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Production and effects of volatile organic compounds during interspecific interactions

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ABSTRACT

Competition between mycelia of saprotrophic cord-forming basidiomycetes occurs both within dead woody resources and in the soil-litter interface, and involves a variety of antagonistic mechanisms including the production of volatile organic compounds (VOCs). The antagonistic potential of VOC profiles from interactions in wood blocks and in soil microcosms was assessed using shared headspace experiments, and the profile of VOCs emitted over the course of interactions elucidated using solid phase microextraction (SPME) with gas chromatography-mass spectrometry (GC–MS). Quantitative and qualitative changes in VOC production occurred in interactions compared to self-pairing controls, with different VOC profiles from fungi growing in wood blocks compared to soil trays. There were both stimulatory and inhibitory effects of VOCs on target mycelial extension rate, hyphal coverage and fractal dimension. VOC-mediated effects were greater in self-pairing controls compared to interactions, and differed depending on the substratum in which the VOC-producing fungi were growing.

1. Introduction

Saprotrophic basidiomycetes are the major agents of primary decomposition in woodland ecosystems, by virtue of their ability to break down complex lignocellulose (Rayner and Boddy, 1988). Whilst the majority of wood decay fungi are confined to the resource which they are decaying, only spreading elsewhere as spores, some are able to grow out as mycelium which aggregate to form persistent mycelial cords (Boddy, 1993, 1999; Rotheray et al. 2008). These cord-forming basidiomycetes are major agents of wood decomposition and nutrient cycling.

Competition by wood decay fungi is effectively competition for space (Boddy, 2000). When a mycelium occupies a volume of wood, it has access to all of the nutrients within that wood, provided that it has appropriate enzymatic ability. Species vary in their antagonistic combative ability, and this is often a major determinant of fungal community structure and dynamics, which in turn has a major effect on decay rate and nutrient cycling, since different species effect decay at different rates (Fukasawa et al., 2011). Antagonistic interactions occur within wood and, in the case of cord-forming fungi, also in the soil when mycelia grow out in search of new resources (Donnelly and Boddy, 1998). The outcome of these interactions can be: deadlock, where neither fungus gains territory; replacement, where one fungus takes over the territory of another; or partial and even mutual replacement (Boddy, 2000). The outcomes vary depending on species combinations, climatic conditions, size and state of decay of the resource, where the interaction is occurring, e.g. wood or soil, and the presence and activity of organisms. Combative interactions are accompanied by production of extracellular enzymes, changes in gross morphology, and the production of antagonistic metabolites such as volatile and diffusible organic compounds (VOCs and DOCs; Griffith et al. 1994; Boddy, 2000; Baldrian, 2004; Heilmann-Clausen and Boddy, 2005; Hynes et al. 2007; Woodward and Boddy, 2008; Evans et al. 2008).

Fungi, like many microorganisms, produce a wide range of VOCs spanning a variety of chemical classes, from short chain alcohols and ketones to high molecular weight aromatic compounds and terpenes (Wheatley and Hackett, 1997; Rösecke et al., 2000; Humphris et al. 2002; Ewen et al. 2004; Ladygina et al., 2006;

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Lemfack et al., 2013). These compounds may be produced constitutively and individual fungi often produce a characteristic profile which can be used in chemotaxonomic identification (Polizzi et al. 2012). This VOC profile is, however, affected by the growth substrates, pH, age, temperature, and by biotic interactions (Chen et al. 1984; Jelen et al., 2002; Wheatley, 2002; Ewen et al. 2004; Wu et al. 2005; Hynes et al. 2007; Evans et al. 2008). Certain VOC profiles are biologically active against mycelia of other fungi, which are physically separated but sharing an atmosphere, inhibiting or stimulating their growth (Dick and Hutchinson, 1966; Schoeman et al., 1996; Mackie and Wheatley, 1999; Evans et al. 2008; Schmidt et al., 2015). The antagonistic potential of a VOC profile is dependent on its chemical composition and the susceptibility of the combatant, with effects often subtle and manifested as changes in growth rate or enzyme activity (Strobel et al. 2001). VOCs may also function as 'infochemicals' signalling the presence of a competitor, or a potential mate, or a larger conspecific mycelium (Wheatley, 2002).

VOC profiles are dynamic, changing during interactions with other fungi both qualitatively and quantitatively. For example, during the interaction between cord-forming fungi *Hypholoma fasciculare* and *Resinicium bicolor* growing on malt broth, 10 VOCs were identified that were not detected in single-species controls (Hynes et al. 2007). Similarly, 8 interaction-specific VOCs were detected during interactions between *Trametes versicolor* and *Stereum gausapatum*; changes in VOCs profiles due to interactions are, however, dependent on the combinations of species involved (Evans et al. 2008). The VOCs were predominantly identified as terpenes (mono- and sesqui-) and aromatic compounds, which are ecologically significant, in some cases possessing antifungal activity which could represent an antagonistic mechanism during interspecific interactions (Viiri et al. 2001).

