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ORIGINAL ARTICLE

Effect of fruiting-related genes on the formation of volatile organic compounds in the mushroom *Schizophyllum commune*

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Abstract

In fungi, little is known about connections between volatile organic compound (VOC) formation and developmental stages that are amongst others triggered by fruiting-related genes (FRGs). We analysed the volatilomes of *Schizophyllum commune* during different developmental stages in a variety of FRG-deletion strains and wild-type strains. The deletion strains Δ*tea1*Δ*tea1*, Δ*wc-2*Δ*wc-2* and Δ*hom2*Δ*hom2* were unable to develop fruiting bodies, and Δ*fst4*Δ*fst4* formed only rudimentary fruiting body structures. Early developmental stages of these strains were dominated by esters, including methyl 2-methylbutanoate, ethyl 2-methylbutanoate, isobutyl 2-methylpropionate, and 2-methylbutyl acetate, of which the last three were not found in the headspace (HS) of the wild-type samples. Compared to the wild type, in the HS of *hom2*^{con} samples, that are able to form fruiting bodies, methyl 2-methylbutanoate was the most abundant substance at early stages (68–81% of the total peak area). In contrast to fruiting body forming strains, Δ*tea1*Δ*tea1*, Δ*wc-2*Δ*wc-2*, Δ*hom2*Δ*hom2* and Δ*fst4*Δ*fst4* showed less sesquiterpenes in the HS. However, the sesquiterpenes found in the HS of FRG-deletion strains, namely, (*E*)-nerolidol, δ-cadinene, L-calamenene, α-bisabolol and β-bisabolene, were not present in *hom2*^{con} or wild-type strains that mainly formed fruiting bodies and barely mycelium. Several sesquiterpenes, including α-guaiene, chamigrene and γ-gurjunene, were only found in presence of fruiting bodies. Our results show remarkable connections between FRGs, fruiting body development and VOC production in *S. commune*, especially counting for sesquiterpenes. Future studies are needed to reveal whether FRGs directly regulates VOC formation or indirectly by changing the phenotype.

Keywords VOCs · Fungi · Development · Deletion mutant · Sesquiterpene · Oxylipin

Introduction

More than 300 distinct fungal volatile organic compounds (VOCs) have been identified so far, being in many cases aroma active (Dickschat 2017) that determine, e.g. the quality of edible fungal fruiting bodies. VOC profiles of several fungi have been analysed so far (Chen and Ho 1986; Costa et al. 2015; Dickschat 2017; Fraatz and Zorn 2011; Kleofas et al. 2015; Rühl et al. 2018; Venkateshwarlu et al. 1999). However, studies investigating volatilomes (the composition of all VOCs produced by a fungus during one time point) are often focusing on one developmental stage ignoring potential dynamics in VOC release. Studies investigating volatilomes during the fungal life cycle are mainly focusing on fruiting bodies of mushrooms (Cho et al. 2006; Combet et al. 2009; Cruz et al. 1997; Li et al. 2016; Mau et al. 1997; Tasaki et al. 2019; Zawirska-Wojtasiak et al. 2007; Zhang et al. 2008) analysing the aroma profiles in context of food quality. Prior to extraction, fruiting bodies are often grinded or otherwise damaged, which can result in unnatural VOC compositions (Combet et al. 2009). Furthermore, the focus on fruiting bodies neglects the role of the mycelium for VOC formation and possible interactions between fruiting bodies and mycelium during development. Studies analysing fungal volatilomes under more natural circumstances are rare. Recently, we established a system consisting of modified crystallizing dishes allowing a non-invasive analysis of fungal VOCs from complete cultures during different

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stages of the fungal life cycle using solid-phase microextraction coupled gas chromatography mass spectrometry (SPME–GC–MS) (Orban et al. 2019). Applying this system on *Cyclocybe aegerita*, remarkable relations between fruiting body development and VOC compositions, especially counting for the release of sesquiterpenes during sporulation, have been depicted (Orban et al. 2020). A subsequent transcriptome study of *C. aegerita* showed that the vegetative mycelium rather than the fruiting body is the origin of the sesquiterpenes (Orban et al. 2021).

