

SOIL BEHAVIOUR OF SULFUR NATURAL FUMIGANTS USED AS METHYL BROMIDE SUBSTITUTES

INGRID ARNAULT, NATHALIE MONDY,
SABINE DIWO and JACQUES AUGER*

*University François Rabelais, IRBI UMR CNRS 6035 and CRITT Innophyt,
Parc de Grandmont, 37200 Tours, France*

(Received 29 September 2002; In final form 3 April 2003)

Methyl bromide is the most widely used and most effective fumigant and is used extensively for soil fumigation. According to the Montreal Protocol of 1991, methyl bromide is categorised as an ozone-depleting chemical and its use is prohibited from 2005.

Many substitutes, such as methyl isothiocyanate and methyl iodide, are not applied as widely as methyl bromide. Moreover, crushed *Allium* spp. plants (garlic, leek and onion) produce thiosulfates (Ti, R–S–SO–R') and related compounds like disulfides, which have the same pesticide activity as methyl bromide. Therefore *Allium* tissues or extracts can be used in biological control and Integrated Pest Management in agriculture.

The successful application of these compounds, and *Allium* tissues and extracts, for biological soil disinfection requires more specific knowledge regarding their subsequent fate in the soil. To obtain this, appropriate analytical methods using IR spectroscopy, SPME (solid-phase micro-extraction) and GC-MS techniques were developed and applied in the laboratory on pure compounds and on *Allium* tissues or extracts.

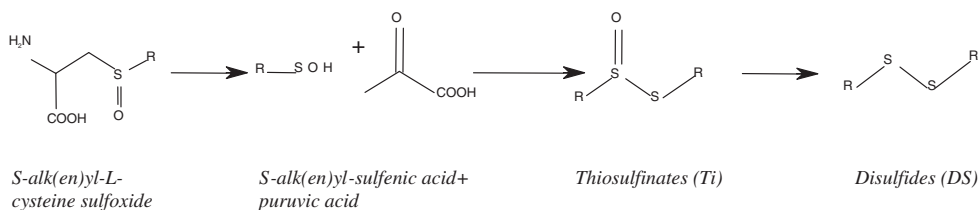
The experiments revealed that thiosulfates are stable in the atmosphere but in soil they are rapidly degraded into disulfides, which are very stable in soil. For that reason and for their general pesticide effect, disulfides are a promising alternative to methyl bromide.

Keywords: Thiosulfates; Disulfides; *Allium*; Soil fumigant; GC-MS; IR

INTRODUCTION

The characteristic flavours and biological activities of *Allium* species are attributable to thiosulfates and related organosulfur compounds formed enzymatically from odourless precursors [1]. When the plant is crushed or cut, intermediates (alk(en)yl sulfenic acids) rearrange rapidly to unstable thiosulfates, Tis (Fig. 1). This enzymatic reaction is different in onion. In fact, 1-propenyl sulfenic acid gives mainly thiopropenal-S-oxide, the lachrymatory factor (LF), and zwiebelanes (Zws), isomers of

*Corresponding author. Fax: +33-0247-367389. E-mail: auger@univ-tours.fr



R=Trans-1-propenyl, allyl, methyl, propyl

FIGURE 1 Biosynthesis of disulfides via *S-alk(en)yl* cysteine sulfoxide in *Allium* spp.

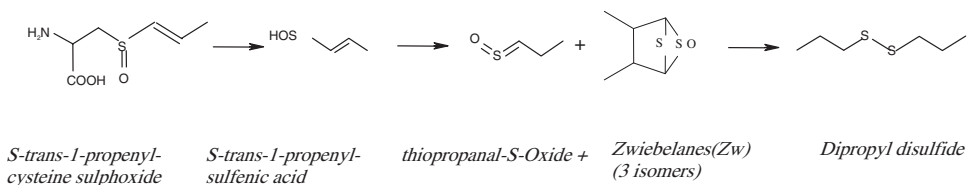


FIGURE 2 Biosynthesis of dipropyl disulfide via *S-trans-1-propenyl* cysteine sulfoxide in onion.

