RESEARCH LETTER

An endophytic/pathogenic *Phoma* sp. from creosote bush producing biologically active volatile compounds having fuel potential

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Abstract

A *Phoma* sp. was isolated and characterized as endophytic and as a pathogen of *Larrea tridentata* (creosote bush) growing in the desert region of southern Utah, USA. This fungus produces a unique mixture of volatile organic compounds (VOCs), including a series of sesquiterpenoids, some alcohols and several reduced naphthalene derivatives. Trans-caryophyllene, a product in the fungal VOCs, was also noted in the VOCs of this pungent plant. The gases of *Phoma* sp. possess antifungal properties and is markedly similar to that of a methanolic extract of the host plant. Some of the test organisms with the greatest sensitivity to the *Phoma* sp. VOCs were *Verticillium*, *Ceratocystis*, *Cercospora* and *Sclerotinia* while those being the least sensitive were *Trichoderma*, *Colletotrichum* and *Aspergillus*. We discuss the possible involvement of VOC production by the fungus and its role in the biology/ecology of the fungus/plant/environmental relationship with implications for utilization as an energy source.

Introduction

Cresote bush, *Larrea tridentata*, is a prominent plant in the Mojave, Sonoran and Chihuahuan deserts of North America. The common name of this zygophyllaceous plant reflects the strong pungent creosote-like odor of its leaves and stems, which becomes even more intense in the organic solvent extracts of its plant parts. This desert plant is drought tolerant and resistant to attack by many plant pests; as such, it and its clones are one of the longest lived plants (Vasek, 1980). It appears that mature plants effectively use sparse water resources and allelopathic effects, which help to explain why young plants fail to appear near the mother plant. This results in a pattern of evenly placed creosote bushes, giving it an overall appearance of having been organized. Furthermore, the substances exuded from its roots inhibit the growth and development of other desert species such as *Ambrosia dumosa* (burro bush).

Examination of the volatile organic compounds (VOCs) by GC-MS of creosote bush revealed the presence of a large number of terpenes, benzene derivatives, ketones, alcohols, hydrocarbons and other hydrocarbon derivatives. Compounds of this type have been implicated as allelochemicals (Fraenkel, 1959; Stamp, 2003). In addition, some may also serve in the overall biology of the plant, especially as it relates to insect and disease tolerance as well as other environmental stresses including drought tolerance (Rice, 1974; Keeling & Bohlmann 2006; Reigosa *et al.*, 2006; Sharkey *et al.*, 2008). Finally, it appears that many of the *Larrea* compounds have potential as fuels, but harvest of the plant per se for this purpose does not appear practical as it is slow growing and is found in rocky and inaccessible areas.

As creosote bush contains many hydrocarbons, it seemed likely that any endophytic fungus associated with this plant may also produce hydrocarbon-like substances that might enable it to cosurvive with such an unusual host in a highly

stressful environment. Thus, the main aim of this study was to determine if any endophytes of creosote bush do exist and if they produce hydrocarbon-like substances that have biological activity and possible potential as fuels. Thus, the rationale for the approach of finding an endophyte-making product similar or identical to its host plant follows the logic relating to an earlier study in which fungal taxol was discovered as a product of an endophytic fungus living in association with Pacific yew, *Taxus brevifolia*, a producer of taxol (Stierle *et al.*, 1993).

We describe the successful recovery of a novel pathogen/endophyte of *L. tridentata* and demonstrate that it produces a plethora of hydrocarbons and hydrocarbon derivatives not only possessing biological activity, but also having potential as a biofuel – Mycodeisel™ (Strobel *et al.*, 2008).

Materials and methods

Fungal isolation and storage

Fungal culture Ut-1 was obtained as an endophyte from a small plant of *L. tridentata*. Tissue samples were excised from several plants growing south of St. George, UT, at 37°03′0672″N, 113°33′1054″W. Isolation procedures followed a previously described protocol (Ezra *et al.*, 2004). Briefly, external tissues were thoroughly exposed to 70% ethanol before excision of internal tissues, which were cultured on standard Petri dishes of water agar. Endophytic fungi growing from the plant tissues were then picked and recultured on potato dextrose agar (PDA). The fungus was stored by placing colonized sterile barley seed, which was subsequently air dried, and then stored at –70 °C. The fungus has been deposited in the living Montana State University mycological collection under acquisition number 2378.