The formation of fruiting bodies is one of the most complex developmental processes in the fungal life cycle (Sánchez-García et al. 2020). It starts with the aggregation of hyphae to form mycelial cords, developing on local spots of the mycelium into hyphal knots by intense branching. The hyphal knots develop successively into fruiting body initials (secondary hyphal knots) (Kües 2000; Kües and Liu 2000; Kües et al. 2018). Cell differentiation results in the formation of bipolar primordia already comprising the plectenchyme as characteristic tissue for mature fruiting bodies. Subsequently, differentiated primordia develop into fruiting bodies primarily due to cell elongation rather than cell differentiation (Kües 2000). In mature fruiting bodies of *Basidiomycota*, karyogamy and meiosis take place in the basidium, leading to the formation of basidiospores that are released during sporulation and marking the beginning of a new life cycle (Oberwinkler 1982). The fruiting process is regulated by environmental factors; e.g. light and low concentrations of CO_2 have been proven to be necessary for proper fruiting body development (Kinugawa et al. 1994; Kües and Navarro-González 2015; Turner 1977; Wessels 1993). Additionally, several gene deletion studies on especially *S. commune* and *Coprinopsis cinerea* have proven that certain genes, so-called fruiting-related genes (FRGs), are essential for the fruiting process (Arima et al. 2004; Kamada et al. 2010; Knabe et al. 2013; Kuratani et al. 2010; Liu et al. 2006; Muraguchi and Kamada 1998; Muraguchi et al. 2008; Murata et al. 1998; Ohm et al. 2010, 2011, 2013; Pelkmans et al. 2017; Terashima et al. 2005). In *S. commune*, induction of fruiting by light is connected to the white collar (WC) complex consisting of the two proteins, WC-1 and WC-2. WC-1 contains a blue light sensing domain and WC-2 functions as a transcription factor. Deletion of *wc-1* and/or *wc-2* results in a blind phenotype under light conditions with symmetrical mycelial growth and being unable to form hyphal knots, primordia or fruiting bodies (Ohm et al. 2013). Inactivation of the homeodomain transcription factor gene *hom2* leads to a comparable phenotype (Ohm et al. 2011). The transcription factor Tea1 is also involved in fruiting body formation, and *tea1* deletion mutants show symmetrically mycelial growth with only scarce formation of flask-like fruiting bodies (Pelkmans et al. 2017). Inactivation of the zinc finger transcription factor gene *fst4* results in mycelial growth comparable to the wild type but without formation of fruiting bodies (Ohm et al. 2011; Pelkmans et al. 2017). Several other FRGs have been identified in *S. commune*, including *bri1*, *c2h2*, *fst3*, *gat1* and *hom1*, regulating different stages of fruiting (Pelkmans et al. 2017). However, connections between fungal FRGs and VOC formation have not been studied so far.

To address this gap, we studied VOC formation in the *S. commune* wild-type dikaryon H4-8; the dikaryotic deletion strains Δ*fst4*Δ*fst4*, Δ*tea1*Δ*tea1*, Δ*wc-2*Δ*wc-2* and Δ*hom2*Δ*hom2* (Ohm et al. 2010, 2011, 2013; Pelkmans et al. 2017); and the monokaryotic *hom2*^{con} strain (Pelkmans et al. 2017). The latter strain, that expresses a constitutive active version of the Hom2 transcription factor, forms flask-like fruiting bodies that do not expose their spore forming gills when exposed to mushroom inducing conditions (presence of blue light and ambient CO_2). We analysed the volatilomes of these *S. commune* strains with various phenotypes during different developmental stages using non-invasive SPME–GC–MS approaches to evaluate the effects of FRGs on volatile formation and to highlight fruiting-specific VOCs.

