C. Romero, E., Zurera, C., Romero, F. (2009). Effect of Biofumigation with *Brassica carinata* and Soil Solarization on *Phytophthora* spp. and Strawberry Yield. *Proc. VIth Internat. Strawberry Symposium:* 969-972.

Schwieterman, M. L., Colquhoun, T. A., Jaworski, E. A., Bartoshuk, L. M., Gilbert, J. L., Tieman, D. M., Odabasi, A. Z., Moskowitz, H. R., Folta, K. M., Klee, H. J., Sims, C. A., Whitaker, V. M., Clark, D. G. (2014). Strawberry Flavor: Diverse Chemical Compositions, a Seasonal Influence, and Effects on Sensory Perception. *PLoS ONE*, 9(2), e88446. doi:10.1371/journal.pone.0088446

Shennan, C., Muramoto, J., Fennimore, S., Mazzola, M., Lazarovits, G. (2013). Nonfumigant strategies for soilborne disease control in California strawberry production systems. *California Strawberry Commission Annual Production Research Report* 2011-2012:145-159.

Sims, G. K., Ellsworth, T. R., Mulvaney, R. L. (1995). Microscale Determination of Inorganic Nitrogen in Water and Soil Extracts. *Communications in Soil Science and Plant Analysis* 6(1-2):303-316.

Van Bruggen, A., Blok, W., Gu, G. (2014). Soil Disinfestation and Food Safety Issues. *Acta Horticulturae (ISHS)* 1044: 437-446.

Williams, L III, Morra, M. J., Brown, P. D., McCaffrey, J. P. (1993), Toxicity of Allyl Isothiocyanate-Amended Soil to Limonius californicus (Mann.) (Coleoptera: Elateridae) Wireworms. *Journal of Chemical Ecology* 19(6): 1033-1046.

Zurera, C., Romero, E., Poras, M., Barrau, C., Romero, F., (2009). In Vitro Suppression of *Phytophthora cactorum* and *Verticillium dahlia* Potential Strawberry Pathogens by *Brassica* Tissues. *Proc. VIth Internat. Strawberry Symposium*: 267-270.

Appendix B:

Table B-1: Treatments and their application rates as used in the 2013-2014 Plant Science Farm Strawberry Field Test.

Treatment Number	Chemical	Rate		Number of Reps
		(lb of Product/acre)	(lb/37.33ft^2) Treated bed area	
1	Control	0	0	6
2	Deactivated Mustard Meal	4000	3.43	6
3	Mustard Meal (Deoiled Overs)	4000	3.43	6
4	Soybean Meal (defatted)	4000	3.43	6
5	Mustard Meal (pellets, Canadian)	4000	3.43	6
6	Molasses	12800	11.0	6
7	Biofence	4000	3.43	6
8	Basamid G (99% a.i.)	400	0.343	6
9	Dried Molasses+Soybean Meal	12800+4000	11.0+3.43	6
10	Dried Molasses+Deoiled Overs	12800+4000	11.0+3.43	6
11	Dried Molasses+Deactivated Mustard Meal	12800+4000	11.0+3.43	6

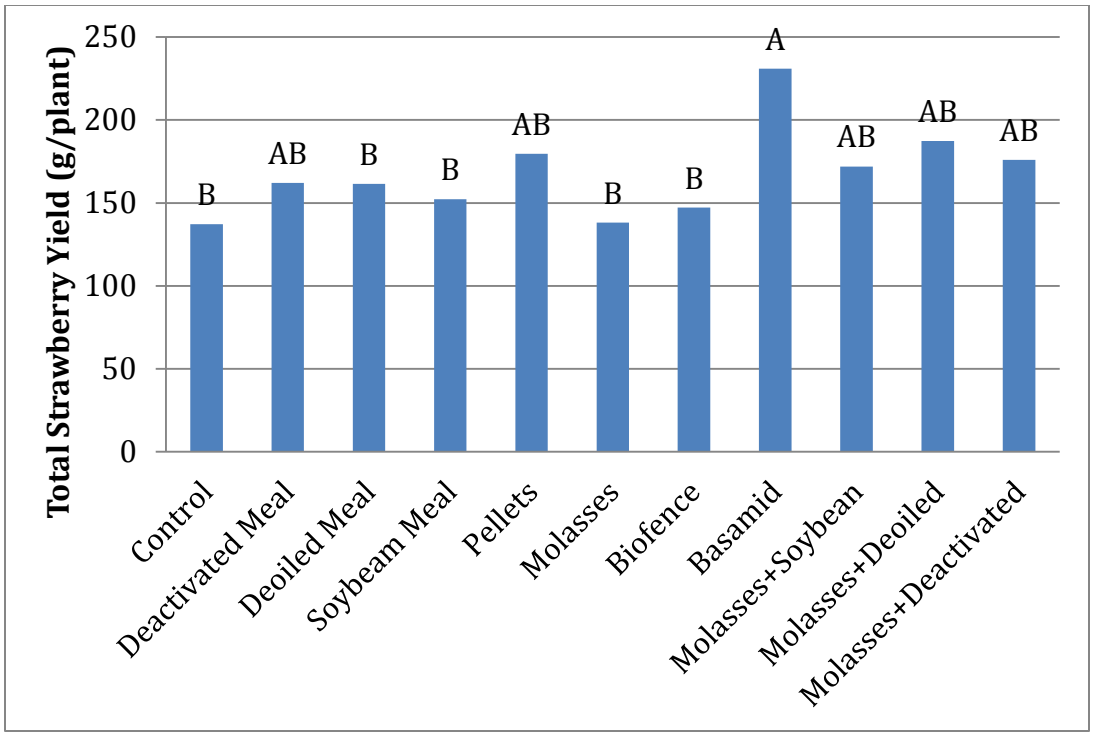

Figure B-1: Mean Total Strawberry Fruit Yield per Plant as affected by treatment. Values are combined means of treatment plots harvested in 2014. Means indicated by different letters are significantly different ($P<0.05$). Fruit was harvested from 28 plants per plot.

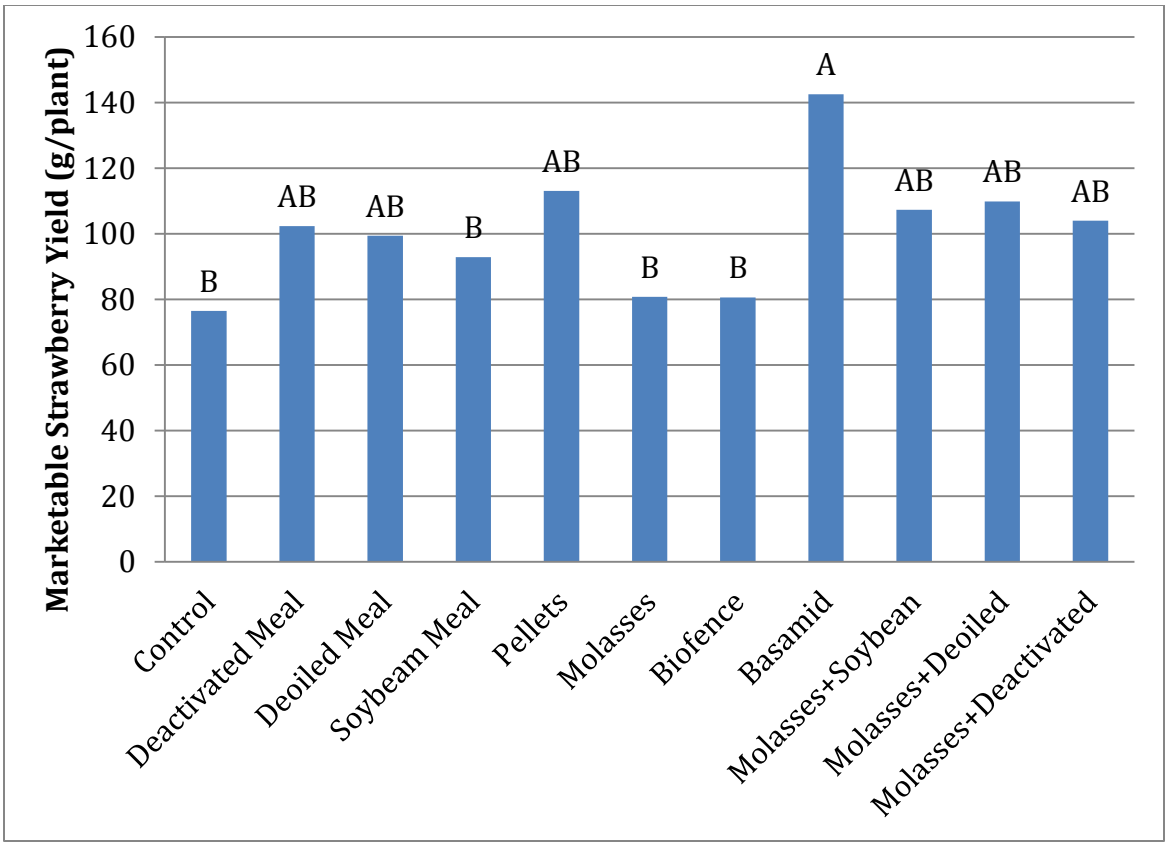

Figure B-2: Mean Marketable Strawberry Fruit Yield per Plant as affected by treatment. Values are combined means of treatment plots harvested in 2014. Means indicated by different letters are significantly different ($P<0.05$). Fruit was harvested from 28 plants per plot.

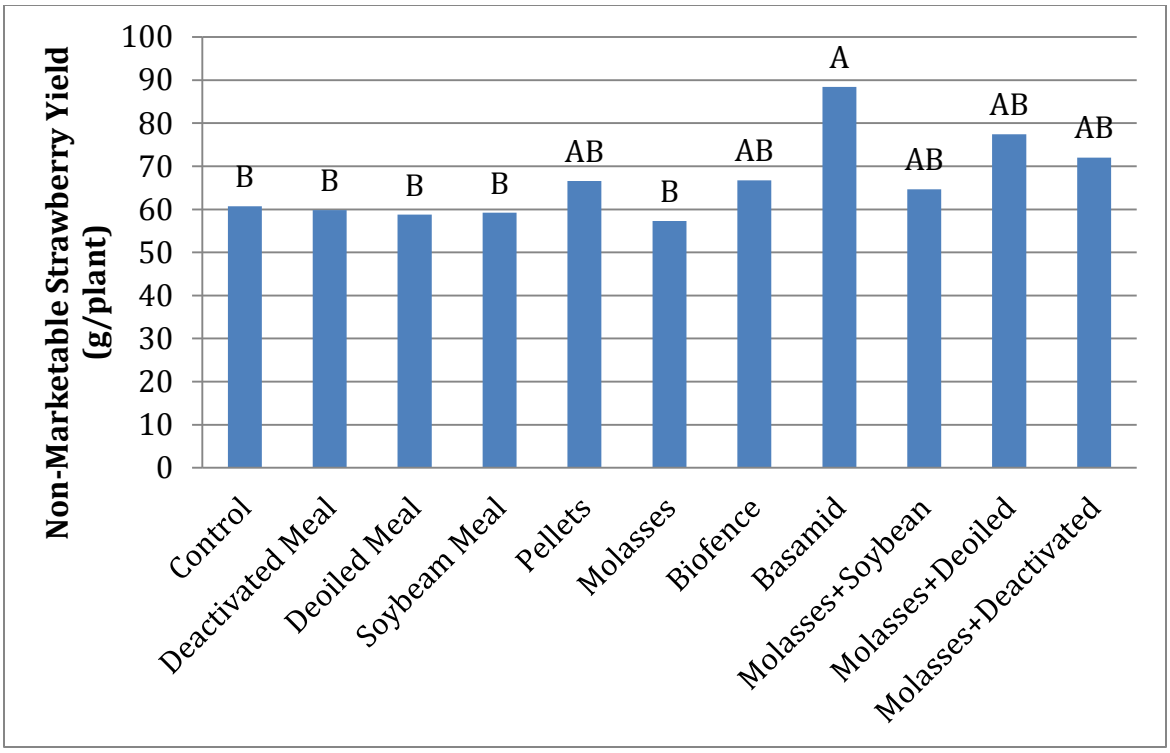

Figure B-3: Mean Non-Marketable Strawberry Fruit Yield per Plant as affected by Treatment. Values are combined means of treatment plots harvested in 2014. Means indicated by different letters are significantly different ($P<0.05$). Fruit was harvested from 28 plants per plot. Non-Marketable refers to the fruit that was rotten, weighed less than 10 grams, and/or was deformed in appearance.