Internal transcribed spacer (ITS)-based phylogenetic analysis

Phylogenetic analysis of the fungal strain was carried out by acquisition of the ITS 5.8S ribosomal gene sequence. The fungus was grown on PDA for 7 days and DNA templates were prepared by using the Prepman Ultra Sample Preparation Reagent (Applied Biosystems) according to the manufacturer's guidelines. The ITS regions of the fungus were amplified with the universal ITS primers ITS1 (5′ TCCGTA GGTGAACCTGCGG 3′) and ITS4 (5′ TCCTCCGCTTATT GATATGC 3′) using PCR. The PCR conditions used were as follows: initial denaturation at 94 °C for 3 min followed by 30 cycles of 94 °C for 15 s, 50 °C for 30 s and 72 °C for 45 s, and a final extension of 72 °C for 5 min. The 50-μL reaction mixture contained 1 × PCR buffer, 200 mM each dNTP, 1.5 mM $MgCl_2$, 10 pmol of each primer, 1–5 ng of DNA and 2.5 U of *Taq* DNA polymerase. The amplified product (5 μL) was visualized on 1% (w/v) agarose gel to confirm the presence of a single amplified band. The amplified products were purified by Amicon Ultra columns (Millipore) and 40–60 ng was used in a 10 μL sequencing reaction using the Big Dye Terminator sequencing kit (v. 3.1). The forward or the reverse primer (3.2 pmol) was used in the cycle sequencing reaction. Twenty cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min were performed and the extension products were purified by ethanol precipitation, dissolved in 15 μL of HiDi formamide, incubated at 95 °C for 1 min and loaded on an ABI Prism 377 Genetic Analyzer (Perkin-Elmer) for sequencing. All the reagents for sequencing were from Applied Biosystems. The amplified products were sequenced and aligned with the sequences in the GenBank database via the BLASTN program (Altschul *et al.*, 1997). Relevant sequences were downloaded and aligned using the MEGALIGN program (DNASTAR, Lasergene) and a phylogenetic tree and distance matrix were constructed according to Guindon & Gascuel (2003).

Scanning electron microscopy (SEM)

SEM was performed on sterile carnation leaves colonized with CI-4 according to the following protocol outlined by Ezra *et al.* (2004). These leaves promoted the production of fungal fruiting structures as they have been sterilized by gamma irradiation. The fungus was grown on carnation leaves for several weeks and then was processed for SEM. The samples were slowly dehydrated in ethanol and then critically point dried, coated with gold and examined with an FEI XL30 scanning electron microscope field emission gun at 5 kV at high-vacuum mode using an Everhart-Thornley detector . A gaseous secondary electron detector was used with a spot size of 3, at 15 kV. The temperature was 4 °C with a chamber pressure which ranged from 5 to 6 T, providing humidity up to 100% at the sample.

Bioassay tests for *Hypoxylon* sp. VOCs against pathogens

The VOCs produced by Ut-1 were tested for inhibitory antimicrobial activity against selected pathogenic fungi according to a bioassay test system described previously for analysis of VOCs produced by *Muscodor albus* (Strobel *et al.*, 2001; Tomsheck *et al.*, 2010). The assays were conducted by removing a 2.5-cm-wide strip of agar from the mid-portion of a standard Petri plate of PDA, creating two isolated halves of agar. The fungus was inoculated onto one semi-circular agar piece and incubated at 23 °C for 10 days to allow for optimum production of volatile compounds. Test pathogens were inoculated onto the semi-circular section of agar opposite the semi-circular section inoculated with Ut-1. The plate was then wrapped with a single piece of parafilm

and incubated at 23 °C for 24 h. Growth of filamentous fungi was quantitatively assessed based on multiple measurements of growth extending from the edge of the inoculum plugs comparable with corresponding controls as described by Strobel *et al.* (2001). All tests were conducted in triplicate.

