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Use of Alliaceae residues to control soil-borne pathogens

Ingrid Arnault[a,*], Christophe Fleurance[b], Frédéric Vey[c], Gaël Du Fretay[d], Jacques Auger[a,e]

[a] CETU Innophyt, Université François Rabelais de Tours, UFR Sciences et Techniques, Avenue Monge, 37200 Tours, France
[b] LCA, le Riou, 41 250 Tour en Sologne, France
[c] Service Régional de la Protection des végétaux du Centre, Laboratoire National de flore pathogène des sols, 93 Rue de Curembourg, 45404 Fleury-les-Aubrais, France
[d] Aber Consulting, 1 Allée Jean Rostand, 33650 Martillac, France
[e] IRBI, UMR CNRS 7261, Université François Rabelais de Tours, UFR Sciences et Techniques, Avenue Monge, 37200 Tours, France

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ABSTRACT

The presence of large amounts of sulfur compounds in the organs of *Allium* species has lead to the suggestion that the residues of this plant family could be used in soil biofumigation. In this paper, we report the preliminary results of laboratory bioassays and field experiments that investigated the biofumigant effects of onion and leek residues. The active molecules in these *Allium* species were determined to be dimethyl disulfide (DMDS) and dipropyl disulfide (DPDS). The results show that onion by-products and DMDS not only had a high level of biofumigant activity, but also stimulated vegetative growth. In the field, when *Allium* by-products were incorporated into the soil, DPDS was frequently released and was detectable for up to one month afterwards. This treatment increased asparagus and strawberry productivity by 15–20%, a result that is comparable to those obtained using *Brassica*-based biofumigation. Given the concordance between the results of the bioassays and those of the preliminary field trials, onion by-products may have practical potential as new biofumigants and could be used as an alternative to methyl bromide. In the agronomic context, it is crucial to develop improved application techniques that reduce the quantity of onion by-products that need to be incorporated into soil.

1. Introduction

Methyl bromide (MB) is an ozone-depleting substance of significant concern that was added to the Montreal Protocol in 1992. Regulations EC2037/00 and EC3093/94 mandated reductions in MB consumption and specifically prohibited the majority of its uses in the European Union starting in 2008. The AlterBromide project (Framework Programme Priority 8.1 Policy-oriented Research Thematic Priority: agriculture and forestry management contract no. 022660; 2006–2009) is a coordinated effort that aims to develop sustainable alternatives to MB for use in soil fumigation and post-harvest. The suitable alternatives listed by the consortium fall into three categories: (1) existing chemicals: chloropicrin, dazomet, dichloropropene, metam sodium, potassium, phosphine, and contact insecticides; (2) new alternative chemicals: ethadinytrile, methyl iodide (whose suitability for development in Europe remains to be confirmed), and sulfuryl fluoride; and (3) sustainable and environmentally safe techniques such as solarization, steam, and biofumigation. In organic farming, biofumigation that relies on the fumigant action of volatile compounds released during biodegradation is used to suppress soil-borne pests and diseases (Brown and Morra, 1997) and control weeds (Al-Khatib et al., 1997). The technique often involves the use of isothiocyanate-generating *Brassica* species (Matthiessen and Kirkegaard, 2006). The biofumigant potential of cruciferous plants is generally assessed by using them as cover crops and then incorporating their residues into the soil (Kirkegaard and Matthiessen, 2004; Motisi et al., 2009). Results show that species vary in their biofumigation efficacy. In order to understand the factors underlying efficacy, Motisi et al. (2010) proposed examining the different biofumigation parameters, such as the period of fumigation, while simultaneously incorporating an epidemiological approach and considering aspects such as inoculum density.