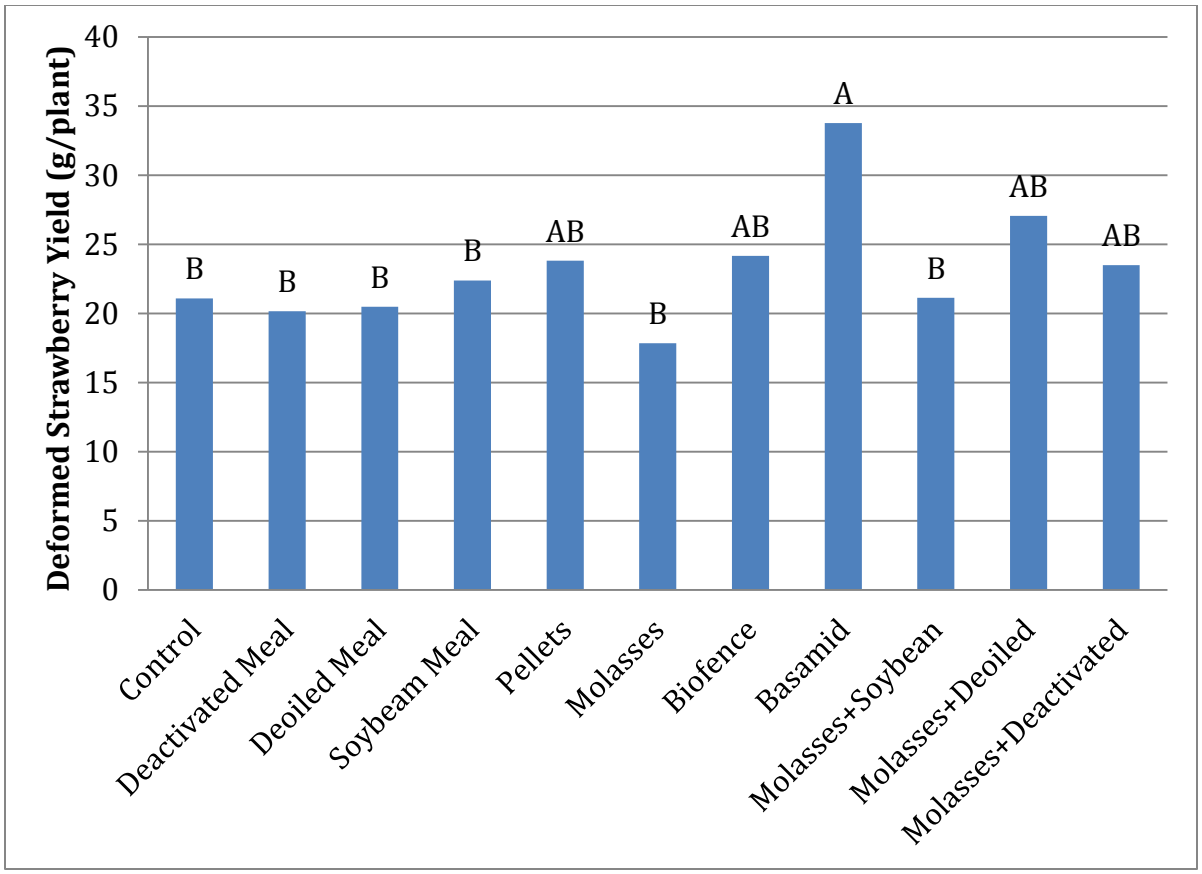

Figure B-4: Mean Deformed Strawberry Fruit Yield per Plant as affected by Treatment. Values are combined means of treatment plots harvested in 2014. Means indicated by different letters are significantly different ($P<0.05$). Fruit was harvested from 28 plants per plot. Deformed refers to the physical appearance of the strawberry as being unable to market.

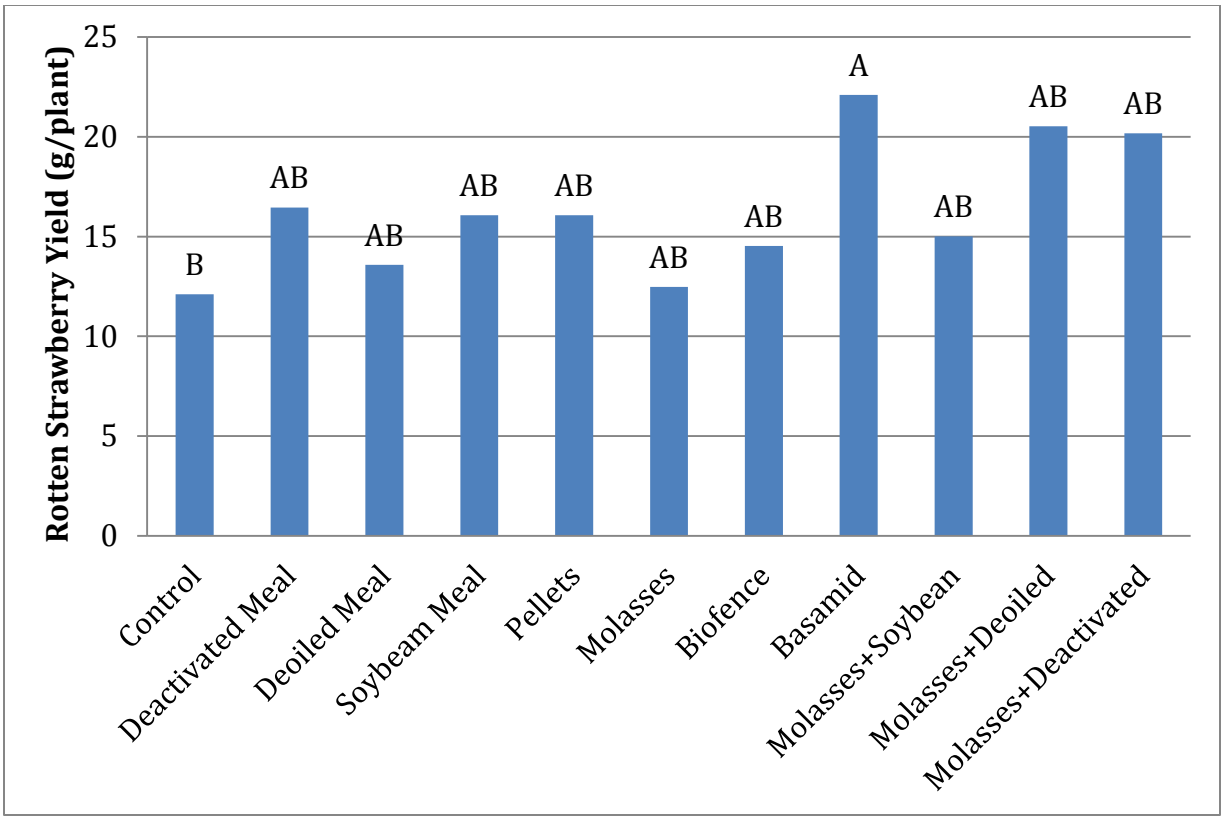

Figure B-5: Mean Rotten Strawberry Fruit Yield per Plant as affected by Treatment. Values are combined means of treatment plots harvested in 2014. Means indicated by different letters are significantly different ($P<0.05$). Fruit was harvested from 28 plants per plot. Rotten refers to blemishes on the fruit that cause it to be unable to market.

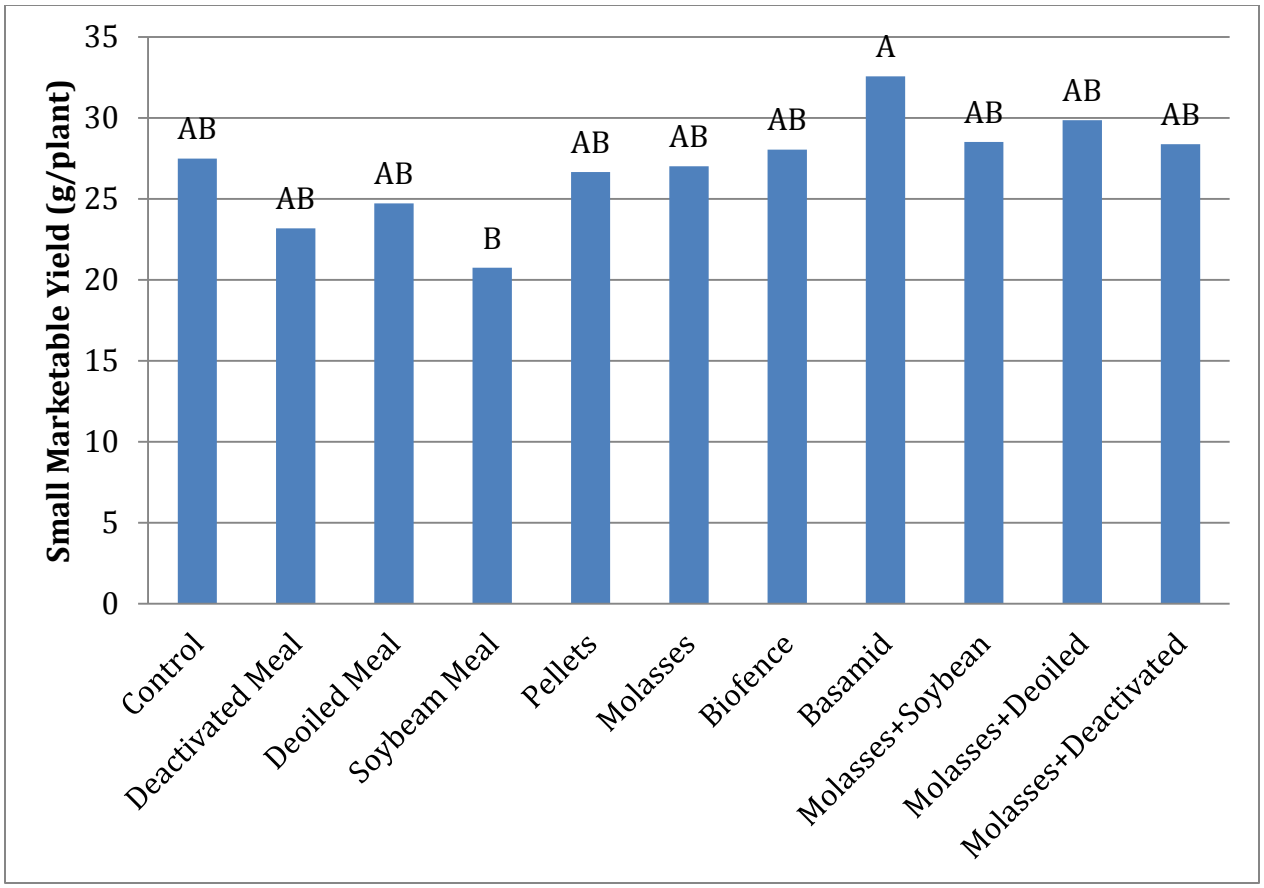

Figure B-6: Mean Small Marketable Strawberry Fruit Yield per Plant as affected by Treatment. Values are combined means of treatment plots harvested in 2014. Means indicated by different letters are significantly different ($P<0.05$). Fruit was harvested from 28 plants per plot. Small marketable strawberries are fruit that do not have physical deformities or blemishes, but are less than 10 grams in weight.

