To the Graduate Council:

I am submitting herewith a dissertation written by Darrell Donaldson Hensley entitled "Sinigrin content and allyl isothiocyanate concentration of whole ground mustard meal and the ability of allyl isothiocyanate to inhibit growth of *Phytophthora parasitica* var. *nicotianae*, *Meloidogyne incognita* and possible factors affecting its action." I have examined the final paper copy of this dissertation for form and content and recommend that it be accepted in the partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant, Soils and Insects.

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Sinigrin content and allyl isothiocyanate concentration of whole ground mustard meal and the ability of allyl isothiocyanate to inhibit growth of *Phytophthora parasitica* var. *nicotianae*, *Meloidogyne incognita* and possible factors affecting its action

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ABSTRACT

Compounds hydrolyzed from decomposition of glucosinolates have the potential to reduce pest populations and possibly control many soilborne diseases. Sinigrin is a glucosinolate commonly found in *Brassica* species. Sinigrin concentrations in whole ground oriental mustard seed meal (OMM) obtained from 12 different lots ranged from 101 to 141 $\mu M \cdot g^{-1}$ of OMM. Allyl isothiocyanate (AITC) concentrations evolving from sinigrin in corresponding seed lots ranged from 17 to 33 $\mu M \cdot g^{-1}$. Conversion efficiency of sinigrin to AITC from these 12 lots was approximately 19 percent. Water volume and soil coverings may affect concentrations of AITC evolving from OMM. Increased amounts of water and use of polyethylene soil coverings negatively affected AITC concentrations.

The concentrations of commercially obtained AITC and AITC evolved from OMM needed to produce 50% and 90% inhibition (IC_{50}, IC_{90}) of black shank (*Phytophthora parasitica* var. *nicotianae*) were calculated. Inhibitory concentrations (IC_{50}, IC_{90}) of AITC were 0.70 and 1.52 $\mu M \cdot L^{-1}$, respectively. Inhibitory concentrations of IC_{50} and IC_{90} of AITC from OMM were 0.99 and 1.72 $\mu M \cdot L^{-1}$, respectively. In a similar experiment, the lethal concentrations (LCs) of commercially available AITC, OMM and OMM mixed with soil were determined for root-knot nematode, (*Meloidogyne incognita* - RKN) eggs. The LC_{50} and LC_{90} for AITC were 0.78 and 1.94 $\mu M \cdot L^{-1}$, respectively. The LC_{50} and LC_{90} for OMM were 0.44 and 1.22 $\mu M \cdot L^{-1}$ and for OMM mixed with soil they were 0.88 and 1.99 $\mu M \cdot L^{-1}$, respectively.

Field tests were conducted to determine the effect of various rates of OMM and commercial fumigants (Telone C-35 and dazomet) on vermiform nematode populations at two locations. Statistical analysis suggested that neither commercially available soil fumigant negatively influenced vermiform nematode populations in the Flethcher or Knoxville location during 2003 or 2004; however, non-significant reductions were observed in nematode populations in plots with Telone or 2,242 $kg^{.}ha^{-1}$ of OMM. Effects from OMM treatments were highly variable and were not significantly different from the control.

Research from multiple laboratory experiments indicated that AITC has the potential to reduce or inhibit growth of both black shank and eggs of RKN. Results from treatments including OMM in field research, was highly variable and more research is needed to determine the extent of efficacy of this material.

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Part I

INTRODUCTION

Brassicaceae, the mustard family, contains approximately 350 genera and over 3,000 species. Seeds of many members of the Brassicaceae are harvested because they are rich in oils that are used for various purposes, such as food, oil, soaps, and lubricants. The genus *Brassica* is in the family Brassicaceae and seeds of some species may contain 40 percent oil on a dry weight basis. Rapeseed or canola (*Brassica napus* L. and *B. campestris* L.) is commonly cultivated in Canada and the United States. Traditional forms of rapeseed and mustard have been reported to have >80 $\mu M \cdot g^{-1}$ glucosinolates in the seed meal. Glucosinolates are secondary metabolites formed within various plants in the mustard and other plant families. Over 120 glucosinolates have been identified. Mustard seed and plants have long been used as spice or as additives to foods to intensify their flavors. Mustard has a pungent odor and acrid flavor, which originates from degradation of glucosinolates. Hydrolysis products are formed when plant tissues containing glucosinolates are damaged and enzymes known as myrosinases come in contact with the glucosinolates. Glucosinolates may degrade to form isothiocyanates. Isothiocyanates are biologically active and are toxic to various soilborne organisms and weed seeds. Allyl isothiocyanate is a major end product of degradation of sinigrin, a glucosinolate commonly found in oriental mustard meal. Allyl isothiocyanate has been reported to have toxic effects on various soil-inhabiting fungi and nematodes.

Methyl bromide (MeBr) is one of the most effective soil fumigants used in agriculture and forestry production. It has been very effective in controlling numerous pests that occur in each of these production areas. Alternative pest control techniques and/or chemicals must be identified because of environmental problems associated with methyl bromide, excessive costs of fumigation and banning (effective 2005) of methyl bromide. Methyl bromide has been used in Tennessee for strawberry (*Fragaria* × *ananassa* Duch.), forestry, floral, nursery, tomato (*Lycopersicon esculentum* Mill.) and tobacco (*Nicotiana tabacum* L.) production. Resistant cultivars and/or the use of alternative pesticides are available to aid in the control of some of the pests previously controlled by MeBr. Growers often reject many of the commonly used methods of pest control, such as; resistant cultivars, fungicides, insecticides, and/or nematicides due to high costs associated with their use. If an alternative to synthetic products is discovered and used by growers, losses could be minimized due to pest infestations which continually attack the economically valuable crops grown in Tennessee.

Since fumigant products have a volatile nature, researchers and producers have investigated use of various methods to improve fumigant efficacy. One method is use of plastic soil coverings made from various compounds, i.e. polyethylene (PE). Polyethylene commonly is used with methyl bromide applications as well a fumigants containing chloropicrin. Soil coverings help reduce loss of volatile products therefore increasing contact time of the fumigant with the targeted pest and improving efficacy.

By-products of glucosinolates are released by many *Brassica* species when these plant residues are tilled into the soil. By-products of glucosinolates are highly volatile and have similar characteristics of several commercially available fumigants. Hydrolysis compounds which evolve from glucosinolates have the potential to reduce pest populations and possibly control several highly recognized destructive diseases that occur in tomato and tobacco production in Tennessee.

Meloidogyne incognita (Kofoid & White) Chitwood, commonly known as the root knot nematode (RKN), have a wide host range and cause large economic losses in many vegetables, especially tomato, if not controlled. Methyl bromide is one of the most effective fumigants currently used for control of this pest; however, alternatives are being sought.

Phytophthora parasitica Dastur var. *nicotianae* (Breda de Haan) Tucker, (black shank of tobacco) is very destructive and can cause entire crop loss if preventative measures are not taken. Methyl bromide was commonly used in ground bed production systems for control of black shank and other pests. Use of methyl bromide is not economically feasible in field production and other methods of control are currently being used for black shank control. The current method of control in the field includes a pre-plant incorporated treatment with mefenoxam (Ridomil Gold) and using varieties with a medium resistance (level of four of resistance) or greater. There have been many instances where targeted fungi have become resistant when fungicide applications have been continuously used, so alternatives should be investigated and available in the event of resistance. Due to the loss of methyl bromide, use of an amendment containing high levels of glucosinolates is an attractive alternative.

Both black shank and RKNs are destructive pests which commonly occur in Tennessee tobacco and vegetable production systems. Due to the foreseen loss of methyl bromide and concerns about other products which may potentially contaminate the environment, efficacy information of AITC is needed in controlled tests. Several factors may effect concentrations of AITC. Information concerning many of these commonly occurring factors and how they may alter ATIC concentrations, may be beneficial in determining effective methods of controlling soilborne pests. Several of the experiments described within this document determine efficacy of AITC on controlling RKNs and black shank and factors that may affect AITC concentrations in controlled environments and field studies.

Part II

LITERATURE REVIEW

A History of Mustard

The mustard seed and plant have been used for many years as a spice or additive to foods to intensify their flavors. Mustard has a pungent odor and acrid flavor. It has been referred to in the New Testament of the Bible as "The Parable of the Mustard Seed" in three different versus with similar wording.

> "The kingdom of heaven is like a mustard seed, which a man took and planted in his garden. Though it is the smallest of all your seeds, yet when it grows, it is the largest of garden plants and becomes a tree, so that the birds of the air come and perch in its branches." (Matthew 13:31, 32, Mark 4:31, 32, Luke 13:18, 19) NIV

The plant mentioned in the Bible may have been referring to one in the Salvadoraceae family, *Salvadora persica* L., which is a relative of evergreens and shrubs that are commonly found in the Middle East, India, Africa and China (Antol, 1999). *Salvadora persica* is commonly called a mustard tree and contains substances similar to that present in mustard. It is not considered a true mustard, however its seeds were harvested for their oils and their seed size is very similar to plants of the Brassicaceae. Seeds of the Brassicaceae are rich in oils that are used for various purposes, i.e. food, oil, soaps, lubricants and as illuminants in lanterns. Seeds of *Brassica* species may contain 40 % oil (dry weight basis) and oils from harvested seeds are used in foods and as lubricants (Downey and Robbelen, 1989). Rapeseed (*Brassica napus* and *B. campestris*) is commonly found cultivated in Canada and the United States and edible cultivars are known as canola where cultivars grown for industrial purposes are known as rapeseed.

Several types of mustards are currently cultivated. Brown mustard, also known as oriental mustard (*B. juncea*), is primarily grown for oil from its seed. Black mustard (*B. nigra*), which has a strong flavor, may cause irritation when it comes in contact with the skin. White mustard (*Sinapis alba* L.) is a milder mustard and is often mixed with black mustards to be used in condiments. Antol (1999) reported that mustard was originally used as a relish, made from ground mustard seed and mixed with unfermented fruit juices.

Brassicaceae

The family Brassicaceae, contains approximately 350 genera and over 3,000 species. The genera *Brassica* is primarily cultivated for its seeds, which are used to manufacture mustard or as a source of edible oil (Fahey, 2001). *Brassica juncea* and *B. nigra* represent two such mustard species. They belong to the kingdom Plantae, subkingdom Tracheobionta, superdivision Spermatophyta, division Magnoliophyta, class Magnoliopsida, subclass Dilleniidae, order Capparales, family Brassicacae, genus *Brassica*, and species *juncea* (taxonomic serial #23059) and *nigra* (taxonomic serial # 23061) (USDA, 2004).

McLeod et al., (2001) reported that although brassicaceous crops are hosts of nematodes, they are poorly invaded and suppress nematode growth and development. Therefore they reduce the risk of nematode population increases in soils where tissues are incorporated as green manure biofumigants. Plants in the Brassicaceae could play an integral role in integrated pest management, if concentrations of glucosinolates contained within them are high enough to control the targeted pest.

The Brassicaceae species are important as vegetable, condiment, fodder, and oilseed crops. They may also be used as a cover crop in crop rotation or as a soil amendment used for biofumigation purposes. The potential of these species to release isothiocyanates from tissues containing glucosinolates and thus reduce pest populations appears very promising.

Brassica species produce sulfur-containing secondary plant metabolites known as glucosinolates. In the presence of myrosinase and water, glucosinolates rapidly convert to isothiocyanates. The isothiocyanates evolving from the glucosinolates appear to be the most potent biofumigant compounds in mustard tissue. Death of target organisms occurs with adequate isothiocyanate concentrations and exposure time.

The major glucosinolate of brown mustard (Mithen, 2001), oriental mustard (*B. juncea*) (Pechacek et al., 2000) and black mustard (*B. nigra*) seed is an aliphatic glucosinolate, sinigrin or 2-propenyl glucosinolate. Brown and Morra (1995) reported that glucosinolates remained fairly stable in defatted meal for extended periods of time, mainly due to low moisture content of the seed meal. Thus, the material has potential in agricultural fumigation.

Various volatile compounds are evolved from mustard glucosinolates when certain conditions exist (Fig. II-1, pg. 34)[1]. An enzymatic hydrolysis of two components found within the tissues produce glucose and sulfate (Shikita, 1999). They further break down into volatile compounds i.e. allyl isothiocyanate (AITC), as well as allyl thiocyanates, nitriles, and epithionitrile (Brown and Morra, 1997) depending on environmental conditions. Environmental conditions with neutral pH produce isothiocyanates, and acidic conditions produce nitriles. Occasionally, other products may be produced from the degradation of sinigrin (Pechacek et al., 1997; Masheshwari et al., 1981). Allyl isothiocyanate is an isothiocyanate and has been identified and reported to

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[1] **Figures and tables are included in the Appendix.**

be formed from hydrolysis of tissues of *Brassica*. Allyl isothiocyanate has characteristics similar to other fumigants and is volatile. The fumigants dazomet (Basamid) and metham-sodium (Vapam) have a similar principle breakdown product, methyl isothiocyanate which has a vapor pressure of 21 at 20 °C (Whitehead, 1978). Methyl isothiocyanate has a reported half-life from 12 hours to 50 days (Hadiri et al., 2003) and the half-life of AITC was reported to range from 20 to 60 h (Borek et al., 1994). Allyl isothiocyanate has a boiling point of 150 °C / 760 mm Hg (low volatility), a melting point of -80 °C and a flash point of 46.1 °C (Rahway, 2001). However, volatility may not be extremely low, because Sarwar et al., (1998) reported that one drop of pure AITC may volatilize within five minutes at room temperature.

Biofumigation

Biofumigation is the term used to describe the suppression of pest or disease organisms by the ITCs released from *Brassica* green manure crops or rotation crops (Angus et al., 1994; Kirkegaard et al., 1993; and Kirkegaard et al., 1996). Green manure is "plant material incorporated into soil while green or at maturity, for soil improvement" (Soil Science Society of America, 2004). The biofumigation definition can be modified by adding "crop residues," since seed meal is processed material and would not be typically considered green manure.

Soil fumigation is a primary method of controlling soilborne pests in high-value crops such as vegetables. Methyl bromide is a commonly used fumigant; however, due to its volatility, it is often used in conjunction with plastic mulch to reduce its loss from soil. Methyl bromide is used for control of a wide range of pests. Biofumigation is a process of pest suppression or elimination and is similar to fumigation. Biofumigation is accomplished by incorporation of plant residues into the soil. Materials released by plant residues either reduce or inhibit targeted pests. Residues of *Brassica* spp. may be used as a biofumigants. Allyl isothiocyanate is the most toxic compound formed from hydrolysis of sinigrin, which is a glucosinolate found in *B. juncea* and possibly the most important compound for use in biofumigation (Brown and Morra, 1997; Kirkegaard and Sarwar, 1998).

Brassica spp., such as rapeseeds, have been investigated for many years for their nutritional levels for use as feed in livestock production. Canola has been defined as a rapeseed cultivar which must have an erucic acid content of less than two percent and less than 30 micromoles of glucosinolates per gram of seed (Berglund and McKay, 2002). Due to some of the detrimental effects of glucosinolates, researchers and seed breeders have attempted to determine methods of reducing their content. Researchers are continuing their efforts to lower levels of glucosinolate content to $< 25\ \mu M \cdot g^{-1}$ in meal (de Quiroz and Mithen, 1996). Also researchers are investigating methods to reduce levels of erucic acid content in crops commonly used for livestock production. Brown and Morra (1997) published a comprehensive review of the impact of breakdown products of glucosinolates and concluded that they may provide positive results since they have the potential to suppress a wide range of soilborne pests.

Plasticulture

Plasticulture production is an agricultural technique that uses plastic materials, raised beds and drip irrigation for high value crops, such as tomatoes and strawberries.

Soil compaction and covering of soils with non-permeable or semi-permeable material may delay or eliminate the loss of fumigants from the soil surface, thus increasing contact time and increasing efficacy of the soil fumigant. The distribution and toxicity of fumigants may be influenced by factors such as temperature, soil moisture, porosity, clay and/or organic matter content of the soil (Goring, 1962). The use of plastic mulches and irrigation may improve efficacy of *Brassica* biofumigant incorporated in soil, since adequate moisture is needed for the hydrolysis process.

In the Southeast, many producers are using embossed polyethylene (PE) plastic mulch in vegetable production. In most situations, irrigation drip tape is being used in conjunction with PE. Fumigation with products containing the active ingredients methyl bromide or methyl isothiocyanate using a soil covering generally provides better pest control than fumigation without covering (Sumner et al., 1978). Covering the soil may be important to reduce escape of highly volatile materials. By increasing the efficiency of the fumigant growers may be able to reduce concentrations of products added to the soil therefore reducing cost. Plastic row coverings are also known or referred to as plastic mulches, soil coverings, plastic tarps, and/or sheeting.

Polyethylene (PE) sheeting is generally inexpensive; however, the product adds additional labor and equipment costs to production and additional cost for removal of material at the end of the production season. Some manufacturers treat PE with UV inhibitors which may extend life of PE for an additional 12 months or longer. The most common type of plastic used in agricultural production is an embossed black polyethylene film or covering. The embossing provides the material with flexibility, allowing it to give or stretch without tearing as easily as flat type films.

Plastic mulches are used for various reasons and have many advantages in agricultural production. Plant mulches are reported to increase yields, reduce soil compaction, reduce fertilizer leaching, reduce evaporation, eliminate root pruning by equipment, and reduces weed populations (Coffey, 1984; Sanders, 1994). They also provide a cleaner crop, reduce spread of diseases, increase soil temperatures in early season, reduce drowning of crop during excessive rainfall, and enhances soil fumigation (Coffey, 1984; Sanders, 1994). However, Daponte (1995) reported that many agricultural films were permeable to volatiles of soil fumigants.

There are several disadvantages of using plasticulture including greater initial cost, removal and disposal costs and greater management needs when used with drip irrigation systems. Controlling soil moisture or use of irrigation is important when using fumigants. It has been a common practice to apply methyl bromide to soils when soil moisture level is highs. The use of *Brassica* tissues incorporated into irrigated soil and covered with PE shows great promise since moisture is needed to achieve the hydrolysis reaction and PE may also aid in the retention of volatile products.

Glucosinolates

Glucosinolates are precursors of the compounds that cause the biting or hot taste, acrid flavors, and pungent odors in *Brassica*-based condiments such as horseradish and mustards. They are a diverse class of sulfur and nitrogen-containing secondary plant metabolites produced in all *Brassica* species, as well as in 500 non-brassicaceous plant species. Examples of families of glucosinolate-producing plants include, but are not limited to, Akaniaceae, Bataceae, Brassicaceae, Bretschneideracea, Capparaceae, Caricceae, Euphorbiaceae, Gyrostemonaceae, Limnanthaceae, Moringaceae, Pentadiplantdraceae, Phytolaceae, Piltosporaceae, Resedaceae, Salvadoraceae, Tovariaceae, and Tropaeolaceae (Fahey et al., 2001; Halkier, 1999).

Although glucosinolates are generally found in all parts of the *Brassica* plant, some types of glucosinolates may be found at higher concentrations within the seed (up to 10 % of dry weight) whereas concentrations in the leaves, stems, and roots are often only a tenth of the seed concentration. Concentrations of glucosinolates differ according to cultivar, tissue type, physiological age, temperature, planting date, plant health (stress) and fertility (Milford et al., 1989; Sang et al., 1984). In *Arabidopsis*, glucosinolates have been found in certain sulfur-containing “S-cells” in the pedicel, located externally to the vascular system (Koroleva et al., 2000). The glucosinolate containing S-cells have translucent vacuoles and are structurally different from the phloem myrosinase cells. In *B. juncea*, the glucosinolate sinigrin is localized in embryos (Kelly et al., 1998).

Glucosinolates are composed of organic anions containing □-D-thioglucose and sulfonated oxime moieties with a variable side chain derived from amino acids (Brown et al., 1994). Generally, glucosinolates are grouped into aliphatic, aromatic and indole, depending on the structure of the amino acid from which they derive. Over 120 glucosinolates have been identified (Fahey et al., 2001.) and as many as 34 have been found in *Arabidopsis thaliana* (L.) Heynh (Kliebenstein et al., 2001).

Glucosinolate Biosynthesis

Glucosinolates are derived from several different amino acids such as aliphatic (methionine, alanine, valine, leucine, isoleucine), aromatic (phenylalanine, tyrosine) or indole (tryptophan) and they differ in structure by these amino acid side-chains. According to McDanell et al. (1988), the biosynthesis of aliphatic glucosinolates and indole glucosinolates in *Brassicas* is not linked but act independently. Variations in fertility, environmental conditions, enzymes, and genetic makeup of the plant affect glucosinolate concentrations in plant tissues. Aliphatic glucosinolates are the major class of glucosinolates found in *Brassicas*. They are biosynthesized from the straight chain amino acid methionine, which is synthesized from intermediates of glycolysis and the pentose phosphate pathways. Biosynthesis of glucosinolates involves a three-step process or chain-elongation cycle. Each pass through the cycle results in the addition of a single methylene group. Up to six cycles of elongation may occur in *A. thaliana* (Textor et al., 2004). The other two steps in glucosinolate biosynthesis include synthesis of the core glucosinolate from the precursor amino acid followed by side chain modifications.

The first reaction of the chain-elongation cycle is catalyzed by the enzyme known as methylthioalkylmalate synthase (MAMS), which condenses a ω-methylthio-2-oxoalkanoic acid with acetyl-CoA. Textor et al. (2004) demonstrated in *A. thaliana* that MAM1 encodes a MAMS, catalyzing the condensing reactions of the first two elongation cycles. Methionine is deaminated to its corresponding 2-oxo acid (4-methylthio-2-oxobutanoic acid), which is condensed with acetyl-CoA to form a malate derivative, [2-(2'-methylthioethyl)malate]. This reaction is followed by an isomerization and oxidative decarboxylation that yields a 2-oxo acid (5-methylthio-2-oxopentanoic acid) with the addition of a methylene group. This compound is then transaminated to form homomethionine. The process forms the C_3 glucosinolates and additional isomerizations and oxidative decarboxylations may form C_4 through C_8 glucosinolates. The identification of the *Gsl-elong* locus controls the methionine side-chain elongation process (Magrath et al., 1994). Sinigrin, the major glucosinolate of *B. juncea*, is produced through only one chain-elongation with a methylene group added and would be considered a C_3 glucosinolate. Research has shown that cytochrome P450, CYP79F1 and CYP79F2 metabolize all chain-elongated methionine derivatives and only long-chain methionine derivatives, respectively (Hansen et al., 2001; Chen et al., 2003).

N-hydroxy amino acids, aldoximes, thiohydroximic acids, and desulfoglucosinolates appear to be precursors of glucosinolates (Du et al., 1995). Enzymes involved in the conversion of amino acids to aldoximes include cytochrome-P450-dependent mono-oxygenases (cytochrome P450), which is the second step in glucosinolate biosynthesis. Cytochromes P450 of the CYP79 family and CYP83Al catalyze the conversion of the amino acids to the aldoximes. CYP83Al was reported to be the aldoxime-metabolizing enzyme in *Arabidopsis* involved in the oxidation of the aldoxime and conjugation with a sulfur donor (Jones et al., 2001). For sinigrin, flavin containing monooxygenases are responsible for conversion of the amino acid to the oxime (Halkier and Du, 1997). The conversion of the oxime to thiohydroximate is a poorly understood step in the biosynthesis of glucosinolates since no biochemical data are available on intermediates involved (Halkier and Du, 1997).

The final step in the development of the glucone pathway is controlled by genetic loci, which is important in forming various glucosinolates by side chain modifications. A 2-oxoglutarate-dependent dioxygenase controls glucosinolate biosynthesis in *Arabidopsis* (Kliebenstein et al., 2001). The *Gsl-oxid* locus controls the oxidation of methylthio to methylsulfinylalkylglucosinolates (C_3 and C_4 basic side chain glucosinolates) and the

Gsl-alk locus controls the removal of the methylsulfinyl residue, which is then followed by the introduction of a double bond (basic side chain C_3 and C_4 atoms), for development of butenylglucosinolate. Alleles at the *Gsl-oh* locus are responsible for the hydroxylation of alkenyl glucosinolates. Magrath et al., 1994, reported that in *Arabidopsis* the *Gsl-ohp* locus controls the conversion of methylsulfinylpropyl to hydroxypropylglucosinolate and *Gsl-alk-A*, *Gsl-alk-C*, and *Gsl-alk-Ar* are responsible for the conversion of methylsulfinylpropyl to propenylglucosinolate (sinigrin). Once formed, the side chain may be modified by desaturation and hydroxylation, producing various types of glucosinolates containing additional carbons.