Material and methods

Fungal strains and growth conditions

The *S. commune* wild-type dikaryon H4-8; the derived dikaryotic deletion strains Δ*fst4*Δ*fst4*, Δ*tea1*Δ*tea1*, Δ*wc-2*Δ*wc-2* and Δ*hom2*Δ*hom2* (Ohm et al. 2010, 2011, 2013; Pelkmans et al. 2017); and the monokaryotic *hom2*^{con} strain (Pelkmans et al. 2017) were routinely grown at 24 °C in the dark on Petri dishes containing minimal medium (MM) with 2.2% glucose and 1.5% agar (van Peer et al. 2009). For investigation of fungal growth and GC–MS analysis, *S. commune* strains were each grown in triplicates at 24 °C in the dark in modified crystallizing dishes (lower dish: 70 mm in diameter, upper dish: 80 mm in diameter; glass pipe attached to the upper dish: outer diameter 16 mm, inner diameter 14 mm) with 16 ml MM containing 2.2% glucose and 1.5% agar (van Peer et al. 2009) and sealed with Parafilm (for a picture of the setup, see Orban et al. (2019)). For *S. commune* wild-type strain H4-8, two different cultivation processes were applied. For the first approach (WT1), the mycelia were cultivated for 8 days after inoculation in the dark as described above. The aeration was then enabled by removing the Parafilm, and the samples were transferred to a climate chamber RUMED 3501 (Rubarth Apparate GmbH, Laatzen, Germany) (24 °C, 95% relative humidity (RH), 12/12 h day/night rhythm) and cultured on glass plates for a further 16 days. For the second approach (WT2), aeration and illumination of the cultures were already applied at day 2, and samples were cultured on glass plates for a

further 22 days in the climate chamber. The *S. commune* strains *hom2*^{con}, Δ*fst4*Δ*fst4*, Δ*wc-2*Δ*wc-2*, Δ*hom2*Δ*hom2* and Δ*tea1*Δ*tea1* were cultivated accordingly to the second approach with the exception that the latter was cultivated for only 14 days in the climate chamber.

Analysis of VOCs by SPME–GC–MS

VOCs were collected by SPME using a divinylbenzene-carboxen-polydimethylsiloxane (50/30 µm DVB/CAR/PDMS) fibre (Agilent Technologies, Santa Clara, CA, USA). Beginning with the transfer of the samples to the climate chamber (see above), VOCs were extracted directly in the HS of the crystallizing dishes for 1 h. This extraction was carried out every second day. For GC–MS analysis, an Agilent Technologies 7890A gas chromatograph equipped with Agilent VF-WAXms column (30 m × 0.25 mm, 0.25 µm) and connected to an Agilent 5975C MSD Triple-Axis mass spectrometer was used. Helium was used as a gas carrier, with a flow rate of 1.2 ml min^{-1}. Mass spectra were acquired in the mass range of 33–300 m/z. Ionization was done by electron impact at 70 eV with an ion source temperature set at 230 °C. The SPME fibre was inserted into the injector of the gas chromatograph for thermal desorption in splitless mode for 1 min, with the injector temperature held at 250 °C. The GC oven temperature was programmed to ramp from 40 °C (hold for 3 min) to 240 °C (hold for 7 min) at 5 °C min^{-1}. Only VOCs were considered that were not found in the MM agar controls. VOCs were identified initially by comparing obtained mass spectra with data from the NIST14 database (National Institute of Standards and Technology, Gaithersburg, MD, USA). Furthermore, linear retention indices of VOCs were determined using a C7–C30 alkane mix solved in hexane (100 µg mL^{-1} each component), of which 1 µL was injected in the GC–MS and analysed with a 1:10 split. Obtained retention indices were compared to published ones or the NIST database. In case of 3-methylfuran, dimethyl trisulfide, 2-methylpropan-1-ol, 3-methylbutan-1-ol, oct-1-en-3-ol, oct-2-en-1-ol, octan-3-one, oct-1-en-3-one, methyl 2-methylbutanoate, ethyl 2-methylbutanoate, α-pinene, β-pinene, β-myrcene, viridiflorene, β-bisabolene and (*E*)-nerolidol, VOC structures were additionally verified by comparison of retention time and mass spectra with authentic standards. Additionally, certain sesquiterpenes were verified using essential oils with known terpene compositions (Agger et al. 2009; Lopez-Gallego et al. 2010): cedrela woods oil (δ-cadinene, L-calamenene) and cubeb oil (α-cubebene, α-copaene, β-cubebene, germacrene D, cadina-1,4-diene). Nine VOCs were tentatively identified as sesquiterpenes, taking their masses and fragmentation patterns into account (Supplementary Fig. S1). However, considering the retention indices and the results from the comparison with the NIST database, for these VOCs, no satisfying matches were found. Hence, they were regarded as not identified sesquiterpenes (n. i. ST).