Table B-2: Mineral nutrients extracted from leaves of 'Chandler' plants in biofumigation and ASD plots[Z].

Treatment	B	Mg	P	S	K	Mn	Fe	Cu	Zn
	----------mg/g dry weight----------								
Control	39.3 a[y,x]	1453 a	2778 a	1072 a	15230 a	132.5 b	8.15 a	4.32 a	20.4 b
Deactivated Meal	40.0 a	1499 a	2523 a	1174 a	15110 a	182.7 ab	15.5 a	4.21 a	21.8 ab
Deoiled Meal	40.5 a	1621 a	2823 a	1286 a	15970 a	180.6 ab	7.62 a	4.03 a	21.1 ab
Soybean Meal	40.9 a	1555 a	2836 a	1223 a	15660 a	153.5 b	9.09 a	4.43 a	22.6 ab
Pellets	42.6 a	1642 a	2913 a	1333 a	16850 a	217.1 ab	15.9 a	4.30 a	24.3 ab
Molasses	41.2 a	1804 a	3031 a	1360 a	16900 a	151.3 b	13.3 a	4.59 a	26.7 ab
Biofence	40.7 a	1493 a	2717 a	981.5 a	15070 a	177.6 ab	4.07 a	4.02 a	22.1 ab
Basamid	41.3 a	1709 a	2911 a	1497 a	15180 a	268.1 a	19.3 a	4.57 a	27.2 a
Molasses +Soybean	42.1 a	1563 a	2710 a	1439 a	17320 a	183.7 a	12.6 a	4.19 a	20.3 b
Molasses +Deoiled	40.5 a	1406 a	2465 a	977.8 a	15750 a	123.6 b	9.15 a	4.34 a	22.5 ab
Molasses +Deactivated	41.5 a	1485 a	2637 a	1139 a	17480 a	122.1 b	5.50 a	4.19 a	20.8 ab

[Z] Samples taken during the third week of fruit harvest.

[Y] Mean separation in columns by Least Squares Means test. Any two means within a column not followed by the same letter are significantly different at $P \leq 0.05$.

[X] Values are combined means from six replications of 28 plants per replicate.

Table B-3: Mineral nutrients extracted from ripe fruit from 'Chandler' plants in bioumigation and ASD plots[Z].

Treatment	B	Na	Mg	P	K	Ca	Mn	Fe	Cu	Zn
	----------mg/g dry weight----------									
Control	30.0 a[y,x]	80.0 ab	1033ab	1904ab	12090ab	1857ab	50.0 b	5.66 b	5.08 ab	15.4 ab
Deactivated Meal	31.5 a	87.3 ab	1126a	2055a	13370a	1664abc	74.8 ab	14.8 ab	4.95 ab	16.2 ab
Deoiled Meal	30.8 a	89.6 ab	1098a	2067a	13510a	1717ab	59.2 b	27.4 a	4.83 ab	15.0 ab
Soybean Meal	26.2 a	66.5 b	818.5b	1522b	9900b	1209c	75.9 ab	6.87 b	3.74 b	13.5 b
Pellets	30.8 a	77.8 ab	1049ab	1945ab	11860ab	1731ab	74.7 ab	11.5 ab	4.89 ab	15.9 ab
Molasses	28.4 a	97.8 a	1069ab	1991ab	13860a	1626abc	50.1 b	14.6 ab	4.94 ab	16.1 ab
Biofence	30.3 a	75.4 ab	1059ab	1962ab	12640ab	1541abc	62.1 b	8.75 ab	4.59 ab	14.6 ab
Basamid®	29.7 a	98.9 a	1072ab	1848ab	11900ab	1961a	90.4 a	9.44 ab	4.13 ab	14.5 ab
Molasses +Soybean	30.8 a	90.4 ab	895.1ab	1732ab	11570ab	1445bc	57.7 b	10.8 ab	5.41 a	13.1 b
Molasses +Deoiled	29.3 a	82.2 ab	988.8ab	1825ab	12420ab	1614abc	55.0 b	13.6 ab	4.81 ab	15.2 ab
Molasses+ Deactivated	28.9 a	96.4 a	1012ab	1896ab	12760ab	1702abc	53.8 b	19.8 ab	4.68 ab	17.7 a

[Z] Samples taken during the third week of fruit harvest.

[Y] Mean separation in columns by Least Squares Means test. Any two means within a column not followed by the same letter are significantly different at $P \leq 0.05$.

[X] Values are combined means from six replications of 28 plants per replicate.

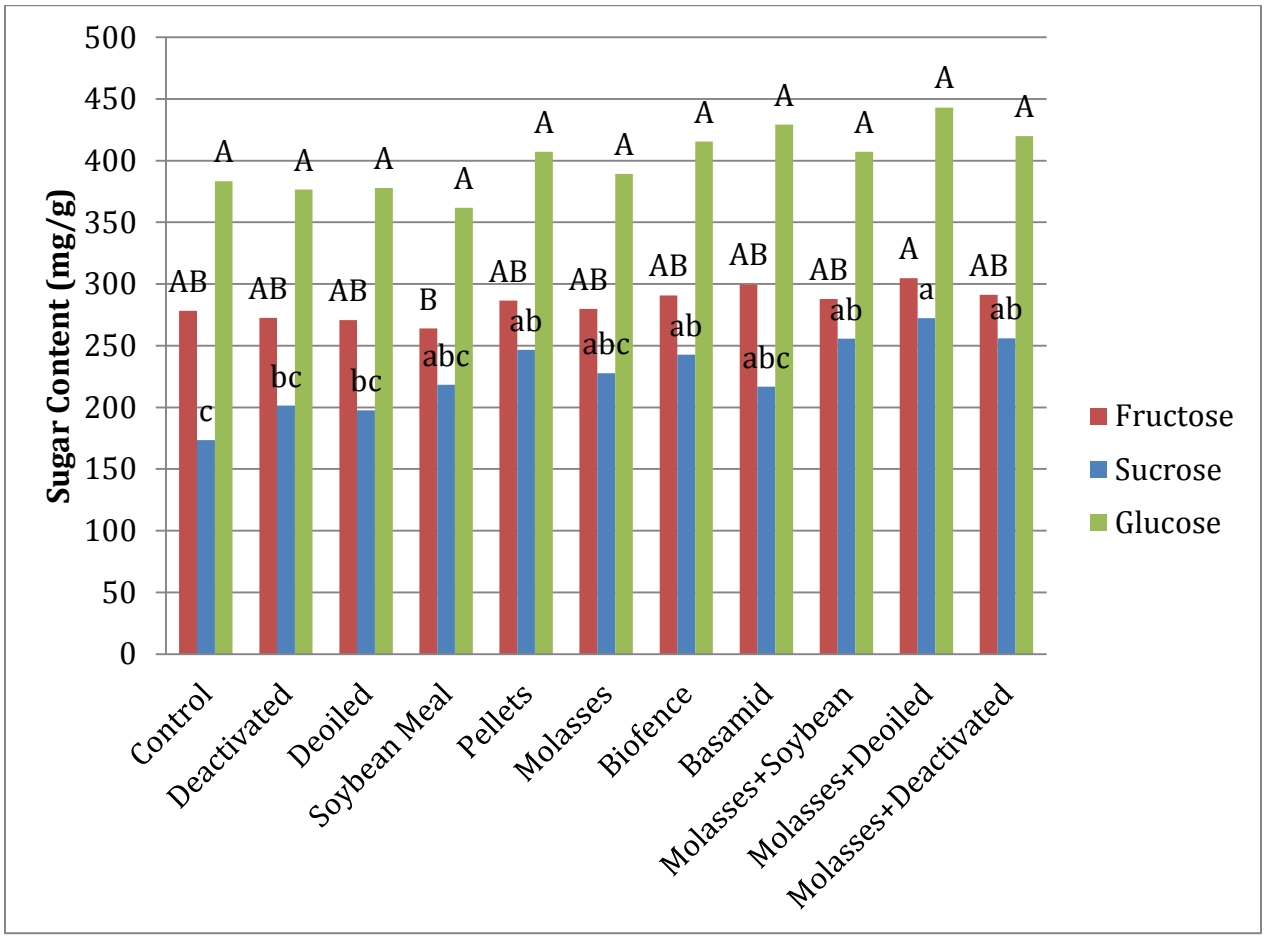

Figure B-7: Glucose (green), fructose (red), and sucrose (blue) content of strawberry fruit tissue as affected by treatment (MM=mustard meal). Mean separation is by Least Squares Means test at $P \leq 0.05$. Mean comparisons of fructose is indicted by capitalized letters and sucrose by lower case letters. (n=6, with fruit from 28 plants per replicate in 2014).

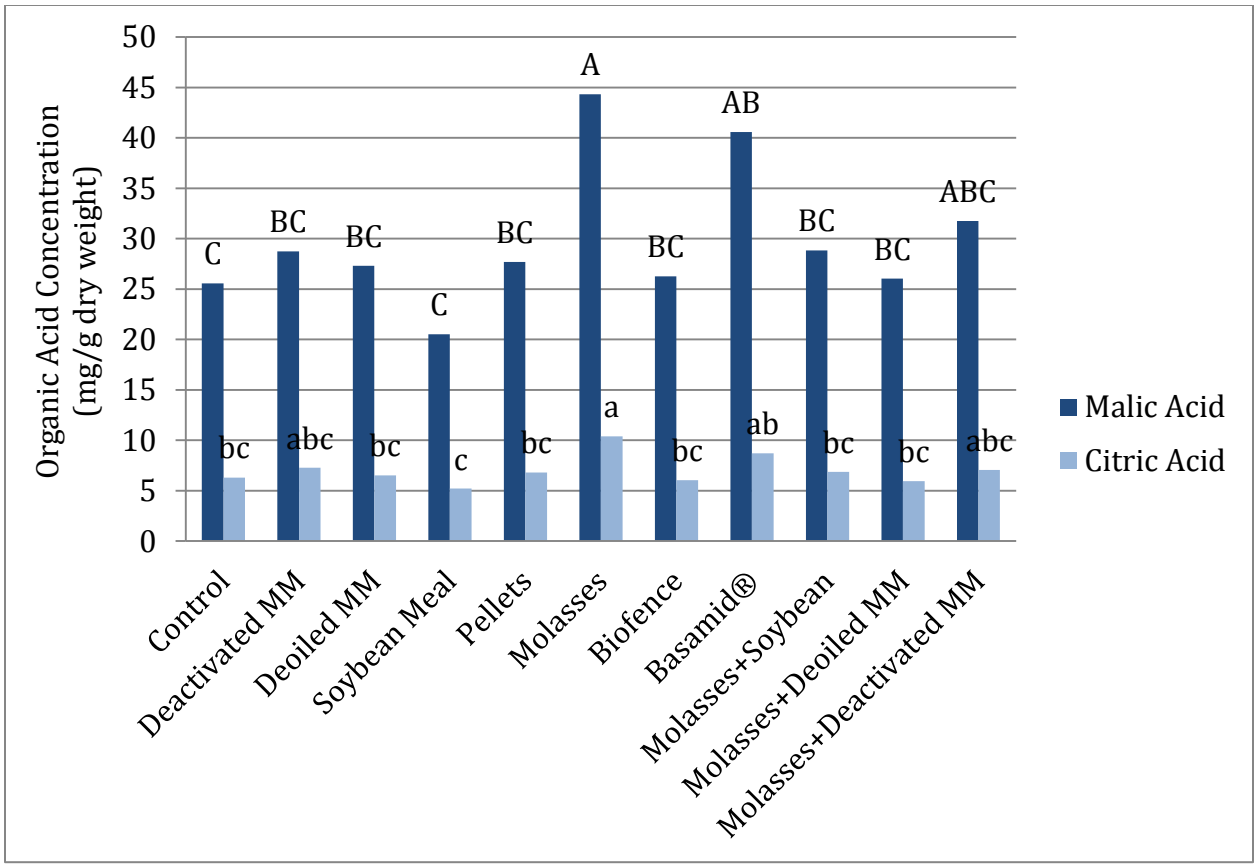

Figure B-8: Citric acid (pale green) and malic acid (dark green) content of strawberry fruit tissue as affected by treatment (MM=mustard meal). Mean separation is by Least Squares Means test at $P \leq 0.05$. Mean comparisons of malic acid is indicted by capitalized letters and citric acid by lower case letters. (n=6, with fruit from 28 plants per replicate in 2014).

