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Use of Alliaceae residues to control soil-borne pathogens

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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). Subproducts such as sugar beets, sugar cane, and wine vinasse have been reported to have an inhibitory effect on soil fungi pathogens

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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

4 μm methylsilicone coating. The carrier gas was helium (99.999%) at 3.5 ml/min and the column temperature program was 5 °C/min from 70 °C 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 ionization mode at 70 eV.

The compounds were identified with their mass spectra, retention time and standard reference DMDS, DPDS and DADS (Sigma, St. Louis, MI) distillated at lab.

2.3. Laboratory tests using the P. ultimum-cucumber system

Soil-borne pathogenic fungi, including various polyphagous species of *Pythium*, *Phytophtora*, and *Fusarium*, have been associated with the pre- and post-emergence damping-off of crop species. *P. ultimum* is a serious problem for several crops, such as tomatoes, sugar beets, and cucumbers, especially when cool and moist conditions prevail, as commonly occurs during sowing. Consequently, the *in vitro* fumigation abilities of ABPs were evaluated using the *P. ultimum*-cucumber system. This experiment sought to characterize the potential of a pathogen-contaminated soil to induce damping-off in a host species and the ability of ABP compounds to control it. To this end, cucumber plants were exposed to ABP-treated or control samples of soil or compost with different pathogen infestation risks. At the end of the bioassay, the number of healthy, necrotic, and dead plants were counted. The experiment had four stages: the preparation of pathogen inocula and *Pythium*-infested compost, sowing, inoculation, and the counting of the plants.

2.3.1. Preparation of pathogen inocula and P. ultimum-infested compost

The *Pythium* isolate used came from the fungi collection of the Regional Services for Crop Protection (La Protection des végétaux, Fleury les Aubrais, France). Two liters of sterilized compost (autoclaved at 105 °C for 1 h, repeated three times and left to rest for 10 days) were inoculated with mycelium from two Petri dishes containing *P. ultimum* grown on PDA for 7 days at 21 °C. The compost was then homogenized at 25 °C over the course of 2 days and used in the experiment three weeks after inoculation took place.

2.3.2. Sowing

Plastic pots (rim diameter and depth of 14 cm) were filled three-quarters full with the prepared *Pythium*-infested compost (about 300 ml). Ten replicates of cucumber plants were seeded and watered until the soil reached saturation. The pots were covered with a plastic dome and placed in a growth chamber (25 °C, 12 h day/12 h night cycle) for 6 days. The fumigation treatment was applied at the end of this period. The duration of fumigation (between 2 days and 2 months) was thus counted starting from this date. In this and all other experiments, the two control treatments were: *Pythium*-free, non-inoculated, sterilized compost and *Pythium*-infested, inoculated, and non-disinfected compost.

Ten pots were used for each ABP and disulfide treatment, and six pots were used for the each of the two controls (*Pythium*-infested and *Pythium*-free).

2.3.3. Inoculation

The treatment compost was prepared by mixing the compost with specific amounts of ABPs and disulfides. DMDS and DPDS were added at a concentration of 10^{-2} mol/l. Three application densities of OBPs and LBPs were used: 120 T/ha, 240 T/ha, and 360 T/ha. These quantities represent, respectively, 95 g, 190 g and 285 g per 900 ml of compost. Next, 1.2 g of sterilized oats mixed with 60 ml of treatment compost were added to the pots after 6 days, and the pots were observed in accordance with the application level and fumigation duration. Eight treatments were tested. Ten replicates of 10 plants were performed for each treatment or density: OBP at 120 T/ha, OBP at 240 T/ha, OBP at 360 T/ha, LBP at 120 T/ha, LBP at 240 T/ha, LBP at 360 T/ha, and DMDS and DPDS at 10^{-2} mol/l. For the ABPs, a phytotoxic control was conducted that consisted of a ABP-treated *Pythium*-free compost.

2.3.4. Plant counts

There were three categories describing plant health: healthy, necrotic, and dead. The relative abundance of necrotic cucumbers indicated the potential risk of soil-borne pathogen infestation. If 0–10% of plants were necrotic, the soil sample presented no infestation risk. If 10–30% of plants were necrotic, infestation risk was low but there could be a significant risk of contamination. If more than 30% of plants were neurotic, the soil was considered to be infested and the disease risk to plants was high.

