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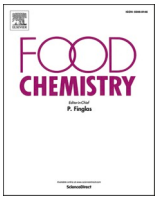

Early detection of dry bubble disease in *Agaricus bisporus* using volatile compounds

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ABSTRACT

Lecanicillium fungicola is a pathogen of the commercial white button mushroom (*Agaricus bisporus*) and is the causal agent of dry bubble disease, which can cause severe economic losses to mushroom growers. Volatile compounds were measured by GC/MS techniques over pure cultures of mycelia on agars, over microcosms of growing mushrooms, and over harvested mushrooms to identify compounds that might give an early warning of the disease. The mushroom strain tested was *Agaricus bisporus*, strain Sylvan A15; either deliberately infected with *L. fungicola* or water as a control. Over microcosms, the appearance of β-copaene, β-cubebene, and α-cedrene coincided with, but did not precede, the earliest visual signs of the disease. Mushrooms with dry bubble symptoms also had high levels of β-barbatene and an unknown diterpene (UK 1821). Over some harvested mushroom sets, high levels of *cis*-α-bisabolene developed as a defence reaction to infection.

1. Introduction

Global production of cultivated mushrooms (and truffles) has been estimated to be 44 million tonnes in 2021 (FAOSTAT, 2023). *Agaricus bisporus*, or the common button mushroom, represents about 40% of worldwide commercial mushroom production (Berendsen et al., 2010). There is currently a lack of genetic diversity in commercial *A. bisporus* strains which increases the risk of a single disease infecting a large volume of worldwide production (Savoie et al., 2013).

The nutrients for growth of *A. bisporus* fruiting bodies are provided by specific mushroom compost. The main raw ingredients of mushroom compost are either a mixture of gypsum and horse manure, or gypsum and chicken manure mixed with wheat straw (Straatsma et al., 1994). The mixed ingredients undergo an initial composting phase (Phase I), followed by semi-pasteurisation and conditioning phases (Phase 2) (Kertesz and Thai, 2018). The next step, the 'spawn run', begins with the inoculation of the compost with a spawn of *A. bisporus* and ends with colonisation of the compost with the vegetative mycelium (Kertesz and Thai, 2018). After the spawn run is complete, the compost is covered with a layer of 'casing' (usually a mixture of peat and lime) and incubated at 25 °C to initiate the formation of fruiting bodies (Eastwood et al., 2013). The vegetative mycelium is allowed to colonise the casing. The formation of mushrooms is triggered by reducing the temperature from 25 °C to 18 °C, and ventilating the growing room in a process known as 'airing' (Eastwood et al., 2013). The mushrooms are subsequently harvested in a series of 2 or 3 batches, known as 'flushes'.

Dry bubble disease, caused by the pathogen *Lecanicillium fungicola*, is a serious and recurrent problem in the commercial cultivation of white button mushrooms. This disease is estimated to cause an annual loss of revenue to mushroom growers of 2–4% (Berendsen et al., 2010). An investigation into the active microbial community in compost using extracted RNA indicated *L. fungicola* was active in compost at the initial house filling stage, and also after the first flush of mushrooms was complete (McGee et al., 2017). Despite this activity, incidence of dry bubble disease throughout the three flushes in the study was below typical levels (McGee et al., 2017). Although spores may be present in the peat used for casing, their germination and growth is inhibited by competing microorganisms (Berendsen et al., 2010). The spores in

Abbreviations: AB, *Agaricus bisporus*; CAC, Compost added at casing; HAC, Heirarchical Agglomerative Clustering; LDA, Linear Discriminant Analysis; LF, *Lecanicillium fungicola*; LOO, Leave-one-out; MEA, Malt extract agar; PCA, Principal Component Analysis; PDA, Potato dextrose agar; StepDisc, Stepwise Discriminant; UK, Unknown; VARCLUS, Variable Clustering; VOC, Volatile organic compound.

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casing remain dormant until *A. bisporus* colonises the casing, causing the spores to germinate and grow alongside the vegetative *A. bisporus* mycelium (Berendsen et al., 2010). Although *L. fungicola* hyphae become attached to the vegetative mycelium of *A. bisporus* in casing, only the fruiting body mycelium of the developing mushrooms is subject to degradation (Calonje et al., 2000). Holmes (1971) found deliberate infection of casing with *L. fungicola* was most effective in inducing dry bubble disease at the point when *A. bisporus* had colonised the casing but not yet formed primordia.

L. fungicola infection of *A. bisporus* may produce different characteristic symptoms depending on the stage of development of the mushroom at infection. These symptoms range from small necrotic lesions on the cap surface (cap spotting), to partial deformation (stipe blow-out) or full deformation (dry bubble). North and Wuest (1993) were able to induce dry bubble or stipe blow-out in mushrooms when primordia were infected. Infection of young pilei led to stipe blow-out. Cap spotting could be induced at any stage in mushroom development. In addition, a mushroom may be infected with *L. fungicola* but not show symptoms (Berendsen et al., 2010).

Real-time monitoring of the infection status of developing mushrooms is reliant on visual inspection. Confirmation of *L. fungicola* infection may take a few days on selective media (Piasecka, Kavanagh, & Grogan, 2011) by which point it is too late to take effective control measures. Radvanyi et al. (2015) have investigated by HS SPME GC/MS the volatile compounds produced over 15 days by pure cultures on Potato Dextrose Agar (PDA) by *A. bisporus* A15, and a number of common mushroom diseases i.e. *L. fungicola* var *fungicola*, *M. perniciosa*, *T. aggressivum f. europaeum*, and an unknown *Trichoderma* species isolated by a mushroom grower. Radvanyi (2023) has also monitored the volatile compounds produced by postharvest button mushrooms (unspecified strain of *A. bisporus*) over 8 days, and also observed the changes in the volatile profile induced by inoculating the postharvest mushrooms with *M. perniciosa* and *T. aggressivum*.

The main hypothesis of this work is that volatile compounds can be used to detect dry bubble disease in white button mushrooms before the appearance of visual symptoms. This information is intended to be used to inform the development of rapid sensing instrumentation for the early detection of *L. fungicola* in mushroom growing systems. It is anticipated that this will enable early interventions that will prevent crop loss due to dry bubble disease.

2. Materials and methods

2.1. HS SPME GC/MS procedure for L. fungicola on PDA and A. bisporus on MEA agars

This experiment was designed to provide a list of what volatile compounds are produced by the organisms *A. bisporus* and *L. fungicola* when grown on agar plates over a 30-day period. Three subcultures (A, B and C) of *Lecanicillium fungicola* (originally obtained from a dry bubble from an Irish mushroom farm) were prepared by cutting 2 mm squares from the growing edge of a colony on Potato Dextrose Agar (PDA) and placing each one inverted in the centre of a PDA plate. The plate was sealed with parafilm, kept in the dark at 20 °C, and tested for VOCs on days 1, 2, 3, 6, 7, 13, 14, 15, 20, 21, 22, 28, 29 and 30. A blank PDA plate was used as a control. A similar protocol was followed for three subcultures of *Agaricus bisporus* (strain Sylvan A15) on Malt Extract Agar (MEA).

On each testing day, a 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre (Sigma-Aldrich Company Ltd., Dorset, England) was exposed over the centre of each culture by lifting the lid of the agar plate just sufficiently to allow entry of the SPME needle. Exposure times were 3 h at 20 °C. The VOCs were measured using a Shimadzu QP-2010 GC/MS (Shimadzu UK Limited, Milton Keynes, UK). The Gas Chromatograph was fitted with an Agilent Crosslab Ultra Inert SPME inlet liner (Agilent Technologies, Inc., Santa Clara, CA) and an Agilent J&W DB-1MS UI GC Column 30 m × 0.25 mm ID × 0.25 μm film thickness (Agilent Technologies, Inc.). Temperature settings were: injection port 270 °C, interface 270 °C, MS ion source 200 °C. The injection port was run in splitless mode with a sampling time of 1 min. The carrier gas was Helium at 1 mL/min. The GC oven temperature program was: Initial 40 °C (hold 1 min); ramp 40°-270 °C at 10 °C per min; hold 270 °C for 10 min. The mass spectrometer was set to monitor the *m/z* range 35–500 from 1.2 to 34 min with a scan speed of 2500. The SPME fibre was cleaned in the GC injection port for 30 min at 270 °C before re-use. Linear retention indices were calculated from separate SPME experiments under the same instrumental conditions with a range of *n*-alkanes from C5-C30 (Sigma-Aldrich Company Ltd., Dorset, England) according to van Den Dool and Kratz (1963). Compounds were identified from their retention index and comparison of their EI-MS spectra with the NIST/EPA/NIH 2011 Mass Spectral Library. Retention indices for the DB-1MS GC column were compared against the Massfinder4 Terpenoid database (Hochmuth Scientific Consulting, Germany). Mass spectra and retention indices were also compared with the Essential Oil GC/MS library of Adams (2007) and peer-reviewed scientific publications. Compounds were quantified by selecting a characteristic ion for each compound (the quant ion) and calculating the peak area of the ion.