TABLE I Abbreviations of Tis, Zws and DSs found in *Allium cepa* (onion) and *Allium porrum* (leek)

Compound	Abbreviation	Name
1	DMTi	Dimethyl thiosulfinate
2	DPTi	Dipropyl thiosulfinate
3	Zw1	Zwiebelane isomer 1
4	Zw2	Zwiebelane isomer 2
5	Zw3	Zwiebelane isomer 3
6	MPDS	Methyl propyl disulfide
7	DPDS	Dipropyl disulfide
8	PPeDS	Propyl propenyl disulfide
9	DPeDs	Dipropenyl disulfide
10	DMDS	Dimethyl disulfide
11	DADS	Diallyl disulfide
12	DATi	Diallyl thiosulfinate

di(propenyl)Ti [2]. We have recently proposed a dual GC-MS method to quantify all these compounds [3]. The degradation of Tis and Zws gives alk(en)yl polysulfides, mainly disulfides, DSs (Fig. 2).

The major degradation products of leek and onion are dipropyl disulfide (DPDS) and dipropenyl disulfide (DPeDS) whereas garlic releases diallyl disulfide (DADS) (Table I). Dimethyl disulfide (DMDS) is also produced by onion and garlic but it is mainly present as a degradation product of crucifers (cabbage, radish) [4,5] and in cooked foods such as coffee [6] and cheese [7].

The toxicity of DSs is equal to methyl bromide for several species of microorganisms, nematodes and insects, with a better sensitivity for lepidopterans and hymenopterans than coleopterans, while Tis are even more toxic than DSs [8] (Table II). Many commercial garlic extracts which contain several polysulfides are used as repellent compounds and insecticides. Furthermore, the biocide effect of crucifers incorporated in

TABLE II Lethal dose (LD₅₀ in 24 h in mg/L) of Ti and corresponding DS

<i>Insect</i>	<i>DMDS</i>	<i>DADS</i>	<i>DMTi</i>	<i>DATi</i>
<i>Ephestia kuehniella</i>	0.2	0.02	0.04	0.02
<i>Bruchidus atrolineatus</i>	0.2	0.6	0.15	0.18
<i>Callosobruchus maculatus</i>	1.1	0.5	0.25	0.16

soil qualified this green manure as a “biofumigant” [9] and many papers reported a fungicidal effect on several pathogens [10,11]. Bending and Lincoln [12] have observed by solid-phase micro-extraction (SPME)-GC-FID that the biofumigant properties of crucifer tissues come from isothiocyanates and DMDS.

Our purpose in this study was to develop an IR and GC-MS method to observe the behaviour in soil of sulfur compounds produced by *Allium* spp. and measure the kinetics of the concentration gradient of these volatiles at different depths and above the soil.

EXPERIMENTAL

Plant Material and Chemicals

Freshly cultivated *Allium* plants originating from organic farming were purchased from a local producer. DMDS, DPDS and DADS were from Sigma (St. Louis, USA) and distilled in the laboratory. Tis were synthesised by oxidation of corresponding DSs with peracetic acid or *meta*-chloro-peroxybenzoic acid (PCPBA) according to the procedure of Cavallito *et al.* [13] and Iberl *et al.* [14].

Instruments and Methods

Based on our previous work [3], GC-MS analysis was carried out on a benchtop Perkin-Elmer Turbomass (Shelton, USA) system with a split-splitless injector and a fused-silica capillary column (10 m × 0.32 mm) with a 4 μm methyl silicone coating. The carrier gas was helium (99.999%) at 3.5 mL/min and the column temperature program was 5°C/min from 70 to 250°C. The injection port temperature was 200°C. The transfer line and the source temperature were maintained at 150°C. Total ion chromatograms and mass spectra were recorded in the electron impact ionisation mode at 70 eV.

The compounds were identified by their mass spectra and retention time.

IR spectra were recorded using a Bio-Rad Digilab, FTS 45A spectrometer equipped with a gas cell of 20 m optical path (Cambridge, MA) according to our previous work [15,16].