Data processing

Only VOCs were considered being found in at least two of the triplicates. For the heatmaps, the peak areas of identified substances were determined and used to calculate the relative amount of the respective VOC. Therefore, each substance of every sample was considered individually, meaning that 100% is related to the highest peak area of the compound measured in a sample during the experiment. The mean value of the relative VOC amounts of the triplicates was calculated for each substance and, afterwards, normalized to 100%. This approach enabled an appropriate overview about the connections between developmental stages and VOC patterns in the HS, whereas a direct comparison of the peak areas is generally difficult due to the lack of a reference quantity. The normalized data were used to generate heatmaps with OriginPro 2020b (OriginLab Corporation, Northampton, MA, USA). For principal component analysis (PCA), each sample was checked for the presence of a specific VOC at each sample time point, and the data was summarized showing if a certain VOC was present during any of the stages or not. This means that the PCA data for a sample is not limited to one time point but represents the volatilomes of all stages. PCA of the volatilome data was carried out with OriginPro 2020b.

Results

Mycelial growth and fruiting body development in *S. commune*

To analyse how mycelium and fruiting body development affect the volatilome of the dikaryotic *S. commune* wild-type strain H4-8, we chose two different cultivation processes. For the first approach (WT1), the mycelia were cultivated for eight days after inoculation in the dark sealed with Parafilm. The aeration was then enabled by removing the Parafilm, and the samples were exposed to a light day/night rhythm. For the second approach (WT2), aeration and illumination of the cultures were already applied at day 2. WT1 led to pronounced growth of mycelia (Fig. 1A). Around day 11, the mycelia developed into hyphal knots, and beginning with day 14, a further development into few but large fruiting bodies was observed (Fig. 1A). Different developmental stages were simultaneously present in the samples, including hyphal knots, primordia and young and mature fruiting bodies. In contrast, WT2 resulted in development of many small fruiting bodies (Fig. 1B) starting to appear already around day 8. Accordingly, these cultures showed less mycelium

