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Allyl Isothiocyanate and Carbon Dioxide Produced during Degradation of *Brassica juncea* Tissue in Different Soil Conditions

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Abstract. **A study was conducted to quantify volatiles generated from Indian mustard (*Brassica juncea* L. Czerniak) tissue incorporated into soils under controlled conditions. Mustard residues were incorporated into noncovered and covered soils that varied by texture, temperature, moisture, pH, or sterility (autoclaved or nonautoclaved). Sandy loam soil had 38% more allyl isothiocyanate (AITC) than clay loam soil. AITC concentration in 45 °C soil was 81% higher than in soil at 15 °C, and 56% higher in covered compared to noncovered treatments. The microbial catabolism of AITC was suggested by the result that AITC concentration in autoclaved soils was over three times that measured in nonautoclaved soils. The highest AITC level detected (1.71 μmol·L^{-1}) occurred in the autoclaved covered soil. Several factors also influenced CO_2 evolution. At 30 or 45 °C, CO_2 concentration was at least 64% higher than at 15°C. The covered soil had over twice the CO_2 found in the noncovered soil, and the nonautoclaved soil treatment yielded twice the CO_2 measured in the autoclaved soil. There were no main effect differences among soil moisture, soil pH, and soil texture treatments for CO_2 concentrations. This information could be helpful in defining ideal soil conditions for field scale experiments. Additionally, this study demonstrates a sampling technique for testing fumigation potential of biofumigation and solarization systems that may have the potential to replace methyl bromide.**

Soil fumigation is widely used in nursery stock, strawberry, tobacco, tomato, and other commodity crop production systems to control nematodes, pathogens, and weeds. Methyl bromide, a broad spectrum soil fumigant, has been one of the main pesticides used for soil fumigation. Methyl bromide was listed in the 1993 Montreal Protocol as an ozone-depleting compound and is banned for use in crop production in the U.S. in 2005 (USDA, 1999). Suitable replacements for methyl bromide are urgently needed.

Biofumigation and solarization are possible solutions to control nematodes, pathogens, and weeds. Biofumigation is the suppression of soilborne pests via toxic compounds released from soil- incorporated *Brassica* tissue (Angus et al., 1994; Sams et al., 1997). Soil solarization is a technique in which a clear polyethylene tarp is used to trap solar energy during periods of high radiation, thereby raising soil temperatures to levels lethal to pathogens (Pullman et al., 1981).

Plants from the Brassicaceae family contain glucosinolates (GLs). Degradation products such as alcohols, aldehydes, isothiocyanates (ITCs), and nitriles are produced upon enzymatic hydrolysis of GLs by myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) (Morra and Kirkegaard, 2002). Residues from *Brassica* crops have been shown to have biotoxic activity against many soilborne pathogens and pests. ITCs, mainly allyl isothiocyanate (AITC), contribute to the majority of toxic effects observed in decomposing *Brassica* tissues (Chew, 1988; Peterson et al., 2001). In closed jars, volatiles from macerated Indian mustard completely suppressed *Pythium ultimum* (Trow) and reduced *Rhizoctonia solani* (Kühn) growth by 72.6% (Charron and Sams, 1999). Soil-incorporated white mustard tissue (*Brassica hirta biennis* L.) has been shown to significantly reduce *Aphanomyces euteiches* (Drechs.) root rot in peas (*Pisium sativum* L.) (Muelichen et al., 1997). Glucosinolate-derived ITCs inhibited pear pathogens such as *Botrytis cinerea* (Pers.: Fr.), *Monilinia laxa* (Aderhold & Ruhland), and *Mucor piriformis* (E. Fisch.) (Mari et al., 1996). Broccoli [*B. olereacea* L. (Botrytis Group)] residues reduced the population of *Verticillium dahliae* (Kleb) microsclerotia in soil (Subbarao and Hubbard, 1996). Indian mustard seed meal suppressed soilborne cereal pathogens when used as an in-furrow treatment for wheat (Kirkegaard et al., 1996). Indian mustard was also shown to suppress masked chaffer beetle larvae (Noble et al., 1998). Turnip-rape (*Brassica napas* L.) can suppress scentless mayweed (*Matricaria inodora* L.) and spiny sowthistle [*Sonchus asper* (L.) Hill] (Peterson et al., 2001). White mustard can reduce emergence of shepherd's purse [*Capsella bursa-pastoris* (L.) Medik], kochia [*Kochia scoparia* (L.) Schrad], and green foxtail [*Setaria viridis* (L.) Beauv.] (Al-Khatib et al., 1997).