Sampling

Allium plants were crushed and the juices were buried in different concentrations at various depths in a can filled with soil. In the same way synthetic liquid sulfur compounds were introduced into soil. These experiments had two objectives: the first to check the presence of Tis and the second to measure the level of DSs; experiments were conducted in soil and in the atmosphere above the soil surface.

We recently reported that the GC analysis of Tis requires an extraction step with an organic solvent as these thermolabile compounds are degraded by the SPME method [3]. We therefore extracted a soil sample with diethyl ether and injected the solution directly into the GC. Then, when no more Tis were present we could extract DSs directly by SPME, which is a very convenient method for kinetic studies. When Tis were present in the atmosphere, we took an atmospheric sample with a gas syringe and analysed it by IR spectroscopy in a gas cell at 1085 cm^{-1} [15].

For SPME, the best extraction of sulfur compounds is offered by a $75\text{ }\mu\text{m}$ carboxen/polydimethylsiloxane fibre [12,17], inserted in the core for 25 min.

RESULTS AND DISCUSSION

Experiments with Pure Tis

Results of Soil Analysis

IR and liquid extraction-GC-MS analysis showed that Tis are totally transformed to DSs, other polysulfides and thiosulfonates (Tos) in about six hours in the soil.

For instance, Fig. 3(B) presents the IR spectra of pure DPTi with the band at 1085 cm^{-1} which is the SO band absorption. Figure 3(A) illustrates the degradation of DPTi after 6 h in soil with the disappearance of the band at 1085 cm^{-1} and the appearance of two SO_2 bands at 1130 and 1320 cm^{-1} from Tos. It is well known that among several mechanisms of degradation, disproportionation of pure Ti leads to a DS and a To.

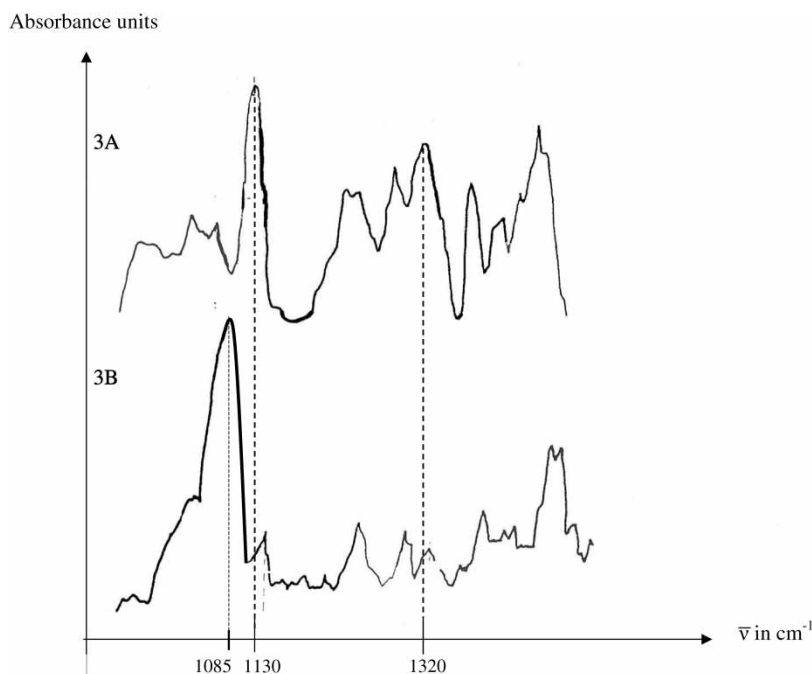


FIGURE 3 IR spectra of (A) pure DPTi and (B) DPTi partially disproportionated.

Results of Atmospheric Analysis

IR analysis allows us to observe the presence of Tis with DSs and other metabolites produced by degradation in soil. The greater the depth at which Tis are incorporated, the more they are degraded in the soil and the less important is the amount of residual Tis found in the atmosphere above the soil.

Experiment with Pure DSs

Results of Soil Analysis

Soil liquid-extraction-GC-MS analysis showed no DS metabolite and demonstrated their long lifespan in soil. Experiments with DPDS showed that this product seems to migrate quickly in soil. Initially, the concentration decreases slowly with depth but it becomes homogeneous in the soil profile within 24 h.