Procedures that are already in place to use and transform agricultural and agro-industrial residues may contribute to the development of biofumigation products. For instance, wastes from the orange juice industry as well as by-products of tomato, pepper, strawberry, and cucumber production show promise in controlling nematodes under laboratory conditions (Piedra Buena et al., 2006, 2007) and protecting against bacterial wilt (Zanón et al., 2011). Sub-products such as sugar beets, sugar cane, and wine vinasse have been reported to have an inhibitory effect on soil fungi pathogens

* Corresponding author. Tel.: +33 247366975; fax: +33 2 47 36 69 75.
E-mail address: ingrid.arnault@univ-tours.fr (I. Arnault).

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in vitro, with wine vinasse's effect being the most pronounced (Santos et al., 2008).

In addition to cruciferous species and cultivars, other plant taxa, and particularly plants of the genus *Allium* (Alliaceae or Liliaceae), may potentially be employed in pest management. However, experiments examining their efficacy have yet to clearly establish optimal application procedures (Thibout and Auger, 2004). The thiosulfinates (Ti) and disulfides (DS) contained in *Allium* extracts act as insecticides, fungicides (Auger et al., 2004; Benkeblia, 2004), acaricides, and nematicides (Gu et al., 2007). More recently, Deberdt et al. (2012) reported that *Allium fistulosum* has antimicrobial properties: it controlled bacterial wilt caused by *Ralstonia solanacearum* when used as a pre-plant soil treatment. Furthermore, Mallek et al. (2007) reported that onion and garlic crop residues reduce the germination of seeds of weedy annual plants.

The pronounced biocidal properties of *Allium* species are tightly linked to the complex biochemistry of the sulfur compounds they contain (Arnault et al., 2010). In addition to common sulfur amino acids such as cysteine, cystine, methionine, glutathione, and related peptide derivatives, *Allium* species contain S-alk(en)yl-cysteine sulfoxides (RCSOs), which are the precursors of the aromatic compounds (Ti and its corresponding DS) associated with these plants. RCSO proportions vary among species, varieties, and plant organs (Keusgen et al., 2002) and are influenced by environmental conditions (Kamenetsky et al., 2005). In the case of garlic, the major RCSO is alliin (S-allyl-L-cysteine sulfoxide), which produces the allicin (diallyl thiosulfinate); allicin is responsible for the characteristic odor of garlic and quickly degrades into diallyl disulfide (DADS). Onions and leeks mainly contain isoalliin (S-1-propenyl-L-cysteine sulfoxide) and propiin (S-propyl cysteine sulfoxide), which yield several thiosulfinates and other volatile sulfur compounds called zwiebelanes. These molecules degrade into dipropyl disulfide (DPDS) (Arnault et al., 2004). In the case of wild *Allium* species like bear's garlic (*Allium ursinum*) and chinese chive (*Allium tuberosum*), the major RCSO is methiin (S-methyl-L-cysteine sulfoxide); methiin degrades into dimethyl thiosulfinate (DMTi), which subsequently degrades into DMDS (dimethyl disulphide). DMDS is also the only disulfide that has been found in soils into which various Brassicaceae plant materials had been incorporated. The enzyme myrosinase hydrolyzes the thioglucoside bonds of glucosinolates, resulting in the production of thiohydroximate-O-sulfonate, an unstable substance that degrades into volatile compounds, namely thiocyanates, nitriles, and isothiocyanates (Cole, 1976). In the case of thiomethylated glucosinolates, corresponding isothiocyanates degrade into DMDS (Chin and Lindsay, 1993). In fact, DMDS was recently implicated in the biofumigant properties of crucifers; the beneficial effects of cruciferous plants in controlling *Verticilium dahliae*, *Fusarium oxysporum*, and *Tylenchulus semipenetrans* were demonstrated to be correlated to the production of DMDS and dimethyl sulphide in the soil (Wang et al., 2009). DMDS in its pure form has also been effective in controlling fungi soil phytopathogens such as *Pythium ultimum* and *Fusarium oxysporum* (Auger et al., 2004; Gerik, 2005) as well as nematodes (Fritsch, 2005). The commercial production of DMDS (Paladin^{TM}) as a replacement for MB was initiated by Arkema. The commercial compound is now sold in the USA and Israël, where it is used to control plant pathogens of vegetable crops (Fritsch, 2005).