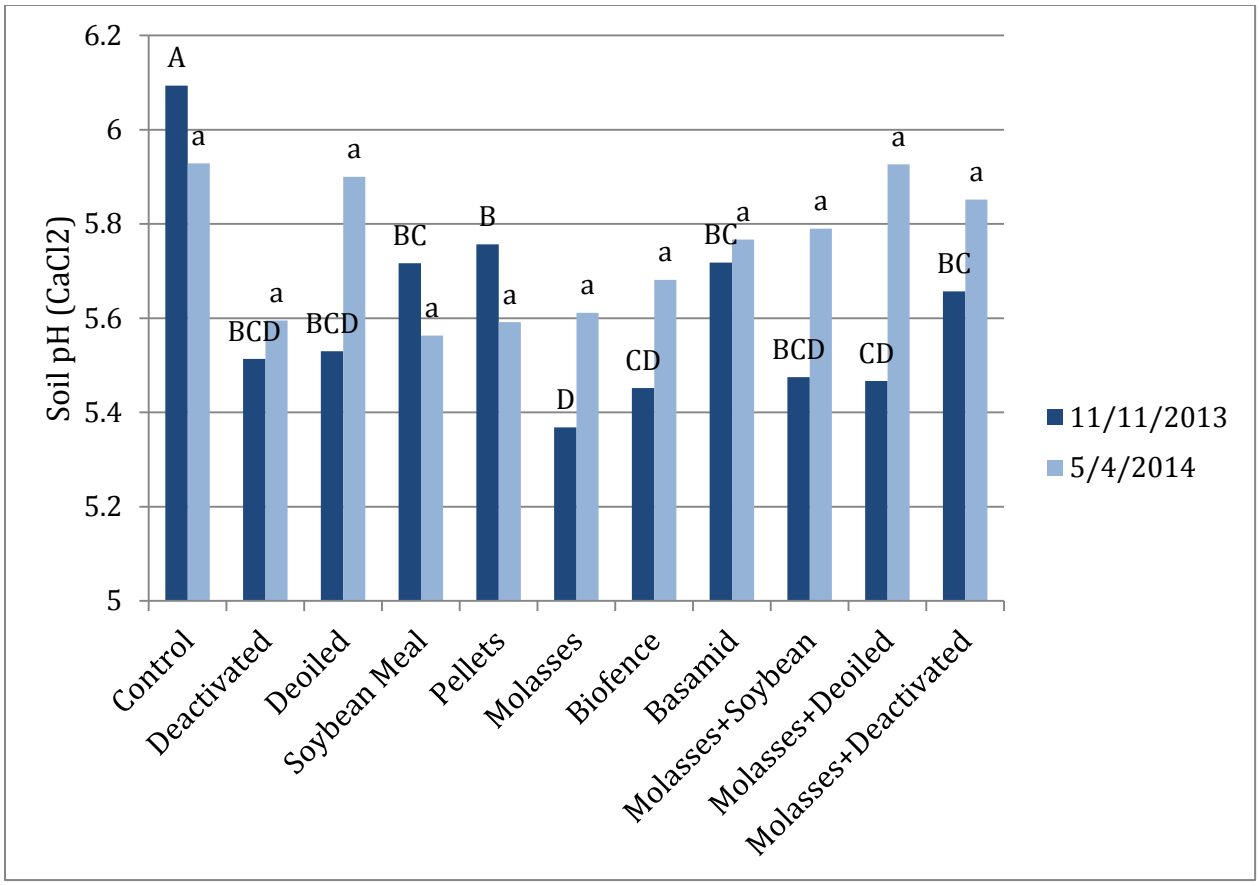

Figure B-9: Soil pH ($CaCl_2$) As Affected by Treatment. Values are combined means of soil samples taken after treatment incorporation in September of 2013 and during harvest in May of 2014. Means Indicated by different letters are significantly different ($P<0.05$).

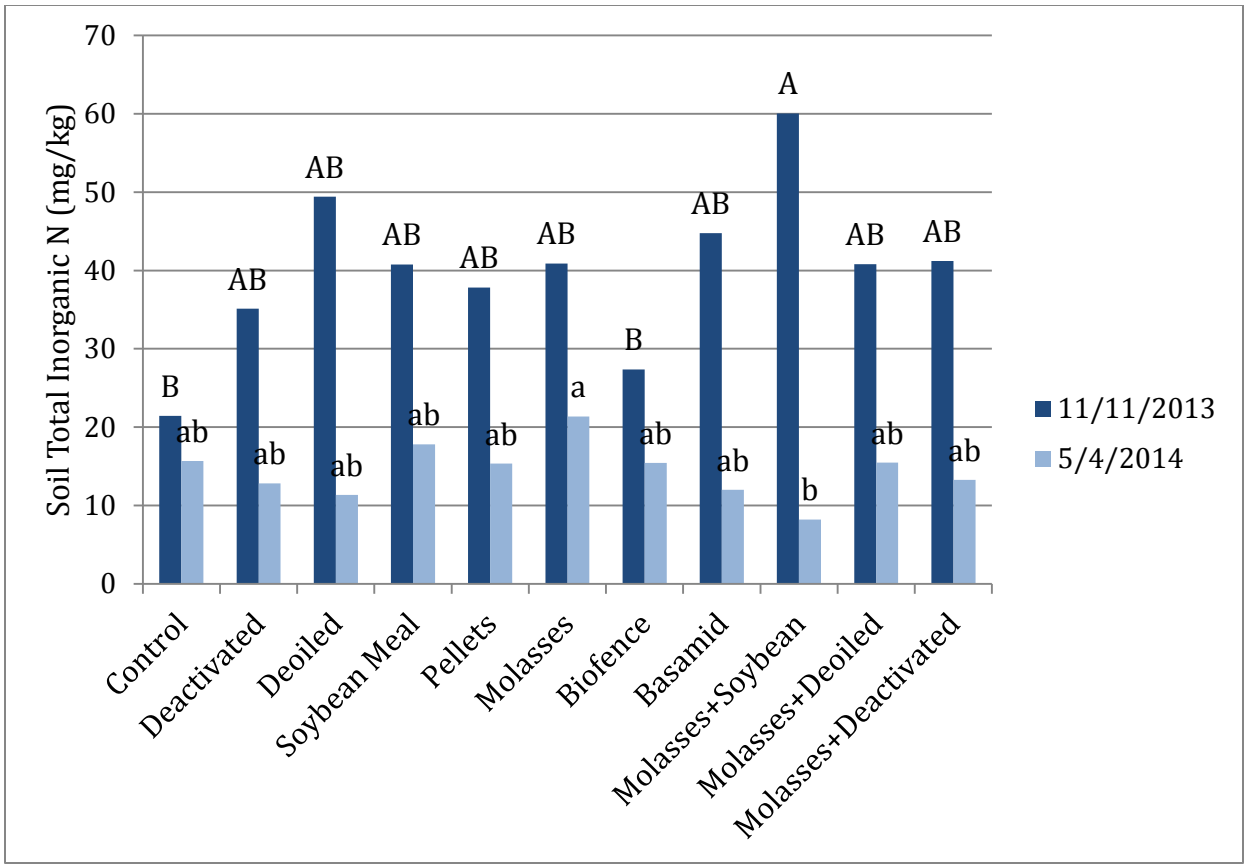

Figure B-10: Soil Total Inorganic N (mg N per kg of soil) As Affected by Treatment. Values are combined means of soil samples taken after treatment incorporation in September of 2013 and during harvest in May of 2014. Means Indicated by different letters are significantly different ($P<0.05$).

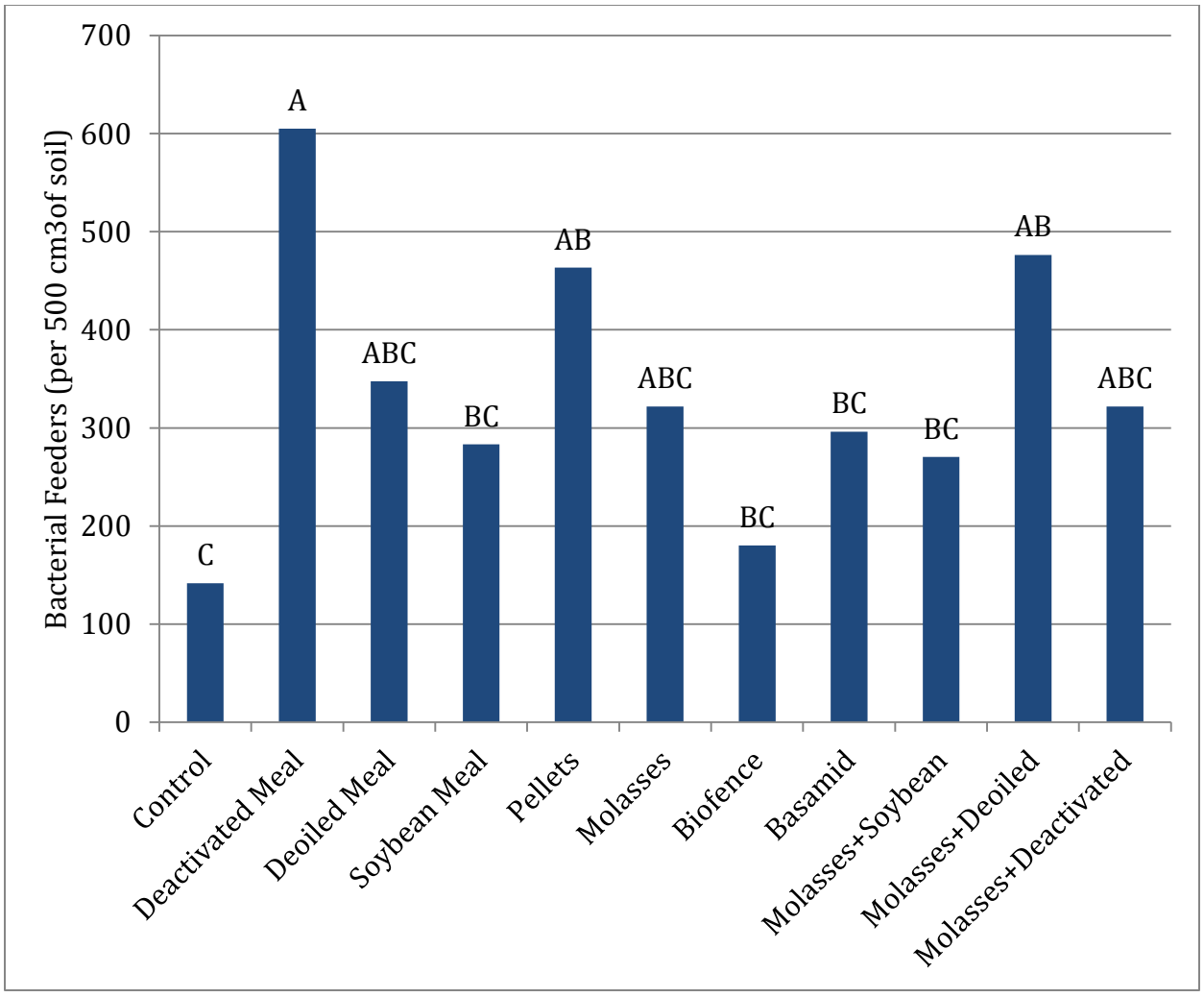

Figure B-11: Bacterial Feeder (Beneficial Nematode) Count (per 500cm^3 of soil) As Affected by Treatment. Values are combined means of soil samples taken after treatment incorporation in September of 2013 and during harvest in May of 2014. Means Indicated by different letters are significantly different ($P<0.05$).