Depending on the treatment application density, plants were counted 2 days and 3 days after fumigation initiation in the case of the DPDS and DMDS treatments. For ABP, plants were counted 15 days, 1 month, and 2 months following the initiation of fumigation.

2.4. Field experiments

2.4.1. Asparagus

Biofumigation using ABP incorporation (75 T/ha) was performed over a three crop cycle using asparagus. The experiment took place in 2006 and 2008 at a research station dedicated to the study of vegetables (Légumes Centre Action, Tour en Sologne, France); environmental conditions varied between years. The experimental design consisted of two blocks composed of 3 rows of asparagus (128 plants, 144 m long). Biofumigation with OBPs and LBPs at a density of 75 T/ha was applied to each block. We also included a conventional control treatment, in which MB was applied. The ABPs were crushed and incorporated into the soil. Asparagus was planted and subject to treatment in April 2006, and commercial yields were measured in April 2008.

2.4.2. Strawberries

The experiment used two soil types taken from fields located at the same research station (Légumes Centre Action, Tour en Sologne, France); local producers have observed that these soils present high and low risks of soil pathogen contamination. The experimental soil was prepared by mixing soil samples with either 100 T/ha of OBPs or LBPs. On April 1st, 2008, 6 days after ABPs had been incorporated into the soil, two strawberries (ELSANTA®) were planted per pot. A 6-day delay is ideal because it is the period over which emission of soil disulfides is maximal, regardless of the application density of ABPs (see results in Section 3.2). The experimental design consisted of 40 pots for each treatment category: OBP, LBP, and control, with three replicates of each. Productivity was calculated by quantifying leaf and fruit development (g/plant). Root development was quantified when the plants were harvested on June 10th 2008. Root development was scored using a number between 0 and 5, where 0 signified that the root was undeveloped while 5 indicated that the root system was well developed.

2.5. Statistical analyses

Differences between two treatments were tested using Student's *t*-tests. Otherwise, the data were analyzed using analysis of variance (ANOVA) and Tukey HSD tests, where $P \leq 0.05$ was considered significant. All analyses were performed using XLSTAT (version 2012.2.02, Addinsoft).

Table 1
Mean concentration of DPDS and DMDS precursors in ABPs (mg/g).

Treatment	DPDS precursors	DMDS precursor
OBP	6.6[a]	0.5[b]
LBP	0.7[b]	0.12[b]

[a] Indicates a significant difference according to Student's *t*-tests ($P \leq 0.05$).
[b] Mean values sharing the same letter do not differ significantly.

3. Results and discussion

3.1. Biochemical analysis of ABPs

The analyses show that OBP and LBP extracts were 92% and 86% composed of DPDS precursors, respectively. The concentrations of DMDS (methiin) and DPDS precursors (propiin and isoalliin) are indicated in Table 1. The results confirm that OBPs and LBPs mostly released DPDS into soil. OBPs are approximately 6 times richer in DPDS and DMDS precursors than are LBPs, which suggests onions have a greater potential to produce DPDS and DMDS in the soil. These data are not surprising considering the nature of these by-products. The onion by-products we used were unmarketable bulbs and the leek by-products were green leaves, which contain smaller amounts of sulfur compounds.

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

The quantity of sulfur compounds emitted by the ABPs incorporated into the soil at densities of 120 T/ha and 240 T/ha were estimated, up to the detection limit of the analytical method. DPDS represented about 85% of the total disulfides found in the soil following OBP and LBP incorporation. Propyl propenyl disulphide, methyl propenyl disulphide, dipropyl trisulfide, dipropenyl disulfide, and DMDS were also detected. This result is not surprising because it is known that methiin, isoalliin, and propiin in onions and leeks degrade into instable molecules, such as thiosulfinates, and may then form alkyl-disulfides with methyl, propyl and isoallyl groups. Fig. 1 shows the amount of DPDS detected in soil following the incorporation of ABPs at two density levels, 120 T/ha and 240 T/ha. DPDS production depended on ABP concentration in the soil; more DPDS was obtained when ABPs were applied at a density of 240 T/ha.