Qualitative analyses of fungal VOCs

Analysis of gases in the air space above the culture grown for 12 days at 23 ± 2 °C on PDA was undertaken using the solid phase microextraction fiber technique (Strobel *et al.*, 2001). First, a baked 'Solid Phase Micro Extraction' syringe (Supelco) consisting of 50/30 divinylbenzene/carburen on polydimethylsiloxane on a stable flex fiber was placed through a small hole drilled in the side of the Petri plate and exposed to the vapor phase for 45 min. The syringe was then inserted into the splitless injection port of a Hewlett Packard 6890 gas chromatograph containing a 30 m × 0.25 mm inner diameter ZB Wax capillary column with a film thickness of 0.50 μm. The column was programmed as follows: 30 °C for 2 min followed by and increase to 220 °C at 5 °C min^{-1}. The carrier gas was ultrahigh-purity helium (local distributor) and the initial column head pressure was 50 kPa. Before trapping the volatiles, the fiber was conditioned at 240 °C for 20 min under a flow of helium gas. A 30-s injection time was used to introduce the sample fiber into the chromatograph. The gas chromatograph was interfaced to a Hewlett Packard 5973 mass-selective detector (mass spectrometer) operating at unit resolution. The spectrometer was scanned at 2.5 scans s^{-1} over a mass range of 35–360 a.m.u. Data acquisition and data processing were performed on the Hewlett Packard CHEMSTATION software system. Initial identification of the compounds produced by the endophyte was made via library comparison using the National Institute of Standards and Technology (NIST) database, and all chemical compounds described in this report use the NIST database chemical terminology. As far as possible, authenticity of each compound identified by GC/MS was reconfirmed by GC/MS of authentic standards. Standard compounds were obtained from Sigma-Aldrich and run in a comparable manner as the fungal samples. Compounds that were not identified on the basis of a match to an authentic standard were tentatively identified (listed) when they yielded a quality score of 60 or better. The experiments were repeated at least twice.

Preparation of *L. tridentata* extracts

Leaves and leaf fragments of 1.0 g of freshly harvested plant material was thoroughly ground with a mortar and pestle in 40 mL methanol. The methanolic solution was decanted and passed through four layers of cheesecloth to remove plant particles. The solution was taken to dryness by flash evaporation at 37 °C and the residue was stored at − 20 °C.

Koch's postulates on Ut-1

A number of creosote plants were selected and transplanted to the Montana State University greenhouse facility. Inoculation of leaves was accomplished by making two to three pin pricks through each of many leaf blades and then flooding the surface with a suspension of 10^7 spores mL^{-1}. Uninoculated leaves were treated in the same manner, but without the introduction of the spore suspension. The leaves were held at 23 °C in 100% relative humidity for 5–7 days and then evaluated for symptom production. Re-isolation of the putative pathogen was accomplised in the same manner as described above for fungal isolation and recovered fungi were evaluated based on cultural and morphological characters.

Results

Over the course of a number of years several sites in the southern deserts of Utah were sampled in May and June for endophytic microorganisms associated with *L. tridentata*, but with no success. In midwinter, the roots, stems and leaves of a number of bushes were sampled in an area south of St. George, UT, and only one fungal endophyte, and no other microorganism, appeared in the root specimens of the symptomless plants that had been sampled. In early spring, close examination of the leaves of many creosote bushes in this area revealed that they were showing disease symptoms, i.e. small necrotic spots having one or more black pustule-like fruiting stuctures (pycnidia) associated with each lesion. From these diseased areas of the leaves it was possible to isolate the same fungus that had been isolated from the symptomless roots of this plant species. Interestingly, cultures of this fungus were odoriferous but not in the same manner as that of the host plant.

The fungus in each case possessed the following cultural and morphological characteristics.

