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Assessing Natural Isothiocyanate Air Emissions after Field Incorporation of Mustard Cover Crop

D. Trott · J. LePage · V. R. Hebert

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Abstract A regional air assessment was performed to characterize volatile natural isothiocyanate (NITC) compounds in air during soil incorporation of mustard cover crops in Washington State. Field air sampling and analytical methods were developed specific to three NITCs known to be present in air at appreciable concentrations during/after field incorporation. The maximum observed concentrations in air for the allyl, benzyl, and phenethyl isothiocyanates were respectively 188, 6.1, and 0.7 μg m^{-3} during mustard incorporation. Based on limited inhalation toxicity information, airborne NITC concentrations did not appear to pose an acute human inhalation exposure concern to field operators and bystanders.

Keywords Biofumigation · NITC · Air quality · Inhalation exposure

In Washington State, an increasingly common practice is to incorporate mustard ground cover (*Brassicaceae*) in rotational patterns with crops such as wheat and potatoes (McGuire 2003). These green manures provide nutrients and organic matter to improve soil tillage. Many of the *Brassicaceae* also have the advantage of emitting volatile sulfur-based allelotoxins, notably isothiocyanates, as a localized de novo response to plant tissue injury from predation or cultivation (Bones and Rossiter 1996; Rosa et al. 1997). Many of these naturally occurring isothiocyanates (NITCs) have biofumigation activity effective to a wide range of soil-borne plant pathogens and nematodes (Kirkegaard and Sarwar 1998; Brown and Morra 1997). NITC biofumigation has been receiving investigative attention as a biological-based integrated pest management alternative to replace or reduce the need for chemical fumigation (Davis et al. 1996; Bending and Lincoln 1999; Olivier et al. 1999; Bhat and Subbareo 2001; Ochiai et al. 2007). There have been concerns, however, that NITC air concentrations can pose an acute inhalation hazard to field operators and bystanders both during and shortly after cover crop soil incorporation. As a group, isothiocyanates are mucous membrane irritants with toxicity associated with increased vapor pressure and inversely with greater structural complexity (Mithen 2001). To address this concern, aliphatic and aromatic NITCs were assessed in air before, during, and 4-days post soil incorporation from a common mixture of oriental and yellow mustard used in Washington State to acquire baseline inhalation exposure data. The specific NITCs assessed in this air assessment were the allyl (AITC), aromatic benzyl (BITC), and 2-phenethyl isothiocyanate (PEITC; Table 1).

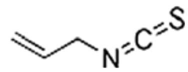
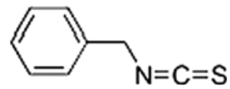
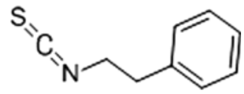
The volatile aliphatic isothiocyanate AITC (commonly known as oil of mustard seed) has been shown to have greater biological activity under field conditions compared to the structurally more complex aromatic NITCs (Matthiessen and Kirkegaard 2006). The simplest and most volatile NITC, methyl isothiocyanate (MITC), has been isolated in the *Capparaceae* (Fenwick et al. 1982) but has not been found in the *Brassicaceae* (Fahey et al. 2001).

Isothiocyanate air emissions resulting from cover crop field incorporation have not been studied to our knowledge,

D. Trott
Environmental Sciences Program,
Washington State University-Tri Cities,
Richland, WA, USA

J. LePage · V. R. Hebert (✉)
Food and Environmental Quality Laboratory,
Department of Entomology, Washington State
University-Tri Cities, Richland, WA 99354, USA
e-mail: vhebert@tricity.wsu.edu

Table 1 Naturally occurring isothiocyanates

Substance	Structure	CAS	Formula
2-Propenyl isothiocyanate (Allyl isothiocyanate)		57-06-7	C_4H_5NS
Benzyl isothiocyanate		622-78-6	C_8H_7NS
2-Phenethyl isothiocyanate (Phenethyl isothiocyanate)		2257-09-2	C_9H_9NS

nor are inhalation toxicity data readily available for NITCs of greater complexity than AITC. Much of the research characterizing isothiocyanate release from *Brassicaceae* has been performed under laboratory conditions with macerated plant tissue. Observed volatile emissions may, therefore, not be reflective of actual agricultural field conditions. Our intent in conducting this single-field assessment is to provide air concentration data to better understand if the potential exists for acute human inhalation risks to field operators and bystanders from exposure to naturally occurring isothiocyanates during and shortly after mustard cover crop incorporation. Attention was given specifically to monitoring AITC, BITC and PEITC concentrations in air since these isothiocyanates have been previously reported in appreciable gas-phase concentrations after mustard tissue injury (Brown and Morra 1997; Smolinska and Horbowicz 1999).

