To the Graduate Council:

I am submitting herewith a thesis written by Andrew Price entitled "Quantification of volatile compounds produced during simulated biofumigation utilizing Indian mustard degrading in soil under different environmental conditions". I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant and Soil Sciences.

Carl E. Sams

Carl E. Sams, Major Professor

We have read this thesis
and recommend its acceptance:

John T. Ammons

David E. Clayton

Accepted for the Council:

Lawrence Winkel

Associate Vice Chancellor and
Dean of The Graduate School

# QUANTIFICATION OF VOLATILE COMPOUNDS PRODUCED DURING SIMULATED BIOFUMIGATION UTILIZING INDIAN MUSTARD DEGRADING IN SOIL UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Andrew Jennings Price
December 1999

AG-VET-MED.
Thesis
99
.P77

# DEDICATION

This thesis is dedicated to my parents

Mrs. Loretta Price

and

Mr. Michael Price

who have given me an invaluable sense of self

ACKNOWLEDGMENTS

There are many people to whom I am very grateful for making my experience at the University of Tennessee memorable. I appreciate the guidance from my Thesis Committee, Carl Sams, Dennis Deyton, and Tom Ammons. I thank the Plant and Soil Science Department for its contribution to my education and its financial support.

To my coworkers Craig and Stephanie I am indebted for your friendship and knowledge. To my brother John and friends Edwin, Sloane, John, Ron, Kathy, Jeff, Danny, Tommy, Vanessa, Cheryl, Aaron and Ryan, thank you for your camaraderie.

I am most grateful to my wife Lesley, for her love and support.

## Abstract

One of the main pesticides used for soil fumigation is methyl bromide, a broad spectrum soil fumigant. Methyl bromide was listed in the 1993 Montreal Protocol as an ozone-depleting compound. Because of this listing, the production of methyl bromide will be frozen at 1991 levels until importation and production are completely halted in the year 2005 (U.S. Clean Air Act). Biofumigation and solarization are strategies that could be inexpensive solutions to control soil pathogens, nematodes and weeds. The objective of this research was to quantify the amount and composition of all volatile compounds, including isothiocyanates (ITC), produced from degradation of two Indian mustards (*Brassica juncea* L.) in a simulated biofumigation strategy under different environmental conditions. The environmental variables studied were soil texture, soil temperature, soil moisture, soil pH, and autoclaved and non-autoclaved soil. Higher concentrations of ITC were detected in biofumigation at higher soil temperature and lower soil moisture. Higher concentrations were also detected in sandy loam textured soils and at lower soil pH. Autoclaved soil had higher ITC concentrations than non-autoclaved soil. Thus soil microbes are implicated in the degradation of ITCs. This information is important in developing a field plan for testing a biofumigation and/or solarization strategy that would meet the requirements of an effective and viable soil fumigant for growers.

## Table of Contents


| CHAPTER | | PAGE |
|---|---|---|
| 1. | INTRODUCTION | 1 |
| 2. | OBJECTIVES | 3 |
| 3. | LITERATURE REVIEW | 4 |
| | *Brassica* Taxonomy and Anatomy | 4 |
| | Glucosinolates | 5 |
| | Biofumigation and Solarization | 8 |
| 4. | METHODS and MATERIALS | 12 |
| | Plant Sample Preparation | 12 |
| | Volatile Sampling and Analysis | 13 |
| | Chromatography | 13 |
| | Soils | 19 |
| | Plant Material | 20 |
| | Experiment 1 | 21 |
| | Experiment 2 | 21 |
| | Experiment 3 | 22 |
| | Experiment 4 | 22 |
| | Experiment 5 | 23 |
| | Experiment 6 | 24 |
| 5. | RESULTS | 25 |
| | Soil Analysis | 25 |
| | Volatile Analysis (Experiments 1-5) | 26 |
| | Experiment 1 | 29 |
| | Experiment 2 | 39 |
| | Experiment 3 | 31 |
| | Experiment 4 | 32 |
| | Experiment 5 | 33 |
| | Experiment 6 | 33 |
| | Volatile Analysis (Experiment 6) | 34 |
| 6. | DISCUSSION | 47 |
| | REFERENCES | 53 |
| | APPENDICES | 59 |
| | VITA | 114 |

## List of Tables


TABLE PAGE

1. Particle Size Analysis for Field Soils....25
2. Particle Size Analysis for Oven Heated, Autoclaved and Field Soils....25
3. Total Organic Carbon for Oven Heated, Autoclaved and Field Soils....26
4. pH Analysis for Field Soils....26
5. Average AITC Concentrations ($\mu$mol/L ± SD) for Experiment 1....30
6. Average AITC Concentrations ($\mu$mol · $L^{-1}$ ± SD) for Temperature Treatments in Sandy Loam Textured Soil....31
7. Average AITC Concentrations ($\mu$mol · $L^{-1}$ ± SD) for Moisture Treatments in Sandy Loam Textured Soil....32
8. Average AITC Concentrations ($\mu$mol · $L^{-1}$ ± SD) for pH Treatments in Clay and Sandy Loam Textured Soil....33
9. Average AITC Concentrations ($\mu$mol · $L^{-1}$ ± SD) for Autoclaved and Non-autoclaved Treatments in Clay and Sandy Loam Textured Soil....34
10. Least square means and standard errors for AITC concentrations ($\mu$mol · $L^{-1}$) for time, texture, temperature, cover and microbe effects....35
11. Least square means and standard errors for AITC concentrations ($\mu$mol · $L^{-1}$) for time * temperature, time * pH, and microbe * cover interactions....37
12. Least square means and standard errors for AITC concentrations ($\mu$mol · $L^{-1}$) for time * microbe, temperature * microbe, and time * cover interactions....38
13. Least square means and standard errors of $CO_2$ (%) for time, temperature, and microbial effects....39
14. Least square means and standard errors for % $CO_2$ for microbe * cover, time * microbe, and temperature * microbe interactions....40

15. Least square means and standard errors for % $CO_2$ for time*cover interactions.....41

16. Least square means and standard errors for sec-butyl ITC and 3-butenyl ITC concentrations ($\mu$mol · $L^{-1}$) for time, temperature, microbe effects.....42

17. Least square means and standard errors for sec-butyl ITC and 3-butenyl ITC concentrations ($\mu$mol · $L^{-1}$) for time*temperature, microbe*cover interactions.....44

18. Least square means and standard errors for sec-butyl ITC and 3-butenyl ITC concentrations ($\mu$mol · $L^{-1}$) for time*microbe and temperature*microbe interactions.....45

19. Least square means and standard errors for sec-butyl ITC and 3-butenyl ITC concentrations ($\mu$mol · $L^{-1}$) for time*cover interaction.....46

## List of Figures

FIGURE

PAGE


1. Standard curve of AITC vs. peak response for clay loam texture soil on GC-FID ..... 15

2. Standard curve of AITC vs. peak response for sandy loam texture soil on GC-FID ..... 16

3. Standard curve of AITC vs. peak response for clay loam texture soil on GC-MS ..... 17

4. Standard curve of AITC vs. peak response for sandy loam texture soil on GC-MS ..... 18

5. Moisture retention curve for clay loam texture soil ..... 27

6. Moisture retention curve for sandy loam texture soil ..... 28

# Chapter 1

## Introduction

Soil fumigation is widely used in strawberry, nursery stock, tomato, and tobacco production to control pathogenic fungi, nematodes, and weeds. One of the main pesticides used for soil fumigation is methyl bromide, a broad spectrum soil fumigant. Methyl bromide was listed in the 1993 Montreal Protocol as an ozone-depleting compound. Because of this listing, the U. S. Clean Air Act bans its use in the year 2005 (U.S. Department of Agriculture, 1999). The U.S. Department of Agriculture estimates that $1.5 billion in crop revenue could be lost every year with the loss of methyl bromide (Wheelwrite, 1997). An environmentally sound and economically feasible alternative for methyl bromide must be in place for growers before the year 2005.

An alternative which could replace methyl bromide and reduce pesticide use would be environmentally desirable. Biofumigation and solarization are soil treatments that could be solutions to control soil pathogens, nematodes and weeds. Biofumigation is the incorporation of plant tissue of mainly *Brassica* species into the soil with the consequently release of toxic compounds which reduce soilborne pests. Solarization is the practice of covering the soil with usually a clear polyethylene sheet that traps solar heat, thereby increasing soil temperature levels high enough to kill some soil pests.

*Brassica* plants contain compounds that break down into volatiles that have toxic effects on soil pathogen populations. These compounds are organic anions called glucosinolates (GLs). Toxic biological effects are due to secondary metabilites formed when GLs degrade. These compounds are produced upon enzymatic degradation by myrosinase

(thioglucoside glucohydrolase, EC 3.2.3.1) when water is present. One class of chemical metabilites, isothiocyanates (ITC), contributes to the majority of toxic effects observed with decomposing *Brassica* tissues (Chew, 1988). However, little information is known about the influence of environmental conditions such as soil moisture, soil temperature, soil pH, soil texture, and microbes on ITC production from different *Brassicas*.

## Chapter 2

## Objectives

The objectives of this research were to quantify the amount and determine the composition of all volatile compounds (including ITCs) produced from degradation of two Indian mustard plant introduction accessions (*Brassica juncea* L.) during simulated biofumigation under different environmental conditions. The environmental variables studied were soil texture, soil temperature, soil moisture, soil pH. Autoclaved and non-autoclaved soil was also used to determine if soil microbes affected compounds released. Simulated plastic mulch used in solarization will also be evaluated as a variable. Soil fertility within each soil texture was held constant for these experiments. This information will be important in developing a field plan for testing a biofumigation and/or solarization system that would meet the requirements of an effective and viable soil fumigant for growers.

# Chapter 3

# Literature Review

## Brassica Taxonomy and Anatomy

The *Brassica* genus belongs to the Kingdom Plantea, in the Division Magnoliophyte, and Class Mignoliopsida. It is in the subclass Dilleniidae, Order Capparales and Family Brassicaceae (Wilson, 1998). A few examples of this genus are cabbage (*Brassica oleracea* var. *capitata* L.), Chinese cabbage (*Brassica campestris* ssp. *pekinesis* L.), turnip (*Brassica rapa* L.), radish (*Raphanus sativus* L.), Brussels sprouts (*Brassica oleracea* var. *gemmifera* DC.), kale (*Brassica oleracea* var. *viridis* L.), broccoli (*Brassica oleracea* var. *botrytis* L.), oilseed rape (*Brassica napus* var. *olifera* L.), and mustard (*Brassica juncea* L.). *Brassica* plants were domesticated 1500 to 2000 B.C. E. and mustard is specifically mentioned in Greek, Roman, and Chinese writings 200 to 500 B.C. E. for its medicinal value (Downey and Robbelen, 1989). *Brassicas* are cool weather plants that germinate and grow at low temperatures (15-18°C) and can be used as winter crops in temperate climates. Most grow well in all types of soils but do best in fertile, heavy soils with high moisture holding capacity (Ludford and Isenberg, 1994).

Indian mustard grows up to a meter in height. It requires 40 - 60 days to mature. It has green leaves with jagged edges. Flowers are yellow and complete flowering may last two to three weeks. Flowering begins at the lowest flower and continues upward with several new flowers opening each day. It is typically two-thirds self-pollinating and one-third insect pollinated. Seeds are produced within a silique and are 1 mm in diameter. Rooting depth is 90-120 cm (Duke, 1984).

**Glucosinolates**

The aromas of the plants found in the *Brassica* genus are due largely to the generation of ITCs from GLs (Fenwick et al., 1983). These compounds are not present in intact plant tissue. ITCs are produced when myrosinase and GLs are brought into contact in the presence of water due to cell membrane damage. GLs are normally present in the plant cell as potassium salts (Peder et. al., 1981). Over 100 GLs are known (Sorensen, 1991). All GLs are amino acid-derived secondary plant products that contain glucose and sulfur but have different side chain R-groups. They are grouped into aliphatic, aromatic, and indole GLs based on whether they are derived from aliphatic amino acids (often methionine, phenylalanine or tyrosine, or tryptophan). Glucosinolate diversity is broadened further by secondary side-chain modifications such as hydroxylations, methylations, oxidations, and desaturations (Sorensen, 1991). Isotopes introduced into glucosinolate metabolism pathways show that they are formed from protein amino acids either directly or after chain extensions (Underhill et. al., 1973).

The biosynthesis of GLs involves three stages. First, the synthesis of chain-elongated amino acid occurs. Next, the glucone present in all GLs is added. The last step is side chain modifications (Underhill et. al., 1973).

The first step in glucosinolate biosynthesis is the conversion of amino acids into oximes, which are then converted into thiohydroximate acids. A study of different species has shown that different enzymes catalyze this reaction and were shown to be cytochrome P450 monooxygenases (Du, 1996). This study was the first to identify the enzymes that catalyze the conversion of amino acids to oximes in the biosynthetic pathway of GLs and of

cyanogenic glucosides. Cyanogenic glucosides are a related group of secondary plant products that have amino acids as precursors and oximes as intermediates. In cyanogenic glucoside biosynthesis, a single, multifunctional cytochrome P450 enzyme catalyzes the conversion of tyrosine to the corresponding oxime in a reaction that involves two consecutive N-hydroxylations, followed by a dehydration and a decarboxylation reaction (Sibbensen, 1995). Microsomes isolated from young green leaves of oilseed rape were used to show that a flavin-containing monooxygenase is involved in the conversion of chain-elongated amino acids to their corresponding oxime (Bennett, 1993). The process included an oxygen and NADPH dependent carbon dioxide released from C-14 labeled homophenylalanine (the precursor of phenylethyl glucosinolate) and dihomomethionine (the precursor of butenyl glucosinolate). The conversion of tryptophan to indole acetaldoxime is the first step in the biosynthesis of both indole glucosinolate and the plant hormone indole acetic acid. Plasma membrane bound peroxidases catalyze the conversion of tryptophan to indole acetaldoxime in seedlings of Chinese cabbage (Mithen, 1995). It was shown that enzymatic activity involved in the formation of oximes from both chain-elongated amino acids and from tryptophan in both oilseed rape and Chinese cabbage decreases with increasing maturity. The peroxidase activity was present in all tissues, whereas the flavin-containing monooxygenase activities were absent from cotyledons and old leaves, as were the corresponding GLs. This correlation between the distribution of flavin containing monooxygenase and peroxidase activities and GLs indicates that flavin containing monooxygenases may be involved in the biosynthesis of aromatic and aliphatic GLs, and peroxidases may be involved in the biosynthesis of indol GLs. There are three different enzymes that catalyze the conversion of

amino acids to oximes in the glucosinolate pathway: cytochrome P450's, flavin-containing monooxygenases, and peroxidases (Halkier and Du, 1997).

The second step of glucosinolate biosynthesis is the conversion of oxime to thiohydroximate. This process is a poorly understood step in the biosynthesis of GLs. Selectively labeled amino acids have shown that the alpha-carbon atom of the amino acid is converted into the thiohydroximate carbon. This also showed that the whole carbon skeleton of the amino acid except for the carboxyl group is incorporated into the glucosinolate (Underhill et. al., 1973). The higher oxidation level of the thiohydroximate is obtained by oxidation of the oxime to an aci-nitro compound, which then functions as the acceptor for an appropriate thiol donor. The biosynthesis continues without cleavage of the bond between the alpha-carbon and the amino nitrogen atom from the parent amino acid. This means that all biosynthesis intermediates must contain nitrogen. Few of the intermediates or enzymes have been isolated between parent amino acids and thiohydroximate acids. The final step of the glucone pathway is an S-glucosylation of the thiohydroximate by a soluble UDPG thiohydroximate glucotransferase to produce the desulphoglucosinolate. The desulphoglucosinolate is then sulfated by a soluble 3'-phosphoadenosine 5'-phosphosulphate (desulphoglucosinolate sulphotransferase) (Halkier and Du, 1997).

The last step of glucosinolate biosynthesis is secondary modifications of the side chains that may occur following glucone development. The methionine-derived aliphatic side chains are modified extensively.

GLs have been detected in all organs of the plant and are located within vacuoles of the cell. Specific GLs vary in concentration between parts of the plant. Glucosinolate

concentration is highest at the points where growth is most active (Josefsson, 1967). Younger leaves of kale may have up to 30 times more GLs than older leaves.

The breakdown of GLs occurs when the plant cells break apart. The lysing of plant cells releases GLs, water, and myrosinase, the enzyme that catalyzes the breakdown reaction. The action of myrosinase forms glucose and sulfur, along with volatile products. Five major volatiles were found in an experiment to determine volatile compounds released by *Brassicas* such as white mustard, black mustard, leafy turnip, rapeseed and garden cress. These volatiles included allyl ITC (AITC), 3-butenyl ITC, benzyl ITC, cis-3-hexen-1-ol, and trans-2-hexenal. AITC was the dominant product. This study used fresh leaf tissue. Volatiles were collected using solid-phase microextraction (SPME) and were identified by gas chromatography-mass spectrometry (Vaugn and Boydston, 1997).

**Biofumigation and Solarization**

Biofumigation describes the practice of soil sterilization by incorporating *Brassica* species plant tissue into soil and its consequent release of bio-toxic compounds. *Brassica* crops have been shown to have biological activity against many soilborne pathogens and pests. Radial growth of *Pythium ultimum* and *Rhizoctonia solani* were suppressed by Indian Mustard in jar experiments (Charron and Sams, in press). *Pythium ultimum* was completely suppressed and killed. *Rhizoctonia solani* growth was 27.4% of the control. AITC comprised over 90% of the volitiles measured by SPME. *Botrytis cinerea* growth was suppressed in a similar jar experiment most effectively by Indian mustard and 'Floriday Broadleaf' mustard (Sams et. al., 1997). White mustard (*Brassica napus olifera* biennis L.)

tissue incorporated into soil has been shown to significantly reduce *Aphanomyces* root rot in peas (Muelichen et al, 1997). Glucosinolate-derived ITC reduced many pear pathogen populations such as *Botrytis cinerea*, *Monilinia laxa*, and *Mucar piriformis* (Mari et al, 1996). Broccoli residue also reduced the population of *Vercillium dahliae* microsclerotia in soil (Subborau and Hubbard, 1996). *Brassica* tissue may also reduce soil populations of nematodes (Mayton et al., 1996). Biological effects are not limited to soil pathogens. Masked Chaffer beetle larvae were killed by Indian mustard amended soil (Noble et. al., 1999). The amount of mustard needed for larvae kill was approximately 4-8% of soil mass. *Brassica* residue has also been shown to reduce weed seed germination (Al-Khatib et al, 1997). Indian Mustard seed meal suppressed soil-borne-cereal pathogens as an in-furrow treatment for wheat (Kirkegaard, et. al., 1996).

Soil solarization is a technique that involves heating soil using a thin clear polyethylene tarp to trap solar heat during periods of high radiation. This technique has been shown to reduce populations of fungi, bacteria, nematodes, insects, and weeds (Stapleton and DeVay, 1986). Prior to being covered the soil is tilled. This allows the tarp to lay flat and close to the surface eliminating any insulating air pockets. The edges of the tarp are covered with soil to avoid heat loss. Sometimes the soil is irrigated before the tarp is placed because wetter soils conduct heat better than dry soils. Usually, the tarp is left on for a period of weeks depending on sunlight intensity to attain pest control at desired depths of up to eight inches. In a study in Alabama, the maximum temperatures attained during soil solarization ranged from 48°C at the soil surface to 34°C eight inches below the soil surface (Himelrick and Dozier, 1991). Tests conducted in two years of strawberry production in California showed

that solarization alone increased strawberry yield 12% over the yield of non-treated plots (Hartz et. al., 1993). Soils in this study reached temperatures above 50°C at the soil surface and 35°C 10 cm below the soil surface. Soil solarization carried out in Greece in plastic green-houses controlled bacterial canker (*Clavibacter michaganensis* subsp. *michiganesis*) so well that the tomato growers in the region now apply the technique commercially (Antoniou, Tjamos et. al., 1995). In northern Florida, research showed that soil solarization decreased densities of *Phytophthora nicotianae* and *P. solanacearum* to depths of 25 and 15 cm respectively (Chllemi and Olson, 1994). In this study, maximum temperatures recorded at depths 5, 15, and 25 cm were 43.8, 38.9, and 36.5°C respectively, in bare soil and 49.5, 46.0, and 41.5°C respectevely, in solarized soil. Solarization for one week reduced the numbers of viable prickly sida (*Sida spinosa* L.), common cocklebur (*Xanthium pensylvanicum* Wallr.), velvet-leaf (*Abutilon theophrasti* Medic.), and spurred anoda (*Anoda crisata* L.)(Egley, 1983). Maximum temperatures in this study at 1.3 cm soil depth reached 69°C for 3 to 4 h in the mid-afternoon. This high temperature did not eliminate dormant weed seeds from the germination zone, but the treatment killed non-dormant seeds which reduced the number of weed seedlings that otherwise would have emerged.

Qualitative and quantitative differences were found in volatiles released from cabbage at different temperatures under simulated solarization (Gamliel and Stapleton, 1993). Also, the propagules from two fungi (*Pythium ultimum* and *Sclerotium rolfsii*) were reduced by 95% by heated cabbage residue amended soil, but were only reduced 25% and 15% respectively by non-heated cabbage residue amended soil. Heated soil treatments amended with cabbage were found to contain ITCs, alcohols, and aldehydes in the soil atmosphere.

Non-heated treatments contained methanethiol, ethanol, and occasionally acetic acid and methanol. In combination with biofumigation, the high temperatures caused by solarization increase the vapor pressure of compounds resulting in greater release into the soil atmosphere. Combining *Brassica* amendments with soil solarization enhances control of pathogens through the combination of thermal killing and enhanced generation of toxic volatile compounds (Gamliel and Stapleton, 1997).

## Chapter 4

## Methods and Materials

The environmental factors in these experiments were soil moisture, soil temperature, soil texture, soil pH, and autoclaved vs. non-autoclaved soil. The contribution of each factor to glucosinolate breakdown and volatile production, primarily ITCs, was evaluated. Experiments were carried out in incubated 500 ml glass jars. Some experiments had covered jars (representing plastic mulch) and uncovered jars (representing normal tillage).

### Plant Sample Preparation

A quantity of 3.15 ± 0.01g of Indian mustard was added to each jar in experiment one. This amount was chosen based on the estimated total plant material grown per hectare (Duke, 97). In experiments two through six, freeze-dried Indian mustard was added (Labconco, Kansas City, MO). If freeze dried material was used, the dry weight equivalent 0.31 ± 0.01g was added. An aliquot of 2.84 ± 0.01g water was added to the freeze dried material to simulate the fresh weight of 3.15g. The same ratio of root, stem, and leaf was added to each jar. Freshly harvested Indian mustard was macerated in a food processor for 30 seconds. Plant material was then manually mixed with a scoupula into soil for 30 seconds in a plastic container and added back to the jar. The Teflon tube was capped with a septum and put in the jar before the soil was added. When the jar was covered, the Teflon lid was added after the tube and before the septum and sealed with a metal ring. The dried material was homogeneously ground and mixed in a blender. Deionized water was added to freeze dried material and mixed for 30 seconds in a 10 mL beaker. The mustard mixture was then

thoroughly mixed with the soil in a plastic container for 30 seconds before being placed back into the jar.

**Volatile Sampling and Analysis**

Volatile samples were taken using Solid Phase Microextraction (SPME)(Supelco Bellefonte, PA). A Teflon tube (length 14.5cm, I.D. 6mm) was inserted through the lid to the bottom of every jar. Thirty-two holes were drilled in each tube below the soil line so that volatiles in the soil atmosphere would diffuse into the tube. The tube was plugged at the bottom with a plastic cap. A septum through which the SPME sampler was inserted was placed into the top of the tube. Jars without simulated solarization were open to the atmosphere and had the same sampling and analysis methods as the simulated solarization jars with lids. The jars that represent solarization with plastic mulch had Teflon lids modified for the tube. The SPME is a pen size device that contains a one-half inch long fused silica fiber probe (100 $\mu$m polydimethylsiloxane coating). When sampling, the fiber is exposed to the atmosphere and adsorbs volatile organic compounds. The SPME probe is then inserted into the injection port of a gas chromatograph (GC). The volatiles desorb from the fiber and are delivered to the GC column.

**Chromatography**

For the first five experiments, a gas chromatograph with a flame-ionization detector (Hewlett-Packard 5890A Series II) was used. The chromatograph parameters were: oven temperature, 60°C ramped 5°C/min to 150°C, injector temperature, 200°C; and flame

ionization detector, 280°C. The chromatograph used was a fused silica EC-WAX capillary column (0.25$\mu$m film thickness, 30m × 0.25mm ID; Alltech, Deerfield, IL). For the last experiment, a Hewlett-Packard (HP) gas chromatograph (5890A Series II) connected to a HP 5972 mass selective detector with the same injector, column, and oven parameters were used. Concentrations of AITC in jar experiments was calculated based on peak areas in relation to the peak area of the standard curves adjusted for each soil volume (See Fig. 1, Fig. 2, Fig. 3, and Fig. 4) . To make the AITC standard curve for both the GC-FID and the GC-MS, ethanol was initially added to 0.15 mL AITC (adjusted for 95% purity) for a 1 mL total volume. One $\mu$L of this mixture was injected into a 500 mL jar for a concentration of 3.3 $\mu$mol · $L^{-1}$ and sampled. For the GC-FID, the AITC mixture was then diluted for eight concentrations. For the GC-MS, the initial AITC mixture was then diluted for five concentrations. GC-MS response factors for sec-butyl ITC and 3-butenyl ITC were assumed to be the same as AITC and concentrations reported are based on this assumption. No authentic standards or response factors were available for sec-butyl ITC and 3-butenyl ITC. Concentrations of $CO_2$ were calculated based on a standard curve made with 1, 3, and 5% standards. Jars with soil and Indian mustard (PI 458934) were spiked with AITC and compared with standards in empty jars to determine recovery rates for both soils.

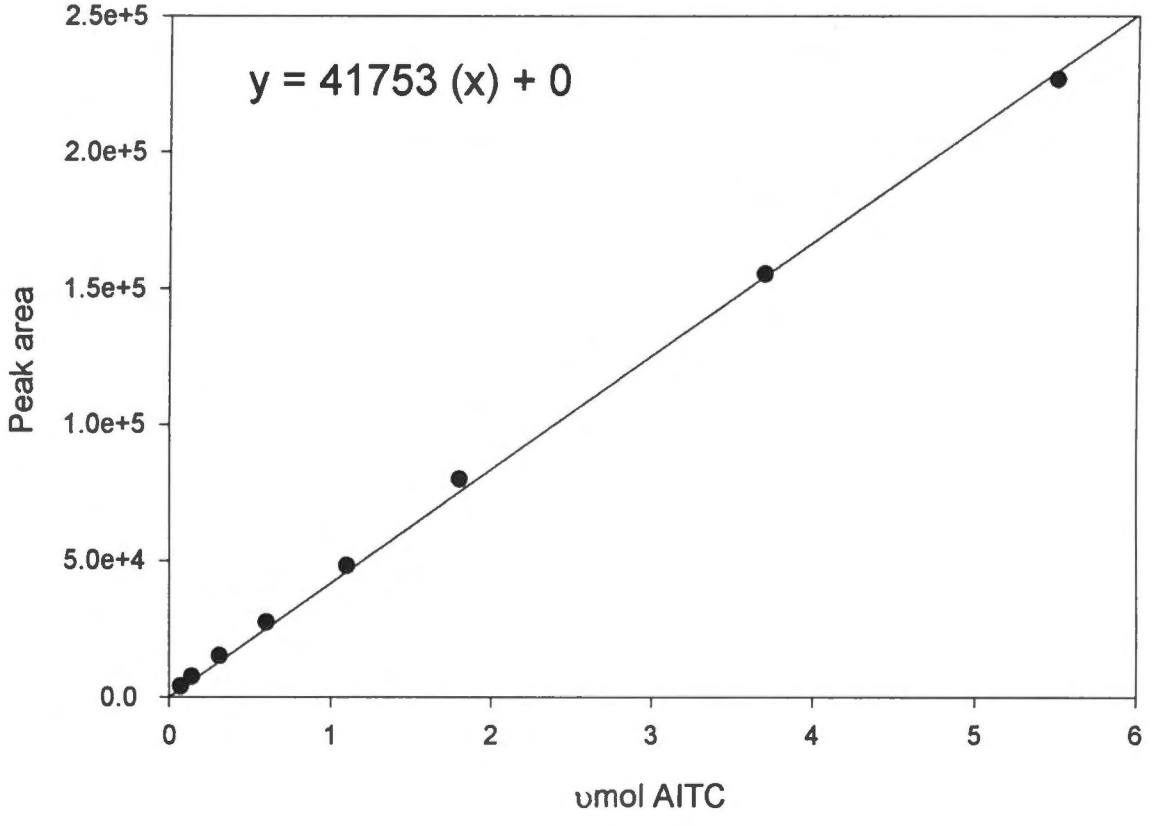



Figure 1. Standard curve of AITC vs. peak response for clay loam texture soil on GC-FID.

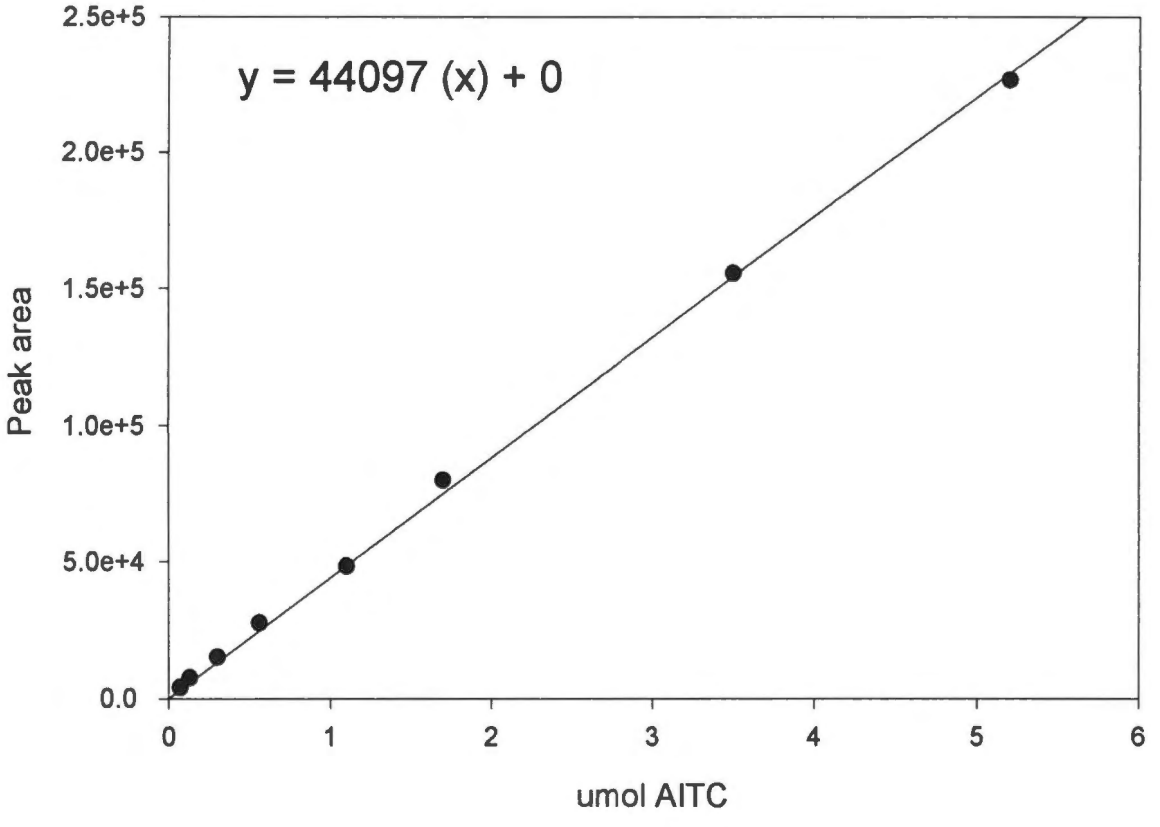



Figure 2. Standard curve of AITC vs. peak response for sandy loam texture soil on GC-FID.