Chapter 3: Simultaneous Extraction of Sinigrin and Allyl Isothiocyanate from Strawberry Soil Following Biofumigation and Anaerobic Soil Disinfestation Treatment

Abstract

Due to the phase-out of methyl bromide, there is a need for alternative, non-chemical fumigation treatments in strawberry production. Biofumigation is an alternative fumigation method that has shown success as non-chemical based alternatives. Biofumigation uses *Brassica* tissues that are rich in glucosinolates in order to control soil pests. Glucosinolates are secondary plant metabolites that are hydrolyzed in contact with water to form isothiocyanates. Isothiocyanates are volatiles that have been found to be effective at fumigating soils. A trial was conducted with 11 pre-plant soil-incorporated treatments arranged in a randomized complete block design with 6 rows (blocks). The biofumigation treatments consisted of deactivated mustard meal, deoiled mustard meal, mustard pellets, and Biofence mustard seed meal. Other treatments included dried molasses, a Basamid® chemical treatment, combination treatments of deactivated mustard meal and molasses, deoiled mustard meal and molasses, and molasses and soybean meal, as well as a control with no treatment. Soil samples were taken at 0hr, 4hr, 9hr, 30hr, 4day, and 9day in order to measure sinigrin, a known glucosinolate in mustard meal, and allyl isothiocyanate, the hydrolysis product of sinigrin simultaneously through an ethyl acetate and acentonitrile extraction which was measured by HPLC. Sinigrin was found in deoiled mustard meal, Biofence, and deactivated mustard meal, but not in mustard pellets. Sinigrin concentrations did not decrease with increased time, as expected. However, sinigrin was not detected in the soil at 4 and 9 days. AITC was only measured at 0hr of analysis in plots treated with mustard pellets. AITC was not detected in the plots in which sinigrin was measured. The inconsistency of this data is due to samples not immediately being taken once treatments laid on field, leading to hydrolysis prior to sampling. Also, samples were

stored for a prolonged period of time in ethyl acetate before analysis took place. In the future, samples should be taken on the day of treatment and then analyzed immediately for sinigrin and AITC.

Introduction

After the phase out of methyl bromide from use as a soil fumigant due to the 2005 Montreal Protocol, farmers are still in need of effective, sustainable soil fumigants. In recent years, many studies have listed biofumigation, the suppression of soil pests and diseases resulting from volatile hydrolysis products released in soil after incorporation of glucosinolate plant material, as a viable treatment option (Morra et al, 2002; Gimsing and Kirkegaard, 2009). *Brassicaceae* plants are rich in glucosinolates, and have been found to be species specific when regarding different glucosinolates (Charron et al., 2005; Kirkegaard and Sarwar, 1999).

Glucosinolates (GSs) are β-thioglycosides (Charron et al., 2004; Hensley et al., 2005) and secondary metabolites which can be found in many plant families. GSs possess limited biological activity until they are hydrolyzed by the enzyme myrosinase (Borek et al., 1995), which is released upon the rupturing of the plant tissue by chewing or tearing (Charron et al., 2004). Myrosinase can be affected by season, temperature, photoperiod that the *Brassica* plant is subjected to during growth (Charron and Sams, 2004; Charron et al., 2005). GSs are hydrolyzed by myrosinase to a variety of compounds, including isothiocyanates (ITCs) (Bones et al., 2006; Charron and Sams, 2004; Gimsing and Kirkegaard, 2006; Kirkegaard and Sarwar, 1998). Allyl isothiocyanate (AITC) has been

regarded as the most toxic ITC when compared to methyl, phenyl, and ethyl ITCs (Walker et al., 1937).

GS and ITC content of soil can help determine effects of volatility of the treatments in the soil environment. Common methods for GS extractions involve boiling water and methanol (Cools and Terry, 2012; Gimsing et al., 2005; Gimsing and Kirkegaard, 2006). However, with these extraction methods, two soil samples are needed due to different methods used for GSs and ITCs. Fahey et al. (1997) found that a triple extraction using acetonitrile (ACN) was effective at measuring GSs. Tsao et al. (2002) also used an ACN extraction to measure both GSs and ITCs simultaneously. While water extractions have been found to be the most cost-effective method of analysis, more room for error arises via loss of volatiles over time if samples are not immediately extracted for analysis upon removal from field (Herzallah and Holley, 2012; Cools and Terry, 2012; Gimsing et al., 2005; Gimsing and Kirkegaard, 2006). Therefore, for storage purposes, a more stable solvent needs to be used for capturing GSs and ITCs for extraction purposes. Mullin (1978) found that methanol, a common solvent used for extraction, can form additional compounds with ITCs and lead to degradation of ITC concentration in storage. Therefore, methanol is not a viable solvent to use for large quantity samples that are not able to be extracted at one time. Prior, studies (unpublished data) in our lab have found ethyl acetate (EA) to be a suitable solvent in which to capture and adequately store volatiles, as well as Borek et al. (1995), who used EA to capture AITC in soils treated with *Brassica* tissues.

In the past, researchers have measured GSs and ITCs separately. GSs have been measured on the HPLC, and ITCs have been measured on the GC (Matthaus and Fiebig,

1996; Charron and Sams, 2004; Gimsing et al., 2005; Gimsing and Kirkegaard, 2006). The HPLC method of analysis has been found to be much quicker than the GC method (Mullin, 1978). Tsao et al. (2000, 2002) were able to adequately extract GSs and ITCs at the same time and quantify all on the HPLC.

The objective of this research was to determine the concentrations of sinigrin and AITC in a soil environment over a period of a week after incorporation of *Brassica* seed meals in order to demonstrate the release of AITC from sinigrin. This relationship will provide knowledge of the volatility and life of the *Brassica* seed meals in a soil environment.

Materials and Methods

Mustard Meal Sources

The mustard meal used in the field experiments are Wisconsin Spice, Inc. brand deactivated mustard meal and deoiled overs (Berlin, Wi), Triumph Italia brand Biofence (Agrium Italia Spa, Livorno, Italy), and Mustard Products and Technologies (MPT) brand mustard pellets (Saskatoon, SK, Canada). The dried molasses was OMALASS from Westway Feed Products LLC (New Orleans, LA). The soybean meal was Hi-Pro brand (Friona, TX)..

Desulfoglucosinolate Analysis of Mustard Meal

The GS analysis was performed using the procedure listed in Charron et al, 2004, with amendments. In brief, a 200-mg sample of each mustard meal was combined with 1.00 mL of benzyl GS solution (1.00 mM) as an internal standard, 2.00 mL methanol, and 0.100 mL of barium-lead acetate (0.600 mM) into a 16 x 100-mm culture tube, vortexed for approximately 10 seconds, and then shaken at 60 rpm for one hour. The samples were

vortexed for approximately 10 seconds and placed into the centrifuge at 2000 g_n for 15 minutes. An aliquot of 0.500 mL of supernatant was added to a 1.00 mL column containing 0.300 mL DEAE A-25 and desulfated by the procedure of Raney and McGregor (1990).

Extracted desulfoglucosinolates (Figure C-1) were separated with an Agilent 1100 Series high performance liquid chromatograph (HPLC) using an Agilent Zorbax Eclipse Plus C18 column, 250x4.6 mm, 5 Micron particle size, and a UV detector at a wavelength of 230 nm. The column temperature was 35°C. A flow rate of 1.5 mL•min-1 was used. The solvent gradient was 100% water for 1 minute. After a 15 min linear gradient to 75% water and 25% acetonitrile, solvent levels were held constant for 5 minutes, and over the final 5 minutes a linear gradient to 100% water was used. Desulfoglucosinolates were identified and quantified by comparison with authentic standards.

2013-2014 Plant Science Farm Strawberry Field Test

A trial was conducted with 11 pre-plant, soil-incorporated treatments arranged in a randomized complete block design with 6 rows (blocks) at the East Tennessee Agricultural Research and Education Center in Knoxville, TN. The soil was a Shady-Whitwell complex originating from loamy alluvium derived from limestone, sandstone, and shale. The biofumigation treatments consisted of deactivated mustard meal, deoiled mustard meal, mustard pellets, and Biofence mustard seed meal. Other treatments included dried molasses as a carbon source for an anaerobic treatment and a Basamid® chemical treatment. Additional combination treatments of deactivated mustard meal and molasses, deoiled mustard meal and molasses, molasses and soybean meal (to lower amendment C:N ratio) were also applied, as well as a control with no treatment. In August of 2013, the

intended field was pretreated with Round-Up® and Basagram twice each in order to eliminate weeds. A perimeter was marked for six plant rows that were 67.1 m long. Beds were then formed at a width of 1.52 m, and the lengths of the rows were divided into 11 plots of 6.10 m length each with a buffer region of 1.22 m at the end of each plot.

On September 18 of 2013, the 11 treatments were applied by hand and incorporated with a rotovator into designated plots, with one treatment for each row (Tables C-1). The treatments were tilled into approximately 0.150 m of the soil depth. The beds were completed with an addition of two John Deere 16 mm, 10 mil wall, 30 cm spacing between emitters drip-tapes per row with a delivery rate of 15 psi and covered immediately with black plastic. The field was then drip irrigated for a little over 32 hours. Soil samples were taken from the top 15.2 cm of soil in order to measure gases coming off of the soil at zero hours, four hours, nine hours, 30 hours, four-days, and eight-days post irrigation initiation. Six soil cores were taken randomly throughout treatment plots in a zig-zag motion and then combined in a clean bowl, mixed, and placed in a 50 mL centrifuge tube to approximately 30.0 mL. Ethyl acetate (EA, 20.0 mL) was immediately poured onto the soil sample, completely covering the sample for storage. Samples were stored in a -20°C freezer until tissue analysis took place.

Sinigrin and AITC Analysis of Field Soil

Approximately 30.0 mL of soil taken from the field and greenhouse experiments at zero hours, four hours, nine hours, 30 hours, four days, and eight days were placed in 50.0 mL centrifuge tubes. EA (20.0 mL) was added to each tube and then the tube was capped for storage in a -20°C Freezer. Samples were thawed, vortexed thoroughly, and centrifuged at 4000 RPM for 10 minutes. The EA was decanted into a graduated cylinder, and the

volume was recorded. Two aliquots of 1.00 mL of the EA was filtered through a 0.200 µm, 13.0 mm syringe filter into 12x32 mm clear standard crimp top vials, capped, and stored in a freezer for HPLC analysis. Acetonitrile (ACN, 20.0 mL) was added to the original 50.0 mL sample tube of soil. The sample was vortexed thoroughly and centrifuged at 4000 RPM for 10 minutes. The ACN was decanted into a graduated cylinder, and the volume was recorded. Two aliquots of 1.00 mL of the ACN solution were filtered through a 0.200 µm, 13 mm syringe filer into 12x32 mm clear standard crimp top vials, capped, and stored in a freezer for HPLC analysis. Two aliquots were taken so that a back-up set can be stored for further analysis.

Mobile phase was pumped at 1.00 mL per minute with a 1:99 Acetonitrile: 0.025M NH_4OAc (volume/volume). This was kept isocratic for 2 minutes, linearly increased to 50:50 (v/v) at 2:30 minutes, kept isocratic until 10 minutes, and then brought down linearly to 1:99 (v/v) until 12 minutes. There was a 2 minute after-run between each sample injection. The Diodaray detector was set at 228 nm for sinigrin and at 242 nm for AITC. 20µL of sample was injected into the column which was held at 40°C. This method was performed on an Agilent 1200 Series HPLC with a Diodaray Detector. The column was an Agilent Zorbax Eclipse XDB-C18, 4.6x50 mm, 1.8 Micron particle size with an Agilent Eclipse XDB-C18, 4.6x12.5 mm, 5 Micron particle size. Sample concentrations of sinigrin and AITC were based on standard curves for both compounds.