2.2. MonoTrap procedure for mushroom microcosms

Mushrooms (*A. bisporus,* strain Sylvan A15) were cultivated in microcosms composed of 3 kg of mushroom compost overlaid with 1.5 kg of mixed CACed casing (5% compost, 95% mushroom casing). They were maintained in an environmental chamber for 21 days using a stepped incubation temperature programme from 25 °C to 18 °C; and the volatile compounds produced on cropping days 3, 6, 9, 14, 17, and 21 were monitored using RCC18 MonoTrap rods (Hichrom Limited, Reading, UK).

On each sampling day, a MonoTrap was suspended above each microcosm, approximately 1 cm from the casing surface to collect volatiles for 24 h. A separate control MonoTrap was used to monitor the air outside the environmental chamber. In the first experiment, *L. fungicola* was applied in water to the microcosms on cropping day 5 to induce dry bubble disease. In the second experiment, the control, the same volume of water was applied at day 5 without *L. fungicola*. After exposure, MonoTraps were stored at 4 °C in individual screw-cap 20 mL headspace vials (Sigma-Aldrich), until day 21, before shipping to the UK for analysis.

The MonoTraps were extracted into 300 μL of analytical grade dichloromethane (Fisher Scientific UK Ltd., Loughborough, England) with 5 min of sonication. The solvent volume was reduced to 10 μL (measured as 13.3 mg on a 4-place balance) by evaporation in a 300 μL clear glass vial insert under a very gentle stream of nitrogen to concentrate the volatiles. Injections were made only once from this small sample. The GC/MS configuration was adjusted for 1 μL splitless liquid injections by replacing the low volume SPME liner with a standard volume injection port liner (Shimadzu UK Limited). The MS program was changed to include a 3 min solvent cut time to avoid saturating the MS with solvent ions. All other instrumental conditions were kept the same. We initially found insufficient volatiles in a single MonoTrap and had to extract the MonoTraps together from 3 microcosms to obtain a satisfactory signal. This prevented statistical estimates of the variance between the same types of microcosm. Linear retention indices were calculated from separate experiments under the same instrumental conditions with a range of *n*-alkanes from C5-C30 in dichloromethane (Sigma-Aldrich Company Ltd.).

2.3. HS SPME GC/MS procedure for harvested mushrooms

Four batches of mushrooms were harvested from trials as described in 2.2 by MBio, Tyholland, Ireland, across a 12-month time period. Each

batch comprised a combination of defective (D), sound (S) or control (C) mushrooms. Defective (D) mushrooms had been deliberately infected with *L. fungicola* and were either mis-shapen (D1 to D7; D11, D12) or had cap spotting (D8 to D10). Sound (S) mushrooms had also been deliberately infected with *L. fungicola*, but showed no visual symptoms of the disease. Control (C) mushrooms were uninfected, and showed no visual symptoms. An unspecified number of the D and S sets had also been treated with a *Bacillus*- based biocontrol agent, or Sporgon® 50 WP, as part of the trial. For testing, there were 12 defective (D) mushrooms sets, 18 sound (S) sets and 7 control (C) sets, which were measured for volatiles 1 or 2 days' post-harvest. Sample set *C*2 had been deliberately aged, and measured once, at 7 days' post-harvest. A repeat measure was made on sets D1 (day 7: D1RPT) and D3 (day 4: D3RPT), which increased the total number of defective sets to 14.

Mushrooms were loaded into a 500 mL Kilner Jar for volatile testing to a mean mass of 55 g (SDev 15 g). A Swagelok fitting in the Kilner top lid permitted entry of the SPME needle via a septum. The exposed SPME fibre was positioned in the headspace, 1 cm from the mushrooms. Exposure times were 3 h at 20 °C. Mushroom physiology is strongly affected by temperature (Eastwood et al., 2013), so this temperature was chosen to approximate that of commercial growing mushrooms, and pre-empt any changes in the volatile profile. Furthermore, the volatile data was also intended to inform the design of rapid sensor technology which would operate at mushroom house temperature (i,e, 25 °C stepped down to 18 °C), Empty Kilner Jars were used as a control. The SPME fibre type and analytical procedure was identical to that described earlier for cultures on agars.

2.4. Statistical analysis

Two datasets were used for the analyses. The dataset for the microcosm experiments comprised: 6 single measures over control mushroom microcosms, 6 single measures over infected (dry bubble) mushroom microcosms, 2 single measures of the lab air outside the control mushroom environmental chamber, and 2 single measures of the lab air outside the infected mushroom environmental chamber.

Statistical analysis of volatile compound data was carried out using the free data-mining software, TANAGRA (Rakotomalala, 2005). Principal Component Analysis (PCA) was carried out on data from the microcosm experiment by normalising the data (mean subtraction and division by the standard deviation) and using the correlation matrix.

The dataset for the harvested mushrooms comprised: 14 single measures for defective mushrooms sets, 18 single measures for sound mushroom sets, and 7 single measures for control mushroom sets.

Using TANAGRA, Linear Discriminant Analysis (LDA) was carried using StepDisc with forward selection and a default F to enter of 3.84 to select variables (unless stated otherwise), The LDA model was tested using a leave-one-out (LOO) error evaluation. Varimax rotation was carried out on the PCA from the harvested mushrooms using the default setting of 2 factors. VARHCA was carried out to cluster variables, using the change in explained variance to stop at the optimal number of clusters. The correlations between variables were calculated using the software, PAST Version 4.05 (Hammer et al., 2001), using Bonferroni correction for probabilities.

3. Results and discussion

3.1. Identified compounds

Table S1 (Supplementary data) is a summary list of 83 volatile compounds identified from chromatograms for pure cultures, harvested mushrooms, and mushroom growing microcosms. The compounds are listed in order of elution from the GC column. Some of these compounds could not be identified; and were given the prefix UK (for 'unknown') and a suffix for the retention index. Tentative identifications are indicated by a question mark. The 'search ions' were specific ions in the mass spectrum of each compound which were useful in identifying individual compounds from the chromatogram. One of these search ions, the quant ion, was chosen to represent the amount of each compound.

81 compounds were detected over harvested mushrooms by SPME; indicated by the symbol 'H' in Table S1 (Supplementary data). The symbol 'LF' indicates compounds found over *L. fungicola* grown on PDA; and the symbol 'AB' indicates compounds found over *A. bisporus* grown on MEA. The 47 compounds that were detected by MonoTraps over microcosms are indicated by the symbol 'M',

The 83 compounds were split into 3 groups by volatility. Acetone was by far the most volatile compound, and was treated as a separate entity. The high-volatility compounds were defined as 1,3-octadiene to 1-undecene in the order they eluted from the GC column. All compounds eluting after 1-undecene were classed as low volatility. The last 10 eluting compounds (from UK 1798 to UK 2075) with a molecular ion of 272 or 274 were defined as 'late-eluting compounds'.

3.2. Pure cultures

Over agar subcultures, individual volatiles over the mycelia peaked and declined at different times over 30 days. Of the high volatility compounds, linalool was the most striking. Linalool peaked at day 3 over *L. fungicola* on PDA at a level approximately 200 times higher than *A. bisporus* on MEA. This remarkable difference did not appear when *L. fungicola* was applied in subsequent experiments with microcosms and harvested mushrooms.