Myrosinase

Myrosinase (thioglucosidase or thioglucoside glucohydrolase, E.C. 3.2.3.1) is an enzyme found in specialized cells known as idioblast or myrosin cells. The idioblast cells are specialized cells which occur in tissue and differ in form, size, or contents from other cells located in the same tissue. They are specialized cells, scattered at low frequency and often as single cells among the other cells in a tissue (Andreasson et al., 2001). In *Arabidopsis*, myrosinase is confined to idioblastic cells in the phloem parenchyma and some cells of developing petals and sepals. Myrosinase has been reported in young guard cells in *B. napus* (Höglund et al., 1991) and has been expressed in both ground tissue and the phloem tissue of *B. napus* (Andreasson et al., 2001). Originally, Luthy and Matile (1984) suggested that myrosinase is localized in the same cells as glucosinolates but in an inactive form. Glucosinolates were later reported to be localized in vacuoles of cells and myrosinase has been reported in myrosin and non-myrosin cells (Bones and Rossiter, 1996). Kelley et al. (1998) reported the presence of glucosinolates in non-myrosin cells of *B. juncea*.

Rask et al. (2000) reported that a complex array of myrosinase genes in *B. napus* contains at least 20 genes divided into three subfamilies, MA, MB, and MC. Myrosinase genes in Arabidopsis are TGG1, TGG2 and TGG3 (Zhang et al., 2002). TGG1 and TGG2 genes are found in complexes together with myrosinase binding proteins (MBP) and the TGG3 gene is considered a pseudogene.

Glucosinolate Degradation

Glucosinolates degrade as the cell ruptures from some type of disruption or tissue damage. Damage may occur due to insect infestation, disease, mechanical damage, or mastication. Glucosinolate combines with myrosinase and water to form the unstable intermediate known as aglycone, which then rearranges to form various products (Fig II-1, pg. 34). The glucosinolate may degrade into products such as epithionitriles, thiocyanates, nitriles, isothiocyanates, or oxazolidine-thione; however, the degradation pathway is dependent on surrounding conditions. For example, Fe+ and an epithiospecifier protein (ESP) are required for the formation of epithionitriles (Tookey, 1973). Nitriles form at pH 4 and isothiocyanates are generally formed at pH 7. Nitrile formation may be enhanced by the presence of ferrous ions (Fenwick et al., 1983), similar to epinitriles. However, at pH 7, isothiocyanates (ITCs) are most abundantly formed. A major compound produced from *B. juncea* tissues is allyl-ITC and it is the active compound which is detrimental to many soilborne pests.

Disease Control

Plant breeding strategies have concentrated on altering the glucosinolate content within mustard and reducing levels in rapeseed. Edible rapeseed, or canola, is processed for oil, for human consumption, with the remaining meal used in livestock rations. Mustard seed is usually harvested for oil content. Researchers and plant breeders have been developing canola cultivars containing low levels of glucosinolates, due to their adverse effects on livestock and has been a major goal in most *Brassica* breeding programs. If *Brassica* tissues were used in IPM programs, tissues containing high levels of glucosinolates would be needed to obtain levels for control of various soilborne pests. When plant tissues containing glucosinolates are added to the soil they form isothiocyanates. Isothiocyanates are responsible for the fumigation ability of these tissues (Charron and Sams, 1999; Harvey et al., 2002). Green manure or seed meal of *Brassica*, when added to soil, has the potential to control various pests. Depending on glucosinolate composition and on the prevalence of hydrolysis products (myrosinase, water) isothiocyanates may be formed. Natural isothiocyanates derived from aromatic and aliphatic glucosinolates are effective biofumigants (Borek et al., 1995). Brown and Morra (1997) compared results of published research and the effects and concentrations of various isothiocyanates reported to achieve inhibition or delay in growth of various microbes. The aliphatic compound, 2-propenyl ITC (sinigrin), is the dominant glucosinolate in the seeds of mustard (*B. juncea*) and was shown to be highly toxic to cereal root pathogens (Sarwar et al., 1998). Bending and Lincoln (1999) suggested the ability of *B. juncea* tissues in soil to control microbes was due to the formation of small quantities of highly toxic ITCs and larger quantities of mildly toxic non-glucosinolate-derived, volatile S-containing compounds produced during decomposition. In an experiment using solarization and cabbage amendments added to soil, control of *Phytophthora* spp. was not successful. This failure may have been due to insufficient amounts of amendment used or lack of proper preparation of the amendment (Coelho et al., 1999). Control of other types of soil-inhabiting pests such as nematodes, using soil amendments, have had similar successes and failures (Halbrendt, 1996). Lack of nematode suppression or inconsistencies among research methodologies may be attributed to differences in glucosinolate concentrations of incorporated amendments (Johnson et al., 1992; Mojtahedi, 1991). Lazzeri et al. (1993) reported that the presence of myrosinase and glucosinolates was toxic to *Heterodera schachtii* Schmidt (sugar beet cyst nematode). Green manures of *Brassica* incorporated into soil at the rate of 10-20 g/kg of soil reduced *Meloidogyne javanica* (Treub) Chitwood populations below the controls (McLeod and Steel, 1999). Potter et al. (1998) reported that the degradation product of gluconasturtiin (2-phenylethyl isothiocyanate) was toxic to a root lesion nematode, *Pratylenchus neglectus* (Rensch,) Filipjev and Schuurmans Stekhoven. Zasada and Ferris (2003) indicated that there was a wide range of toxicity among the isothiocyanates and they suggested that slight differences in chemical structure may have profound differences in nematicidal effects. Their research showed that aromatic ITCs were more toxic than aliphatic ITCs to *Tylenchlus semipenetrans* Cobb and *M. javanica*. Their research further showed that *T. semipenetrans* was more sensitive to tested ITCs than was *M. javanica*, which may have been due to the wide host range and distribution of *M. javanica*.

Various experiments have shown that ITCs, especially volatile materials (allyl isothiocyanate) from *B. juncea*, have a potential use in agricultural production systems. With the loss of the commonly used fumigant, methyl bromide, alternatives are being sought. Use of *Brassica* amendments provides an environmentally friendly, sound method of pest control without raising fears of environmental contamination by the public.

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Appendix - II

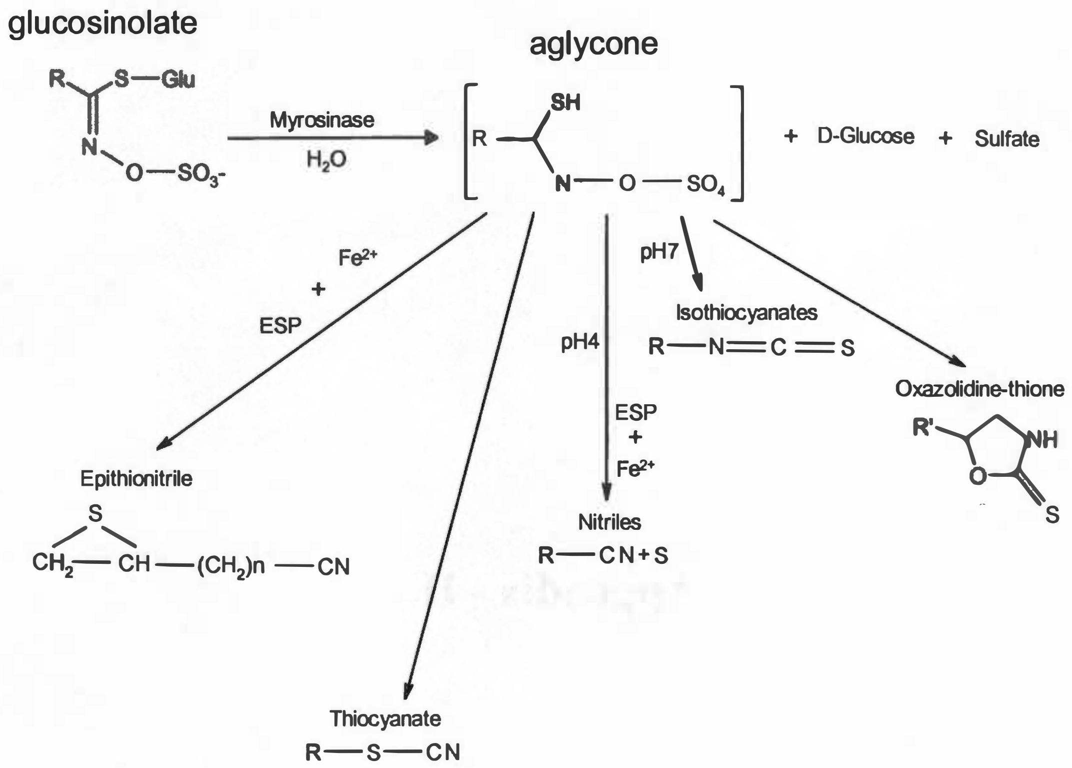

Figure II-1. Degradation pathway of glucosinolates (modified from Brown and Morra, 1997).

Part III

GLUCOSINOLATE AND ALLYL ISOTHIOCYANATE CONTENT OF ORIENTAL MUSTARD MEAL

Abstract

Glucosinolates are secondary plant metabolites found in various species which may produce only one or may contain multiple glucosinolates. When plant tissues are damaged the glucosinolates may come in contact with enzymes known as myrosinases. Reaction of the two materials forms volatile products known as isothiocyanates (ITCs). Isothiocyanates have been reported as being toxic to various organisms. Glucosinolate content of plants may be affected by various factors such as harvest date, season grown, fertility, location, and many other environmental and biological factors. The knowledge of concentration of ITCs produced by plant tissues intended for use in crop production as biofumigants would be beneficial to researchers in determining quantities needed to be applied to fields to obtain efficacious control. Experiments were designed to determine the concentrations of glucosinolates in several lots of whole ground oriental mustard seed meal (*Brassica juncea*) and concentrations of ITC's evolved from these same tissues. Sinigrin concentrations of whole ground oriental mustard seed meal obtained from 12 different lots ranged from 101 to 141 $\mu M \cdot g^{-1}$ of seed meal. Allyl isothiocyanate (AITC) concentrations evolving from corresponding seed lots ranged from 17 to 33 $\mu M \cdot g^{-1}$. Conversion efficiency of sinigrin to AITC from these 12 lots was approximately 23 %.

Introduction

Glucosinolates are secondary plant metabolites found in various plant species. One to several glucosinolates may be found in a given plant species. One of the main glucosinolates found in the genera *Brassica* is sinigrin (2-propenyl-glucosinolate), also called allyl glucosinolate (Sang et al., 1984). Sinigrin has been reported in higher concentrations than any other glucosinolate detected in *B. juncea* and may be the only glucosinolate detected depending on the cultivar tested (Bending and Lincoln, 1999; Kirkegaard and Sarwar, 1998). When plant tissues are damaged and glucosinolates are exposed to the enzyme myrosinase, which occurs within the cell, the glucosinolates are hydrolyzed and form products that are toxic to various organisms. Allyl isothiocyanate (AITC) is a primary product formed from the hydrolysis reaction of sinigrin. AITC is highly volatile, and has been reported to be toxic to insects, fungi, bacteria (Bending and Lincoln, 2000), weeds and in some cases, livestock (Brabban and Edwards, 1994). Kirkegaard and Sarwar (1999) reported that glucosinolate levels varied among several cultivars of *B. juncea*, and ranged from 10 to 21 $\mu M \cdot g^{-1}$ of tissue. Hanley et al. (1983) reported that 100 grams of dried seed of *B. juncea* yielded up to one gram of sinigrin.

Glucosinolate concentrations of plants are affected by several factors such as season planted, harvest date, growing conditions, site (Rosa et al., 1997), and fertility (Chen and Anderasson, 2001). Porter et al. (1991) reported that glucosinolate concentrations of *B. napus* changed with leaf age and peak concentrations appeared approximately 40 days after planting and declined thereafter. There was a wide variability of glucosinolate content within one cultivar of *Brassica* (Yu et al., 2003). However, the variability in glucosinolate content should result in similar variations within evolution of allyl isothiocyanate, if optimum conditions exist. They reported that allyl isothiocyanate represented the greatest percentage (54.8% - 68.8%) of all isothiocyanates contained in oil obtained from *B. juncea*. Rodregues and Rosa (1999) reported that broccoli contained high levels of glucosinolates, but levels declined when heads were stored at room temperature. Increased nitrogen levels lowered glucosinolate levels

(Fenwick et al., 1983) and addition of sulfur to sulfur-deficient soils increased glucosinolate levels (Booth et al., 1991).

Glucosinolates should be converted to ITCs on an equimolar basis (Bending and Lincoln, 1999), but conversion is not 100%. Warton et al. (2001) reported that the molar amount of the ITC produced by hydrolysis was lower than the amount of the corresponding glucosinolate present in plant tissues. Morra and Kirkegaard (2002) assumed a 10% conversion of glucosinolates to ITCs. Actual conversion of glucosinolates to isothiocyanates can range from 10 to 60% (Borek et al., 1997), depending on chemical structure of the glucosinolate and environmental conditions (Brown et al., 1996). Glucosinolate concentrations of plant material intended to be used as a biofumigants is very critical. Higher glucosinolate content should result in a correspondingly higher level of isothiocyanates and more effective biofumigant.

To achieve effective control from the addition of *Brassica* tissues, glucosinolate concentration should be determined prior to field use. Having prior knowledge of glucosinolate content and blending of tissues obtained from different harvest dates and locations may aid in quality control of the product, allowing for consistent levels of glucosinolates to be amended to the soil.

The objective of this experiment was to determine the sinigrin and AITC content of several lots of oriental mustard meal (OMM). The supplier of the mustard meal indicated that the lots had different harvest dates and were harvested from different locations.

Materials and Methods

Source of Oriental Mustard Meal

Mustard meal (whole ground oriental mustard seed/hull, 8% moisture content) was obtained from Poulenger USA, Lakeland Fla. It was shipped in 22.68 kg moisture resistant lined paper bags. The supplier indicated that contents of the bags were prepared from different lots, had varying harvest dates, and were harvested from different locations. Information on planting and harvest dates was not provided by the supplier. Samples were placed into one-liter Nalgene containers (square HDPE bottles, 1 L, 2114-0032, Nalge Nunc International, Rochester, N.Y.) and stored at -40 °C until use in each experiment.

Glucosinolate Analysis of Oriental Mustard Meal

Glucosinolate concentration (sinigrin) was quantified with modified procedures of methods used by the Canadian Grain Commission (McGregor, 1990). Mustard meal (200 mg) was placed into a 10-mL vial. Methanol (2 mL) was added and followed with 1 mL of glucotropaeolin (1 mM) (Bioraf Denmark Foundation, Aakirkeby, Denmark) as an internal standard solution. Glucotropaeolin is a glucosinolate not commonly found in *B. juncea* and therefore served as an independent internal standard (McGregor, 1990). Finally, 0.1 mL of barium lead acetate (0.6 M) was added. Methanol and barium lead acetate are inhibitors of the myrosinase enzyme found in *Brassica* tissue. The mixture was shaken for one hour on a Vortex-Genie 2 shaker (Model #g-560, Scientific Industries Inc., Bohemia, N.Y.) at a setting of 6, followed by centrifugation at 2000 g_n for 10 min. in a Centra-MP4R Centrifuge (International Equipment Company (IEC), Needham

Heights, Mass.) fitted with an IEC 224 swing bucket rotor (IEC). The supernatant was then transferred to a diethylaminoethyl (DEAE) Sephadex A25 (Sigma-Aldrich, St. Louis, Mo.) column, where it was purified and extracted by ion exchange chromatography by washing with 1.8 mL 67% methanol, 1.8 mL deionized water (ddH_2O), 0.9mL pyridine-acetate (0.02 M), and 0.05 mL purified sulfatase. Sulfatase removes the thiol group from the glucosinolate backbone. After 24 hours, the sample was eluted from the column with 0.9 mL ddH_2O. DEAE columns were regenerated for reuse by rinsing with 1 mL sodium acetate (1 M), 1 mL sodium hydroxide (0.5 M), 1 mL hydrochloric acid (0.5 M), and 3.6 mL of nanopure water respectively.

The samples were analyzed by high performance liquid chromatography (HPLC). The HPLC was an HP1050 Series pump with a variable Wavelength 1050 Series Detector (Hewlett Packard, Palo Alto, Calif.), with detection wavelength set to 227 nm. The column was a 250 x 4.60 mm 5 µm C18 Phenomenex ODS Hypersil (Phenomenex Corp., Torrance, Calif.). Mobile phase solvents were Burdick and Jackson HPLC grade water (Honeywell Burdick & Jackson, Muskegon, Mich.) and Fisher HPLC grade acetonitrile (Fisher Scientific, Atlanta, Ga.). During the 30 minute run-time with a solvent flow of 1 mL/min (with a 50 µL sample injection), the solvents progressed in a gradient from 100% HPLC grade water, to 75% HPLC grade water: 25% actetonitrile, and returned back to 100% HPLC grade water.

Identification of desulfonated glucosinolate peaks was elucidated by running the desulfonated glucosinolate standards glucoiberin, progoitrin, epiprogoitrin, sinigrin, sinalbin, gluconapin, gluconasturtiin, and neoglucobrassicin on the HPLC by the above method and comparing retention times of standards to mustard meal samples.

Metabolites of mustard meal samples and internal standards prepared as described above were separated and quantified by UV absorption at 227 nm. Analysis of the sinigrin and internal standard peaks were performed with the Chemstation Software Module, Version 6.03 (Hewlett Packard, Palo Alto, Calif.). Concentrations of sinigrin were compared to levels of glucotropaeolin and calculated with methods described by McGregor (1990).

Sulfatase Preparation

Seventy milligrams of unpurified sulfatase (Sigma-Aldrich) were placed into a centrifuge tube. Three mL of nanopure water were added in order to dissolve the sulfatase, followed by the addition of 3 mL of absolute ethanol. The mixture was centrifuged for 20 minutes at 2000 g_n on a Centra-MP4R Centrifuge with an IEC 224 swing bucket rotor (IEC). The supernatant was decanted into a second vial, the precipitate was discarded, and 9 mL of absolute ethanol was added to the supernatant. The solution was centrifuged for 15 minutes at 2000 g_n on the Centra-MP4R Centrifuge using an IE 224 swing bucket rotor (IEC). The supernatant liquid was discarded and the remaining precipitate was dissolved into 2.5 mL dd-H_2O. This final step provided the purified sulfatase, which was then frozen at -40 °C until use.

Statistical Analysis of Glucosinolate Concentrations

Statistical analysis of data was performed with SAS system for Windows, version 9.00. Proc Mixed Models were used and the statistical analysis was a completely random design. All data fell into a normal distribution range and all were tested with a criterion of $\alpha = 0.05$. Means separation for lots was generated with PROC MIXED (SAS Institute, Cary, N.C., 2002) using the PDMIX800 macro (Saxton, 2004) are listed in Table III-1[1], pg. 54.

Analysis of AITC Evolution from Oriental Mustard Meal

A wide-mouth, threaded, glass Mason jar with screw-on lid and retaining ring (Alltrista Corp., Ball/Kerr, Broomfield, Colo.) was used to determine the concentration of AITC. The volume of the jars was determined to be 490-mL. Each glass jar was capped with a 0.79 mm-thick Teflon lid. A threaded retaining ring was screwed down to hold the Teflon lid in place. Previously made stock solutions of AITC were dispensed into the glass jars with a 10-μL syringe (Hamilton 1701 series point style no. 2, fixed-needle, no. 80075, Hamilton, Reno, Nev.) through a 3.175-mm-diameter hole that was previously made in the Teflon lid. Labeling tape (Fisherbrand Colored Label Tape 19mm width, cat no. 11-880-5C, Fisher Scientific, Pittsburgh, Pa.) was immediately placed over hole. The tape was pierced using a Hamilton 26s needle (7748-19, Hamilton). This procedure was done to keep tape adhesive off of the sampling syringe that was later used for headspace sampling. This method helped minimize clogging of the Merlin Microseal septum (Agilent Technologies, Santa Clara, Calif., part no. 5182-3442). The hole was immediately covered with another piece of Fisher labeling tape to minimize the escape of gas. Headspace samples were taken with a Hamilton Gastight series 1705, 50-μL beveled HP style 2 tip syringe (#80930, Hamilton). Head space sampling was conducted at timed increments after a stock solution was injected into the glass jar. Allyl isothiocyanate

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[1] Figures and tables are included in the Appendix.

mixtures were injected into jars at timed intervals so evolution time would be identical for each jar. Jars were incubated at 22 ± 2 °C before sampling.

The syringe was placed into the injection port of a Hewlett Packard 5890a Series II (Hewlett Packard Company, Palo Alto, Calif.) gas chromatograph (GC-FID). The inlet port was fitted with a Merlin Microseal septum (Merlin Instrument Company, Halfmoon, Calif., kit no. 304, septum no. 2218) for manual injections. The column was a 60 m x 0.53 mm, with a 0.25 μ film (J&W Scientific, Folsom, Calif., DB1301, part no. 125-1361). The inlet and outlet temperatures were 200 and 250 °C, respectively. The oven parameters were programmed for 60°C for 1 min., and then increased by 5 °C per minute to a maximum of 150 °C. Detector response was quantified based on the equation for the AITC standard curve. Analysis of the allyl isothiocyanate peaks were performed with Chemstation Software Module, Version 6.03 (Hewlett Packard, Palo Alto, Calif.).

Statistical Analysis

This experiment was a completely randomized design repeated four times with six concentrations of AITC and two repeated hourly measurements that were averaged together. Allyl isothiocyanate concentration standards ranged from 0.5 to 33.33 $\mu M \cdot L^{-1}$. Commercially available allyl isothiocyanate (AITC, 95% purity) – C_4H_5NS (377430-100G, Sigma-Aldrich Corp., St. Louis, Mo.) was mixed in 95% HPLC grade hexane (439177, Sigma-Aldrich Corp., St. Louis, Mo.) to prepare stock solutions. Serial dilutions were prepared by mixing AITC with hexane to form various concentrations to obtain data points. Results from PC-SAS linear regression analysis (SAS, ver 9.0, 2002, Cary, N.C.) of AITC was used to provide a predicted AITC level of mustard meal

(R^2=0.99). The equation [$y = 1083.16 - (-260.09) / x$] was used to determine the μM concentration of one gram of OMM (Fig. III-1, pg. 55), where y = HPGC counts and x = AITC concentration. Samples were analyzed using PROC MIXED (PC SAS, Widows ver.9.0., Cary, N.C.) and means were generated using PDMIX800 macro (Saxton, 2003).

Preparing a Standard Curve using AITC

Commercially available allyl isothiocyanate (AITC, 95% purity) – C_4H_5NS (377430-100G, Sigma-Aldrich) was mixed in 95% HPLC grade hexane (439177, Sigma-Aldrich) to prepare stock solutions. Serial dilutions were prepared by mixing AITC with hexane to form various concentrations to obtain a standard curve. Dilutions of AITC ranged from 0.05 $\mu M \cdot L^{-1}$ to 33.33 $\mu M \cdot L^{-1}$ and were replicated four times. Peak measurements taken at hours one and two were averaged. Linear regression was used to produce the equations for quantifying AITC production and for describing the relationship between AITC release and OMM. A standard curve was developed with serial dilutions of AITC and hexane. The various concentrations of AITC were prepared and injected into 490-mL jars. During this time peak area readings were taken from the GC-FID.

Statistical Analysis and Linear Regression of Commercial AITC

The linear regression equation ($y = mx+b$) for the commercially available AITC was determined to be x = GC reading peak area - (-260.09) / 1083.16. Peak GC readings obtained from the OMM were placed into the formula to determine the predicted concentrations of AITC evolving from the jars. A prediction equation for AITC levels is shown (Fig. III-1, pg. 55).

Results and Discussion

In developing economically feasible control alternatives, it is essential that alternative materials intended for use provide reliable, predictable results. Data from this experiment showed that AITC levels followed a similar trend as that of glucosinolate levels. The AITC analysis did not measure total AITC production, but measured the relative concentration at the time of sampling. In preliminary testing, peaks of AITC were observed at one and two hours after introduction of AITC or mixing of dry meal with water. Quantification of AITC was determined by averaging peaks measured in containers at one hour after introduction into the container and again at hour two. To determine ability of fumigant to kill a targeted pest, most fumigants are measured by concentration over time. This experiment was conducted in a similar manner, where AITC concentrations were measured for a given time.

The glucosinolate levels of bags ranged from 101.44 to 129.30 $\mu M \cdot g^{-1}$ of mustard meal. Allyl isothiocyanate levels of the mustard meal ranged from 17.14 to 33.63 $\mu M \cdot L^{-1}$. Bags containing high levels of sinigrin also contained high levels of AITC, following similar tends of the sinigrin samples. However, this experiment did not provide results indicating an equilmolar conversion. The method used to detect AITC detects relative concentrations of AITC at timed intervals and does not measure total concentrations. Thus may account for the lower rate of AITC measured in the containers. The concentrations of sinigrin and AITC obtained from testing several bags of oriental mustard meal are shown in Table III-1, pg. 54.