The amount of DPDS present in the soil following ABP incorporation followed the same trend for all treatments. DPDS production was maximal 6 days post-incorporation, regardless of the application density, and concentrations had decreased to undetectable levels after 30 days had passed. In addition, the levels of total sulfur compounds, 2-propenyl propyl disulphide, 2-propenyl methyl disulphide, dipropyl trisulfide, di-2-propenyl disulfide, and DMDS released over the 30 days were measured. OBPs applied at a density of 240 T/ha produced higher concentrations of total sulfur compounds than did other treatments; LBP at 240 T/ha was the second highest. In conclusion, all treatments showed fungicidal potential over the course of a month, and OBPs applied at a density of 240 T/ha seemed to most effectively control soil-borne pathogens. Thirty days had passed before the chromatographic profiles showed evidence of DPDS-derived compounds.

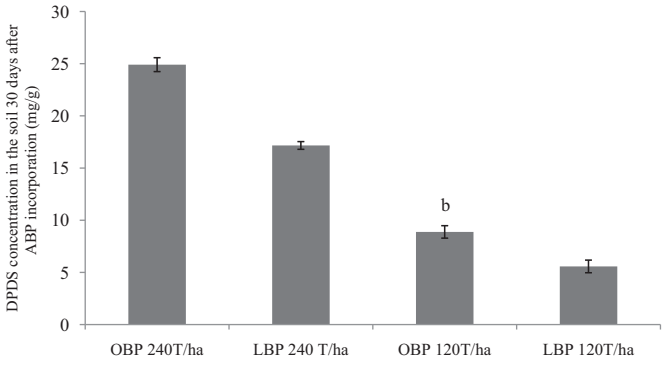

Fig. 1. Total DPDS concentration in the soil 30 days after ABP biofumigation. Onion (OBP) and leek (LBP) by-products were applied at two different densities, 240 T/ha and 120 T/ha. Values are means ± standard errors.

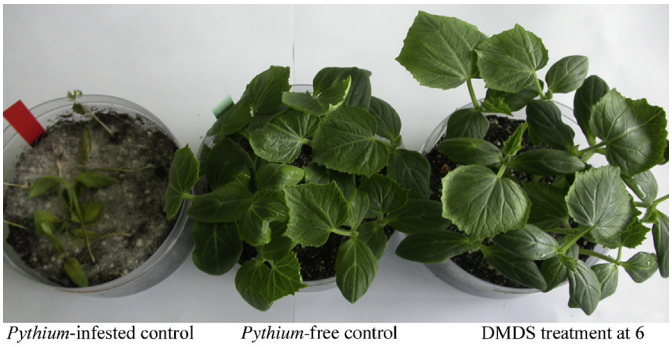

Photo 1. Stimulatory effect of DMDS on plant growth.

3.3. Laboratory tests using the P. ultimum-cucumber system

3.3.1. Tests with sulfur compounds

Table 2 shows the fumigant ability of DMDS and DPDS at the same treatment densities after 2, 3, and 7 days. From 2 to 7 days, DMDS was the most effective; 100% of plants in DMDS-treated soil were healthy, a result that was statistically similar to that of the *Pythium*-free control. At 2 and 6 days, the results for plants in DPDS-treated soil were statistically similar to those for the plants in *Pythium*-infested control soil; there was no evidence of a DPDS-induced biofumigant effect. However, a small biofumigant effect was observed at 7 days, with 20% of plants remaining healthy.

The same trend was seen for necrotic plants; plants in DMDS-treated soil showed no symptoms of disease between 2 and 7 days. However, after two days, the plants in DPDS-treated soil did not show the same low level of infestation as the plants in *Pythium*-free soil, which probably indicated that contamination was in progress. After three days, there were fewer necrotic plants than after 2 days (23% and 44%, respectively). Since DPDS is less volatile than DMDS, DPDS had probably not spread evenly throughout the soil after only 2 days. From 6 to 7 days, plants in DPDS-treated soil were infested, and there was potentially a high risk of disease-caused damping-off.