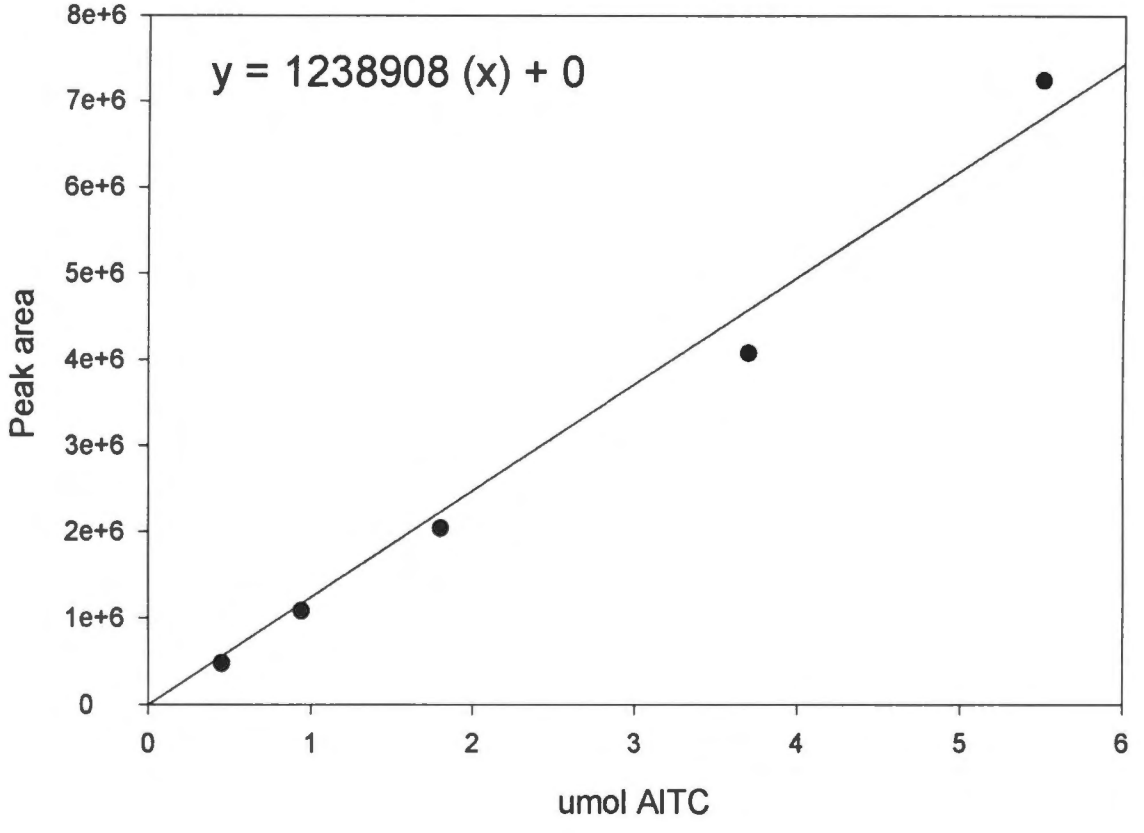



Figure 3. Standard curve of AITC vs. peak response for clay loam texture soil on GC-MS.

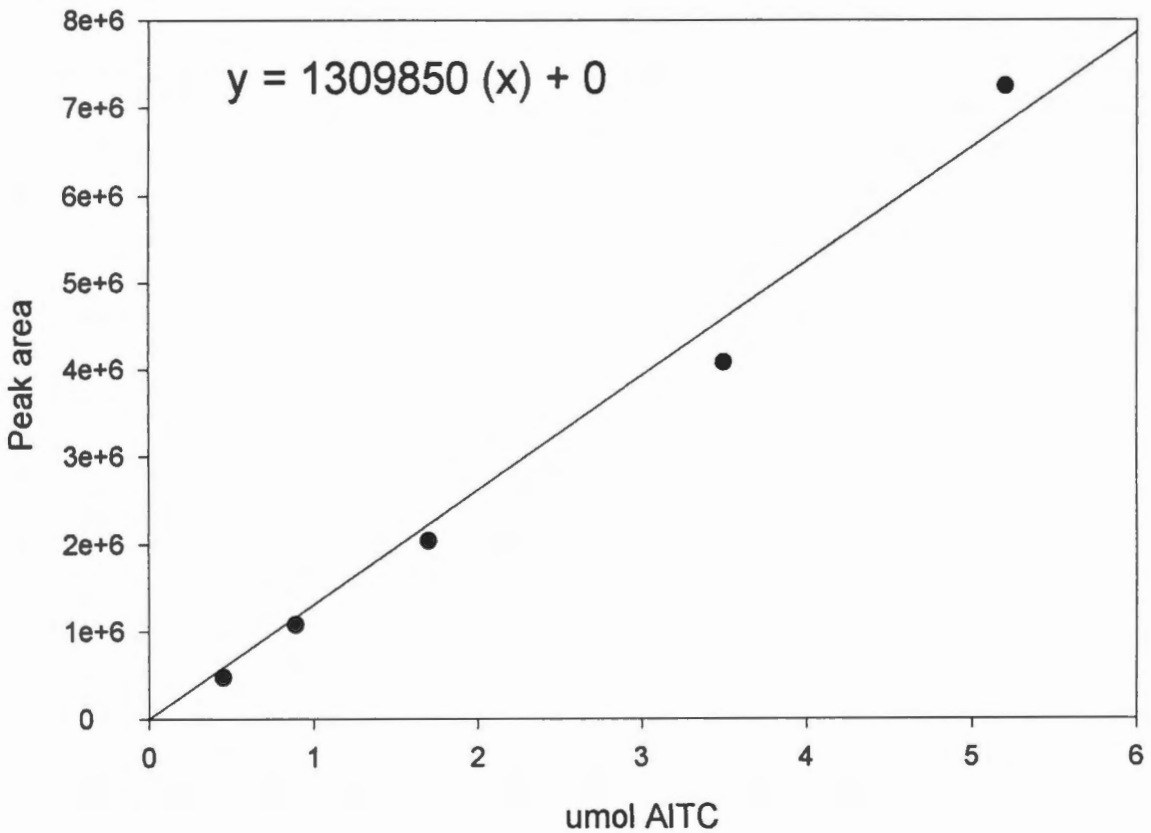



Figure 4. Standard curve of AITC vs. peak response for sandy loam texture soil on GC-MS.

The experiments were conducted in incubators which provided consistant environmental conditions. Jars were stored in darkness, and relative humidity was monitored with a Thermo-Hydro instrument (Baxter, McGraw Park, IL) and maintained at 50 ± 10%.

**Soils**

Two soil textures were used, a Waynesboro clay loam and a Sequatchie fine sandyy loam. The Waynesboro clay loam is described in the Knox County Soil Survey (Roberts et. al. 1955) as found on hilly areas and having been severely eroded so that most of the original surface soil has been lost. The plow layer is reddish firm but friable clay loam material. The fertility and organic matter for these soils are low. The Sequatchie fine sandy loam is a light brown sandy soil found on low river terraces. This soil is moderately fertile but low in organic matter. All soil was collected from the U.T. Plant Science farm, Knoxville, TN. The soils were prepared by air drying at room temperature and sieving through a #20 mesh sieve. The soil textures were determined by a particle size analysis method using Stoke's law described by Day, 1965 (see Appendix I.): A cylinder in a water bath contained the sample suspension (sandys removed). A sub-sample was then removed, dried and weighed. The weight of the sample corresponded with the relationship between settling velocity and particle diameter. The percentage of silt and clay was then calculated using Stoke's law. Particle size and total carbon analysis was also run on oven heated soil, autoclaved soil, and field soil to see if differences existed among these treatments. The pH of both soils was determined as described by Thomas, 1996 (see Appendix II.). A glass electrode pH meter was used to measure both deionized water pH and $CaCl_2$ pH. Lime requirements for both soils were

determined by a method by Adams and Evans, 1962 (see Appendix III.) A water retention curve was created for each soil using a method by Klute, 1986 (see Appendix IV.) This method involved using a pressure cell apparatus connected to a regulated gas pressure source. Pressure cells are subjected to a sequence of pressure equilibria and weight changes are recorded. The water content was calculated using the change in weight, and a moisture retention curve was constructed. The curves were prepared for each soil to show what amount of water to add to attain three moisture levels. The moisture was monitored by weighing the jars (containing soil and plant material). Deionized water was added to keep the jars at the selected moisture. A sample of 350 ± 1g of soil was placed in each 500 mL glass jar.

**Plant Material**

Two plant introduction accessions (PIs) of Indian mustard were used for the first experiment. The two PIs were 458928 and 458934. Only PI 458934 was used for experiments 2, 3, 4, 5, and 6. Both PIs were used by Mayton et al. (1996) to determine the correlation of fungicidal activity of *Brassica* species with allyl ITC production. The accessions were acquired from the USDA-ARS Regional Plant Introduction Station, Ames, IA. The Indian mustard used was grown outside at the U.T. Plant Science Farm in fertile soil and were watered.

**Experiment 1**

The first experiment was designed to determine the relationships among PIs, soil textures, soil microbes, simulated plastic mulch cover, and ITC production. The design was a completely randomized design with factorial treatment assignments and repeated measures. The treatments were clay loam and sandy loam textured soil, heat sterilized and non-heat sterilized soil, uncovered and covered containers, and two Indian mustard PIs. Each combination had three replications sampled at 0.25, 4, 8, and 24 h after initial mixing. The sterilized soil was treated with a dry heat method described by Wolf and Skipper, 1994 (see appendix V.). This method involves heating soil at 200°C for 24 h. In this experiment soil temperature was held at 30°C, and a soil moisture of 60 ± 3% of field capacity was created and maintained by adding water as jar weight decreased. Duncan mean separations were used for testing using SAS statistical software, "PROC GLM" procedure (SAS Institute, 1996).

**Experiment 2**

The second experiment was designed to determine the relationships among soil temperature, simulated plastic mulch cover, and ITC production. The design was a completely randomized design with factorial treatment assignments and repeated measures. The treatments were three soil temperatures (15, 30, and 45°C), and uncovered verses covered soil. Each combination had three replications and were sampled at 0.25, 4, 8, and 24 h after initial mixing. Only sandy loam textured soil and 458934 Indian mustard PI was used. One temperature was 45°C, a soil temperature attained through solarization techniques of soil sterilization (Himelrick and Dozier, 1991). The second temperature was 30°C, a soil

temperature attained during hot summer days. The last temperature was 15°C, the temperature at the low end of the range at which biofumigation would be carried out. A soil moisture of 60% ± 3% of field capacity was created and maintained by adding water as jar weight decreased. Duncan mean separations were used for testing using SAS statistical software, "PROC GLM" procedure (SAS Institute, 1996).

**Experiment 3**

The third experiment was designed to determine the relationships among soil moisture content, simulated plastic mulch cover, and ITC production. The experiment used a completely randomized design with factorial treatment assignments and repeated measures. The treatments were three moistures (field capacity, 60% field capacity, and permanent wilting point), and uncovered and covered soil. Each combination had three replications and was sampled at 0.25, 4, 8, and 24 h after initial mixing. This experiment involved one soil and 458934 freeze dried Indian mustard PI. The moisture levels were prepared and determined by gravimetric methods with distilled water to ± 3% maintained by adding water as jar weight decreased. The temperature of the soil was held at 30°C. Duncan mean separations were used for testing using SAS statistical software, "PROC GLM" procedure (SAS Institute, 1996).

**Experiment 4**

The fourth experiment was conducted to determine if soil pH affects the production of volatiles from Indian mustard degradation. The design was a completely randomized

design with factorial treatment assignments and repeated measures. The treatments were two soil textures (clay and sandy loams) at field and adjusted to around 7.0 pH. Each combination had three replications and were sampled at 0.25, 4, 8, and 24 h. Freeze dried 458934 Indian mustard PI was used. A portion of both soil textures were adjusted to a pH around 7.0 using Adams-Evans buffer method (see Appendix III). This was compared to the pH as found in the field which was around 6.0. Soil moisture was held at 60% of field capacity and maintained by adding water as jar weight decreased. The temperature of the soil was held at 30°C. Duncan mean separations were used for testing using SAS statistical software, "PROC GLM" procedure (SAS Institute, 1996).

**Experiment 5**

The fifth experiment was conducted to determine if production of ITC from Indian mustard degradation in autoclaved soil differed from that in non-autoclaved soil. The design was a completely randomized design with factorial treatment assignments and repeated measures. The treatments were autoclaved and non-autoclaved soil. This experiment used sandy loam textured soil and freeze dried 458934 Indian mustard PI. A portion of the soil was autoclaved using the sterilization method described by Wolf and Skipper (see Appendix VI). This was compared to the non-autoclaved soil, as found in the field. Soil moisture was held at 60% of field capacity and maintained by adding water as jar weight decreased. The temperature of the soil was held at 30°C. Duncan mean separations were used for testing using SAS statistical software, "PROC GLM" procedure (SAS Institute, 1996).

**Experiment 6**

The sixth experiment combined the previous five experiments into one design to determine if the treatment combinations affect volatiles released by Indian mustard degradation. The design was an incomplete block with fractional factorial treatment assignments and repeated measures. The treatments were soil texture (clay loam and sandy loam), soil cover (covered and uncovered), soil temperature (15°C, 30°C, and 45°C), soil moisture (permanent wilting point, 60% field capacity, and field capacity), soil pH (adjusted and field), and autoclaved verses non-autoclaved soil. Samples were taken at 0.25, 4, 8, and 24 h. Treatment combinations were obtained by using the SAS statistical software "OPTEX" (SAS Institute, 1996). Soil moisture was maintained by adding water as jar weight decreased. Indian mustard PI 458934 was used. LSD mean separations were used for testing ($P \leq 0.05$) using SAS statistical software, "PROC MIXED" procedure (SAS Institute, 1996).

# Chapter 5

## Results

### Soil analysis

The clay loam textured soil had 25% sandy fraction, 47% silt fraction, and 28% clay fraction. The sandy loam textured soil had 68% sandy fraction,23% silt fraction, and 9% clay fraction ( Table 1). Particle size and total carbon analysis was also run on oven heated soil, autoclaved soil, and field soil (Tables 2 and 3). The clay loam textured soil had a deionized water pH of 5.64 and a $CaCl_2$ pH of 5.06. The sandy loam textured soil had a deionized water pH of 5.74 and a $CaCl_2$ pH of 5.60 (See Table 4). The lime requirement for the clay loam to adjust to a pH of 7 from a buffered pH of 7.34 was calculated to be 0.91 g lime per pound of soil. The lime requirement for the sandy loam to adjust to a pH of 7 from a buffered pH of 8.01 was calculated to be 0.2 g lime per pound of soil. The pH of the limed clay texture soil was 6.8 and the pH of the limed sandy loam texture soil was 6.9.

Table 1. Particle size analysis for field soils.

| Soil Texture | Fraction (%) | | |
|---|---|---|---|
| | sandy | silt | clay |
| Clay loam | 25 | 47 | 28 |
| Sandy loam | 68 | 23 | 9 |

Table 2. Particle size analysis for oven heated, autoclaved and field soils.

| Soil Treatment | Clay loam fraction (%) | | | sandy loam fraction (%) | | |
|---|---|---|---|---|---|---|
| | sandy | silt | clay | sandy | silt | clay |
| Field | 25 | 45 | 29 | 61 | 25 | 14 |
| Autoclaved | 25 | 43 | 30 | 61 | 25 | 14 |
| Oven heated | 26 | 44 | 29 | 61 | 25 | 14 |

Table 3. Total organic carbon for oven heated, autoclaved and field soils.

| Soil Treatment | % Total Carbon (g/100 g) | |
|---|---|---|
| | clay loam | sandy loam |
| Field | 0.97 | 0.55 |
| Autoclaved | 0.98 | 0.54 |
| Oven heated | 0.56 | 0.31 |

Table 4. pH analysis for field soils.

| Soil Texture | pH | |
|---|---|---|
| | $H_2O$ | $CaCl_2$ |
| Clay loam | 5.64 | 5.06 |
| Sandy loam | 5.74 | 5.60 |

Moisture retention curves were constructed for both soil textures (Fig.5 and Fig. 6). Field capacity was assumed to be -33 kPa and permanent wilting point -1500 kPa. Using the moisture retention curve for clay loam, 22% and 8.5% moistures were found for field capacity and permanent wilting point, respectively. The sandy loam texture had moistures of 14% and 4.5% respectfully for field capacity and the permanent wilting point.

**Volatile analysis** (experiments 1-5)

AITC was the only volatile identified by GC-FID in experiments 1 - 5 by comparing its retention time with an authentic standard and by comparing its mass spectra with published mass spectra (Ohashi, M., et. al. 1963).

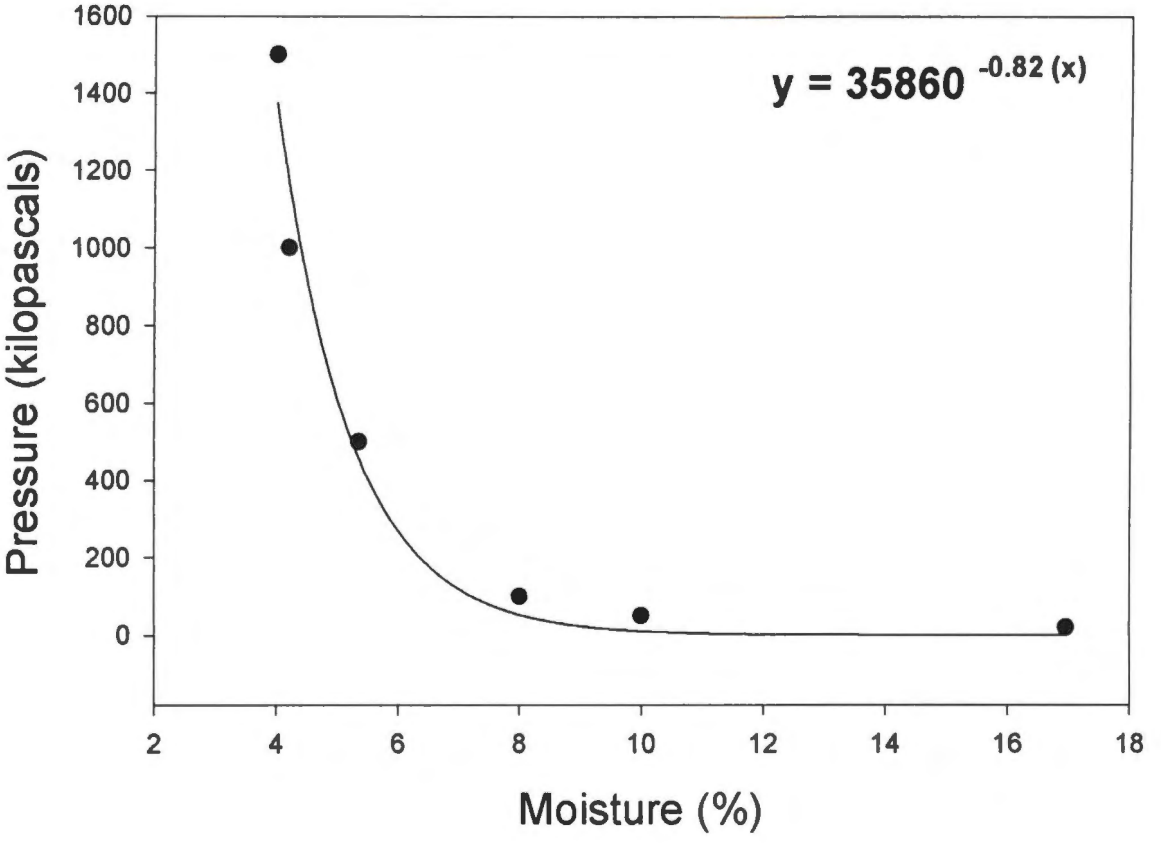



Figure 5. Moisture retention curve for clay loam texture soil.

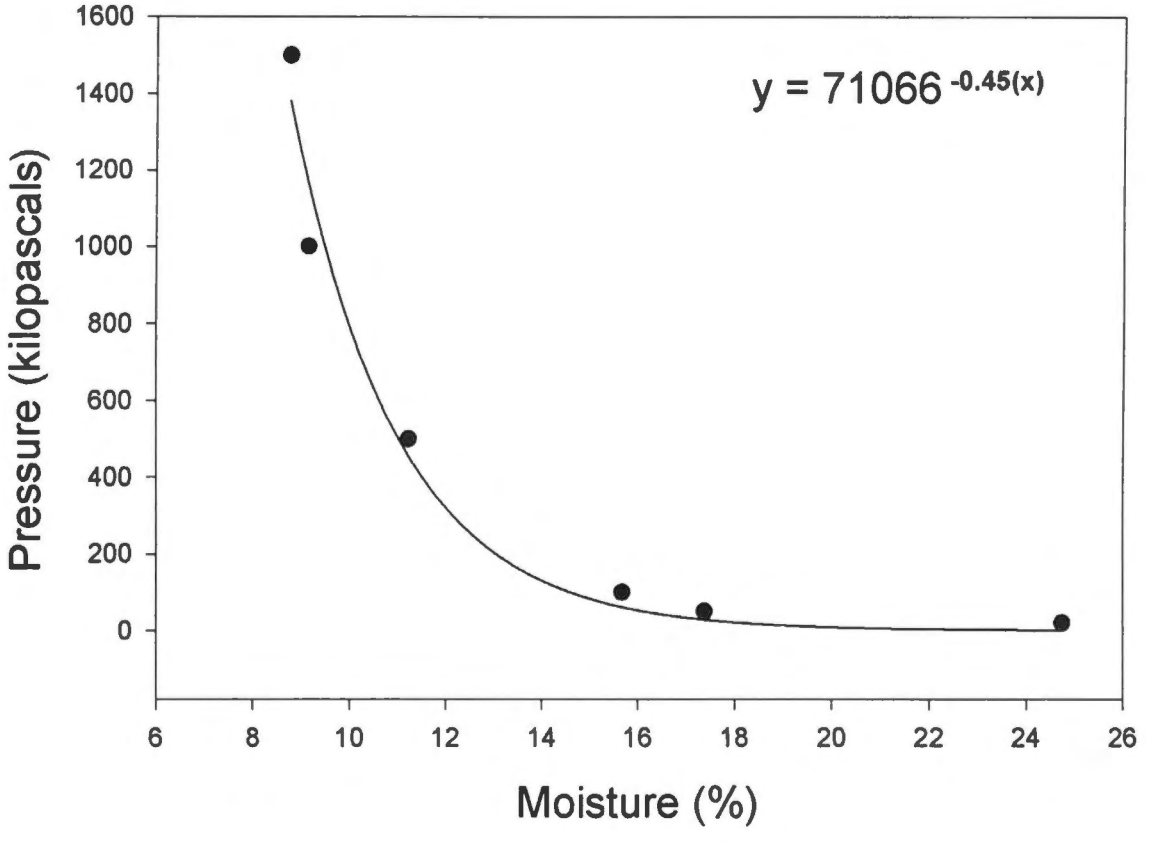



Figure 6. Moisture retention curve for sandy loam texture soil.

**Experiment 1**

No treatments differed ($P>0.05$), and there was large variability between replications. Standard deviations sometimes exceeded means (Table 5). The extreme variations between replication might have been due to the variations in glucosinolate levels, myrosinase levels, or myrosinase activity. Plants used for each replication were different ages and had been subjected to different environmental factors. This most likely caused the plants to have varying glucosinolate levels that would effect the concentration of ITC released. These results showed that a different method for mustard preparation and introduction into the jar-soil system was needed. A method involving freeze drying Indian mustard was then utilized. A single source of freeze-dried mustard sufficient for all remaining experiments was prepared. This ensured that plant material was more homogeneous across treatments.

**Experiment 2**

The effects of soil temperature and simulated plastic mulch cover on production of AITC were evaluated. At 0.25 h, the 15°C soil treatment yielded significantly lower AITC concentration than the 45°C treatment (Table 6). The 15°C and the 30°C treatments were not different, nor were the 30°C and the 45°C treatments. At 4, 8, and 24 h, the 15°C treatment yielded significantly higher concentrations than the 30°C and 45°C treatments. The 30°C and 45°C treatments did not differ at these sampling times. Soil cover was not different at fifteen minutes. However, it was different at 4, 8, and 24 h with covered being approximately twice greater than the uncovered. The three soil temperature treatments differed significantly across time with the 15°C treatment yielding higher concentrations than the 30°C treatment, and lower than the 45°C treatment.

Table 5. Average AITC concentrations ($\mu$mol/L ± SD) for experiment 1. Two Indian mustard PIs were used in two soil types (clay loam and sandy loam). Jars were covered or uncovered and half were oven-heat sterilized.

| Treatments | | | | Time (h) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Cultivar | Soil | Cover | Oven-sterilized | 0.25 | 4 | 8 | 24 | 48 | 72 |
| 1[y] | Clay | Yes | Yes | 0.26 ± 0.13 | 0.10 ± 0.12 | 0.05 ± 0.06 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 1 | Clay | Yes | No | 0.32 ± 0.18 | 0.14 ± 0.02 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 1 | Clay | No | Yes | 0.33 ± 0.15 | 0.08 ± 0.03 | 0.30 ± 0.01 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 1 | Clay | No | No | 0.35 ± 0.17 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 1 | Sandy | Yes | Yes | 0.35 ± 0.01 | 0.20 ± 0.20 | 0.56 ± 0.93 | 0.28 ± 0.47 | 0.05 ± 0.08 | 0.00 ± 0.00 |
| 1 | Sandy | Yes | No | 0.37 ± 0.12 | 0.35 ± 0.58 | 0.30 ± 0.50 | 0.06 ± 0.11 | 0.03 ± 0.05 | 0.01 ± 0.02 |
| 1 | Sandy | No | Yes | 0.14 ± 0.10 | 0.08 ± 0.08 | 0.07 ± 0.07 | 0.07 ± 0.07 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 1 | Sandy | No | No | 0.20 ± 0.11 | 0.03 ± 0.04 | 0.01 ± 0.02 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 2[x] | Clay | Yes | Yes | 0.26 ± 0.34 | 0.12 ± 0.19 | 0.19 ± 0.31 | 0.15 ± 0.25 | 0.03 ± 0.05 | 0.01 ± 0.02 |
| 2 | Clay | Yes | No | 0.18 ± 0.15 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 2 | Clay | No | Yes | 0.20 ± 0.14 | 0.08 ± 0.13 | 0.07 ± 0.11 | 0.02 ± 0.03 | 0.02 ± 0.01 | 0.00 ± 0.00 |
| 2 | Clay | No | No | 0.23 ± 0.14 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 2 | Sandy | Yes | Yes | 0.39 ± 0.19 | 0.13 ± 0.18 | 0.17 ± 0.26 | 0.16 ± 0.26 | 0.02 ± 0.04 | 0.01 ± 0.02 |
| 2 | Sandy | Yes | No | 0.27 ± 0.20 | 0.02 ± 0.02 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 2 | Sandy | No | Yes | 0.42 ± 0.13 | 0.19 ± 0.31 | 0.12 ± 0.20 | 0.02 ± 0.03 | 0.01 ± 0.01 | 0.00 ± 0.00 |
| 2 | Sandy | No | No | 0.24 ± 0.18 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |

[z] AITC means calculated with 3 replications.
[y] Indian Mustard PI:458928
[x] Indian Mustard PI:458934.

Table 6. Average AITC concentrations ($\mu$mol · $L^{-1}$ ± SD) for temperature treatments in sandy loam textured soil. This experiment involved three temperature levels, and one freeze dried Indian mustard PI (458934). Only differences in temperature treatments are shown by grouping.

| Treatments | | Time (h) and Duncan grouping | | | |
|---|---|---|---|---|---|
| | Temperature (°C) | 0.25 | 4 | 8 | 24 |
| Covered | | | | | |
| | 15 | 0.58 ± 0.15 b | 0.56 ± 0.17 a | 0.39 ± 0.15 a | 0.05 ± 0.04 a |
| | 30 | 0.60 ± 0.01 ab | 0.20 ± 0.10 b | 0.05 ± 0.02 b | 0.01 ± 0.01 b |
| | 45 | 0.73 ± 0.18 a | 0.16 ± 0.16 b | 0.04 ± 0.02 b | 0.01 ± 0.01 b |
| Uncovered | | | | | |
| | 15 | 0.52 ± 0.24 b | 0.35 ± 0.12 a | 0.17 ± 0.07 a | 0.03 ± 0.01 a |
| | 30 | 0.57 ± 0.14 ab | 0.09 ± 0.06 b | 0.02 ± 0.00 b | 0.00 ± 0.00 b |
| | 45 | 0.77 ± 0.10 a | 0.17 ± 0.16 b | 0.04 ± 0.02 b | 0.01 ± 0.01 b |

**Experiment 3**

The effects of soil moisture and simulated plastic mulch cover on production of AITC were evaluated. At 0.25 h, the field capacity soil treatment had significantly lower AITC concentration than the 60% field capacity and the permanent wilting point treatments (Table 7). The 60% field capacity and the permanent wilting point treatments were not different. At 4, 8, and 24 h, none of the moisture treatments differed significantly. The AITC concentration of covered soil did not differ from uncovered soil for fifteen minutes but was different after 4, 8, and 24 h with the covered being approximately twice greater than the uncovered. Two of the soil moisture treatments differed significantly across time with the permanent wilting point treatment yielding higher concentrations than the field capacity treatment. The 60% field capacity did not differ across time from the other treatments.

Table 7. Average AITC concentrations ($\mu$mol · $L^{-1}$ ± SD) for moisture treatments in sandy loam textured soil. This experiment involved three moisture levels, and one freeze dried Indian mustard PI (458934). Only differences in moisture are shown by grouping.

| Treatments | | Time (h) and Duncan grouping | | | |
|---|---|---|---|---|---|
| | Moisture | 0.25 | 4 | 8 | 24 |
| Covered | | | | | |
| | permanent wilting point | 0.65 ± 0.06 a | 0.38 ± 0.15 a | 0.09 ± 0.06 a | 0.03 ± 0.01 a |
| | 60% field capacity | 0.53 ± 0.08 a | 0.50 ± 0.25 a | 0.13 ± 0.07 a | 0.03 ± 0.01 a |
| | field capacity | 0.36 ± 0.10 b | 0.26 ± 0.11 a | 0.07 ± 0.03 a | 0.02 ± 0.00 a |
| Uncovered | | | | | |
| | permanent wilting point | 0.69 ± 0.16 a | 0.14 ± 0.02 a | 0.05 ± 0.02 a | 0.03 ± 0.01 a |
| | 60% field capacity | 0.71 ± 0.09 a | 0.15 ± 0.07 a | 0.04 ± 0.02 a | 0.02 ± 0.02 a |
| | field capacity | 0.47 ± 0.24 b | 0.10 ± 0.06 a | 0.02 ± 0.02 a | 0.01 ± 0.01 a |

**Experiment 4**

The effects of soil pH and soil texture on production of AITC were evaluated. Soil pH treatments did not differ for either soil texture ($P>0.1$). Soil texture was different at fifteen minutes, 4, 8, and 24 h with the sandy loam texture being approximately one-third greater than the clay loam textured soil at 0.25 h (Table 8). At 4 h the field pH sandy loam textured soil was six times greater than the field pH clay loam textured soil. At 8 h the field pH sandy loam texture was four times greater than the field pH clay loam soil.