There are several factors that may have affected the conversion of sinigrin to AITC. Variations of AITC concentration may be due to sorption processes within the meal due to the presence of proteins or sorption to glass jars and/or Teflon surfaces. Also, possible losses due to permeation at the sampling site or through the Teflon seal may have occurred reducing the total AITC concentration.

Other factors that may have influenced AITC concentrations include incomplete hydrolysis, possible decomposition of allyl isothiocyanate, formation of non-AITC hydrolysis products and/or activity of proteins or amino acids present within the tissues (Warton et al., 2001). Also, if tissues of the mustard meal are not ground thoroughly, water may not have been able to penetrate the tissues to react with the glucosinolate and myrosinase to form AITC. The decomposition of glucosinolates in mustard was improved by using a cellulolytic pretreatment (Szakacs-Dobozi et al., 1988). This method allowed glucosinolates to come in contact with myrosinases, therefore increasing release of AITC from samples. The efficiency of the conversion that occurred when testing of glucosinolates and AITC is provided in Table III-1, pg. 54. The mean conversion or efficiency rate obtained using this method of analysis was approximately 23.11%.

A 15% conversion rate was reported when testing *Brassica* tissues (Borek et al., 1997). Bending and Lincoln (1999) suggested that AITC evolving from *B. juncea* leaf tissue added to soil produced less than 0.1% of that which could potentially have been produced from glucosinolate hydrolysis. Mixed results may have been due to methods selected by other researchers which varied slightly from methods used in AITC analysis reported within this document; however, glucosinolate levels reported from rapeseed meal (*Brassica napus*) tested by Borek et al., (1997) were similar to singrin levels measured in meal of *Brassica juncea* lots tested in this research. The measured soil ITC concentration release efficiency was reported by Morra and Kirkegaard (2002) at 1% when entire plant portions of *Brassica napus* were added to the soil. Differences observed in total measured ITC levels measured by other researchers may have been due to effects from sorption or decomposition of glucosinolates or ITCs into other compounds.

This research demonstrates that the procedures selected for glucosinolate and AITC analysis resulted in a better conversion than previously reported research. Thus the GC-FID procedure may have had a greater sensitivity or less loss of AITC occurred when sinigrin degraded into AITC in these experiments. High levels of glucosinolates present in tested OMM may provide adequate levels of AITC that may control various soilborne pests. Thus with more accurate information obtained from these analytical methods it may be possible to better predict the precise amount of tissue needed to provide adequate control of targeted pest. Therefore reducing input cost of materials containing AITC which are used for control.

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Appendix - III

Table III-1. Mean and standard error of sinigrin and allyl isothiocyanate content of whole ground oriental mustard meal (*Brassica juncea*) lots.

Lot no.	Sinigrin (GS)[z] ($\mu M \cdot g^{-1}$)		Allyl isothiocyanate (AITC) ($\mu M \cdot g^{-1}$)		% Conversion efficiency
1	129.31 ± 1.43	ab[y]	32.28 ± 1.35	bc	24.96
2	126.85 ± 11.79	b	30.20 ± 1.83	bcd	23.80
3	112.68 ± 3.08	cd	25.64 ± 1.16	def	22.75
4	111.23 ± 4.77	cd	25.40 ± 1.52	ef	22.83
5	141.10 ± 2.73	a	34.30 ± 1.08	b	24.30
6	109.00 ± 2.22	cd	21.30 ± 1.98	fg	19.54
7	141.70 ± 4.80	a	40.14 ± 2.62	a	28.32
8	118.97 ± 4.74	bc	27.98 ± 2.19	cde	23.51
9	119.62 ± 4.40	bc	28.04 ± 1.48	cde	23.44
10	118.62 ± 5.83	bc	26.26 ± 1.47	de	22.13
11	101.45 ± 0.61	d	20.28 ± 0.89	g	19.99
12	109.56 ± 2.73	cd	23.94 ± 1.40	efg	21.85

[z] Sinigrin and AITC concentration samples obtained from 12 lots of whole ground oriental mustard meal (0.5 g) subsamples. Analysis of variance indicated that lot means of GS and AITC differed at *P* □0.0001. Means are of three replicates of GS and four replicates of AITC.

[y] Mean separation was conducted using Proc Mixed and mean separation using PDMIX800 macro (Saxton, 2003). Means with different letters are significantly different at *P* □0.05.

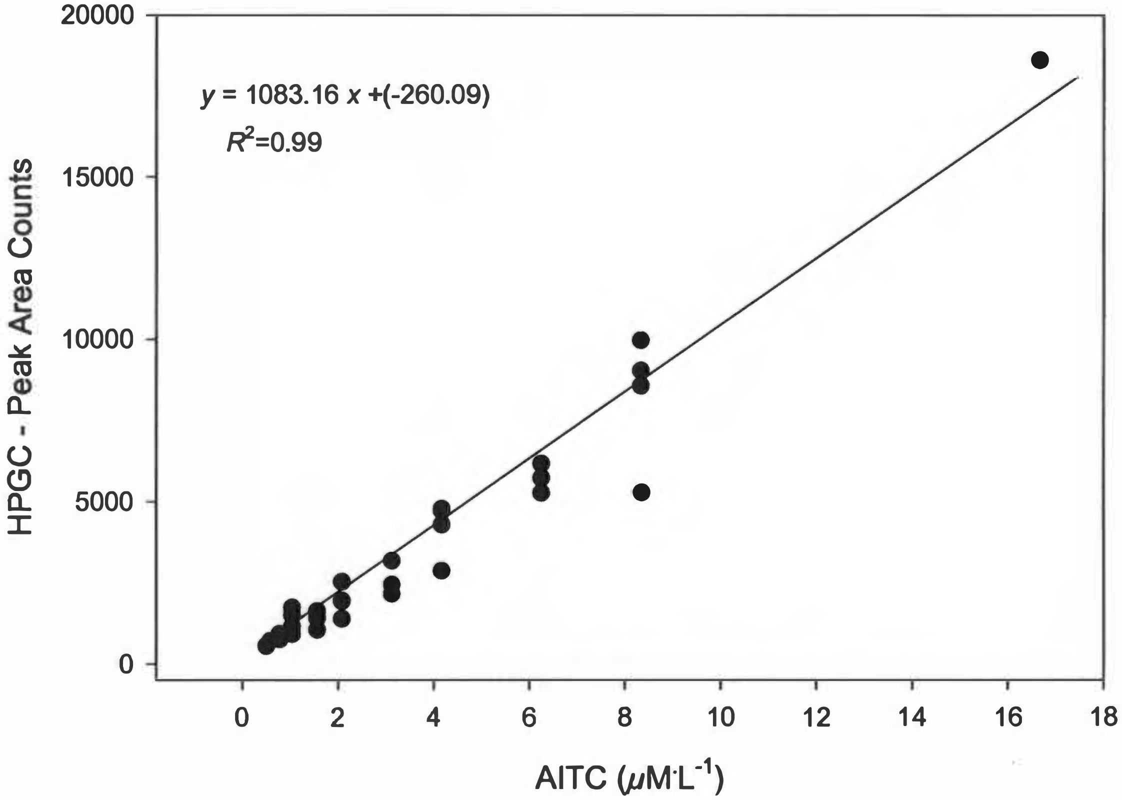

Figure III-1. Linear regression of concentrations of commercially available allyl isothiocyanate (AITC) quantified from head space-volume against peak area counts obtained from a Hewlett Packard high performance gas chromatograph with a flame ionization detector (HPGC-FID).

Part IV

EFFECT OF ALLYL ISOTHIOCYANATE ON *PHYTOPHTHORA*

Abstract

Phytophthora parasitica var. *nicotianae* is a pathogen of tobacco causing a disease commonly known as black shank. *Brassica* tissues form hydrolysis products from biochemicals found within the cells known as glucosinolates. Many glucosinolates have been reported to occur within various plant species, but they most commonly occur in the Brassicaceae. One primary glucosinolate found in *Brassica* is sinigrin. Allyl isothiocyanate (AITC) is a by-product of sinigrin that is released by many *Brassica* spp. when plant residues are tilled into the soil. Hydrolysis compounds from decomposition of whole ground oriental mustard seed meal (OMM) have the potential to reduce populations of soilborne diseases that occur in tobacco fields in Tennessee. Results from experiments included within this document have demonstrated that greater concentrations of ITC evolving from OMM are required to provide control similar to commercially available AITC. The percent inhibition or inhibition concentrations (IC_{50}s and IC_{90}s) of volatiles from OMM and commercially available AITC were determined. The IC_{50} of mustard meal was 0.99 $\mu M \cdot L^{-1}$ and that of AITC was 0.70 $\mu M \cdot L^{-1}$. The IC_{90} of mustard meal and allyl isothiocyanate were 1.72 $\mu M \cdot L^{-1}$ and 1.52 $\mu M \cdot L^{-1}$, respectively.

Introduction

Phytophthora parasitica Dastur var. *nicotianae* (Breda de Haan) Tucker causes black shank of tobacco (*Nicotiana tabacum* L.) (MacKenzie et al., 1983). Black shank has been managed in the field with the soil fumigant methyl bromide. Alternative pest control techniques and/or chemicals must be identified because of the environmental problems associated with methyl bromide (Thomas, 1996) and impending loss of methyl bromide for crop protection in the United States (U.S. EPA, 1993). *Brassica* tissues form hydrolysis products from glucosinolates within the cells. Sinigrin is one primary glucosinolate found in *Brassica.* Allyl isothiocyanate (AITC), derived from sinigrin, is released by decomposition of *Brassica* when plant residues are amended to soil. Allyl isothiocyanate has the potential to limit many types of pathogens and possibly control soilborne diseases (Rosa et al., 1997). Thus, AITC may reduce occurrence of black shank in tobacco.

Suppression of several soilborne fungi by propenyl ITC and 2-phenylethyl ITC was superior to that of the synthetic fumigant methyl-ITC (metham sodium) (Sarwar et al., 1998). This research suggested that by-products of *Brassicas* could play an important role in suppression of plant pathogens. However, fungi differ in methods of survival and growth. Some fungi produce spores that are resistant to extreme temperatures or other harsh environmental factors while others may not survive similar conditions.

Since fungi have different strategies of survival and growth, as well as cell walls of varying composition, their ability to survive in harsh environments may differ. Higher concentrations of active materials may be required to inhibit or reduce growth of the targeted pest depending upon its growth stage. Brown and Morra (1997) reported that fungicidal concentrations of ITCs differed by an order of magnitude among different fungal species. Thus, it is imperative to determine the minimum concentration of AITC needed to control the targeted organism. Information obtained through laboratory or greenhouse experiments can be utilized in determining concentrations that are needed in field studies to obtain adequate control of all forms present within the soil.

Materials and Methods

Phytophthora parasitica var. *nicotianae* causes a disease of tobacco commonly known as black shank of tobacco. The objective of this experiment was to determine the effect of AITC on *Phytophthora parasitica* var. *nicotianae* by the addition of commercially available allyl isothiocyanate and whole ground oriental mustard meal (*Brassica juncea*).

Phytophthora parasitica var. *nicotianae* was isolated from infected tobacco obtained from the Tobacco Experiment Station, Greeneville, Tennessee. Infected tissue was placed in Petri dishes containing approximately 10 mL of Difco (Midland, Mich.) corn meal agar (CMA) amended with benomyl (25 ppm), ampicillin (100 ppm), and pentachloronitrobenzene (100 ppm). Petri dishes were incubated overnight at room temperature. Extended hyphal tips from small portions of infected tissue were removed and transferred to new dishes of CMA. The culture was allowed to grow for several days and a 4.8 mm diameter brass cork borer (S50166C, Fisher Scientific, Pittsburgh, Pa.) was used to cut CMA plugs which were then transferred to five, 10.16-cm sterilized clay pots containing sterilized media, composed of soil and sand. Clay pots and media were sterilized in an autoclave at 120 °C and 1.4 kg / cm^2 for two hours. The five clay pots containing one, 2-month old plant of a black shank-susceptible tobacco cultivar, R403. Plants were grown in the greenhouse for approximately five weeks until discoloration of the stem was observed. Discolored tissue was microscopically examined for hyphae and determined to have characteristics of *Phytophthora*. The isolate was transferred to another greenhouse-grown plant to repeat the process. The *Phytophthora* isolate was maintained throughout the experiment on oat meal agar (25 grams rolled oats, 12 grams agar, 1 L deionized water).

Commercially available allyl isothiocyanate (AITC, 95% purity) – C_4H_5NS (377430-100G, Sigma-Aldrich Corp., St. Louis, Mo.) was mixed in 95% HPLC grade hexane (439177, Sigma-Aldrich., St. Louis, Mo.) to prepare stock solutions. Serial dilutions were prepared by mixing AITC with hexane to form various concentrations to obtain a linear equation. Dilutions of AITC ranged from 0.05 $\mu M \cdot L^{-1}$ to 33.33 $\mu M \cdot L^{-1}$ with four replications and peak measurements taken on hours one and two were averaged. A linear equation was developed using serial dilutions of AITC and hexane (Fig. IV-1, pg. 72)[1]. The various concentrations of AITC were prepared and injected into 490-mL jars. During this time peak area readings were taken from a Hewlett Packard high performance gas chromatograph with a flame ionization detector (HPGC-FID) (Hewlett Packard, model 5890A, Series II, Palo Alto, Calif.).

Linear regression (Fig. IV-1, pg. 72) was used to estimate AITC concentrations ($y = m * 910.38 + 121.45$) evolving from treatments. The value for "y" was equal to the peak reading obtained from the HPGC-FID.

A wide-mouth, threaded, glass Mason jar with screw-on lid and retaining ring (Alltrista Corp., Ball/Kerr, Broomfield, Colo.) was used to determine concentration of AITC. A 6.35-mm diameter hole was drilled on one side of the jar approximately 38.1 mm from the top edge. The volume of the pint jars were determined to be 490-mL. Each glass jar was capped with 0.79 mm thick Teflon lid. A threaded retaining ring was screwed down to hold the Teflon lid in place. Previously made stock solutions of AITC

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[1] Figures and tables are located in the Appendix.

were used as treatments and dispensed into the glass jars using a Hamilton 1701 series point style no. 2, fixed-needle, 10-μL syringe (no. 80075, Hamilton, Reno, Nev.). Treatments containing mustard meal mixed with deionized water (dd-H_2O) on a w/v basis were added to the glass jars. Labeling tape (Fisherbrand Colored Label Tape 19mm width, cat no. 11-880-5C, Fisher Scientific, Pittsburgh, Pa.) was immediately placed over holes in the side of the jar. The tape on the side of the jar was pierced using a Hamilton 26s needle (7748-19, Hamilton). This procedure was done to keep tape adhesive off of the sampling syringe that was later used for headspace sampling. This method helped minimize clogging of the Merlin Microseal septum (Agilent Technologies, Santa Clara, Calif., part no. 5182-3442). The hole was immediately covered with another piece of Fisher labeling tape to reduce the escape of gas. Headspace samples were taken using a Hamilton Gastight series 1705, 50-μL beveled HP style 2 tip syringe (#80930, Hamilton). Head space sampling was conducted at timed increments after AITC stock solution or mustard meal was added to the glass jar. Jars were incubated at 22 °C ± 2, prior to sampling.

Since mustard meal samples varied slightly in the amount of AITC that evolved from each sample, samples were added to jars containing various meal weights and peak readings from the GC-FID were used to calculate the AITC concentration. Dry weight of meal used for this experiment ranged from 0.01 grams to 0.08 grams of tissue. Mustard meal treatments were weighed and placed into 5-mL polystyrene beakers. DD-H_2O was added to each beaker containing meal such that a ratio of 1:1 w/v was prepared.

Dilutions of commercially available AITC were used to compare their affects with those of mustard meal. A 10-µl syringe (no. 701 Hamilton) was used to place AITC into jars and the injection site was immediately covered with adhesive tape and then punctured and re-covered with an additional piece of tape.

Headspace samples were drawn with a syringe as mentioned above and placed into the injection port of a Hewlett Packard 5890a Series II (Hewlett Packard, Palo Alto, Calif.) gas chromatograph (GC-FID). The inlet port was fitted with a Merlin Microseal septum (Merlin Instrument Company, Halfmoon, Calif., kit no. 304, septum no. 2218) for manual injections. The capillary column was a 60 m x 0.53 mm with a 0.25 μ film (J&W Scientific, Folsom, Calif., DB1301, cat. no. 125-1361). The inlet and outlet temperatures were 200 and 250 °C, respectively. The oven parameters were programmed for 60 °C for one minute, and then increased by 5 °C per minute to a maximum of 150 °C. Detector response was quantified based on the equation obtained from the AITC regression equation. Analysis of the allyl isothiocyanate peaks were performed using Chemstation Software Module, Version 6.03 (Hewlett Packard, Palo Alto, Calif.).

A 1000-mL Erlenmeyer flask containing 750 ml deionized H_2O (dd-H_20) with 15 grams of corn meal agar were placed into a Steromaster autoclave (Consolidated, Boston, Mass.) at 120 °C at 1.4 kg / cm^2 for 30 minutes. Prepared medium was allowed to cool (50 °C) and 20 mL was poured into sterilized petri dishes (95 x 15 mm, Fisher brand Media-Miser dishes, Pittsburgh, Pa.). After the agar had solidified, an agar plug (5 mm diameter) containing fungal growth was placed onto the center of recently prepared agar dish. Petri dishes were inoculated with *P. parasitica* and incubated for 15 hours at 20 °C to allow the fungi to grow onto the agar surface. Polystyrene 5-mL Micro-beakers (2-544-30, Fisher Scientific, Pittsburgh, Pa.) containing OMM with dd-H_2O or containing 0.5 mL dd-H_2O were lowered into all jars containing OMM treatments.

Petri dishes containing CMA and colonies of *P. parasitica* were inverted onto the top of the glass jars. Parafilm was cut into sections 153 mm x 12.7 mm wide (Parafilm M, American National Can, Chicago, Ill.) and wrapped around the edge of the inverted petri disk and jar to minimize seepage of volatile gases.

Treatments were applied and holes were immediately covered with a 13-mm wide piece of adhesive tape (Fisher brand, Colored Label Tapes, 11-880A, Pittsburgh, Pa.) after the addition of treatments. The tape was punctured with a 22s needle (Agilent Technologies, Cary, N.C.). The tape was then covered with an additional piece of tape to eliminate escape of gasses from the jar. Headspace atmosphere was sampled each hour for two hours. The GC-FID peak readings were taken on one and two hours were averaged for each treatment for further statistical analysis. Petri dishes remained inverted on jars for seven days after inoculation and measurements were made of hyphal growth on the fifth day. Two perpendicular measurements of the diameter growth were averaged and adhesive tape was removed from the holes. Dishes with no growth were inspected again at the seventh day to confirm the lack of growth.

The radial growth of *Phytophthora* or percent inhibition was measured using a formula similar to one used by Mari et al., 1993: (Ø control – Ø treatment)/ Ø control x 100 = IC, where IC = inhibition concentration and Ø = growth.

Statistical Analysis

Probit analysis is a type of regression analysis used when the success/trial at any X is actually the discretely observed outcome of a continuous underlying normal distribution of probabilities. The cumulative normal distribution function (the "probit") is used in these cases. The running model, generating estimated probits (which are actually z scores), and estimated probabilities and confidence intervals are modifications of the PROC LOGIT (SAS Institute, Cary, N.C., Windows ver. 9.0). The effective concentration needed to achieve the IC_{50} and IC_{90} were analyzed using PROC PROBIT (SAS Institute, Cary, N.C., Windows Ver. 9.0) (Table IV-1 and IV-2, pg. 74 and 75).

The experiments were analyzed as completely randomized designs. The experiment using allyl isothiocyanate was repeated nine times. The experiment using oriental mustard meal was repeated six times. A test for normality was run on all data and the data within data sets were normally distributed. Analysis was performed using Proc Probit to obtain an IC_{50} and IC_{90} for the two materials (Fig. IV-2, pg. 73).

Results and Discussion

Preliminary testing (data not shown) suggested that AITC levels peaked within one to two hours after addition of mustard meal and/or allyl isothiocyanate into the jars. Therefore, two hours of data were averaged for these two experiments to provide an estimate of peak value or AITC concentration. In preliminary experiments, AITC concentrations from both meal and commercially available AITC began to decline after four to five hours after placement into jars. It is difficult to measure the exact ITC concentration to which the fungus or other organisms were exposed in the headspace experiments and thus, direct comparisons were obtained using averages of peak sampling from jar headspace tests. Results from these experiments indicated that meal treatments required slightly higher AITC peaks than commercially obtained AITC to provide similar control.

The predicted lethal concentrations of AITC on *P. parasitica* are listed in Fig. IV-2, pg. 73. The IC_{50} of mustard meal was 0.99 $\mu M \cdot L^{-1}$ and that of AITC was 0.70 $\mu M \cdot L^{-1}$. The IC_{90} of mustard meal and allyl isothiocyanate were 1.72 $\mu M \cdot L^{-1}$ and 1.52 $\mu M \cdot L^{-1}$, respectively. Thus, the commercial AITC may provide more effective control against the pathogen than mustard meal at lower concentrations. The fiducial limits of mustard meal and allyl isothiocyanate are listed in Table IV-1 and IV-2, pg. 74 and 75. The fiducial limits are confidence intervals or inverse confidence limits, which are calculated using the effective dose or inhibition concentration (IC). The AITC treatments were repeated nine times and the mustard meal experiment was repeated six times. Several factors may have affected levels of inhibition. There may have been adsorption affects by proteins and amino acids contained within the mustard meal as suggested by Kawakishi and Kaneko (1987). They indicated that regardless of pH, the major product of AITC decomposition in buffered aqueous media is allylamine (AA) which may not effect growth of *Phytophthora* as significantly as AITC. Samples containing meal were mixed in water whereas commercial AITC only contained hexane. The commercial AITC was diluted with hexane, which may have not affected the decomposition of AITC. Thus the AITC evolving from meal may have been decomposed or may have been bound to proteins present within the tissue. If this was occurring within the test, AITC levels would probability have been reduced. Mari et al. (2002) reported similar results, in which AITC concentration evolving from defatted meal was less effective than similar

AITC concentration evolving from commercially obtained sinigrin. In another experiment, inhibition of mycelial growth of *Sclerotium rolfsii* by AITC alone required a higher IC than equivalent AITC concentration derived from *Brassica juncea* (Harvey et al., 2002).

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Appendix - IV

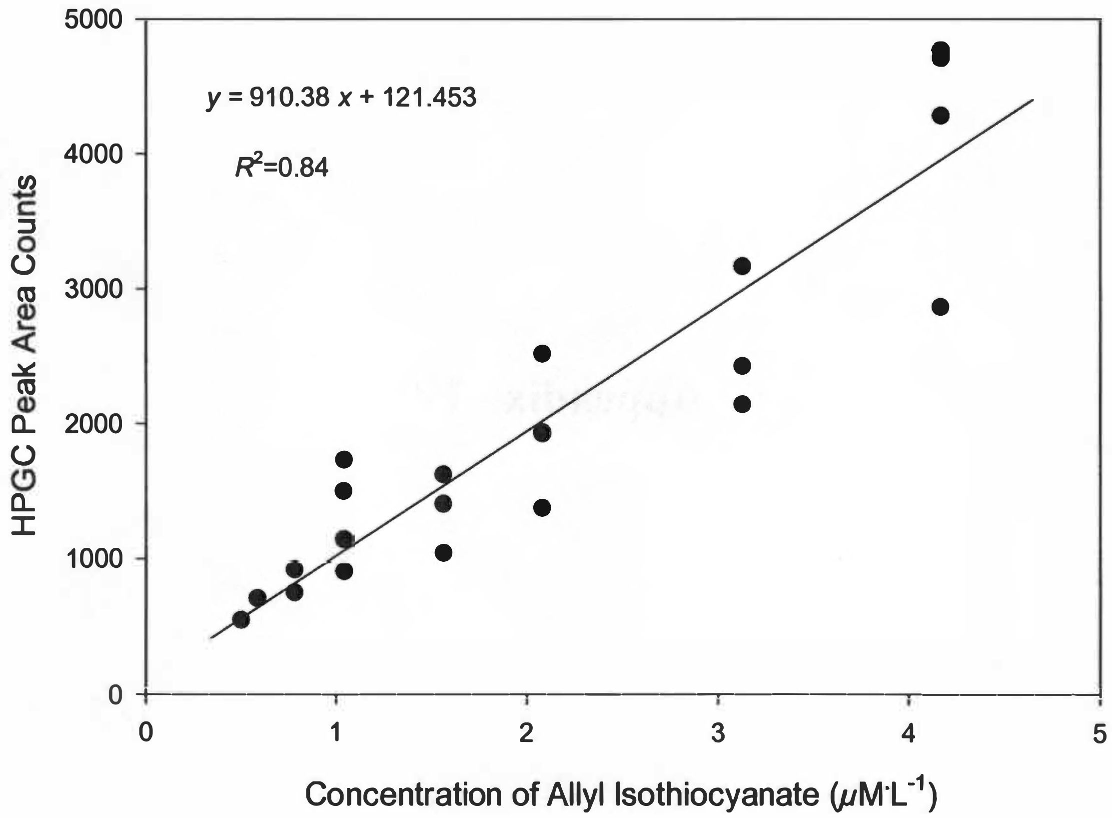

Figure IV-1. Linear regression of the concentration of commercially available allyl isothiocyanate quantified from the head space-volume against peak area counts obtained from a Hewlett Packard high performance gas chromatograph with a flame ionization detector (HPGC-FID).