Results of Atmospheric Analysis

Initially, the linearity of the SPME fibre was tested during the experiment for the reference compounds DMDS, DPDS and DADS in the range 1–5 mg and the correlation coefficients (R^2) were 0.9815, 0.9999 and 0.9979, respectively.

SPME-GC-MS analysis showed that DSs are progressively absorbed in soil to a great depth. Absorption of DPDS in soil at the concentration of 64 $\mu\text{g/g}$ of soil is shown in Fig. 4: upto 28% of DPDS was progressively absorbed in 1 day but 2% of the initial amount of DSs remain in the atmosphere above for 7 days. Thereafter, we did not find any metabolites. Nevertheless, the DMDS reaction with radical OH^\bullet is reported in many papers [18,19] to lead to degradation products such as methane sulfonic acid (MSA) and SO_2 . However no such metabolites were detected with our method.

In Water

Ramakrishnan *et al.* [20] studied the persistence in water of DADS, the major component providing insecticidal activity to garlic oil, and observed that the

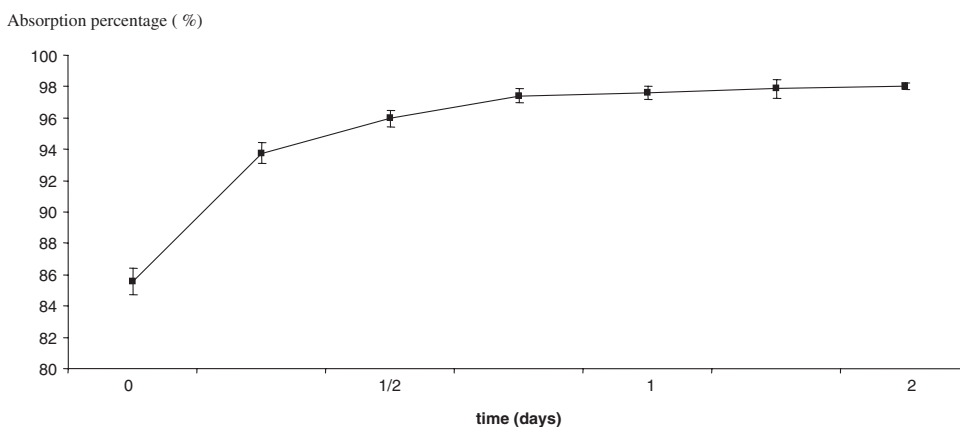


FIGURE 4 Absorption percentage (%) of pure DPDS in soil with time (days). Four measurements were used to calculate means.

insecticidal effect is depleted by 84% in 24 h. This loss of effect is probably due to a conversion of DADS from the liquid phase into the gas phase as DSs are very stable in an aqueous medium.

Experiments with *Allium* Tissues or Extracts

Results of Soil Analysis

No report exists on the degradation of *Allium* tissue extracts in soil. However, Coventry *et al.* [21] studied the effect of composted onion waste on destroying any sclerotia in order to disinfect the soil. They detected methyl mercaptan, *n*-propyl mercaptan and DPDS in onion waste and our results are similar. Several papers also report the metabolism of crucifer tissues during degradation in soil. In crucifers, some of the sulfur compounds are produced from methyl cysteine sulphoxide by the same enzymatic reaction as *Allium* species via DMTi [5]. Bending and Lincoln [12] found DMDS in soil amended by crucifer. Chin and Lindsay [22] also investigated the volatile sulfur compounds from decomposed cabbage tissue and identified dimethyl sulfide, DMDS, dimethyl trisulfide, hydrogen sulfide, carbonyl sulfide and methyl mercaptan.

Figure 5 shows chromatograms of Tis and Zws produced by leek or onion and extracted after one hour's maceration. The three Zws are mostly present in onion and DPTi is the major Ti from leek. They can be quantified by GC-MS in selected ion recording mode [23]. The analytical results showed that DPDS was the major compound in soil after 6 h.