We noted no phytotoxic effects of DMDS; instead, it seemed to stimulate plant growth. At 6 days, cucumbers in DMDS-treated soil were significantly bigger than control cucumbers. The visual difference was obvious (Photo 1). This effect may be related to the multiple properties of DMDS. DMDS has previously been implicated in multitrophic interactions in the soil. Many plant-associated bacteria, especially root endophytic bacteria, have been found to interact closely with plants, namely affecting their metabolism. Some nonpathogenic *F. oxysporum* strains exhibit antagonistic activity toward pathogenic *F. oxysporum* isolates. When the former strains live in association with their community of ectosymbiotic bacteria, they produce active volatiles. Within this community, the most important members are *Serratia* spp. and *Achromobacter* spp. The major compound emitted by one *Achromobacter* strain was DMDS (94% of volatiles emitted); it also represents 2.5% of the volatiles emitted by a *Serratia* strain (Minerdi et al., 2011).

Table 2
Mean percentages of healthy, necrotic, and dead plants occurring in DMDS and DPDS treatments.

Duration of fumigation	Plant appearance	DMDS	DPDS	*Pythium*-free control	*Pythium*-infested control
2 days	Dead	0^{e}	50^{c}	0^{e}	78^{b}
	Necrotic	0^{d}	44^{b}	0^{d}	22^{c}
	Healthy	**100**^{a}	6^{c}	**100**^{a}	0^{c}
3 days	Dead	0^{e}	77^{b}	0^{e}	98^{a}
	Necrotic	0^{d}	23^{c}	0^{d}	2^{c}
	Healthy	**100**^{a}	0^{c}	**100**^{a}	0^{c}
6 days	Dead	0^{e}	59_{c}	0^{e}	100^{a}
	Necrotic	0^{d}	41^{b}	0_{d}	0^{c}
	Healthy	**100**^{a}	0^{c}	**100**^{a}	0^{c}
7 days	Dead	0^{e}	18^{d}	0^{e}	**100**^{a}
	Necrotic	0^{a}	62^{a}	0^{d}	0^{d}
	Healthy	**100**^{a}	20^{b}	**100**^{a}	0^{c}

^{a,b,c,d,e} Different letters indicate statistical differences according to Tukey's HSD test ($P \leq 0.05$). Mean values ($n = 10$) in the same row for each health category (dead, necrotic, or healthy) sharing the same letter do not differ significantly. 2d = 2 days, 3d = 3 days.
The number in bold indicate the highest value.

Table 3
Mean percentages of healthy plants grown in soils biofumigated with ABPs.

ABP concentration in the soil	Duration of fumigation	OBP	LBP	Pythium-free control	Pythium-infested control
120 T/ha	15 days	1^{ef}	0^{f}	**100**^{a}	0^{f}
	1 month	48^{bcd}	38^{cde}	**100**^{a}	17^{def}
	2 months	**82**^{a}	32^{cde}	**100**^{a}	2^{ef}
240 T/ha	15 days	74^{ab}	48^{bcd}	**100**^{a}	0^{f}
	1 month	**94**^{a}	64^{abc}	**100**^{a}	15^{def}
360 T/ha	15 days	44^{bcd}	48^{bcd}	**100**^{a}	0^{f}
	1 month	75^{ab}	17^{def}	**100**^{a}	17^{def}

^{a,b,c,d,e,f} Different letters in superscript indicate statistical differences according to Tukey's HSD test ($P \leq 0.05$). Mean values ($n = 10$) sharing the same letter do not differ significantly.

In another example, two broad-range antagonistic rhizobacteria *Pseudomonas fluorescens* and *Serratia plymuthica* were able to suppress *Agrobacterium*-caused crown gall tumors in tomatoes *via* volatile compounds (Dandurishvili et al., 2011). DMDS was the major volatile emitted by *S. Plymuthica*, plays a more minor role in *P. fluorescens* emissions, and strongly inhibits *A. Tumefaciens* and *A. vitis* growth. This fungistatic property is perhaps correlated to the stimulatory effect observed.