The soil texture treatments differed significantly across time with the sandy loam texture soil yielding higher concentrations than the clay loam soil. Also, the pH treatments differed significantly across time with field soil pH yielding higher concentrations than the adjusted soil pH.

Table 8. Average AITC concentrations ($\mu$mol · $L^{-1}$ ± SD) for pH treatments in clay and sandy loam textured soil. This experiment involved two soils, each at two different pHs, and one freeze dried Indian mustard PI (458934). Only differences at field pH between the two soil textures are shown by grouping.

| Treatments | | Time (h) and Duncan grouping | | | |
|---|---|---|---|---|---|
| | pH | 0.25 | 4 | 8 | 24 |
| Clay loam | | | | | |
| | 5.6 | 0.42 ± 0.03 a | 0.11 ± 0.02 b | 0.03 ± 0.00 b | 0.01 ± 0.01 b |
| | 6.8 | 0.53 ± 0.14 | 0.31 ± 0.14 | 0.08 ± 0.02 | 0.03 ± 0.00 |
| Sandy loam | | | | | |
| | 5.7 | 0.63 ± 0.14 a | 0.63 ± 0.05 a | 0.14 ± 0.02 a | 0.03 ± 0.00 a |
| | 6.9 | 0.53 ± 0.14 | 0.31 ± 0.14 | 0.08 ± 0.02 | 0.03 ± 0.01 |

**Experiment 5**

The effects of autoclaved verses non-autoclaved soil and soil texture on production of AITC were evaluated. At 0.25 h, 4, 8, and 24 h, the sterilized soil treatment had significantly higher AITC concentration than the unsterilized (Table 9). The soil texture treatments differed significantly across time with the sandy loam texture treatment yielding higher concentrations than the clay treatment. Also, the autoclaved treatment differed significantly across time yielding higher concentrations than the non-autoclaved treatment.

**Experiment 6**

The effects of soil texture (clay loam and sandy loam), soil cover (covered and uncovered), soil temperature (15°C, 30°C, and 45°C), soil moisture (permanent wilting point, 60% field capacity, and field capacity), soil pH (adjusted and field), and soil microbes (non-autoclaved and autoclaved) on production of seven volatile compounds were evaluated.

Table 9. Average AITC concentrations ($\mu$mol · $L^{-1}$ ± SD) for autoclaved and non-autoclaved treatments in clay and sandy loam textured soil. Indian mustard PI (458934). Only differences between autoclaved and non-autoclaved soils are shown by grouping.

| Treatments | | Time (h) and Duncan grouping | | | |
|---|---|---|---|---|---|
| | | 0.25 | 4 | 8 | 24 |
| Clay loam | | | | | |
| | Unsterile | 0.36 ± 0.13 b | 0.20 ± 0.04 b | 0.04 ± 0.01 b | 0.02 ± 0.00 b |
| | Sterile | 0.61 ± 0.12 a | 1.30 ± 0.08 a | 1.24 ± 0.01 a | 0.90 ± 0.04 a |
| Sandy loam | | | | | |
| | Unsterile | 0.52 ± 0.04 b | 0.70 ± 0.12 b | 0.23 ± 0.09 b | 0.04 ± 0.02 b |
| | Sterile | 0.87 ± 0.18 a | 2.06 ± 0.23 a | 2.05 ± 0.33 a | 1.67 ± 0.25 a |

**Volatile Analysis (Experiment 6)**

Volatile compounds detected by GC-MS included AITC, carbon dioxide, sec-butyl ITC, and 3-butenyl ITC. Allyl-ITC, sec-butyl ITC, and 3-butenyl ITC were identified by comparing their mass spectra with published mass spectra (Ohashi, M., Wilson, J. M., and Djerassi, C, 1963). $CO_2$ and AITC were identified by comparing their mass spectra to those of authentic standards.

All treatments and two-way interactions were analyzed. The only three-way and higher interactions analyzed where those involving time, due to limitations of the statistical model. None of the three-way interactions were significant at $P \leq 0.05$. Significant factors are discussed for Allyl-ITC, sec-butyl ITC, 3-butenyl ITC and $CO_2$.

Soil texture was significant across time with the sandy loam texture soil yielding higher AITC concentrations than the clay loam texture soil(Table 10). The 15°C soil treatment yielded significantly lower AITC concentration across time than the 45°C treatment. The 15°C and the 30°C treatments were not different, nor were the 30°C and the 45°C treatments across time. There were no differences among moisture and pH treatments

Table 10. Least square means and standard errors for AITC concentrations ($\mu$mol · $L^{-1}$) for time, texture, temperature, cover and microbe effects. Indian mustard PI 458934.

| Treatments | | Lsmean | Std. Error | LSD group |
|---|---|---|---|---|
| Time (h) | | | | |
| | 0.25 | 0.80 | 0.07 | A |
| | 4 | 0.88 | 0.07 | A |
| | 8 | 0.67 | 0.07 | B |
| | 24 | 0.41 | 0.07 | C |
| Texture | | | | |
| | clay | 0.58 | 0.07 | B |
| | sandy | 0.80 | 0.07 | A |
| Temperature (°C) | | | | |
| | 15 | 0.48 | 0.09 | B |
| | 30 | 0.71 | 0.09 | A |
| | 45 | 0.87 | 0.09 | A |
| Cover | | | | |
| | yes | 1.05 | 0.07 | A |
| | no | 0.32 | 0.07 | B |
| Soil Autoclaved | | | | |
| | yes | 1.06 | 0.07 | A |
| | no | 0.32 | 0.07 | B |

across time. Covered soil yielded significantly higher AITC across time than the uncovered. The autoclaved soil differed significantly across time yielding higher concentrations of AITC than the non-autoclaved soil. The treatment interactions time*temperature, time*pH, microbe*cover, time*microbe, temperature*autoclaved, and time*cover were significant ($P \leq 0.05$). The time*temperature interaction yielded higher concentrations of AITC in the 45°C and 30°C treatments than the 15°C treatment at 15 min. However, the 15°C treatment maintained its initial concentration released over 24 h. The 45°C and 30°C treatments lost AITC concentration quickly over time. The time*pH interaction differed with the limed soil

yielding higher AITC concentration at 0.25 h than the field soil. At four and eight hours the field soil yielded higher AITC concentration than the limed soil. At twenty-four hours, there was no differences between treatments. The microbe*cover interaction differed with the autoclaved covered treatment yielding higher concentration of AITC than autoclaved uncovered, non-autoclaved covered, and the non-autoclaved uncovered treatments. The time*microbe interaction differed with autoclaved treatment yielding increasing AITC concentrations with increasing time. The non-autoclaved treatment lost AITC concentration over time. The temperature*microbe interaction differed with the autoclaved 45°C, 35°C, and 15°C treatments yielding higher AITC concentrations than the non-autoclaved treatments at 15 min. However, the non-autoclaved treatments maintained AITC concentrations at all temperatures. The time*cover interaction differed with the covered treatment yielding increasing concentrations of AITC than the uncovered with increasing time. The uncovered treatments had decreasing AITC concentrations with increasing time (Tables 11 and 12).

Soil texture treatments were not significantly different for $CO_2$ concentration. Soil temperature was significant with the 15°C treatment yielding a lower $CO_2$ concentration than either the 30°C and 45°C treatments across time (Table 13). There were no concentration differences between the 30°C and the 45°C treatments across time. Soil moisture and soil pH treatments were not significant for $CO_2$ concentration. Covered soil yielded twice the $CO_2$ concentration as uncovered. The autoclaved treatment differed significantly across time yielding lower concentrations of $CO_2$ than the non-autoclaved treatment.

Table 11. Least square means and standard errors for AITC concentrations ($\mu$mol · $L^{-1}$) for time*temperature, time*pH, and microbe*cover interactions. Indian mustard PI 458934.

| Treatments | | Lsmean | Std. Error | LSD group |
|---|---|---|---|---|
| Time (h) | Temperature (°C) | | | |
| 0.25 | 15 | 0.44 | 0.11 | FGH |
| 0.25 | 30 | 0.85 | 0.11 | BCD |
| 0.25 | 45 | 1.11 | 0.11 | AB |
| 4 | 15 | 0.36 | 0.11 | H |
| 4 | 30 | 0.43 | 0.11 | GH |
| 4 | 45 | 0.45 | 0.11 | FGH |
| 8 | 15 | 0.55 | 0.11 | EFGH |
| 8 | 30 | 0.91 | 0.11 | ABC |
| 8 | 45 | 1.17 | 0.11 | A |
| 24 | 15 | 0.57 | 0.11 | DEFG |
| 24 | 30 | 0.67 | 0.11 | DEF |
| 24 | 45 | 0.76 | 0.11 | CDE |
| Time | pH | | | |
| 0.25 | limed | 0.94 | 0.09 | A |
| 0.25 | field | 0.66 | 0.09 | CD |
| 4 | limed | 0.36 | 0.09 | F |
| 4 | field | 0.46 | 0.09 | EF |
| 8 | limed | 0.85 | 0.09 | AC |
| 8 | field | 0.90 | 0.09 | AB |
| 24 | limed | 0.67 | 0.09 | BDE |
| 24 | field | 0.66 | 0.09 | CD |
| Soil Autoclaved | Cover | | | |
| yes | no | 0.40 | 0.10 | B |
| no | no | 0.25 | 0.10 | B |
| yes | yes | 1.71 | 0.10 | A |
| no | yes | 0.40 | 0.10 | B |

Table 12. Least square means and standard errors for AITC concentrations ($\mu$mol · $L^{-1}$) for time*microbe, temperature*microbe, and time*cover interactions. Indian mustard PI 458934.

| Treatments | | Lsmean | Std. Error | LSD group |
|---|---|---|---|---|
| Time (h) | Autoclaved | | | |
| 0.25 | yes | 0.93 | 0.09 | C |
| 0.25 | no | 0.67 | 0.09 | D |
| 4 | yes | 0.76 | 0.09 | D |
| 4 | no | 0.05 | 0.09 | F |
| 8 | yes | 1.39 | 0.09 | A |
| 8 | no | 0.40 | 0.09 | E |
| 24 | yes | 1.13 | 0.09 | B |
| 24 | no | 0.20 | 0.09 | EF |
| Temperature (°C) | Autoclaved | | | |
| 15 | yes | 0.63 | 0.12 | B |
| 15 | no | 0.32 | 0.12 | BC |
| 30 | yes | 1.16 | 0.12 | A |
| 30 | no | 0.27 | 0.12 | C |
| 45 | yes | 1.37 | 0.12 | A |
| 45 | no | 0.37 | 0.12 | BC |
| Time (h) | Cover | | | |
| 0.25 | no | 0.70 | 0.09 | C |
| 0.25 | yes | 0.90 | 0.09 | C |
| 4 | no | 0.05 | 0.09 | E |
| 4 | yes | 0.77 | 0.09 | C |
| 8 | no | 0.36 | 0.09 | D |
| 8 | yes | 1.39 | 0.09 | A |
| 24 | no | 0.18 | 0.09 | E |
| 24 | yes | 1.15 | 0.09 | B |

Table 13. Least square means and standard errors of $CO_2$ (%) for time, temperature, and microbial effects. Indian mustard PI 458934.

| Treatments | | Lsmean | Std. Error | LSD group |
|---|---|---|---|---|
| Time (h) | | | | |
| | 0.25 | 1.74 | 0.29 | B |
| | 4 | 2.35 | 0.29 | C |
| | 8 | 2.59 | 0.29 | AB |
| | 24 | 3.62 | 0.29 | A |
| Temperature (°C) | | | | |
| | 15 | 1.86 | 0.30 | B |
| | 30 | 3.07 | 0.30 | A |
| | 45 | 2.80 | 0.30 | A |
| Soil Autoclaved | | | | |
| | yes | 1.71 | 0.26 | B |
| | no | 3.44 | 0.26 | A |

The treatment interactions microbe∗cover, time∗microbe, temperature∗autoclaved, and time∗cover were significant ($P \leq 0.05$). The microbe∗cover interaction differed with the non-autoclaved covered treatment yielding higher concentration of $CO_2$ than autoclaved covered, autoclaved uncovered, or the non-autoclaved uncovered treatments. The time∗microbe interaction differed with the non-autoclaved treatment by yielding increasing $CO_2$ concentrations with increasing time. The autoclaved treatments had constant $CO_2$ concentrations with increasing time. The temperature∗microbe interaction differed with the autoclaved 45°C and 30°C treatments yielding higher $CO_2$ concentrations than the 15°C autoclaved treatment and all non-autoclaved treatments. The non-autoclaved treatments had a constant $CO_2$ concentration at all temperature treatments. The time∗cover interaction differed with the covered treatment yielding increasing concentrations of $CO_2$ over time and the uncovered yielding constant concentrations over time (Tables 14 and 15).

Table 14. Least square means and standard errors for % $CO_2$ for microbe*cover, time*microbe, and temperature*microbe interactions. Indian mustard PI 458934.

| Treatments | | | | |
|---|---|---|---|---|
| | | Lsmean | Std. Error | LSD group |
| Soil Autoclaved | Cover | | | |
| yes | no | 1.61 | 0.33 | B |
| no | no | 1.63 | 0.33 | B |
| yes | yes | 1.82 | 0.33 | B |
| no | yes | 5.25 | 0.33 | A |
| Time (h) | Autoclaved | | | |
| 0.25 | yes | 1.73 | 0.37 | C |
| 0.25 | no | 1.75 | 0.37 | C |
| 4 | yes | 1.59 | 0.37 | C |
| 4 | no | 3.13 | 0.37 | B |
| 8 | yes | 1.59 | 0.37 | C |
| 8 | no | 3.60 | 0.37 | A |
| 24 | yes | 1.94 | 0.37 | C |
| 24 | no | 5.29 | 0.37 | A |
| Temperature (°C) | Autoclaved | | | |
| 15 | yes | 1.79 | 0.39 | B |
| 15 | no | 1.92 | 0.39 | B |
| 30 | yes | 1.64 | 0.39 | B |
| 30 | no | 4.50 | 0.39 | A |
| 45 | yes | 1.70 | 0.39 | B |
| 45 | no | 3.90 | 0.39 | A |

Table 15. Least square means and standard errors for % $CO_2$ for time*cover interactions. Indian mustard PI 458934.

| Treatments | | Lsmean | Std. Error | LSD group |
|---|---|---|---|---|
| Time (h) | Cover | | | |
| 0.25 | no | 1.66 | 0.37 | C |
| 0.25 | yes | 1.83 | 0.37 | C |
| 4 | no | 1.63 | 0.37 | C |
| 4 | yes | 3.09 | 0.37 | B |
| 8 | no | 1.49 | 0.37 | C |
| 8 | yes | 3.69 | 0.37 | A |
| 24 | no | 1.71 | 0.37 | C |
| 24 | yes | 5.53 | 0.37 | A |

Soil texture treatments were not significant for sec-butyl ITC and 3-butenyl ITC concentration. Soil Temperature was significant with the 15°C treatment yielding lower concentration of sec-butyl ITC and 3-butenyl ITC than either the 30°C and 45°C treatments across time (Table 16). There were no yield differences between the 30°C and the 45°C treatments across time. Soil moisture was not significant for either ITCs. Covered treatment had higher sec-butyl ITC and 3-butenyl ITC concentration than uncovered across time. The autoclaved treatment differed significantly across time yielding higher concentrations of sec-butyl ITC and 3-butenyl ITC than the non-autoclaved treatment.

The treatment interactions time*temperature, microbe*cover, time*microbe, temperature*autoclaved, and time*cover were significant ($P \leq 0.05$). The time*temperature

Table 16. Least square means and standard errors for sec-butyl ITC and 3-butenyl ITC concentrations ($\mu$mol · $L^{-1}$) for time, temperature, microbe effects. Indian mustard PI 458934.

| Treatments | | sec-butyl ITC | | | 3-butenyl ITC | | |
|---|---|---|---|---|---|---|---|
| | | Lsmean | Std. Error | LSD group | Lsmean | Std. Error | LSD group |
| Time (h) | | | | | | | |
| | 0.25 | 0.64 | 0.05 | AB | 0.023 | 0.003 | A |
| | 4 | 0.70 | 0.05 | C | 0.014 | 0.003 | C |
| | 8 | 0.60 | 0.05 | A | 0.011 | 0.003 | B |
| | 24 | 0.35 | 0.05 | B | 0.005 | 0.003 | BC |
| Temperature (°C) | | | | | | | |
| | 15 | 0.57 | 0.05 | A | 0.004 | 0.004 | C |
| | 30 | 0.61 | 0.05 | A | 0.013 | 0.004 | B |
| | 45 | 0.53 | 0.05 | A | 0.023 | 0.004 | A |
| Soil Autoclaved | | | | | | | |
| | yes | 0.69 | 0.04 | A | 0.021 | 0.003 | A |
| | no | 0.45 | 0.04 | B | 0.006 | 0.003 | B |

interaction differed with higher temperatures yielding higher concentrations of sec-butyl ITC and 3-butenyl ITC at 15 min. and 4 h. However, at 8 and 24h the lower temperatures yielded higher concentrations of sec-butyl ITC and 3-butenyl ITC than the high temperature. The microbe∗cover interaction differed with the autoclaved covered treatment yielding higher concentration of sec-butyl ITC and 3-butenyl ITC than autoclaved uncovered, non-autoclaved covered, and the non-autoclaved uncovered treatments. The time∗microbe interaction differed with the autoclaved treatment yielding increasing sec-butyl ITC and 3-butenyl ITC concentrations at 15min., 4 and 8 h. sample times. The non-autoclaved treatment lost sec-butyl ITC and 3-butenyl ITC concentrations with increasing time. The temperature∗microbe

interaction differed with the autoclaved treatments yielding increasing sec-butyl ITC and 3-butenyl ITC concentrations at higher temperatures. The non-autoclaved treatments yielded lower sec-butyl ITC and 3-butenyl ITC concentrations as temperature increased. The time*cover interaction differed with the covered treatment yielding increasing concentrations of sec-butyl ITC and 3-butenyl ITC until 8 h and then decreasing. The uncovered treatments lost sec-butyl ITC and 3-butenyl ITC concentrations over time (Tables 17, 18 and 19).

Table 17. Least square means and standard errors for sec-butyl ITC and 3-butenyl ITC concentrations ($\mu$mol · $L^{-1}$) for time*temperature, microbe*cover interactions. Indian mustard PI 458934.

| Treatments | | sec-butyl ITC | | | 3-butenyl ITC | | |
|---|---|---|---|---|---|---|---|
| | | Lsmean | Std. Error | LSD group | Lsmean | Std. Error | LSD group |
| Time (h) | Temperature (°C) | | | | | | |
| 0.25 | 15 | 0.59 | 0.07 | BCD | 0.007 | 0.005 | DEFG |
| 0.25 | 30 | 0.72 | 0.07 | ABC | 0.024 | 0.005 | B |
| 0.25 | 45 | 0.61 | 0.07 | ABCD | 0.037 | 0.004 | A |
| 4 | 15 | 0.59 | 0.07 | DE | 0.004 | 0.005 | G |
| 4 | 30 | 0.78 | 0.07 | EF | 0.014 | 0.004 | FG |
| 4 | 45 | 0.72 | 0.07 | F | 0.025 | 0.004 | DEF |
| 8 | 15 | 0.65 | 0.07 | BCD | 0.005 | 0.004 | EFG |
| 8 | 30 | 0.59 | 0.07 | A | 0.010 | 0.005 | CDE |
| 8 | 45 | 0.55 | 0.07 | AB | 0.017 | 0.005 | BC |
| 24 | 15 | 0.48 | 0.07 | ABC | 0.000 | 0.004 | EFG |
| 24 | 30 | 0.32 | 0.07 | BCD | 0.004 | 0.004 | DEFG |
| 24 | 45 | 0.24 | 0.07 | CD | 0.013 | 0.005 | BCD |
| Autoclaved | Cover | | | | | | |
| yes | no | 0.26 | 0.06 | C | 0.008 | 0.004 | B |
| no | no | 0.26 | 0.06 | C | 0.004 | 0.004 | B |
| yes | yes | 1.12 | 0.06 | A | 0.034 | 0.004 | A |
| no | yes | 0.65 | 0.06 | B | 0.008 | 0.004 | B |

Table 18. Least square means and standard errors for sec-butyl ITC and 3-butenyl ITC concentrations ($\mu$mol · $L^{-1}$) for time*microbe and temperature*microbe interactions. Indian mustard PI 458934.

| Treatments | | sec-butyl ITC | | | 3-butenyl ITC | | |
|---|---|---|---|---|---|---|---|
| | | Lsmean | Std. Error | LSD group | Lsmean | Std. Error | LSD group |
| Time (h) | Autoclaved | | | | | | |
| 0.25 | yes | 0.68 | 0.06 | ABC | 0.028 | 0.004 | A |
| 0.25 | no | 0.60 | 0.06 | BC | 0.018 | 0.004 | BC |
| 4 | yes | 0.78 | 0.06 | CD | 0.025 | 0.004 | CD |
| 4 | no | 0.61 | 0.06 | E | 0.004 | 0.004 | E |
| 8 | yes | 0.73 | 0.06 | A | 0.019 | 0.004 | AB |
| 8 | no | 0.47 | 0.06 | BC | 0.002 | 0.004 | DE |
| 24 | yes | 0.58 | 0.06 | AB | 0.012 | 0.004 | B |
| 24 | no | 0.13 | 0.06 | D | 0.000 | 0.004 | E |
| Temperature (°C) | Autoclaved | | | | | | |
| 15 | yes | 0.63 | 0.07 | ABC | 0.005 | 0.005 | C |
| 15 | no | 0.53 | 0.07 | BCD | 0.003 | 0.005 | C |
| 30 | yes | 0.76 | 0.07 | A | 0.020 | 0.005 | B |
| 30 | no | 0.45 | 0.07 | CD | 0.005 | 0.005 | C |
| 45 | yes | 0.69 | 0.07 | AB | 0.037 | 0.005 | A |
| 45 | no | 0.38 | 0.07 | D | 0.009 | 0.005 | BC |

Table 19. Least square means and standared errors for sec-butyl ITC and 3-butenyl ITC concentrations ($\mu$mol · $L^{-1}$) for time*cover interaction. Indian mustard PI 458934.

| Treatments | | sec-butyl ITC | | | 3-butyl ITC | | |
|---|---|---|---|---|---|---|---|
| | | Lsmean | Std. Error | LSD group | Lsmean | Std. Error | LSD group |
| Time (h) | Cover | | | | | | |
| 0.25 | no | 0.57 | 0.06 | B | 0.017 | 0.004 | BC |
| 0.25 | yes | 0.71 | 0.06 | B | 0.029 | 0.004 | A |
| 4 | no | 0.29 | 0.06 | D | 0.006 | 0.004 | E |
| 4 | yes | 1.10 | 0.06 | B | 0.023 | 0.004 | CD |
| 8 | no | 0.15 | 0.06 | C | 0.002 | 0.004 | DE |
| 8 | yes | 1.05 | 0.06 | A | 0.019 | 0.004 | AB |
| 24 | no | 0.04 | 0.06 | D | 0.000 | 0.004 | E |
| 24 | yes | 0.67 | 0.06 | A | 0.012 | 0.004 | B |

# Chapter 6

## Discussion

AITC detected by GC-FID in the first five experiments and compounds detected by GC-MS in experiment six (AITC and 3-butenyl ITC) were compounds detected by Vaughn and Boydston (1997). However, sec-butyl ITC, also detected in experiment six, was not detected by Vaughn and Boydston (1997). In a study evaluating decomposition of *Brassica juncea* in soil by Bending and Lincoln (1999) detected AITC, but no other ITCs. They also detected methanethiol, dimethyl-sulphide, carbon-disulphide, and dimethyl-disulphide; methanethiol was the dominant headspace compound throughout their analyses. Bending and Lincoln also found that AITC concentrations in a clay loam textured soil was one-sixth of those found in a sandy loam textured soil which is similar to results found in this study. No glucosinolate hydrolysis products were found by direct extraction of soil. This suggests that no products were being adsorbed by the soil. Bending and Lincoln (1999) used a 75 $\mu$m carboxen-polydimethylsiloxane coated fiber that was allowed to equilibrate for 12 minutes in the sample container. Various coatings and capacities on SPME fibers may bind different compounds and concentrations. Longer adsorption times may cause different results due to preferential binding. Also, the sample preparation differed in that fresh Indian mustard leaves were cut into 1 cm squares. Freeze dried Indian mustard in this study was finely ground and may have allowed more myrosinase enzyme and GLs to come into contact and release higher levels of ITCs. GLs and myrosinase are stored in separate vacuoles in the plant cell. Freeze drying and subsequent grinding would allow these components to come together at higher

amounts than in normal plant tissue decay. As fresh mustard decays, GLs and myrosinase enzymes themselves may be degraded by microbes before the reaction leading to ITC production occurs.

Results from the second experiment showed a relationship between soil temperature and AITC concentration. ITC concentration increased with increasing temperature. ITCs partitioning into one phase of the three-phase soil system (vapor, aqueous, and solid) is dependent on their solubility in water (Brown and Morra, 1997). For methyl ITC in re-packed soil columns, gas phase diffusion was the most important factor for the loss of methyl ITC from soil due to the strong partitioning into the gas phase (Frick et. al., 1998). The rate of movement of ITCs to the soil surface and into the atmosphere may be enhanced by higher temperatures due to higher kinetic energies of the compound. By covering the soil in this study, the rate of diffusion from the soil was reduced and higher concentrations of AITC were detected.

Volatilization of AITC into the gas-phase is dependent on its vapor pressure within the soil. Higher soil temperature will result in higher vapor pressures of compounds within the soil liquid or adsorbed onto soil solids. This will cause higher volatilization rates of AITC in the system. Higher volatilization rates will increase the mass flow of AITC from the soil by increasing the diffusion gradient. Reductions in the diffusion of AITC away from soil may be achieved by avoiding biofumigation under high temperatures. However, increased volatilization may be desired for maximum gas-phase AITC concentrations within the soil for pest control. By increasing temperatures and covering the soil, both desired effects may be attained.

Temperature may affect myrosinase activity and subsequent AITC release. Thermal inactivation of myrosinase has been measured at temperatures of 30°C (Ludikhuyze et. al., 1999). Although the temperatures in this study reached 45°C, the glucosinolate-myrosinase reaction may have predominately occurred in the beaker in which Indian mustard and water were combined (at room temperature), thus reducing or eliminating any reduction in myrosinase activity due to high temperature.

Results from the third experiment showed that soil moisture influenced AITC concentration. At 0.25 h after treatment, the wettest soil (field capacity) had lower AITC concentration than the drier soils. This was probably due to the soil pores being filled with water and blocking AITC from diffusing throughout the soil as quickly. According to Van den Berg et. al. (1999), substantial reduction of volatilization rate of methyl ITC can be expected by increasing water content in soil. The permanent wilting point treatment yielded higher concentrations of AITC across time than the field capacity treatment. Reductions in the volatilization and diffusion of AITC from the soil may be achieved by biofumigating under moist soil conditions. Higher concentrations may be attained by fumigating under dryer soil conditions.

The results from the fourth experiment showed that there is a relationship between AITC production and soil pH and soil texture. Across time, the field soil pH (mean of 5.7 for both textures) yielded higher concentrations of AITC than the adjusted soil pH (mean of 6.9 for both textures). This result contradicts those of Borek et. al. (1995) which found no correlation between soil pH (ranging from 4.35 to 9.10) in six soils and the disappearance of AITC.

Soil texture affected AITC concentration measured. AITC levels were higher in the sandy loam textured soil compared to the clay loam textured soil. This may be due to the higher organic carbon content of the clay loam soil to which AITC could adsorb or react. Adsorption of methyl ITC to soil increased with increasing organic matter (Smelt and Leistra, 1974). Adsorption of AITC to soil constituents decreases AITC gas-phase concentration. Biofumigation in soils with high clay content may be less effective than sandyy textured soils.

Results from the fifth experiment showed that there was a relationship between AITC levels and whether or not the soil was autoclaved. AITC concentrations in the autoclaved treatment were up to 41 times greater than the non-autoclaved treatments at 24 h. This is most likely due to microbial degradation of AITC in the non-autoclaved soil. Organic matter and particle size distribution did not change in soil that was autoclaved. These results are different than those found in a study by Borek et. al. (1995) in which autoclaved soil treatments did not change disappearance rates of allyl ITC. There is little published literature about this relationship.

The sixth experiment showed that there were many relationships among the different volatiles and the combined environmental factors in the previous experiments. All of the results of the previous five experiments that were statistically significant were also significant in the sixth experiment. This is due to the fractional factorial design that increased statistical power to find differences among combined treatments. Most of the trends for AITC were repeated in the sec-butyl ITC and 3-butenyl ITC results.

Autoclaved soils had lower $CO_2$ concentrations and higher AITC concentrations than non-autoclaved soils. Microbes may have degraded AITC in the non-autoclaved soil. The

most important result may be that $CO_2$ concentrations were inversely proportional to AITC concentrations when comparing autoclaved vs. non-autoclaved soils.

At the 6 leaf stage, leaves and stems of Indian mustard PI 458934 had an allyl GL concentration of 31.8 $\mu$mol/g and at flowering, 43.5 $\mu$mol/g (unpublished data). By adding 0.31 g of mustard, the maximum theoretical concentration of AITC in the 0.5 L jar should be 23 $\mu$mol/L. The range of AITC recovery for most experiments was between 0.87% and 8.7% of this theoretical concentration. The recovery rate calculated by using AITC spiked samples yielded less than 0.1% of the theoretical AITC yield for both soils. This may be due to the AITC standard reacting differently in the soil. The difference in projected recovery rates and actual concentrations may be due to AITC adsorbing onto soil particles.

The results in summary suggest that higher yields of ITC may be obtained in biofumigation at higher soil temperature, and lower soil moisture. Sandy loam textured soils and lower soil pH may also yield higher ITC concentrations. By covering biofumigated soils, higher ITC concentrations will be maintained. Soil microbes may play a role in the degredation of ITCs.

Biofumigation and solarization may be an environmentally sound and economically feasible alternative for methyl bromide. The problem with replacing such a major pesticide used in crop production with a biofumigation system by the year 2005 is that not enough field research has been done to show actual crop yield response due to decreased but not eliminated pathogen densities. The results in this experiment may help in the development of such field plans. Literature about biofumigation focuses mostly on pathogenic responses to breakdown products of glucosinolates, or on glucosinolate breakdown products themselves.

A particular crop's pathogen response to *Brassica* bi-products is irrelevant unless the same results are found in field yield studies. If field plans for a particular crop such as strawberry, nursery stock, tomato, and tobacco are to be implemented by the year 2005, much field research must take place. Growers might implement cropping practices that are shown to be profitable. Questions concerning toxicity of *Brassica* tissue degradation products to crops, and affects of continued biofumigation in cropping systems remain unanswered. Also, cost-benefit studies have not been done to show the real monetary gain of a biofumigation system for a particular crop. Until research produces recommended field plans for growers, biofumigation will remain only a "possible" alternative strategy to the loss of methyl bromide.

# References

## References


Adams, F. and C. E. Evans. 1962. A rapid method for measuring lime requirement of Red-Yellow Podzolic soils. Soil Sci. Soc. Am. Proc. 26:355-357.

Al-Khatib, K. C. Libbey, and R. Boydston, R. 1997. Weed suppression with *Brassica* green manure crops in green pea. Weed Science. 45:439-445.