Results

Standard Curves

Sinigrin concentration was quantified using standards purchased from Sigma Aldrich (St. Louis, MO). A standard curve was constructed using increasing amounts of sinigrin in acetonitrile and resulted in a linear relationship between the concentration and the peak area (R^2=0.999; Figure C-2). AITC concentration was quantified using standards purchased from Sigma Aldrich (St. Louis, MO). AITC was mixed with ethyl acetate in order to make standard solutions at significant concentrations. The standard curve of AITC provided a linear relationship between the concentration of AITC and the peak area of the resulting chromatogram (R^2=0.997; Figure C-3).

Desulfoglucosinolate Analysis of Mustard Meal

The desulfonated sinigrin content of the mustard meal treatments used in the field differed significantly ($P<0.05$; Figure C-4). Deoiled mustard meal had significantly more sinigrin (157 mg/g) than Biofence (114 mg/g) and deheated mustard meal (8.10 mg/g). The expected desulfonated sinigrin content of the samples taken in the field was 8.26 mg/g for Biofence, 0.590 mg/g for deheated mustard meal, 11.4 mg/g for deoiled mustard meal, and 10.7 for mustard pellets (Figure C-5).

Sinigrin and AITC Analysis of Field Soil

Sinigrin was not detected in plots treated with only mustard pellets or deheated mustard meal (Table C-2). Sinigrin was found most in plots treated with a combination of deoiled mustard meal and dried molasses; however, sinigrin was not found consistently among replications or treatment times. Concentrations also did not always decrease with increasing sampling times as expected. The plots treated with deoiled mustard meal had a

larger sinigrin concentration at the four hour sampling time than at the zero hour sampling time. Sinigrin was not detected in the field at four and nine days sampling times.

AITC was only detected in plots treated with mustard pellets (Table C-2). AITC was found in the largest concentration when sampled at time zero, and trace amounts were measured at four hours. Sinigrin was not detected in these plots. Plots treated with deheated mustard meal, deoiled mustard meal, and Biofence did contain sinigrin; however, there was no evidence of AITC in these plots at the times samples.

Discussion

While capture of sinigrin was inconsistent among replications, the sinigrin content that was captured in the field was similar to the expected concentration calculated based upon desulfonated sinigrin analysis of the mustard meals. The inconsistency of the concentrations of sinigrin and AITC in the soil could be due to inaccuracies with irrigation in the field at the beginning of the experiment. Water was not immediately introduced after treatment incorporation tillage and plastic mulch installation due to pump malfunction. The treatments sat in the field overnight for approximately 17 hours before watering began the following day. During this time, hydrolysis could have occurred, resulting in a decrease in sinigrin and AITC concentrations by the time irrigation began and further sampling took place. Morra et al. (2002) reported that sinigrin hydrolysis occurred immediately after tissue incorporation into soil due to tilling. They measured ITC concentrations higher than 0.800 nmol/g soil at 2 hours post irrigation time. Gimsing et al. (2006) detected ITCs at their highest concentration (~90nmol/g soil) immediately after tissue incorporation (approximately 30 minutes). They were able to detect GSs and ITCs

for up to several days. While it is hard to compare biofumigation experiments due to different treatment designs and locations, similar patterns can be drawn from different experiments. Price et al. (2005) measured AITC concentrations in soil treated with *Brassica juncea* to be 19% greater when captured at 0.25 hour and 4 hours than when captured at 8 hours, and 95% more at 0.25 hour and 4 hours than 24 hours. However, they were still able to detect AITC at 24 hours. Based on the findings of Gimsing et al. (2006), we should have still been able to detect trace concentrations of sinigrin and AITC in our soil even after letting it sit overnight.

Also, irrigation occurred for approximately 32 hours due to the installation of the new pump. The increase in water in the soil could have mobilized the sinigrin and AITC in the soil profile, beyond where sampling took place. Soil cores taken at 30 hours and beyond were affected by this increased watering time.

A sampling error could have occurred if the treatments were not tilled into the soil uniformly. Also, the increased storage time of the samples in the -20°C freezer could have led to an extraction error. Warton et al. (2001) reported that increased storage time reduced the concentrations of GSs and ITCs captured from *Brassica* tissue samples. EA has not been studied as a storage solvent for GS or ITC extraction over long time periods. Gimsing and Kirkegaard (2006) did not mention storing their soil samples when they conducted their ITC extractions with EA. It is possible that the GSs and ITCs in the soil solution could have reacted with the ethyl acetate to form secondary products over the storage period. ITCs are reported to be reactive compounds (Borek et al., 1995). Borek et al. (1995) reported that increased organic carbon content of soil decreases the ITC half-life

in soil. Our experiment combines carbon rich molasses with mustard meal treatment in order to reach anaerobic environments. It is possible that the mixtures reacted; however, it is most likely the increased time in field before sampling as well as the prolonged period before extraction led to decreased capture of sinigrin and AITC.

Repeating this experiment in a controlled environment, such as a greenhouse, can help alleviate irrigation errors that occurred in the field. Irrigation, weather, and pests can all potentially negatively affect volatile sampling in the field environment, and greenhouses can greatly decrease their negative impacts. Irrigation is easier to control in a greenhouse due to more direct delivery and smaller plot sizes. Greenhouses shield plants from harmful effects from weather, such as increased precipitation or heat. Pest potential is decreased in a greenhouse setting due to protection from the outside environment decreasing access to most field pests, such as geese that can rip the plastic mulch, releasing the volatiles. A greenhouse plan of this experiment can be found in on page 106-107 of Appendix C.

Conclusion

The results of this experiment have demonstrated the presence of sinigrin and AITC in soil after mustard meal application. While concentrations were inconsistent, we were able to extract sinigrin from plots treated with deoiled mustard meal, Biofence, and deactivated mustard meal. We were also able to extract AITC from plots treated with mustard pellets. For the future, we should focus on the effects of ethyl acetate on sinigrin and AITC concentrations for an observed period of time. Samples should be analyzed in a shorter period of time post collection. Also, this experiment should be repeated in a

controlled, greenhouse environment in order to account for any inconsistencies that may have occurred in the field.

References:

Bones, A. M., Rossiter, J. T. (2006). The enzymic and chemically induced decomposition of glucosinolates. *Phytochemistry* 67(11): 1053-1067.

Borek, V., Morra, M. J., Brown, P. D., McCaffrey, J. P. (1995). Transformation of the Glucosinolate-Derived Allelochemicals Allyl Isothiocyanate and Allylnitrile in Soil. *Journal of Agricultural and Food Chemistry* 43(7): 1935-1940.

Charron, C. S., Sams, C. E. (2004). Glucosinolate content and myrosinase activity in rapid-cycling *Brassica oleracea* grown in a controlled environment. *Journal of the American Society for Horticultural Science* 129(3): 321-330.

Charron, C. S., Saxton, A. M., Sams, C. E. (2005). Relationship of climate and genotype to seasonal variation-myrosinase system. I. Glucosinolate content in ten cultivars of *Brassica oleracea* grown in fall and spring seasons. *J. Sci. Food Agric.* 85: 671-681.

Cools, K., Terry, L. A. (2012). Comparative study between extraction techniques and column separation for the quantification of sinigrin and total isothiocyanates in mustard seed. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* 901: 115-118.

Fahey, J. W., Zhang, Y., Talalay, P. (1997). Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proceedings of the National Academy of Sciences of the United States of America* 94(19): 10367-10372.

Gimsing, A. L., Kirkegaard, J. A. (2005). Extraction and determination of Glucosinolates from soil. *Journal of Agricultural and Food Chemistry* 53(25): 9663-9667.

Gimsing, A. L., Kirkegaard, J. A. (2006). Glucosinolate and isothiocyanate concentration in soil following incorporation of *Brassica* biofumigants. *Soil Biology & Biochemistry* 38(8): 2255-2264.

Gimsing, A.L., Sorensen, J. C., Tovgaard, L., Jorgensen, A. M. F., Hansen, H. C. B. (2006). Degradation kinetics of Glucosinolates in soil. *Environmental Toxicology and Chemistry* 25(8): 2038-2044.

Gimsing, A. L., Kirkegaard, J. A. (2009). Glucosinolates and biofumigation: fate of glucosinolates and their hydrolysis products in soil. *Phytochemistry Reviews* 8(1): 299-310.

Hensley, D.D. (2005). Glucosinolate and Allyl Isothiocyanate Content of Oriental Mustard Meal. Doctoral Dissertation. University of Tennessee, Knoxville: 35-55.

Herzallah, S., Holley, R. (2012). Determination of sinigrin, sinalbin, allyl- and benzyl isothiocyanates by RP-HPLC in mustard powder extracts. *Lwt-Food Science and Technology* 47(2): 293-299.

Kirkegaard, J. A., Sarwar, M (1998). Biofumigation potential of *Brassicas* - I. Variation in glucosinolate profiles of diverse field-grown *Brassicas*. *Plant and Soil* 201(1): 71-89.

Kirkegaard, J. A., Sarwar, M. (1999). Glucosinolate profiles of Australian canola (*Brassica napus annua* L.) and Indian mustard (*Brassica juncea* L.) cultivars: implications for biofumigation. *Aust. J. Agric. Res*. 50(3): 315-324.

Matthaus, B., Fiebig, H. J. (1996). Simultaneous determination of isothiocyanates, indoles, and oxazolidinethiones in myrosinase digests of rapeseeds and rapeseed meal by HPLC. *Journal of Agricultural and Food Chemistry* 44(12): 3894-3899.

Morra, M. J., Kirkegaard, J. A. (2002). Isothiocyanate release from soil-incorporated *Brassica* tissues. *Soil Biology and Biochemistry* 34:1683-1690.

Mullin, W.J. (1978). High-performance liquid chromatography and gas chromatography of organic isothiocyanates and their methanol-isothiocyanate addition compounds. *J. Chromatogr.* 155:198-202.

Price, A. J., Charron, C. S., Saxton, A. M., Sams, C. E. (2005). Allyl Isothiocyanate and Carbon Dioxide Produced during Degradation of *Brassica juncea* Tissue in Different Soil Conditions. *HortScience* 40(6): 1734-1739.

Raney, J. P., McGregor, D. I. (1990). Determination of glucosinolate content by gas liquid chromatography of trimethylsilyl derivatives of desulfated Glucosinolates. *Proc. Oil Crops Network*: 21-23.

Tsao, R., Yu, Q., Friesen, I., Potter, J., Chiba, M. (2000). Factors Affecting the Dissolution and Degradation of Oriental Mustard-Derived Sinigrin and Allyl Isothiocyanate in Aqueous Media. *J. Agric. Food Chem.* 48(5):1898-1902.

Tsao, R., Yu, Q., Potter, J., Chiba, M. (2002). Direct and simultaneous analysis of sinigrin and allyl isothiocyanate in mustard samples by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry* 50(17): 4749-4753.

Walker, J. C., Morell, S., Foster, H. H. (1937). Toxicity of Mustard Oils and Related Sulfur Compounds to Certain Fungi. *American Journal of Botany* 24(8):536-541.

Warton, B., Matthiessen, J. N., Shackleton, M. A. (2001). Glucosinolate Content and Isothiocyanate Evolution-Two Measures of the Biofumigation Potential of Plants. *J. Agric. Food Chem.* 49:5244-5250.

Appendix C:

Table C-1: Treatments and their application rates as used in the 2013-2014 Plant Science Farm Strawberry Field Test.