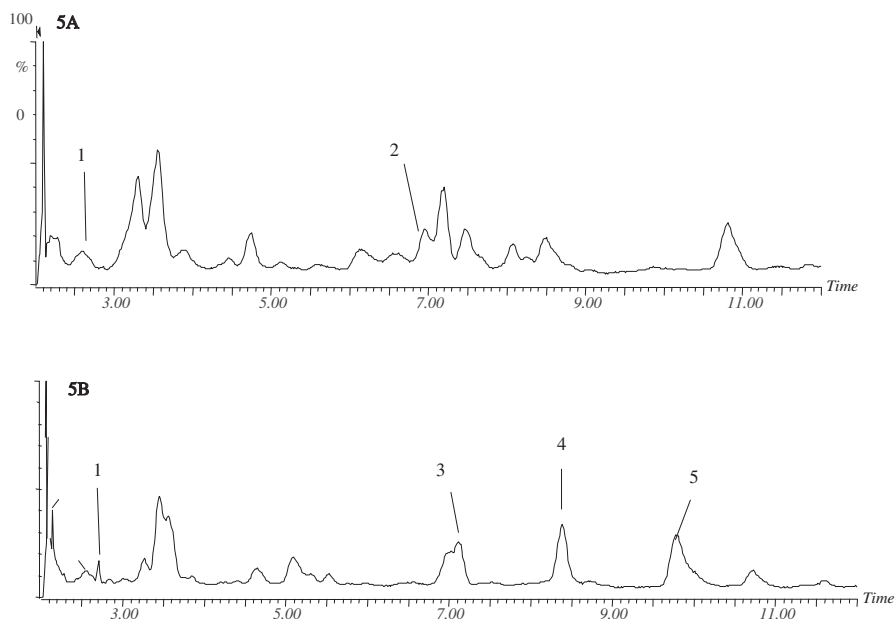


FIGURE 5 TIC of sulfur volatiles of (A) leek and (B) onion extracted by ether after one hour's maceration. Refer to Table I for the identity of sulfur compounds.

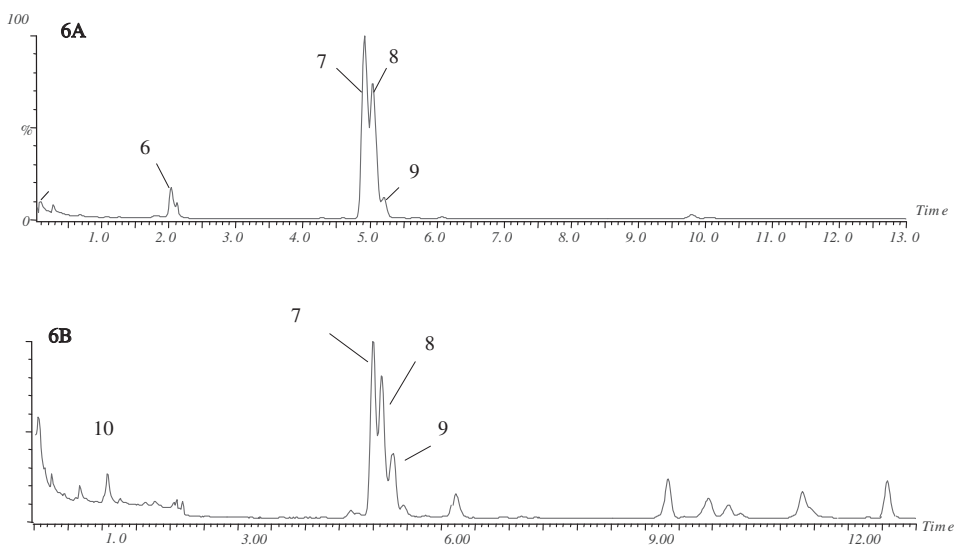


FIGURE 6 TIC of sulfur volatiles of (A) leek and (B) onion extracted by SPME after one hour's maceration. Refer to Table I for the identity of sulfur compounds.