Solarization has been shown to reduce populations of bacteria, fungi, insects, nematodes, and weeds (Pullman et al., 1981; Stapleton and DeVay, 1986). In an experiment in Alabama, the maximum temperatures attained during soil solarization ranged from 48 °C at the soil surface to 34 °C 20 cm deep (Himelrick and Dozier, 1991). Experiments conducted during two years of strawberry production in California showed that solarization increased strawberry yield 12% over the yield of nonsolarized plots (Hartz et al., 1993). Hartz et al. (1993) reported that soil temperatures exceeded 50 °C at the soil surface and 35 °C 10 cm below the surface. In Greece, soil solarization has been commercially adapted to control bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis* E.F. Smith) in greenhouse-grown tomato (Antoniou et al., 1995). In northern Florida, soil solarization decreased densities of *Phytophthora nicotianae* (Breda de Haan) and *P. solanacearum* to depths of 25 and 15 cm, respectively (Chellemi and Olson, 1994). Chellemi and Olson reported maximum temperatures in bare soil of 43.8, 38.9, and 36.5 °C and in solarized soil 49.5, 46.0, and 41.5 °C at depths of 5, 15, and 25 cm, respectively. Egley (1983) reported that solarization for one week reduced the numbers of viable prickly sida (*Sida spinosa* L.), common cocklebur (*Xanthium strumarium* Wallr.), velvetleaf (*Abutilon theophrasti* Medic.), and spurred anoda (*Anoda cristata* L.) seeds. Maximum temperature in this experiment at 1.3 cm soil depth reached 69 °C for 3 to 4 h in the mid-afternoon. This temperature did not eliminate dormant weed seed from the germination zone, but the treatment killed germinated seed which reduced the number of weed seedlings that otherwise would have emerged.

Biofumigation and solarization may be combined to improve efficacy. Qualitative and quantitative differences were found in volatiles released from cabbage (*Brassica oleracea* L.) incorporated into soil at temperatures typical of solarized soil (Gamliel and Stapleton, 1993). Also, this cabbage residue reduced propagules from *Pythium ultimum* and *Sclerotium rolfsii* by 95% when soil was heated, but no more than 25% without heat application. Heated soil amended with cabbage was found to contain alcohols, aldehydes, and ITCs in the soil air. Nonheated treatments contained methanethiol, ethanol, and occasionally acetic acid and methanol. In combination with biofumigation, solarization would increase the vapor pressure of compounds resulting in greater volatile release into the soil. Due to elevated volatile release, combining *Brassica* amendments with soil solarization can enhance the control of pathogens through the combination of thermal killing and the enhanced generation of toxic volatile compounds (Gamliel and Stapleton, 1997).

However, information about the influence of soil conditions (moisture, temperature, pH, texture) and microbes on ITC production from *Brassica* tissue is somewhat limited. AITC was found to be the predominant product formed by sinigrin (allyl GL) decomposition in soil or in ammonium acetate extracts from soils regard-

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less of soil characteristics (Borek et al.,1994). Morra and Kirkegaard (2002) reported that ITC release was greater in waterlogged fine sandy loam soil than in soil at –32 kPa .

The main objective of this research was to determine effects of soil texture, temperature, moisture, and pH on volatiles produced from the degradation of Indian mustard during simulated biofumigation. A second objective was to determine if the presence of soil microbes affected the volatiles released. Finally, the trapping effect of solarization was evaluated. No published research shows the relationship between the variables listed above in a comprehensive study that combines multiple environmental variables and uses one detection method. This information could be helpful in developing field sampling techniques as well as defining ideal field conditions for testing biofumigation and solarization systems for soil fumigation efficacy.

Materials and Methods

Experimental design. The experimental design was a balanced incomplete block with three replications with fractional factorial treatment assignments and repeated measures. The treatments were soil texture (clay loam and sandy loam), soil cover (covered with a teflon lid to simulate plastic mulch used in solarization, and noncovered), soil temperature (15, 30, and 45 °C), soil moisture (permanent wilting point, 60% field capacity, and field capacity), soil pH (soil adjusted to a pH of 7.0 and field pH), and soil sterilization (autoclaved and nonautoclaved soil). Repeated measures were taken at 0.25, 4, 8, and 24 h. Treatment combinations were obtained from the Optex procedure (SAS Institute, 1996). The experiment was conducted in 500-mL glass jars in darkness within incubation chambers that provided consistent environmental conditions. Relative humidity was maintained at 50% ± 10%.