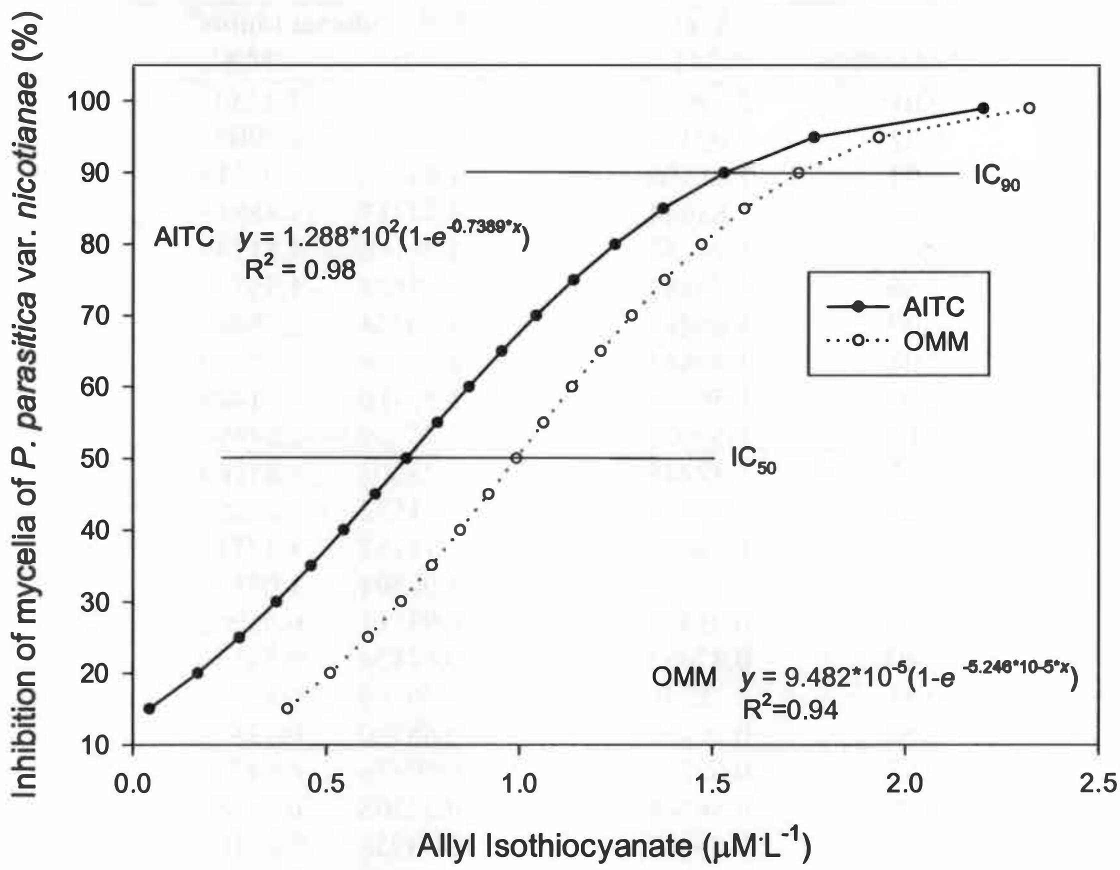

Figure IV-2. Inhibition of mycelial growth of *Phytophthora parasitica* var. *nicotianae* by known concentrations of allyl isothiocyanate (AITC) from commercially available AITC and whole ground oriental mustard meal (OMM). The inhibition concentrations IC_{50} and IC_{90} (concentrations resulting in 50% and 90% inhibition, respectively) were calculated from the equations produced by nonlinear regression. Means for data are based on $n = 82$ for AITC, $n = 55$ for OMM.

Table IV-1. Fiducial limits of AITC on *Phytophthora parasitica* var. *nicotianae.*

	AITC	95% Fiducial Limits[z]	
Probability[y]	($\mu M \cdot L^{-1}$)	MIN	MAX
0.01	2.19955	2.13189	2.27303
0.02	2.02476	1.96395	2.09074
0.03	1.91386	1.85735	1.97513
0.04	1.83044	1.77713	1.88819
0.05	1.76257	1.71186	1.81749
0.06	1.70481	1.65629	1.75733
0.07	1.65417	1.60754	1.70460
0.08	1.60883	1.56388	1.65740
0.09	1.56759	1.52416	1.61448
0.10	1.52962	1.48759	1.57499
0.15	1.37245	1.33602	1.41164
0.20	1.24754	1.21532	1.28205
0.25	1.14037	1.11152	1.17112
0.30	1.04413	1.01804	1.07177
0.35	0.95496	0.93111	0.98002
0.40	0.87033	0.84824	0.89333
0.45	0.78846	0.76764	0.80989
0.50	0.70789	0.68780	0.72828
0.55	0.62731	0.60739	0.64724
0.60	0.54544	0.52508	0.56551
0.65	0.46082	0.43938	0.48165
0.70	0.37164	0.34846	0.39388
0.75	0.27540	0.24980	0.29971
0.80	0.16823	0.13941	0.19537
0.85	0.04332	0.01027	0.07422

[z] Probit Analysis on average concentration ($\mu M \cdot L^{-I}$) produced by commercially available allyl isothiocyanate (AITC) to inhibit mycelial growth of Phytophthora.

[y] The probability, is the percent mycelial growth when using the stated concentration of AITC. The fiducial limits are confidence intervals of the inhibition concentration (IC). The IC_{90} of AITC for *P. parasitica* var. *nicotianae* is 1.52 $\mu M \cdot L^{-1}$. Output produced using SAS Proc Probit (SAS, ver.9.0, Windows PC).

Table IV-2. Fiducial limits of AITC from mustard meal on *Phytophthora parasitica* var. *nicotianae.*

	AITC	95% Fiducial Limits[z]	
Probability[y]	(μM·L^{-1})	MIN	MAX
0.01	2.31878	2.24877	2.39565
0.02	2.16339	2.10023	2.23265
0.03	2.06480	2.00594	2.12929
0.04	1.99063	1.93497	2.05157
0.05	1.93030	1.87721	1.98837
0.06	1.87895	1.82803	1.93460
0.07	1.83393	1.78490	1.88747
0.08	1.79362	1.74626	1.84529
0.09	1.75695	1.71110	1.80695
0.10	1.72320	1.67872	1.77166
0.15	1.58348	1.54452	1.62574
0.20	1.47242	1.43760	1.51002
0.25	1.37715	1.34561	1.41100
0.30	1.29159	1.26273	1.32235
0.35	1.21231	1.18562	1.24052
0.40	1.13708	1.11210	1.16321
0.45	1.06430	1.04057	1.08882
0.50	0.99266	0.96971	1.01607
0.55	0.92103	0.89834	0.94383
0.60	0.84824	0.82526	0.87098
0.65	0.77301	0.74916	0.79626
0.70	0.69373	0.66837	0.71810
0.75	0.60818	0.58062	0.63433
0.80	0.51290	0.48235	0.54159
0.85	0.40185	0.36726	0.43404
0.90	0.26212	0.22188	0.29929
0.91	0.22838	0.18669	0.26682
0.92	0.19171	0.14844	0.23157
0.93	0.15140	0.10636	0.19283
0.94	0.10638	0.05933	0.14960
0.95	0.05503	0.00565	0.10032

[z] Probit Analysis on average concentration (μM·L^{-1}) of allyl isothiocyanate (AITC) produced from mustard meal to inhibit mycelial growth of Phytophthora.

[y] The probability, is the percent mycelial growth when using the stated concentration of AITC from mustard meal. The fiducial limits are confidence intervals of the inhibition concentration (IC). The IC$_{90}$ of AITC from mustard meal for *P. parasitica* var. *nicotianae* is 1.72 μM·L^{-1}. Output produced using SAS Proc Probit (SAS, ver.9.0, Windows PC).

Part V

Effect of Allyl Isothiocyanate on *Meloidogyne incognita*

Abstract

Root-knot nematodes, *Meloidogyne* spp., are economically important plant pathogens that are often managed by cultural practices, synthetic nematicides, soil fumigants, and resistant cultivars. Each method of control has its advantages and disadvantages. The cultural practice of rotation is not always feasible for small farms due to limited space. Synthetic nematicides may result in harmful effects on non-target organisms, residue build up, and/or contamination of ground water or run off. Methyl bromide fumigation is one of the more widely used and effective soil treatments for the management of root-knot nematodes. However, the use of methyl bromide is being restricted due to environmental and human health concerns. Prolonged use of resistant crop cultivars often results in nematode populations that eventually overcome resistance by forming new races. Naturally occurring products are generally perceived as environmentally friendly and should be sought to evaluate their efficacy to develop methods of effective control without contaminating the environment with synthetic materials.

In greenhouse studies, the lethal concentrations (LCs) of commercially available allyl isothiocyanate (AITC), whole ground oriental mustard meal (OMM) and OMM mixed with soil were determined for root knot nematode, *Meloidogyne incognita* (RKN) eggs. The LC$_{50}$ and LC$_{90}$ for AITC were 0.78 and 1.94 $\mu M{\cdot}L^{-1}$ respectively. The LC$_{50}$ and LC$_{90}$ for OMM alone were 0.44 and 1.22 $\mu M{\cdot}L^{-1}$ and for OMM mixed with soil at 33 Pa they were 0.88 and 1.99 $\mu M{\cdot}L^{-1}$, respectively. The use of *Brassica* amendments as biofumigants may be a solution to the problem of reducing pest populations. Data from

experiments conducted in this research indicated that volatile products produced from commercially obtained AITC and by-products of *Brassica* tissues can affect the viability of nematode eggs.

Introduction

The use of organic amendments for reducing phytoparasitic nematode populations has resulted in failures as well as successes (Halbrendt, 1996). Successful management of soilborne pests requires knowledge of available materials and the mode of action of the materials. Glucosinolate profiles differ among plant species and their isothiocyanate derivatives and other compounds derived from decomposition differ in toxicity to bacteria, fungi, insects (Williams et al., 1993) and nematodes (Zasada and Ferris, 2003). Lack of nematode suppression or inconsistencies among research results may be attributed to differences in glucosinolate concentrations of incorporated amendments (Johnson et al., 1992; Mojtahedi, 1991). The addition of fresh *Brassica napus* did not affect the population densities of plant parasitic nematodes (Johnson et al., 1992) whereas Potter et al. (1998) reported that different cultivars of *B. napus* provided differing levels of control. Allyl isothiocyanate was highly toxic to the free-living nematode *Caenorhabditis elegans* (Donkin et al., 1995). Reduction of *Meloidogyne incognita* and *M. javanica* was negligible after incorporation of rapeseed green manures (Johnson et al., 1992). Also, Mojtahedi et al. (1991) indicated that *B. napus* amendments did not reduce populations of *M. chitwoodi*. Breakdown products from four of six tested glucosinolates were highly toxic to *Heterodera schachtii* (Lazzeri et al., 1993). McLeod and Steel (1999) reported that using green manures of *Brassica* at the rate of 10-20 $g{\cdot}kg^{-1}$ of soil lowered *Meloidogyne javanica* populations. The degradation product of gluconasturtiin (2-phenylethyl isothiocyanate) was toxic to the root lesion nematode, *Pratylenchus neglectus* (Potter et al., 1998). Halbrendt (1996) stated that organic amendments were variable in abilities to control plant parasitic nematodes and that more research is needed to determine their efficacy.

Brassica crops used as either green manures or as seed meal amendments may control soilborne pests. However, the use of soil incorporated seed meal may be more attractive to growers than the use of green manures. *Meloidogyne* spp. can feed and reproduce on Brassicaceae intended for use in biofumigation (Bernard and Montgomery-Dee, 1993). One benefit of using ground oriental mustard seed meal (*Brassica juncea)* for control of plant parasitic nematodes is that there is no prior opportunity for the targeted pest to feed on the added material. This eliminates a possible food source which could increase nematode populations. It is important to obtain data that is reliable for use in determining proper amounts of the product to use for control of targeted pests. Data obtained in the laboratory and greenhouse are beneficial for estimating the approximate rates to be used in field testing. Allyl isothiocyanate (AITC) is a major end product that is produced from decomposing *Brassica* tissues and may provide adequate control of root-knot nematodes.

The objective of this experiment is to determine the efficacy of AITC alone, AITC produced by mustard meal and efficacy of mustard meal when mixed with soil in controlling *Meloidogyne incognita.* The research was conducted in a greenhouse to determine if nematode populations were affected by AITC in a controlled environment.

Materials and Methods

Source of Oriental Mustard Meal

Mustard meal (whole ground oriental mustard seed/hull, 8% moisture content) was obtained from Poulenger USA, Inc., Lakeland Fla. It was shipped in 22.68 kg moisture resistant lined paper bags. The supplier indicated that contents of the bags were prepared from different lots which had varying harvest dates and were harvested from different locations. Information on planting and harvest dates was not provided by the supplier. Samples were placed into one-liter Nalgene containers (square HDPE bottles, 1 L, 2114-0032, Nalge Nunc International, Rochester, N.Y.) and stored at -40 °C until use in each experiment.

Oriental Mustard Meal and AITC Concentrations

A wide-mouth, threaded, glass Mason jar with screw-on lid and retaining ring (Alltrista Corp., Ball/Kerr, Broomfield, Colo.) was used to determine concentration of AITC. The volume of the jars were determined to be 490-mL. Each glass jar was capped with 0.79 mm thick Teflon lid. A threaded retaining ring was screwed down to hold the Teflon lid in place. Previously made stock solutions of AITC were dispensed into the glass jars using a Hamilton 1701 series point style no. 2, fixed-needle, 10-μL syringe (no. 80075, Hamilton, Reno, Nev.) through a 3.175 mm diameter hole that was previously made in the Teflon lid. Labeling tape (Fisherbrand Colored Label Tape 19-mm width, cat no. 11-880-5C, Fisher Scientific, Pittsburgh, Pa.) was immediately placed over hole. The tape was pierced using a Hamilton 26s needle (7748-19, Hamilton). This procedure was done to keep tape adhesive off of the sampling syringe that was later used for headspace sampling. This method helped minimize clogging of the Merlin Microseal septum (cat no. 5182-3442, Agilent Technologies, Santa Clara, Calif.). The hole was immediately covered with another piece of Fisher labeling tape to reduce the escape of gas. Headspace samples were taken using a Hamilton Gastight series 1705, 50-μL beveled HP style 2 tip syringe (#80930, Hamilton). Head space sampling was conducted at timed increments after a stock solution was injected into the glass jar. Allyl isothiocyanate mixtures were injected into jars at timed intervals so evolution time would be identical for each jar. Mustard meal was placed into a 5-mL polystyrene microbeaker mixed with 1:1 w/v ratio of dd-H_2O and the mixture was placed into the jars. Jars were incubated at approximately 22 ± 2 °C before sampling.

The syringe was placed into the injection port of a Hewlett Packard 5890a Series II (Hewlett Packard Company, Palo Alto, Calif.) high performance gas chromatograph with a flame ionization detector (GC-FID). The inlet port was fitted with a Merlin Microseal septum (Merlin Instrument Company, Halfmoon, Calif., kit no. 304, septum no. 2218) for manual injections. The column was a 60 m x 0.53 mm, 0.25 μ film (J&W Scientific, Inc., Folsom, Calif. part no. 125-1361, DB1301). The inlet and outlet temperatures were 200 and 250 °C, respectively. The oven parameters were programmed for 60 °C for 1 min., and then increased by 5 °C per minute to a maximum of 150 °C. Detector response was quantified based on the equation for the AITC standard curve. Analysis of the allyl isothiocyanate peaks were performed using Chemstation Software Module, Version 6.03 (Hewlett Packard, Palo Alto, Calif.).

Extraction of Root-Knot Eggs for Inoculum

Eggs of root knot nematodes (RKN) were extracted from roots of 'Rutgers' tomato plants previously colonized by the nematodes. Procedures were modified from those used by Hussey and Barker, 1973. Galled roots of tomato plants were cut into 3-4 cm length pieces and placed into a 500-mL glass beaker. Tap water (320 mL) was added, followed by 60 mL of sodium hypochlorite (Chlorox Ultra 6%, The Chlorox Co., Oakland, Calif.). Galled roots and the sodium hypochlorite solution were stirred with a spatula for one minute. The mixture was occasionally agitated for three additional minutes. The mixture was then poured over stacked wet sieves, the top sieve being a 200 mesh (75-µm pore size) and the lower a 500-mesh sieve (25-µm pore size). Sieves were rinsed with tap water (approximately 29 °C) for 30 seconds to remove any remaining sodium hypochlorite. The 500-mesh sieve was held at a 45° angle over the sink and was backwashed using a wash bottle containing tap water. As eggs and debris were gently washed towards the lower section of the sieve, the eggs and sediment were poured and rinsed into a 100-mL graduated beaker. The beaker was filled to the 100 mL reference with tap water. After use of the sieves, they were backwashed, rinsed, and placed onto greenhouse benches into direct sunlight to assist in desiccation of any remaining eggs to eliminate contaminating future tests.

Quantitation of Root-Knot Nematode Eggs

Extracted eggs were poured into a 100-mL graduated beaker (referred to as stock solution). The stock solution was stirred with the point of a 5-mL glass pipette with a vacuum siphon attached. The stock solution (3 mL) was drawn into a pipette and then distributed into a 7.62 cm x 2.54 cm x 2.54 cm polystyrene counting chamber (Ward's, Rochester, N.Y.), which will be referred to as a counting chamber (CC). An additional 7 mL of tap water was added to fully cover the surface of the CC. The CC was gently swirled to evenly distribute the solution of eggs across the grids of the chamber. Prior to adding eggs into the CC, a grid (16 equal sections) was inscribed on the bottom of the chamber. After determining the number of eggs in the suspension, the suspension was stirred with a pipette and then siphoned into the pipette to obtain enough suspension to acquire adequate egg numbers to develop galls on roots systems. Approximately 4,000 eggs were placed onto a Whatman 9.0-cm ashless filter paper (no. 42, cat. no. 1442090, Fisher Scientific, Pittsburgh, Pa.) an folded into quarters to form a cone. Nematode suspension was added to the filter paper, which was placed on top of a 100-mL beaker and excess moisture was allowed to drip from the filter paper for five minutes. A 5-mL polystyrene beaker containing measured mustard meal was lowered into the jar. Deionized H_2O (dd-H_2O) was added to the meal at a ratio of 1:1 w/v mustard meal weight (g) to dd-H_2O volume (mL). Other treatments included various concentrations of stock solutions of commercially obtained AITC prepared as described in Part III and injected into glass jars after the addition of filter paper containing RKN eggs. AITC concentrations ranged from 2.08 to 4.17 $\mu M \cdot L^{-1}$. Also, various amounts of ground oriental mustard meal (OMM) from 0.03 g to 0.06 g per container were used. Filter paper cones containing RKN eggs were lowered into glass jars for comparison of headspace of AITC and mustard meal. Empty 5-mL polystyrene beakers and corresponding dd-H_2O were included in jars with AITC samples to reduce effects of differences due to the polystyrene containers and moisture in jars containing OMM.

Headspace Analysis of AITC

Headspace analysis measurements were conducted using methods and materials described above. Concentrations of AITC alone were diluted with hexane to range from 2.08 to 4.17 $\mu M \cdot L^{-1}$ and then added to the jars. The peak headspace volume readings obtained from the HPGC-FID were compared to the standard curve equation from Fig. IV-1, pg. 72, to determine actual AITC concentration within each jar. The purpose for this was that the addition of filter paper and nematode eggs may affect AITC concentration evolving within the container and actual measured concentrations may not correspond to diluted concentrations injected into the container. Headspace peaks were measured at one and two hours after injection then averaged for all experiments. Experiment 1 included three replications and experiment two included four replications of the treatments which consisted of OMM mixed with 100 g at 33 Pa of Sequatchie fine sandy loam soil. The moisture level had previously been determined and the field capacity of the soil was used within this experiment (Price, 1999). The jars remained sealed for 18 h after which filter cones containing RKN eggs were transferred to 15.24-cm pots.

Tomato Transplants

Rutgers tomato plants were grown for approximately six weeks in the greenhouse in 5.08-cm pots containing builder's sand. Plants were then transferred into 15.24-cm pots containing builder's sand and then were infested with approximately 4,000 root-knot nematode eggs per potted plant. Whatman filter paper containing RKN eggs was placed directly under the root mass of plants. The containers were then filled with builder's sand within a half inch from the top. After 30 days, plants were removed from the containers and root masses were rated on a scale of 0-10 (Zeck, 1971), where 0 = no galling, 1 = very few small galls, 2 = slight increase over 1, 3 = numerous small galls, some of which have grown together, 4 = numerous small galls, some larger galls are present and the majority of the roots are functioning, 5 = approximately 25% of the root system is not functioning due to severe galling, 6 = up to 50% of the root system is not functioning due to severe galling, 7 = 75% of the root system is not functioning is heavily galled and lost for production, 8 = No healthy roots are left; however the plant is still green, 9 = The root system is completely galled and roots are rotting, and 10 = is the highest level of infestation where the plant and roots are dead.

Statistical Analysis

The data for each experiment were analysis using SAS PROC PROBIT (SAS Institute, Cary N.C., 2002). Amount of root tissue invaded by RKNs was measured using the Zeck Scale (Zeck, 1971). The experiments used a completely randomized design with treatments of varying levels of AITC and an untreated control. All experiments were tested for normality and data fell within acceptable ranges of normality.

Results and Discussion

Experiment I included both commercially available AITC and OMM. Volatile AITC concentrations were measured in headspace from jars on the same day (see Tables V-1, V-2 and V-3, pg. 97, 98 and 99)[1]. Experiment II included pre weighted OMM mixed with dry soil brought to 33 Pa (to obtain field capacity conditions with dd-H_2O, as

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[1] Figures and tables are listed in the Appendix.

described in Price, 1999) and weighed 100 g at 33 Pa. Experiment II testing of OMM mixed with soil was conducted several weeks after the first experiment. Headspace peak readings were measured on hours one and two after AITC alone or OMM was introduced into the jars and hourly measurements were averaged for all experiments.

In experiment I, predicted headspace values of AITC and OMM to achieve a LC_{50} were 0.78, 0.44 $\mu M{\cdot}L^{-1}$, respectively and to achieve an LC_{90} 1.94, 1.22 $\mu M{\cdot}L^{-1}$, respectively. Commercially available AITC concentrations fell in the range of normality, as indicated by a Shapiro-Wilk test (0.927). Mustard meal fell outside of the range of normality with a Shapiro-Wilks test for normality of 0.806, which means the null hypothesis may be accepted, meaning there was a large variance. The differences of concentration for both tests were very significant ($P = <0.001$). A comparison of the LC_{50} and LC_{90}s of AITC, OMM and OMM mixed with soil is shown in Fig. V-1, pg. 96. Tables V-1 and V-2, on pg. 97 and 98, list the probabilities of the LC_{50} and LC_{90} of both commercially obtained AITC and OMM.

In experiment II, the LC_{50} and LC_{90} for OMM mixed with soil at 33 Pa were 0.88 $\mu M{\cdot}L^{-1}$ and 1.99 $\mu M{\cdot}L^{-1}$, respectively. The Normality test fell in an acceptable range with a value of 0.967. The test was very significant with a value $P= <0.001$. The LC_{50} and LC_{90}s of OMM mixed in soil are listed in a probability table predicting the values (Table V-3, pg. 99). The predicted value of AITC, mustard meal and mustard meal mixed with soil to obtain mortality of RKN eggs is shown in Fig. V-1, pg. 96. Tables V-1, V-2 and V-3, pg. 97, 98 and 99, provide probability levels obtained from using statistical analysis, Proc Probit (SAS Institute, Cary, N.C.).