Results of Atmospheric Analysis

Figure 6 shows the profile of volatiles from leek and onion extracted by SPME after 6 h. The major compound was DPDS produced by DPTi and LF degradation. In soil, we observed the same results after 7 days; fatty acids and thiols produced in anaerobic conditions were identified in the atmosphere above soil.

IR analysis showed that the band at 1085 cm^{-1} was still present in the atmosphere above the soil after several days, which means that remaining Tis are not degraded in air.

CONCLUSIONS

SPME-GC-MS for monitoring sulfur compounds in soil and IRFT for the analysis of the atmosphere above soil are very convenient methods for investigating *Allium* fumigant behaviour.

Fumigation of soil by Tis as pure compounds or present in fresh *Allium* tissues or extracts is mainly considered to be due to the action of DSs because the primarily produced compounds, such as Tis, are very unstable in soil whereas gaseous Tis in the atmosphere are stable. The DSs remaining in soil are not rapidly degraded.

In consequence, we can affirm that DSs are the real Ti metabolites in the soil, and that they have a sufficient lifespan to provide the expected pesticide effect.

References

- [1] E. Block, D. Putman and S.H. Zhao, *J. Agric. Food Chem.*, **40**, 2431 (1992).
- [2] S. Ferary and J. Auger, *J. Chrom. A*, **750**, 63 (1996).

- [3] I. Arnault, N. Mondy, F. Cadoux and J. Auger, *J. Chrom. A*, **896**, 117–124 (2000).
- [4] A.J. McLeod and G. McLeod, *J. Food Sci.*, **35**, 44–750 (1970).
- [5] A. Kjaer, J.O. Madsen, Y. Maeda, Y. Ozawa and Y. Uda, *Agric. Biol. Chem.*, **42**(9), 1715–1728 (1978).
- [6] J.C. Casey, R. Self and T. Swain, *Nature*, **4909**, 885 (1963).
- [7] B. Jaillais, V. Bertrand and J. Auger, *Talanta*, **48**, 747–753 (1999).
- [8] J. Auger, Proceeding of 25th IOBC meeting (Greece) *IOBC Bulletin*, **25**, 295–306 (2000).
- [9] M.G. Angus, P.A. Gardner, J.A. Kirkegaard and J.M. Desmarchelier, *Plant Soil*, **162**, 107–112 (1994).
- [10] M.K.Y. Chan and R.C. Close, *New Zealand J. Agric. R.*, **30**, 225–233 (1987).
- [11] P.B. Adams, *Phytopathology*, **61**, 93–97 (1971).
- [12] G.D. Bending and S. Lincoln, *Soil Biol. Biochem.*, **31**, 695–703 (1999).
- [13] C.J. Cavallito, J.S. Buck and C.M. Suter, *J. Am. Chem. Soc.*, **66**, 1952–1954 (1944).
- [14] G. Iberl Winkler, B. Müller and K. Knobloch, *Planta Med.*, **56**, 320–326 (1990).
- [15] J. Auger, F.X. Lalau-Keraly and C. Belinsky, *Chemosphere*, **21**, 837–843 (1990).
- [16] S. Ferary, J. Auger and A. Touché, *Talanta*, **43**, 349–357 (1996).
- [17] A.T. Nielsen and S. Jonsson, *J. Chromatogr. A*, **963**, 57–64 (2002).
- [18] C. Arsene, I. Barnes, K.H. Becker and R. Mocanu, *Atmos. Environ.*, **35**, 3769–3780 (2001).
- [19] I. Barnes, K.H. Becker and N. Mihalopolous, *J. Atmos. Chem.*, **18**, 267–289 (1994).
- [20] V. Ramakrishnan, G.J. Chintalvar and A. Banerji, *Chemosphere*, **18**, 1525–1529 (1989).
- [21] E. Coventry, R. Noble, A. Mead and J.M. Whipps, *Soil Biol. Biochem.*, **34**, 1037–1045 (2002).
- [22] H.W. Chin and R.C. Lindsay, *J. Food Sci.*, **58**(4), 835–839 (1993).
- [23] N. Mondy, D. Duplat, J.P. Christides, I. Arnault and J. Auger, *J. Chrom. A*, **963**, 89–93 (2002).