Soils. Two soils were used, a Waynesboro clay loam (Fine, kaolinitic, thermic Typic Paleudults) and a Sequatchie fine sandy loam (Fine-loamy, siliceous, semiactive, thermic Humic Hapludults), both collected from the University of Tennessee Plant Science farm, Knoxville, Tenn. (Roberts et al., 1955). The soils were air dried at room temperature for approximately 40 h and sieved through a #20 mesh sieve. The soil textures were determined by a particle size analysis method using Stoke's law as described by Day (1965). A glass electrode pH meter was used to measure both deionized aqueous pH and $CaCl_2$ pH. Lime requirements for both soils were determined by the Adams and Evans (1962) method to determine the amount of lime required for the pH 7.0 treatment. Water retention curves were created for each soil using the pressure plate method by Klute (1986) to indicate the amount of water needed to attain field capacity, 60% field capacity, and permanent wilting point. Field capacity was assumed to be -33 kPa and permanent wilting point –1500 kPa. Soil moisture was maintained by adding water as jar weight decreased. Autoclaved soils were sterilized using the method described by Wolf and Skipper (1994). A sample of 350 ± 1 g of soil was placed in each 500-mL glass jar.

Plant material. Indian mustard plant introduction accession 458934 was acquired from the USDA–ARS Regional Plant Introduction Station, Ames, Iowa. Mustard used in this study was grown in the field at the University of Tennessee Plant Science Farm and harvested at the six- to eight-leaf growth stage.

Plant sample preparation. A consistent method for mustard tissue preparation was needed to ensure homogeneous mustard tissue introduction into the jar-soil system across replications and treatments. A method that involved freeze drying mustard tissue was determined to be the procedure that consistently yielded isothiocyanates in their respective ratios as compared to fresh materials (unpublished data). However, other volatiles such as alcohols and aldehydes were lost in this process. A similar method was used by Warton et al. (2001b) when preparing *Brassica* tissue for GL determination and ITC production following hydrolysis. Plant tissue was freeze dried (Labconco, Kansas City, Mo.) and homogenized manually, thereby ensuring that the same proportion of root, stem, and leaf tissue was added to each jar. To simulate tissue fresh weight of 3.15 g, 0.31 ± 0.01 g of freeze dried material was combined with 2.84 ± 0.01 mL of deionized water and mixed for 30 s. This amount of mustard was chosen based on an estimated total plant material that could be present per hectare if grown in the field (Duke, 1997). The mustard mixture was then thoroughly mixed with the soil in a plastic container for 30 s before being mixed into the soil within the jar.

Volatile sampling. Volatile samples were collected for 45 s by solid phase microextraction consisting of a fiber with a 100 µm polydimethylsiloxane (PDMS) coating (SPME; Supelco Bellefonte, Pa.). This coating functions by noncompetitive absorption and has a larger linear range than fibers that use competitive adsorption. A preliminary experiment indicated that this fiber was not saturated by AITC or CO_2 at detectable concentrations in this experiment. A Teflon tube (length 14.5cm., I.D. 6mm) was inserted through the soil to the bottom of every jar. Thirty-two 2-mm holes were drilled in each tube so that when inserted into the soil in the jar, the holes were 2.5 cm below the soil line, allowing volatiles in the soil air to diffuse into the tube. A septum through which the SPME sampler was inserted was placed into the top of each tube. For the cover treatments, a Teflon lid was installed and sealed with a threaded metal ring.

Chromatography. A Hewlett-Packard (HP) gas chromatograph (model 5890A Series II; Hewlett-Packard Co., Palo Alto, Calif.) using a fused silica EC-WAX capillary column (0.25 µm film thickness, 30 m × 0.25 mm ID; Alltech, Deerfield, Ill.) was connected to a HP 5972 mass selective detector (GC–MS). The chromatograph oven temperature was initially set to 60 °C, ramped 5 °C·min^{-1} to 150 °C, with the injector temperature at 200 °C. Allyl ITC, sec-butyl ITC, and 3-butenyl ITC were identified by comparing their mass spectra with published mass spectra (Ohashi et al., 1963). Carbon dioxide and AITC were identified by comparing their mass spectra to those of authentic standards.