Previously reported methods for nematode control using ITCs and *Brassica* tissues used various procedures for control and data was reported in different fashions. It is difficult to compare the results obtained from tests above with other experiments reported to date. The LC_{50} and LC_{90} for commercial AITC on *Tylenchulus semipenetrans* to be 0.02 and 0.04 $\mu M \cdot mL^{-1}$ and for *M. javanica* 0.10 and 0.29 $\mu M \cdot mL^{-1}$, respectively Zasada and Ferris (2003). In their experiments, sand was added to PVC tubes that could be sealed, 1 mL of water and an aqueous solution of AITC, which was added to obtain the results. In another report Buskov et al. (2002) reported 100% mortality of *Globodera rostochiensis* Cv. Woll after it was exposed to one mg/mL AITC for 40 hours and only 20% mortality occurred, if exposed for 16 hours. In another experiment, Mojtahedi et al. (1991) added 40 g of rapeseed tissue to 500 g of soil and incubated it for one month, greatly reducing *M. chitwoodi* populations. Mojtahedi et al. (1993) reported that 20 g of leaf tissue added to 515 g soil evolved approximately 2 μM of ITC per gram of soil. They estimated the ED_{50} (effective dose) to be 23 mg of rapeseed tissue per gram of soil to control *M. chitwoodi.*

Past research has shown that by-products of glucosinolates do have the potential to control various plant parasitic nematodes; however variations in the soil environment may have a role in determining their efficacy. Experiments conducted in this study determined the concentration of AITC needed to control RKN in a controlled environment when using both commercially available AITC, and AITC which has evolved from OMM. Treatments including OMM mixed with soil required slightly higher AITC concentrations to provide similar control as AITC alone. Information concerning the efficacy of OMM mixed with soil suggests that similar results may occur in field soils with similar soil composition, when applying similar concentrations of AITC as tested in the laboratory. With this knowledge, researchers may be better prepared to determine the proper amount of *Brassica* tissue needed to achieve effective control when applied to similar soil systems.

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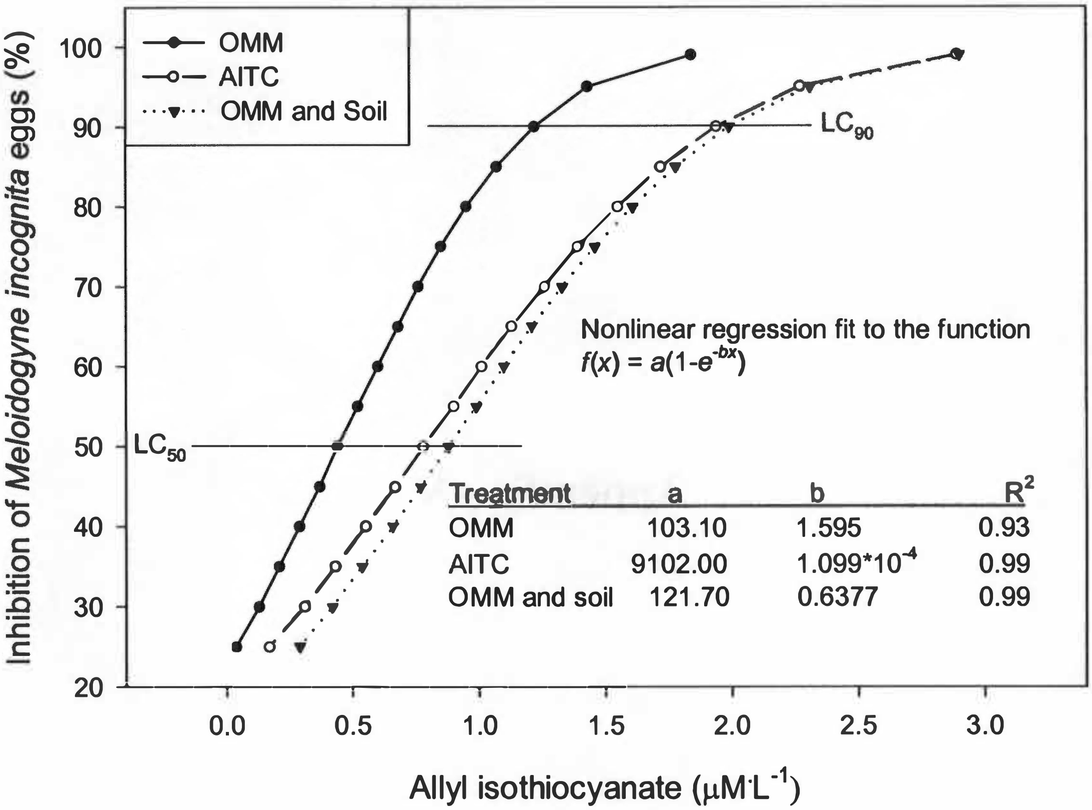
Appendix - V



Treatment	a	b	R^2
OMM	103.10	1.595	0.93
AITC	9102.00	1.099×10^{-4}	0.99
OMM and soil	121.70	0.6377	0.99

Figure V-1. Lethal concentration (LC) of commercially available allyl isothiocyanate (AITC), oriental mustard meal (OMM), and OMM mixed with soil at moisture level of 33 Pa, to the eggs of *Meloidogyne incognita*. Means of data are based on *n* = 30 for OMM, *n* = 33 for AITC, and *n* = 47 for OMM mixed with soil. The results of parameter estimates for the nonlinear regression model to which data were fit are given in the insert table above.

Table V-1. Fiducial limits of commercially available AITC on *Meloidogyne incognita*.

	AITC	95% Fiducial Limits^z	
Probability^y	($\mu M \cdot L^{-1}$)	MIN	MAX
0.01	2.89573	2.55095	3.40082
0.02	2.64867	2.34167	3.09524
0.03	2.49192	2.20836	2.90189
0.04	2.37400	2.10772	2.75679
0.05	2.27808	2.02559	2.63904
0.06	2.19644	1.95545	2.53904
0.07	2.12486	1.89375	2.45157
0.08	2.06076	1.83832	2.37343
0.09	2.00247	1.78774	2.30253
0.10	1.94882	1.74102	2.23744
0.15	1.72666	1.54548	1.97002
0.20	1.55010	1.38674	1.76082
0.25	1.39863	1.24708	1.58481
0.30	1.26260	1.11795	1.43046
0.35	1.13655	0.99434	1.29139
0.40	1.01694	0.87294	1.16353
0.45	0.90122	0.75142	1.04389
0.50	0.78733	0.62800	0.92997
0.55	0.67344	0.50112	0.81952
0.60	0.55772	0.36916	0.71031
0.65	0.43811	0.23017	0.60005
0.70	0.31206	0.08143	0.48610
0.75	0.17603	- 0.08106	0.36512
0.80	0.02456	- 0.26381	0.23220

[z] Probit Analysis on average allyl isothiocyanate (AITC) concentration ($\mu M \cdot L^{-1}$) produced by commercially available AITC to reduce galling on tomato caused by *Meloidogyne incognita*.

[y] The probability equals the percentage of total population of nematodes forming galls on tomato after using the stated concentration of AITC. Output produced using SAS Proc Probit (SAS, ver.9.0, Windows PC).

Table V-2. Fiducial limits of oriental mustard meal on *Meloidogyne incognita*.

Probability[y]	AITC ($\mu M \cdot L^{-1}$)	95% Fiducial Limits[z] MIN	MAX
0.01	1.84907	1.59648	2.21500
0.02	1.68508	1.45642	2.01306
0.03	1.58104	1.36708	1.88541
0.04	1.50277	1.29956	1.78970
0.05	1.43910	1.24440	1.71209
0.06	1.38491	1.19726	1.64621
0.07	1.33740	1.15576	1.58862
0.08	1.29485	1.11846	1.53720
0.09	1.25616	1.08440	1.49056
0.10	1.22055	1.05293	1.44776
0.15	1.07309	0.92115	1.27202
0.20	0.95589	0.81424	1.13452
0.25	0.85535	0.72047	1.01861
0.30	0.76506	0.63428	0.91650
0.35	0.68139	0.55242	0.82387
0.40	0.60200	0.47275	0.73797
0.45	0.52519	0.39369	0.65683
0.50	0.44959	0.31395	0.57893
0.55	0.37400	0.23231	0.50291
0.60	0.29719	0.14754	0.42749
0.65	0.21779	0.05819	0.35127
0.70	0.13413	- 0.03765	0.27262
0.75	0.04384	- 0.14269	0.18936

[z] Probit Analysis on average allyl isothiocyanate (AITC) concentration ($\mu M \cdot L^{-1}$) produced by oriental mustard meal to reduce gall formation on tomato plants caused by *Meloidogyne incognita.*

[y] The probability equals the percentage of total population of nematodes forming galls on tomato after using the stated concentration of AITC from oriental mustard meal. Output produced using SAS Proc Probit (SAS, ver.9.0, Windows PC).

Table V-3. **Fiducial limits of oriental mustard meal mixed with soil on *Meloidogyne incognita.***

Probability[y]	AITC ($\mu M \cdot L^{-1}$)	95% Fiducial Limits[z] MIN	MAX
0.01	2.90801	2.56987	3.38087
0.02	2.67066	2.36555	3.09567
0.03	2.52006	2.23565	2.91499
0.04	2.40678	2.13775	2.77925
0.05	2.31463	2.05798	2.66896
0.06	2.23619	1.98998	2.57520
0.07	2.16742	1.93027	2.49309
0.08	2.10584	1.87672	2.41964
0.09	2.04984	1.82794	2.35293
0.10	1.99829	1.78296	2.29158
0.15	1.78487	1.59587	2.03851
0.20	1.61524	1.44580	1.83874
0.25	1.46972	1.31566	1.66876
0.30	1.33903	1.19726	1.51763
0.35	1.21794	1.08583	1.37930
0.40	1.10302	0.97810	1.25004
0.45	0.99185	0.87155	1.12730
0.50	0.88243	0.76401	1.00918
0.55	0.77302	0.65347	0.89407
0.60	0.66184	0.53789	0.78035
0.65	0.54693	0.41507	0.66619
0.70	0.42583	0.28230	0.54921
0.75	0.29515	0.13581	0.42617
0.80	0.14962	- 0.03034	0.29219

[z] Probit Analysis on average allyl isothiocyanate (AITC) concentration ($\mu M \cdot L^{-1}$) produced by oriental mustard meal mixed in 100 grams of soil at 33 Pa to reduce gall formation on tomato plants caused by *Meloidogyne incognita.*

[y] The probability equals the percentage of total population of nematodes forming galls on tomato after using the stated concentration of AITC from oriental mustard meal when mixed with soil. Output produced using SAS Proc Probit (SAS, ver.9.0, Windows PC).

Part VI

COMPARISON OF TEFLON AND POLYETHYLENE

Abstract

Huge losses occur every year in crop production from plant pathogens, and insect and weed infestations. Alternative methods of control of these pests that are environmental friendly and economically feasible are being sought by researchers and producers. Within the last few years, researchers have been reviewing the effects of biological control agents because they are relatively safe to use and to the environment. They are natural and often do not cause adverse effects to non-targeted pests. Incorporation of *Brassica* tissues into soil is one method of controlling many pathogens, as well as using a natural product. Different methods of application and soil coverings may affect the efficacy of soil amendments. Information is needed about a commonly used soil covering containing polyethylene (PE) to determine if it may have adverse effects on the retention in soil or efficacy of gaseous materials produced by *Brassica* tissues. Two experiments were conducted comparing the evolution of AITC from different weights of seed meal of *Brassica juncea* were conducted over time comparing Teflon to PE coverings. Containers with Teflon coverings over seed meal resulted in significantly higher peak GC-FID readings than containers with PE coverings.

Introduction

Due to the loss of methyl bromide (MeBr), alternatives are being investigated for the control of various soil inhabiting pests. Large losses often occur from infection by plant pathogens or heavy infestations of insects and/or weeds. Use of *Brassica* tissues in greenhouse and laboratory settings have resulted in control of various pests, however, mixed results have occurred in the field environments (Halbrendt, 1996). Many factors may affect the efficacy of soil applied fumigants. Gan et al., (1996) showed that several factors (soil type, water content, and bulk density) affected behavior of MeBr volatilization rate from soil. Since various factors affect rates of volatilization, they therefore affect the time and concentration that the active ingredient will be in contact with the targeted organism.

The distribution and toxicity of fumigants may be influenced by factors such as temperature, soil moisture, air space, clay and/or organic matter content of the soil (Goring, 1962). Soil compaction and covering of soils with non-permeable or semi permeable soil coverings may delay or eliminate the loss of fumigants from the soil surface, increasing contact time therefore possibly increasing efficacy of the soil fumigant. The combination of using plastic mulches and irrigation may improve efficacy of *Brassica* tissues incorporating within the soil because adequate moisture is required to achieve an effective chemical reaction.

Allyl isothiocyanate vapors may control many spoilage organisms when applied to a modified atmosphere packaging used in the food preservation industry (Isshiki et al., 1992). This method is similar to use of plasticulture. Over the past few years, the use of plastic mulches has been termed plasticulture. Plasticulture production is an agricultural technique that uses plastic materials, raised beds and drip irrigation for high valued crops, such as vegetable production. In Tennessee, many producers use polyethylene (PE) plastic mulch in vegetable production (Coffey, 1984). The most commonly used type of PE is embossed PE that resists tears to a greater extent than non-embossed PE. In most situations, irrigation drip tape is used in conjunction with the covering.

The use of polyethylene (PE) tarping is not essential when using the fumigant 1,3-dichloropropene, because of the nature of the product, but tarping is recommended. However, fumigation of soil with methyl bromide normally requires a covering for effective control of young weeds and weed seeds (Lembright, 1990). When chloropicrin is used, the soil is covered to avoid eye irritation caused by low concentrations of the compound that volatilize and enter into the atmosphere. Soil covering may not be necessary when using dazomet which breaks down into methyl isothiocyanate (MITC) (Donald, 1986).

There have been various reports concerning the ability of soil coverings to retain volatile materials. Allyl isothiocyanate has a moderate ability to permeate films made of polyethylene and polypropylene with permeability inversely proportional to film thickness (Sekiyama et al., 1995). Covering the soil may be important to reduce escape of highly volatile materials. Fumigation using a soil covering generally provides better pest control than fumigation without a soil covering (Sumner et al., 1978). By increasing the efficiency of the fumigant, growers may be able to reduce concentrations of products added to the soil, therefore possibly reducing cost and environmental effects.

Polyethylene and other tested plastic mulches have a high capacity to sorb fumigant vapors, such as methyl bromide, chloropicrin and 1,3-dichloropropene (Papiernik et al, 1999). The films tested had the behaviors of sorption and desorption, but the sorption was largely reversible, with little retention of the fumigant on the plastic covering when there was a large concentration gradient. Inexpensive permeable tarps may be sufficient to provide adequate control because the sorptive properties of the film will provide a barrier to the rapid flux of vapors. However, the films may not decrease total emissions of fumigant vapors to the atmosphere.

The main reason to use plastic mulches is to retain fumigants in close association with the targeted pests. Control of vapor emission through use of field components such as soil coverings may be essential to facilitate proper utilization of *Brassica* tissues and their activity. However, knowledge of reactions or properties of covering materials is needed to better utilize these materials.

The objective of these experiments was to compare AITC concentrations in glass jars sealed with Teflon or Polyethylene (PE).

Materials and Methods

AITC Evolution from Oriental Mustard Meal

A wide-mouth, threaded, glass Mason jar with screw-on lid and retaining ring (Alltrista Corp., Ball/Kerr, Broomfield, Colo.) was used to determine concentration of AITC. The volume of the jars were determined to be 490-mL. Each glass jar was capped with 0.79 mm thick Teflon lid. A threaded retaining ring was screwed down to hold the Teflon lid in place. Previously made stock solutions of AITC were dispensed into the glass jars using a Hamilton 1701 series point style no. 2, fixed-needle, 10-µL syringe (no. 80075, Hamilton, Reno, Nev.) through a 3.175-mm diameter hole that was previously made in the Teflon lid. Labeling tape (Fisherbrand Colored Label Tape 19mm width, cat no. 11-880-5C, Fisher Scientific, Pittsburgh, Pa.) was immediately placed over hole. The tape was pierced using a Hamilton 26s needle (7748-19, Hamilton). This procedure was done to keep tape adhesive off of the sampling syringe that was later used for headspace sampling. This method helped minimize clogging of the Merlin Microseal septum (Agilent Technologies, Santa Clara, Calif. cat. no. 5182-3442). The hole was immediately covered with another piece of Fisher labeling tape to reduce the escape of gas. Headspace samples were taken using a Hamilton Gastight series 1705, 50-μL beveled HP style 2 tip syringe (#80930, Hamilton). Head space sampling was conducted at timed increments after a stock solution was injected into the glass jar. Allyl isothiocyanate mixtures were injected into jars at timed intervals so evolution time would be identical for each jar. Jars were incubated at 22 ± 2 °C before sampling.

The syringe was placed into the injection port of a Hewlett Packard 5890a Series II (Hewlett Packard Company, Palo Alto, Calif.) gas chromatograph (GC-FID). The inlet port was fitted with a Merlin Microseal septum (Merlin Instrument Company, Halfmoon, Calif., kit no. 304, septum no. 2218) for manual injections. The column was a 60 m x 0.53 mm (J&W Scientific, Inc., Folsom, Calif.. DB1301, cat. no. 125-1361). The inlet and outlet temperatures were 200 and 250 °C, respectively. The oven parameters were programmed for 60 °C for one min., and then increased by 5 °C per minute to a maximum of 150 °C. Detector response was quantified based on the equation for the AITC standard curve [$y = 910.38\ (x) + 121.45$] which had an $R^2 = 0.84$. Analysis of the allyl isothiocyanate peaks were performed using Chemstation Software Module, Version 6.03 (Hewlett Packard, Palo Alto, Calif.).

A Hewlett Packard high performance gas chromatograph with a flame ionization detector (HPGC-FID) was used to detect relative peaks of AITC concentrations in containers. Mustard meal (0.5 grams) was placed into a 5-mL polystyrene microbeaker (cat no. 2-544-30, Fisher Scientific, Pittsburgh, Pa.) that was placed into the glass Mason jar. Deionized water (dd-H_2O) (0.5 mL) was pipetted into the microbeakers with the pre-weighed mustard meal. The jars were immediately sealed with either Teflon or PE after mixture ratio of 1:1 w/v of meal and dd-H_2O. A 6.35 mm x 12.7 mm piece of adhesive tape was placed in the center of a precut 152.4 mm x 152.4 mm square of embossed PE. The PE was pulled firmly across a Teflon lid to reduce gathering and a lid retaining ring was screwed down tightly on the jar. The Teflon lid contained a 9.525 mm diameter hole which was located directly over the top of the taped section of the PE. The adhesive tape and PE were pierced with a 22s needle. This method helped reduce adhesive from accumulating on the sampling syringe needle. The hole was covered with an additional piece of adhesive tape to reduce loss of gas. AITC headspace sampling was conducted on the hour and repeated measures were made up to five hours for experiment I and repeated measures were made up to six hours in experiment II. In experiment II, the rate of both water volume (0.25 mL), and meal weight (0.25 g) were reduced and sampling time (repeated measures) was increased to six hours.

Statistical Design and Analysis

Two experiments were conducted using a completely randomized design, containing two treatments (Teflon and PE) and three replications. Data were analyzed using Proc ANOVA and Duncan's Means Separation (SAS, Institute, Cary, N.C.). Samples of AITC in the headspace of jars were collected as repeated measures (sampling taken from the identical container over five hours for expt. I and six hours for expt. II) over five hours for experiment I and over six hours in experiment II.

Results and Discussion

In preliminary testing, AITC headspace peaks declined over time, and usually declining within four hours. In both experiments, treatments including Teflon lids, yielded the greatest headspace peak measurements and AITC concentrations reduced over time of sampling. Jars with Teflon lids had significantly higher peak readings than treatments including PE lids. The *P* values were <0.0001 and <0.006 for the five hour and six hours tests, respectively (Fig. VI-1 and VI-2, pg. 114 and 115)[1]. Each test fell in an acceptable range of normality with values of 0.97.

The first experiment was the five hour test (*DF* = 1, 20) that resulted in significance by treatment and hour by treatment interaction. Data exhibited a bell shaped curve for the Teflon treatments (Fig. VI-1, pg. 114). This was evident in preliminary testing, suggesting that the mixture of myrosinase, glucosinolate and water produce peak AITC concentrations within two to three hours after mixing and generally declines thereafter. When reviewing treatment effects an analysis using Duncan's means separation test revealed differences and means of AITC concentrations were higher for Teflon at 17.20 $\mu M \cdot L^{-1}$ and 13.46 $\mu M \cdot L^{-1}$ for PE.

In the second experiment (*DF* = 1, 24), AITC concentrations were measured in containers with Teflon and PE covering over six hours using less meal at 0.25grams meal. This experiment did have AITC peaks that started declining after the fifth hour (Fig. VI-2, pg. 115). The AITC concentration means from the Duncan's means

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[1] Figures are included in the Appendix.

separation test showed significant differences with 8.27 $\mu M \cdot L^{-1}$ for Teflon and 6.79 $\mu M \cdot L^{-1}$ for PE.

There have been numerous reports that various plastic materials are permeable to gases (Wang et al., 1997). A copolymer film, polyvinylidene chloride with polyvinyl chloride or PVDC/PVC (Saran Wrap) was not a good barrier against AITC vapor and sorption of AITC occurred (Lim and Tung, 1997). Also, PVDC/PVC retains AITC levels for a slightly longer period of time, therefore aiding in control of spoilage organisms. When using high rates (3,000 ppm) of AITC, in polyethylene containers, AITC was absorbed within several hours (Sekiyama, et al., 1995). Polypropylene did not absorb AITC at similar rates. Allyl isothiocyanate vapor had moderate ability to permeate polyethylene and polypropylene, with permeability inversely proportional to film thickness. When in gaseous form AITC may permeate both PE and PP; however, experiments I and II were designed to determine the affects of PE absorption of AITC. In conclusion, polyethylene film is a product that is commonly used in vegetable production and may affect concentrations of AITC under field conditions. These experiments suggested that AITC sorption occurs with PE, however, no information was obtained to determine if the process was reversible or irreversible. More research is needed to determine if adsorption or adsorption of AITC is occurring with PE coverings and to determine if PE provides more effective control of targeted pest when used as a soil covering versus other available coverings. Materials which have characteristics similar to that of Teflon should be investigated to determine their efficacy in retaining volatile products within treated areas; however, costs of these materials should be considered.

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Appendix - VI

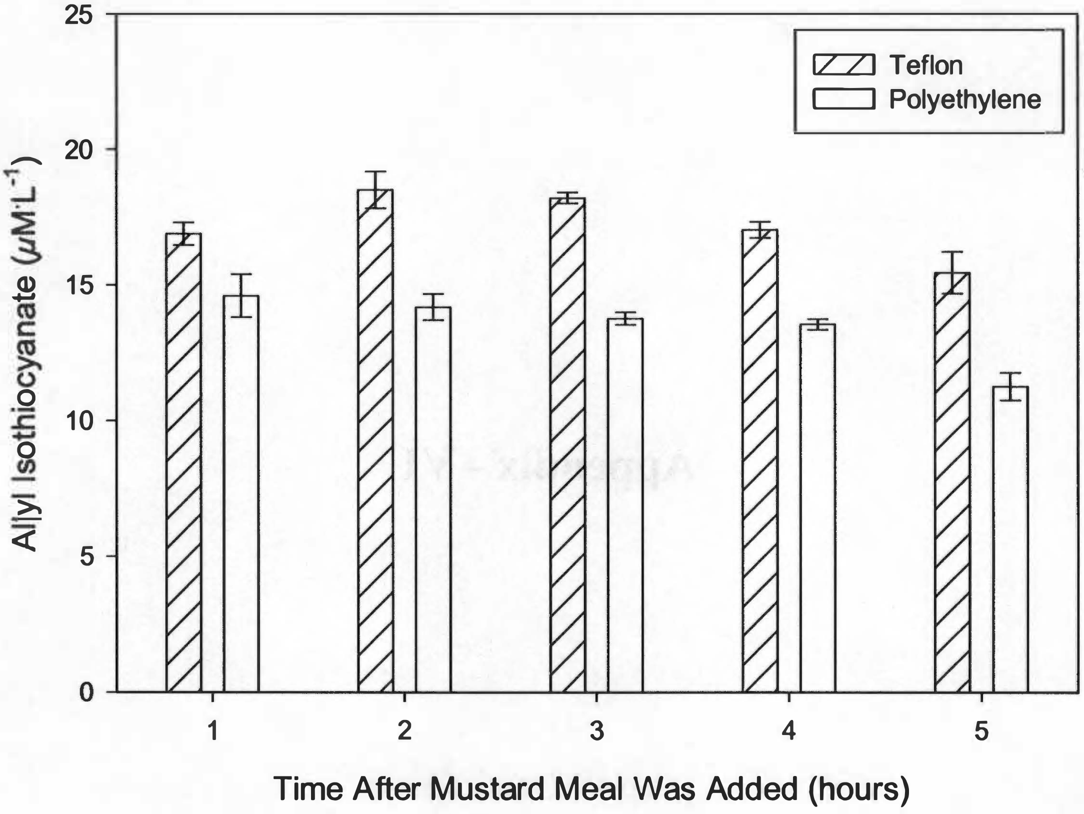

Figure VI-1. Mean (±SE) peak readings of allyl isothiocyanate (AITC) from whole ground oriental mustard meal (0.5g). A Hewlett Packard high performance gas chromatograph with a flame ionization detector was used to detect AITC concentrations in glass Mason jars with different coverings (Teflon and polyethylene) sampled using repeated measures (hours). Formula used to determine μM concentration was; $y = 910.38\ (x) + 121.45$, with an $R^2 = 0.84$.