Antoniou, P. P., E. C. Tjamos, M. T. Andreou, and C. G. Panagopoulos. 1995. Effectiveness, modes of action and commercial application of soil solarization for control of *clavibacter michiganensis* subsp. *michiganensis* of tomato. Acta Horticulture. 119-128.

Bending, G., and S. Lincoln. 1999. Characterisation of volatile sulpher-containing compounds produced during decomposition of *Brassica juncea* tissue soil. Soil Biology and Biochemistry. 31:695-703.

Bennett, R. 1993. Aldoxime-forming microsomal enzyme systems involved in the biosynthesis of glucosinolates in oilseed rape leaves. Plant Physiol. 102:1307-1312.

Borek, V., M. J. Morra, P. D. Brown, and J. P. McCaffrey. 1995. Transformation of the glucosinolate-derived allelochemicals allyl isothiocyanate allylnitrile in soil. Food Chem. 43:1935-1940.

Brown, P. D., and M. J. Morra. 1997. Control of soil-borne plant pests using glucosinolate-containing plants. Advances in Agronomy. 61:167-231.

Brown, P. D., M. J. Morra, and V. Borek. 1997. Gas chromatography of allelochemicals produced during glucosinolates in soil. J. Agric. Food Chem. 42:2029-2034.

Charron, C. S. and C. E. Sams. 1999. Inhibition of *Pythium ultimum* and *Rhizoctonia solani* by shredded leaves of *Brassica* species. J. Hort. Sci.

Chellemi, D. O., and S. M. Olson. 1994. Effects of soil solarization and fumigation on survival of soilborne pathogens of tomato in northern Florida. Plant Disease. 78:1167-1172.

Chew, F. S. 1988. Biological effects of glucosinolates in: biologically active products. potential use in agriculture. American Chemical Society. 155-181.

Day. P. R. 1965. Particle fractionation and particle-size analysis. Methods of Soil Analysis. Part I. Agronomy. 9:545-567.

Downey, R. K. and G. Robbelen. 1989. *Brassica* Species. Oil Crops of The World. McGraw Hill.

Du, L. and B. A. Halkier. 1996. Isolation of a microsomal enzyme system involved in glucosinolate biosynthesis from seedlings of *Tropaeolum majus* L. Plant Physiol. 111:831-837.

Duke, J. A. 1984 Handbook of energy crops. http://www.hort.purdue.edu/newcrop/duke_energy/Brassica_juncea#Germplasm.

Egley, G. 1983. Weed seed and seedling reduction by soil solarization with transparent polyethylene sheets. Weed Science. 31:404-409.

Fenwick, G. R., R. K. Heaney, and W. J. Mullin. 1983. Glucosinolates and their breakdown products in food and plants. Food Science and Nutrition. 18:123.

Frick, A., B. J. Zebarth, and S. Y. Szeto. 1998. Behavior of the soil fumigant methyl isothiocyanate in repacked soil columns. J. Environ. Qual. 27:1158-1169.

Gamliel, A. and J. J. Stapleton. 1993. Characterization of antifungal volatile compounds evolved from solarization soil amended with cabbage residues. Phytopathology. 83:899-905.

Gamliel, A. and J. J. Stapleton. 1997. Improvement of soil solarization with volatile compounds generated from organic amendments. *Phytoparasitica.* 25:31S-38S.

Halkier, A. and L. Du. 1997. The biosynthesis of glucosinolates. Elsevier Science Ltd. 11:425-431.

Hartz, T. K., J. E. DeVay, and C. L. Elmore. 1993. Solarization is an effective soil disinfestation technique for strawberry production. Hortscience. 28:104-106.

Himelrick, D.G. and W. A. Dozier Jr. 1991. Soil fumigation and soil solarization in strawberry production. Advances in Strawberry Production. 10:12-27.

Josefsson, E. 1967. Distribution of thioglucosides in different parts of *Brassica* plants. Phytochemistry. 6:1617.

Kirkegaard, J. A., P. T. W. Wong, J. M. Desmarchelier, and M. Sarwar. 1996. Suppression of soilborne cereal pathogens and inhibition of wheat germination by mustard seed meal. Proc. 8th Austral. Agron. Conf. Toowoomba. 353-356.

Klute, A. 1986. Methods of soil analysis. Amer. Soc. of Agron., Inc. Madison, Wisconson.

Ludford, P. M. and F. M. R. Isenberg. 1994. Brassica Crops. Postharvest Physiology of Vegetables. Cornell University, New York.

Ludikhuyze, L., V. Ooms, C. Weemaes, and M. Hendrickx. 1999. Kinetic study of the irreversible thermal and pressure inactivation of myrosinase from broccoli (*Brassica oleracea* L. Cv. Italica). J. Agric. Food Chem. 47:1794-1800.

Mari, M., R. Iori, and A. Marchi. 1996. Bioassays of glucosinolate-derived isothiocyanates against postharvest pear pathogens. Plant Pathology. 45:753-760.

Mayton, H. S., C. Olivier, S. F. Vaughn, and R. Loria. 1996. Correlation of fungicidal activity of Brassica species with allyl isothiocyanate production in macerated leaf tissue. Phytopathology. 86:267-271.

Mithen, R. 1995. Genetics of aliphatic glucosinolates. III. Side chain structure of aliphatic glucosinolates in *Arabidopis thaliana*. Heredity. 74:210-215.

Muehlchen, A.M., R. E. Rand, and J. L. Park. 1997. Evaluation of crucifer green manures for controlling Aphanomyces root rot of peas. Plant Disease. 74:651-654.

Noble, R. R. P., C. S. Charron, and C. E. Sams. 1998. Toxicity of Indian mustard and allyl isothiocyanate to Masked Chaffer beetle larvae. (Abstr.)

Ohashi, M., J. M. Wilson, and C. Djerassi. 1963. Mass spectra of isothiocyanates. Acta. Chem. Scand. 17:2143-2157.

Peder, Olesen, and Larsen. 1981. Glucosinolates. The Biochemistry of Plants.

Roberts, W., B. C. Nichols, J. N. Odom, M. H. Gallatin, and L. E. Odom. 1955. Soil Survey, Knox County Tennessee. Soil Conservation Service. United States Department of Agriculture.

Sams, C. E., C. S. Charron, and C. O. Chardonnet. 1997. The potential for using *Brassicas* as an alternative to methyl bromide in controlling soilborne diseases. Annul. Intl. Conf. On Methyl Bromide Alternatives and Emmissions Reductions. (19)1-(19)3.

SAS Institute. 1996. SAS/STAT user's guide, version 6, 4th ed., vol. 1. SAS Inst., Cary, N.C.

Sibbesen, O. 1995. Cytochrome P-450 is a multifuntional heme-thiolate enzyme catalyzing the conversion of L-tyrosine to p-hydroxphenylacetaldehyde oxime in the biosynthesis of the cyanogenic glucoside dhurrin in *Sorghum bicolor* L. J. Biol. Chem. 270:3506-3511.

Smelt, J. H., and M. Leistra. 1974. Conversion of metham-sodium to methyl isothiocyante and basic data on the behavior of methyl isothiocyante in soil. Pestic. Sci. 5:401-407.

Sorensen, H. 1991. Glucosinolate structure-properties-function, in canola and rapeseed. Van Nostrand Reinhold. 9:149-172.

Stapleton, J. J., and J. E. DeVay. 1986. Soil solarization: a non-chemical approach for management of plant pathogens and pests. Crop Protection. 5:190-198.

Subbarao, K. V., and J. C. Hubbard. 1996. Interactive effects of broccoli residue and temperature on *Verticillium dahliae* Microsclerotia in soil and on wilt in cauliflower. Phytopathology. 86:1303-1310.

Underhill, E. W., L. R. Wetter, and M. D. Chisholm. 1973. Biosynthesis of glucosinolates. Biochem. Soc. Symp. 38:303-326.

U. S. Department of Agriculture. 1999. Administration extends deadline on MeBr ban to 2005. Methyl Bromide Alternatives. 5:1.

Van den Berg, F., J. H. Smelt, T. I. Boesten, and W. Teunissen. 1999. Volatilization of methyl isothiocyanate from soil after application of metam-sodium with two techniques. J. Environ. Qual. 28:918-928.

Vaughn, S. F., and R. A. Boydston. 1997. Volatile allelochemicals released by crucifer green manures. J. of Chemical Ecology. 23,9:2107-2116.

Wheelwrite, J. 1997. The berry and the poison. Smithsonian. 27:40-51.

Wilson, H. 1998. Taxonomy of flowering plants http://csdl.tamu.edu/FLORA/Wilson/tfp/dil/brapage.htm

# Appendices

## Appendix I.

### Particle-size Analysis

(Day, Methods of Soil Analysis. Soil Science Society of America 1994.)

### Materials:

Beakers
Bronze stirrer
Drying oven
Fleakers
Hotplate
Pipet
Reagents
Scale
Sieve shaker
Sedimentation cylinder
Set of sieves
Thermometer
Watch glasses
Water bath

### Procedure:

Organic matter removal

•Weigh approximately 11 grams of soil into a 300 mL fleaker and record weight to the nearest 0.001 mg. Add 50 mL of deionized water to fleaker. Add 10 mL of hydrogen peroxide to fleaker. Heat fleaker on a hotplate at 80 °C for one hour (or until reaction has stopped) while adding hydrogen peroxide (5 mL) to facilitate chemical reaction. Heat fleaker in drying oven until sample is dry.

Chemical dispersion

•Weight fleaker after drying to the nearest 0.001 mg. Add 10 mL of Na-hexametaphosphate and shake for eight hours.

Separation of sand size particles

•Pour the suspended sample through a 300 mesh sieve into a 1205 mL sedimentation cylinder. The sieve is then placed on a plastic funnel held by a stand above the cylinder. The sand is washed thoroughly on the sieve with distilled water. Transfer the sand sized particles into a tared beaker, dry (105 °C), and weigh. The sand size particles are then

transferred to the top of a stack of sieves arranged from top to bottom with decreasing size in the following order: 1000, 500, 250, 106, 53 microns, and pan. Shake the sieves on a sieve shaker for five minutes. Weigh each sand fraction and the residual silt and clay that passed through the 53 micron (270 mesh) sieve to the nearest 0.01 gram.

Pipet method for silts and clays

•Sedimentation analysis is based on the relationship between settling velocity and particle diameter. The pipet method is a direct sampling method. Small sub-samples are taken at a depth *h*, at time *t*, in which all particles coarser than *X* have settled out. Using Stokes' Law, settling times for the clay fraction (less than 2 microns) can be calculated.

Stokes' Law: $t = 18\, nh / [g\,(p_s - p_l)X^2]$

The clay and silt fraction of the sample was washed through a 300 mesh sieve and into a 1205 mL sedimentation cylinder. Distilled water is then added to bring the cylinder to volume. Place the cylinder containing the silt and clay suspension into a water bath and cover with a watch glass. Allow the suspension to stand several hours to let the temperature equilibrate. After equilibration, stir the suspension thoroughly for at least 30 seconds using an up-and-down motion. Note the time at the completion of stirring and the temperature of the water bath. After 3 minutes 40 seconds, sample the suspension for the silt fraction at the depth of 10 cm with a pipet. Withdraw a 25 mL sample in about 12 seconds. Discharge the sample into a tared and numbered weighing beaker. Rinse the pipet with distilled water into the same beaker. Evaporate the water in the beaker, and dry the silt at105 °C. Cool the beaker in a desiccator, and weigh. Repeat sampling technique at 6 hours 10 minutes for clay fraction (do not re-stir).

## Appendix II.

### Soil pH Determination

(Thomas, Methods of Soil Analysis. Soil Science Society of America 1994.)

### Materials:

pH meter equipped with glass and reference electrode
50 mL beakers
Pipet
Standard buffers, pH 7 and pH 4
Deionized water
0.01 M $CaCl_2$ solution

### Proceedure:

Weigh 10 g of air-dry soil into a 50 mL beaker. Add 10 mL of deionized water to the soil in the beaker and mix. Let mixture stand for 10 minutes. Swirl the suspension in the beaker and insert the electrode. Stay consistant with depth of electrode. Read pH meter and record as $pH_w$. Rinse the electrode with distilled water. Repeat the proceedure again but use 0.01 M $CaCl_2$ instead of water to make the soil suspension. Read pH meter and record as $pH_{CaCl2}$.

## Appendix III.

### LIME REQUIREMENT

(Adams and Evans, Methods of Soil Analysis. Soil Science Society of America, 1994.)

**Materials:**

pH meter equipped with glass and reference electrode
Standard buffers, pH 7 and pH 4
Deionized water
50 mL beakers
Buffer solution at pH 8 ± 0.1 consisting of the following made up in 1-liter solution: *p*-nitrophenol 20g, Boric acid (H3BO3) 15g, Potassium chloride (KCL) 74g, and Potassium hydroxide (KOH) 10.5g.

**Procedure:**

Measure 20g of air-dry soil into a 50 mL beaker. Add 20 mL of distilled water and stir with a glass rod for one hour. Read soil-water pH on standardized pH meter. To each cup add 20 mL of buffer solution stirring twice at 10 minute intervals. Read soil-buffer pH. Select lime requirement from table in reference.

## Appendix IV.

### Determination of Water Retention

(Klute, Methods of Soil Analysis. Soil Science Society of America 1994.)

**Materials:**

Distilled water
Pressure cell apparatus (high and low pressures)
Weighing scale

**Procedure for low pressure cell:**

Fill tempe cell sleeve with soil. Place sleeve into a reservoir of distilled water until sample is saturated. Attach end caps, and weigh entire tempe cell, and record weight to the nearest 0.001 mg. Connect tempe cell to the regulated gas pressure source. Carry pressure cells through a sequence of low pressure equilibria, and determine its weight at each. At the final equilibrium, determine the final water content. Back calculate the water contents at all previous pressures using the change in weight.

**Procedure for high pressure cell:**

Weigh approximately one tablespoon of the soil sample into a ring on the wetted ceramic plate in the pressure cell apparatus. Replicate six times for each soil texture. Thoroughly wet each sample with distilled water. Secure lid on apparatus. Carry pressure cell through a sequence of high pressure equilibria, and determine each sample weight at each. At the final equilibrium, determine the final water content. Back calculate the water contents at all previous pressures using the change in weight.

**Calculations:**

Field capacity:

After equilibrium at 33 kPa, weigh sample to nearest 0.01 mg. Dry sample in oven at 105 °C until weight does not change. The weight difference is the moisture held at field capacity for each sample weight.

Permanent wilting point:

After equilibrium at 1500 kPa, weigh sample to nearest 0.01 mg. Dry sample in oven at 105 °C until weight does not change. The weight difference is the moisture held at permanent wilting point for each sample weight.

## Appendix V.

### Soil sterilization by dry heat

(Wolf and Skipper, Methods of Soil Analysis. Soil Science Society of America 1994.)

**Materials:**

Aluminum foil
Containers for soil
Oven capable of temperatures greater than 200 °C

**Procedure:**

Spread soil to a depth of 2.5 cm in a shallow container. Cover soil with aluminum foil to prevent subsequent contamination. Heat soil at 200 °C for 24 hours. Verify sterility by inoculating growth media and confirming the absence of microbial growth. Store soil in a air-tight container.

## Appendix VI.

**Determination of glucosinolate content by gas liquid chromatography of trimethylsilyl derivatives of desulfated glucosinolates**
(Raney and McGregor, 1990. Selected methods for glucosinolate analysis, Agriculture Canada)

**Reagents:**

Acetone
Barium actetate
Benzyl glucosinolate (tetramethyl ammonium salt)
Dimethylformamide
Ethanol, absolute
Glacial acetic acid
Hydrochloric acid
Lead acetate
Methanol
1-Methyl imidazole
N, O-bis (Trimethylsilyl) tetrafluoroacetamide
Pyridine
Sephadex DEAE A25
Sinigrin (allyl glucosinolate)
Sodium acetate
Sodium hydroxide
Sulfatase
Trimethylsilylchlorosilane

**Preparation:**

Benzyl glucosinolate, 1 mM

- Weigh 24.1 mg of benzyl glucosinolate, tetreamethyl ammonium salt, into a 50 mL volumetric flask, and fill to volume with water.

Barium-lead acetate, 0.6 M

- Weigh 7.66 g of barium acetate and 11.38 g lead acetate into a 100 mL volumetric flask, and fill to volume with water.

DEAE Sephadex column, 0.2 mL

- Cut a 1 mL plastic syringe at the 0.2 mL mark. Insert a disk of porous polyethylene cut from a sheet with a #1 cork bore into the bottom of the syringe. Fill this part of the syringe with DEAE Sephadex A-25 pre-swollen in water.

Methanol, 67%

- Transfer 67 mL of methanol to a 100 mL volumetric flask, and fill to volume with water.

Pyridine acetate, 0.02 M

- Transfer 2 mL pyridine and 1.5 mL glacial acetic acid to a 1000 mL volumetric flask, and fill to volume with water.

Purification of sulfatase

- Weigh 70 mg of sulfatase into a centrifuge tube. Add 3 mL of water and swirl to dissolve sulfatase, minimizing formation of foam as much as possible. Add 3 mL absolute ethanol and mix. Centrifuge at 2000 g for 15 minutes. Discard the supernatant and dissolve the precipitant in 2.5 mL water. Store frozen and thaw just before use.

Methanol, 60%

- Transfer 60 mL of methanol to a 100 mL volumetric flask, and fill to volume with water.

Sodium acetate, 1 M

- Weigh 8.2 g of sodium acetate into a 100 mL volumetric flask, and fill to volume with water.

Sodium hydroxide, 0.5 M

- Weigh 2 g of sodium hydroxide into a 100 mL volumetric flask. Dissolve and fill to volume with water.

Hydrochloric acid, 0.5 M

- Place 4.3 mL of concentrated hydrochloric acid in a 100 mL volumetric flask containing water, and fill to volume with water.

**Procedure:**

Glucosinolate Extraction

Weigh 200 mg of oil-extracted meal into a 4 mL vial and record weight to the nearest 0.1 mg.

**Note**: This is a critical measurement. Be accurate.

Add 2 mL of methanol, 1 mL 1 mM benzyl glucosinolate standard solution and 0.1 mL of 0.6 M barium-lead acetate, cap vial, and mix.

**Note**: The order of addition of these reagents is essential. Water must not contact the meal in the absence of methanol. The addition of benzyl glucosinolate is a critical measurement. Be accurate.

Shake vials in a Eberbach shaker (200 strokes per minute) for one hour. Centrifuge at
2000 g for 10 minutes.

Sulfatase Hydrolysis

Transfer approximately 105 mL of the supernatant to a 0.2 mL DEAE Sephadex A25 column. Wash column with 1.8 mL 67% methanol, 1.8 mL water, and 1 mL 0.02 M pyridine-acetate. Add 0.05 mL of purified sulfatase to the column, cap and let stand overnight at room temperature. Elute column twice with 0.45 mL 60% methanol into a 1 mL autosampler vial or 4 mL screw-cap vial. Regenerate column after use by washing
with 1 mL 1 M sodium acetate, 1 mL 0.5 M sodium hydroxide and 1 mL 0.5 M hydrochloric acid followed by two washes of 1.8 mL water.

Derivatization

Place the vial in a dry block heater at 60 °C and take to dryness with a stream of air passing over the sample. Remove the vials when they are dry. Immediately before addition, premix acetone, dimethylformamide, N, O-bis (Trimethylsilyl) tetrafluoroacetamide, trimethylsilylchlorosilane, and 1-methylimidazole (40:20:20:2:1). Add 400 micro liters of the derivatization mixture to the vial, cap immediately with a Teflon lined cap, and let
stand for 15 minutes at room temperature.

Chromatographic Conditions

The parameters are injector temperature, 300 °C, detector temperature, 300 °C, and oven temperature, 245 °C ramped to 281 °C over 13 minutes.

## Calculation and Reporting of Results

Area glucosinolate / area benzyl glucosinolate standard * response factor * 0.86 * 1000 / 100 = micro moles per gram oil extracted meal. The response factor is the ratio of the carbon number of the trimethylsilyl derivative of the glucosinolate to the carbon number of the trimethylsilyl derivative of the internal standard (benzyl glucosinolate = 29). The factor 0.86 adjusts for the response of benzyl glucosinolate relative to allyl glucosinolate. The factor 1000 / 100 converts to a gram basis (1000 mg) the weight of oil-extracted meal (100 mg) from which the glucosinolates were extracted.

## Appendix VII.

## Modification of prior method for determination of glucosinolate content in *Brassica* leaves, stems, and roots

**Procedure:**

Glucosinolate extraction

Weigh 500 mg of freeze dried leaf material into a 4 mL vial, and record weight to the nearest 0.1 mg. Add 6 mL of methanol, 1 mL 1mM benzyl glucosinolate standard solution, and 0.3 mL of 0.6 M barium-lead acetate. Cap vial and mix.

## Appendix VIII.

The following pages contain the ANOVA tables from SAS statistical software output and raw data for experiments 1-6. The experiment number corresponds with the experiments in the text.

### Experiment 1

General Linear Models Procedure

Class Level Information

| Class | Levels | Values |
|---|---|---|
| PLANT | 2 | 1 2 |
| SOIL | 2 | 1 2 |
| COVER | 2 | 1 2 |
| MICRO | 2 | 1 2 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| PLANT | 1 | 12201842 | 12201842 | 0.13 | 0.7282 |
| SOIL | 1 | 93138766 | 93138766 | 0.96 | 0.3434 |
| COVER | 1 | 91928049 | 91928049 | 0.94 | 0.3464 |
| MICRO | 1 | 93456636 | 93456636 | 0.96 | 0.3426 |
| REP | 1 | 116601721 | 116601721 | 1.20 | 0.2909 |
| PLANT*SOIL | 1 | 22452984 | 22452984 | 0.23 | 0.6379 |
| PLANT*COVER | 1 | 101888010 | 101888010 | 1.05 | 0.3224 |
| PLANT*MICRO | 1 | 73144125 | 73144125 | 0.75 | 0.3996 |
| REP*PLANT | 1 | 142323756 | 142323756 | 1.46 | 0.2452 |
| SOIL*COVER | 1 | 52733265 | 52733265 | 0.54 | 0.4730 |
| SOIL*MICRO | 1 | 8520203 | 8520203 | 0.09 | 0.7713 |
| REP*SOIL | 1 | 40761421 | 40761421 | 0.42 | 0.5272 |
| COVER*MICRO | 1 | 10996888 | 10996888 | 0.11 | 0.7414 |
| REP*COVER | 1 | 4449145 | 4449145 | 0.05 | 0.8335 |
| REP*MICRO | 1 | 108103808 | 108103808 | 1.11 | 0.3085 |
| REP*REP | 1 | 16556848 | 16556848 | 0.17 | 0.6858 |
| PLANT*SOIL*COVER | 1 | 120780248 | 120780248 | 1.24 | 0.2827 |
| PLAN*SOIL*COVE*MICRO | 4 | 85927447 | 21481862 | 0.22 | 0.9226 |
| REP*PLA*SOI*COV*MICR | 11 | 196430422 | 17857311 | 0.18 | 0.9964 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| PLANT | 1 | 152899709 | 152899709 | 1.57 | 0.2292 |
| SOIL | 1 | 5122039 | 5122039 | 0.05 | 0.8216 |
| COVER | 1 | 31099827 | 31099827 | 0.32 | 0.5802 |
| MICRO | 1 | 35666369 | 35666369 | 0.37 | 0.5539 |
| REP | 1 | 31023565 | 31023565 | 0.32 | 0.5806 |
| PLANT*SOIL | 1 | 197735 | 197735 | 0.00 | 0.9646 |
| PLANT*COVER | 1 | 14448231 | 14448231 | 0.15 | 0.7054 |
| PLANT*MICRO | 1 | 51581724 | 51581724 | 0.53 | 0.4777 |
| REP*PLANT | 1 | 142323756 | 142323756 | 1.46 | 0.2452 |
| SOIL*COVER | 1 | 11303236 | 11303236 | 0.12 | 0.7379 |
| SOIL*MICRO | 1 | 36663697 | 36663697 | 0.38 | 0.5485 |
| REP*SOIL | 1 | 40761421 | 40761421 | 0.42 | 0.5272 |

| | | | | | |
|---|---|---|---|---|---|
| COVER*MICRO | 1 | 223769 | 223769 | 0.00 | 0.9624 |
| REP*COVER | 1 | 4449145 | 4449145 | 0.05 | 0.8335 |
| REP*MICRO | 1 | 108103808 | 108103808 | 1.11 | 0.3085 |
| REP*REP | 1 | 16556848 | 16556848 | 0.17 | 0.6858 |
| PLANT*SOIL*COVER | 1 | 33500 | 33500 | 0.00 | 0.9854 |
| PLAN*SOIL*COVE*MICRO | 3 | 32911988 | 10970663 | 0.11 | 0.9513 |
| REP*PLA*SOI*COV*MICR | 7 | 165270526 | 23610075 | 0.24 | 0.9670 |
| SOIL*COVER*MICRO | 1 | 973199 | 973199 | 0.01 | 0.9217 |
| REP*SOIL*COVER*MICRO | 3 | 30449465 | 10149822 | 0.10 | 0.9563 |
| REP*COVER*MICRO | 1 | 710432 | 710432 | 0.01 | 0.9330 |

Experiment 1

| Cultivar | Soil type | Top | Microbe status | Rep |
|---|---|---|---|---|
| 1 = 458928 | 1 = clay loam | 1 = covered | 1 = sterilized | 1 |
| 2 = 458934 | 2 = sand loam | 2 = uncovered | 2 = unsterilized | 2 |
| | | | | 3 |

| sample | Compound | .25 hour | 4 hour | 8 hour | 24 hour | 48 | 96 |
|---|---|---|---|---|---|---|---|
| 11111 | AITC | 14257 | 10039 | 4922 | 605 | 554 | 0 |
| 11112 | AITC | 4647 | 1035 | 565 | 0 | 0 | 0 |
| 11113 | AITC | 14360 | 1035 | 791 | 278 | 0 | 0 |
| | **AVER** | **11088** | **4036.333** | **2092.667** | **294.3333** | **184.6667** | **0** |
| | **STDEV** | **4554.669** | **4244.526** | **2002.767** | **247.2601** | **261.1581** | **0** |
| | **STERR** | **2629.639** | **2450.578** | **1156.298** | **142.7557** | **150.7797** | **0** |
| 11121 | AITC | 22445 | 1577 | 662 | 261 | 0 | 0 |
| 11122 | AITC | 8854 | 0 | 0 | 0 | 0 | 0 |
| 11123 | AITC | 9901 | 196 | 0 | 0 | 0 | 0 |
| | **AVER** | **13733.33** | **591** | **220.6667** | **87** | **0** | **0** |
| | **STDEV** | **6174.89** | **701.7839** | **312.0698** | **123.0366** | **0** | **0** |
| | **STERR** | **3565.075** | **405.1751** | **180.1736** | **71.0352** | **0** | **0** |
| 11211 | AITC | 17192 | 4552 | 1399 | 474 | 0 | 0 |
| 11212 | AITC | 6803 | 3500 | 1403 | 0 | 0 | 0 |
| 11213 | AITC | 18151 | 2018 | 989 | 300 | 0 | 0 |
| | **AVER** | **14048.67** | **3356.667** | **1263.667** | **258** | **0** | **0** |
| | **STDEV** | **5138.397** | **1039.454** | **194.2255** | **195.7754** | **0** | **0** |
| | **STERR** | **2966.655** | **600.1291** | **112.1362** | **113.031** | **0** | **0** |
| 11221 | AITC | 15071 | 551 | 226 | 0 | 0 | 0 |
| 11222 | AITC | 7589 | 0 | 0 | 0 | 0 | 0 |
| 11223 | AITC | 21513 | 784 | 382 | 0 | 0 | 0 |
| | **AVER** | **14724.33** | **445** | **202.6667** | **0** | **0** | **0** |
| | **STDEV** | **5689.732** | **328.7258** | **156.8212** | **0** | **0** | **0** |
| | **STERR** | **3284.968** | **189.7899** | **90.54076** | **0** | **0** | **0** |
| 12111 | AITC | 16396 | 1286 | 621 | 407 | 0 | 0 |
| 12112 | AITC | 10135 | 17743 | 69048 | 34591 | 5819 | 0 |
| 12113 | AITC | 17762 | 7041 | 2906 | 554 | 231 | 0 |
| | **AVER** | **14764.33** | **8690** | **24191.67** | **11850.67** | **2016.667** | **0** |
| | **STDEV** | **3320.596** | **6818.974** | **31731.93** | **16079.96** | **2690.309** | **0** |
| | **STERR** | **1917.147** | **3936.937** | **18320.44** | **9283.767** | **1902.336** | **0** |

| | | | | | | |
|---|---|---|---|---|---|---|
| 12121 | AITC 10564 | 734 | 319 | 0 | 0 | 0 |
| 12122 | AITC 15739 | 42812 | 36370 | 7630 | 3390 | 1766 |
| 12123 | AITC 20647 | 802 | 402 | 0 | 0 | 0 |
| | **AVER 15650** | **14782.67** | **12363.67** | **2543.333** | **1130** | **588.6667** |
| | **STDEV 4116.849** | **19819.75** | **16975.07** | **3596.816** | **1598.061** | **832.5004** |
| | **STERR 2376.864** | **11442.94** | **9800.564** | **2076.623** | **1130** | **588.6667** |
| 12211 | AITC 11184 | 457 | 0 | 0 | 0 | 0 |
| 12212 | AITC 2847 | 0 | 0 | 0 | 0 | 0 |
| 12213 | AITC 10976 | 3435 | 1803 | 333 | 0 | 0 |
| | **AVER 8335.667** | **1297.333** | **601** | **111** | **0** | **0** |
| | **STDEV 3882.002** | **1523.029** | **849.9424** | **156.9777** | **0** | **0** |
| | **STERR 2241.275** | **879.3212** | **490.7144** | **90.63112** | **0** | **0** |
| 12221 | AITC 4246 | 0 | 0 | 0 | 0 | 0 |
| 12222 | AITC 5926 | 139 | 0 | 0 | 0 | 0 |
| 12223 | AITC 12991 | 684 | 160 | 0 | 0 | 0 |
| | **AVER 7721** | **274.3333** | **53.33333** | **0** | **0** | **0** |
| | **STDEV 3789.043** | **295.1839** | **75.42472** | **0** | **0** | **0** |
| | **STERR 2187.605** | **170.4245** | **43.54648** | **0** | **0** | **0** |
| 21111 | AITC 0 | 329 | 180 | 61 | 0 | 0 |
| 21112 | AITC 28481 | 851 | 478 | 215 | 0 | 0 |
| 21113 | AITC 6427 | 15244 | 23851 | 18934 | 3898 | 1733 |
| | **AVER 11636** | **5474.667** | **8169.667** | **6403.333** | **1299.333** | **577.6667** |
| | **STDEV 12196.78** | **6911.248** | **11089.04** | **8860.742** | **1837.535** | **816.944** |
| | **STERR 7041.813** | **3990.211** | **6402.263** | **5115.752** | **1299.333** | **577.6667** |
| 21121 | AITC 6094 | 328 | 172 | 0 | 0 | 0 |
| 21122 | AITC 15552 | 839 | 453 | 100 | 0 | 0 |
| 21123 | AITC 2574 | 0 | 0 | 0 | 0 | 0 |
| | **AVER 8073.333** | **389** | **208.3333** | **33.33333** | **0** | **0** |
| | **STDEV 5479.99** | **345.2255** | **186.7125** | **47.14045** | **0** | **0** |
| | **STERR 3163.874** | **199.3161** | **107.7985** | **27.21655** | **0** | **0** |
| 21211 | AITC 2559 | 111 | 62 | 0 | 0 | 0 |
| 21212 | AITC 15217 | 414 | 232 | 0 | 0 | 0 |
| 21213 | AITC 9011 | 10116 | 8851 | 2212 | 690 | 0 |
| | **AVER 8929** | **3547** | **3048.333** | **737.3333** | **230** | **0** |
| | **STDEV 5167.932** | **4646.631** | **4103.692** | **1042.747** | **325.2691** | **0** |
| | **STERR 2983.707** | **2682.734** | **2369.268** | **602.0301** | **230** | **0** |
| 21221 | AITC 2979 | 162 | 72 | 0 | 0 | 0 |
| 21222 | AITC 12402 | 467 | 231 | 0 | 0 | 0 |
| 21223 | AITC 14489 | 740 | 335 | 0 | 0 | 0 |
| | **AVER 9956.667** | **456.3333** | **212.6667** | **0** | **0** | **0** |
| | **STDEV 5006.98** | **236.088** | **108.1491** | **0** | **0** | **0** |
| | **STERR 2890.781** | **136.3055** | **62.4399** | **0** | **0** | **0** |
| 22111 | AITC 22111 | 809 | 425 | 0 | 0 | 0 |
| 22112 | AITC 22411 | 1412 | 684 | 119 | 0 | 0 |
| 22113 | AITC 7493 | 14943 | 20764 | 20486 | 2679 | 1795 |
| | **AVER 17338.33** | **5721.333** | **7291** | **6868.333** | **893** | **598.3333** |
| | **STDEV 6962.779** | **6525.348** | **9527.436** | **9629.267** | **1262.893** | **846.1711** |
| | **STERR 4019.962** | **3767.412** | **5500.668** | **5559.46** | **893** | **598.3333** |
| 22121 | AITC 5864 | 194 | 0 | 0 | 0 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 22122 | AITC 22485 | 1673 | 846 | 353 | 0 | 0 |
| 22123 | AITC 7471 | 611 | 195 | 0 | 0 | 0 |
| | **AVER 11940** | **826** | **347** | **117.6667** | **0** | **0** |
| | **STDEV 7485.247** | **622.6444** | **361.7154** | **166.4058** | **0** | **0** |
| | **STERR 4321.609** | **359.4839** | **208.8365** | **96.07443** | **0** | **0** |
| 22211 | AITC 14509 | 312 | 140 | 0 | 0 | 0 |
| 22212 | AITC 25105 | 1113 | 570 | 277 | 0 | 0 |
| 22213 | AITC 16444 | 24013 | 15599 | 2474 | 959 | 0 |
| | **AVER 18686** | **8479.333** | **5436.333** | **917** | **319.6667** | **0** |
| | **STDEV 4607.149** | **10988.83** | **7188.234** | **1106.758** | **452.0769** | **0** |
| | **STERR 2659.938** | **6344.403** | **4150.129** | **638.9869** | **319.6667** | **0** |
| 22221 | AITC 4836 | 252 | 127 | 0 | 0 | 0 |
| 22222 | AITC 7632 | 171 | 0 | 0 | 0 | 0 |
| 22223 | AITC 19703 | 1115 | 344 | 0 | 0 | 0 |
| | **AVER 10723.67** | **512.6667** | **157** | **0** | **0** | **0** |
| | **STDEV 6451.136** | **427.1958** | **142.0305** | **0** | **0** | **0** |
| | **STERR 3724.565** | **246.6416** | **82.00136** | **0** | **0** | **0** |