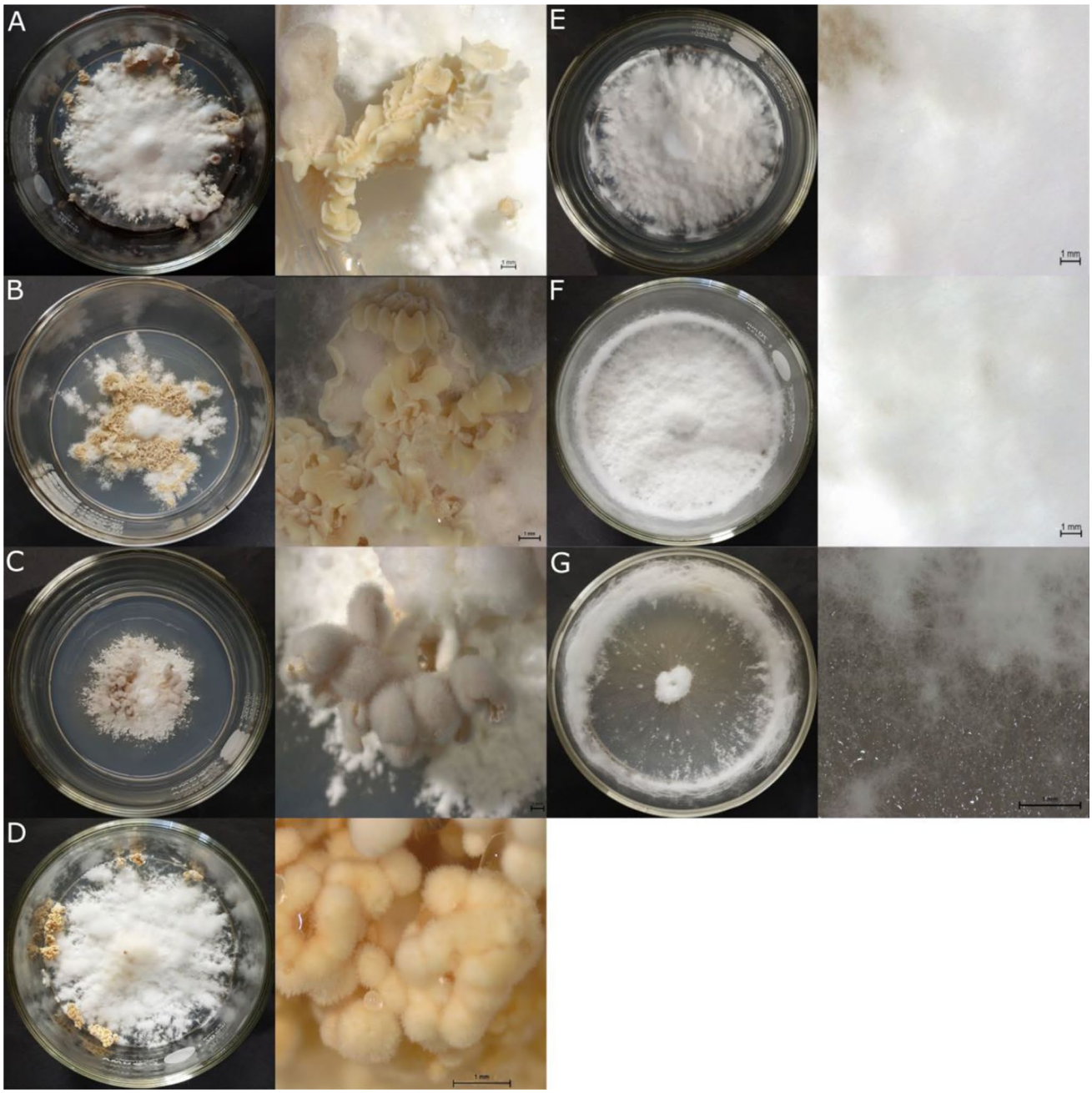


Fig. 1 Morphology and fruiting body formation of *S. commune* wild-type samples and derived mutants. Samples were grown at 24 °C in the dark in modified crystallizing dishes with MM and sealed with Parafilm. Except for WT1 (**A**, details see below), the Parafilm was removed 2 days after inoculation, and the samples were transferred to a climate chamber (24 °C, 95% rH, 12/12 h day/night rhythm) and cultured a further 22 days. **A** Wild-type dikaryon H4-8 (WT1) with mycelia cultivated for eight days after inoculation in the dark sealed with Parafilm. Afterwards, aeration and light exposure were applied as described above and samples were cultivated for a further 16 days. **B** Wild-type dikaryon H4-8 (WT2). **C** Monokaryon *hom2*.^{con}. **D** Dikaryon Δ*fst4* Δ*fst4*. **E** Dikaryon Δ*tea1* Δ*tea1*. **F** Dikaryon Δ*wc-2* Δ*wc-2*. **G** Dikaryon Δ*hom2* Δ*hom2*

and more fruiting bodies compared to WT1 (Fig. 1A, B). Moreover, mycelia of WT2 showed a more irregular growth than that of WT1 samples. All *S. commune* mutant strains were analysed using the second cultivation approach to increase the chances of fructification. Strain *hom2*^{con} grew more regular and slower than wild-type mycelia using the WT2 approach and started to develop bulging fruiting bodies around day 10 that only in some cases slightly began to expose their gills at late stages around day 20 (Fig. 1C). The Δ*fst4*Δ*fst4* dikaryon showed, like the WT2 mycelium,