For *L. fungicola* on PDA there was an initial peak for 1-octen-3-ol on day 1, followed by a rapid decline to a low level over 5 days. 3-octanone was more abundant and more persistent, peaking on day 3, and declining to a low level on day 28. The development of 1-octen-3-ol and 3-octanone over *A. bisporus* on MEA was different, with both compounds rising to a peak before day 20, with 1-octen-3-ol more prominent. Subsequent volatile measurements on sets of harvested mushrooms in Kilner jars always found 3-octanone to be far more prominent than 1-octen-3-ol (data not shown), irrespective of whether the mushroom was infected with *L. fungicola* or not, and despite efforts to avoid tissue damage when loading the jars. Tissue damage to developing or post-harvest mushrooms releases 8-carbon volatile compounds in an enzymatic process. 3-octanone is the most prominent compound released by light damage (slicing), whilst 1-octen-3-ol is prominent after homogenisation (Combet et al., 2009; Costa et al., 2013).

L. fungicola on PDA was a prolific producer of low volatility compounds. The low volatility compounds tended to peak at day 21, and then decline to day 30. Large amounts of the sesquiterpene β-barbatene was produced, together with 9 late-eluting compounds with a molecular ion of 272. *L. fungicola* notably produced α-muurolene and γ-cadinene, but low levels were also detected over *A. bisporus*. There were few low volatility compounds present over *A. bisporus* but absent over *L. fungicola*. The most abundant of these was the chlorinated compound: benzene, 1,2,4,5-tetrachloro-3,6-dimethoxy-, which has been reported before over *A. bisporus* mycelium by Grove (1981), and over intact harvested mushrooms (*A. bisporus*) by Radvanyi (2023).

3.3. Microcosms

Visual symptoms of *L. fungicola* infection (dry bubble phenotype) were first seen in the infected microcosms in the emerging pins on day 14, and also in the developing mushrooms on days 17 and 21. An image comparing healthy mushrooms with dry bubble infected mushrooms grown in the microcosms is shown in Figure S1 (Supplementary data). Visual symptoms of *L. fungicola* infection were absent from the uninfected microcosms. The volatiles detected over microcosms comprised 14 high volatility and 33 low volatility compounds. The laboratory air samples, which acted as a control, had similar levels of low volatility compounds to those over uninfected and infected microcosms, with the exception of linalool.

PCA was applied to visualise the changes in the 47 volatile compounds over the microcosms and laboratory air over 21 days. Fig. 1 is a plot of Principal component 2 (PC2) against Principal component 1 (PC1). accounting together for 60% of the variance in the dataset. The PCA axis loadings are shown in Table S2 (Supplementary data). The points in Fig. 1 labelled LF14S, LF17S and LF21S were associated with visual signs of *L. fungicola* infection, and correlated negatively with PC2. The majority of the volatiles correlated with PC1, including β-barbatene and linalool. Eight volatile compounds that correlated negatively with PC2 were candidate markers for detection of *L. fungicola* infection over CACed casing. These were: α-muurolene, γ-cadinene, α-cedrene, α-cubebene, β-cubebene, (+)-sativene, β-copaene, and UK 1821. The mass spectrum of UK 1821 was very similar to that presented by Liu et al. (2022) for the diterpene, cycloaraneosene (see Supplementary data, Figure S2).

Fig. 2 shows there was a high background level of low volatility compounds originating from the CACed casing on day 3, which declined with time over the uninfected control mushrooms. The addition of *L. fungicola* in water on day 5 to create infected microcosms carried with it volatiles that were still detectable on day 6. However, these added volatiles dissipated by day 9. This change in volatiles was also seen in the PCA plot (Fig. 1) as an increase along PC1 from LF6 to LF9. The direction of this increase was not the same as that for the negative progress along PC2 for the mushrooms with *L. fungicola* symptoms (LF14S to LF21S). This indicated different volatile profiles for the *L. fungicola* inoculum and the infected mushrooms. In Fig. 2 there was a rise in low volatility compounds on day 21 over the infected microcosms, by which time the disease was already well-established.

β-barbatene was the most abundant low volatility compound measured over microcosms-representing more than a third of the combined signal from the 33 low volatility compounds at all stages. The development of β-barbatene over the microcosms is shown in Fig. 3, and the behaviour mirrored that of the total low volatility compounds in

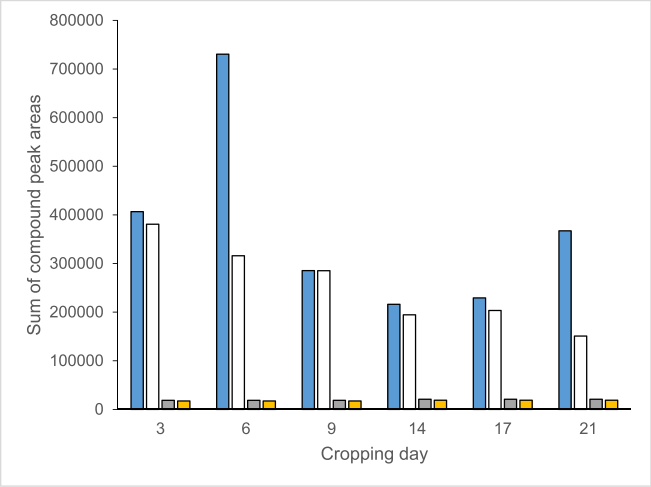

Fig. 2. The sum of 33 low volatility compounds found over microcosms of growing mushrooms –effect of infection with *L. fungicola* on cropping day 5. *L. fungicola* infected mushrooms, blue: uninfected control mushrooms, white; lab air outside *L. fungicola* chamber, grey; lab air outside uninfected chamber; yellow.

Fig. 2. In uninfected microcosms there was a high initial level of β-barbatene on day 3, which originated from the CACed casing. The application of *L. fungicola* in water on day 5 created a spike in the level of β-barbatene on day 6, but the addition of water alone had no effect. This indicated the inoculum contained β-barbatene. This β-barbatene spike dissipated by day 9. On cropping day 21 the level of β-barbatene rose again over infected microcosms, accompanied by visual signs of dry bubble disease.