Treatment Number	Chemical	Rate		Number of Reps
		(lb of Product/acre)	(lb/37.33ft^2) Treated bed area	
1	Control	0	0	6
2	Deheated Mustard Meal	4000	3.43	6
3	Mustard Meal (Deoiled Overs)	4000	3.43	6
4	Soybean Meal (defatted)	4000	3.43	6
5	Mustard Meal (pellets, Canadian)	4000	3.43	6
6	Molasses	12800	11.0	6
7	Biofence	4000	3.43	6
8	Basamid G (99% a.i.)	400	0.343	6
9	Dried Molasses+Soybean Meal	12800+4000	11.0+3.43	6
10	Dried Molasses+Deoiled Overs	12800+4000	11.0+3.43	6
11	Dried Molasses+Deheated Mustard Meal	12800+4000	11.0+3.43	6

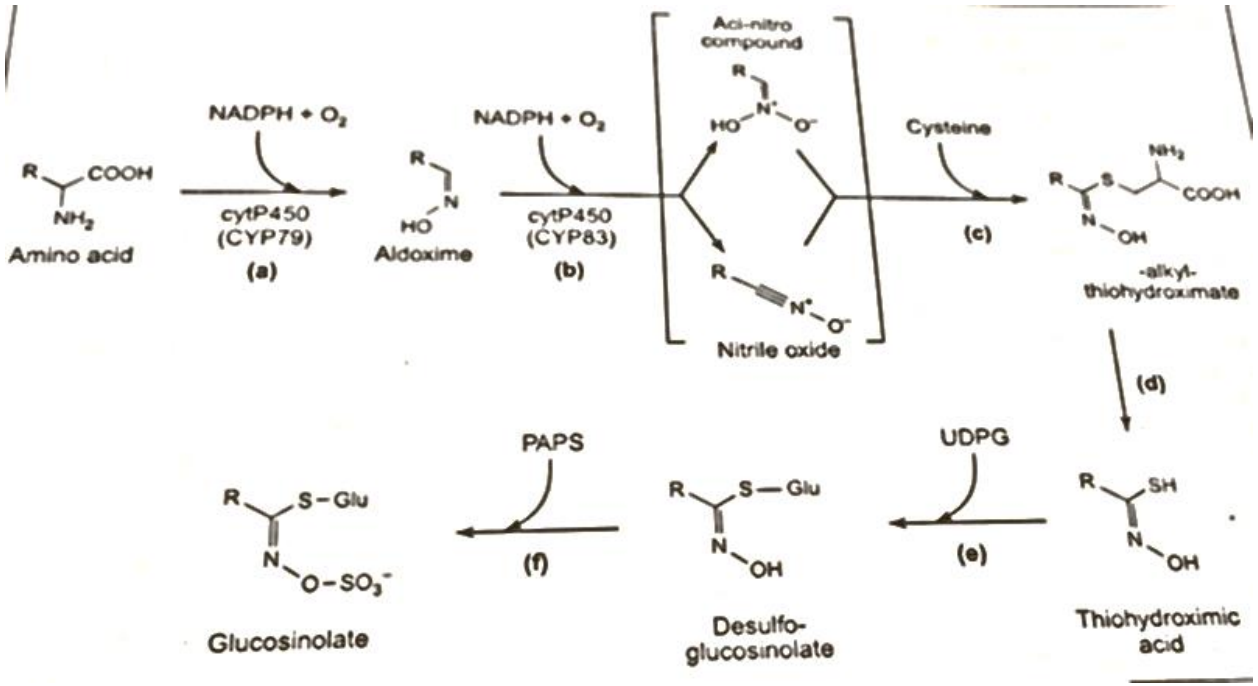

Figure C-1: Desulfoglucosinolate production (Quinsac and Ribaillier, 1991)

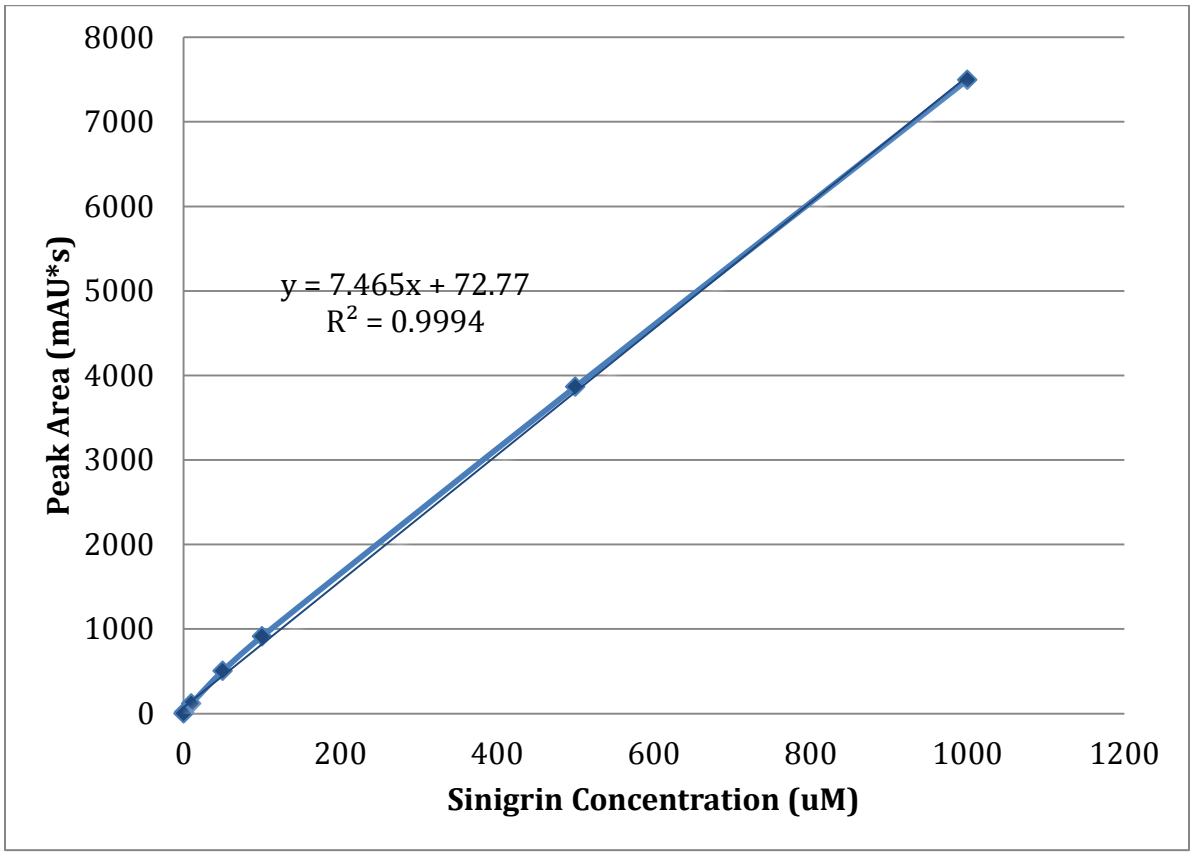

Figure C-2: Sinigrin standard curve. Sigma Aldrich chemical standard used with acetonitrile.

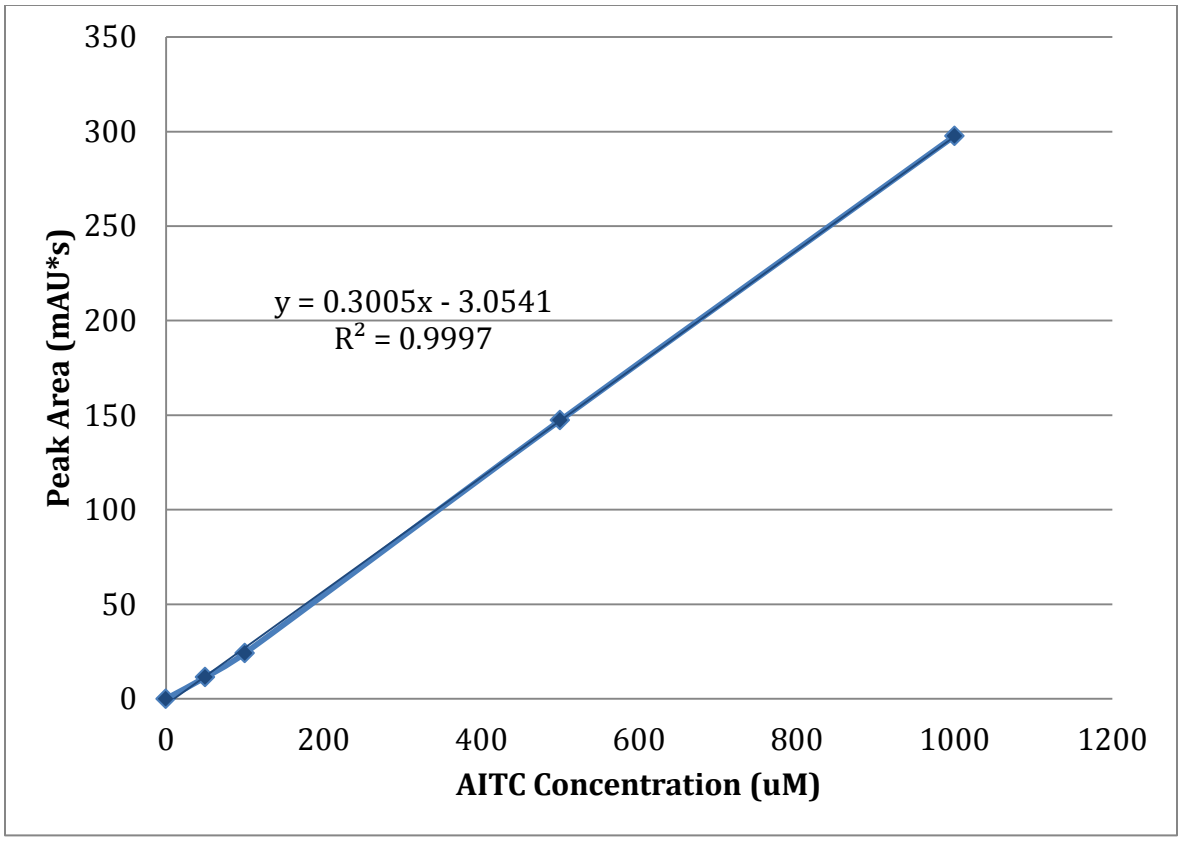

Figure C-3: Allyl isothiocyanate standard curve. Sigma Aldrich chemical standard used with ethyl acetate.

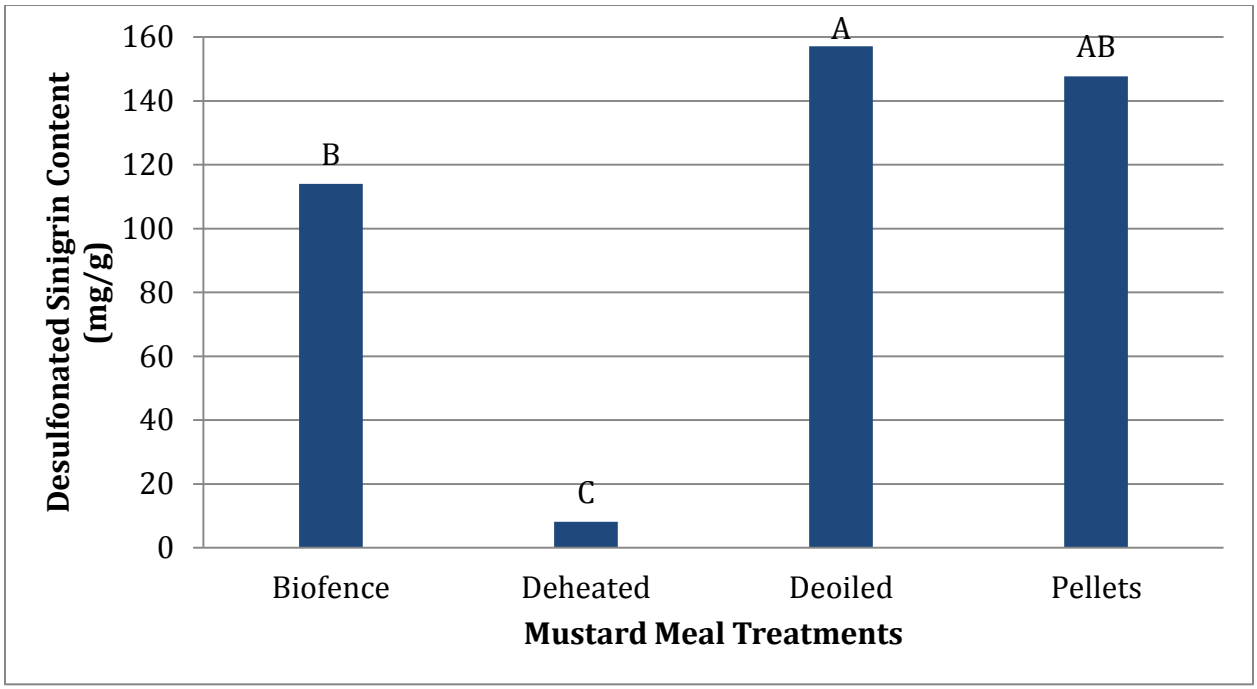

Figure C-4: Desulfonated sinigrin content (mg/g) of the mustard meal treatments used in field experiment. Values are combined means of six replications. Means indicated by different letters are significantly different ($P<0.05$).