Materials and Methods

NITC air emission evaluations were conducted on a 102-acre center pivot-irrigated circle in Benton County, Washington starting October 25, 2008. This field was seeded in the summer with a mixture of oriental (*Brassica juncea*) and yellow mustard (*Sinapis alba*) and routinely irrigated. At maturity (late flowering stage), the mustard was systematically flail chopped by tractor followed by disking within approximately 20-min. Sufficient residue of mustard was left on the field to minimize surface erosion. Before field incorporation, four activated charcoal air sampling masts (receptors) were positioned at ca. 90° and ca. 10–20 m from the crop circle perimeter. A fifth receptor with co-located charcoal cartridges was positioned at the center of the circle. The four perimeter receptors consisted of a single SKC air sampling unit (SKC Model 224-PCXR8) placed at the base of a ring-stand with a vertical crossbar set at 1 m above ground. The 600 mg activated charcoal-filled glass cartridges were horizontally positioned on the crossbar and shielded from ultraviolet light and precipitation using a 1" PVC sleeve. The receptors were operated at an air flow rate of ca. 2 L min^{-1} for intervals of approximately 4 h on the day before mustard incorporation (−1 day), on the day of incorporation during the first 1/4 of field disking (A1), during the last 1/4 completion of disking (A2), immediately post disking (0), and once daily for 4-days following the green manure incorporation. Air sampling was routinely performed during the warmest daytime period of the day, averaging 12.5°C but ranging from 4 to 19°C during the sampling intervals.

At the start and end of all sampling collections, air flow measurements were taken and recorded. At the end of each sampling period, the collected charcoal cartridges were placed on blue ice and immediately taken to the Washington State University-Food and Environmental Quality Laboratory where they were stored at −80°C until analysis. Wind speed and direction data together with precipitation data was gathered from the WSU-AgWeatherNet weather station located 3 miles from the study site in Benton City, WA. A Hobo weather station was sited near the field as well for comparison. A trip blank was routinely shipped and stored with each set of air samples and later extracted with the sample set. On the last day of the study, a mixed AITC-BITC-PEITC standard was fortified to a charcoal cartridge and accompanied the samples to and from the field. This spiked trip spike was treated in a similar manner, stored, and extracted/analyzed as part of the data set. To assess trapping efficiency and potential breakthrough, a field spiked fortification set, including non-fortified charcoal cartridge controls, was performed under similar field-air sampling conditions outdoors at the WSU Tri-Cities campus located 14 miles from the field location. A storage stability study for the three examined NITCs was conducted over 408 days beginning February 2008.

The analytical method for charcoal extraction/quantitation of AITC, BITC, and PEITC was modified based on a regulatory procedure used for MITC (California Department of Pesticide Regulations 1994). The exposed charcoal cartridges were solvent extracted using a 1:1 mixture of carbon disulfide: ethyl acetate (i.e., 50% carbon disulfide in ethyl acetate) followed by sonication. The extract was filtered through a 0.45 µm Whatman® Teflon® membrane, then vialed for gas chromatography determination. A Varian Star 3400CX gas chromatograph (Walnut Creek, CA) using thermionic-specific detection (TSD) with an 8200CX Autosampler was employed for residue detection and quantification. An EC-Wax chromatographic column, 15 m × 0.53 mm, 1.2 µm-film-thickness (AllTech), was used in analyte separation. Ultrapure helium at ca 2–4 mL min^{-1} served as the carrier gas. The initial column temperature of 55°C was ramped to 175°C by 20°C min^{-1} increments, then to 225°C by 15°C min^{-1} increments, and held for 5 min. The injector temperature was increased from an initial 55–225°C and held for the entire run, and the injection volume was 1 µL. The hydrogen, air and makeup gas flows were set at 3–4 mL min^{-1}, ca. 170 mL min^{-1}, and 25–30 mL min^{-1}, respectively. Retention times for AITC, BITC and PEITC were approximately 4.6, 9.6 and 10.4 min, respectively and were verified against reference standards (Sigma Aldrich). The analytical method was validated in triplicate at three fortification levels covering the range of anticipated NITC concentrations in air. Stock solutions were prepared from reference solutions to use for field spiking and working standards in appropriate dilutions. Concentrations of the selected NITCs from solvent extracted charcoal cartridges were calculated by linear regression using a spreadsheet program (Microsoft Excel®) from at least four external standards, and external calibration standards bracketed for every 2 or 3 samples in the analytical set.

Results and Discussion

The analytical method for the measurement of the selected NITCs in receptor samples was found to be rugged with a method limit of quantitation (mLOQ) of 0.5 µg m^{-3} for AITC, BITC and PEITC. Laboratory fortification percent recoveries run with each set of solvent extracted field samples ranged from 84% to 118% for AITC, 73%–97% for BITC, and 77%–111% for PEITC. All combined laboratory method recoveries fell within 10% standard deviation. Linearity as measured by linear regression correlation (R^2) of a minimum of four standards among the range of encountered air concentrations for all analytical sets was ≥0.98.

For field fortification determinations, air was sampled at a rate of 2 L min^{-1} for ca. 6 h outside the WSU-Tri Cities facility, the longest field sampling time before cartridge collection. The recovery results from field-spiked fortifications (n = 2) ranged from 89% to 93% (AITC), 80%–86% (BITC), and 86%–92% (PEITC). The maximum storage interval to solvent extraction/GC determination was 149 days. A stability study conducted after 408 days frozen storage indicates good stability for AITC and PEITC (89% for each NITC). However, BITC appeared to be less stable with 64% recovery. The NITC air concentration data were not adjusted for possible losses during frozen storage.