The 8 low volatility compounds identified as markers in the PCA are

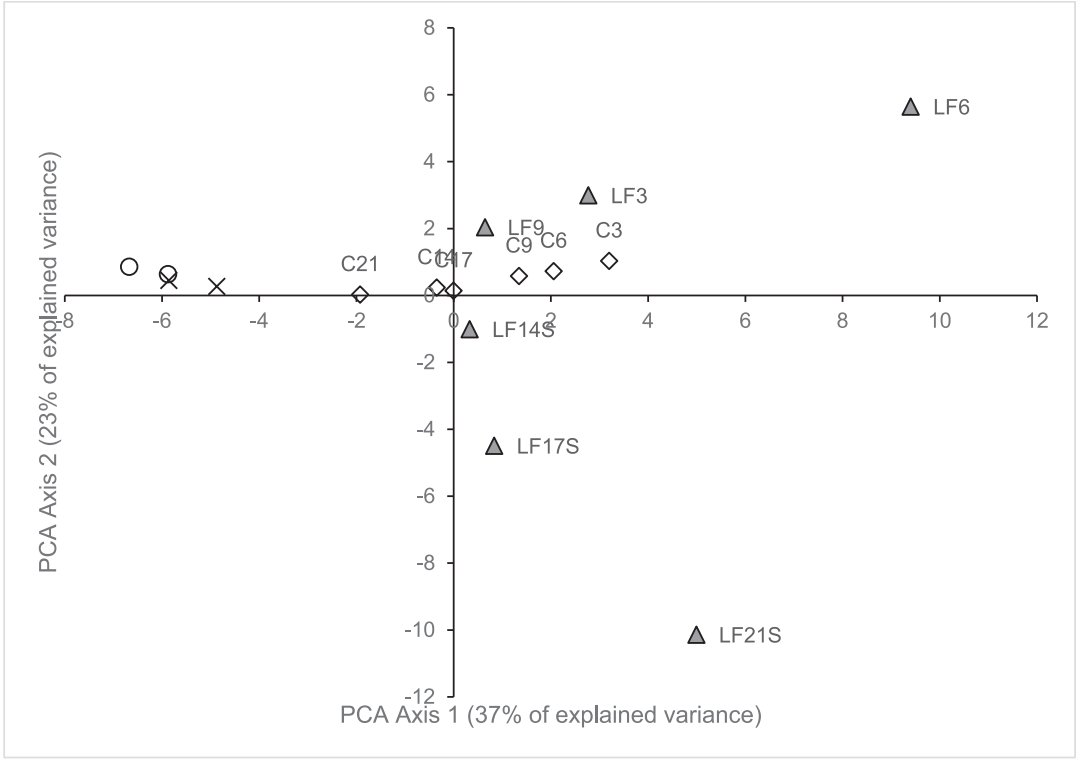

Fig. 1. Principal component analysis of 47 volatile compounds found over 21 days over microcosms of growing mushrooms-the effect of infection at day 5 with *L. fungicola*. Mushrooms infected with *L. fungicola*-LF- suffix S for symptoms, triangles: uninfected control mushrooms-C, diamonds; lab air outside *L. fungicola* chamber, circles; lab air outside uninfected chamber, crosses.

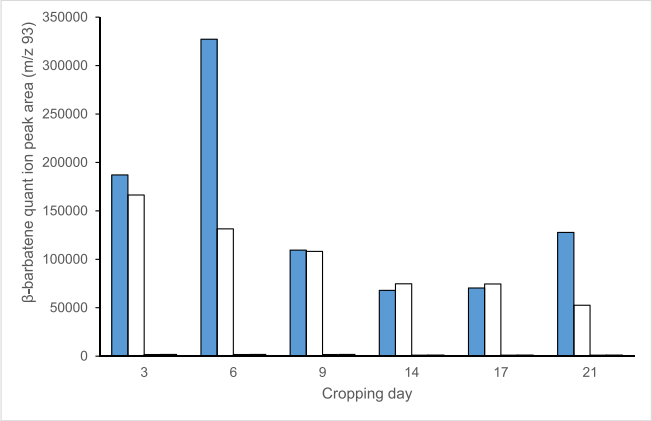

Fig. 3. The level of volatile β-barbatene detected over *L. fungicola* infected (LF) and uninfected (C) growing mushroom microcosms over 21 days. *L. fungicola* infected mushrooms, blue: uninfected control mushrooms, white; lab air outside *L. fungicola* chamber, grey; lab air outside uninfected chamber; yellow.

shown as bar charts in Figs. 4 and 5. In Fig. 4, α-muurolene was the most abundant low volatility marker for symptoms of *L. fungicola* infection at day 21, but it was also present at moderate levels over uninfected microcosms. γ-cadinene was also present over uninfected microcosms, and was a weak marker at day 21. α-cubebene was detected only on day 21 over infected microcosms. α-cedrene was found only over infected microcosms on days 14 to 21, coincident with the visual symptoms of the disease. Although α-cedrene was specific to infected microcosms, the quant ion peak areas for this compound were very small (914 to 2,224).

In Fig. 5, UK 1821 was notable for its abundance on cropping day 21. Both UK 1821 and (+)-sativene had the drawback of being present over uninfected microcosms. UK 1821 was also present in the lab air. β-copaene and β-cubebene were excellent markers because they were found only over infected microcosms on days 14 to 21; coincident with the visual symptoms of the disease, and were present in higher amounts than α-cedrene (Fig. 4).

In summary, instrumental detection of β-copaene, β-cubebene and α-cedrene coincided with visual detection of the disease, but did not provide an earlier warning of it.

3.4. Harvested mushrooms

3.4.1. High abundance compounds

Following the microcosm experiment, 39 sets of harvested mushrooms were tested to determine which volatile compounds discriminated mushrooms showing visual symptoms of dry bubble disease (dry bubble, cap-spotted) from those that did not. The harvested mushroom sets comprised 3 main groups. There were 14 infected and visually defective (D) sets; comprising 11 sets of dry bubble mushrooms (D1, D1RPT, D2-D3, D3RPT, D4-D7, D11-D12) and 3 sets of cap-spotted mushrooms (D8, D9, D10). There were 18 sets of infected but visually sound (S1-S18) mushrooms, and 7 sets of uninfected control mushrooms (C1-C7).

81 volatiles were identified. The volatiles were subdivided by volatility into the 3 classes used earlier with microcosms to give: acetone, 20 high volatility compounds, and 60 low volatility compounds. The stacked bar chart in Figure S3 (Supplementary data) shows the summed quant ion peak areas against the 39 sets of mushrooms, together with the split by volatility. The figure illustrates the heterogeneous nature of the volatiles over the harvested mushrooms. Control set *C*2 was measured later than other control mushroom sets, at 7 days' post-harvest.

There were 5 major compounds detected with instances of peak areas greater than 1,000,000. These abundant compounds were: acetone, 3-octanone, β-barbatene, *cis*-α-bisabolene, and UK 1821. 3-octanone has been reported to be the main 8-carbon volatile over intact mushrooms before harvest (Combet et al., 2009). *Cis*-α-bisabolene has been reported before over commercial *A. bisporus* mushrooms by Costa et al. (2015), Feng et al. (2021) and Radvanyi (2023). UK 1821 and β-barbatene were observed over microcosms but no *cis*-α-bisabolene had been detected.

3.4.2. LDA with high abundance compounds

An attempt was made using the 5 abundant compounds to distinguish defective (dry bubble and cap-spotted) and regular (sound and control mushrooms) by 2-Class Linear Discriminant Analysis (LDA). The attempt failed because the quant ion data was highly skewed. The quant ion data was log 10 transformed, using a value of 0 to represent non-detections. Using forward selection and an F to enter of 3.84, StepDisc selected two variables: UK 1821 and *cis*-α-bisabolene. The LDA (1-way MANOVA: Wilk's lambda = 0.2044; Rao's F (2,36) = 70.04, p = 0.0000) produced the following two discrimination functions. Defective = (2.673 × UK 1821) – (0.415 × *cis*-α -bisabolene) – 7.351. Regular =

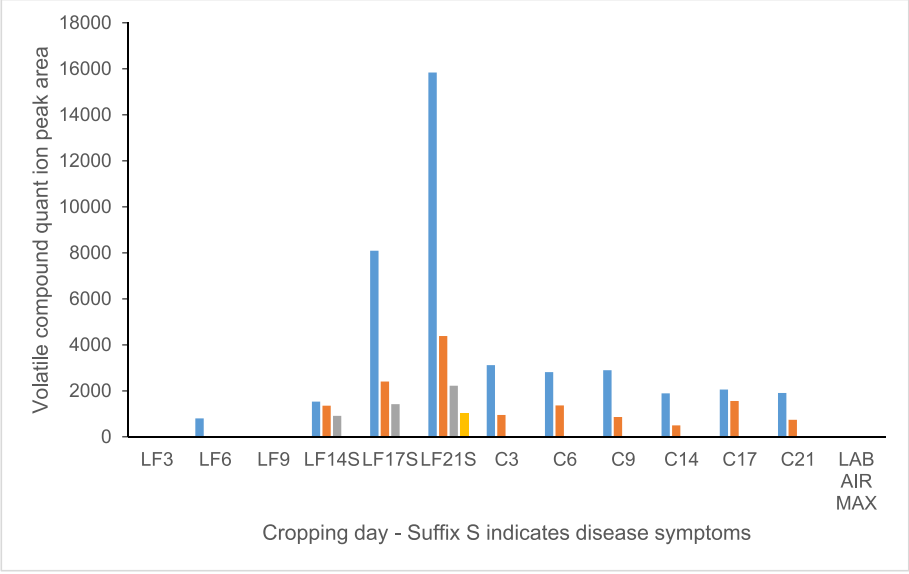

Fig. 4. The development of 4 identified volatile marker compounds for *L. fungicola* over infected (LF) and uninfected (C) growing mushroom microcosms over 21 days. α-Muurolene (*m/z* 161), blue; γ-cadinene (*m/z* 161), orange; α-cedrene (*m/z* 119), grey; α-cubebene (*m/z* 161), yellow.