3.3.2. Tests with ABP

The percentages of healthy plants in ABP-treated soil observed over the 2 months of the experiment at the 3 different application densities (120 T/ha, 240 T/ha, 360 T/ha) are shown in Table 3. OBP biofumigation was more effective than LBP biofumigation, and the values in bold highlight the highest values that were found in statistical group a. Furthermore, when comparing the effect of the same application densities of OBP and LBP over the same amount of time, OBP biofumigation was always more effective. The most effective treatment was OBP applied at 240 T/ha over 1 month; it was statistically equivalent to the *Pythium*-free group. For LBP, the highest percentage of healthy plants (64%) was obtained when 240 T/ha was applied over one month. The number of necrotic plants suggests the same conclusion (Table 4). Only OBP biofumigation at a level of 240 T/ha over a period of one month resulted in 10% or fewer necrotic plants, which indicated limited crop damage risk. The values for this group were statistically equivalent to those for the *Pythium*-free control. More than 30% of LBP-treated plants were necrotic, which indicated that pathogen contamination risk was high. When we compared the number of dead plants (Table 5), the conclusions were different. All OBP treatments (except one) were statistically similar to the *Pythium*-free control. This result signifies that plant mortality was not necessary observed even when the soil was infested with *Pythium* spp. This bioassay was used to evaluate the potential of a given soil to induce damping-off in plants following treatment with ABSs, and necrosis appeared to be the most reliable indicator of the effect on the plants.

We also explored the correlation between DPDS concentration in ABPs, fumigation duration, and fumigation efficacy using the concentration-time (Ct) product (Anms, 1998). We found that the percentage of healthy plants increased over time and with DPDS

Table 4
Mean percentage of necrotic plants in ABP-biofumigated soil.

ABP concentration in soil	Duration of fumigation	OBP	LBP	*Pythium*-free control	*Pythium*-infested control
120 T/ha	15 days	41^{ab}	44^{ab}	**0**^{c}	3^{abc}
	1 month	47^{ab}	51^{a}	**0**^{c}	15^{bc}
	2 months	18^{bc}	45^{ab}	**0**^{c}	2^{c}
240 T/ha	15 days	26^{abc}	45^{ab}	**0**^{c}	23^{abc}
	1 month	**6**^{c}	32^{abc}	**0**^{c}	3^{c}
360 T/ha	15 days	52^{a}	43^{ab}	**0**^{c}	23^{abc}
	1 month	19^{bc}	38^{ab}	**0**^{c}	3^{c}

^{a,b,c} Different letters in superscript indicate statistical differences according to Tukey's HSD test ($P \leq 0.05$). Mean values ($n = 10$) sharing the same letters do not differ significantly. Values in bold highlight values that are similar to the healthy control (group c).

Table 5
Mean percentages of dead plants in ABP-biofumigated soil.

ABP concentration in the soil	Duration of fumigation	OBP	LBP	*Pythium*-free control	*Pythium*-infested control
120 T/ha	15 days	49^{c}	56^{c}	**0**^{e}	7^{b}
	1 month	**5**^{e}	11de	**0**^{e}	85^{a}
	2 months	**0**^{e}	23^{d}	**0**^{e}	98^{a}
240 T/ha	15 days	**0**^{e}	**7**^{e}	**0**^{e}	77^{c}
	1 month	**0**^{e}	**4**^{e}	**0**^{e}	97^{a}
360 T/ha	15 days	**4**^{e}	9de	**0**^{e}	77^{c}
	1 month	**6**^{e}	45^{c}	**0**^{e}	97^{a}

^{a,b,c,d,e} Different letters indicate statistical differences according to Tukey's HSD test ($P \leq 0.05$). Mean values ($n = 10$) sharing the same letters do not differ significantly. The number in bold indicate the highest value.

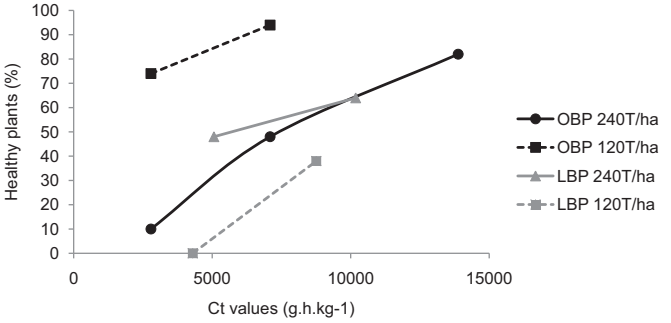

Fig. 2. Relationship between DPDS concentration in ABPs × time (Ct) and the percentage of healthy plants.