The concentrations of AITC in the jars were calculated based on chromatographic peak areas in relation to standard curves. To generate the AITC standard curve, ethanol was added to 0.15 mL AITC (adjusted for 95% purity) for a 1 mL total volume. One microliter of the ethanol-AITC mixture was injected into a 500-mL jar to yield a concentration of 3.3 µmol·L^{-1} and sampled. The initial AITC mixture was then diluted for a total of five different concentrations. Concentrations of CO_2 were calculated based

Table 1. Least square means and standard errors for AITC concentrations for time, soil texture, soil temperature, soil cover and soil sterilization (autoclaved and nonautoclaved), aqueous soil pH, and soil moisture main treatment effects.[z]

	AITC concn (µmol·L^{-1})	
Treatment	Mean	SE
Time (h)		
0.25	0.80 a	0.07
4	0.88 a	0.07
8	0.67 b	0.07
24	0.41 c	0.07
Texture		
Clay	0.58 b	0.07
Sandy	0.80 a	0.07
Temperature (°C)		
15	0.48 b	0.09
30	0.71 ab	0.09
45	0.87 a	0.09
Soil cover		
Yes	1.05 a	0.09
No	0.32 b	0.09
Soil autoclaved		
Yes	1.06 a	0.07
No	0.32 b	0.07
Aqueous soil pH		
5.7	0.67 a	0.07
7.0	0.71 a	0.07
Soil moisture		
Field capacity	0.58 a	0.09
60% Field capacity	0.71 a	0.09
Permanent wilting point	0.78 a	0.09

[z]Means within a treatment with no common letter differ by LSD ($P \leq 0.05$).

on a standard curve made with 1%, 3%, and 5% standards. Jars with soil and Indian mustard were spiked with AITC and compared with standards in empty jars to determine recovery rates for both soils.

Statistical analysis. Analysis of variance was performed on all data for volatile analysis. The fractional factorial allowed all main and two-way interaction treatment effects to be tested. Lack of independence due to repeated measures was addressed with an autoregressive correlation structure. LSD mean separations ($P \leq 0.05$), converted to letter groups by the PDMIX612 macro (Saxton,1998), were used for interpretation of significant fixed effects as determined by the Mixed procedure (SAS Institute, 1996).

Results

Soil analysis. The clay loam textured soil contained 25% sand, 47% silt, and 28% clay. The sandy loam textured soil contained 68% sand, 23% silt, and 9% clay. The clay loam soil had an aqueous pH of 5.6 and a $CaCl_2$ pH of 5.1 while the sandy loam soil had an aqueous pH of 5.7 and a $CaCl_2$ pH of 5.6. Field capacity and permanent wilting point was 22% and 8.5% gravimetric water content, respectively, for the clay loam soil. The sandy loam soil had field capacity and permanent wilting point moistures of 14% and 4.5% gravimetric water content, respectively.

Volatile analysis. Volatile compounds detected by GC–MS included AITC, carbon dioxide (CO_2), sec-butyl ITC, and 3-butenyl ITC. All treatments and two-way interactions were analyzed. The only three-way and higher interactions analyzed were those involving time, due to limitations of the statistical model. No three-way interactions were significant at $P \leq 0.05$. Main treatment effects and two-way interactions for AITC and CO_2 are discussed. Significant factors for sec-butyl ITC and 3-butenyl ITC are not discussed; however, most of the trends for AITC were repeated in the sec-butyl ITC and 3-butenyl ITC results (data not shown).

Main treatment effects for AITC. The sampling time effect was significant for AITC concentration with 0.25 and 4 h treatments yielding at least 19% more AITC than the 8 h treatment, and 95% more than the 24 h treatment (Table 1). AITC concentration at 24 h was 48% lower than the mean concentration of all earlier sampling times. The sandy loam soil produced 38% higher AITC concentration than the clay loam soil. Temperature had a significant influence, resulting in an AITC concentration in 45°C soil that was 81% higher than in 15°C soil. The 15 and 30°C soil treatments were not significantly different, nor were the 30°C and the 45°C soil treatments. On a percentage basis, covering or autoclaving soil had the greatest influence on AITC concentration; covered soil had over three times the concentration of AITC that was measured in noncovered soil, and similarly, AITC concentration in autoclaved soil was over three times that measured in nonautoclaved soil. There were no main effect differences among soil moisture and pH treatments.