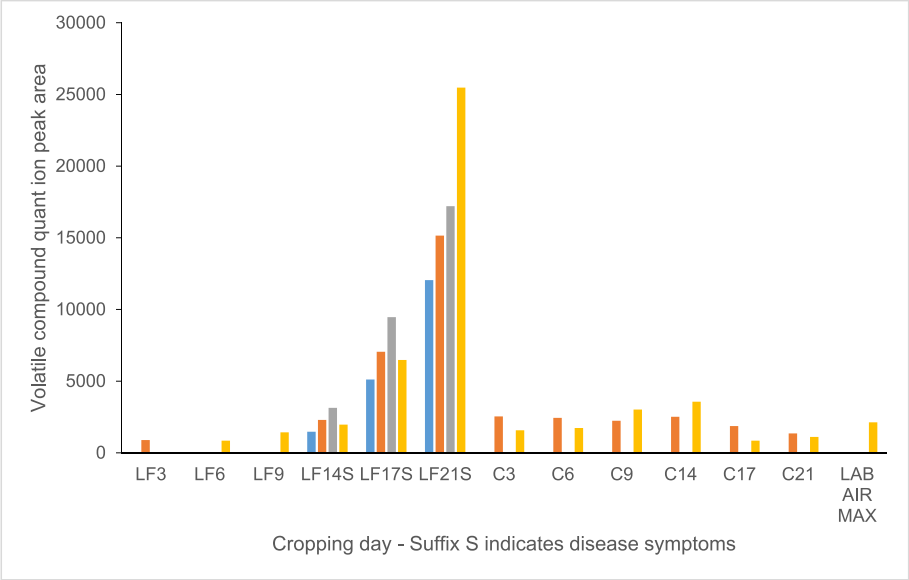

Fig. 5. The development of 3 more identified and 1 unidentified volatile marker compounds for *L. fungicola* over infected (LF) and uninfected (C) growing mushroom microcosms over 21 days. β-cubebene (*m/z* 161), blue: (+)-sativene (*m/z* 108), orange; β-copaene (*m/z* 161), grey; UK 1821 (*m/z* 133), yellow.

(-0.149 × UK 1821) + (1.264 × *cis*-α -bisabolene) – 2.514. The leave-one-out (LOO) error rate was 0.0513 (2 sets out of 39).

The quant ion peak areas for *cis*-α-bisabolene and UK 1821 over all mushroom sets are presented in Table S3 (Supplementary data) in their non-log transformed form. Sound mushroom sets S1 to S3, and set S3 in particular, appeared to be outliers within the set of sound mushrooms. Set S3 had levels of late eluting compounds more typical of dry bubble sets. In particular, sound set S3 had a higher level of UK 1821 than any dry bubble set. In contrast, sound set S3 had an extraordinary level of *cis*-α-bisabolene, compared to all other mushroom sets. This tendency to a high level of *cis*-α-bisabolene was also notable within a subset of the sound mushroom sets. These factors appear to have guided the selection of the log-transformed variables by StepDisc. It is suggested that high levels of *cis*-α-bisabolene were being developed as a defence reaction to infection by *L. fungicola*.

An attempt to use the 5 abundant compounds to discriminate dry bubble mushrooms from all other mushroom sets led to the same 2 variables being selected by StepDisc. However, the LDA gave an unsatisfactory resubstitution error rate of 0.1056 and leave-one-out (LOO) error rate of 0.1538 (6 sets out of 39).

3.4.3. LDA with all compounds

A 2-Class LDA was attempted between defective (dry bubble and cap-spotted) sets and regular (sound and control) sets using all 81 compounds. Using forward selection and an F to enter of 6.00, the 5 compounds selected by StepDisc were: (-)-gymnomitra-3(15),4-diene, UK 1821, *trans*-cadina-1,4-diene, trichodiene and phenylethyl alcohol. The discrimination between groups was significant (MANOVA: Wilk's lambda = 0.0124; Rao's F (2,36) = 1437.5, p = 0.0000). The classification functions are shown in Table S4 (Supplementary data). A LOO error rate of 0.0769 was obtained; assigning 2 out of 14 defective mushroom sets incorrectly to the regular group, and 1 regular mushroom set incorrectly to the defective group.

The dry bubble sets were then compared by 2-Class LDA to the combined sound, control and cap-spotted sets. Using forward selection and an F to enter of 3.84, the 2 compounds selected by StepDisc were: UK 1415 and *trans*-cadina-1,4-diene. The discrimination between groups was significant (MANOVA: Wilk's lambda = 0.0273; Rao's F (5,33) = 84.68, p = 0.0000). The discrimination functions were: Dry Bubble = (111.40 × UK 1415) + (-6.70 × *trans*-cadina-1,4-diene) – 184.87; and Non Dry Bubble = (-1.23 × UK 1415) + (0.36 × *trans*-cadina-1,4-diene) – 0.42. The LDA gave a zero leave-one-out (LOO) error rate. This was because UK 1415 was exclusive to dry bubble sets as shown in Table S3 (Supplementary data). UK 1415 was tentatively identified as acora-3,7 (14)-diene; based on a mass spectrum from Adams (2007) and a retention index of 1415 from Antoniotti et al. (2019). When UK 1415 was excluded, UK 1888 and UK 1618, selected by StepDisc, gave a LOO error rate of 0.0256. This was due to UK 1888 being present only in dry bubble sets and set S3. UK 1415 and UK 1888 were the two best discriminating compounds between dry bubble mushroom sets and the other 3 types.

3.4.4. PCA with Varimax rotation

Principal component analysis (PCA) was next applied to all 81 log transformed mushroom volatiles. The plot of PC2 vs. PC1, after Varimax rotation, is shown in Fig. 6. 30% of the variance was explained by rotated axis PC1, and 30% by rotated axis PC2. The most notable feature in Fig. 6 was the dry bubble mushroom sets had the highest positive scores along rotated axis PC1, but sound sample set S3 was close to the dry bubble sets. Aged control set C2 and cap-spotted set D9 also approached the dry bubble sets. The rotated PCA loadings are shown in Table S5 (Supplementary data). In general, rotated axis PC1 correlated well with late eluting compounds (including the high abundance compound UK 1821); and correlated best with two low-abundance compounds; UK 1415 (r = 0.923) and UK 1888 (r = 0.923). There was also a negative correlation (−0.585) between the high abundance compound *cis*-α-bisabolene and rotated axis PC1. Rotated axis PCA2 correlated best with 1-octen-3-ol (r = 0.846), and to a lesser degree, with 3-octanone (r = 0.796).

UK 1415 was present in all mushroom sets with dry bubble symptoms, but absent from cap-spotted and regular mushrooms. UK 1888 was present only in dry bubble sets and sound set S3. *Cis*-α bisabolene was absent from most (all but two) of the dry bubble sets. Repeating the PCA with exclusions of UK 1415, UK 1888 or *cis*-α-bisabolene, demonstrated that only *cis*-α-bisabolene was essential to separate set S3 from the dry bubble sets.