The biological activity of VOCs indicates that they will have a variety of effects on the local microbial community (Ramirez et al., 2009). VOCs are likely to accumulate within the enclosed vessels of woody tissue, and in the insulated litter layer. This could form a zone around the producer that inhibits certain elements of the microbial community, whilst stimulating others. The effects on the community would be further altered by changes to VOC profiles stimulated by interspecific interactions. To understand fully the effects a mycelium is having on decay community structure, an accurate profile of its metabolites is needed. To achieve this, an ecologically relevant substratum must be used. Previous studies have examined the production and antifungal effects of VOCs during interactions on artificial media (Hynes et al. 2007; Evans et al. 2008), and others have compared VOC profiles during single-species growth on different substrata (e.g. Fäldt and Jonsell, 1999; Ewen et al. 2004). However, no previous work has been performed on VOC production and effects during interactions in natural substrata. The present study aimed to determine: (1) the effect of VOCs produced by cord-forming basidiomycetes during interactions and growth alone in natural substrata (wood blocks and across soil) on the growth of remote 'target' mycelia sharing the same atmosphere; and (2) how VOC production changes during growth in natural substrata, using GC–MS (gas chromatography-mass spectrometry). Further, following the findings on artificial media we hypothesised that different species combinations would produce different VOC profiles, which would be different from the profile of the fungi growing alone. We also hypothesised that the VOC profile of a fungus would be altered by the availability of different chemical precursors within different growth substrata, and that this would affect any inhibitory activity of the fungal volatiles.

2. Methods

2.1. Preparation of inocula

Cultures of five cord-forming wood decay basidiomycetes (*Hypholoma fasciculare, Phanerochaete velutina, Phallus impudicus, Resinicium bicolor and Phlebiella vaga*), obtained from the Cardiff University Culture Collection (previously isolated from fruit bodies or mycelial cords), were maintained on 2% malt agar (MA; 20 g l^{-1} malt extract, 15 g l^{-1} agar; Lab M, Lancs, UK) in the dark at 20 °C. Freshly felled beech (*Fagus sylvatica*) wood blocks were sterilised by freezing at −20 °C, then autoclaving 3 times at 24 h intervals. Pure fungal cultures were inoculated onto 2% MA in either 2 l conical flasks (2 × 2 × 2 cm blocks) or 14 cm diameter Petri dishes (2 × 2 × 1 cm and 4 × 2 × 1 cm blocks; Greiner Bio-one Ltd, UK). Once mycelial growth covered the agar surface sterilised wood blocks were added: 25 blocks to the conical flasks and 20 blocks to the 14 cm Petri dishes, and incubated in the dark at 20 °C for 8 weeks.

2.2. Preparation of soil trays

Soil was collected to 20 cm depth from mixed deciduous woodland (Coed Beddick, Tintern, UK). Soil was sieved through 10 mm mesh, air-dried, sieved through 4 mm and 2 mm meshes, then frozen overnight at −20 °C to kill any soil microfauna. The soil matric potential was adjusted to −0.012 MPa (determined by the method of Fawcett and Collins-George, 1967), and 200 g wet soil was evenly compacted into either square (24 × 24 cm) or circular (14 cm) lidded bioassay trays (Nunc-Gibco, Paisley, UK) to a depth of 5 mm. Colonised wood blocks were scraped free of adhering mycelium and placed on the soil surface; either as a central, single inoculum, or by placing two blocks in the tray corners diagonally opposite each other (8 cm from each corner, and 9 cm apart from each other). Where interactions were set up, timing of addition of slower-growing competitors was staggered to ensure that mycelia met in the centre of the tray. Trays were incubated at 20 °C in the dark and sprayed with distilled water weekly to maintain water potential.

2.3. Determination of interaction outcomes

Interaction outcomes were determined by measuring the proportion of each block occupied by different colonisers. Blocks were split in half using a sterile chisel, pieces of wood (2 mm^3) were excised from 4 regions within each block, representing each of 25% intervals progressing outward from the interaction zone (the surface touching the competitor block in wood block interactions), or the initial point of contact (in soil tray interactions). Excised fragments were inoculated onto 2% MA and incubated at 20 °C until mycelium had emerged and could be identified morphologically. Interactions were recorded as deadlock (where neither competitor gained territory from the other), or replacement (where one competitor completely replaced the other).

2.4. Effects of VOCs produced during interactions in wood

Colonised wood blocks (4 × 2 × 1 cm) of *H. fasciculare* and *R. bicolor* were fixed, abutting each other along their thin edges, in the centre of a 14 cm Petri dish using 1 cm long stainless steel nails. Self-pairings of each species and blank controls were also set up. Four 1 mm holes were made in the side of each dish for aeration, and the lidless dishes placed above a similar dish containing sterile distilled water and incubated in the dark at 20 °C for 14 d (interactions) or 7 d (controls). The bottom dishes were subsequently