Treatment interactions for AITC. Time × temperature, soil sterilization (autoclaved or nonautoclaved) × cover, time × soil sterilization, temperature × soil sterilization, and time × cover were significant for AITC concentration at $P \leq 0.01$ (Tables 2 and 3). The time × temperature interaction data showed that the AITC concentration in the 15°C treatment was 0.44 μmol·L^{-1} at 0.25 h, and did not significantly change over time. The AITC concentration in the 30°C treatment was 0.85 μmol·L^{-1} at 0.25 h and did not change significantly until at 24 h, it decreased to 0.43 μmol·L^{-1}. The initial AITC concentration at 0.25 h (1.11 μmol·L^{-1}) in the 45°C treatment did not change significantly at

Table 2. Least square means and standard errors for AITC concentrations for time × temperature, time × pH, and soil sterilization (autoclaved and nonautoclaved) × cover treatment interactions.[z]

		AITC concn (μmol·L^{-1})	
Treatment		Mean	SE
Time (h)	Temperature (°C)		
0.25	15	0.44 fgh	0.11
0.25	30	0.85 bcd	0.11
0.25	45	1.11 ab	0.11
4	15	0.55 efg	0.11
4	30	0.91 abc	0.11
4	45	1.17 a	0.11
8	15	0.57 defg	0.11
8	30	0.67 def	0.11
8	45	0.76 cde	0.11
24	15	0.35 h	0.11
24	30	0.43 gh	0.11
24	45	0.45 fgh	0.11
Time	pH		
0.25	Limed	0.94 a	0.09
0.25	Field	0.66 cd	0.09
4	Limed	0.85 ac	0.09
4	Field	0.90 ab	0.09
8	Limed	0.67 bde	0.09
8	Field	0.66 cd	0.09
24	Limed	0.36 f	0.09
24	Field	0.46 ef	0.09
Soil autoclaved	Cover		
Yes	No	0.40 b	0.10
No	No	0.25 b	0.10
Yes	Yes	1.71 a	0.10
No	Yes	0.40 b	0.10

[z]Means within an interaction with no common letter differ by LSD ($P \leq 0.05$).

Table 3. Least square means and standard errors for AITC concentrations for time × soil sterilization (autoclaved and nonautoclaved), temperature × soil sterilization, and time × cover treatment interactions[z].

		AITC concn (μmol·L^{-1})	
Treatment		Mean	SE
Time (h)	Autoclaved		
0.25	Yes	0.93 c	0.09
0.25	No	0.67 d	0.09
4	Yes	1.39 a	0.09
4	No	0.36 e	0.09
8	Yes	1.13 b	0.09
8	No	0.20 ef	0.09
24	Yes	0.76 d	0.09
24	No	0.05 f	0.09
Temperature (°C)	Autoclaved		
15	Yes	0.63 b	0.12
15	No	0.32 bc	0.12
30	Yes	1.16 a	0.12
30	No	0.27 c	0.12
45	Yes	1.37 a	0.12
45	No	0.37 bc	0.12
Time (h)	Cover		
0.25	No	0.70 c	0.09
0.25	Yes	0.90 c	0.09
4	No	0.36 d	0.09
4	Yes	1.39 a	0.09
8	No	0.18 e	0.09
8	Yes	1.15 b	0.09
24	No	0.05 e	0.09
24	Yes	0.77 c	0.09

[z]Means within a treatment with no common letter differ by LSD ($P \leq 0.05$).

4 h, but decreased to 0.76 μmol·L^{-1} at 8 h and decreased again to 0.45 μmol·L^{-1} at 24 h.

The soil sterilization × cover interaction data showed that the autoclaved covered treatment had a higher concentration of AITC (1.71 μmol·L^{-1}) than the autoclaved noncovered, nonautoclaved covered, and the nonautoclaved noncovered soil treatments (0.40, 0.25, and 0.40 μmol·L^{-1}, respectively). The autoclaved soil treatment had a concentration of 0.93 μmol·L^{-1} at 0.25 h, which increased to 1.39 μmol·L^{-1} at 4 h, decreased to 1.13 μmol·L^{-1} at 8 h, and decreased again to 0.76 μmol·L^{-1} at 24 h. The nonautoclaved treatment had an AITC concentration of 0.67 μmol·L^{-1} at 0.25 h, which decreased to 0.36 μmol·L^{-1} at 4 h and reached its lowest level of 0.05 μmol·L^{-1} at 24 h. The autoclaved soil treatments had higher AITC concentrations as temperature increased, rising from 0.63 to1.37 μmol·L^{-1} as temperature increased from 15 to 45 °C. There were no significant differences between the nonautoclaved treatments at different soil temperatures.

The time × cover interaction data showed that the noncovered treatment had an AITC concentration of 0.70 μmol·L^{-1} at 0.25 h, which decreased to 0.36 μmol·L^{-1} at 4 h, then decreased again to 0.18 μmol·L^{-1} at 8 h. In the covered treatment, AITC concentration increased from 0.90 μmol·L^{-1} at 0.25 h to 1.39 μmol·L^{-1} at 4 h, then decreased to 1.15 μmol·L^{-1} at 8 h and 0.77 μmol·L^{-1} at 24 h.