There is consequently an important need for research investigating the biocidal effects of *Allium* products or subproducts and thus the potential of these plants to serve as biopesticides and, in particular, biofumigants. The onion is the second most highly produced vegetable in the world, and France is the top European producer of dehydrated onions. Nevertheless, the agro-industrial by-products (or subproducts) of edible *Allium* species like the onion, garlic and leek are not considered to have much value. To explore the fungicidal potential of *Allium* by-products (ABPs), we designed a three-year study whose main objective was to investigate the innovative technique of employing onion and leek by-products (respectively OBPs and LBPs) in biofumigation. The work described here sought to: (1) identify the active compounds released in the soil after ABP incorporation; (2) quantify the *in vitro* fungicidal effects of ABPs and related pure active compounds in the context of a plant-pathogen system; and (3) evaluate the biofumigant activity of OBPs and LBPs on the soil-borne pathogens of asparagus and strawberry crops, systems in which MB was traditionally used.

2. Materials and methods

2.1. Biochemical analysis of Allium by-products (ABPs)

We used unmarketable onions bulbs as our OBPs and waste peels as our LBPs; the green leaves of leeks are known to contain fewer sulfur compounds as a result of their lower dry matter content. Levels of DPDS (propiin and isoalliin) and DMDS (methiin) precursors present in OBPs and LBPs were quantified. We used an ion-pair high-performance liquid chromatography (HPLC) method developed using garlic samples (Arnault et al., 2003) and that has successfully been applied in analyses of compounds from other *Allium* species (Arnault et al., 2010). Analysis were performed with a Waters 616 pump and DAD 996 diode-array detector (Waters Corporation, Milford, MA, USA). Compounds were separated on a 150 mm × 3 mm i.d. × 3 μm particle C18 Hypurity Elite Thermo Quest column, at 38 °C (Thermo Hypersil Division, Keystone, Bellefonte, PA, USA) and quantified with UV at 208 nm. The column flow was 0.4 ml/min. The mobile phase consisted of: (a) 20 mM sodium dihydrogen phosphate +10 mM heptane sulfonic acid-pH 2.1 (adjusted withorthophosphoric acid 85%); and (b), acetonitrile −20 mM sodium dihydrogen phosphate +10mMheptane sulfonic acid pH 2.1 (50/50).

The vegetable material was crushed in a mixture of methanol and water (ratio of 9:1) to which 0.05% formic acid was added. Ten replicates of 1 kg of each residue were analyzed; levels of isoalliin (S-1-propenyl-L-cysteine sulfoxide) and propiin (S-propyl-cysteine sulfoxide) were quantified to evaluate the release potential of DPDS and DMDS.

2.2. Sampling and analyses of sulfur compounds following ABP incorporation into the soil

Intermediate sulfur compounds (Ti, zwiebelanes) are unstable and degrade at the time of ABP incorporation into the soil. The major stable metabolite produced in the soil is DPDS (Arnault et al., 2004). To characterize DPDS behavior in the soil, solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC/MS) must be used. SPME is the preferred method when extracting volatile compounds from compost containing ABPs (see Section 2.3.3 for details on the preparation of the soil mixture), and GC/MS allows a clear separation and identification of the compounds present. To sample sulfur compounds present in the soil atmosphere, a sampling chamber was used. A glass tube (1 cm in diameter × 5 cm in length) was inserted into the soil mixture until it was 1 cm from the bottom and was placed against the glass surface around a hole (1 mm in diameter), which is necessary for the insertion of the SPME needle. Four application densities were analyzed using laboratory tests: OBPs at 240 T/ha (tons/hectare), OBPs at 120 T/ha, LBPs at 240 T/ha, and LBPs at 120 T/ha. Three replicates of each were performed.

GC–MS analysis was carried out on a benchtop Perkin-Elmer Turbomass (Shelton, Co., USA) system with a split–splitless injector and a fused-silica capillary column (10 m × 0.32 mm) with a