irregular growth but it grew faster (Fig. 1D). In contrast to previous reports (Ohm et al. 2010; Pelkmans et al. 2017), Δ*fst4*Δ*fst4* started around day 16 to develop spherical, fruiting body-like structures that elongated and branched with ongoing cultivation (Fig. 1D, Supplementary Fig. S2). The absence of fruiting body formation by Δ*fst4*Δ*fst4* is likely explained by the lower light intensity that was used in the past showing that it can be important to apply different cultivation conditions to investigate the growth potential of fungal (deletion) strains. The Δ*tea1*Δ*tea1* dikaryon mycelia showed a radial growth of aerial mycelia with irregular surface that grew faster than WT2 mycelia (Fig. 1E), but the few clusters of fruiting bodies that were observed previously (Pelkmans et al. 2017) did not develop. The Δ*wc-2*Δ*wc-2* samples formed dense and symmetrical mycelia without formation of fruiting bodies (Fig. 1F). The Δ*hom2*Δ*hom2* dikaryon showed the fastest growth of all samples covering the plates already at day 6 with thin, symmetrically growing mycelia, which formed aerial hyphae especially at the outer edge of the samples (Fig. 1G). No formation of fruiting bodies was observed for Δ*hom2* Δ*hom2* samples.

VOCs in the HS of *S. commune*

By cultivating the *S. commune* wild type using two different approaches, one with pronounced mycelial growth as well as fruiting body development (WT1) and another resulting in early and pronounced fruiting body growth (WT2), we wanted to highlight fruiting-specific VOCs. For WT1, alcohols and esters as well as 3-methylfuran were the dominant VOCs in the HS of mycelial stages between day 8 and 12 decreasing with ongoing cultivation and with methyl 2-methylbutanoate being the most abundant substance (~ 70% of the total peak area at early stages) (Fig. 2). Beginning with the appearance of fruiting bodies at day 14, various sesquiterpenes were detected in the HS of WT1 peaking at day 18 with development of mature fruiting bodies (Fig. 2). At these late stages, α-guaiene, γ-gurjunene and δ-cadinene were the most dominant VOCs in the HS (Supplementary Fig. S3). Interestingly, some sesquiterpenes such as viridiflorene, germacrene D and cadina-1,4-diene were only detectable for a short period around day 18 whereas others, including α-guaiene, γ-gurjunene and chamigrene, remained present in the HS throughout all following sampling days (Fig. 2).

Comparable with WT1, the HS of early developmental stages between days 4 and 8 of WT2 was dominated by alcohols and esters. At these stages, methyl 2-methylbutanoate was again the most abundant VOC (~ 68% of the total peak area) (Fig. 3A). It seems that these VOCs are associated with mycelial growth. Oct-1-en-3-ol and several sesquiterpenes appeared in the HS beginning with the development of fruiting bodies around day 8 and peaked at day 12 during maturation of fruiting bodies with γ-gurjunene and α-guaiene being the most abundant VOCs with ~ 34% and ~ 25% of the total peak area. Comparable to WT1, these sesquiterpenes remained present in the HS of the samples with ongoing cultivation. However, in contrast to WT1, remarkably less different sequiterpenes were detected in the HS. Considering the less pronounced mycelial growth of WT2, it could be that certain sesquiterpenes, such as α-guaiene, γ-gurjunene and chamigrene, are formed in fruiting bodies, whereas others, including α-cubebene, α-copaene and viridiflorene, might require in addition to mature fruiting bodies the presence of mycelium. To further investigate the connections between fungal growth and VOC formation, we analysed VOCs of *S. commune* deletion mutants with different phenotypes.

In early stages of *hom2*^{con} between days 2 and 8, alcohols and esters, including 2-methylpropan-1-ol, 3-methylbutan-1-ol and methyl 2-methylbutanoate, dominated the volatilomes, and their amounts decreased with ongoing fruiting body development (Fig. 3B). Like for WT2, sesquiterpenes, such as α-guaiene, γ-gurjunene and chamigrene, were detected beginning with the appearance of fruiting bodies at day 10 matching the assumption that these sesquiterpenes are formed in fruiting bodies (Fig. 3B). The amounts of the sesquiterpenes successively increased along with the further development of the fruiting bodies and peaked around day 22. However, despite showing pronounced mycelial growth and the presence of fruiting bodies, certain sesquiterpenes found in WT1, like α-cubebene, were not detected in *hom-2*^{con}. This could be related to the fact that the monokaryotic *hom2*^{con} strain is able to form fruiting bodies, but these are not fully developed in contrast to the wild type.