Table 4. Least square means and standard errors of CO_2 (%) for time, temperature, and soil sterilization (autoclaved and nonautoclaved) treatment effects.[z]

Treatment	CO_2 concn (%)	
	Mean	SE
Time (h)		
0.25	1.74 c	0.30
4	2.27 bc	0.30
8	2.58 b	0.30
24	3.62 a	0.30
Temperature (°C)		
15	1.84 b	0.31
30	3.02 a	0.31
45	2.80 a	0.31
Soil autoclaved		
Yes	1.72 b	0.28
No	3.39 a	0.28
Soil covered		
Yes	3.53 a	0.28
No	1.57 b	0.28
Soil moisture		
Field capacity	2.51 a	0.31
60% Field capacity	2.82 a	0.31
Permanent wilting point	2.33 a	0.31
Aqueous soil pH		
5.7	2.53 a	0.28
7.0	2.58 a	0.28

[z]Means within a treatment with no common letter differ by LSD ($P \leq 0.05$).

Table 5. Least square means and standard errors for CO_2 (%) for soil sterilization (autoclaved and nonautoclaved) × cover, time × soil sterilization, and temperature × soil sterilization treatment interactions.[z]

Treatment		CO_2 concn (%)	
		Mean	SE
Soil autoclaved	Cover		
Yes	No	1.62 b	0.33
No	No	1.53 b	0.33
Yes	Yes	1.81 b	0.33
No	Yes	5.25 a	0.33
Time (h)	Autoclaved		
0.25	Yes	1.74 c	0.37
0.25	No	1.75 c	0.37
4	Yes	1.59 c	0.37
4	No	2.95 b	0.37
8	Yes	1.58 c	0.37
8	No	3.57 b	0.37
24	Yes	1.95 c	0.37
24	No	5.28 a	0.37
Temperature (°C)	Autoclaved		
15	Yes	1.79 b	0.39
15	No	1.90 b	0.39
30	Yes	1.65 b	0.39
30	No	4.38 a	0.39
45	Yes	1.71 b	0.39
45	No	3.90 a	0.39

[z]Means within an interaction with no common letter differ by LSD ($P \leq 0.05$).

Main treatment effects for CO_2. The sampling time effect was significant for CO_2 concentration. The 4, 8, and 24 h treatments had at least 30% more CO_2 than the 0.25 h treatment (Table 4). The CO_2 concentrations were similar at 4 and 8 h (2.27% and 2.58%, respectively) as well as 8 and 24 h (2.58% and 3.62%, respectively). The soil temperature effect was significant with the 30 and 45 °C treatments yielding at least 52% more CO_2 than the 15 °C soil treatment. Covered soil had more than twice the CO_2 measured in noncovered soil. Nonautoclaved soil produced 97% more CO_2 than autoclaved soil. There were no main effect differences for soil moisture, soil pH, and soil texture treatments.

Treatment interactions for CO_2. Soil sterilization × cover, time × soil sterilization, temperature × soil sterilization, and time × cover were significant for CO_2 production at $P \leq 0.01$ (Tables 5 and 6). The soil sterilization × cover data showed that the nonautoclaved covered soil treatment had about three times as much CO_2 as autoclaved covered, autoclaved noncovered, or nonautoclaved noncovered soil treatments. CO_2 concentrations in autoclaved treatments were stable across time, varying only from 1.6% to 2.0%. In the nonautoclaved treatments, however, CO_2 concentrations increased significantly from 1.8% at 0.25 h to 5.3% at 24 h. CO_2 concentrations did not vary with temperature in autoclaved soils, but in nonautoclaved soils, the CO_2 concentration at 15 °C was 1.9%, about half of that at 30°C (4.4%) and 45 °C (3.9%). In covered soil, CO_2 concentration increased with increasing sampling time, from 1.8% at 0.25 h to 5.5% at 24 h. In noncovered soil, CO_2 did not vary significantly with time and ranged from 1.5 to 1.7%.

Discussion

The AITC and 3-butenyl ITC detected by GC–MS are GL hydrolysis products similar to those detected by Vaughn and Boydston (1997). However, sec-butyl ITC, also detected within this experiment, was not detected by Vaughn and Boydston (1997) possibly due to differing sampling or tissue preparation techniques. Peterson et al. (2001) detected AITC, sec-butyl, and 3-butenyl ITC released from decomposing turnip rape (*Brassica napus* L.). In a study evaluating decomposition of *B. juncea* in soil, Bending and Lincoln (1999) detected AITC, but no other ITCs. Bending and Lincoln also detected methanethiol, dimethyl-sulphide, carbon-disulphide, and dimethyl-disulphide; methanethiol was the dominant headspace compound throughout their analyses, none of which we detected. Again, differences in volatiles detected are likely due to the tissue preparation method used in this experiment and/or differing sampling techniques. In addition, it is well documented that GL levels can vary within a species and between cultivars, which would lead to varying GL degradation products (Kirkegaard and Sarwar, 1998).