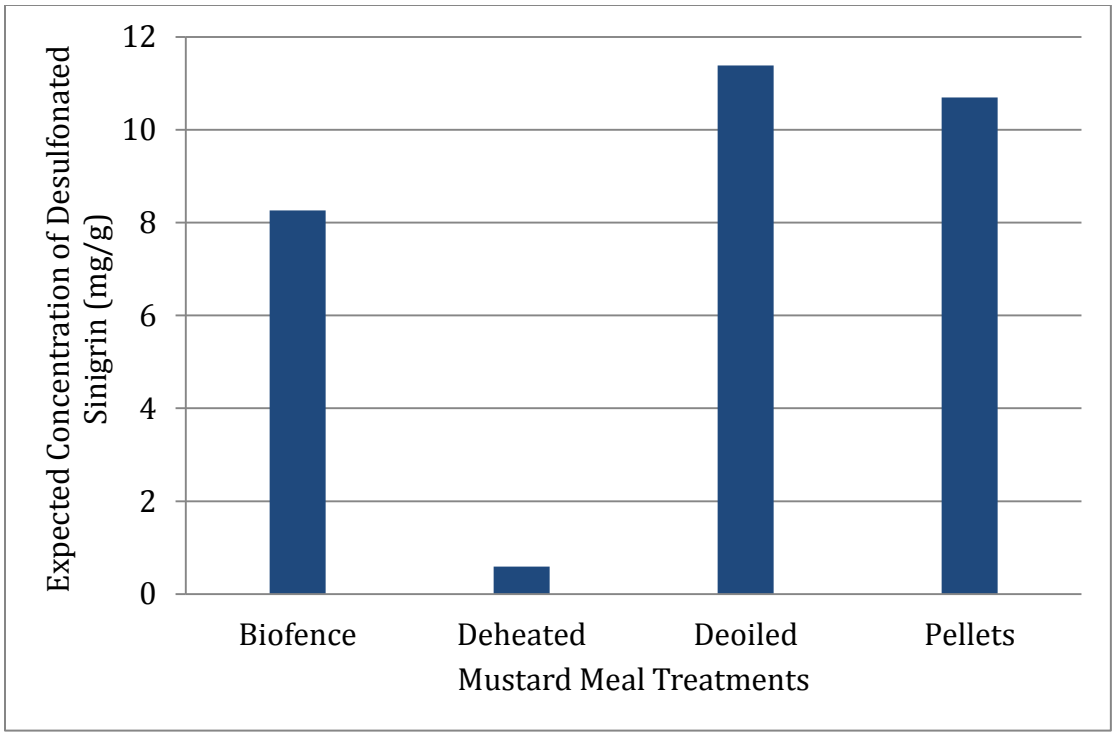

Figure C-5: Expected sinigrin content of mustard meal treatments used in field calculated based on desulfonated sinigrin content of these meals (Figure C-4).

Table C-3: Sinigrin and AITC concentrations (with standard errors) of soil samples taken in field post treatment application. Samples were taken after application (0hr), four hours, nine hours, 30 hours, 4days, and 9days post irrigation. Values are means of six replications.

	Sinigrin						**AITC**					
	µMol/g											
Treatment	**0hr[z]**	**4hr[y]**	**9hr[x]**	**30hr[v]**	**4day[u]**	**9day[t]**	**0hr[z]**	**4hr[y]**	**9hr[x]**	**30hr[v]**	**4day[u]**	**9day[t]**
Control	0	0	0	0	0	0	0	0	0	0	0	0
Deheated Mustard Meal	0	0	0	0	0	0	0	0	0	0	0	0
Deoiled Mustard Meal	22.72±50.81	32.58±72.84	14.78±33.05	0	0	0	0	0	0	0	0	0
Soybean Meal	0	0	0	0	0	0	0	0	0	0	0	0
Mustard Pellets	0	0	0	0	0	0	0.86±32.58	0	0	0	0	0
Dried Molasses	0	0	0	0	0	0	0	0	0	0	0	0
Biofence	16.44±36.77	0	23.47±52.48	0	0	0	0	0	0	0	0	0
Basamid	0	0	0	0	0	0	0	0	0	0	0	0
Dried Molasses+Soybean Meal	0	0	0	0	0	0	0	0	0	0	0	0
Dried Molasses+Deoiled Mustard Meal	66.22±95.59	29.41±65.75	17.3±38.69	14.57±32.58	0	0	0	0	0	0	0	0
Dried Molasses+Deheated Mustard Meal	15.79±35.31	16.93±37.85	0	0	0	0	0	0	0	0	0	0

[z]Approximately 1 hour after treatment application.
[y]Approximately 21 hours after treatment application.
[x]Approximately 30 hours after treatment application.
[v]Approximately 47 hours after treatment application.
[u]Approximately 5 days after treatment application.
[t]Approximately 10 days after treatment application.

Proposal for a greenhouse experiment repeating field experiment in a controlled environment.

The four highest yielding field treatments plus a control will be set up in a randomized complete block design with replication. Each row will contain one of the treatments being tested. Therefore, each row will be considered a replicate of each other. SAS 9.3 will determine where to place each treatment, in order to take away bias and have a proper randomized block design.

Field soil will be retrieved from the ETREC Plant Science Farm Unit. This will then be taken back to the greenhouse and mixed with sand in a cement mixer. This mixture will then be added to all of the 30 pots being used. Specified containers will be inoculated with *Phytophthora cactorum* obtained from Dr. Bonnie Ownley's plant pathogen laboratory. Treatments will be applied to each pot. An initial soil sample of each plot will then be taken and the pots will be covered with black plastic to simulate plasticulture in the field. Irrigation will be applied to the pots, and soil samples will be taken at 2, 4, 6, 24, and 48 hours post irrigation application in order to measure sinigrin and allyl isothiocyanate volatiles. Irrigation will run for the minimal time needed to saturate each pot. In the interim, strawberry plants will be ordered from the supplier. Once plugs arrive, they should not be planted into the pots until two weeks have passed from fumigation. Once plants have established, fertigation shall begin as aforementioned. Plants will then be managed by checking for health and pests until ready to harvest fruit. When fruiting begins, harvest will take place twice a week. At each harvest, fruit weights and yield of marketable strawberries, ones that are over 10 grams in weight, will be measured. In the middle of harvest season, leaf samples will be collected for nutrition analysis of each plant. After

harvest, whole plant samples will be collected for biomass analysis. Soil samples will then be taken in order to measure nutrient levels in soils and to measure pathogens in the soil.

Stake emitters will be placed in the soil near plants so that water will be easily accessed by the roots. This system will be attached to a dose meter, so that every pot will receive the same amount of irrigation. Irrigation will be applied weekly from the irrigation dose meter. Before planting, a 2:1 mixture of 10-10-10 and 0-20-20 fertilizer will be used in a concentrated solution in order to conserve space. Once plants are established in pots, the strawberries will receive a weekly alternation between a soluble 20-20-20 mixture and calcium nitrate. At harvest, the strawberries will receive a weekly alternation of calcium nitrate and potassium nitrate. The dose meter will then concentrate the mixture to a daily requirement for each plant. Each plant will receive the same amount of fertigation.

Most strawberry pests will be shielded by the greenhouse. However, thrips and whiteflies can be common in a shared greenhouse setting. Yellow and blue monitoring tape will be placed throughout the house to check for their occurrence. If a problem arises, *Neoseiulus cucumeris* and *Dlephastus catalinae* will be introduced in order to control their populations.

Chapter 4: Conclusion

In regard to total strawberry fruit yield, biofumigation and ASD treatments on their own were not comparable to the chemical treatment of Basamid; however, combining biofumigation and ASD treatments provided a yield that was significantly the same as Basamid®. More importantly, marketable yield harvested from plots treated with all biofumigation (minus Biofence), ASD, and combination treatments were statistically equal to those harvested from plots treated with Basamid®. Therefore, any would be a viable alternative to chemical treatments if marketable yield is the main concern. Leaf mineral analysis did not vary enough among treatments for one treatment to be considered superior. While fruit mineral analysis had differences, the differences were not consistent in regard to a better treatment. Fruit quality with respect to carbohydrate analysis differed from the combination treatment of deoiled meal and molasses, providing a carbohydrate content superior to some of the other treatments. ASD treatment of molasses yielded fruit with higher organic acid content, while also decreasing the soil pH at treatment incorporation but not at harvest. These two observations could be related. Further research on the effects of higher soil pH from ASD treatments on strawberry fruit organic acid concentrations should be performed. For this analysis, the increase in organic acid content of the strawberry fruit could cause a bitter taste that may be detrimental to consumptive sales. The addition of soybean meal to the ASD treatment of molasses was able to increase the concentration of inorganic N in the soil solution after incorporation; however, when measured at harvest, the concentration of total inorganic N was significantly lower than most other treatments. Parasitic nematodes were equally not present in plots treated with the alternative methods; however, beneficial nematodes were found in these plots. Overall, combining ASD with biofumigation is effective at increasing

total yield; however, for all other aspects of strawberry production, each treatment is efficient on their own in comparison to the chemical treatment of Basamid®. The results of this experiment have demonstrated the presence of sinigrin and AITC in soil after mustard meal application. While concentrations were inconsistent, we were able to extract sinigrin from plots treated with deoiled mustard meal, Biofence, and deactivated mustard meal. We were also able to extract AITC from plots treated with mustard pellets. For the future, we should focus on the effects of ethyl acetate on sinigrin and AITC concentrations for an observed period of time. Samples should be analyzed in a shorter period of time post collection. Also, this experiment should be repeated in a controlled, greenhouse environment in order to account for any inconsistencies that may have occurred in the field.

Vita

Jennifer Renee' Wheeler was born in Knoxville, TN to the parents of Dennis and Angie Merriman. She is the last of three daughters, Mary, Lindsay, and Caroline, and one son, Andrew. She attended Karns Elementary, Primary, Middle, and High Schools in Knoxville, TN. After graduation, she continued her education at Pellissippi State Community College in order to work on undergraduate pre-requisites. She then attended the University of Tennessee, Knoxville where she studies Environmental and Soil Sciences. During her time as an undergraduate, she worked for Dr. Carl Sams in a plant physiology lab and became excited about plant sciences and sustainable agriculture. She obtained a Bachelor's of Science degree from the University of Tennessee, Knoxville in May 2013 in Environmental and Soil Sciences. She accepted a graduate research assistantship at the University of Tennessee, Knoxville in Plant Sciences in order to study sustainable production of strawberries. Jennifer graduated with a Master's of Science degree in Plant Sciences in May 2016. She is continuing research at the University of Tennessee, Knoxville as a Research Associate II.