The dikaryotic Δ*fst4*Δ*fst4,* able to develop rudimentary fruiting bodies, showed remarkable differences in its VOCs profiles compared with WT1, WT2 and *hom2*^{con} (Figs. 1D and 3C). Like for wild-type samples, early mycelial stages from days 4 to 8 were dominated by esters, including methyl 2-methylbutanoate (most abundant substance at these stages with ~ 70% of the total peak area), ethyl 2-methylbutanoate, isobutyl 2-methylpropionate and 2-methylbutyl acetate. However, the last three esters were not found in the HS of the wild-type samples. Additionally, the sulphur containing VOCs dimethyl disulphide and dimethyl trisulfide could be detected in the HS of Δ*fst4*Δ*fst4* samples at days 2 and 4, but were not present in the wild-type samples or *hom2*^{con}. We did not detect in Δ*fst4*Δ*fst4* certain sesquiterpenes, like α-guaiene, which were associated in WT1, WT2 and *hom2*^{con} with fruiting body development, which supports the assumption that the formation of certain sesquiterpenes requires the presence of fully mature fruiting bodies. Instead, three sesquiterpenes, namely, (*E*)-nerolidol, L-calamenene and α-bisabolol, were present in the HS of Δ*fst4*Δ*fst4* at days 14 and 16, simultaneously with the appearance of the fruiting body structures of which the last two were also detected in WT1 (Figs. 2 and 3C).