At the six-leaf stage, leaves and stems of Indian mustard PI 458934 had an allyl GL concentration of 31.8 μmol·g^{-1} and at flowering, 43.5 μmol·g^{-1} (data not shown). By adding 0.31 g of mustard, the maximum theoretical concentration

Table 6. Least square means and standard errors for CO_2 (%) for time × cover treatment interactions.[z]

Treatment		CO_2 concn (%)	
		Mean	SE
Time (h)	Cover		
0.25	No	1.66 c	0.37
0.25	Yes	1.83 c	0.37
4	No	1.46 c	0.37
4	Yes	3.09 b	0.37
8	No	1.49 c	0.37
8	Yes	3.68 b	0.37
24	No	1.71 c	0.37
24	Yes	5.53 a	0.37

[z]Means within an interaction with no common letter differ by LSD ($P \leq 0.05$).

of AITC in the 0.5-L jar would have been 23 μmol·L^{-1}. The range of AITC recovery for most experiments was between 1.0% and 7.4% of this theoretical concentration. The recovery rate calculated by using AITC spiked soil samples yielded <0.1% of the theoretical AITC yield for both soils, perhaps since AITC standard does not undergo a hydrolysis process compared to AITC evolving from sinigrin in plant tissue. Warton et al. (2001b) found that the molar amount of ITC produced by hydrolysis is lower than the amount of corresponding GL present in plant tissue, possibly due to incomplete hydrolysis. Morra and Kirkegaard (2002) found that 1% or less of the ITC predicted from tissue GL concentration was measured in soil amended with rapeseed or mustard. The Indian mustard in this study was finely ground after freeze drying. Since GLs and myrosinase are stored in separate vacuoles within the plant cell, freeze drying and subsequent grinding could allow these reagents to react more efficiently than during normal plant tissue decay. Because this study used homogenized freeze dried tissue, we effectively reduced the inherent variability that would have been introduced using fresh tissue and were able to evaluate treatment effects that otherwise would have been nondetectable.

Our results revealed that AITC concentration generally decreased with increasing time. This result is similar to those found by Borek et al. (1994), Brown et al. (1991, 1994), Morra and Kirkegaard (2002), and Peterson et al. (2001) and supports the knowledge that activity of ITCs on soil organisms and seeds is very short in duration.

AITC concentration was also related to soil texture. AITC levels were higher in the sandy loam soil compared to the clay loam soil. Similarly, Bending and Lincoln (1999) found that gas phase AITC concentrations in a clay loam soil were lower than in a sandy loam soil. This result may be due to the higher organic carbon content of clay loam soil to which AITC could adsorb or react. Soil adsorption of methyl ITC (MITC), a similar compound to AITC, has been shown to increase with increasing organic matter (Matthiessen et al., 1996; Smelt and Leistra, 1974). Since adsorption of AITC to soil constituents decreases AITC gas phase concentration, biofumigation in soils with high clay content may be less effective compared to sandy textured soils. Also, diffusion may be slower in clay soil due to smaller pore space and increased tortuosity.

Soil temperature influences AITC concentration. The partitioning of ITCs into phases of the three-phase soil system (vapor, aqueous, and solid) is dependent on their solubility in water (Brown and Morra, 1997). For MITC, 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; Van den Berg et al., 1999). However, soil temperature was shown not to influence soil sorption of MITC (Matthiessen et al., 1996). Because we found that soil temperature influenced AITC concentrations detected, the rate of partitioning of AITC between the vapor and aqueous phases was likely the most influential factor determining the rate of AITC volatilization. AITC escape from the aqueous phase may be enhanced by higher temperatures due to higher kinetic energies.

Also, higher concentrations of AITC were detected within covered treatments, probably due to reduced diffusion from the soil. Because solarization systems increase soil temperatures, both trapping and higher AITC vaporization effects would likely be realized.