## Experiment 2

General Linear Models Procedure

Class Level Information

| Class | Levels | Values |
|---|---|---|
| TEMP | 3 | 15 30 45 |
| COVER | 2 | no yes |
| TIME | 4 | 0.25 4 8 24 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TEMP | 2 | 0.3096117 | 0.1548058 | 36.48 | 0.0001 |
| COVER | 1 | 0.0554572 | 0.0554572 | 13.07 | 0.0015 |
| REP | 1 | 0.0056584 | 0.0056584 | 1.33 | 0.2606 |
| TIME | 3 | 3.8601862 | 1.2867287 | 303.23 | 0.0001 |
| TEMP*COVER | 2 | 0.0333257 | 0.0166628 | 3.93 | 0.0348 |
| REP*TEMP | 2 | 0.0274466 | 0.0137233 | 3.23 | 0.0587 |
| COVER*TIME | 3 | 0.0585478 | 0.0195159 | 4.60 | 0.0121 |
| REP*COVER | 1 | 0.0676229 | 0.0676229 | 15.94 | 0.0006 |
| REP*REP | 1 | 0.0025292 | 0.0025292 | 0.60 | 0.4483 |
| REP*TIME | 3 | 0.1769468 | 0.0589823 | 13.90 | 0.0001 |
| TEMP*COVER*TIME | 12 | 0.6002678 | 0.0500223 | 11.79 | 0.0001 |
| REP*COVER*TIME | 3 | 0.0392564 | 0.0130855 | 3.08 | 0.0484 |
| REP*TEMP*COVER | 2 | 0.0237905 | 0.0118952 | 2.80 | 0.0823 |
| REP*TEMP*COVER*TIME | 12 | 0.0728067 | 0.0060672 | 1.43 | 0.2253 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TEMP | 2 | 0.0036115 | 0.0018057 | 0.43 | 0.6587 |
| COVER | 1 | 0.0184571 | 0.0184571 | 4.35 | 0.0488 |
| REP | 1 | 0.0010392 | 0.0010392 | 0.24 | 0.6256 |
| TIME | 3 | 0.9313896 | 0.3104632 | 73.16 | 0.0001 |
| TEMP*COVER | 2 | 0.0050877 | 0.0025439 | 0.60 | 0.5578 |
| REP*TEMP | 2 | 0.0252612 | 0.0126306 | 2.98 | 0.0718 |
| COVER*TIME | 3 | 0.0477253 | 0.0159084 | 3.75 | 0.0258 |
| REP*COVER | 1 | 0.0639549 | 0.0639549 | 15.07 | 0.0008 |
| REP*REP | 1 | 0.0027010 | 0.0027010 | 0.64 | 0.4335 |
| REP*TIME | 3 | 0.1279859 | 0.0426620 | 10.05 | 0.0002 |
| TEMP*COVER*TIME | 12 | 0.1188156 | 0.0099013 | 2.33 | 0.0409 |
| REP*COVER*TIME | 3 | 0.0357677 | 0.0119226 | 2.81 | 0.0632 |
| REP*TEMP*COVER | 2 | 0.0223567 | 0.0111783 | 2.63 | 0.0943 |
| REP*TEMP*COVER*TIME | 12 | 0.0728067 | 0.0060672 | 1.43 | 0.2253 |

Experiment 2

temperature
1 = 15
2 = 30
3 = 45

cover
1 = no
2 = yes

| temp | cover | rep | | AITC conc. 15 min | 4 hour | 8 hour | 24 hour |
|---|---|---|---|---|---|---|---|
| | 1 | 1 | 1 | 0.795678 | 0.261718 | 0.149874 | 0.018709 |
| | 1 | 1 | 2 | 0.452639 | 0.310996 | 0.125201 | 0.027734 |
| | 1 | 1 | 3 | 0.326712 | 0.48772 | 0.249904 | 0.034696 |
| | | | avg | 0.525009 | 0.353478 | 0.174993 | 0.027046 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | stdev | 0.242715 | 0.118839 | 0.066037 | 0.008016 |
| | | sterr | 0.140131 | 0.068612 | 0.038126 | 0.004628 |
| 1 | 2 | 1 | 0.427104 | 0.405651 | 0.29519 | 0.02592 |
| 1 | 2 | 2 | 0.613851 | 0.537315 | 0.308048 | 0.034311 |
| 1 | 2 | 3 | 0.720412 | 0.743565 | 0.568973 | 0.096106 |
| | | avg | 0.587122 | 0.562177 | 0.390737 | 0.052112 |
| | | stdev | 0.14847 | 0.170323 | 0.154491 | 0.03833 |
| | | sterr | 0.085719 | 0.098336 | 0.089195 | 0.02213 |
| 2 | 1 | 1 | 0.712883 | 0.051818 | 0.022042 | 0 |
| 2 | 1 | 2 | 0.599859 | 0.061093 | 0.023562 | 0 |
| 2 | 1 | 3 | 0.426718 | 0.152074 | 0.022813 | 0 |
| | | avg | 0.57982 | 0.088328 | 0.022806 | 0 |
| | | stdev | 0.144131 | 0.0554 | 0.00076 | 0 |
| | | sterr | 0.083214 | 0.031985 | 0.000439 | 0 |
| 2 | 2 | 1 | 0.600993 | 0.144386 | 0.03456 | 0 |
| 2 | 2 | 2 | 0.615461 | 0.141393 | 0.036851 | 0 |
| 2 | 2 | 3 | | 0.322221 | 0.063814 | 0.025353 |
| | | avg | 0.608227 | 0.202667 | 0.045075 | 0.008451 |
| | | stdev | 0.01023 | 0.103548 | 0.016269 | 0.014638 |
| | | sterr | 0.005907 | 0.059784 | 0.009393 | 0.008451 |
| 3 | 1 | 1 | 0.834637 | 0.029866 | 0 | 0 |
| 3 | 1 | 2 | 0.843663 | 0.044765 | 0.021362 | 0 |
| 3 | 1 | 3 | 0.660453 | 0.046738 | 0.02109 | 0 |
| | | avg | 0.779584 | 0.040456 | 0.014151 | 0 |
| | | stdev | 0.103269 | 0.009224 | 0.012256 | 0 |
| | | sterr | 0.059623 | 0.005326 | 0.007076 | 0 |
| 3 | 2 | 1 | 0.75123 | 0.065379 | 0.028006 | 0 |
| 3 | 2 | 2 | 0.906524 | 0.077239 | 0.03837 | 0 |
| 3 | 2 | 3 | 0.552668 | 0.356192 | 0.064789 | 0.022405 |
| | | avg | 0.736807 | 0.16627 | 0.043722 | 0.007468 |
| | | stdev | 0.177368 | 0.164584 | 0.018966 | 0.012936 |
| | | sterr | 0.102404 | 0.095023 | 0.01095 | 0.007468 |

## Experiment 3

General Linear Models Procedure

Class Level Information

| Class | Levels | Values |
|---|---|---|
| MOIST | 3 | 60%field field wilt |
| COVER | 2 | no yes |
| TIME | 4 | 0.25 4 8 24 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| MOIST | 2 | 0.0640274 | 0.0320137 | 3.63 | 0.0435 |
| COVER | 1 | 0.1280879 | 0.1280879 | 14.51 | 0.0010 |
| REP | 1 | 0.0157380 | 0.0157380 | 1.78 | 0.1955 |
| TIME | 3 | 2.9446726 | 0.9815575 | 111.19 | 0.0001 |
| MOIST*COVER | 2 | 0.0128937 | 0.0064468 | 0.73 | 0.4931 |
| REP*MOIST | 2 | 0.0011897 | 0.0005948 | 0.07 | 0.9350 |
| MOIST*TIME | 6 | 0.1487708 | 0.0247951 | 2.81 | 0.0349 |
| REP*COVER | 1 | 0.0696747 | 0.0696747 | 7.89 | 0.0102 |
| COVER*TIME | 3 | 0.2800896 | 0.0933632 | 10.58 | 0.0002 |
| REP*REP | 1 | 0.1188451 | 0.1188451 | 13.46 | 0.0013 |
| REP*TIME | 3 | 0.2365216 | 0.0788405 | 8.93 | 0.0005 |
| REP*MOIST*COVER | 2 | 0.0107811 | 0.0053906 | 0.61 | 0.5519 |
| MOIST*COVER*TIME | 6 | 0.0100816 | 0.0016803 | 0.19 | 0.9763 |
| REP*COVER*TIME | 3 | 0.0720397 | 0.0240132 | 2.72 | 0.0690 |
| REP*MOIST*TIME | 6 | 0.0731132 | 0.0121855 | 1.38 | 0.2662 |
| REP*MOIST*COVER*TIME | 6 | 0.0159793 | 0.0026632 | 0.30 | 0.9293 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| MOIST | 2 | 0.0152910 | 0.0076455 | 0.87 | 0.4344 |
| COVER | 1 | 0.0103215 | 0.0103215 | 1.17 | 0.2913 |
| REP | 1 | 0.1045528 | 0.1045528 | 11.84 | 0.0023 |
| TIME | 3 | 1.0380875 | 0.3460292 | 39.20 | 0.0001 |
| MOIST*COVER | 2 | 0.0037008 | 0.0018504 | 0.21 | 0.8125 |
| REP*MOIST | 2 | 0.0004435 | 0.0002217 | 0.03 | 0.9752 |
| MOIST*TIME | 6 | 0.0692490 | 0.0115415 | 1.31 | 0.2951 |
| REP*COVER | 1 | 0.0540894 | 0.0540894 | 6.13 | 0.0215 |
| COVER*TIME | 3 | 0.1310453 | 0.0436818 | 4.95 | 0.0089 |
| REP*REP | 1 | 0.1199823 | 0.1199823 | 13.59 | 0.0013 |
| REP*TIME | 3 | 0.2300512 | 0.0766837 | 8.69 | 0.0005 |
| REP*MOIST*COVER | 2 | 0.0121245 | 0.0060623 | 0.69 | 0.5137 |
| MOIST*COVER*TIME | 6 | 0.0121093 | 0.0020182 | 0.23 | 0.9629 |
| REP*COVER*TIME | 3 | 0.0736177 | 0.0245392 | 2.78 | 0.0651 |
| REP*MOIST*TIME | 6 | 0.0733860 | 0.0122310 | 1.39 | 0.2642 |
| REP*MOIST*COVER*TIME | 6 | 0.0159793 | 0.0026632 | 0.30 | 0.9293 |

Experiment 3

moisture
1 = field capacity
2 = 60% of field capacity
3 = permanent wilting point

cover
1 = uncovered
2 = covered

| moisture | cover | rep | AITC conc. 15 min | 4 hour | 8 hour | 24 hour |
|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 0.539311 | 0.071706 | 0.018142 | 0 |
| 1 | 1 | 2 | 0.669773 | 0.168356 | 0.046919 | 0.023017 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | 1 | 3 | 0.195705 | 0.05921 | 0 | 0.019253 |
| | | avg | 0.468263 | 0.099757 | 0.021687 | 0.01409 |
| | | stdev | 0.24489 | 0.059736 | 0.02366 | 0.012347 |
| | | sterr | 0.141387 | 0.034489 | 0.01366 | 0.007128 |
| 1 | 2 | 1 | 0.290723 | 0.273148 | 0.049663 | 0.021181 |
| 1 | 2 | 2 | 0.482119 | 0.509944 | 0.100324 | 0.024469 |
| 1 | 2 | 3 | 0.327664 | 0 | 0.061818 | 0.028596 |
| | | avg | 0.366835 | 0.261031 | 0.070602 | 0.024748 |
| | | stdev | 0.101533 | 0.255188 | 0.026448 | 0.003716 |
| | | sterr | 0.05862 | 0.147333 | 0.01527 | 0.002145 |
| 2 | 1 | 1 | 0.804204 | 0.07903 | 0.026782 | 0 |
| 2 | 1 | 2 | 0.717759 | 0.219244 | 0.064948 | 0.0371 |
| 2 | 1 | 3 | 0.622083 | 0.143865 | 0.035966 | 0.019593 |
| | | avg | 0.714682 | 0.14738 | 0.042565 | 0.018898 |
| | | stdev | 0.0911 | 0.070173 | 0.01992 | 0.01856 |
| | | sterr | 0.052596 | 0.040514 | 0.011501 | 0.010715 |
| 2 | 2 | 1 | 0.617321 | 0.228247 | 0.060208 | 0.02592 |
| 2 | 2 | 2 | 0.519355 | 0.711273 | 0.190104 | 0.043676 |
| 2 | 2 | 3 | 0.465292 | 0.555253 | 0.13468 | 0.031408 |
| | | avg | 0.533989 | 0.498258 | 0.128331 | 0.033668 |
| | | stdev | 0.077064 | 0.246505 | 0.06518 | 0.009091 |
| | | sterr | 0.044493 | 0.14232 | 0.037632 | 0.005249 |
| 3 | 1 | 1 | 0.861623 | 0.122208 | 0.068712 | 0.037531 |
| 3 | 1 | 2 | 0.67567 | 0.157789 | 0.044515 | 0.020591 |
| 3 | 1 | 3 | 0.536227 | 0.12951 | 0.029934 | 0.016872 |
| | | avg | 0.691173 | 0.136502 | 0.047721 | 0.024998 |
| | | stdev | 0.163251 | 0.018793 | 0.019587 | 0.011012 |
| | | sterr | 0.094253 | 0.01085 | 0.011308 | 0.006358 |
| 3 | 2 | 1 | 0.720979 | 0.241694 | 0.059483 | 0.026464 |
| 3 | 2 | 2 | 0.628138 | 0.545842 | 0.164138 | 0.044289 |
| 3 | 2 | 3 | 0.611039 | 0.346554 | 0.058598 | 0.019639 |
| | | avg | 0.653385 | 0.37803 | 0.094073 | 0.030131 |
| | | stdev | 0.059159 | 0.154498 | 0.06068 | 0.012727 |
| | | sterr | 0.034155 | 0.089199 | 0.035034 | 0.007348 |

## Experiment 4

General Linear Models Procedure

Class Level Information

| Class | Levels | Values |
|---|---|---|
| SOIL | 2 | 1 2 |
| REP | 3 | 1 2 3 |
| PH | 2 | 1 2 |
| TIME | 4 | 15 24 4 8 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| SOIL | 1 | 6.446E+08 | 6.446E+08 | 57.31 | 0.0001 |
| PH | 1 | 8.858E+07 | 8.858E+07 | 7.88 | 0.0082 |
| REP | 2 | 5.428E+07 | 2.714E+07 | 2.41 | 0.1047 |
| TIME | 3 | 3.364E+09 | 1.121E+09 | 99.70 | 0.0001 |
| SOIL*TIME | 3 | 4.830E+08 | 1.610E+08 | 14.31 | 0.0001 |
| PH*TIME | 3 | 1.276E+08 | 4.255E+07 | 3.78 | 0.0192 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| SOIL | 1 | 6.446E+08 | 6.446E+08 | 57.31 | 0.0001 |
| PH | 1 | 8.858E+07 | 8.858E+07 | 7.88 | 0.0082 |
| REP | 2 | 5.428E+07 | 2.714E+07 | 2.41 | 0.1047 |
| TIME | 3 | 3.364E+09 | 1.121E+09 | 99.70 | 0.0001 |
| SOIL*TIME | 3 | 4.830E+08 | 1.610E+08 | 14.31 | 0.0001 |
| PH*TIME | 3 | 1.276E+08 | 4.255E+07 | 3.78 | 0.0192 |

Experiment 4

soil texture
1 = sand
2 = clay

pH
1 = field
2 = adjusted

| | | | | AITC | | |
|---|---|---|---|---|---|---|
| soil | ph | rep | 15 min | 4 hour | 8 hour | 24 hour |
| 1 | 1 | 1 | 0.50377577 | 0.630066 | 0.159149 | 0.026873 |
| 1 | 1 | 2 | 0.59521056 | 0.578838 | 0.110733 | 0.032565 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | 1 | 3 | 0.78259292 | 0.677801 | 0.142096 | 0.0332 |
| | | avg | 0.62719308 | 0.628901 | 0.137326 | 0.030879 |
| | | stdev | 0.14213343 | 0.049492 | 0.024558 | 0.003484 |
| | | sterr | 0.08206078 | 0.028574 | 0.014179 | 0.002012 |
| 1 | 2 | 1 | 0.48284464 | 0.16847 | 0.048257 | 0.02084 |
| 1 | 2 | 2 | 0.42445064 | 0.316756 | 0.088759 | 0.033812 |
| 1 | 2 | 3 | 0.69394743 | 0.454044 | 0.090233 | 0.033971 |
| | | avg | 0.53374757 | 0.31309 | 0.07575 | 0.029541 |
| | | stdev | 0.1417761 | 0.142823 | 0.023821 | 0.007535 |
| | | sterr | 0.08185447 | 0.082459 | 0.013753 | 0.00435 |
| 2 | 1 | 1 | 0.3769789 | 0.124829 | 0.032644 | 0.014801 |
| 2 | 1 | 2 | 0.43905827 | 0.088425 | 0.025196 | 0 |
| 2 | 1 | 3 | 0.43390894 | 0.108519 | 0.032453 | 0.014322 |
| | | avg | 0.4166487 | 0.107258 | 0.030098 | 0.009708 |
| | | stdev | 0.0344514 | 0.018235 | 0.004246 | 0.008411 |
| | | sterr | 0.01989052 | 0.010528 | 0.002452 | 0.004856 |
| 2 | 2 | 1 | 0.39058271 | 0.028693 | 0.015592 | 0 |
| 2 | 2 | 2 | 0.59974134 | 0.044548 | 0.072282 | 0 |
| 2 | 2 | 3 | 0.40423443 | 0.052595 | 0.017196 | 0 |
| | | avg | 0.46485282 | 0.041945 | 0.035023 | 0 |
| | | stdev | 0.11701613 | 0.012162 | 0.032277 | 0 |
| | | sterr | 0.0675593 | 0.007022 | 0.018635 | 0 |

## Experiment 5

General Linear Models Procedure

Class Level Information

| Class | Levels | Values |
|---|---|---|
| SOIL | 2 | 1 2 |
| STERILE | 2 | 1 2 |
| TIME | 4 | 4 8 15 24 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| SOIL | 1 | 2.300233 | 2.300233 | 48.05 | 0.0001 |
| STERILE | 1 | 13.829758 | 13.829758 | 288.92 | 0.0001 |
| REP | 1 | 0.043577 | 0.043577 | 0.91 | 0.3472 |
| TIME | 3 | 1.709245 | 0.569748 | 11.90 | 0.0001 |
| SOIL*TIME | 3 | 0.290074 | 0.096691 | 2.02 | 0.1308 |
| STERILE*TIME | 3 | 2.549512 | 0.849837 | 17.75 | 0.0001 |
| REP*TIME | 3 | 0.065771 | 0.021924 | 0.46 | 0.7135 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| SOIL | 1 | 2.300233 | 2.300233 | 48.05 | 0.0001 |
| STERILE | 1 | 13.829758 | 13.829758 | 288.92 | 0.0001 |
| REP | 1 | 0.043577 | 0.043577 | 0.91 | 0.3472 |
| TIME | 3 | 0.084103 | 0.028034 | 0.59 | 0.6288 |
| SOIL*TIME | 3 | 0.290074 | 0.096691 | 2.02 | 0.1308 |
| STERILE*TIME | 3 | 2.549512 | 0.849837 | 17.75 | 0.0001 |
| REP*TIME | 3 | 0.065771 | 0.021924 | 0.46 | 0.7135 |

Experiment 5

soil texture
1 = sand
2 = clay

autoclaved
1 = no
2 = yes

| soil | sterile | rep | | AITC conc. 15 min | 4 hour | 8 hour | 24 hour |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | | 0.56511781 | 0.8047259 | 0.315486 | 0.0598 |
| 1 | 1 | 2 | | 0.52182688 | 0.569177 | 0.16085 | 0.038415 |
| 1 | 1 | 3 | | 0.48660907 | 0.7387124 | 0.217294 | 0.022337 |
| | | | AVG | 0.52451792 | 0.7042051 | 0.23121 | 0.040184 |
| | | | STDEV | 0.03932349 | 0.1215067 | 0.078252 | 0.018794 |
| | | | STERR | 0.02270343 | 0.0701519 | 0.045179 | 0.010851 |
| 1 | 2 | 1 | | 1.05916502 | 2.1591718 | 2.180851 | 2.088782 |
| 1 | 2 | 2 | | 0.85468399 | 1.8071978 | 1.681883 | 1.233485 |
| 1 | 2 | 3 | | 0.70562623 | 2.227816 | 2.296528 | 1.688732 |
| | | | AVG | 0.87315842 | 2.0647285 | 2.053088 | 1.670333 |
| | | | STDEV | 0.17749196 | 0.2256536 | 0.326634 | 0.427945 |
| | | | STERR | 0.10247503 | 0.1302812 | 0.188582 | 0.247074 |
| 2 | 1 | 1 | | 0.51296913 | 0.1839389 | 0.029531 | 0.015544 |
| 2 | 1 | 2 | | 0.30254113 | 0.1726343 | 0.034872 | 0.013269 |
| 2 | 1 | 3 | | 0.27454315 | 0.2408929 | 0.049625 | 0.017292 |
| | | | AVG | 0.36335114 | 0.1991554 | 0.038009 | 0.015368 |
| | | | STDEV | 0.13032701 | 0.036585 | 0.010408 | 0.002018 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | STERR | 0.07524433 | 0.0211224 | 0.006009 | 0.001165 |
| 2 | 2 | 1 | 0.62702081 | 1.2535147 | 1.228343 | 0.933849 |
| 2 | 2 | 2 | 0.71911 | 1.3936963 | 1.248126 | 0.854358 |
| 2 | 2 | 3 | 0.48648001 | 1.2424736 | 1.233708 | 0.90827 |
| | | AVG | 0.61087028 | 1.2965615 | 1.236726 | 0.898826 |
| | | STDEV | 0.11715293 | 0.0843021 | 0.010231 | 0.040578 |
| | | STERR | 0.06763827 | 0.0486718 | 0.005907 | 0.023428 |

## Experiment 6

The MIXED Procedure for **AITC**

Class Level Information

| Class | Levels | Values |
|---|---|---|
| TIME | 4 | 0.25 4 8 24 |
| GROUP | 9 | 1 2 3 4 5 6 7 8 9 |
| COVER | 2 | no yes |
| TEMP | 3 | 15 30 45 |
| MOIST | 3 | 60field field wilt |
| PH | 2 | adjus field |
| SOIL | 2 | clay sand |
| MICROB | 2 | steril unsteril |

Tests of Fixed Effects

| Source | NDF | DDF | Type III F | Pr > F |
|---|---|---|---|---|
| TIME | 3 | 181 | 24.14 | 0.0001 |
| COVER | 1 | 30 | 59.42 | 0.0001 |
| TIME*COVER | 3 | 181 | 19.74 | 0.0001 |
| TEMP | 2 | 30 | 5.94 | 0.0067 |
| TIME*TEMP | 6 | 181 | 3.97 | 0.0009 |
| COVER*TEMP | 2 | 30 | 1.82 | 0.1799 |
| MOIST | 2 | 30 | 1.52 | 0.2349 |
| TIME*MOIST | 6 | 181 | 1.77 | 0.1071 |
| COVER*MOIST | 2 | 30 | 1.46 | 0.2479 |
| TEMP*MOIST | 4 | 30 | 0.99 | 0.4299 |
| PH | 1 | 30 | 0.14 | 0.7102 |
| TIME*PH | 3 | 181 | 4.02 | 0.0085 |
| COVER*PH | 1 | 30 | 0.26 | 0.6140 |
| TEMP*PH | 2 | 30 | 0.36 | 0.7035 |
| MOIST*PH | 2 | 30 | 0.78 | 0.4683 |
| SOIL | 1 | 30 | 5.17 | 0.0303 |
| TIME*SOIL | 3 | 181 | 1.04 | 0.3752 |
| COVER*SOIL | 1 | 30 | 2.77 | 0.1065 |
| TEMP*SOIL | 2 | 30 | 0.35 | 0.7072 |
| MOIST*SOIL | 2 | 30 | 0.00 | 0.9997 |
| PH*SOIL | 1 | 30 | 0.67 | 0.4192 |
| MICROB | 1 | 30 | 60.13 | 0.0001 |
| TIME*MICROB | 3 | 181 | 16.46 | 0.0001 |
| COVER*MICROB | 1 | 30 | 37.26 | 0.0001 |
| TEMP*MICROB | 2 | 30 | 5.23 | 0.0112 |
| MOIST*MICROB | 2 | 30 | 0.18 | 0.8400 |
| PH*MICROB | 1 | 30 | 0.30 | 0.5851 |
| SOIL*MICROB | 1 | 30 | 0.47 | 0.4992 |

The MIXED Procedure for $CO_2$

Class Level Information

| Class | Levels | Values |
|---|---|---|
| TIME | 4 | 0.25 24 4 8 |
| GROUP | 9 | 1 2 3 4 5 6 7 8 9 |
| COVER | 2 | no yes |
| TEMP | 3 | 15 30 45 |
| MOIST | 3 | 60field field wilt |
| PH | 2 | adjus field |
| SOIL | 2 | clay sand |
| MICROB | 2 | steril unsteril |

Tests of Fixed Effects

| Source | NDF | DDF | Type III F | Pr > F |
|---|---|---|---|---|
| TIME | 3 | 181 | 13.94 | 0.0001 |
| COVER | 1 | 30 | 45.59 | 0.0001 |
| TIME*COVER | 3 | 181 | 13.26 | 0.0001 |
| TEMP | 2 | 30 | 6.85 | 0.0036 |
| TIME*TEMP | 6 | 181 | 4.00 | 0.0009 |
| COVER*TEMP | 2 | 30 | 6.72 | 0.0039 |
| MOIST | 2 | 30 | 0.82 | 0.4507 |

| Source | NDF | DDF | Type III F | Pr > F |
|---|---|---|---|---|
| TIME*MOIST | 6 | 181 | 0.67 | 0.6759 |
| COVER*MOIST | 2 | 30 | 0.87 | 0.4282 |
| TEMP*MOIST | 4 | 30 | 0.04 | 0.9969 |
| PH | 1 | 30 | 0.15 | 0.6993 |
| TIME*PH | 3 | 181 | 0.33 | 0.8049 |
| COVER*PH | 1 | 30 | 0.04 | 0.8458 |
| TEMP*PH | 2 | 30 | 0.61 | 0.5496 |
| MOIST*PH | 2 | 30 | 0.61 | 0.5509 |
| SOIL | 1 | 30 | 3.22 | 0.0828 |
| TIME*SOIL | 3 | 181 | 0.15 | 0.9299 |
| COVER*SOIL | 1 | 30 | 1.29 | 0.2642 |
| TEMP*SOIL | 2 | 30 | 0.84 | 0.4428 |
| MOIST*SOIL | 2 | 30 | 0.54 | 0.5910 |
| PH*SOIL | 1 | 30 | 0.06 | 0.8076 |
| MICROB | 1 | 30 | 37.73 | 0.0001 |
| TIME*MICROB | 3 | 181 | 10.72 | 0.0001 |
| COVER*MICROB | 1 | 30 | 36.16 | 0.0001 |
| TEMP*MICROB | 2 | 30 | 8.84 | 0.0010 |
| MOIST*MICROB | 2 | 30 | 0.82 | 0.4494 |
| PH*MICROB | 1 | 30 | 0.01 | 0.9181 |
| SOIL*MICROB | 1 | 30 | 1.12 | 0.2993 |

The MIXED Procedure for **sec-butyl ITC**

Class Level Information

| Class | Levels | Values |
|---|---|---|
| TIME | 4 | 0.25 4 8 24 |
| GROUP | 9 | 1 2 3 4 5 6 7 8 9 |
| COVER | 2 | no yes |
| TEMP | 3 | 15 30 45 |
| MOIST | 3 | 60field field wilt |
| PH | 2 | adjus field |
| SOIL | 2 | clay sand |
| MICROB | 2 | steril unsteril |