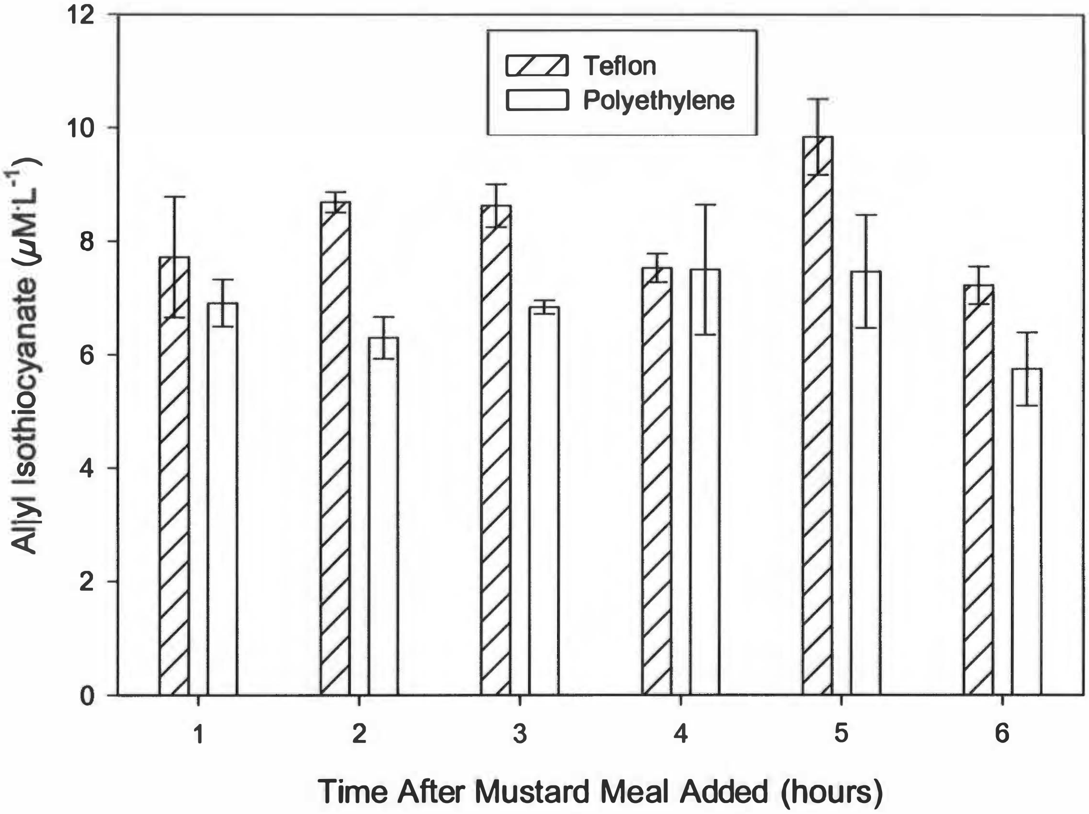

Figure VI-2. Mean (±SE) peak concentration of allyl isothiocyanate (AITC) evolving from containers with different coverings (Teflon or Polyethylene (PE). Each container included whole ground oriental mustard meal (0.25g). Sampling occurred over a six hour period. Formula used to determine μM concentration was; $y = 910.38\ (x) + 121.45$, with an $R^2 = 0.84$.

Part VII

EFFECTS OF MOISTURE AND RELATIVE HUMIDITY ON ALLYL ISOTHIOCYANATE EVOLUTION

Abstract

In order to incorporate *Brassica* tissues that have volatile decomposition compounds, such as allyl isothiocyanate (AITC) into production systems, it is necessary to understand the potential interactive effects of the covering materials and the effects that moisture may have when these compounds are added to the soil. The mechanical and barrier properties of soil coverings may have profound affects on the sorption of relatively low molecular weight compounds. Also, it has been a common practice to moisten soils prior to fumigation with methyl bromide and other commonly used compounds. The use of *Brassica* tissues incorporated within the soil, irrigated and covered with polyethylene (PE) shows great promise since moisture is needed to achieve the hydrolysis of glucosinolates found in *Brassica* tissues. Using plasticulture may also aid in the retention of volatile products produced by these tissues. Two experiments were conducted to determine the effects of moisture on concentrations of AITC evolved from whole ground oriental mustard seed meal (*Brassica juncea*). Various amounts of de-ionized water were added to known amounts of seed meal. Treatments with higher volumes of water resulted in lower AITC peak readings. There were no statistical differences observed between jars containing different relative humidities, therefore relative humidity had no effect on AITC concentration. However, jars containing 75% relative humidity consistently had lower AITC concentration than jars with 23% relative humidity. A possible reason for this may have been from condensation forming on the underside of the Teflon surface of containers with higher humidity, therefore sorption may have had an effect on concentration measured.

Introduction

Fumigants have been applied to soil using various techniques and involving different formulations of active ingredients (Goring, 1962). Knowledge of the characteristics of the active ingredient can help determine the best method of application. *Brassica* tissues contain glucosinolates and enzymes known as myrosinases. Toxic materials may be formed from hydrolysis, if adequate moisture is available and tissues of plants containing glucosinolates become damaged. Major degradation products of glucosinolates in soil are organic isothiocyanates, nitriles and sulfur contain compounds (Borek et al., 1995). Half-lives of allyl isothiocyanates applied to six soils were determined using gas chromatographic analysis of ethyl acetate extracts and half-life of allyl isothiocyanate (AITC) ranged from 20 to 60 hours. AITC transformation into non-active products increased with reduced soil moisture and higher temperatures and occurred more rapidly in soils containing greater concentrations of organic carbon (Borek et al., 1995). They also reported that as soil moisture levels increased the half-life of AITC increased. Allyl isothiocyanate in both liquid and gaseous forms showed similar antibacterial activities against three test species *Salmonella* Montvideo, *Escherichia coli* 0157:H7, and *Listeria monocytogenes* Scott A (Lin et al., 2000). Since moisture appears to affect efficacy of AITC on several organisms, it would be beneficial to determine the effect of moisture conditions on the levels of AITC produces in modified environments especially since moisture is needed to achieve an affective release of AITC from tissues containing glucosinolates.

The objective of the following experiments was to determine the effect of humidity and water concentration on AITC alone and AITC concentrations evolving from oriental mustard meal (OMM) when exposed to environments with varying moisture conditions.

Materials and Methods

AITC Evolution from Oriental Mustard Meal

A wide-mouth, threaded, glass Mason jar with screw-on lid and retaining ring (Alltrista Corp., Ball/Kerr, Broomfield, Colo.) was used to determine concentration of AITC. The volume of the jars were determined to be 490-mL. Each glass jar was capped with 0.79 mm thick Teflon lid. A threaded retaining ring was screwed down to hold the Teflon lid in place. For experiment I, a previously made stock solution of AITC 4.16 $\mu M \cdot L^{-1}$ was dispensed into the glass jars using a Hamilton 1701 series point style no. 2, fixed-needle, 10-μL syringe (no. 80075, Hamilton, Reno, Nev.) through a 3.175-mm diameter hole that was previously made in the Teflon lid. In experiment 1, 0.5, 1.0, 5.0 and 15.0 mL of water was added to 0.25 g of OMM. Labeling tape (Fisherbrand Colored Label Tape 19mm width, cat no. 11-880-5C, Fisher Scientific, Pittsburgh, Pa.) was immediately placed over hole. The tape was pierced using a Hamilton 26s needle (Hamilton, Reno, Nev., cat. no. 7748-19). This procedure was done to keep tape adhesive off of the sampling syringe that was later used for headspace sampling. This method helped minimize clogging of the Merlin Microseal septum (Agilent Technologies, Santa Clara, Calif., cat. no. 5182-3442). The hole was immediately covered with another piece of Fisher labeling tape to reduce the escape of gas. Headspace samples were taken using a Hamilton Gastight series 1705, 50-μL beveled HP style 2 tip syringe (#80930, Hamilton). Head space sampling was conducted at timed increments after a stock solution was injected into the glass jar or after OMM was added. Allyl isothiocyanate and OMM were placed into jars at timed intervals so evolution time would be identical for each jar. Jars were incubated at approximately 22 ± 2 °C before sampling.

The syringe was placed into the injection port of a Hewlett Packard 5890a Series II (Hewlett Packard Company, Palo Alto, Calif.) gas chromatograph with a flame ionization detector (GC-FID). The inlet port was fitted with a Merlin Microseal septum (Merlin Instrument, Halfmoon, Calif., kit no. 304, septum no. 2218) for manual injections. The column was a 60 m x 0.53 mm, 0.25 μ film (J&W Scientific, Folsom, Calif., DB1301, cat. no. 125-1361). The inlet and outlet temperatures were 200 and 250°C, respectively. The oven parameters were programmed for 60 °C for 1 min., and then increased by 5 °C per minute to a maximum of 150 °C. Detector response was quantified based on the equation for the AITC standard curve [$y = 910.38 (x) + 121.45$] which had an $R^2 = 0.84$. Analysis of the allyl isothiocyanate peaks were performed using Chemstation Software Module, Version 6.03 (Hewlett Packard, Palo Alto, Calif.). Experiments were repeated the following day.

Experiment I, Relative Humidity

Two treatments were used that included room relative humidity levels measured at 23% and levels modified to 75%. Polystyrene 5-mL microbeakers (cat no. 2-544-30, Fisher Scientific, Pittsburgh, Pa.) were placed into glass Mason jars. Room humidity was measured with a Hobo data logger (Onset, Inc. model H08-003-02, Pocasset, Mass.). Levels of 75% relative humidity were achieved by methods suggested by the manufacturer of Hobo data logger. This method suggested adding four grams of table salt followed by 4 mL of water. Deionized water (dd-H_2O) and table salt (NaCl) were added at rates suggested by Onset, Inc., the manufacturer of the Hobo data logger, to polystyrene 5-mL microbeakers (cat no. 2-544-30, Fisher Scientific, Pittsburgh, Pa.) and placed into glass Mason jars. The experiment was repeated. Commercially available AITC at 4.125 $\mu M \cdot L^{-1}$ concentration was injected into jars. One application was made to each jar and injections to jars were spaced on seven minute intervals to allow exact on hour sampling increments. Sampling occurred one hour after injection and then again two hours after injection.

Statistical Design of Experiment I, Relative Humidity

The experiment was a completely random design with two treatments (23% relative humidity and 75% relative humidity), and four replications with repeated measures taken at hours one and two. This experiment was repeated and data was compiled (2 blocks) and analyzed as one test (DF=1,1). Data were analyzed using Proc Mixed Model in PC SAS (SAS Institute, Cary, N.C.). Analysis for normality was tested and data was in acceptable ranges for normality.

Experiment II, Water and *Brassica*

Samples of 0.25 grams of ground whole oriental mustard meal (OMM) *Brassica juncea* (PI 458928) was placed into the glass Mason jars. Water (dd-H_2O) was applied as treatments at the rates of 0.5 mL, 1 mL, 5 mL and 15 mL with a battery operated 1 mL micropipette. Jars were immediately sealed with a Teflon cap and screw-on lid. AITC levels were measured after two and three hours. The experiment was repeated the following day.

Statistical Design of Experiment II, Water and *Brassica* Tissue

The experiment was a completely random design with four water treatments, two days (blocks), and two repeated measures at hours two and three after mixing with water. (DF=3,1). Statistical analysis using Proc Mixed in PC SAS (SAS Institute, Cary, N.C.) was used. Analysis for normality was performed and data fell in an acceptable range of normality.

Results and Discussion

In experiment I, AITC from headspace analysis of treatments containing a relative humidity of 75% had a mean of 2.16 $\mu M \cdot L^{-1}$, while treatments with 23% relative humidity had a mean of 3.64 $\mu M \cdot L^{-1}$. However when statistically analyzed, there was no differences between the two treatments (Fig. VII-1, pg. 130)[1]. This may have been due to possible condensation formation on the underside of Teflon lids. Thus, to determine the efficacy of AITC in different humidity conditions may require additional testing to be performed and containers should be thoroughly inspected for any possible build up of condensation.

In experiment II, treatments containing meal with 1.0 and 0.5 mL of dd-H_2O added had the highest AITC peaks and were statistically different from treatments containing larger volumes of water (5 and 15 mL). The GC-FID peak area of treatments containing amounts of 5 and 15 mL of water were significantly lower. The average mean

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[1] Figures are listed in the Appendix.

peak count for the 1.0 mL water treatment was 2.36 $\mu M \cdot L^{-1}$ whereas the 15 mL treatment mean average peak count was 0.99 $\mu M \cdot L^{-1}$ (Fig. VII-2, pg. 131).

Both experiments suggest that moisture plays a role in AITC concentrations evolving from pure forms of AITC as well as from oriental mustard meal; however the efficacy of these concentrations of volatiles may or may not be affected. Delaquis and Sholberg, (1997) reported that the gaseous form of AITC had a higher antibacterial potency than the liquid form. Furuya and Isshiki, (2001) suggested that the effective concentration of AITC can be reduced under high humidity conditions. However, AITC had stronger activity on tested bacteria in high humidity than lower humidity conditions. Vapor pressures of allyl isothiocyanate at relative humidities of 0 and 80 percent were similar and vapor pressure of AITC could be controlled when diluted with non-volatile oils (Sekiyama et al., 1994). If non-volatile oils have an effect on vapor pressure, greater concentrations of water used in experiment 2, may have had similar effects resulting in lower GC-FID peak counts. Evaporation behavior is not affected by high humidities (Sekiyama et al., 1994), however, unseen condensation may have formed on the underside of container lids, thus resulting in sorption and therefore resulting in lower AITC concentrations. Another possible reason of lower measured concentrations of AITC in the headspace may have been due to OMM being exposed to greater concentrations of water and possible degradation of glucosinolates in aqueous solution into other products. Water is needed for a hydrolysis reaction however, excess water may bound sites or diluted the substrate / enzyme system therefore eliminating and/or slowing evolution of ATIC. This could be similar to what Borek et al., (1995) reported where soil moisture had an effect on AITC half-life, as moisture levels increased half-life of AITC increased. To determine if this was occurring, sampling would need to be taken over longer periods of time as well as flushing the system of all AITC to determine total AITC formed from OMM. This was not performed due to time constraints and limitations of items needed to perform these tasks. Information concerning effects of moisture can be very beneficial to researchers by assisting to predict concentrations of AITC needed in a field environment where moisture levels can not be as easily controlled.

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Appendix -VII

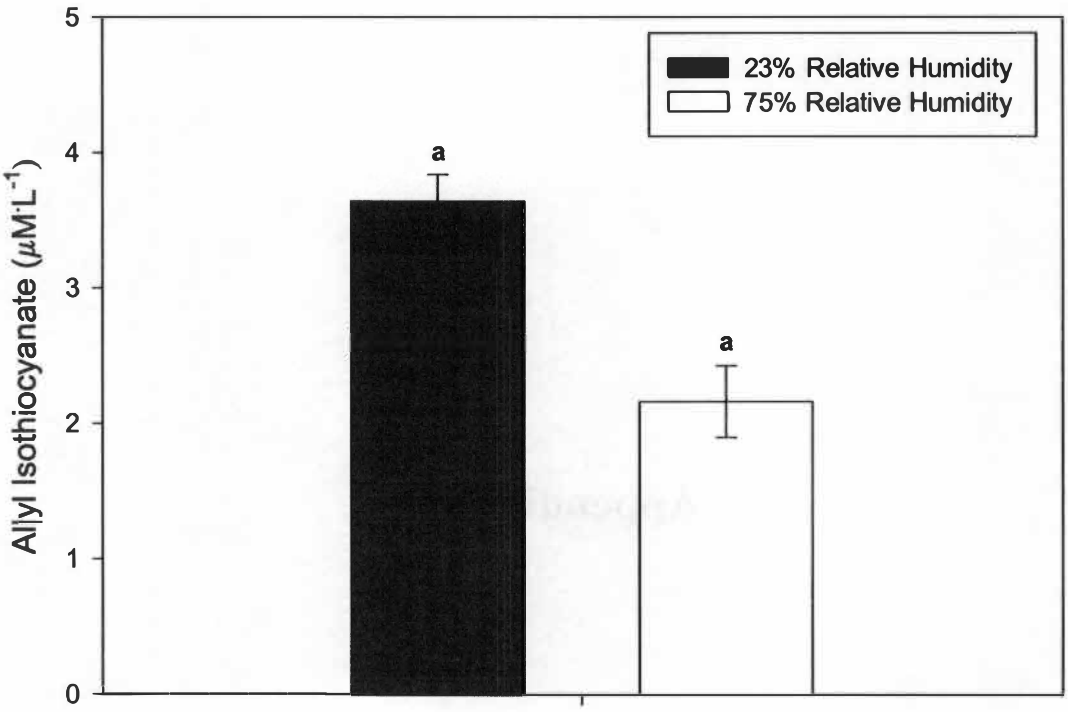

Figure VII-1. Mean (±SE) allyl isothiocyanate (AITC) concentration in glass containers with different relative humidity (23% and 75%). AITC (4.16 µM.L-1) was applied to each container. Analysis conducted using SAS, PROC MIXED where F= 17.51 and P=0.1493. Formula used to determine final µM concentration was; $y = 910.38 (x) + 121.45$ with an $R^2 = 0.84$.

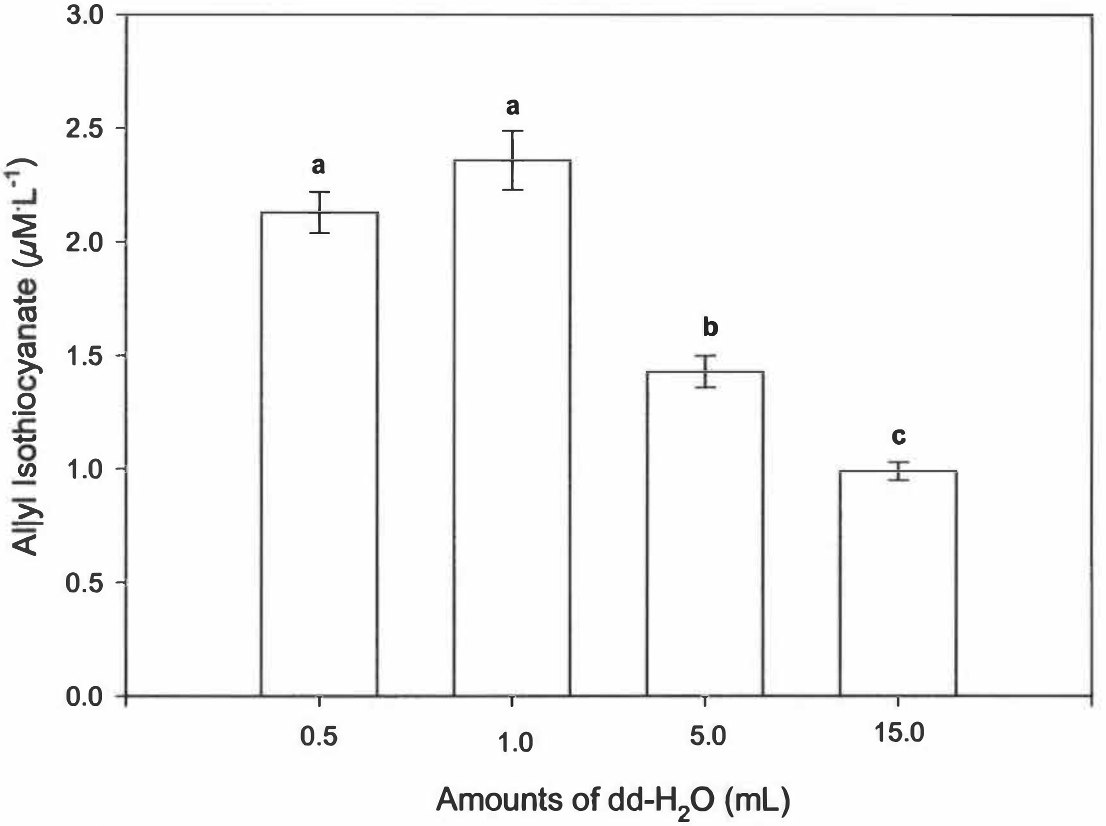

Figure VII-2. Concentrations of allyl isothiocyanate (AITC) evolving from whole ground mustard meal (0.25g) in different amounts of deionized water. Mean (±SE) AITC peak readings affected by the addition of water. Analysis was conducted using SAS, PROC MIXED, F=55.13 and P=0.004. Bars with different letters are significantly different at P<0.01, Duncan's multiple range test. Formula used to determine μM concentration from a standard curve was; $y = 910.38\ (x) + 121.45$, with an $R^2 = 0.84$.

Part VIII

FIELD TESTS FOR NEMATODE AND *PHYTOPHTHORA* CONTROL USING *BRASSICA* AMENDMENTS

Abstract

Tobacco and tomato production are beneficial to Tennessee's economy. Both crops are plagued with soilborne diseases. Black shank has been reported in every county where tobacco is grown. In tomato production as well as the production of many other vegetable crops, the southern root-knot nematode, *Meloidogyne incognita*, has been reported to reduce yields and cause severe losses. Methyl bromide has been used in various cropping situations and is very effective for various soilborne pathogens. However its use will be severely restricted by 2005. Other fumigants are being investigated, but their full potential in tomato and tobacco production areas of Tennessee are not fully known. The addition of *Brassica* amendments is another alternative to methyl bromide. *Brassica* tissues have been reported to produce toxic gases when mixed with soil and adequate moisture is available. Degradation products of *Brassica* tissues have been investigated in the laboratory and have had variable results in the field.

The purpose of this research was to investigate the efficacy of *Brassica* amendments in field situations. Weather conditions during evaluation caused some severe problems in tobacco production sites, however, in one test, high rates of *Brassica* amendments were correlated with better stand counts. In tomato research, areas previously infested with the root-knot nematode were rotated by growers, so other sites were selected. Newer selected sites had few if any root knot nematodes present, so effects of *Brassica* amendments on total vermiform nematode populations were evaluated. Results from use of high rates of *Brassica* tissues in one test suggested that tissues have the potential to reduce total nematode populations.

Introduction

Tennessee ranked fifth among 16 states in tobacco production in the United States. Both burley and dark fired tobacco *Nicotiana tabacum* L., are produced in the state. During 1997, Tennessee harvested 51,000 acres of burley tobacco with a state average of 1,830 lbs of product produced per acre. The total value of burley tobacco production was valued at $176,020,000 for 1997 with growers receiving an average of $1.88 per pound. (Danekas, 1998, Tennessee Department of Agriculture)

During 1998, approximately 3,400 acres of tomatoes were planted, of which 3,200 acres were harvested. The average yield per acre was 250 cwt. (Danekas, 1998). Cash receipts from farm marketing of tomatoes in Tennessee during 1998 were valued at $30,400,000. During 1998, record level prices ($38.00 per cwt.) and the second highest yield on record resulted in excellent returns to growers of fresh market tomatoes. Acreage in fresh market tomatoes has steadily declined from 1991s high of 5,100 acres planted and 1998s acreage of 3,400. The 1998 growing season resulted in the lowest acreage planted since 1976.

Tobacco and tomato production made up approximately 8.6% of the 1997 farm cash receipts (total of receipts also included livestock) for the 1997 season and both crops are considered a large portion of the agricultural economy in Eastern Tennessee (Tennessee Department of Agriculture, 1998).

Soil fumigation is an important agricultural practice for controlling soil-borne diseases, soil-inhabiting arthropods, and weed seeds (Ben-Yephet and Frank, 1989; Malathrakis, 1989; Mason-Sedun, 1986; Matthiesen et al., 1996). Fumigation usually is selected when growers are not able to rotate into other non-infested areas due to limited production acreage.

Methyl bromide is one of the most effective soil fumigants used in agriculture and forestry production, because it provides simultaneous control of numerous pests. Methyl bromide has been used in several production areas of Tennessee (Burgess et al, 2004, Kearney and Coffey, 1982, Sperry, 1997). Areas of crop production in which methyl bromide has been utilized include: strawberry (Sperry, 1997), forestry (Cordell, 1989), floral (McDonald, 1993), nursery (Juzwik, 1996), tomato (Kearney and Coffey, 1982) and tobacco production (Burgess et al., 2004). Because of environmental problems (Thomas, 1996, USDA-EPA) associated with methyl bromide, excessive costs of fumigation, and an international ban on usage (effective 2005), alternative pest control techniques and/or chemicals must be identified. Use of resistant cultivars and/or other pesticides sometimes aid in the control of pests of these crops. However, growers often reject resistant cultivars and/or high costs associated with fungicide, insecticide / nematicide usage.