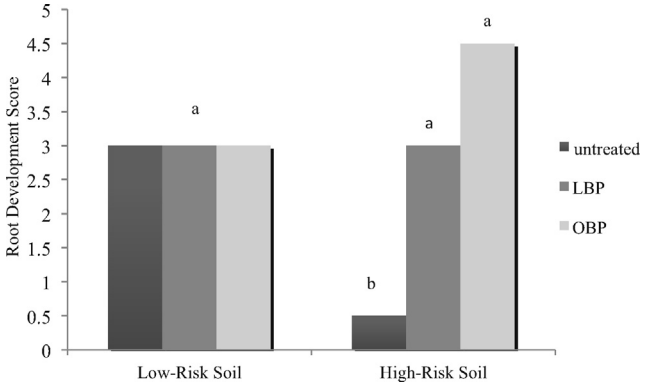

Fig. 3. Root development scores for strawberry plants grown in soil at high *versus* low risk for fungal infestation. Soils were treated with onion (OBP) or leek (LBP) by-products or left untreated (nd). Different letters indicate statistical differences according to Tukey's HSD test ($P \leq 0.05$).

concentration for all treatments (Fig. 2). The efficacy of ABPs is clearly correlated with the application density of ABPs (and thus the dose of DPDS) and fumigation duration. However, this correlation was not established between the treatments. Each ABP should be considered as a single fumigant with its own Ct product. It is not surprising because the quantitative behavioral responses of ABPs may also be affected by the chemical nature of all the fumigant molecules present, such as the concentration of DMDS.

Thus, the ability of ABPs to disinfect soils has been demonstrated *in vitro*. The application density and the chemical characteristics of the plant by-products used as well as the duration of fumigation are the principal considerations when designing a successful biofumigation technique. OBPs were more efficient biofumigants than LBPs because OBPs produced more disulfide compounds in the soil than did the LBPs. Our results suggest that the disulfide release efficiencies associated with individual ABPs depend on the plant species, the plant organ used, and the application density. The concentration of the volatile compounds DMDS and DPDS declined more slowly over the first days following incorporation, which may be related to their absorption by the soil. Furthermore, they persisted in soil for 30 days without producing other, further metabolites.

3.4. Field experiments

3.4.1. Asparagus

The highest yield was obtained with the reference chemical (10.6 T/ha). OBP biofumigation (9.4 T/ha) resulted in an intermediate yield that was statistically different from that obtained from untreated soil. The lowest yields were associated with the LBP treatment (8.3 T/ha) and the untreated soil (8.2 T/ha). Yield was increased by 15% and 31% using OBP and MB fumigation, respectively, compared to untreated soil. These results concur with those obtained from the *in vitro* bioassay, which indicated that OBP biofumigation was effective. However, the field experiment used a lower OBP dosage than did the *in vitro* bioassay (75 T/ha *versus* 240 T/ha).

3.4.2. Strawberries

Fig. 3 shows the development scores of strawberry plant roots in control and ABP-treated soils at low *versus* high risk for fungal infestation. In low-risk soil, there was no statistical difference between the untreated soil group and the OBP and LBP fumigation groups. Root development in high-risk soil was statistically different among the three treatments. Roots were poorly developed in untreated, high-risk soil while LBP fumigation resulted in root development patterns similar to those observed in low-risk soil. OBP fumigation obtained the best results; plants in OBP-treated soil had more highly developed root systems than plants in low-risk soil. These results also concur with the bioassay results for DMDS in suggesting that the OBP treatment has a stimulatory effect on the root system.

Productivity is presented in Table 6. In low-risk soil, productivity in the OBP, LBP, and the untreated groups was statistically similar. In contrast, the trend in high-risk soil was different. Results from LBP-treated and untreated soil were similar, whereas the OBP treatment resulted in a significantly higher level of productivity, similar to that observed in low-risk soil. Once again, the results support the biofumigant properties of OBPs; productivity was increased by about 21%. These results are comparable with the results of biofumigation efforts directed against strawberry pathogens employing *Brassica* spp. (Mattner et al., 2008): a similar increase in productivity of about 19% was seen (Lazzeri et al., 2003).

Table 6
Strawberry crop productivity (in g per plant, total mass of fruits and leaves) following ABP fumigation of soil types at low and high risk for fungal infestation.