AITC levels tended to be higher in autoclaved soil than nonautoclaved soil. Microbial degradation of AITC in the nonautoclaved soil probably accounted for lower levels of AITC. Although Borek et al. (1995) determined that autoclaved soil treatments did not change disappearance rates of AITC, Warton et al. (2001a) documented enhanced biodegradation by microbes of MITC in soils where metham sodium had been extensively used compared to soils with no metham sodium history. *Rhodococcus* spp. and *Bacillus* spp. were both implicated in the enhanced biodegradation of MITC. Warton et al. (2003) demonstrated that AITC degraded more rapidly in soil that also degraded MITC at higher rates than in nondegrading soil, perhaps as a consequence of the structural similarity of AITC and MITC.

Our results did not show differences in AITC concentrations among soil moisture treatments. Morra and Kirkegaard (2002) found that extractable ITC concentrations were greater in waterlogged fine sandy loam soil as compared to soil at –32 kPa. They cited increased water availability for glucosinolate hydrolysis as one reason for this result. Additionally, their technique of water-saturating the soil and tightly capping the tubes containing the soil prevented the partitioning of AITC into the gas phase, and therefore would have increased extractable ITCs compared to soil with free pore space. Our data indicates that gas phase ITC concentration does not vary significantly with soil moisture content at or less than field capacity. Our results concerning the effects of soil pH agreed with those of Borek et al. (1995) who found no correlation between AITC concentration and soil pH (ranging from 4.35 to 9.10) in six soils.

Because our sampling technique allowed us to measure CO_2, we were able to evaluate the influences of time, soil temperature, soil sterilization, and soil cover on CO_2 concentration. CO_2 concentration increased with increasing time. Similar results were reported by Reicosky et al. (1999) following soil disturbance. Because *Brassica* tissue used in this study was mixed thoroughly into the soil, it is not surprising that we observed a similar trend.

Relatively lower soil temperatures resulted in lower CO_2 concentrations in this study. This result may be attributed to lower respiration or organic carbon decomposition by soil microbes. Similar results were observed by Torbert et al. (2001) who showed that CO_2 emission was 32% higher in 30°C soil compared to 20 °C soil over a 30 d period and was 42% higher after 60 d.

The CO_2 concentration in covered soil was twice that obtained in noncovered soils, probably because CO_2 could not diffuse out of the covered jars. Covering soil immediately after incorporating *Brassica* tissue could be of particular importance because Reicosky et al. (1999) showed that soil disturbance greatly increases CO_2 emissions from soil due to enhanced biological activity. Enhanced soil biological activity was shown to be due to increases in soil temperature as well as oxygen available to soil microbes. Since biofumigation systems using *Brassica* tissue rely mainly on tillage to incorporate residue, covering the soil may reduce the amount of oxygen available to microbes that may use ITCs as a carbon source. However, the subsequent increase in soil temperature could enhance microbial activity which may in turn cause ITC concentrations to decrease. An alternative nontillage biofumigation method that uses a rotary tiller (Peterson et al., 2001) or rolling stalk chopper such as the types used by many Brazilian growers (Derpsch et al., 1991; Raper et al., 2003), or use of a membrane-disrupting herbicide such as paraquat to desiccate *Brassica* tissue before trapping with plastic, would reduce the amount of soil disturbance before planting and may prevent increased microbial activity.

The lower CO_2 concentrations measured in autoclaved compare to nonautoclaved soils indicate that at least half of CO_2 evolved was likely from microbial activity. Also, concentrations of AITC were much higher in autoclaved than in nonautoclaved soils. Lower AITC in nonautoclaved soils may be due to soil microorganisms using AITC as a carbon source.

Soil water content did not influence CO_2 concentration in this study. This result differs from that observed by Prior et al. (1997) who showed that CO_2 evolution initially increased under lower soil moisture potentials. Again, difference in results may be due to differing soil chemistry or microbiology. Lastly, in agreement with Torbert (1995), soil texture did not influence CO_2 concentration.

These data in summary suggest that higher concentrations of volatile AITC may be obtained in biofumigation at higher soil temperature.

Sandy loam textured soils may yield higher volatile AITC concentrations compared to clay loam soils. By covering biofumigated soils, maximum AITC concentrations would be maintained. This research also indicates that soil microbes play a role in the degradation of AITC. Solid phase microextraction is a viable method for detecting ITCs and likely for field work evaluating GL breakdown products.

Biofumigation and solarization may be environmentally sound and economically feasible alternatives for methyl bromide. One difficulty in replacing such a widely used pesticide with a biofumigation system is that insufficient field research has been conducted to quantify the effects of *Brassica* amendments on crop yield and the soil microbial community. The results in this experiment could help in the development of field scale experiments and methods for volatile monitoring, and subsequently, field plans for growers to use biofumigation as part of a program to control soilborne pathogens.

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