Methyl bromide (Bromo-gas) manufactured by Great Lakes Chemical is manufactured in formulations that consist of 67% methyl bromide and 33% chloropicrin. This formulation is used in conventional tobacco plant beds and in field tomato production systems. Methyl bromide is among one of the most important pre-plant soil fumigants available (Noling and Beck, 1994). However, to obtain effective control of pests in either cropping system, methyl bromide must be used at rates that are not feasible, due to current pricing. To reduce input costs growers have selected other methods of pest control. In tobacco production, many producers use resistant varieties in combination with mefenoxam to aid in control of black shank (Burgess et al., 2004; Csinos et al., 1994; Nesmith et al., 1982). In tomato production methyl bromide is no longer applied as a broadcast application but applied in rows and injected under polyethylene mulch to help reduce damage caused by several soilborne diseases and weed infestations (Kearney and Coffey, 1982; Locascio et al., 1997; Malathrakis, 1989).

Methyl bromide (BROMO-GAS), chloropicrin, dazomet (BASAMID), and sodium methyldithiocarbamate (VAPAM, metham, metham sodium, metam sodium, SMDC) are some of the more recently used and highly recognized fumigants which have been used in agriculture and forestry production systems (Gilreath et al., 1994, Thomas, 1996). Other products which have been used in the past include the following; Vorlex (methyl isothiocyanate 20%, 1,3-dichloropropene and chlorinated C_3 hydrocarbons, 80%; Nor-Am), Telone (1,3-dichloropropene 92%, chloropicrin 2% and related C_3 hydrocarbons; DowElanco) Mylone (3,5-dimethyl-tetrahydrol-1,2,3,2 H-thiadiazine-2-thione) and Telone C17 (1,3-dichloropropene 76.3%, chloropicrin 17.1%, and inert ingredients 6.6%; DowElanco). Often the active ingredients of fumigants are mixtures of chemicals and are used with other control techniques (Miller and Clark, 1970; Overman, 1986). Each fumigant mentioned has the potential to be effective in controlling various fungi, bacteria, nematodes, insects, and weed species found in many of the production systems commonly utilized in East Tennessee (Gilreath et al., 1994; Miller and Clark, 1970; Overman and Jones, 1986; Thomas, 1986).

Dazomet is commercially available as Basamid and is registered for use in turf, nursery production, and tobacco seed beds. It is considered an alternative to methyl bromide and the active ingredient (tetrahydro-3,5-dimethyl-2H,1,3,5-thiadizine-2-thione) breaks down to methyl isothiocyanate (MITC). Basamid is manufactured as a granular formulation that is extremely stable until applied to moist soil and in water has a half-life ranging from 1.46 to 8.6 hours depending on pH. MITC accounts for the majority of the fumigants activity, however other products may form. Gases and volatile liquids formed by this material diffuse upward through the soil air spaces forming toxic compounds affecting various living organisms (Juzwik, 1994).

Dazomet was as effective as methyl bromide in controlling *Phytophthora*, *Pythium,* and *Fusarium* (McElroy, 1985). Metam-sodium at 935 $L \cdot ha^{-1}$ and dazomet applied at 387 $kg \cdot ha^{-1}$ performed well in controlling a *Pythium* spp. and *Fusarium solani*, however control was somewhat lacking for *Phytophthora parasitica* var. *nicotianae* (Csinos et al., 1997).

Alternative pest control products are being investigated due to the loss of methyl bromide and alternatives often pursued by researchers include materials that are environmentally friendly. Current research includes investigations of the efficacy of *Brassica* tissues and their ability to inhibit or reduce various pest populations. The research is focusing on hydrolysis products of the glucosinolates present in *Brassica* tissues and the efficacy of the active degradation product, allyl isothiocyanate (AITC). AITC is production from sinigrin, a glucosinolate that is released by many *Brassica* species when these plant residues are amended to soil.

Brassica juncea (PI 458928) is known to have high levels of aliphatic glucosinolates, with one major glucosinolate being sinigrin. Compounds formed from hydrolysis of sinigrin have the potential to reduce populations and possibly control two highly recognized destructive diseases that occur in tomato (*Lycopersicon esculentum*

Mill.) and tobacco (*Nicotiana tabacum* L.) production in Tennessee. Other products such as dazomet and Telone C-35 have been used in crop production; however, efficacy for weed control is lacking in geographic areas where tomato and tobacco production occurs in Tennessee.

Black shank (*Phytophthora parasitica* var. *nicotianae*) of tobacco is an annual problem in tobacco production areas of Tennessee. This disease can cause drastic yield loss if preventative measures are not established. Most commonly utilized practices for control of this disease include the following: rotation and use of a soil applied fungicide in conjunction with resistant varieties. Growers infrequently rotate due to limited production sites, so the fungicide mefenoxam commonly known as Ridomil Gold is used for control of black shank with varieties with a level of resistance of four or greater are used (Burgess et al., 2004). Black shank has been reported to overwinter in soil as chlamydospores and these spores may persist from season to season if an adequate host is available (Erwin, et al. 1983).

The addition of *Brassica* amendments would also be considered as adding organic matter to the soil. The addition of organic matter to soil influences diverse and important biological activities (Widmer et al., 2002). Hodges (1991) suggested that organic matter added to the soil improves soil structure, erosion control, water relationships, buffering capacity, is an energy source for microbes, and suppresses of some plant pathogens. Populations of plant parasitic nematodes decreased and saprophytic nematode populations increased with the addition of organic matter (Mojtahedi et al., 1991). The mode of action of *Brassica* amendments is purportedly by production of AITC. AITC has been reported to control plant parasitic nematodes, however the effect on total vermiform nematode populations has not been reported to date.

This research was conducted to determine if compounds released from the breakdown of cruciferous plant materials are capable of effectively controlling tobacco black shank and vermiform nematodes in tomato production, and to compare the effects of *Brassica* tissues on vermiform nematode populations with other alternative fumigants used in tomato production.

Materials and Methods

Mustard meal (whole ground oriental mustard seed/hull) was obtained from Poulenger USA, Inc., Lakeland Fla. for years 2002 and 2003. It was shipped in 22.67 kg moisture resistant plastic lined paper bags. Whole ground mustard meal (OMM) was obtained from Wisconsin Spice, Inc., Berlin, Wisconsin, for plots established in 2004.

In the tobacco experiments, black shank had been previously reported in all field plots. In all tomato field experiments, tomato production had been previously established over several years, but very few RKNs were observed in samples collected at all locations. Populations of all vermiform (worm like, no cysts or swollen) nematodes were determined in the samples.

Experiment I, Tobacco Field 2002.

During 2002, a tobacco research plot was established in Grainger County on a private farm located in the community of Blaine, Tenn. During the same year a duplicate plot was established on the Tobacco Experiment Station Located in Greeneville, Tenn.

The statistical design was a randomized complete split block design containing two tobacco varieties NC129 and TN90. The field plots contained four rows measuring 9.14 m x 1.22 m with 3.05 m alleys between blocks. Each field received typical herbicide applications for tobacco production. Herbicide applications included pendimethalin (Prowl) and clomazone (Command) and transplants in trays were drenched with imidacloprid (Admire) insecticide. The 2002 season was very difficult for most growers as little if any rainfall occurring during the season. The Grainger County site did not receive rainfall until after August 1. No plants survived. On the Tobacco Experiment Station, the experiment only received two irrigations with an overhead system. The majority of plants died by mid to season end. No data was obtained from either location.

Experiments II and III, Tobacco Field Experiments during 2003 and 2004.

During 2003 and 2004, only one location for each year was used. The sites were located at the Tobacco Experiment Station located in Greeneville, Tenn. The soil type for this location was classified as a Decatur silty clay loam, clayey, Kaolinitic, thermic Rhodic Paleudults. Each design was a randomized complete block design. Herbicide applications included pendimethalin (Prowl) and clomazone (Command) at labeled rates during 2003. No herbicide was used during 2004. In both years transplants in trays were drenched with the insecticide imidacloprid (Admire) at labeled rates. Mefenoxam marketed as Ridomil Gold was used for comparison, since it is normally used by growers in conjunction with black shank resistant (tolerant) varieties with levels of resistance of four or greater.

During 2003 for experiment II, the tobacco variety NC129 (level two resistance for black shank) was selected. The treatments included the following: control, Ridomil Gold 1.17 $L{\cdot}ha^{-1}$ pre-plant incorporated (ppi) followed by 1.17 $L{\cdot}ha^{-1}$ applied a layby, Ridomil 2.34 $L{\cdot}ha^{-1}$ ppi followed by 1.17 $L{\cdot}ha^{-1}$ at layby, Ridomil Gold 1.17 $L{\cdot}ha^{-1}$ ppi, OMM at 227 $kg{\cdot}ha^{-1}$, OMM at 907 $kg{\cdot}ha^{-1}$, OMM at 454 $kg{\cdot}ha^{-1}$, Ridomil Gold 1.17 $L{\cdot}ha^{-1}$ with 227 $kg{\cdot}ha^{-1}$ of OMM. Ridomil Gold was applied with a CO_2 backpack spray with a two nozzle sprayer that adequately covered the plot area. Mustard meal was applied by hand and evenly spread over the plot area. Mustard meal and mefenoxam treatments were applied and tilled into the top 10.16 cm of soil. Rain (<0.64 cm) occurred within an hour of treatment. The plots were not covered. Plots contained four rows at 1.07 m row spacing. Plot length was 9.14 m with 3.05 m alley ways. Plants were visually inspected and plants that turned yellow or had wilting symptoms were recorded as positive for black shank. Plant stand counts were made at six and again at eight weeks after transplanting.

During 2004 for experiment IV, the variety Clays 403 was selected, drip irrigation and 1.52-m wide, 1-mL thick embossed PE sheeting was used to cover the plots soon after treatments were made. A tractor with a Rain-Flo raised-bed plastic mulch applicator (model 2600, East Earl, Pa.) was used to apply the plastic in a hilled or raised bed fashion. Edges of the plastic were covered with soil. Treatments included; control, Ridomil Gold pre-plant-incorporated at 2.34 $L{\cdot}ha^{-1}$ rate, and rates per acre basis of 4483.39 $kg{\cdot}ha^{-1}$ or 2241.69 $kg{\cdot}ha^{-1}$ of OMM. Ridomil Gold was applied with a CO_2 backpack sprayer with a two nozzle boom. Treated area measured 0.91 m wide by 6.10 m long for the finished plot size. Alley ways between treatments were 2.44 m in length.

Water was applied for eight hours after coverage of PE sheeting and eight hours the following day to achieve 253,887 $L \cdot ha^{-1}$ on day one followed by an additional 253,887 $L \cdot ha^{-1}$ on day two.

Experiments IV and V, Tomato Field Experiments at Fletcher NC, 2003 -2004

The objective was to determine the effect of compost materials in plasticulture tomato production. The effects of OMM, compost, and Telone C35, a methyl bromide alternative were also evaluated for nematode suppression. Treatments and field design were not changed in the Fletcher, NC location from 2003 to 2004. Soil type for this location is classified as a Cordus loam, fine-loamy, mixed, mesic, Fluvaquentic, Dystrudepts. Tomato research plots were established on the North Carolina Horticultural Research Station in spring of 2003 (Experiment IV) and again in 2004 (Experiment V). Four blocks (replicates) were designed running north and south adjacent to the creek located on the south side of the experiment station in a sandy loam soil. The statistical design of the plots was a randomized complete block design containing six treatments, totaling 24 plots. Plots were approximately 7.62 m in length. Beds were 68.58 cm wide on 1.52 m centers. Tomato cv. "Celebrity" was used. Treatments included; a control, Telone C-35, compost at 67.21 metric tons per hectare, OMM at 1120.84 $kg \cdot ha^{-1}$, OMM at 2241.68 $kg \cdot ha^{-1}$, and OMM at 1120.84 $kg \cdot ha^{-1}$ with compost at rate as described above.

Compost consisted of mushroom composted obtained from Monterey Mushroom, Loudon, Tenn. Compost and OMM treatments were tilled into the soil at a 15.24 cm depth and beds were reformed during laying of plastic sheeting soon after mixing of treatment materials. Telone C-35 (7.10 lbs -1,3-dichloropropene, 0.46 kg - chloropicrin per liter of Telone C-35, Dow Agro Sciences, Indianapolis, Ind.) was applied at 327.28 $L \cdot ha^{-1}$ rate, when plastic was laid down. Compost was applied at rate of 67.21 $tonnes \cdot ha^{-1}$. Compost and mustard treatments were tilled into the bed at a 15.24 cm depth and beds were reformed at the time of laying of plastic. During the 2003 experiment IV, compost and OMM was applied on May 14 and May 25 during 2004 experiment V. All plots were irrigated with drip irrigation for four hours on date of treatment application (253,887 $L \cdot ha^{-1}$) followed an eight hour water application (253,887 $L \cdot ha^{-1}$) on the following day. Irrigation occurred throughout the season.

Experiment VI and VII, Tomato Field Experiments - Knoxville, Tennessee Location 2003 and 2004.

Tomato research plots were established on the Knoxville Experiment Station in fall of 2002 and the fall of 2003. The second year of the experiment was moved several yards from the initial location. Each experiment contained four blocks (replicates), which were designed running north and south adjacent to the Tennessee River. During the spring of 2003 (Experiment VI), the variety 'Celebrity' was used and during 2004 (Experiment VII) the variety 'Mountain Fresh' was selected. Control plots and alley ways were seeded with rye. All plots were limed to raise pH to 6.5 to 7.0. *Brassica juncea* ISCI.20 was used on all *Brassica* treatments. *Brassica* was seeded at 18 g per 7.62 m by 1.22 m for each plot containing *Brassica.*

In experiment VI, the plots were taken from a testing site in which an ongoing research project was being conducted. The statistical design of the plots consisted of a randomized complete block design containing five treatments, totaling 20 plots. The data was collected from treatments for each block of the original test site which included four blocks and 32 treatments. However, only five treatments were utilized in this study containing applications of the following materials including the following: control, 1120.84 $kg \cdot ha^{-1}$ OMM, 2241.68 $kg \cdot ha^{-1}$ OMM, 4483.38 $kg \cdot ha^{-1}$ OMM, and dazomet (Basamid) at 180.45 $kg \cdot ha^{-1}$.

In experiment VII, nine treatments were evaluated in a randomized complete block design that included the following: control, 560.42 $kg \cdot ha^{-1}$ OMM, 1120.84 $kg \cdot ha^{-1}$ OMM, 2241.68 $kg \cdot ha^{-1}$ OMM, Spring grown *Brassica* that was mowed and incorporated into the soil with 217.58 $kg \cdot ha^{-1}$ of granular dazomet (Basamid, Certis or BASF corporation), *Brassica* grown in the Fall season that was incorporated into the soil, *Brassica* grown in the Fall season and incorporated with 1120.84 $kg \cdot ha^{-1}$ of OMM, 67.21 $tonnes \cdot ha^{-1}$ of compost and the last treatment included 67.21 $tonnes \cdot ha^{-1}$ compost with 1120.84 $kg \cdot ha^{-1}$ of OMM. Dazomet was incorporated into the moistened soil at 21 days prior to bed formation. Overhead irrigation for eight hours was used to water-seal the beds and to keep the soil moist during a critical period for volatilization. Fumigated beds were re-tilled to aid in release of possible remaining fumigant. Beds were formed during tillage. Compost and/or OMM were applied and tilled into the soil and immediately following treatment, plots were covered with plastic mulch.

Tomato transplants were planted through the plastic at 44.45 cm intervals. After transplanting, a drip irrigation system was used for retaining moisture levels and applying fertilizers. During the season the plants received trickle irrigation equal to 2.54 cm per week. Fertigation through the drip irrigation was applied within one week of transplanting and a starter solution of 9-45-15 was applied at the rate of 8.09 $kg \cdot ha^{-1}$.

Field locations and treatments changed from 2003 and 2004 on the Knoxville Experiment Station. For each field test, nematode sampling occurred prior to treatments and three weeks after treatments.

Nematode Sampling

A 10.16-cm diameter soil auger was used to obtain soil core samples from each treatment area. Six soil cores were taken from each plot at a depth of 12.7 cm. Soil from each plot was placed into a 18.92-L plastic bucket and the soil cores were thoroughly mixed to obtain a homogeneous sample. One pint of soil was removed from the bucket and placed into a marked plastic bag and stored after departure from the field at 4 °C until nematode analysis. Soil samples were held no longer than 15 days in cold storage until populations could be determined.

A North Carolina-style elutriator (Byrd et al. 1976) was used for extracting and determining nematode populations from soil samples taken at each location. This technique incorporates soil into an aqueous solution of water and allows nematodes to be rinsed free from the soil and washed into mesh retaining screens. After elutriation, vermiform nematodes were placed into a grided counting dish to determine populations per 100 cm^3 of sampled soil. A dissection stereo microscope was used for identification purposes. In all locations, few if any root knot nematodes (RKN) were observed, so total nematode populations were counted. Readings reported from each sample included total vermiform nematodes present, including both plant-parasitic as well as free-living nematodes.

Results and Discussion

Results of the Tobacco Field Plots – 2002 – Experiment I.

The 2002 growing season was one of the worst seasons for tobacco production in Tennessee's history. The Grainger County field did not receive any rainfall after planting until the end of August. The Greeneville plot received irrigation twice after planting however, irrigation systems were utilized in other production areas later in the season. Each location was considered a failure.

Results of the Tobacco Field Plot – 2003 and 2004 – Experiment II and III.

Experiment II, during the 2003 season, rain occurred soon after treatments were made. However, rainfall may not have been adequate to activate the *Brassica* amendments. The variety NC129 was utilized in this field trial. NC129 has a level two black shank resistance on a scale of 0 to 10, with 0 having no resistance. The treatment containing OMM at the rate of 1120.84 $kg \cdot ha^{-1}$ had the lowest percent (32.08% stand) survival at eight weeks following transplant. This application rate was followed by the control (39.17% stand), Ridomil 1.17 $L \cdot ha^{-1}$ ppi with 560 $kg \cdot ha^{-1}$ OMM (40.75%), OMM at 560 kg ha^{-1} (45.10%), Ridomil 1.17 $L \cdot ha^{-1}$ ppi (47.97%), Ridomil 2.34 $L \cdot ha^{-1}$ ppi followed by 1.17 $L \cdot ha^{-1}$ layby (60.13%), OMM at 2241.68 $kg \cdot ha^{-1}$ (69.41%) and Ridomil 1.17 L ppi followed by 1.17 $L \cdot ha^{-1}$ at layby (74.65%) (Fig. VIII-1, pg. 162)[1].

Mefenoxam (Ridomil Gold) is highly water-soluble and heavy rainfall may leach the active ingredient through the soil profile. In previous experiments conducted on the Experiment Station split applications of mefenoxam provided higher numbers of

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[1] Figures are listed in the Appendix.

surviving plants and greater yields when compared to similar levels of mefenoxam applied only once at pre-plant incorporated (ppi) (data not published). Split applications are recommended by The University of Tennessee (Burgess et al., 2004) if field have a history of heavy infection from black shank.

Moisture levels may have been sufficient to activate OMM at 2241.68 kg$\cdot$ha^{-1} treatment because stand levels were similar to that of the mefenoxam treatments. Stand counts for the 2241.68 kg$\cdot$ha^{-1} OMM were not different from the Ridomil Gold 1.17 L$\cdot$ha^{-1} ppi + 1.17 L$\cdot$ha^{-1} layby or Ridomil Gold 2.34 L$\cdot$ha^{-1} ppi and 1.17 L$\cdot$ha^{-1} layby, but were different from stands of the Ridomil Gold 1.17 L$\cdot$ha^{-1} ppi and Ridomil Gold 1.17 L$\cdot$ha^{-1} ppi with 560.42 kg$\cdot$ha^{-1} of meal.

In experiment III, the field plot was irrigated to increase efficacy of *Brassica* amendment activation as well as develop vigorous plants. However, throughout the season the plot continually received rainfall reducing possible stress on the plant and reducing visual signs of yellowing and wilting. No plants appeared to have black shank symptoms up to six weeks after transplanting.

Experiments IV and V, Tomato production at Fletcher, NC.

In experiment IV conducted in 2003, the nematode populations were slightly lower after treatments except the compost alone were higher. There was no significant difference among nematode populations prior to treatment (P=0.14). Nematode populations were normally distributed (Shapiro-Wilk scale, W=0.96). Nematode populations after treatment, were statistically different when comparing treatments comprised of compost or 2241.68 kg$\cdot$ha^{-1} OMM to the Telone treatment. Other treatments were not statistically different from compost or 2241.68 $kg \cdot ha^{-1}$ OMM or Telone treatments. Means of nematode populations before and after treatments were made are shown (Fig. VIII-2, pg. 163).

In experiment V, conducted during 2004, all treatments except for the OMM with compost, had reduced nematode populations. Nematode populations were higher in the OMM with compost treatment with a mean of more than 3,880 vermiform nematodes after treatment. During statistical analysis, data failed the analysis for normality, Shapiro-Wilk scale (W=0.802). However, treatments containing OMM with compost were statistically different (P=0.074) from all other treatments with an increase in nematode populations after treatment with an average mean of 6,806 nematodes per 100 cm^3 of soil. The untreated control, Telone and OMM at 2,242 $kg \cdot ha^{-1}$ treatments had lower nematode populations. Means of nematode populations before and after treatments are shown (Fig. VIII-3, pg. 164).

Results of Nematode Control in 2003 Knoxville Location, Experiment VI.

There were no differences among treatments (P=0.37). Nematode populations were distributed normally. The 1120.84 $kg \cdot ha^{-1}$ rate of OMM had the greatest reduction of nematodes after treatment followed by OMM at 2241.68 $kg \cdot ha^{-1}$, then OMM 4483.38 $kg \cdot ha^{-1}$, dazomet and then the control. Nematode populations in the treatment including spring planted *Brassica* remained low prior to planting and after planting. The OMM at 1121 kg $L \cdot ha^{-1}$ treatment had the greatest reduction in nematode population of all treatments (Fig. VIII-4, pg. 165).

Results of Nematode Control in 2004 Knoxville Location, Experiment VII.

There were no significant differences in nematode populations after application among treatments (*P*=0.59). Nematode counts were distributed normally. The control had the greatest increase in nematode populations followed by plots with spring *Brassica* with dazomet. All other treatments had a reduction in nematode populations with compost treatments and OMM at 560.42 $kg \cdot ha^{-1}$ having the greatest reductions following treatment. Other treatments had lower nematode populations than the control (Fig. VIII-5, pg. 166).

General Discussion

Moisture plays an important role in efficacy of soil applied fumigants (Goring, 1962). Also, covering the soil with polyethylene sheeting may prolong the contact time of fumigants with the targeted pest. In the tobacco experiments, rainfall was limited in 2002 (Experiment I) possibly resulting in ineffective activation of *Brassica* amendments as well as causing crop failure. During 2003 (Experiment III), rainfall did occur throughout early to mid-season, however late season rainfall was limited. Inadequate rainfall may have been responsible for the poor control observed. Treatments containing lesser amounts of OMM may have not released high enough concentrations of AITC to provide adequate control. During 2004, plasticulture was used in experiment IV to help maintain moisture levels, and enhance activation of *Brassica* amendments. However, during 2004, rainfall occurred weekly, often two to four times a week throughout the growing season. Free water aids in spreading *Phytophthora*, but increased soil moisture may have reduced stress of the plant and appearance of symptoms. Variable results from these experiments suggests that moisture content and possibility of uneven distribution of inoculum may have played roles in variable results, but tests should be investigated to determine efficacy of AITC since control was observed in 2003 (experiment II) when using higher rates of OMM. Often a previous year's treatment may persist or other synergistic effects may occur, these affects may have an influence on following production season's nematode populations.