Colonies on PDA are 50–55 mm after 8 days at 23 °C, olivaceous to greenish olivaceous, forming concentric rings, later turning completely black due to formation of pycnidia; aerial mycelium is almost absent, margin is regular and reverse concolorous. Conidiomata are pycnidial, solitary (sub-)globose to broadly ellipsoidal, glabrous or with some hyphal outgrows, on the agar surface and immersed, later forming concentric rings, 120–200 × 113–145 μm. Ostioles (one to three) are nonpapillate sometimes slightly papillate, circular to oval and 20–25 μm in diameter. The pycnidial wall is pseudoparenchymatous, composed of angular cells and comprises two to four layers. Conidiogenous cells are phialidic, simple, smooth-walled, hyaline, flask-shaped and somewhat isodiametric. Conidia are ellipsoidal to ovoid or

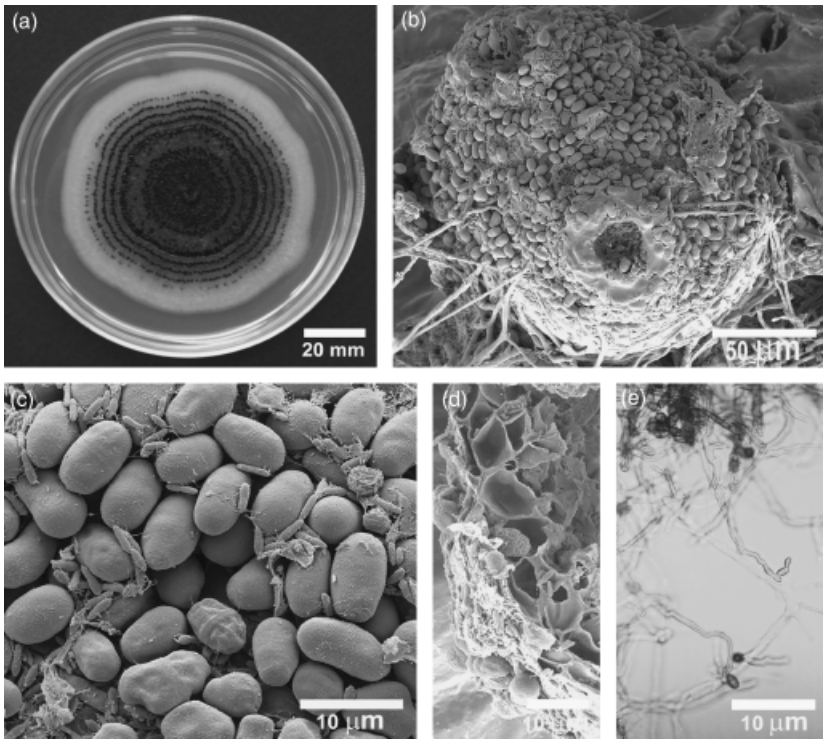

Fig. 1. *Phoma* sp. (a) Colony grown on PDA after 15 days. (b) Scanning electron micrograph of mature pycnidium with subtending mycelium developed on PDA. (c) Scanning electron micrograph of conidia. (d) Scanning electron micrograph of pycnidial wall consisting of three to four layers of textura angularis. (e) Mycelial mat with short conidiophores developed on PDA as per light microscopy.

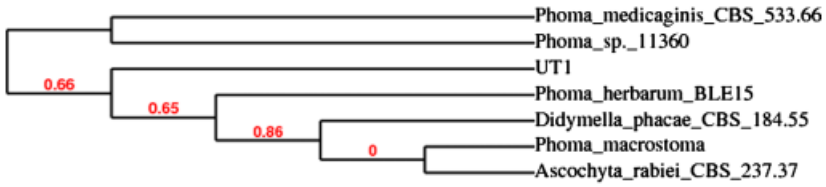

Fig. 2. Phylogenetic position of *Phoma* sp. Ut-1 (Guindon & Gascuel, 2003). Numbers designate branch support values.

subcylindrical, thin and smooth-walled, hyaline, aseptate to septate, extremely variable in size [(5) 5.5–9.5 (10) µm ($x = 7.05$, SD = 1.18, $n = 30$) × (3) 3.5–4.5 (5) µm ($x = 4.26$, SD = 0.64, $n = 30$)] and rarely guttulate (Fig. 1).

Collectively, these morphological features strongly support the placement of the present isolate as a species of *Phoma* Sacc. emend. Boerema & G.J. Bollen (Fig. 1). Furthermore, ITS sequence data showed that the endophyte is a strain of the genus *Phoma* (Fig. 2). The ITS 5.8S ribosomal gene showed a maximum homology of 99.2% with *Phoma herbarum* strain BLE15 and *Phoma* sp. strain 11360. The endophyte also exhibited 99% sequence homology with *Phoma medicaginis* strain CBS 533, *Phoma macrostoma*, *Ascochyta rabiei* (*Phoma rabiei*) strain CBS 237.37 and *Didymella phacae* CBS strain 184.55, as presented in the distance matrix chart (Fig. 2).