3.4.5. PCA of dry bubble and control mushrooms

PCA was applied to 11 dry bubble mushroom sets and 7 control mushroom sets, using the log transformed quant ion peak areas for 81 compounds, to enable a comparison to be made with the microcosm experiment. A plot of PC2 against PC1 is shown in Figure S4 (Supplementary data) and the PCA axis loadings are shown in Table S6

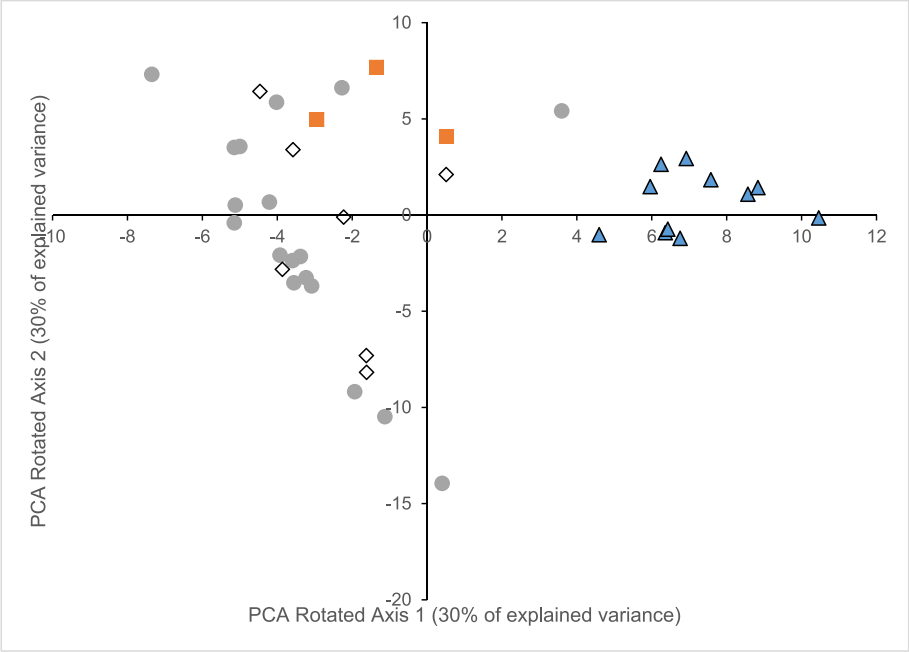

Fig. 6. PCA plot (Varimax rotation) of 4 types of harvested mushroom (dry bubble, cap-spotted, sound, control) based on log transformed quant ion peak areas for 81 volatile compounds. Infected by *L. fungicola* giving dry bubble symptoms, triangles: infected with cap spotting symptoms, squares; sound i.e. infected but with no visual symptoms, circles; uninfected control mushrooms, diamonds. Sound set S3 (circle), aged control set *C*2 (diamond), and cap-spotted set D9 (square) occupy the top right quadrant close to the 11 dry bubble sets.

(Supplementary data). There was a strong positive correlation between β-barbatene and axis PC1 (r = 0.968) and a weak negative correlation with *cis*-α-bisabolene (r = -0.372). UK 1821 also correlated with PC1 to a lesser degree (r = 0.851). Of most interest was the remaining compounds previously identified in the microcosm experiment seemed to form a group with similar correlations to PC1 (range: r = 0.864——0.905). This was investigated using variable clustering.

3.4.6. *Variable clustering (VARHCA) of dry bubble mushrooms*

The normal quant ion peak areas were used to carry out a VARHCA clustering of a subset of 30 of the volatile compounds over 11 dry bubble mushroom sets. This subset consisted of low volatility compounds in the range α-cubebene to UK 1756, with a further exclusion of compounds with more than one zero value over 11 sets of dry bubble mushrooms. Three clusters were obtained, and each cluster was named after the compound with the largest mean peak area in each cluster i.e. the α-muurolene cluster, the β-barbatene cluster, and the δ-cadinene cluster. The compounds in each cluster are shown in Table S7 (Supplementary data). The correlation of each compound to the named compound in each cluster is also shown. The α-muurolene cluster contained 6 of the compounds previously identified in the microcosm experiment, including the 3 compounds selected as marker compounds for early detection of dry bubble disease on cropping day 14. The β-barbatene cluster contained 10 compounds which, with the exception of UK 1465 and γ-muurolene, appeared to have a common precursor, the bisabolyl cation (Hong & Tantillo, 2014). There were 4 compounds (isobazzanene, cuparene (which is an oxidation product of cuprenene), trichodiene, and β-acoradiene) which correlated well with β-barbatene in sub-cluster 1, and which were previously observed to be present over pure cultures of *L. fungicola* and absent over pure cultures of *A. bisporus* (see Table S1, Supplementary data). These 5 compounds probably resulted from the activity of a β-barbatene synthase from *L. fungicola*. The δ-cadinene cluster contained γ-cadinene, which was noted in the microcosm experiment, but this compound was not significantly correlated to δ-cadinene.

3.4.7. *Late eluting compounds over dry bubble mushrooms*

Nine compounds with a molecular weight of 272 (UK 1798 to UK 2014) appeared over the 11 harvested sets of mushrooms with dry bubble symptoms; and agar cultures indicated their origin to be *L. fungicola* (see Table S1, Supplementary data). An additional compound (UK 2075) with a molecular weight of 274 was detected only over harvested mushrooms but had not been seen over pure cultures or the microcosms. Only UK 1821 and UK 1881 had earlier been detected over microcosms of growing mushrooms. Whilst UK 1821 was noted in Section 3.3 as a late potential marker compound for dry bubble disease over microcosms, UK 1881 was not identified as a marker because it was detected at similar levels over both healthy and diseased mushrooms, and in the laboratory air. To obtain more detail, correlations were sought between the levels of late eluting compounds over harvested dry bubble mushrooms.

Table S8 (Supplementary data) shows the correlation coefficients between 8 pairs of the 10 late eluting compounds over the 11 sets of dry bubble mushrooms, obtained using the data from Table S3 (Supplementary data). Two sets (UK 1798 and UK 1875) were initially excluded from the analysis for having too many zero values. A group of 5 late eluting compounds were significantly correlated with one another. These were: UK 1827, UK 1866, UK 1881, UK 1932 and UK 2014. UK 1821, UK 1888, and UK 2075 were not significantly correlated with any of the others.

UK 1821 is possibly cycloaraeosene from a mass spectrum by Liu et al. (2022) and a retention index of 1843 on a HP-5MS column by Pemberton et al. (2017). Cycloaraeosene is the volatile precursor to sordarin and related compounds with known antifungal activity (Liu et al., 2022). It is possible that cycloaraneosene may be involved in the pathology of *L. fungicola* against other fungi.

Three of the group of 5 inter-correlated late eluting compounds were tentatively identified. UK 1827 is possibly fusicocca-2,10(14)-diene from a mass spectrum by Toyomasu et al. (2007). UK 1881 is possibly δ-araneosene from a mass spectrum and retention index of 1882 on a HP-5MS GC column by Bian et al. (2018). UK 2014 is possibly β-araneosene from a mass spectrum and retention index of 2025 on a HP-5MS

GC column by Rinkel et al. (2019). The last three compounds were originally detected together by Sassa et al. (2004) in flask culture of the fungus *Phomopsis amygdali* to produce Fusicoccin A.

Fusicoccadiene (fusicocca-2,10(14)-diene) has no reported biological activity, but it is the precursor to Fusicoccin A in *Phomopsis amygdali* and Brassicicene A in *Alternaria brassicicola* (de Boer & de Vries-van Leeuwen, 2012). Both these fungi are plant pathogens. The Brassicicenes from *Alternaria brassicicola* have been tested by disc biodiffusion assay against several fungi, but no antifungal activity was found (MacKinnon et al., 1999). The activity of Fusicoccin A against fungi has not been tested. Fusicoccin A has a number of effects on plants, including an auxin-like stimulation of growth and an increase in hydrogen-ion extrusion by cells (de Boer & de Vries-van Leeuwen, 2012). Bunney et al. (2003) found the application of Fusicoccin A from *Phomopsis amygdali* to frog embryos during early development caused a disruption of left–right asymmetry (ataxia). The target of Fusicoccin A is the 14–3-3 family of proteins. Since these proteins are common in plants, animals and fungi; it is feasible that Fusicoccin A, if produced by *L. fungicola*, may have a role in the development of dry bubble symptoms in *A. bisporus*.

3.4.8. *Cis*-α-bisabolene

Masunaga et al. (2023) recently identified 9 gene candidates for sesquiterpene synthases in *A. bisporus* H97. They were able to functionally express 3 of these in *Saccharomyces cerevisiae* in flask culture. These were two sesquiterpene synthases for δ-cadinene (AbSTS-05 and AbSTS-07) and one for *cis*-α-bisabolene (AbSTS-09). A chromatogram of the compounds captured in the headspace over the AbSTS-09 culture showed a clear peak for *cis*-α-bisabolene, and a number of very minor peaks, which the authors did not attempt to identify. It was predicted that this terpene synthase, or a variant, would be present in the *A. bisporus* Sylvan A15 strain used in the present work.