Over the field sampling period, the ca. 4-h time weighted average (TWA) NITC air concentrations, particularly for the AITC, were observed above the mLOQ during and shortly after completion of field operations. The highest observed NITC concentrations occurred within the center of the crop circle during tractor flail-chopping and disking with observed maximum receptor air concentrations of 188, 6.1, and 0.7 µg m^{-3}, respectively, for AITC, BITC, and PEITC. Measured NITC emissions sharply attenuated after completion of field mustard incorporation and were below quantifiable levels at all receptors through the succeeding 3 days. The observation of peak NITC concentrations during green manure incorporation was anticipated since NITC production is enzymatically catalyzed on tissue injury (Fenwick et al. 1982; Rosa et al. 1997; Rask et al. 2000; Fahey et al. 2001). Moreover, it was not surprising to observe higher AITC receptor concentrations relative to BITC, and PEITC. At 25°C, the aliphatic allyl isothiocyanate and the aromatic benzyl-and 2-phenylethyl isothiocyanates are respectively 5, 1,700 and 2,300 times less volatile than MITC (Boublik et al. 1984).

Substantial variation (ca. 12-fold) was observed among the two cartridges that were co-located at Site 5 (center pivot) during monitoring period A1 (Fig. 1). This was the period where we observed the highest concentrations for all NITCs. Pump variability cannot explain this difference in measured NITC concentrations. What may be a source of variation was orientation of the adsorbent cartridges on the receptor mast. The cartridges air in-take was at ca. 90° orientation to each other. Wind direction may have been a contributing factor in the discrepancy of air concentrations among these two cartridges. One possible explanation for this disparity among samples may lie in the activity around the sample sites that affected air flows, such as would occur from tractors passing close to the sample. On all other sampling events (−1, A2, 0, 1, 2, 3, and 4-days post green mustard incorporation), the air concentrations from these two co-located samples were in agreement. The weather on the last day of air sampling changed from the warm and breezy weather pattern that held from the beginning of the study, to a clouded sky and light precipitation (0.25 mm). This light precipitation coincided with a reoccurrence of AITC at all receptor locations at levels above the mLOQ (Fig. 1). Higher surface moisture on the 4th day likely enhanced emission of NITCs from previously unhydrolyzed glucosinolates in surface mustard residues and at the

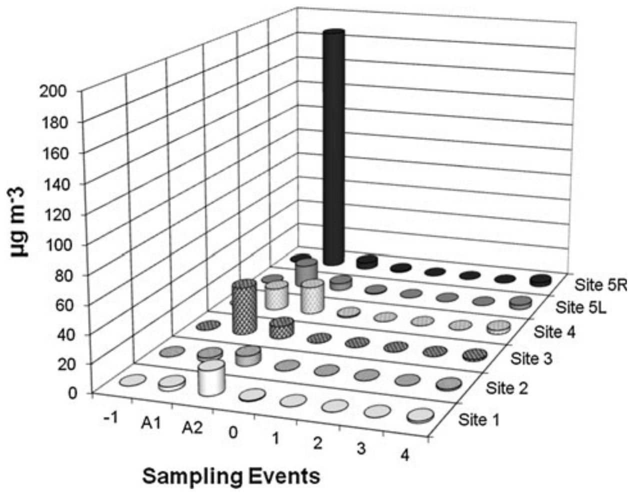


Fig. 1 Allyl isothiocyanate field receptor air concentrations

immediate soil surface. The reoccurrence of NITC emissions was particularly evident for the more volatile AITC. Since air monitoring was not continuously performed, the observed within and near-field emissions may not represent the highest NITC air concentrations that can be encountered during and after mustard green manure field incorporation. Moreover, appreciable variation in NITC concentrations should be anticipated given different weather and soil moisture conditions at mustard incorporation.

Of the above three NITCs assayed in air, toxicity information is only available for AITC. Indirect estimates from experimentally derived oral and dermal lethal dose 50% (LD_{50}) animal toxicological studies have been developed for AITC by the Department of Energy for setting Temporary Emergency Exposure Limits (TEELs, Department of Energy 2008). The highest measured AITC concentration (188 µg m^{-3}, see Fig. 1) taken during flail chopping/disking operations was sevenfold below an estimated threshold concentration of 1.4 mg m^{-3}. This TEEL threshold was established to represent a concentration in air below which most people should experience no appreciable risk or health effect.

Based on TEELs, this single field assessment indicates concentrations in air for the most volatile mustard NITC, AITC, does not pose an appreciable human health inhalation hazard to occupational workers or bystanders during the warmest part of the day of field operations. Since reported values were 4-h time-weighted averaged concentrations, maximum shorter term exposure concentrations would have to appreciably greater to trigger a concern based on established TEEL values. The reliability, however, for using these evacuation-based estimates for acute human inhalation health risk assessment should be viewed with reservation since they are derived animal lethal dose values from other routes of exposure. Moreover, this single regional study should be viewed as providing preliminary inhalation exposure data since NITC concentrations will likely vary appreciably in different growing regions and under different growing conditions.

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