Tests of Fixed Effects

| Source | NDF | DDF | Type III F | Pr > F |
|---|---|---|---|---|
| TIME | 3 | 182 | 21.11 | 0.0001 |
| COVER | 1 | 30 | 154.27 | 0.0001 |
| TIME*COVER | 3 | 182 | 26.04 | 0.0001 |
| TEMP | 2 | 30 | 0.77 | 0.4704 |
| TIME*TEMP | 6 | 182 | 2.64 | 0.0177 |
| COVER*TEMP | 2 | 30 | 0.54 | 0.5878 |
| MOIST | 2 | 30 | 8.22 | 0.0014 |
| TIME*MOIST | 6 | 182 | 1.69 | 0.1257 |
| COVER*MOIST | 2 | 30 | 10.07 | 0.0005 |
| TEMP*MOIST | 4 | 30 | 0.67 | 0.6170 |
| PH | 1 | 30 | 0.11 | 0.7478 |
| TIME*PH | 3 | 182 | 2.44 | 0.0661 |
| COVER*PH | 1 | 30 | 1.22 | 0.2772 |
| TEMP*PH | 2 | 30 | 2.06 | 0.1451 |
| MOIST*PH | 2 | 30 | 0.56 | 0.5783 |
| SOIL | 1 | 30 | 7.59 | 0.0099 |
| TIME*SOIL | 3 | 182 | 1.14 | 0.3353 |
| COVER*SOIL | 1 | 30 | 5.38 | 0.0273 |
| TEMP*SOIL | 2 | 30 | 1.05 | 0.3622 |
| MOIST*SOIL | 2 | 30 | 0.08 | 0.9240 |
| PH*SOIL | 1 | 30 | 1.72 | 0.1996 |
| MICROB | 1 | 30 | 22.80 | 0.0001 |
| TIME*MICROB | 3 | 182 | 5.84 | 0.0008 |
| COVER*MICROB | 1 | 30 | 22.36 | 0.0001 |
| TEMP*MICROB | 2 | 30 | 2.12 | 0.1374 |
| MOIST*MICROB | 2 | 30 | 1.06 | 0.3587 |
| PH*MICROB | 1 | 30 | 0.38 | 0.5436 |
| SOIL*MICROB | 1 | 30 | 0.80 | 0.3770 |

The MIXED Procedure for **3-butenyl ITC**

Class Level Information

| Class | Levels | Values |
|---|---|---|
| TIME | 4 | 0.25 4 8 24 |
| GROUP | 9 | 1 2 3 4 5 6 7 8 9 |
| COVER | 2 | no yes |
| TEMP | 3 | 15 30 45 |
| MOIST | 3 | 60field field wilt |
| PH | 2 | adjus field |
| SOIL | 2 | clay sand |
| MICROB | 2 | steril unsteril |

Tests of Fixed Effects

| Source | NDF | DDF | Type III F | Pr > F |
|---|---|---|---|---|
| TIME | 3 | 181 | 16.53 | 0.0001 |
| COVER | 1 | 30 | 17.90 | 0.0002 |
| TIME*COVER | 3 | 181 | 0.73 | 0.5371 |
| TEMP | 2 | 30 | 9.74 | 0.0006 |
| TIME*TEMP | 6 | 181 | 2.11 | 0.0539 |
| COVER*TEMP | 2 | 30 | 2.84 | 0.0741 |
| MOIST | 2 | 30 | 2.62 | 0.0895 |
| TIME*MOIST | 6 | 181 | 0.60 | 0.7330 |
| COVER*MOIST | 2 | 30 | 2.09 | 0.1414 |
| TEMP*MOIST | 4 | 30 | 0.96 | 0.4449 |
| PH | 1 | 30 | 0.06 | 0.8124 |
| TIME*PH | 3 | 181 | 3.22 | 0.0241 |
| COVER*PH | 1 | 30 | 0.05 | 0.8217 |
| TEMP*PH | 2 | 30 | 0.12 | 0.8840 |
| MOIST*PH | 2 | 30 | 1.49 | 0.2418 |
| SOIL | 1 | 30 | 2.99 | 0.0939 |
| TIME*SOIL | 3 | 181 | 0.17 | 0.9134 |
| COVER*SOIL | 1 | 30 | 3.05 | 0.0910 |
| TEMP*SOIL | 2 | 30 | 1.62 | 0.2146 |
| MOIST*SOIL | 2 | 30 | 0.19 | 0.8273 |
| PH*SOIL | 1 | 30 | 0.26 | 0.6144 |
| MICROB | 1 | 30 | 19.62 | 0.0001 |
| TIME*MICROB | 3 | 181 | 1.95 | 0.1239 |
| COVER*MICROB | 1 | 30 | 9.17 | 0.0050 |
| TEMP*MICROB | 2 | 30 | 5.09 | 0.0125 |
| MOIST*MICROB | 2 | 30 | 0.49 | 0.6200 |
| PH*MICROB | 1 | 30 | 1.70 | 0.2019 |
| SOIL*MICROB | 1 | 30 | 0.84 | 0.3671 |

Experiment 6

ITCs in umol/L

| obs | time | group | cover | temp | moist | PH | soil | microb | AITC | % CO2 | sec-butyl | 3-butenyl |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.25 | 4 | yes | 45 | wilt | adjus | sand | unsteril | 1.7747 | 1.325687 | 1.24091 | 0.06004 |
| 1 | 4 | 4 | yes | 45 | wilt | adjus | sand | unsteril | 0.7757 | 2.396482 | 1.08721 | 0.01833 |
| 1 | 8 | 4 | yes | 45 | wilt | adjus | sand | unsteril | 0.1252 | 2.933393 | 0.6807 | 0 |
| 1 | 24 | 4 | yes | 45 | wilt | adjus | sand | unsteril | 0.0275 | 7.605086 | 0 | 0 |
| 2 | 0.25 | 4 | yes | 45 | 60field | field | clay | steril | 0.9197 | 0.910411 | 0.60623 | 0.03406 |
| 2 | 4 | 4 | yes | 45 | 60field | field | clay | steril | 2.0371 | 0.853191 | 1.01272 | 0.06778 |
| 2 | 8 | 4 | yes | 45 | 60field | field | clay | steril | 1.5659 | 0.912151 | 0.82512 | 0 |
| 2 | 24 | 4 | yes | 45 | 60field | field | clay | steril | 1.2452 | 0.911979 | 0.82412 | 0.0407 |
| 3 | 0.25 | 4 | yes | 30 | wilt | field | sand | steril | 0.779 | 1.003308 | 0.77611 | 0.0161 |
| 3 | 4 | 4 | yes | 30 | wilt | field | sand | steril | 1.9296 | 0.962347 | 1.47799 | 0.04458 |
| 3 | 8 | 4 | yes | 30 | wilt | field | sand | steril | 1.8703 | 0.898898 | 1.42702 | 0.04619 |
| 3 | 24 | 4 | yes | 30 | wilt | field | sand | steril | 1.3783 | 0.775995 | 1.03596 | 0 |
| 4 | 0.25 | 4 | yes | 30 | field | field | clay | steril | 0.1248 | 1.12838 | 0.14024 | 0 |
| 4 | 4 | 4 | yes | 30 | field | field | clay | steril | 0.714 | 1.027966 | 0.48619 | 0.01022 |
| 4 | 8 | 4 | yes | 30 | field | field | clay | steril | 0.5709 | 0.82385 | 0.48722 | 0 |
| 4 | 24 | 4 | yes | 30 | field | field | clay | steril | 0.4147 | 0.312542 | 0.54378 | 0 |
| 5 | 0.25 | 4 | no | 45 | field | adjus | sand | steril | 0.9419 | 0.937969 | 0.49322 | 0.0259 |
| 5 | 4 | 4 | no | 45 | field | adjus | sand | steril | 0.0062 | 0.924029 | 0.00712 | 0 |
| 5 | 8 | 4 | no | 45 | field | adjus | sand | steril | 0.0731 | 1.144447 | 0.0335 | 0 |
| 5 | 24 | 4 | no | 45 | field | adjus | sand | steril | 0.0098 | 1.280753 | 0.00161 | 0 |
| 6 | 0.25 | 4 | no | 30 | wilt | adjus | clay | unsteril | 0.2606 | 0.837532 | 0.25559 | 0.00972 |
| 6 | 4 | 4 | no | 30 | wilt | adjus | clay | unsteril | . | . | . | . |
| 6 | 8 | 4 | no | 30 | wilt | adjus | clay | unsteril | 0.0019 | 0.90459 | 0 | 0 |
| 6 | 24 | 4 | no | 30 | wilt | adjus | clay | unsteril | 0 | 1.177352 | 0 | 0 |
| 7 | 0.25 | 4 | no | 30 | 60field | field | sand | unsteril | 0.9638 | 1.03529 | 1.05535 | 0.01474 |
| 7 | 4 | 4 | no | 30 | 60field | field | sand | unsteril | 0.1523 | 0.783105 | 0.27934 | 0 |
| 7 | 8 | 4 | no | 30 | 60field | field | sand | unsteril | 0 | 0.737934 | 0.07499 | 0.00225 |
| 7 | 24 | 4 | no | 30 | 60field | field | sand | unsteril | 0.013 | 1.263118 | 0.00701 | 0 |
| 8 | 0.25 | 4 | no | 15 | wilt | field | clay | steril | 0.474 | 0.920742 | 0.79736 | 0.01632 |
| 8 | 4 | 4 | no | 15 | wilt | field | clay | steril | 0.2277 | 0.741435 | 0.24498 | 0 |
| 8 | 8 | 4 | no | 15 | wilt | field | clay | steril | 0.1523 | 0.678502 | 0.15707 | 0 |
| 8 | 24 | 4 | no | 15 | wilt | field | clay | steril | 0.0944 | 1.101446 | 0.09079 | 0 |
| 9 | 0.25 | 8 | no | 45 | wilt | adjus | sand | steril | 1.9636 | 1.196276 | 0.71179 | 0.05998 |
| 9 | 4 | 8 | yes | 45 | wilt | adjus | sand | steril | 3.9913 | 1.243744 | 1.21937 | 0.14231 |
| 9 | 8 | 8 | yes | 45 | wilt | adjus | sand | steril | 3.4356 | 1.036965 | 1.04058 | 0.11913 |
| 9 | 24 | 8 | yes | 45 | wilt | adjus | sand | steril | 1.9525 | 0.914685 | 0.53979 | 0.08316 |
| 10 | 0.25 | 8 | yes | 30 | 60field | field | clay | unsteril | 0.4278 | 2.095517 | 0.22905 | 0.01387 |
| 10 | 4 | 8 | yes | 30 | 60field | field | clay | unsteril | 0.0547 | 7.60863 | 0.31846 | 0 |
| 10 | 8 | 8 | yes | 30 | 60field | field | clay | unsteril | 0.0153 | 12.73138 | 0.05282 | 0 |
| 10 | 24 | 8 | yes | 30 | 60field | field | clay | unsteril | 0.0076 | 12.21816 | 0.00314 | 0 |
| 11 | 0.25 | 8 | yes | 15 | field | adjus | sand | unsteril | 0.9581 | 1.479563 | 0.41634 | 0.03006 |
| 11 | 4 | 8 | yes | 15 | field | adjus | sand | unsteril | 0.2164 | 1.999485 | 0.34235 | 0 |
| 11 | 8 | 8 | yes | 15 | field | adjus | sand | unsteril | 0.0462 | 1.450953 | 0.48843 | 0 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | 24 | 8 | yes | 15 | field | adjus | sand | unsteril | 0.0411 | 2.263161 | 0.02068 | 0 |
| 12 | 0.25 | 8 | no | 45 | 60field | adjus | sand | unsteril | 1.9799 | 1.584273 | 0.62174 | 0.07279 |
| 12 | 4 | 8 | no | 45 | 60field | adjus | sand | unsteril | 0.0618 | 1.332646 | 0.0495 | 0 |
| 12 | 8 | 8 | no | 45 | 60field | adjus | sand | unsteril | 0.0298 | 1.387589 | 0.00466 | 0 |
| 12 | 24 | 8 | no | 45 | 60field | adjus | sand | unsteril | 0 | 0.918014 | 0 | 0 |
| 13 | 0.25 | 8 | no | 45 | field | field | clay | unsteril | 0.2988 | 1.289237 | 0.0691 | 0.00984 |
| 13 | 4 | 8 | no | 45 | field | field | clay | unsteril | . | . | . | . |
| 13 | 8 | 8 | no | 45 | field | field | clay | unsteril | 0.01 | 1.8783 | 0.01771 | 0 |
| 13 | 24 | 8 | no | 45 | field | field | clay | unsteril | 0.0033 | 0.991881 | 0 | 0 |
| 14 | 0.25 | 8 | no | 30 | field | adjus | clay | steril | 0.5595 | 1.291106 | 0.21499 | 0.0165 |
| 14 | 4 | 8 | no | 30 | field | adjus | clay | steril | 1.028 | 0.960801 | 0.34248 | 0.0146 |
| 14 | 8 | 8 | no | 30 | field | adjus | clay | steril | 0.5937 | 0.715596 | 0.26836 | 0.00547 |
| 14 | 24 | 8 | no | 30 | field | adjus | clay | steril | 0.1007 | 1.932556 | 0.03236 | 0 |
| 15 | 0.25 | 8 | no | 15 | wilt | field | clay | unsteril | 0 | 1.869064 | 0 | 0 |
| 15 | 4 | 8 | no | 15 | wilt | field | clay | unsteril | 0.2623 | 1.003802 | 0.1516 | 0 |
| 15 | 8 | 8 | no | 15 | wilt | field | clay | unsteril | 0.1241 | 0.826664 | 0.08142 | 0 |
| 15 | 24 | 8 | no | 15 | wilt | field | clay | unsteril | 0.0544 | 0.88223 | 0 | 0 |
| 16 | 0.25 | 8 | no | 15 | 60field | field | sand | steril | 0.8051 | 1.255321 | 0.36432 | 0.02047 |
| 16 | 4 | 8 | no | 15 | 60field | field | sand | steril | 0.5255 | 1.05228 | 0.17121 | 0.00974 |
| 16 | 8 | 8 | no | 15 | 60field | field | sand | steril | 0.346 | 1.083317 | 0.09024 | 0.0036 |
| 16 | 24 | 8 | no | 15 | 60field | field | sand | steril | 0.0828 | 0.826922 | 0.01106 | 0 |
| 17 | 0.25 | 6 | yes | 45 | 60field | adjus | clay | unsteril | 0.7527 | 1.194171 | 0.0633 | 0.03359 |
| 17 | 4 | 6 | yes | 45 | 60field | adjus | clay | unsteril | . | . | . | . |
| 17 | 8 | 6 | yes | 45 | 60field | adjus | clay | unsteril | 0.09 | 9.599072 | 0.88132 | 0 |
| 17 | 24 | 6 | yes | 45 | 60field | adjus | clay | unsteril | 0.0223 | 23.10207 | 0.05592 | 0 |
| 18 | 0.25 | 6 | yes | 30 | wilt | adjus | clay | steril | 1.8261 | 1.190133 | 1.40315 | 0.06975 |
| 18 | 4 | 6 | yes | 30 | wilt | adjus | clay | steril | 2.093 | 0.862105 | 1.40978 | 0.03997 |
| 18 | 8 | 6 | yes | 30 | wilt | adjus | clay | steril | 1.9609 | 1.410529 | 1.62371 | 0.02458 |
| 18 | 24 | 6 | yes | 30 | wilt | adjus | clay | steril | 1.3293 | 1.526924 | 1.48399 | 0.00853 |
| 19 | 0.25 | 6 | yes | 30 | 60field | field | sand | steril | 1.8633 | 1.141912 | 0.81133 | 0.05762 |
| 19 | 4 | 6 | yes | 30 | 60field | field | sand | steril | 4.1924 | 1.278519 | 2.04686 | 0.13158 |
| 19 | 8 | 6 | yes | 30 | 60field | field | sand | steril | 3.9596 | 1.537341 | 2.11348 | 0.11648 |
| 19 | 24 | 6 | yes | 30 | 60field | field | sand | steril | 3.44 | 1.694074 | 2.05014 | 0.09298 |
| 20 | 0.25 | 6 | yes | 15 | wilt | field | sand | unsteril | 0.1894 | 1.305196 | 0.49675 | 0.00413 |
| 20 | 4 | 6 | yes | 15 | wilt | field | sand | unsteril | 0.2164 | 1.999485 | 0.34235 | 0 |
| 20 | 8 | 6 | yes | 15 | wilt | field | sand | unsteril | 1.0244 | 2.340529 | 1.80227 | 0.01493 |
| 20 | 24 | 6 | yes | 15 | wilt | field | sand | unsteril | 0.682 | 1.881221 | 1.86162 | 0.00994 |
| 21 | 0.25 | 6 | yes | 15 | field | adjus | sand | steril | 0.4983 | 1.124858 | 0.56348 | 0.01105 |
| 21 | 4 | 6 | yes | 15 | field | adjus | sand | steril | 1.2734 | 1.353287 | 1.03085 | 0.01877 |
| 21 | 8 | 6 | yes | 15 | field | adjus | sand | steril | 1.3914 | 2.023498 | 1.32698 | 0.01974 |
| 21 | 24 | 6 | yes | 15 | field | adjus | sand | steril | 1.1295 | 1.464291 | 1.30947 | 0.00792 |
| 22 | 0.25 | 6 | no | 45 | field | field | clay | steril | 0.7619 | 1.28449 | 0.50864 | 0.02913 |
| 22 | 4 | 6 | no | 45 | field | field | clay | steril | 1.7405 | 0.837532 | 0.96603 | 0.05914 |
| 22 | 8 | 6 | no | 45 | field | field | clay | steril | 1.0324 | 1.076251 | 0.71209 | 0.03606 |
| 22 | 24 | 6 | no | 45 | field | field | clay | steril | 0.1037 | 2.342226 | 0.03765 | 0 |
| 23 | 0.25 | 6 | no | 30 | field | adjus | sand | unsteril | 0.7342 | 1.320059 | 0.93366 | 0 |
| 23 | 4 | 6 | no | 30 | field | adjus | sand | unsteril | 0.1416 | 1.092918 | 0.39533 | 0 |
| 23 | 8 | 6 | no | 30 | field | adjus | sand | unsteril | 0.0271 | 1.413901 | 0.11315 | 0 |
| 23 | 24 | 6 | no | 30 | field | adjus | sand | unsteril | 0.0086 | 1.120777 | 0 | 0 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | 0.25 | 6 | no | 15 | 60field | adjus | clay | steril | 0.3749 | 5.365294 | 1.14263 | 0 |
| 24 | 4 | 6 | no | 15 | 60field | adjus | clay | steril | 0.0805 | 0.956269 | 0.08792 | 0 |
| 24 | 8 | 6 | no | 15 | 60field | adjus | clay | steril | 0.0201 | 1.120949 | 0.02003 | 0.00243 |
| 24 | 24 | 6 | no | 15 | 60field | adjus | clay | steril | 0.0098 | 1.32079 | 0.01612 | 0 |
| 25 | 0.25 | 9 | yes | 45 | field | adjus | clay | unsteril | 0.6368 | 0.99579 | 0.29182 | 0.02699 |
| 25 | 4 | 9 | yes | 45 | field | adjus | clay | unsteril | 0.1529 | 5.396353 | 0.57193 | 0.00157 |
| 25 | 8 | 9 | yes | 45 | field | adjus | clay | unsteril | 0 | 10.49945 | 0.26422 | 0 |
| 25 | 24 | 9 | yes | 45 | field | adjus | clay | unsteril | 0 | 8.453466 | 0 | 0 |
| 26 | 0.25 | 9 | yes | 30 | wilt | field | clay | unsteril | 0.5389 | 1.392379 | 0.64982 | 0.02094 |
| 26 | 4 | 9 | yes | 30 | wilt | field | clay | unsteril | 0.551 | 5.69678 | 1.09985 | 0.01117 |
| 26 | 8 | 9 | yes | 30 | wilt | field | clay | unsteril | 0.0725 | 7.151771 | 0.81704 | 0 |
| 26 | 24 | 9 | yes | 30 | wilt | field | clay | unsteril | 0.0164 | 11.01918 | 0 | 0 |
| 27 | 0.25 | 9 | yes | 30 | field | field | sand | unsteril | 0.2674 | 0.061752 | 0.27181 | 0.00724 |
| 27 | 4 | 9 | yes | 30 | field | field | sand | unsteril | 0.6588 | 5.593058 | 1.14075 | 0.00828 |
| 27 | 8 | 9 | yes | 30 | field | field | sand | unsteril | 0.0732 | 8.525399 | 0.90261 | 0 |
| 27 | 24 | 9 | yes | 30 | field | field | sand | unsteril | 0.0121 | 16.76534 | 0 | 0 |
| 28 | 0.25 | 9 | yes | 15 | 60field | field | sand | unsteril | 0.2267 | 1.388642 | 0.53377 | 0.00258 |
| 28 | 4 | 9 | yes | 15 | 60field | field | sand | unsteril | 0.8149 | 2.005005 | 1.43259 | 0.01244 |
| 28 | 8 | 9 | yes | 15 | 60field | field | sand | unsteril | 0.7205 | 2.021501 | 1.45764 | 0.01232 |
| 28 | 24 | 9 | yes | 15 | 60field | field | sand | unsteril | 0.4244 | 1.721825 | 0 | 0 |
| 29 | 0.25 | 9 | yes | 15 | field | field | clay | steril | 0.696 | 1.260541 | 0.59074 | 0.02361 |
| 29 | 4 | 9 | yes | 15 | field | field | clay | steril | 1.0345 | 1.466138 | 0.77963 | 0.01883 |
| 29 | 8 | 9 | yes | 15 | field | field | clay | steril | 0.9766 | 1.319007 | 0.76878 | 0.01398 |
| 29 | 24 | 9 | yes | 15 | field | field | clay | steril | 0.8887 | 1.253839 | 0.79162 | 0 |
| 30 | 0.25 | 9 | no | 45 | 60field | field | clay | unsteril | 1.1026 | 1.406534 | 0.64822 | 0.03452 |
| 30 | 4 | 9 | no | 45 | 60field | field | clay | unsteril | 0.5026 | 1.206006 | 0.3561 | 0.00848 |
| 30 | 8 | 9 | no | 45 | 60field | field | clay | unsteril | 0.2394 | 1.341989 | 0.09277 | 0 |
| 30 | 24 | 9 | no | 45 | 60field | field | clay | unsteril | 0.0714 | 1.280967 | 0 | 0 |
| 31 | 0.25 | 9 | no | 30 | 60field | adjus | sand | steril | . | . | . | . |
| 31 | 4 | 9 | no | 30 | 60field | adjus | sand | steril | 0.6685 | 1.017033 | 0.47987 | 0.01428 |
| 31 | 8 | 9 | no | 30 | 60field | adjus | sand | steril | 0.205 | 1.09367 | 0.18357 | 0 |
| 31 | 24 | 9 | no | 30 | 60field | adjus | sand | steril | 0 | 1.455205 | 0.00873 | 0 |
| 32 | 0.25 | 9 | no | 15 | wilt | adjus | sand | unsteril | . | . | . | . |
| 32 | 4 | 9 | no | 15 | wilt | adjus | sand | unsteril | 0.2452 | 1.094272 | 0.54787 | 0 |
| 32 | 8 | 9 | no | 15 | wilt | adjus | sand | unsteril | 0.1102 | 1.262925 | 0.26479 | 0 |
| 32 | 24 | 9 | no | 15 | wilt | adjus | sand | unsteril | 0.0415 | 1.601993 | 0 | 0 |
| 33 | 0.25 | 2 | yes | 30 | field | adjus | sand | steril | 1.2005 | 1.25646 | 0.78298 | 0.03869 |
| 33 | 4 | 2 | yes | 30 | field | adjus | sand | steril | 2.3911 | 1.800567 | 1.32663 | 0 |
| 33 | 8 | 2 | yes | 30 | field | adjus | sand | steril | 2.2246 | 2.493052 | 1.36836 | 0.04073 |
| 33 | 24 | 2 | yes | 30 | field | adjus | sand | steril | 1.2787 | 4.111369 | 1.06583 | 0 |
| 34 | 0.25 | 2 | yes | 15 | wilt | adjus | clay | unsteril | 0.5574 | 2.149215 | 1.08719 | 0.02233 |
| 34 | 4 | 2 | yes | 15 | wilt | adjus | clay | unsteril | 0.7322 | 3.160577 | 1.26615 | 0.00977 |
| 34 | 8 | 2 | yes | 15 | wilt | adjus | clay | unsteril | 2.2743 | 2.030522 | 1.45931 | 0.03905 |
| 34 | 24 | 2 | yes | 15 | wilt | adjus | clay | unsteril | 0.0695 | 3.440879 | 1.04741 | 0 |
| 35 | 0.25 | 2 | yes | 15 | 60field | field | clay | steril | 0.4888 | 1.344953 | 0.53846 | 0.01615 |
| 35 | 4 | 2 | yes | 15 | 60field | field | clay | steril | 1.1119 | 1.351633 | 0.86019 | 0.01819 |
| 35 | 8 | 2 | yes | 15 | 60field | field | clay | steril | 1.1038 | 3.044505 | 0.94699 | 0.01369 |
| 35 | 24 | 2 | yes | 15 | 60field | field | clay | steril | 1.0647 | 4.08467 | 0.98032 | 0.01008 |
| 36 | 0.25 | 2 | no | 30 | wilt | field | clay | steril | 0.95 | 1.761153 | 0.69258 | 0.03448 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 36 | 4 | 2 | no 30 | wilt field | clay | steril | 0.7576 | 1.442275 | 0.43744 | 0.02202 |
| 36 | 8 | 2 | no 30 | wilt field | clay | steril | 0.3968 | 3.053375 | 0.22126 | 0 |
| 36 | 24 | 2 | no 30 | wilt field | clay | steril | 0.0359 | 3.62431 | 0.0117 | 0 |
| 37 | 0.25 | 2 | no 30 | 60field adjus | clay | unsteril | 0.6751 | 1.627339 | 0.61315 | 0.01899 |
| 37 | 4 | 2 | no 30 | 60field adjus | clay | unsteril | 0.0282 | 1.816805 | 0.24281 | 0.00156 |
| 37 | 8 | 2 | no 30 | 60field adjus | clay | unsteril | 0 | 2.321348 | 0.02696 | 0 |
| 37 | 24 | 2 | no 30 | 60field adjus | clay | unsteril | 0 | 3.928496 | 0 | 0 |
| 38 | 0.25 | 2 | no 15 | wilt adjus | sand | steril | 0.2764 | 1.418605 | 0.20985 | 0 |
| 38 | 4 | 2 | no 15 | wilt adjus | sand | steril | 0.1493 | 2.117512 | 0.08806 | 0 |
| 38 | 8 | 2 | no 15 | wilt adjus | sand | steril | 0.0821 | 1.583049 | 0.02577 | 0 |
| 38 | 24 | 2 | no 15 | wilt adjus | sand | steril | 0.0101 | 2.022274 | 0 | 0 |
| 39 | 0.25 | 2 | no 15 | field field | sand | unsteril | 0.2337 | 1.731405 | 0.58005 | 0.00736 |
| 39 | 4 | 2 | no 15 | field field | sand | unsteril | 0.4786 | 1.730416 | 0.57127 | 0.00727 |
| 39 | 8 | 2 | no 15 | field field | sand | unsteril | 0.2843 | 2.383637 | 0.41978 | 0 |
| 39 | 24 | 2 | no 15 | field field | sand | unsteril | 0.0238 | 2.48751 | 0.08805 | 0 |
| 40 | 0.25 | 2 | no 45 | wilt field | sand | steril | 1.1234 | 1.29437 | 0.74056 | 0.03452 |
| 40 | 4 | 2 | no 45 | wilt field | sand | steril | 0.2982 | 2.247847 | 0.1865 | 0.01277 |
| 40 | 8 | 2 | no 45 | wilt field | sand | steril | 0.085 | 1.804949 | 0.03569 | 0 |
| 40 | 24 | 2 | no 45 | wilt field | sand | steril | 0.0164 | 1.8693 | 0 | 0 |
| 41 | 0.25 | 5 | yes 45 | wilt field | clay | steril | 1.1033 | 4.485126 | 0.63772 | 0.03167 |
| 41 | 4 | 5 | yes 45 | wilt field | clay | steril | 3.246 | 1.925103 | 1.81619 | 0.09652 |
| 41 | 8 | 5 | yes 45 | wilt field | clay | steril | 2.7512 | 0.251541 | 1.67518 | 0.08958 |
| 41 | 24 | 5 | yes 45 | wilt field | clay | steril | 1.6185 | 4.094293 | 1.12116 | 0.05717 |
| 42 | 0.25 | 5 | yes 45 | 60field field | sand | unsteril | 0.8054 | 4.670511 | 0.71685 | 0.02554 |
| 42 | 4 | 5 | yes 45 | 60field field | sand | unsteril | 0.8672 | 5.050948 | 1.46352 | 0 |
| 42 | 8 | 5 | yes 45 | 60field field | sand | unsteril | 0.1598 | 5.075434 | 0.95404 | 0 |
| 42 | 24 | 5 | yes 45 | 60field field | sand | unsteril | 0.0334 | 10.35234 | 0.05096 | 0 |
| 43 | 0.25 | 5 | yes 45 | field adjus | clay | steril | 0.7843 | 2.754666 | 0.40565 | 0.02541 |
| 43 | 4 | 5 | yes 45 | field adjus | clay | steril | 2.6744 | 3.144361 | 1.26028 | 0 |
| 43 | 8 | 5 | yes 45 | field adjus | clay | steril | 2.2569 | 3.473634 | 1.27489 | 0 |
| 43 | 24 | 5 | yes 45 | field adjus | clay | steril | 1.3749 | 6.14537 | 1.07735 | 0 |
| 44 | 0.25 | 5 | yes 30 | wilt adjus | sand | unsteril | 2.7694 | 3.125996 | 3.12263 | 0.09469 |
| 44 | 4 | 5 | yes 30 | wilt adjus | sand | unsteril | 1.1487 | 5.250875 | 2.5903 | 0.01715 |
| 44 | 8 | 5 | yes 30 | wilt adjus | sand | unsteril | 0.4456 | 5.197779 | 1.67274 | 0 |
| 44 | 24 | 5 | yes 30 | wilt adjus | sand | unsteril | 0.1885 | 12.59031 | 0.09261 | 0 |
| 45 | 0.25 | 5 | yes 15 | 60field adjus | sand | steril | 0.6928 | 2.935713 | 0.61283 | 0 |
| 45 | 4 | 5 | yes 15 | 60field adjus | sand | steril | 1.158 | 3.637047 | 1.04264 | 0 |
| 45 | 8 | 5 | yes 15 | 60field adjus | sand | steril | 1.0988 | 3.18966 | 1.04937 | 0 |
| 45 | 24 | 5 | yes 15 | 60field adjus | sand | steril | 1.1542 | 2.939494 | 1.27965 | 0 |
| 46 | 0.25 | 5 | yes 15 | field field | clay | unsteril | 0.1057 | 1.94205 | 0.20475 | 0 |
| 46 | 4 | 5 | yes 15 | field field | clay | unsteril | 0.3298 | 3.535902 | 0.36913 | 0 |
| 46 | 8 | 5 | yes 15 | field field | clay | unsteril | 0.3024 | 4.543055 | 0.43289 | 0 |
| 46 | 24 | 5 | yes 15 | field field | clay | unsteril | 0.1375 | 5.26765 | 0.54431 | 0 |
| 47 | 0.25 | 5 | no 45 | field adjus | sand | unsteril | 0.7011 | 2.170995 | 0.30041 | 0.01456 |
| 47 | 4 | 5 | no 45 | field adjus | sand | unsteril | 0.4033 | 2.196984 | 0.2641 | 0.0021 |
| 47 | 8 | 5 | no 45 | field adjus | sand | unsteril | 0.2279 | 2.540585 | 0.06411 | 0.00289 |
| 47 | 24 | 5 | no 45 | field adjus | sand | unsteril | 0.0641 | 2.774169 | 0 | 0 |
| 48 | 0.25 | 5 | no 30 | field field | sand | steril | 0.3447 | 2.104195 | 0.37449 | 0.00902 |
| 48 | 4 | 5 | no 30 | field field | sand | steril | 0.4996 | 3.096441 | 0.45588 | 0 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 48 | 8 | 5 | no | 30 | field | field | sand | steril | 0.3206 | 2.902206 | 0.30938 | 0 |
| 48 | 24 | 5 | no | 30 | field | field | sand | steril | 0.0614 | 2.915501 | 0.03648 | 0 |
| 49 | 0.25 | 3 | yes | 45 | field | field | sand | steril | 1.6361 | 2.350903 | 0.89421 | 0.07872 |
| 49 | 4 | 3 | yes | 45 | field | field | sand | steril | 4.075 | 1.966106 | 1.33707 | 0.00879 |
| 49 | 8 | 3 | yes | 45 | field | field | sand | steril | . | . | . | . |
| 49 | 24 | 3 | yes | 45 | field | field | sand | steril | 2.4417 | 1.934811 | 1.10303 | 0.07693 |
| 50 | 0.25 | 3 | yes | 30 | 60field | adjus | sand | unsteril | 0.5228 | 3.831669 | 0.47339 | 0 |
| 50 | 4 | 3 | yes | 30 | 60field | adjus | sand | unsteril | 0.4339 | 4.940267 | 0.9861 | 0.00239 |
| 50 | 8 | 3 | yes | 30 | 60field | adjus | sand | unsteril | 0.0514 | 5.038082 | 0.54245 | 0 |
| 50 | 24 | 3 | yes | 30 | 60field | adjus | sand | unsteril | 0 | 17.70329 | 0.02272 | 0 |
| 51 | 0.25 | 3 | yes | 15 | wilt | adjus | clay | steril | 0.5694 | 5.264708 | 0.9736 | 0 |
| 51 | 4 | 3 | yes | 15 | wilt | adjus | clay | steril | 0.9792 | 1.972936 | 1.09076 | 0.01457 |
| 51 | 8 | 3 | yes | 15 | wilt | adjus | clay | steril | 0.9437 | 1.537986 | 1.21882 | 0.01298 |
| 51 | 24 | 3 | yes | 15 | wilt | adjus | clay | steril | 0.775 | 1.374337 | 1.18797 | 0.00489 |
| 52 | 0.25 | 3 | no | 45 | wilt | field | sand | unsteril | 0.9994 | 1.386644 | 0.75716 | 0 |
| 52 | 4 | 3 | no | 45 | wilt | field | sand | unsteril | 0.0919 | 1.758962 | 0.11687 | 0.00198 |
| 52 | 8 | 3 | no | 45 | wilt | field | sand | unsteril | 0.0228 | 1.308181 | 0 | 0 |
| 52 | 24 | 3 | no | 45 | wilt | field | sand | unsteril | 0.004 | 1.377645 | 0 | 0 |
| 53 | 0.25 | 3 | no | 45 | 60field | adjus | clay | steril | 1.4566 | 1.484374 | 0.71998 | 0.06885 |
| 53 | 4 | 3 | no | 45 | 60field | adjus | clay | steril | 0.5409 | 1.57944 | 0.24481 | 0.0161 |
| 53 | 8 | 3 | no | 45 | 60field | adjus | clay | steril | 0.1006 | 1.073351 | 0.0609 | 0 |
| 53 | 24 | 3 | no | 45 | 60field | adjus | clay | steril | 0 | 1.568937 | 0 | 0 |
| 54 | 0.25 | 3 | no | 30 | wilt | adjus | sand | steril | 1.1841 | 1.45832 | 0.82365 | 0.0297 |
| 54 | 4 | 3 | no | 30 | wilt | adjus | sand | steril | 0.5897 | 1.2004 | 0.27531 | 0.01393 |
| 54 | 8 | 3 | no | 30 | wilt | adjus | sand | steril | 0.17 | 1.275018 | 0.07877 | 0 |
| 54 | 24 | 3 | no | 30 | wilt | adjus | sand | steril | 0.021 | 1.435724 | 0 | 0 |
| 55 | 0.25 | 3 | no | 30 | field | field | clay | unsteril | 0.2529 | 1.548145 | 0.27626 | 0 |
| 55 | 4 | 3 | no | 30 | field | field | clay | unsteril | 0.1646 | 1.731426 | 0.34493 | 0 |
| 55 | 8 | 3 | no | 30 | field | field | clay | unsteril | 0.0293 | 1.695943 | 0.18977 | 0 |
| 55 | 24 | 3 | no | 30 | field | field | clay | unsteril | 0 | 1.764676 | 0.00722 | 0 |
| 56 | 0.25 | 3 | no | 15 | field | adjus | clay | unsteril | 0.3877 | 1.960178 | 0.62772 | 0.01938 |
| 56 | 4 | 3 | no | 15 | field | adjus | clay | unsteril | 0.2846 | 1.152265 | 0.37066 | 0 |
| 56 | 8 | 3 | no | 15 | field | adjus | clay | unsteril | 0.04 | 1.446163 | 0.17821 | 0 |
| 56 | 24 | 3 | no | 15 | field | adjus | clay | unsteril | 0 | 1.656614 | 0 | 0 |
| 57 | 0.25 | 1 | yes | 45 | field | field | sand | unsteril | 0.5977 | 1.718582 | 0 | 0.02895 |
| 57 | 4 | 1 | yes | 45 | field | field | sand | unsteril | 0.6894 | 5.128745 | 1.01534 | 0.00992 |
| 57 | 8 | 1 | yes | 45 | field | field | sand | unsteril | 0.1168 | 6.444788 | 0.72674 | 0 |
| 57 | 24 | 1 | yes | 45 | field | field | sand | unsteril | 0.03 | 2.856176 | 0 | 0 |
| 58 | 0.25 | 1 | yes | 30 | 60field | adjus | clay | steril | 2.0372 | 1.567348 | 1.44711 | 0.08729 |
| 58 | 4 | 1 | yes | 30 | 60field | adjus | clay | steril | 3.3251 | 2.257104 | 1.64162 | 0 |
| 58 | 8 | 1 | yes | 30 | 60field | adjus | clay | steril | 2.8531 | 2.274889 | 1.59264 | 0 |
| 58 | 24 | 1 | yes | 30 | 60field | adjus | clay | steril | 1.7217 | 1.657903 | 1.34922 | 0 |
| 59 | 0.25 | 1 | yes | 15 | wilt | field | sand | steril | 0.9098 | 1.854243 | 1.28555 | 0 |
| 59 | 4 | 1 | yes | 15 | wilt | field | sand | steril | 1.4491 | 1.632494 | 1.61683 | 0.00404 |
| 59 | 8 | 1 | yes | 15 | wilt | field | sand | steril | 1.6005 | 1.989432 | 1.67571 | 0.00321 |
| 59 | 24 | 1 | yes | 15 | wilt | field | sand | steril | 1.4042 | 1.417402 | 1.46361 | 0 |
| 60 | 0.25 | 1 | no | 45 | wilt | adjus | clay | unsteril | 0.9671 | 1.967631 | 0.72618 | 0 |
| 60 | 4 | 1 | no | 45 | wilt | adjus | clay | unsteril | 0.0462 | 1.737354 | 0.11763 | 0 |
| 60 | 8 | 1 | no | 45 | wilt | adjus | clay | unsteril | 0.0227 | 1.719398 | 0.02218 | 0 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 60 | 24 | 1 | no 45 | wilt adjus | clay | unsteril | 0 | 1.33924 | 0 | 0 |
| 61 | 0.25 | 1 | no 45 | 60field field | sand | steril | 1.1229 | 1.711064 | 0.73395 | 0.03634 |
| 61 | 4 | 1 | no 45 | 60field field | sand | steril | 0.5689 | 1.488197 | 0.24071 | 0.01082 |
| 61 | 8 | 1 | no 45 | 60field field | sand | steril | 0.1873 | 1.223447 | 0.06181 | 0.00808 |
| 61 | 24 | 1 | no 45 | 60field field | sand | steril | 0.0183 | 1.78609 | 0 | 0 |
| 62 | 0.25 | 1 | no 15 | 60field field | clay | unsteril | 0.4176 | 2.770862 | 0.78722 | 0 |
| 62 | 4 | 1 | no 15 | 60field field | clay | unsteril | 0.2884 | 1.687544 | 0.40157 | 0 |
| 62 | 8 | 1 | no 15 | 60field field | clay | unsteril | 0.2098 | 1.700367 | 0.32208 | 0 |
| 62 | 24 | 1 | no 15 | 60field field | clay | unsteril | 0.074 | 1.713813 | 0.14471 | 0 |
| 63 | 0.25 | 1 | no 15 | 60field adjus | sand | unsteril | 0.2609 | 2.764203 | 0.60443 | 0 |
| 63 | 4 | 1 | no 15 | 60field adjus | sand | unsteril | 0.2403 | 2.091264 | 0.39274 | 0 |
| 63 | 8 | 1 | no 15 | 60field adjus | sand | unsteril | 0.1342 | 1.646455 | 0.32052 | 0 |
| 63 | 24 | 1 | no 15 | 60field adjus | sand | unsteril | 0.0045 | 1.35913 | 0.04127 | 0 |
| 64 | 0.25 | 1 | no 15 | field adjus | clay | steril | 0.0904 | 2.455721 | 0.0739 | 0 |
| 64 | 4 | 1 | no 15 | field adjus | clay | steril | 0.316 | 3.639925 | 0.0094 | 0 |
| 64 | 8 | 1 | no 15 | field adjus | clay | steril | 0.3604 | 1.432889 | 0.29702 | 0.00174 |
| 64 | 24 | 1 | no 15 | field adjus | clay | steril | . | . | 0.13513 | . |
| 65 | 0.25 | 7 | yes 45 | wilt field | clay | unsteril | 0.931 | 1.910003 | 0.92396 | 0.04161 |
| 65 | 4 | 7 | yes 45 | wilt field | clay | unsteril | 0.7083 | 6.193741 | 1.03795 | 0.01705 |
| 65 | 8 | 7 | yes 45 | wilt field | clay | unsteril | 0.1757 | 6.461477 | 0.81335 | 0 |
| 65 | 24 | 7 | yes 45 | wilt field | clay | unsteril | 0.0114 | 12.58487 | 0.02521 | 0 |
| 66 | 0.25 | 7 | yes 45 | 60field adjus | sand | steril | 1.0018 | 1.177889 | 0.79684 | 0.02461 |
| 66 | 4 | 7 | yes 45 | 60field adjus | sand | steril | 2.9513 | 1.240286 | 1.62714 | 0.09756 |
| 66 | 8 | 7 | yes 45 | 60field adjus | sand | steril | 2.4279 | 1.404536 | 1.47462 | 0.07335 |
| 66 | 24 | 7 | yes 45 | 60field adjus | sand | steril | 1.367 | 1.683678 | 0.93445 | 0.03884 |
| 67 | 0.25 | 7 | yes 30 | field adjus | clay | unsteril | 0.2022 | 1.364628 | 0.35504 | 0 |
| 67 | 4 | 7 | yes 30 | field adjus | clay | unsteril | 0.0842 | 5.528256 | 0.35954 | 0 |
| 67 | 8 | 7 | yes 30 | field adjus | clay | unsteril | 0.0095 | 6.874176 | 0.12602 | 0 |
| 67 | 24 | 7 | yes 30 | field adjus | clay | unsteril | 0 | 9.58191 | 0 | 0 |
| 68 | 0.25 | 7 | yes 15 | 60field adjus | clay | unsteril | 0.1453 | 1.556157 | 0.36914 | 0 |
| 68 | 4 | 7 | yes 15 | 60field adjus | clay | unsteril | 0.4497 | 1.908736 | 0.67082 | 0 |
| 68 | 8 | 7 | yes 15 | 60field adjus | clay | unsteril | 0.2062 | 2.406792 | 0.70002 | 0 |
| 68 | 24 | 7 | yes 15 | 60field adjus | clay | unsteril | 0.0297 | 1.93698 | 0.5678 | 0 |
| 69 | 0.25 | 7 | no 45 | wilt adjus | clay | steril | 0.6188 | 1.225616 | 0.38563 | 0.01808 |
| 69 | 4 | 7 | no 45 | wilt adjus | clay | steril | 0.2653 | 1.001289 | 0.08976 | 0 |
| 69 | 8 | 7 | no 45 | wilt adjus | clay | steril | 0.0719 | 0.905686 | 0.01796 | 0 |
| 69 | 24 | 7 | no 45 | wilt adjus | clay | steril | 0.009 | 2.010374 | 0 | 0 |
| 70 | 0.25 | 7 | no 30 | wilt field | sand | unsteril | 0.5335 | 1.274889 | 0.40181 | 0.01392 |
| 70 | 4 | 7 | no 30 | wilt field | sand | unsteril | 0.0638 | 1.25399 | 0.05999 | 0 |
| 70 | 8 | 7 | no 30 | wilt field | sand | unsteril | 0.0049 | 1.118564 | 0.00344 | 0 |
| 70 | 24 | 7 | no 30 | wilt field | sand | unsteril | 0.0022 | 1.553193 | 0 | 0 |
| 71 | 0.25 | 7 | no 30 | 60field field | clay | steril | 0.3167 | 1.028717 | 0.29689 | 0 |
| 71 | 4 | 7 | no 30 | 60field field | clay | steril | 0.2581 | 1.283416 | 0.18166 | 0 |
| 71 | 8 | 7 | no 30 | 60field field | clay | steril | 0.0725 | 1.382026 | 0.08312 | 0 |
| 71 | 24 | 7 | no 30 | 60field field | clay | steril | 0.0141 | 2.238052 | 0.00893 | 0 |
| 72 | 0.25 | 7 | no 15 | field field | sand | steril | 0.3797 | 1.204158 | 0.50258 | 0 |
| 72 | 4 | 7 | no 15 | field field | sand | steril | 0.5846 | 1.46936 | 0.44175 | 0 |
| 72 | 8 | 7 | no 15 | field field | sand | steril | 0.3621 | 1.315312 | 0.30515 | 0.00253 |
| 72 | 24 | 7 | no 15 | field field | sand | steril | 0.3215 | 1.487961 | 0.24066 | 0 |