No *Phoma* sp. previously has been reported from this plant either as an endophyte or as a pathogen. The genus *Phoma* sp., as typified by *P. herbarum* (Boerema 1964), is a complex and heterogeneous assemblage of more than 3000 infrageneric taxa (Monte *et al.*, 1991). It has been considered to be one of the largest fungal genera, consisting of taxa inhabiting soil, organic debris and water, as well as species that parasitize other fungi, lichens, insects and vertebrates. In addition, a substantial proportion of the taxa are associated with plant material as primary pathogens. In the case of isolate Ut-1, it appears that the fungus can exist in the host plant as both an endophyte and a pathogen under some circumstances. It was possible to show pathogenicity of the organism on inoculated leaves of the host, yielding necrotic spots. Also, subsequently it was possible to successfully reisolate the causal agent using standard procedures followed by identification of the organism on the basis of its morphological features (Fig. 1).

Production of VOCs by *Phoma* sp.

When *Phoma* sp. was grown on PDA for 10–12 days and the headspace was examined for VOC content the most significant observation was that at least 15 compounds appeared whose mass was 204 and whose chemical assignment was that of a sesquiterpene, with α-humulene (or

Table 1. The VOCs of a 12-day-old culture of *Phoma* sp. as determined by GC/MS

Compound	Retention time (min)	Response	Quality	Molecular weight (g mol^{-1})
2-Butanone	3.191	1.02	72*	72
Ethanol	3.670	27.1	83*	46
1-Propanol, 2-methyl-	6.159	1.09	90*	74
Unknown	7.789	1.14	40	172
1-Butanol, 3-methyl-	7.937	25.3	78*	88
1H-3a, 7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-,[3r-(3α,3αβ,7β,8aα)]-	10.624	59.5	87	204
Benzene, 1,3,5-tris (1-methylethyl)-	10.866	2.30	58	204
β-Chamigrene	11.186	1.70	62	204
Longipinene	11.745	2.65	97	204
trans-Caryophyllene	11.925	22.8	72*	204
α-Longipinene	11.998	13.3	87	204
(+)-Aromadendrene	12.204	5.05	60	204
β-Ylangene	12.380	6.48	86	204
Unknown	12.854	1.20	50	80
Unknown	12.955	11.2	58	204
(+)-Aromadendrene	13.079	26.2	91	204
4-(3-Butenyl)-1,2,3,6,7,7a-hexahydro-7a-methyl-5H-inden-5-one	13.142	3.36	83	204
Unknown	13.199	4.08	45	161
Unknown	13.467	48.8	49	222
1-Hexene, 2-(P-anisyl)-4-methyl-	13.918	18.9	80	204
Epi-bicyclosequiphellandrene	14.027	1.72	83	204
1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-octahyro-3,5,5-trimethyl-9-methylene-,(4as-*cis*)-	14.126	26.6	99	204
β-Selinene	14.230	3.16	64	204
δ-Guaiene	14.411	15	76	204
α-Humulene	14.542	194	91*	204
α-amorphene	14.629	3.18	99	204
Unknown	14.698	12.2	49	204
β-Himachalene	14.910	3.52	99	204
Cadinene	14.936	2.49	64	204
Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	15.069	3.74	96	204
2,3,4-Trimethyl-4-hydroxy-1,4-dihyronaphthalenone	15.128	6.62	83	202
6,10,11,11-Tetramethyl-tricyclo[6.3.0.1(2,3)]undec-7-ene	15.426	4.24	95	204
Unknown	15.488	6.04	38	181
Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1s-*cis*)-	16.315	1.16	96	202
Unknown	16.374	1.83	50	222
Unknown	16.838	1.68	–	–
Benzeneethanol	17.204	1.60	95*	122
Naphthalene, decahydro-, *cis*-	18.689	1.37	64	138
β-Selinene	19.269	1.14	94	204
Unknown	21.003	1.45	46	152
Unknown	21.186	2.37	46	220

The 'Quality' column indicates the confidence with which compound identity can be assumed (an asterisk indicates that an authentic standard compound yielded the same mass data and retention time as the fungal compound). The 'Response' column indicates the relative amounts of the compound detected.