Cis-α-bisabolene has previously been identified in uninfected *A. bisporus* mushrooms by HS-SPME, but the reported levels vary. In developing mushrooms (strain A15 from the USA) Feng et al. (2021) detected trace levels of *cis*-α-bisabolene (0.01–0.02 μg/g) during growth but none at all at the final cap-opening stage. This appears to agree with the present work with uninfected mushrooms grown in microcosms, where *cis*-α-bisabolene was not detected with MonoTraps. In harvested mushrooms (unspecified strain of *A. bisporus*, Italian supermarket) Costa et al. (2015) detected a low level (0.03% of 51 total volatiles by GC/FID peak area) of *cis*-α-bisabolene. The highest levels of *cis*-α-bisabolene in harvested mushrooms (unspecified strain of *A. bisporus*, Hungarian producer) were reported by Radvanyi (2023) who found that this compound declined from 2.6% to 0.5 % of total volatiles by GC/MS peak area over 8 days of storage.

In the present work, the levels of *cis*-α-bisabolene over harvested mushrooms were calculated using the summed quant ion peak areas for all volatile compounds. Mean % *cis*-α-bisabolene levels from the whole dataset were: 1.1 % (control), 4.8% (sound) and 0.3% (defective). The mean level over the sound mushroom sets exceeded literature values for harvested mushroom. In particular, sound set S3 had an extraordinary level of 25% *cis*-α-bisabolene.

It is possible that excess *cis*-α-bisabolene production by *A. bisporus* is a fungal defence reaction to *L. fungicola*, which prevents the development of dry bubble disease. *Cis*-α-bisabolene has never been investigated as an anti-fungal agent, but it seems possible, since Wirth et al., (2021) demonstrated that the structurally-related compounds; β-bisabolene (mixed with *trans*-γ-bisabolene) and (-)-(1R,2S)-β-bisabolol, were inhibitory as airborne molecules to the growth of several fungi. A proposed defence reaction (production of β-bisabolene) has been noted in the mushrooms *Pleurotus ostreatum* and *Pleurotus eryngii* in response to volatile organic compounds emitted by the bacterium, *Paenibacillus peoriae* strain M48F (Orban et al., 2023). Furthermore, Gramss (2020) found that mycelial contact between the brown rot basidiomycete *Fomitopsis pinicola*, and the white rot fungus *Kuehneromyces mutabilis* caused *K. mutabilis* to produce large amounts of *trans*-α-bisabolene and β-bisabolene. It was suggested by Gramss (2020) that this had an antimicrobial effect against *F. pinicola*.

4. Conclusions

The main hypothesis of this work was that volatile compounds can be used to detect dry bubble disease in white button mushrooms before the appearance of visual symptoms. Three potential volatile marker compounds for infected mushrooms with dry bubble symptoms grown in microcosms were identified as β-copaene, β-cubebene, and α-cedrene. The hypothesis was rejected since these compounds were not detected before the first appearance of visual symptoms in the emerging pins, which occurred on cropping day 14, but were detected at the same time.

β-barbatene and an unidentified diterpene (UK 1821 – suspected to be cycloaraneosene) developed strongly on the final cropping day (day 21) over infected mushrooms that developed dry bubble symptoms, but there was a large confounding contribution of β-barbatene from the CACed casing, and minor confounding contributions of UK 1821 from the CACed casing and laboratory air. Harvested mushrooms that had developed dry bubble symptoms had high levels of β-barbatene, UK 1821, or both. A few infected but visually sound harvested mushrooms had levels of these two compounds in excess of some defective (dry bubble and cap-spotted) mushrooms, accompanied by high levels of *cis*-α bisabolene; a compound only previously identified in *A. bisporus*. In harvested mushrooms, a simple LDA using just *cis*-α-bisabolene and UK 1821 distinguished defective mushrooms (dry bubble and cap-spotted) from regular mushrooms (infected but visually sound, and control mushrooms) with a LOO error rate of 0.0513. It was hypothesised that *cis*-α-bisabolene production by *A. bisporus* was a defence reaction to infection by *L. fungicola*. This phenomenon is worthy of further investigation with a view to a protective treatment against dry bubble disease.

CRediT authorship contribution statement

William Hayes: Methodology, Formal analysis, Investigation, Writing – original draft. **Cathy Keenan:** Methodology, Formal analysis, Investigation, Resources, Writing – review & editing, Project administration. **Jude Wilson:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Bukola Adenike Onarinde:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.137518.

References

Adams, R. P. (2007). *Identification of essential oil components by gas chromatography/mass spectrometry* (4th Ed). Carol Stream, Illinois, USA: Allured Publishing Corporation.

Antoniotti, S., Filippi, J. J., Francesco, I. N., & Ramilijaona, J. (2019). Biotechnological manufacture of vetiveryl esters. *U.S. Patent Application No. 10,323,210 (B2)*. U.S. Patent and Trademark Office, Washington, DC. https://image-ppubs.uspto.gov/dirsearch-public/print/downloadPdf/10323210.

Berendsen, R. L., Baars, J. J. P., Kalkhove, S. I. C., Lugones, L. G., Wosten, H. A. B., & Bakker, P. A. H. M. (2010). *Lecanicillium fungicola*: Causal agent of dry bubble disease in white button mushroom. *Molecular Plant Pathology, 11*(5), 585–595. https://doi.org/10.1111/j.1364-3703.2010.00627.x

Bian, G., Rinkel, J., Wang, Z., Lauterbach, L., Hou, A., Yuan, Y., … Dickschat, J. S. (2018). A Clade II-D fungal chimeric diterpene synthase from *Colletotrichum gloeosporioides* produces dolasta-1(15),8-diene. *Agnewandte Chemie International Edition, 57*, 15887–15890. https://doi.org/10.1002/anie.201809954

Bunney, T. D., de Boer, A. H., & Levin, M. (2003). Fusicoccin signaling reveals 14–3-3 protein function as a novel step in left-right patterning during amphibian embryogenesis. *Development, 130*(20), 4847–4858. https://doi.org/10.1242/dev.00698

Calonje, M., Mendoza, C. G., Cabo, A. P., Bernardo, D., & Novaes-Ledieu, M. (2000). Interaction between the mycoparasite *Verticillium fungicola* and the vegetative mycelial phase of *Agaricus bisporus*. *Mycological Research, 104*(8), 988–992. https://doi.org/10.1017/S0953756299002154

Combet, E., Henderson, J., Eastwood, D. C., & Burton, K. S. (2009). Influence of Sporophore development, damage, storage, and tissue specificity on the enzymic formation of volatiles in mushrooms (*Agaricus bisporus*). *Journal of Agricultural and Food Chemistry, 57*, 3709–3717. https://doi.org/10.1021/jf8036209

Costa, R., de Grazia, S., Grasso, E., & Trozzi, A. (2015). Headspace-solid-phase microextraction-gas chromatography as analytical methodology for the determination of volatiles in wild mushrooms and evaluation of modifications occurring during storage. *Journal of Analytical Methods in Chemistry, 2015*, Article 951748. https://doi.org/10.1155/2015/951748

Costa, R., Tedone, L., De Grazia, S., Dugo, P., & Mondello, L. (2013). Multiple headspace-solid-phase microextraction: An application to quantification of mushroom volatiles. *Analytica Chimica Acta, 770*, 1–6. https://doi.org/10.1016/j.aca.2013.01.041

de Boer, A. H., & de Vries-van Leeuwen, I. J. (2012). Fusicoccanes: Diterpenes with surprising biological functions. *Trends in Plant Science, 17*(6), 360–368. https://doi.org/10.1016/j.tplants.2012.02.007

Eastwood, D. C., Herman, B., Noble, R., Dobrovin-Pennington, A., Sreenivasaprasad, S., & Burton, K. S. (2013). Environmental regulation of reproductive phase change in *Agaricus bisporus* by 1-octen-3-ol, temperature and CO_2. *Fungal Genetics and Biology, 55*, 54–66. https://doi.org/10.1016/j.fgb.2013.01.001

FAOSTAT, F. (2023). Crops and livestock products. *Statistics Division, Food and Agriculture Organization of the United Nations: Rome, Italy*. Retrieved from: https://www.fao.org/faostat/en/#data/QCL/visualize. Accessed August 24, 2023.