In an experiment conducted by Wang et al., 2002, the incorporation of *Crotalaria juncea* into soil increased populations of bacterivorous nematodes in short-term field tests. Results reported in tests conducted at the Fletcher location during 2003 (compost treatment) and 2004 (compost with 1120.84 $kg^{.}ha^{-1}$ meal), had some similar increases as Wang et al., (2002). Compost treatment was effective against plant-parasitic nematodes by raising levels of omnivorous and predator nematodes (Lopez-Perez et al., 2003). Increases in nematode populations occurred in 2003, with compost and again in 2004 consisting of treatments of compost with 1121 $kg^{.}ha^{-1}$ OMM. In this same test in 2004, *Brassica* amendments at high rates reduced total nematode populations to 0 in three of the four replications of the treatment consisting of OMM at 2242 $kg^{.}ha^{-1}$ and in treatments consisting of 2242 $kg^{.}ha^{-1}$ of OMM. During 2004, in experiment 5, Telone C-35 reduced total nematode populations, however effects were not significant. Stirling and Stirling (2003) reported that the addition of *Brassica* amendments reduced RKN levels, but not those of free-living nematodes. They attributed the ineffectiveness on free-living nematodes to low levels of AITC in which these nematodes were not affected and RKN were suppressed. Free-living nematodes populations tend to increase as food sources increase (Lopez-Perez et al., 2003), however this may have not been the case in experiments conducted at Knoxville locations or in some of the treatments at the Fletcher locations. Reductions in nematode numbers may have been caused by various factors since the environment was drastically changing in the spring to summer transition. Samples taken prior to treatments were taken during cooler temperatures (in soils without PE coverings), than samples taken after treatments (when the PE was applied). The soil covering may have produced an environment that was not as conducive to the free-living nematode populations, therefore reducing the populations. Bacterial-feeding nematode populations oscillate from day to day, similar as oscillating bacterial populations found within the soil community (Zelenev et al., 2004). There research demonstrated the bacterial feeding nematode populations did not simply increase in a sigmoid fashion after the addition of a organic amendment but populations changed daily and the changes were in a wave like pattern.

There was a great degree of variability in vermiform nematode populations within replications of all treatments for all locations. Many factors may have been responsible for these variations and therefore, if more testing is considered, more replications and subsampling may aid in reducing these variables. Due to these variations more research should be conducted to determine the affects of OMM and commercially available fumigants on vermiform nematode populations. As discussed earlier the addition of plant residues may increase some nematode populations while reducing others. Since plant parasitic nematode populations at these locations were extremely low, to none existent, total vermiform nematode populations were evaluated.

In experiments attempting to control black shank, one season of field data generated from OMM treatments and their effects are not sufficient to determine if by-products of OMM provide adequate control of this disease. Therefore, more research should be conducted to investigate OMM's potential to control disease.

The goal for integrated pest management is to incorporate various methods of control and to reduce pest populations to acceptable levels, while attempting to select methods which have the least effect on the environment. If future testing of *Brassica* tissues provides information concerning the control of plant pests while having no or limited effects on other soil inhabiting organisms, this natural product would be attractive to an integrated pest management program as well as an alternative for methyl bromide.

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Appendix – VIII

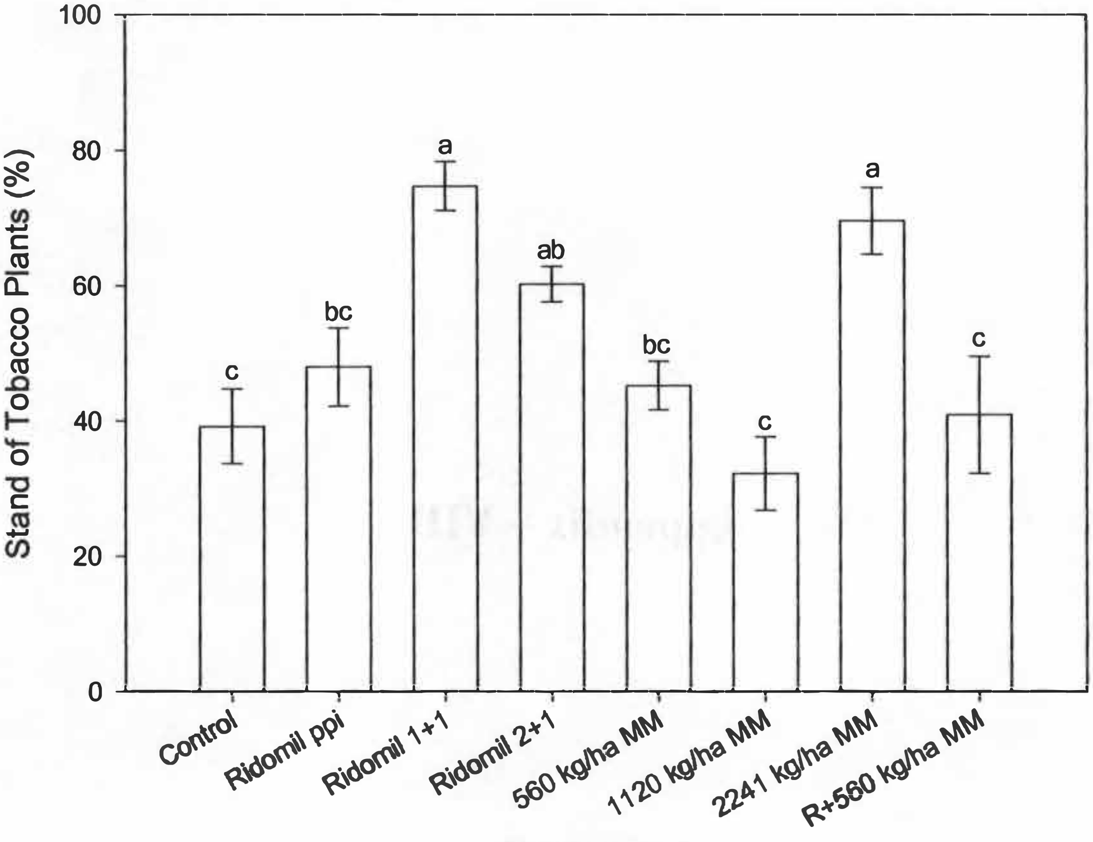

Figure VIII-1. Mean (±SE) stand counts of tobacco with different soil treatments. The treatments included an untreated control, Ridomil 1.17 $\mathrm{L{\cdot}ha^{-1}}$ ppi, Ridomil 1.17 $\mathrm{L{\cdot}ha^{-1}}$ ppi and again at layby, Ridomil 2.34 $\mathrm{L{\cdot}ha^{-1}}$ ppi and 1.17 $\mathrm{L{\cdot}ha^{-1}}$ layby, mustard meal (MM) 560 $\mathrm{kg{\cdot}ha^{-1}}$, MM 1120 $\mathrm{kg{\cdot}ha^{-1}}$, MM 2241 $\mathrm{kg{\cdot}ha^{-1}}$ and Ridomil 1.17 $\mathrm{L{\cdot}ha^{-1}}$ with 560 $\mathrm{kg{\cdot}ha^{-1}}$ MM. Analysis conducted with SAS using PROC ANOVA, $Pr>F=0.001$, $F=8.24$, $n=24$. Bars with similar letters are not significantly different ($P=0.05$) using Duncan's mean separation.

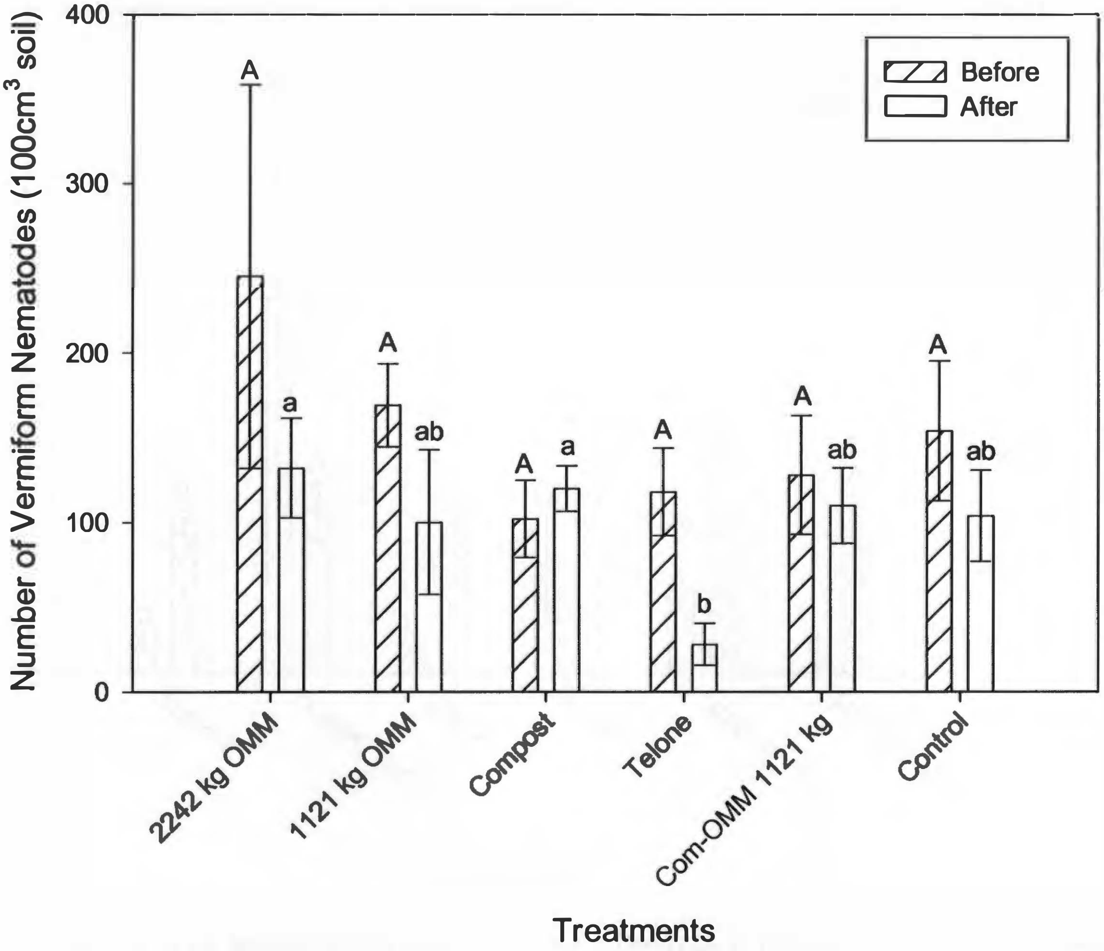

Figure VIII -2. Mean (±SE) vermiform nematode field populations before and after treatments during 2003 at Fletcher, NC. Treatments included, 2,242 $kg\cdot ha^{-1}$ whole ground mustard meal (OMM), 1,121 $kg\cdot ha^{-1}$ OMM, 67.21 $tonnes\cdot ha^{-1}$ compost, Telone at 327.28 $L\cdot ha^{-1}$, and compost at 67.21 $tonnes\cdot ha^{-1}$ with 1,121 $kg\cdot ha^{-1}$ of OMM and the control (no treatment). Soil samples were taken prior to treatment and again two weeks after treatment. Bars with hatched marks represent nematode populations before treatment and empty bars represent nematode populations after treatment. The analysis of data before and after treatment was conducted using SAS, PROC GLM, values for Before F=0.90 and $Pr>F$=0.5012, After was F=1.90 and $Pr>F$=0.1436, for treatments. There were no statistical differences Before or After treatments; however, analysis was conducted using Duncan's mean separation and tested at (P = 0.05) to aid in determining what treatments should be considered in future studies. Bars with different patterns were tested separately. Bars with like letters are not significantly different.

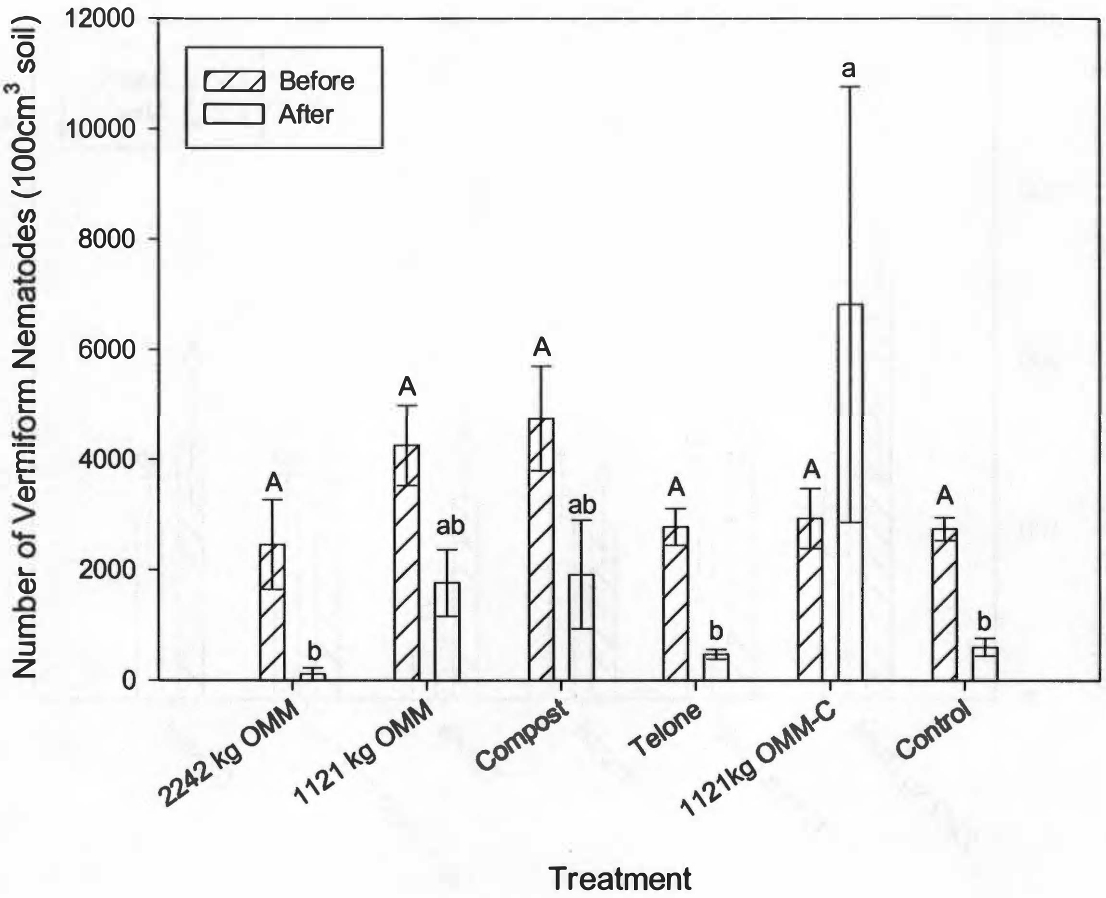

Figure VIII-3. Mean (±SE) vermiform nematode populations during 2004 before and after treatments at Fletcher, NC. Treatments included; mustard meal (OMM) at 2242 $kg^{.}ha^{-1}$, OMM at 1121 $kg^{.}ha^{-1}$, compost at 67.21 $tonnes^{.}ha^{-1}$, Telone at 327.28 $L^{.}ha^{-1}$, compost at 67.21 $tonnes^{.}ha^{-1}$ with 1121 $kg^{.}ha^{-1}$ OMM, and a control (no treatment) in the field test. Bars with hatched marks represent nematode populations before treatment and empty bars represent nematode populations after treatment. The analysis of data before and after treatment was conducted using SAS, PROC GLM, values for Before F=2.10 and Pr>F=0.1130, and After was F=2.19 and Pr>F=0.1003, for treatments. There were no statistical differences Before or After treatments; however, analysis was conducted using Duncan's mean separation and tested at (P = 0.05) to aid in determining future treatments to be used. Bars with like letters are not significantly different. Empty bars were tested separately from bars with hatching.

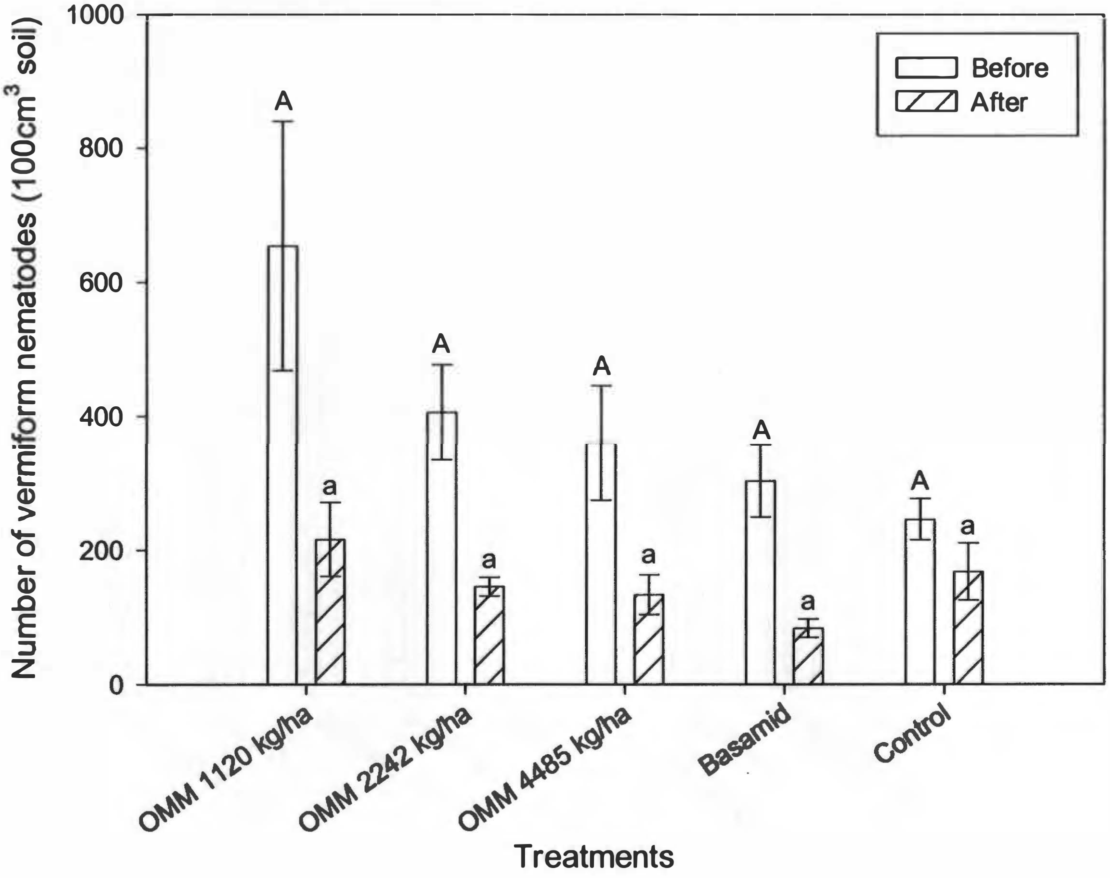

Figure VIII-4. Mean (±SE) vermiform nematode populations during 2003 before and after treatments at the Knoxville Experiment Station. Treatments were 1120 kg·ha^{-1} mustard meal (OMM), OMM at 2242 kg·ha^{-1}, OMM at 4485 kg·ha^{-1}, Basamid at 180.45 kg·ha^{-1} and a control (no treatment). Bars with hatched marks represent nematode populations after treatment and empty bars represent nematode populations before treatment. The analysis of data before and after treatment was conducted using SAS, PROC GLM, values for Before F=2.44 and Pr>F=0.0925, and After was F=1.91 and Pr>F=0.1613, for treatments. Bars with like letters are not significantly different. Bars with different patterns were tested separately.

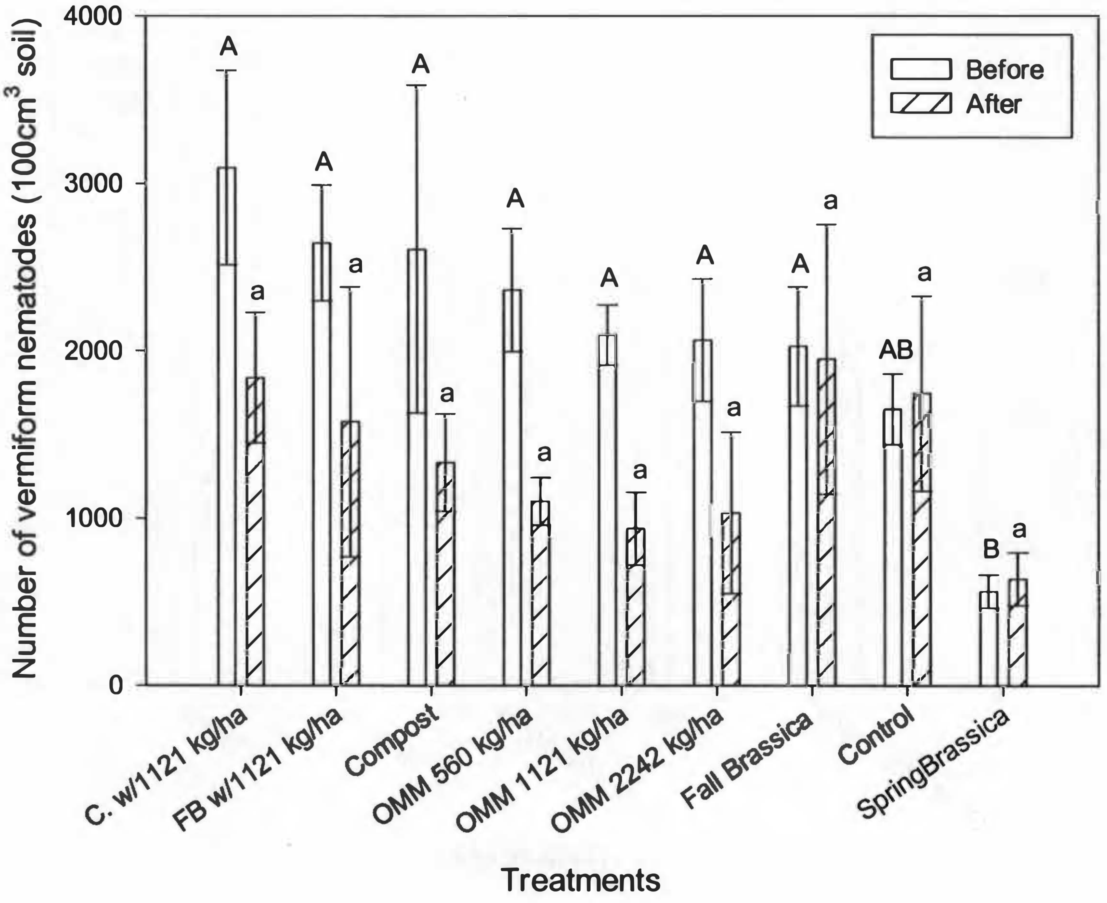

Figure VIII-5. Mean (±SE) vermiform nematode populations during 2004 before and after treatments at Knoxville, TN. Treatments were 67.21 tonnes·ha^{-1} of compost with 1121 kg·ha^{-1} mustard meal (OMM), previously planted fall brassica with additional 1121 kg·ha^{-1} OMM, 67.21 tonnes compost·ha^{-1}, OMM at 560 kg·ha^{-1}, OMM at 1121 kg·ha^{-1}, OMM at 2242 kg·ha^{-1}, previously planted fall *Brassica* incorporated into soil four weeks prior to initial sampling, control (no treatment) and spring planted *Brassica.* Bars with hatched marks represent nematode populations after treatment and empty bars represent nematode populations before treatment. The analysis of data before and after treatment was conducted using SAS, PROC GLM, values for Before F=2.48 and Pr>F=0.0372, and After was F=0.78 and Pr>F=0.6271, for treatments. Analysis conducted separately (before, after) with Duncan's mean separation to aid in determining future treatments. Tested at (P = 0.05). Bars with like letters are not significantly different. Bars with different patterns were tested separately.

Vita

Darrell Donaldson Hensley was born in Memphis, Tennessee in 1961. He attended Memphis Harding Academy and graduated from Germantown High School in 1979. He attended college at Memphis State University, now known as the University of Memphis and completed requirements for a Bachelor of Science in Agriculture Science at the University of Tennessee at Martin. He was employed at Memphis Packing Company in Memphis for a brief period and then worked for the University of Tennessee West Tennessee Experiment Station as an Extension Assistant in the Department of Entomology and Plant Pathology in 1984. Following this employment, Mr. Hensley pursued and obtained a Master of Science Degree in 1988 in Plant Pathology from The University of Tennessee. Mr. Hensley married his wife Greta during his pursuit of this degree. After obtaining a Master of Science degree, Mr. Hensley was re-hired by the University of Tennessee, Agricultural Extension Service as an Extension Assistant and was promoted several times. His areas of responsibility included the National Pesticide Impact Assessment Program (NAPIAP) and disease control in burley tobacco. He currently coordinates Tennessee's Pest Management Information Network and is Co-chair of Extension's Agroterrorism Team. Mr. Hensley and his wife had one child, Kaitlin in 1992. Mr. Hensley began working on a Doctor of Philosophy in Plant and Soil Sciences while employed in the department of Entomology and Plant Pathology in a full-time position as an Assistant Extension Specialist. During his studies Mr. Hensley and his wife had another child named Samuel. Mr. Hensley completed requirements for his Doctor of Philosophy in 2004.