Soil type	Untreated soil	LBP	OBP
Low-risk soil	169^{a}	160^{a}	158^{a}
High-risk soil	134^{b}	134^{b}	163^{a}

^{a,b} Different letters indicate statistical differences according to Tukey's HSD test ($P \leq 0.05$).

3.5. Perspectives

These results, especially if they are confirmed at an industrial scale, suggest interesting practical perspectives for *Allium* by-products. Certainly, they may serve as an environmentally friendly alternative to MB in the control of soil-borne fungi. The biofumigant activity observed when ABPs were applied in field and *in vitro* conditions may be related to the activity of thiosulfinates, which are very active volatile compounds that degrade into disulfides (see Section 1). Indeed, thiosulfinates, which result from the hydrolyzation of raw vegetable material in the soil, are the most fungicidal sulfur molecules of *Allium* species (Auger et al., 2004; Thibout and Auger, 2004) and rapidly degrade into disulfides.

In practice, ABP application densities between 240 T/ha and 100 T/ha are impractical in the field because the by-product layer is as thick as 10 cm before it is incorporated into soil. Further studies have to focus on the most effective incorporation strategies so as to maximize the release of disulfides from OBPs into the soil and thus achieve high disulfide concentrations. OBPs should take a form that can be easily incorporated into the soil but that simultaneously preserves the amino acid precursors of DMDS, DPDS, and alliinase; forms such as concentrated powders or dried pellets should be investigated. The hydrolyzation of dehydrated biocidal plants could have an effect similar to that of ABP incorporation into soil, in addition to, or as an alternative to green manure (Mallek et al., 2007; Lazzeri et al., 2004). Therefore, it may be that the organic matter content provided by ABPs would be sufficient, especially when biofumigation is combined with other techniques, for example, plastic covers. Heating a soil covered with plastic film and to which the appropriate organic material has been added accelerates a chain reaction of chemical and microbial degradation events, which leads to the generation of toxic compounds in the vapor and liquid soil phase. The generation of toxic compounds increases with increasing temperature. These compounds accumulate under the plastic mulch, and their toxicity to soil flora and fauna, primarily soil-borne plant pathogens, is enhanced (Gamiel, 2000).

Furthermore, the effect of post-incorporation irrigation and water soil content has to be studied. Excess water could facilitate the hydrolysis of precursors into disulfides, but some RCSOs would probably remain unhydrolyzed due to incomplete tissue pulverization or other limitations to hydrolysis (Gimsing and Kirkegaard, 2006).

The dispersion of disulfides and RCSOs in the soil could play a role in biofumigant activity. For Brassicacea, water transports or dissolves isothiocyanates and glucosinolates into layers below the level of incorporation (Gimsing and Kirkegaard, 2006).

Finally, the search for biopesticides should be directed toward plant varieties and/or species that contain high levels of disulfides. Wild species with high levels of DMDS are particularly interesting because: (1) the pure product Paladin™ is now used by producers and (2) its biocidal activity toward phytopathogens, nematodes, and insects has been demonstrated. For example, *Allium vineale* (wild garlic), *A. ursinum* (bear's garlic), *Allium saxatile*, *Allium grosum*, *Allium schoenoprasum* (wild chive), and *A. tuberosum* (chinese chive) extracts contain more than 50% MCSOs (Keusgen et al., 2002).

Whereas cystein derivatives are highly specific to *Allium*, this study suggests that other residues containing disulfides may have practical value. DMDS can be produced by several different pathways and is present in many plants, such as asparagus, neem, shiitake, and the invasive saline marsh plant *Spartina alterniflora*.

4. Conclusion

Using *Allium* residues to biofumigate soils is a new direction in the search for alternatives to MB. This study combined laboratory and field experiments to investigate the practical potential of *Allium* compounds and concludes that onion by-products have a significant effect on soil-borne pathogens. Their efficiency is linked to the higher concentration of disulfides diffused through the soil following incorporation, and DMDS was found to be more effective than DPDS. The stimulatory effect on vegetative growth was probably due to the presence of DMDS in soil, and this result should be studied further. However, more studies will be required to evaluate the effect of ABP biofumigation on beneficial microflora like mycorrhizae and soil fertility.

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