## Appendix IX.

The following pages contain the ANOVA tables from SAS statistical software output for experiments comparing glucosinolate concentrations in air dried Indian mustard (PI 458928) and freeze dried mustard. An experiment was also done comparing glucosinolate concentrations in oven dried (60°C) and freeze dried Indian mustard of the same PI. For each experiment, 10 leaves were halved down the mid-vein. One half was either air or oven dried, the other half was freeze dried. Glucosinolates were measured using a modified method by Raney and McGregor (1990), see Appendix VI and VII . Mean concentrations are $\mu$mol/g. Significant differences were found in allyl glucosinolates, methoxy glucosinolates, total glucosinolates, and total alipatic glucosinolates between air dried and freeze dried samples. The only differences between oven dried and freeze dried was indole glucosinolates and total indole glucosinolates. Results show that oven drying Indian mustard may be a viable way of processing Indian mustard plant material before storage. This does assume, however, that myrosinase enzyme activity is not effected.

General Linear Models Procedure for air dried vs. freeze dried mustard

**Allyl glucosinolate**

Class Level Information

| Class | Levels | Values |
|---|---|---|
| REP | 10 | 1 2 3 4 5 6 7 8 9 10 |
| TMT | 2 | air dried freeze dried |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 187.73643 | 20.85960 | 2.74 | 0.0748 |
| TMT | 1 | 158.87321 | 158.87321 | 20.85 | 0.0014 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 187.73643 | 20.85960 | 2.74 | 0.0748 |
| TMT | 1 | 158.87321 | 158.87321 | 20.85 | 0.0014 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 22.407 | 10 | freeze dried |
| B | 16.770 | 10 | air dried |

**Gluconasturtiin**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 6.4233968 | 0.7137108 | 21.69 | 0.0001 |
| TMT | 1 | 0.1232450 | 0.1232450 | 3.75 | 0.0850 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 6.4233968 | 0.7137108 | 21.69 | 0.0001 |
| TMT | 1 | 0.1232450 | 0.1232450 | 3.75 | 0.0850 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 1.61560 | 10 | freeze dried |
| A | 1.45860 | 10 | airdried |

**Indole Glucosinolates**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 0.3023030 | 0.0335892 | 2.87 | 0.0658 |
| TMT | 1 | 0.0116645 | 0.0116645 | 1.00 | 0.3439 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 0.3023031 | 0.0335892 | 2.87 | 0.0658 |
| TMT | 1 | 0.0116645 | 0.0116645 | 1.00 | 0.3439 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 0.42150 | 10 | freeze dried |
| A | 0.37320 | 10 | air dried |

**Methoxy Glucosinolates**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 8 | 0.0525899 | 0.0065737 | 1.37 | 0.3468 |
| TMT | 1 | 0.0333062 | 0.0333062 | 6.92 | 0.0338 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 8 | 0.0578792 | 0.0072349 | 1.50 | 0.3020 |
| TMT | 1 | 0.0333062 | 0.0333062 | 6.92 | 0.0338 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 0.13333 | 9 | freeze dried |
| B | 0.05200 | 8 | air dried |

**Total Glucosinolates**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 204.98550 | 22.77617 | 2.93 | 0.0625 |
| TMT | 1 | 175.26160 | 175.26160 | 22.55 | 0.0010 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 204.98550 | 22.77617 | 2.93 | 0.0625 |
| TMT | 1 | 175.26160 | 175.26160 | 22.55 | 0.0010 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 24.564 | 10 | freeze dried |
| B | 18.643 | 10 | air dried |

**Total Aliphatic Glucosinolates**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 210.01924 | 23.33547 | 2.97 | 0.0605 |
| TMT | 1 | 167.84639 | 167.84639 | 21.34 | 0.0013 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 210.01924 | 23.33547 | 2.97 | 0.0605 |
| TMT | 1 | 167.84639 | 167.84639 | 21.34 | 0.0013 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 24.022 | 10 | freeze dried |
| B | 18.228 | 10 | air dried |

**Total Indole Glucosinolates**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 0.4093490 | 0.0454832 | 2.80 | 0.0704 |
| TMT | 1 | 0.0805181 | 0.0805181 | 4.96 | 0.0529 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 0.4093490 | 0.0454832 | 2.80 | 0.0704 |
| TMT | 1 | 0.0805181 | 0.0805181 | 4.96 | 0.0529 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 0.54160 | 10 | freeze dried |
| A | 0.41470 | 10 | air dried |

General Linear Models Procedure for oven dried vs. freeze dried mustard

**Allyl glucosinolate**

Class Level Information

| Class | Levels | Values |
|---|---|---|
| REP | 10 | 1 2 3 4 5 6 7 8 9 10 |
| TMT | 2 | oven dried freeze dried |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 565.47998 | 62.83111 | 2.31 | 0.1143 |
| TMT | 1 | 46.97113 | 46.97113 | 1.73 | 0.2214 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 565.47998 | 62.83111 | 2.31 | 0.1143 |
| TMT | 1 | 46.97113 | 46.97113 | 1.73 | 0.2214 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 25.718 | 10 | freeze dried |
| A | 22.653 | 10 | oven dried |

**Gluconasturtiin**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 7.4828988 | 0.8314332 | 27.08 | 0.0001 |
| TMT | 1 | 0.0749088 | 0.0749088 | 2.44 | 0.1527 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 7.4828988 | 0.8314332 | 27.08 | 0.0001 |
| TMT | 1 | 0.0749088 | 0.0749088 | 2.44 | 0.1527 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 1.56180 | 10 | freeze dried |
| A | 1.43940 | 10 | oven dried |

**Indole Glucosinolate**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 0.0897799 | 0.0099755 | 3.69 | 0.0398 |
| TMT | 1 | 0.0595125 | 0.0595125 | 22.02 | 0.0016 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 0.0984630 | 0.0109403 | 4.05 | 0.0308 |
| TMT | 1 | 0.0595125 | 0.0595125 | 22.02 | 0.0016 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 0.21670 | 10 | freeze dried |
| B | 0.11311 | 9 | oven dried |

**Methoxy Glucosinolate**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 6 | 0.0016534 | 0.0002756 | 0.87 | 0.6218 |
| TMT | 1 | 0.0008640 | 0.0008640 | 2.73 | 0.2403 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 6 | 0.0019592 | 0.0003265 | 1.03 | 0.5682 |
| TMT | 1 | 0.0008640 | 0.0008640 | 2.73 | 0.2403 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 0.04750 | 6 | freeze dried |
| A | 0.03225 | 4 | oven dried |

**Total glucosinolates**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 642.81366 | 71.42374 | 2.47 | 0.0969 |
| TMT | 1 | 55.04562 | 55.04562 | 1.91 | 0.2008 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 642.81366 | 71.42374 | 2.47 | 0.0969 |
| TMT | 1 | 55.04562 | 55.04562 | 1.91 | 0.2008 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 27.525 | 10 | freeze dried |
| A | 24.207 | 10 | oven dried |

**Total Aliphatic Glucosinolates**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 646.87655 | 71.87517 | 2.50 | 0.0947 |
| TMT | 1 | 50.80397 | 50.80397 | 1.76 | 0.2169 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 646.87655 | 71.87517 | 2.50 | 0.0947 |
| TMT | 1 | 50.80397 | 50.80397 | 1.76 | 0.2169 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 27.280 | 10 | freeze dried |
| A | 24.092 | 10 | oven dried |

**Total Indole Glucosinolate**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 0.0852954 | 0.0094773 | 2.43 | 0.1127 |
| TMT | 1 | 0.0789369 | 0.0789369 | 20.25 | 0.0020 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 9 | 0.0984255 | 0.0109362 | 2.80 | 0.0806 |
| TMT | 1 | 0.0789369 | 0.0789369 | 20.25 | 0.0020 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 0.24520 | 10 | freeze dried |
| B | 0.12733 | 9 | oven dried |

Glucosinolate study for freeze vs. oven vs. air dried Indian mustard

Treatment
1=air dried
2=freeze dried that goes w/air dried
3=oven dried
4= freeze dried that goes w/oven dried

| rep | Tmt | Leaf Wt. | sinigrin | gluconas | 3indolyl | umol/g 4-methoxy | total | aliph | indol |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 202.6 | 11.81753 | 1.528096 | 0.632014 | 0.063469 | 14.0411 | 13.34562 | 0.695482 |
| 2 | 1 | 200 | 19.23004 | 2.001321 | 0.349355 | 0.027854 | 21.60857 | 21.23136 | 0.377209 |
| 3 | 1 | 201.6 | 18.73421 | 1.967446 | 0.408515 | 0.053135 | 21.16331 | 20.70166 | 0.46165 |
| 4 | 1 | 201.3 | 14.99098 | 1.269801 | 0.476405 | 0.039393 | 16.77657 | 16.26078 | 0.515797 |
| 5 | 1 | 199 | 14.51925 | 1.085384 | 0.164167 | 0 | 15.76881 | 15.60464 | 0.164167 |
| 6 | 1 | 201.6 | 13.20977 | 1.882171 | 0.590624 | 0.089772 | 15.77234 | 15.09194 | 0.680396 |
| 7 | 1 | 201 | 21.71359 | 1.250581 | 0.255013 | 0.031935 | 23.25112 | 22.96417 | 0.286947 |
| 8 | 1 | 195.5 | 13.71257 | 0.977935 | 0.346493 | 0.027709 | 15.06471 | 14.69051 | 0.374201 |
| 9 | 1 | 201.4 | 22.95313 | 2.2779 | 0.238683 | 0.082715 | 25.55242 | 25.23103 | 0.321397 |
| 10 | 1 | 198.6 | 16.81371 | 0.346171 | 0.270886 | 0 | 17.43077 | 17.15988 | 0.270886 |
| 1 | 2 | 195.4 | 19.28676 | 2.027391 | 0.658798 | 0.152412 | 22.12536 | 21.31416 | 0.811209 |
| 2 | 2 | 198.4 | 25.1874 | 2.153573 | 0.300055 | 0.078771 | 27.7198 | 27.34097 | 0.378826 |
| 3 | 2 | 198.1 | 20.57294 | 2.258775 | 0.411337 | 0.325198 | 23.56825 | 22.83171 | 0.736535 |
| 4 | 2 | 203.1 | 24.09478 | 1.63784 | 0.60234 | 0.092581 | 26.42754 | 25.73262 | 0.694921 |
| 5 | 2 | 196.4 | 23.37119 | 1.585371 | 0.443734 | 0.053893 | 25.45419 | 24.95656 | 0.497628 |
| 6 | 2 | 199.9 | 23.8252 | 1.639654 | 0.398513 | 0.083173 | 25.94653 | 25.46485 | 0.481685 |
| 7 | 2 | 201 | 27.22808 | 1.213758 | 0.211479 | 0.070606 | 28.72393 | 28.44184 | 0.282085 |
| 8 | 2 | 195.5 | 13.71257 | 0.836544 | 0.290843 | 0.048741 | 14.8887 | 14.54912 | 0.339584 |
| 9 | 2 | 201.4 | 22.50684 | 2.373067 | 0.334896 | 0.293615 | 25.50842 | 24.87991 | 0.628511 |
| 10 | 2 | 196.5 | 24.27948 | 0.429282 | 0.562931 | 0 | 25.2717 | 24.70877 | 0.562931 |
| 1 | 3 | 198.7 | 13.37232 | 0.281902 | 0.041202 | 0 | 13.69543 | 13.65422 | 0.041202 |
| 2 | 3 | 197.3 | 23.98485 | 1.501191 | 0.082639 | 0.030449 | 25.59913 | 25.48604 | 0.113088 |
| 3 | 3 | 200.4 | 43.31968 | 2.537489 | 0 | 0 | 45.85717 | 45.85717 | 0 |
| 4 | 3 | 199.2 | 19.28498 | 2.111762 | 0.10441 | 0 | 21.50115 | 21.39674 | 0.10441 |
| 5 | 3 | 197.9 | 31.91896 | 1.816384 | 0.147011 | 0.041054 | 33.92341 | 33.73535 | 0.188066 |
| 6 | 3 | 198.2 | 18.46644 | 0.572691 | 0.030733 | 0.025221 | 19.09509 | 19.03914 | 0.055955 |
| 7 | 3 | 197.8 | 18.23487 | 0.890909 | 0.2009 | 0 | 19.32668 | 19.12578 | 0.2009 |
| 8 | 3 | 202.8 | 15.02815 | 1.52824 | 0.153713 | 0 | 16.71011 | 16.55639 | 0.153713 |
| 9 | 3 | 198.5 | 21.44973 | 1.834593 | 0.125315 | 0 | 23.40964 | 23.28433 | 0.125315 |
| 10 | 3 | 198.7 | 21.47129 | 1.318845 | 0.131861 | 0.032629 | 22.95462 | 22.79013 | 0.16449 |
| 1 | 4 | 204.5 | 21.95231 | 0.461308 | 0.081514 | 0.016749 | 22.51188 | 22.41362 | 0.098263 |
| 2 | 4 | 204.8 | 26.8329 | 1.765206 | 0.177673 | 0.082519 | 28.8583 | 28.5981 | 0.260191 |
| 3 | 4 | 197.5 | 30.86417 | 2.053793 | 0.114038 | 0 | 33.032 | 32.91796 | 0.114038 |
| 4 | 4 | 197.6 | 23.47629 | 2.477989 | 0.396433 | 0.052201 | 26.40291 | 25.95428 | 0.448634 |
| 5 | 4 | 202.4 | 26.50613 | 1.710025 | 0.228575 | 0 | 28.44472 | 28.21615 | 0.228575 |
| 6 | 4 | 199.2 | 26.00214 | 0.852748 | 0.099606 | 0.036358 | 26.99085 | 26.85489 | 0.135964 |
| 7 | 4 | 195.5 | 22.82442 | 1.036816 | 0.331841 | 0 | 24.19308 | 23.86124 | 0.331841 |
| 8 | 4 | 201 | 26.83735 | 1.80675 | 0.279928 | 0 | 28.92403 | 28.6441 | 0.279928 |
| 9 | 4 | 200.3 | 21.31795 | 1.988962 | 0.193373 | 0.055791 | 23.55607 | 23.30691 | 0.249165 |
| 10 | 4 | 198.1 | 30.56913 | 1.463904 | 0.263261 | 0.041344 | 32.33764 | 32.03304 | 0.304605 |

## Appendix X.

The following pages contain the ANOVA tables from SAS statistical software output for experiments comparing volatiles released from fresh Indian mustard (PI 458934) and freeze dried mustard. Ten leaves were halved down the mid-vein. One half was macerated fresh and 10 g was added to a 500 mL jar, the other half was freeze dried and ground. The relative fresh weight of freeze dried material and moisture was added to a 500 mL jar and compared to fresh mustard . Volatiles were measured using SPME. This data was for comparison purposes only, means are reported as peak areas. Results show that freeze drying may causes alcohols and aldehydes to break down and not be detected. However, isothiocyanate areas increased.