α-caryophyllene) being the most predominant VOC (Table 1). Furthermore, *trans*-caryophyllene is also present in the fungal VOC headspace and it too is a major VOC in the volatiles of *L. tridentata* (G. Strobel, unpublished data). Also of interest is the presence of a number of reduced naphthalene derivatives such as those with retention times of 15.06, 15.12, 16.31 and 18.68 min (Table 1). Reduced naphthalene compounds of this type have been reported from *M. albus* (Strobel *et al.*, 2001). GC/MS analyses of diesel fuel from all parts of the world have revealed the presence of reduced and sometimes derivatized naphthalenes of the general type produced by *Phoma* sp. (Adams & Richmond, 1951; G. Strobel, unpublished data). Likewise, benzene and its derivatives are found in diesel fuels worldwide (Adams &

Table 2. The VOCs of a 15-day-old culture of *Phoma* sp. as determined by GC/MS

Compound	Retention time (min)	Response	Quality	Molecular weight (g mol^{-1})
Heptane	1.62	10.50	87*	100
Acetaldehyde	1.73	12.30	72*	44
Heptane, 2, 4-dimethyl	2.16	10.40	72*	128
Unknown	2.41	–	–	–
Acetic acid, ethyl ester	3.13	58.0	80*	88
Ethanol	3.81	411.9	78*	46
Propanoic acid, 2-methyl-	4.18	9.3	87*	116
Butanoic acid, 2-methyl, ethyl ester	5.53	13.9	94*	130
Butanoic acid, 3-methyl-, ethyl ester	5.77	9.8	94*	130
2-Butanol	6.28	50.4	90*	74
1-Butanol, 3-methyl-, acetate	6.65	15.44	90*	130
1-Butanol	7.09	10.21	91*	74
1-Butanol, 3-methyl-	8.03	556.9	90*	88
Acetic acid	12.16	71.71	90*	90
Propanoic acid, 2-methyl-	13.56	12.3	91*	88
Benzenethanol	17.42	73.02	95*	122
Dodecanoic acid	23.29	9.12	92	200
Hexadecanoic acid	13.467	11.97	94	256

The organism was grown in a brown bottle for 5 days then the cap sealed for the remaining 10 days of incubation at 23 °C. The 'Quality' column indicates the confidence with which compound identity can be assumed (and asterisk indicates that an authentic standard compound yielded the same mass data and retention time as the fungal compound). The 'Response' column indicates the relative amount of the compound detected.

Richmond, 1951). At least one benzene derivative is found in the *Phoma* sp. headspace at 10.86 min, and benzeneethanol (= phenylethyl alcohol) is also present at 17.2 min. The latter is a common VOC product of these endophytic fungi (Strobel *et al*., 2007). Other products of interest include alcohols and ketones, which undoubtedly contribute to the biological activity of the organism (Strobel *et al*., 2001).

When the *Phoma* sp. was grown on PDA in a regular atmosphere for 5 days and then the container sealed to yield a limited oxygen environment for 10 days, the VOCs found in the headspace were entirely different (Table 2). For instance the most abundant products were 1-butanol, 2-methyl and ethanol. Smaller quantities of the following compounds were also detected: butanoic acid, 2-methyl-ethyl ester; butanoic acid, 3-methyl-ethyl ester; 1 propanol, 2-methyl; and propanoic acid, 2-methyl ethyl ester and ethyl acetate. Interestingly, none of the terpenes appeared, suggesting that they require greater amounts of oxygen to form.

Antifungal activity of the *Phoma* sp. VOCs

As the organism produced a plethora of organic substances and emitted an aromatic odor it seemed logical to test the cultures for activities of the headspace VOCs. Unlike the VOC activity of many *Muscodor* spp., this endophyte did not kill any test fungus (Table 2; Strobel *et al*., 2001). To this end, the test fungus giving the greatest response to the *Phoma* sp. VOCs was *Phytophthora palmivora* with approximately 50% inhibition (Table 3). *Verticillium dahliae*, *Ceratocystis ulmi* and *Cercospora beticola* also were reasonably strongly inhibited by the fungal VOCs. On the other hand, some fungi were not affected at all, including *Trichoderma viride* and *Colletotrichum lagenarium* (Table 3).