Feng, T., Yang, M., Ma, B., Zhao, Y., Zhuang, H., Zhang, J., & Chen, D. (2021). Volatile profiles of two genotype *Agaricus bisporus* species at different growth stages. *Food Research International, 140*, Article 109761. https://doi.org/10.1016/j.foodres.2020.109761

Gramss, G. (2020). Aspects determining the dominance of *Fomitopsis pinicola* in the colonization of deadwood and the role of the pathogenicity factor oxalate. *Forests, 11*(3), Article 290. https://doi.org/10.3390/f11030290

Grove, J. F. (1981). Volatile compounds from the mycelium of the mushroom *Agaricus bisporus*. *Phytochemistry, 20*(8), 2021–2022. https://doi.org/10.1016/0031-9422(81)84057-5

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica, 4*(1), 9.

Holmes, J. (1971). Effect of time of inoculation on incidence and control of *Verticillium* disease of the commercial mushroom. *The Plant Disease Reporter, 55*(7), 643–645. Retrieved from https://babel.hathitrust.org/cgi/pt?id=chi.23658013&view=1up&seq=16 Accessed April 11, 2023.

Hong, Y. J., & Tantillo, D. J. (2014). Branching out from the bisabolyl cation. Unifying mechanistic pathways to barbatene, bazzanene, chamigrene, chamipinene, cumacrene, cuprenene, dunniene, isobazzanene, iso-γ-bisabolene, isochamigrene, laurene, microbiotene, sesquithujene, sesquisabinene, thujopsene, trichodiene, and widdradiene sesquiterpenes. *Journal of the American Chemical Society, 136*(6), 2450–2463. https://doi.org/10.1021/ja4106489

Kertesz, M. A., & Thai, M. (2018). Compost bacteria and fungi that influence growth and development of *Agaricus bisporus* and other commercial mushrooms. *Applied Microbiology and Biotechnology, 102*, 1639–1650. https://doi.org/10.1007/s00253-018-8777-z

Liu, S. H., Sun, J. L., Hu, Y. L., Zhang, L., Zhang, X., Yan, Z. Y., Guo, X., Guo, Z. K., Jiao, R. H., Zhang, B., Tan, R. X., & Ge, H. M. (2022). Biosynthesis of sordarin revealing a Diels–Alderase for the formation of the norbornene skeleton. *Angewandte Chemie International Edition, 61*, Article e202205577. https://doi.org/10.1002/anie.202205577

MacKinnon, S. L., Keifer, P., & Ayer, W. A. (1999). Components from the phytotoxic extract of *Alternaria brassicicola*, a black spot pathogen of canola. *Phytochemistry, 51*(2), 215–221. https://doi.org/10.1016/S0031-9422(98)00732-8

Masunaga, N., Kitaoka, T., & Ichinose, H. (2023). Biocatalyst collection and heterologous expression of sesquiterpene synthases from basidiomycetous fungi: Discovery of a novel sesquiterpene hydrocarbon. *Microbial Biotechnology, 16*(3), 632–644. https://doi.org/10.1111/1751-7915.14204

McGee, C. F., Byrne, H., Irvine, A., & Wilson, J. (2017). Diversity and dynamics of the DNA- and cDNA-derived compost fungal communities throughout the commercial process for *Agaricus bisporus*. *Mycologia, 109*(3), 475–484. https://doi.org/10.1080/00275514.2017.1349498

North, L. H., & Wuest, P. J. (1993). The infection process and symptom expression of *Verticillium* disease of *Agaricus bisporus*. *Canadian Journal of Plant Pathology, 15*(2), 74–80. https://doi.org/10.1080/07060669309500829

Orban, A., Jerschow, J. J., Birk, F., Suarez, C., Schnell, S., & Ruhl, M. (2023). Effect of bacterial volatiles on the mycelial growth of mushrooms. *Microbiological Research, 266*, Article 127250. https://doi.org/10.1016/j.micres.2022.127250

Pemberton, T. A., Chen, M., Harris, G. G., Chou, W. K. W., Duan, L., Koksal, M., … Christianson, D. W. (2017). Exploring the influence of domain architecture on the catalytic function of diterpene synthases. *Biochemistry, 56*(14), 2010–2023. https://doi.org/10.1021/acs.biochem.7b00137

Piasecka, J., Kavanagh, K., & Grogan, H. (2011). Pests and diseases: Detection of sources of *Lecanicillium* (*Verticillium*) *fungicola* on mushroom farms. In *Conference paper presentation at the Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products (ICMBMP7), Arcachon, France* (pp. 479–484). https://www6.bordeaux-aquitaine.inrae.fr/mycsa_eng/International-conferences-we-organised/Proceedings-ICMBMP7.

Radvanyi, D. (2023). Smelling the difference: separation of healthy and infected button mushrooms via microbial volatile organic compounds. *Heliyon, 9*(1), Article e12703. https://doi.org/10.1016/j.heliyon.2022.e12703

Radvanyi, D., Gere, A., Jokai, Z., & Fodor, P. (2015). Rapid evaluation technique to differentiate mushroom disease-related moulds by detecting microbial volatile organic compounds using HS-SPME-GC-MS. *Analytical and Bioanalytical Chemistry, 407*(2), 537–545. https://doi.org/10.1007/s00216-014-8302-x

Rakotomalala, R. (2005). *TANAGRA: Un logiciel gratuit pour l'enseignement et la recherché*. In: Actes de EGC'2005, RNTI-E-3, *2*, 697–702. https://editions-rnti.fr/?procid=4587. Accessed April 11, 2023.

Rinkel, J., Kollner, T. G., Chen, F., & Dickschat, J. S. (2019). Characterisation of three terpene synthases for β-barbatene, β-araneosene and nephthenol from social amoebae. *Chemical Communications, 55*, 13255–13258. https://doi.org/10.1039/C9CC07681F

Sassa, T., Kenmoku, H., Nakayama, K., & Kato, N. (2004). Fusicocca-3(16),10(14)-diene, and β- and δ-araneosenes, new fusicoccin biosynthesis-related diterpene hydrocarbons from *Phomopsis amygdali*. *Bioscience, Biotechnolology and Biochemistry, 68*(7), 1608–1610. https://doi.org/10.1271/bbb.68.1608

Savoie, J. M., Foulongne-Oriol, M., Barroso, G., & Callac, P. (2013). Genetics and genomics of cultivated mushrooms, application to breeding of agarics. In F. Kempken (Ed.), *The Mycota. Volume 11: Agricultural applications* (pp. 3–33). Berlin: Springer.

Straatsma, G., Samson, R. A., Olijnsma, T. W., Camp, H. J. M. O. D., Gerrits, J. P. G., & Van Griensven, L. J. L. D. (1994). Ecology of thermophilic fungi in mushroom compost, with emphasis on *Scytalidium thermophilum* and growth stimulation of *Agaricus bisporus* mycelium. *Applied and Environmental Microbiology, 60*(2), 454–458. https://doi.org/10.1128/aem.60.2.454-458.1994

Toyomasu, T., Tsukahara, M., Kaneko, A., Niida, R., Mitsuhashi, W., Dairi, T., … Sassa, T. (2007). Fusicoccins are biosynthesized by an unusual chimera diterpene synthase in fungi. *PNAS, 104*(9), 3084–3088. https://doi.org/10.1073/pnas.0608426104

van Den Dool, H., & Kratz, P. D. (1963). A generalization of the retention index system including linear temperature programmed gas – liquid partition chromatography. *Journal of Chromatography A, 11*, 463–471. https://doi.org/10.1016/S0021-9673(01)80947-X

Wirth, S., Krause, K., Kunert, M., Broska, S., Paetz, C., Boland, W., & Kothe, E. (2021). Function of sesquiterpenes from *Schizophyllum commune* in interspecific interactions. *PLoS ONE, 16*(1), Article e0245623. https://doi.org/10.1371/journal.pone.0245623