General Linear Models Procedure for fresh vs. freeze dried

Class Level Information

| Class | Levels | Values |
|---|---|---|
| TMT | 2 | dried fresh |
| REP | 10 | 1 2 3 4 5 6 7 8 9 10 |

**Unknown**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 603615.2 | 603615.2 | 0.87 | 0.3780 |
| REP | 9 | 5047783.1 | 560864.8 | 0.81 | 0.6229 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 485703.7 | 485703.7 | 0.70 | 0.4268 |
| REP | 9 | 5047783.1 | 560864.8 | 0.81 | 0.6229 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 914.9 | 9 | dried |
| A | 557.9 | 10 | fresh |

**Z-3-Hexenal**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 990103.18 | 990103.18 | 23.79 | 0.0012 |
| REP | 9 | 368785.77 | 40976.20 | 0.98 | 0.5141 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 1024562.7 | 1024562.7 | 24.62 | 0.0011 |
| REP | 9 | 368785.8 | 40976.2 | 0.98 | 0.5141 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 457.19 | 10 | fresh |
| B | 0.00 | 9 | dried |

**Sec-butyl Isothiocyanate**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 1057788.8 | 1057788.8 | 5.36 | 0.0492 |
| REP | 9 | 3400521.3 | 377835.7 | 1.92 | 0.1863 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 990073.1 | 990073.1 | 5.02 | 0.0554 |
| REP | 9 | 3400521.3 | 377835.7 | 1.92 | 0.1863 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 1712.8 | 9 | dried |
| B | 1240.2 | 10 | fresh |

**Unknown**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 38145068 | 38145068 | 44.62 | 0.0002 |
| REP | 9 | 69527056 | 7725228 | 9.04 | 0.0025 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 30412486 | 30412486 | 35.58 | 0.0003 |
| REP | 9 | 69527056 | 7725228 | 9.04 | 0.0025 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 5898.6 | 9 | dried |
| B | 3060.8 | 10 | fresh |

**Z-3 Hexenol**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 1867.9 | 1867.9 | 0.01 | 0.9103 |
| REP | 9 | 1027028.2 | 114114.2 | 0.83 | 0.6124 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 1063.8 | 1063.8 | 0.01 | 0.9323 |
| REP | 9 | 1027028.2 | 114114.2 | 0.83 | 0.6124 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 445.9 | 10 | fresh |
| A | 426.0 | 9 | dried |

**AITC**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 1.469E+11 | 1.469E+11 | 185.95 | 0.0001 |
| REP | 9 | 9.942E+09 | 1.105E+09 | 1.40 | 0.3235 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 1.383E+11 | 1.383E+11 | 175.13 | 0.0001 |
| REP | 9 | 9.942E+09 | 1.105E+09 | 1.40 | 0.3235 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 205543 | 9 | dried |
| B | 29470 | 10 | fresh |

**3-butenyl Isothiocyanate**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 2991262 | 2991262 | 0.43 | 0.5300 |
| REP | 9 | 36822081 | 4091342 | 0.59 | 0.7766 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 2483322 | 2483322 | 0.36 | 0.5663 |

| | | | | | |
|---|---|---|---|---|---|
| REP | 9 | 36822081 | 4091342 | 0.59 | 0.7766 |

| Duncan Grouping | Mean | N | TMT | |
|---|---|---|---|---|
| | A | 3857 | 10 | fresh |
| | A | 3063 | 9 | dried |

Volatile study for fresh vs. freeze dried Indian mustard

| | | | Peak areas | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Rep 8.2 | | 8.34 | 9.024 | 11.227 | 12.326 | 13.541 | 13.928 |
| fresh | 1 | 301.97 | 277.46 | 1208.75 | 918.16 | 486.21 | 22606.56 | 4323.41 |
| fresh | 2 | 330.77 | 377.86 | 1758.04 | 2934.50 | 355.24 | 22910.72 | 4661.77 |
| fresh | 3 | 434.72 | 288.69 | 1310.32 | 3917.26 | 251.59 | 22863.69 | 3175.60 |
| fresh | 4 | 1398.88 | 1088.27 | 1701.40 | 3198.60 | 314.25 | 55479.61 | 4759.50 |
| fresh | 5 | 177.28 | 209.40 | 1303.13 | 611.23 | 1755.87 | 7637.08 | 3958.75 |
| fresh | 6 | 260.83 | 196.02 | 145.92 | 4899.80 | 305.77 | 37978.13 | 3102.39 |
| fresh | 7 | 1018.60 | 742.17 | 1176.06 | 3170.17 | 127.44 | 45818.78 | 3442.17 |
| fresh | 8 | 616.15 | 388.46 | 813.46 | 1014.23 | 128.65 | 15109.62 | 2545.77 |
| fresh | 9 | 481.35 | 409.84 | 1420.98 | 3863.47 | 496.89 | 18850.00 | 4017.36 |
| fresh | 10 | 558.74 | 593.72 | 1564.35 | 6080.94 | 236.77 | 45446.19 | 4585.65 |
| | average | 557.93 | 457.19 | 1240.24 | 3060.84 | 445.87 | 29470.04 | 3857.24 |
| | stdev | 379.20 | 279.22 | 472.83 | 1785.98 | 477.14 | 15637.79 | 758.06 |
| | sterr | 119.91 | 88.30 | 149.52 | 564.78 | 150.89 | 4945.10 | 239.72 |
| | Rep | | | | | | | |
| dried | 1 | . | . | . | . | . | . | . |
| dried | 2 | 939.63 | 0.00 | 2164.80 | 6224.94 | 611.66 | 214554.55 | 3358.97 |
| dried | 3 | 996.23 | 0.00 | 2753.37 | 9038.69 | 410.91 | 236875.00 | 8403.57 |
| dried | 4 | 446.93 | 0.00 | 2170.11 | 6176.82 | 351.96 | 292774.02 | 0.00 |
| dried | 5 | 653.00 | 0.00 | 2136.81 | 2136.81 | 381.98 | 200053.26 | 7330.29 |
| dried | 6 | 236.98 | 0.00 | 1354.47 | 7456.86 | 468.79 | 222515.90 | 4457.46 |
| dried | 7 | 434.62 | 0.00 | 1385.08 | 3823.57 | 249.36 | 175500.74 | 0.00 |
| dried | 8 | 665.77 | 0.00 | 1106.92 | 2778.46 | 521.35 | 172734.62 | 4012.88 |
| dried | 9 | 171.50 | 0.00 | 1397.67 | 7326.94 | 419.69 | 177092.23 | 0.00 |
| dried | 10 | 3689.46 | 0.00 | 945.96 | 8124.22 | 418.39 | 157787.44 | 0.00 |
| | average | 914.90 | 0.00 | 1712.80 | 5898.59 | 426.01 | 205543.08 | 3062.57 |
| | stdev | 1078.07 | 0.00 | 608.92 | 2440.74 | 102.68 | 41921.56 | 3301.40 |
| | sterr | 340.92 | 0.00 | 192.56 | 771.83 | 32.47 | 13256.76 | 1043.99 |

## Appendix XI.

The following pages contain the ANOVA tables from SAS statistical software output for experiments comparing volatiles released from fresh Indian mustard (PI 458928) and freeze dried mustard. Ten leaves were halved down the mid-vein. One half was macerated fresh and 10 g was added to a 500 mL jar, the other half was freeze dried. The relative fresh weight of freeze dried material and moisture was added to a 500 mL and compared to fresh mustard . Volatiles were measured using SPME. This data was for comparison purposes only, means for the ratio of every compound compared to AITC are reported. The results show that the mean ratios of all compounds changed in comparison to AITC after freeze drying.

ratio of fresh vs. freeze

**Unknown**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 0.0011026 | 0.0011026 | 11.98 | 0.0086 |
| REP | 9 | 0.0004678 | 0.0000520 | 0.56 | 0.7941 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 0.0011552 | 0.0011552 | 12.55 | 0.0076 |
| REP | 9 | 0.0004678 | 0.0000520 | 0.56 | 0.7941 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 0.020290 | 10 | fresh |
| B | 0.005033 | 9 | dried |

**Z-3-Hexanal**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 0.0013738 | 0.0013738 | 57.15 | 0.0001 |
| REP | 9 | 0.0002172 | 0.0000241 | 1.00 | 0.5033 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 0.0013869 | 0.0013869 | 57.69 | 0.0001 |
| REP | 9 | 0.0002172 | 0.0000241 | 1.00 | 0.5033 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 0.017030 | 10 | fresh |
| B | 0.000000 | 9 | dried |

**Unknown**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 0.0118216 | 0.0118216 | 10.73 | 0.0113 |
| REP | 9 | 0.0098698 | 0.0010966 | 0.99 | 0.5083 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 0.0114660 | 0.0114660 | 10.40 | 0.0121 |
| REP | 9 | 0.0098698 | 0.0010966 | 0.99 | 0.5083 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 0.05819 | 10 | fresh |
| B | 0.00823 | 9 | dried |

**Sec-butyl Isothiocyanate**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 0.0295061 | 0.0295061 | 33.85 | 0.0004 |
| REP | 9 | 0.0203671 | 0.0022630 | 2.60 | 0.0969 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 0.0336182 | 0.0336182 | 38.56 | 0.0003 |
| REP | 9 | 0.0203671 | 0.0022630 | 2.60 | 0.0969 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 0.10818 | 10 | fresh |
| B | 0.02926 | 9 | dried |

**Z-3-Hexenol**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 0.0046453 | 0.0046453 | 1.72 | 0.2266 |
| REP | 9 | 0.0217329 | 0.0024148 | 0.89 | 0.5695 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 0.0047955 | 0.0047955 | 1.77 | 0.2199 |
| REP | 9 | 0.0217329 | 0.0024148 | 0.89 | 0.5695 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 0.03346 | 10 | fresh |
| A | 0.00214 | 9 | dried |

**AITC**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 0 | 0 | . | . |
| REP | 9 | 0 | 0 | . | . |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 0 | 0 | . | . |
| REP | 9 | 0 | 0 | . | . |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 1.000 | 9 | dried |
| B | 1.000 | 10 | fresh |

**3-butenyl Isothiocyanate**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 0.1260918 | 0.1260918 | 14.79 | 0.0049 |
| REP | 9 | 0.0875260 | 0.0097251 | 1.14 | 0.4317 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TMT | 1 | 0.1175963 | 0.1175963 | 13.79 | 0.0059 |
| REP | 9 | 0.0875260 | 0.0097251 | 1.14 | 0.4317 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 0.17771 | 10 | fresh |
| B | 0.01456 | 9 | dried |

## Appendix XII.

The following pages contain the ANOVA tables from SAS statistical software output for experiments comparing AITC released from fresh Indian mustard in oven-sterilized and autoclaved soils. AITC was measured using SPME. This data was for comparison purposes only, means for AITC peak areas are reported. The results show that mean peak areas are not significantly different between the two soil treatments.

General Linear Models Procedure

Class Level Information

| Class | Levels | Values |
|---|---|---|
| REP | 2 | 1 2 |
| TMT | 2 | 1 2 |

**0.25 Hours**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 1 | 460714028 | 460714028 | 3.26 | 0.0963 |
| TMT | 1 | 1068639 | 1068639 | 0.01 | 0.9322 |
| REP*TMT | 1 | 76566875 | 76566875 | 0.54 | 0.4761 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 1 | 460714028 | 460714028 | 3.26 | 0.0963 |
| TMT | 1 | 1068639 | 1068639 | 0.01 | 0.9322 |
| REP*TMT | 1 | 76566875 | 76566875 | 0.54 | 0.4761 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 13589 | 8 | 2 |
| A | 13072 | 8 | 1 |

**4 Hours**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 1 | 745235401 | 745235401 | 2.30 | 0.1550 |
| TMT | 1 | 414611044 | 414611044 | 1.28 | 0.2797 |
| REP*TMT | 1 | 555285660 | 555285660 | 1.72 | 0.2147 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 1 | 745235401 | 745235401 | 2.30 | 0.1550 |
| TMT | 1 | 414611044 | 414611044 | 1.28 | 0.2797 |
| REP*TMT | 1 | 555285660 | 555285660 | 1.72 | 0.2147 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 17463 | 8 | 2 |
| A | 7282 | 8 | 1 |

**8 Hours**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 1 | 745235401 | 745235401 | 2.30 | 0.1550 |
| TMT | 1 | 414611044 | 414611044 | 1.28 | 0.2797 |
| REP*TMT | 1 | 555285660 | 555285660 | 1.72 | 0.2147 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 1 | 745235401 | 745235401 | 2.30 | 0.1550 |
| TMT | 1 | 414611044 | 414611044 | 1.28 | 0.2797 |
| REP*TMT | 1 | 555285660 | 555285660 | 1.72 | 0.2147 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 17463 | 8 | 2 |
| A | 7282 | 8 | 1 |

**24 Hours**

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 1 | 314909525 | 314909525 | 0.82 | 0.3876 |
| TMT | 1 | 586736946 | 586736946 | 1.54 | 0.2466 |
| REP*TMT | 1 | 513376927 | 513376927 | 1.34 | 0.2762 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 1 | 290211181 | 290211181 | 0.76 | 0.4061 |
| TMT | 1 | 316449511 | 316449511 | 0.83 | 0.3865 |
| REP*TMT | 1 | 513376927 | 513376927 | 1.34 | 0.2762 |

| Duncan Grouping | Mean | N | TMT |
|---|---|---|---|
| A | 15832 | 7 | 2 |
| A | 3384 | 6 | 1 |

steam vs. heat sterilized

Soil tmt
texture
1 = clay 1= heat
2 = sand 2= steam

| soil | tmt | rep | .25 hour | 4 hour | 8 hour | 24 hour | 48 |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 14655 | 5598 | 1351 | 0 | 0 |
| 1 | 1 | 2 | 15889 | 1397 | 814 | 0 | 0 |
| 1 | 1 | 3 | 17082 | 9279 | 2605 | 984 | 0 |
| 1 | 1 | 4 | 17377 | 16590 | 4438 | 1166 | 0 |
| 1 | 2 | 1 | 7132 | 8664 | 3591 | 676 | 0 |
| 1 | 2 | 2 | 13410 | 19257 | 15231 | 2732 | 0 |
| 1 | 2 | 3 | 9357 | 13090 | 8350 | 776 | 0 |
| 1 | 2 | 4 | 54672 | 79706 | 75444 | 30180 | 6798 |
| 2 | 1 | 1 | 5162 | 661 | 0 | 0 | 0 |
| 2 | 1 | 2 | 6085 | 627 | 0 | 0 | 0 |
| 2 | 1 | 3 | 20620 | 23462 | 10300 | 1949 | 1052 |
| 2 | 1 | 4 | 7708 | 645 | 796 | 0 | 0 |
| 2 | 2 | 1 | 8843 | 9764 | 5507 | 691 | 0 |
| 2 | 2 | 2 | 2916 | 1558 | 0 | 0 | 0 |
| 2 | 2 | 3 | 6287 | 5057 | 1742 | 0 | 0 |
| 2 | 2 | 4 | 6096 | 2611 | 962 | 0 | 0 |

## Appendix XIII.

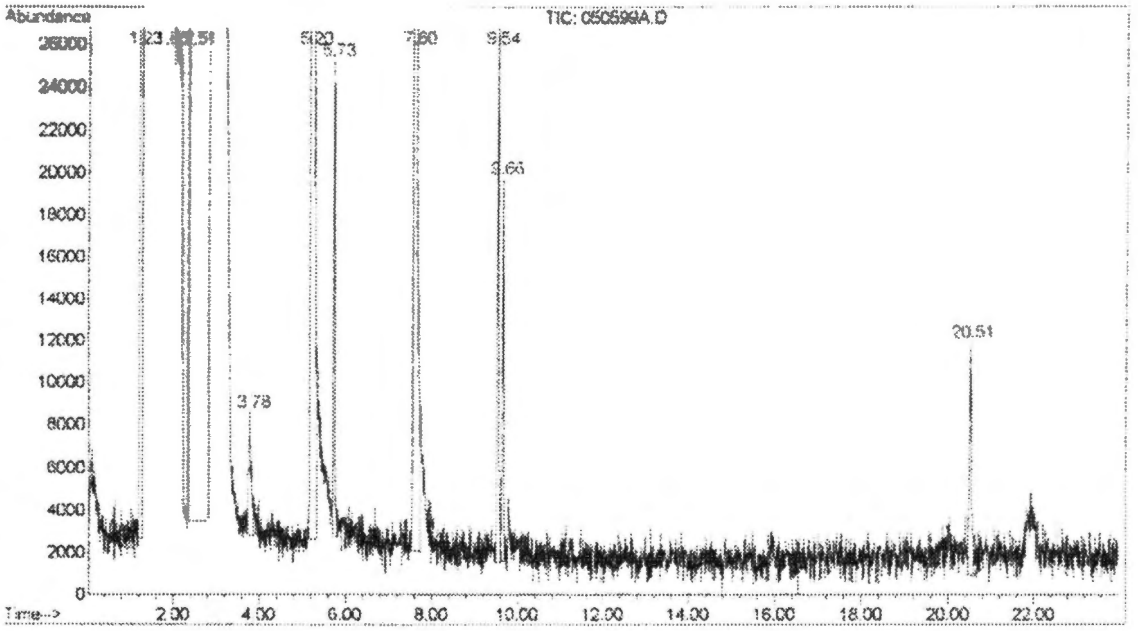
Estimated bacteria counts for clay loam and sandy loam textured soils. Autoclaved soils produced no colonies of fungus or bacteria.

| soil texture | rep | bacteria population estimated | fungi colonies estimated |
|---|---|---|---|
| sand | 1 | $8.0*10^2$ | 18 |
| sand | 2 | $8.0*10^2$ | 24 |
| sand | 3 | $8.4*10^2$ | 26 |
| | | | |
| clay | 1 | $8.79*10^2$ | 7 |
| clay | 2 | $8.0*10^2$ | 0 |
| clay | 3 | $1.0*10^3$ | 0 |

## Appendix XIV.

The following pages contain chromatograms and spectra for observation 41, group 5, within experiment 6. The first page is the chromatogram for this sample and retention time of 10 peaks. The second page shows the spectra for AITC. The third page shows the spectra for sec-butyl ITC. The fourth page shows the spectra for 3-butenyl ITC. The fifth page shows the chromatogram for freeze dried Indian mustard with no soil. The sixth page shows a SPME blank sample. The last page is a spectra for AITC detected (top) and an authentic standard (bottom).

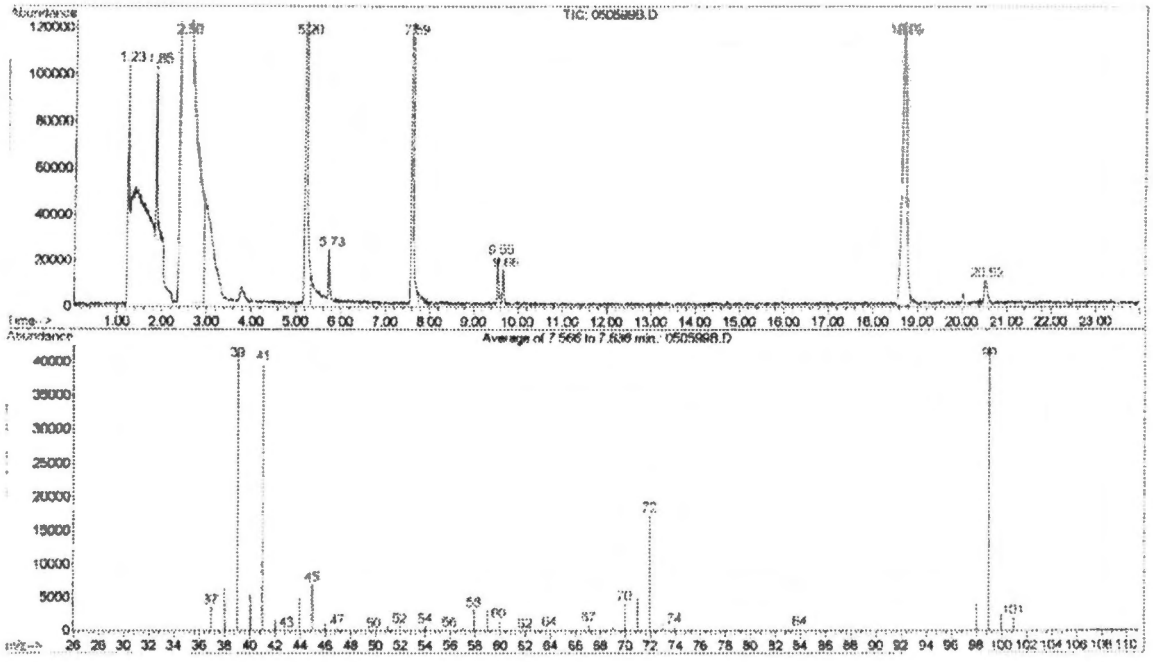
File : E:\050599A.D
Operator :
Acquired : 5 May 1999 8:11 using AcqMethod DEFAULT
Instrument : 5972 - In
Sample Name: obs. 41, group 5, 15 min
Misc Info :
Vial Number: 1



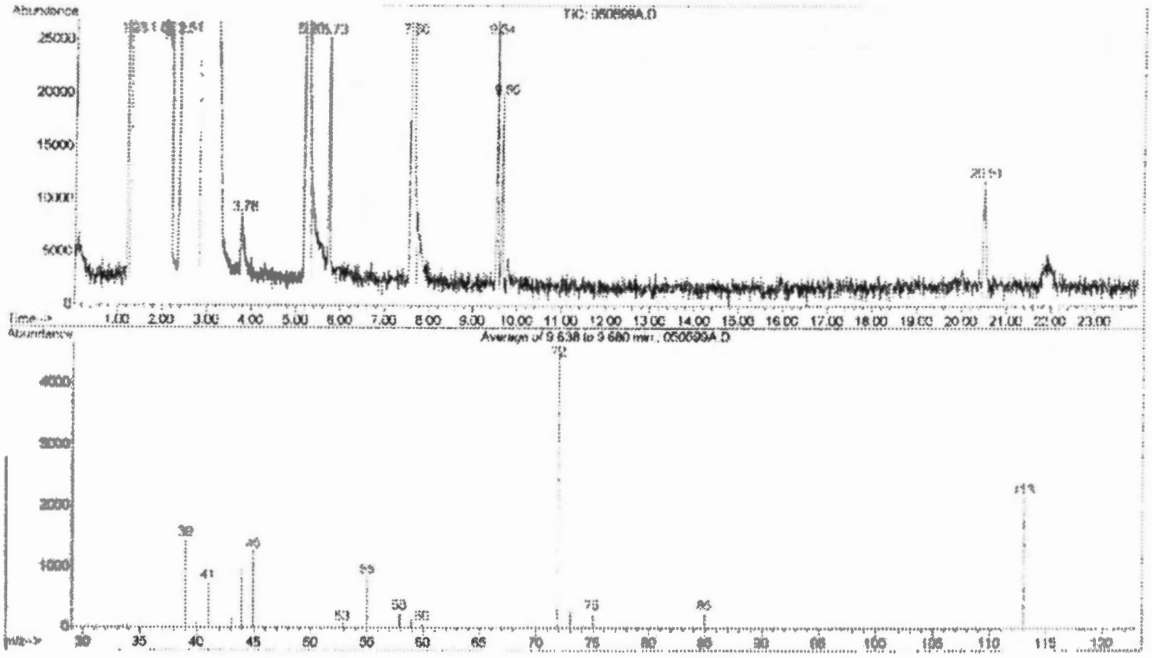

TIC: 050599A.D
obs. 41, group 5, 15 min

| Peak# | Ret Time | Type | Width | Area | Start Time | End Time |
|---|---|---|---|---|---|---|
| 1 | 1.226 | rBV | 0.092 | 266487 | 1.190 | 1.282 |
| 2 | 1.855 | rVB | 0.106 | 60177 | 1.834 | 1.940 |
| 3 | 2.505 | rBV | 0.474 | 6308864 | 2.350 | 2.823 |
| 4 | 3.778 | rBV | 0.120 | 24093 | 3.735 | 3.855 |
| 5 | 5.199 | rBV | 0.177 | 790129 | 5.149 | 5.326 |
| 6 | 5.729 | rVB | 0.120 | 58366 | 5.693 | 5.814 |
| 7 | 7.595 | rBV | 0.191 | 1308666 | 7.539 | 7.729 |
| 8 | 9.539 | rBV | 0.113 | 61022 | 9.490 | 9.603 |
| 9 | 9.659 | rVB | 0.106 | 42093 | 9.610 | 9.716 |
| 10 | 20.510 | rVB | 0.141 | 43899 | 20.454 | 20.595 |

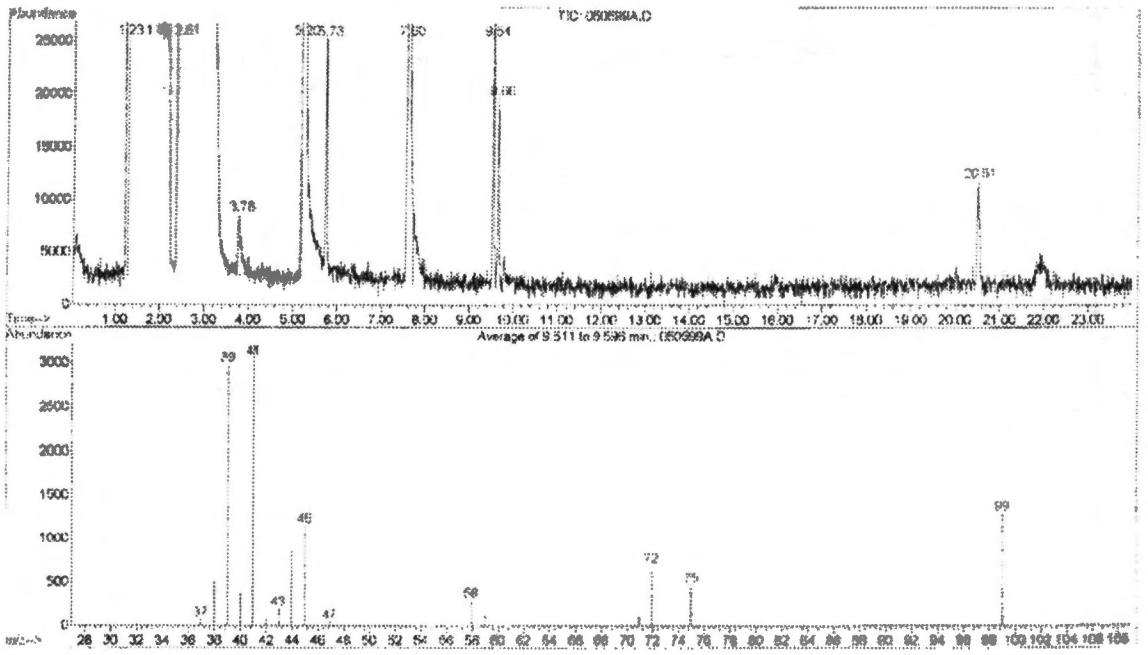
File : C:\HPCHEM\1\DATA\050599B.D
Operator :
Acquired : 5 May 1999 8:40 using AcqMethod DEFAULT
Instrument : 5972 - In
Sample Name: obs. 42, group 5, 15 min
Misc Info :
Vial Number: 1



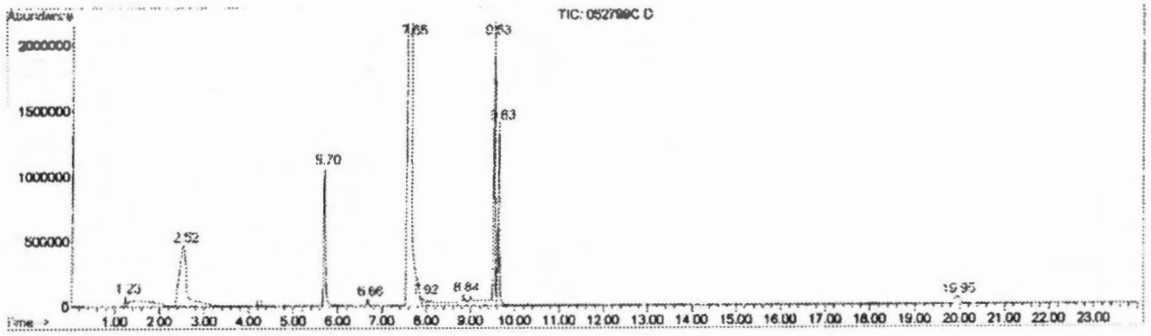
File : E:\050599A.D
Operator :
Acquired : 5 May 1999 8:11 using AcqMethod DEFAULT
Instrument : 5972 - In
Sample Name: obs. 41, group 5, 15 min
Misc Info :
Vial Number: 1



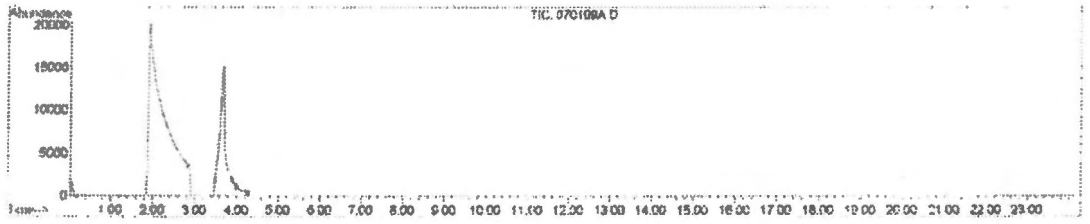
File : E:\050599A.D
Operator :
Acquired : 5 May 1999 8:11 using AcqMethod DEFAULT
Instrument : 5972 - In
Sample Name: obs. 41, group 5, 15 min
Misc Info :
Vial Number: 1



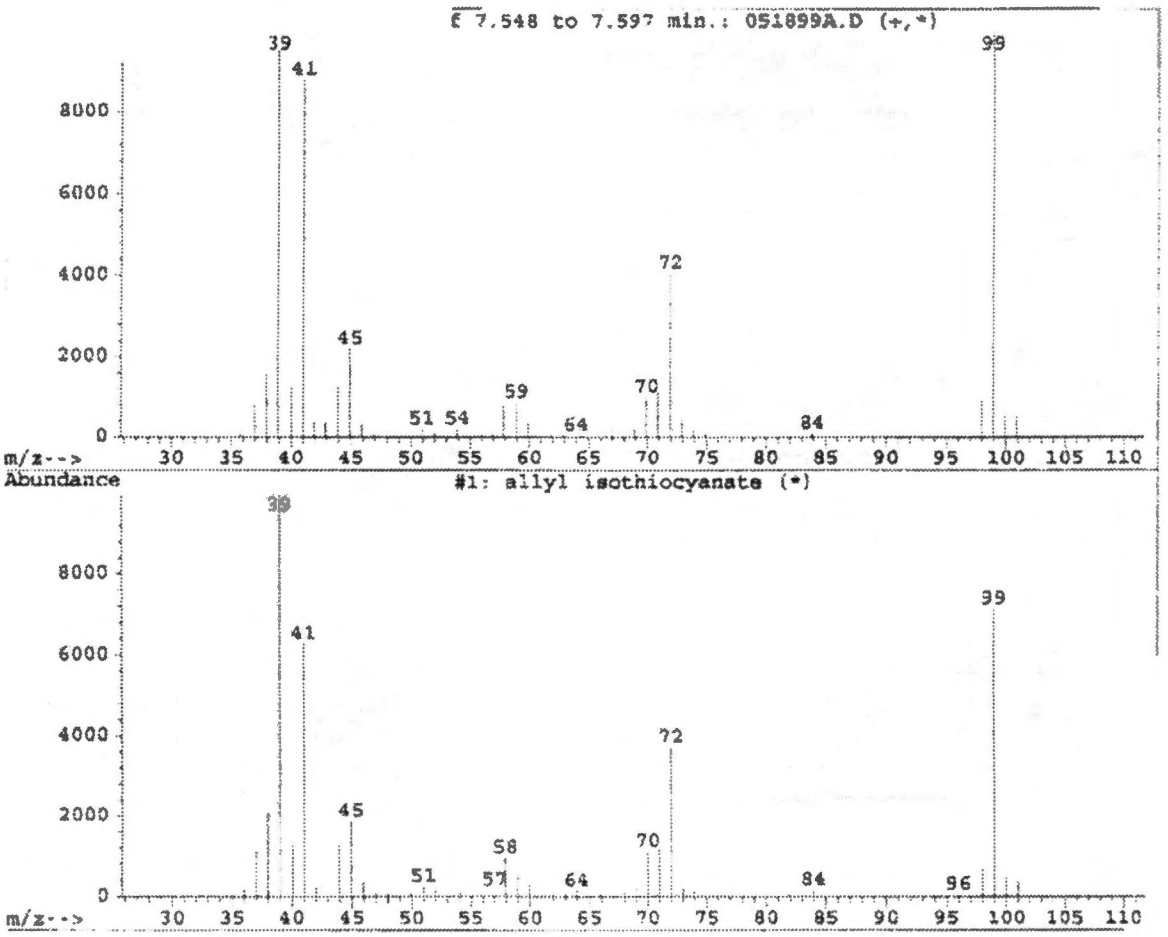
File : E:\052799C.D
Operator :
Acquired : 27 May 1999 12:12 using AcqMethod DEFAULT
Instrument : 5972 - In
Sample Name: PI:458934
Misc Info :
Vial Number: 1



File : C:\HPCHEM\1\DATA\070199A.D
Operator : ejp
Acquired : 1 Jul 1999 14:37 using AcqMethod SPME
Instrument : GC/MS Ins
Sample Name: SPME blank
Misc Info :
Vial Number: 1



Library Searched
Quality
ID



## VITA

Andrew Price was born in Knoxville, Tennessee on September 17, 1975. He attended public schools in Grainger and Knox Counties, and graduated from Carter High School in 1992. He entered The University of Tennessee, Knoxville in fall 1992 where in December 1996 he received the Bachelor of Science Degree in Plant and Soil Sciences. He then entered the Master's program in Plant and Soil Sciences at The University of Tennessee, officially receiving his degree in December, 1999. He is presently working on his Doctorate of Philosophy in Crop Science at North Carolina State University.

1152 7266 31
04•27•00 VP MAB
INFORMATION CONSORTIUM, INC