Crude *L. tritendata* extract residue (50 mg) was placed on a PDA plate and challenged (small agar blocks with the test organism placed within 1–1.5 cm of the plant extract) with many of the same pathogens as per the fungal VOC test. Within 24 h it was obvious that the residue was expressing inhibitory activity against some of these fungi. The same test fungi that were not inhibited by the *Phoma* sp. VOCs likewise were not affected by the plant extract (Table 2). However, in the case of the plant extract, the most sensitive test fungi were *V. dahliae* and *Sclerotinia sclerotiorum* and they too were inhibited by the VOCs of *Phoma* sp., but never at the 100% level as with *V. dahliae* (Table 2). The results indicate, as was initially pointed out, that plants enriched in hydrocarbons, especially terpenoids, seem to possess anti-pest properties.

Discussion

Endophytes producing a plethora of VOCs appear uncommon; in an unpublished survey of over 40% of 87 endophytes of oil palm there were no detectable fungal VOCs and about 20% produced only one to three VOCs while the remainder produced between three and eight (Green, Synthetic Genomics Co., La Jolla, CA). However, we show here that an endophyte/pathogen found in a plant known for

Table 3. The biological activity of the VOCs of *Phoma* sp. against a range of plant-associated fungi

Test fungus	Percentage inhibition over control after 24-h exposure to *Phoma* sp.	Percentage inhibition over control after 24-h exposure to 50 mg of *L. tritdentata* extract
Aspergillus flavus	0.0	0.0
Botrytis cinerea	8.0 ± 11	27.6 ± 3.7
Ceratocystis ulmi	44 ± 22	38.6 ± 8.0
Cercospora beticola	19.9 ± 5.6	44.3 ± 8.0
Colletotrichum lagenarium	0.0	0.0
Fusarium solani	10.3 ± 7.6	40.3 ± 8.0
Phytium ultimum	6.4 ± 2.5	39 ± 25
Phytophthora palmivora	53.3 ± 28.6	15 ± 10.8
Sclerotinia sclerotiorum	17.9 ± 3.9	83 ± 28
Trichoderma viride	0.0	0.0
Verticillum dahliae	32.7 ± 13.1	100.0

The tests were conducted as described in Materials and methods. In addition, a methanolic extract of *Larrea tridentata* was examined on PDA using the same test fungi.
The data are the mean ± SD of three observations.

volatile hydrocarbon production can also produce a plethora of volatile hydrocarbons when in an isolated state, and some of the molecules are quite complex (Table 1).

The largest class of natural substances, the terpenoids, also makes up the largest number of volatile compounds detected by GC/MS as produced by *Phoma* sp., an endophyte on creosote bush (Table 1). In the case of *Phoma* sp. it appears that the terpenoids produced are limited to those in the category of sesquiterpenoids, although other chemical classes are also represented (Table 1). Other VOCs, as expected, are produced when the organism is grown under microaerophilic conditions (Table 2). It would appear that this is only one case out of many that may exist in nature in which a microbial endophyte may mimic the biochemistry of its host in order to survive the conditions of a stressful environment. Although both the host and the endophyte do produce at least one hydrocarbon in common, namely *trans*-caryophyllene, the most abundant fungal product is *cis*-caryophyllene or humulene (Table 1). Although the products of both the host and the endophyte are antifungal, it remains to be seen what the role of each of these sets of products might be in the defense of the host in its native state and what role they play in the ability of the host and its endophyte/pathogen to survive a relatively harsh environment. The myriad of VOCs, such as alcohols, and other reduced products of this organism have potential as bio-fuels.

The endophytic/pathogenic nature of *Phoma* sp. may not be unique to this organism. Other endophytic species, *Pestalotiopsis* spp., are well-known plant pathogens of tropical plants yet can be readily found as endophytes. The age, nutritional status and general environment of the plant more or less dictate the outcome of the host/microorganism relationship, as experimentally demonstrated by Madar *et al.* (1991).

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