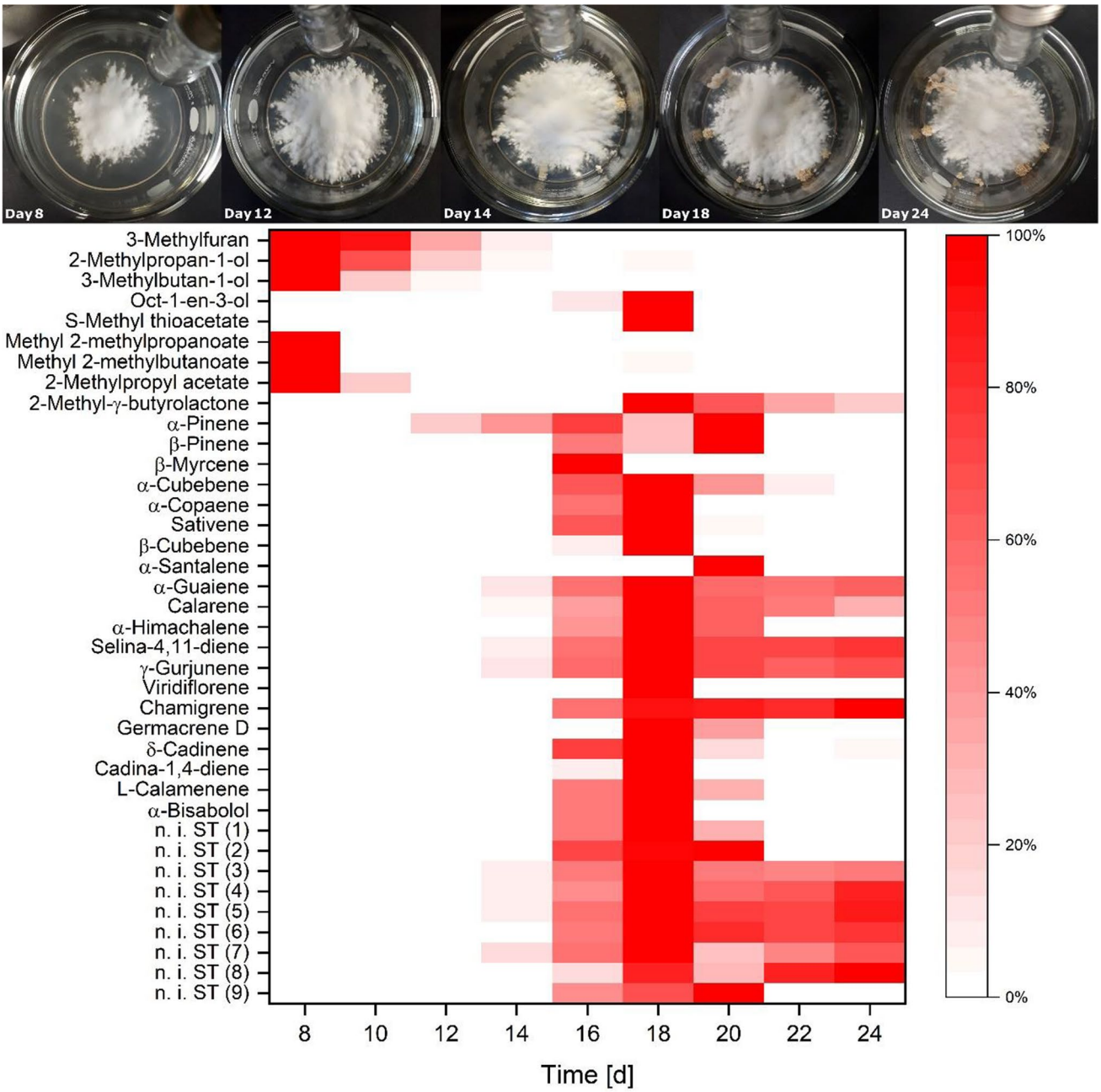


Fig. 2 VOCs of the dikaryotic wild type strain *S. commune* H4-8 cultivated accordingly to the first cultivation approach (WT1). Hyphal knots appeared around day 11, at day 12 first primordia grew and first mature fruiting bodies developed about day 16. Shown is the development of the relative amounts of VOCs between days 8 and 24 after inoculation. The percent disclosure refers to each substance individually, meaning that 100% is related to the highest peak area of the compound measured during the experiment. Samples were grown in triplicates on MM at 24 °C in the dark in modified crystallizing dishes sealed with Parafilm. Eight days after inoculation, the Parafilm was removed, and the samples were transferred to a climate chamber (24 °C, 95% rH, 12/12 h day/night rhythm) and cultured for a further 16 days. VOCs were collected by SPME from day 8 onwards: volatiles were absorbed directly from the HS of the crystallizing dish for 1 h and analysed by GC–MS. n. i. ST: not identified sesquiterpene

Comparable to VOC patterns of Δ*fst4*Δfst4, esters (mainly methyl 2-methylbutanoate, ~ 80% of the total peak area) and dimethyl trisulfide could be detected in the HS of early stages, mainly between days 2 and 6, of the *S. commune* deletion strains Δ*wc-2*Δ*wc-2* and Δ*hom2*Δ*hom2* (Fig. 4A, B). For Δ*wc-2*Δ*wc-2*, the sesquiterpenes β-bisabolene, δ-cadinene and L-calamenene could be detected in late stages around day 22 (Fig. 4A), whereas for Δ*hom2*Δ*hom2* δ-cadinene, (*E*)-nerolidol and α-bisabolol were detectable around day 12 (Fig. 4B). For Δ*tea1*Δ*tea1*,

no sesquiterpenes could be detected in the HS of samples (Supplementary Fig. S4). However, at days 2 and 4, the monoterpenes β-myrcene and α-terpineol were present in the HS of Δ*tea1*Δ*tea1* of which the latter could not be found in wild-type samples (Supplementary Fig. S4). Taking into account that β-bisabolene, δ-cadinene, L-calamenene, (*E*)-nerolidol and α-bisabolol were present in the HS of *S. commune* strains unable to develop fruiting bodies, it seems that the formation of these sesquiterpenes, in contrast to others mentioned above, does not require the presence of fruiting bodies.

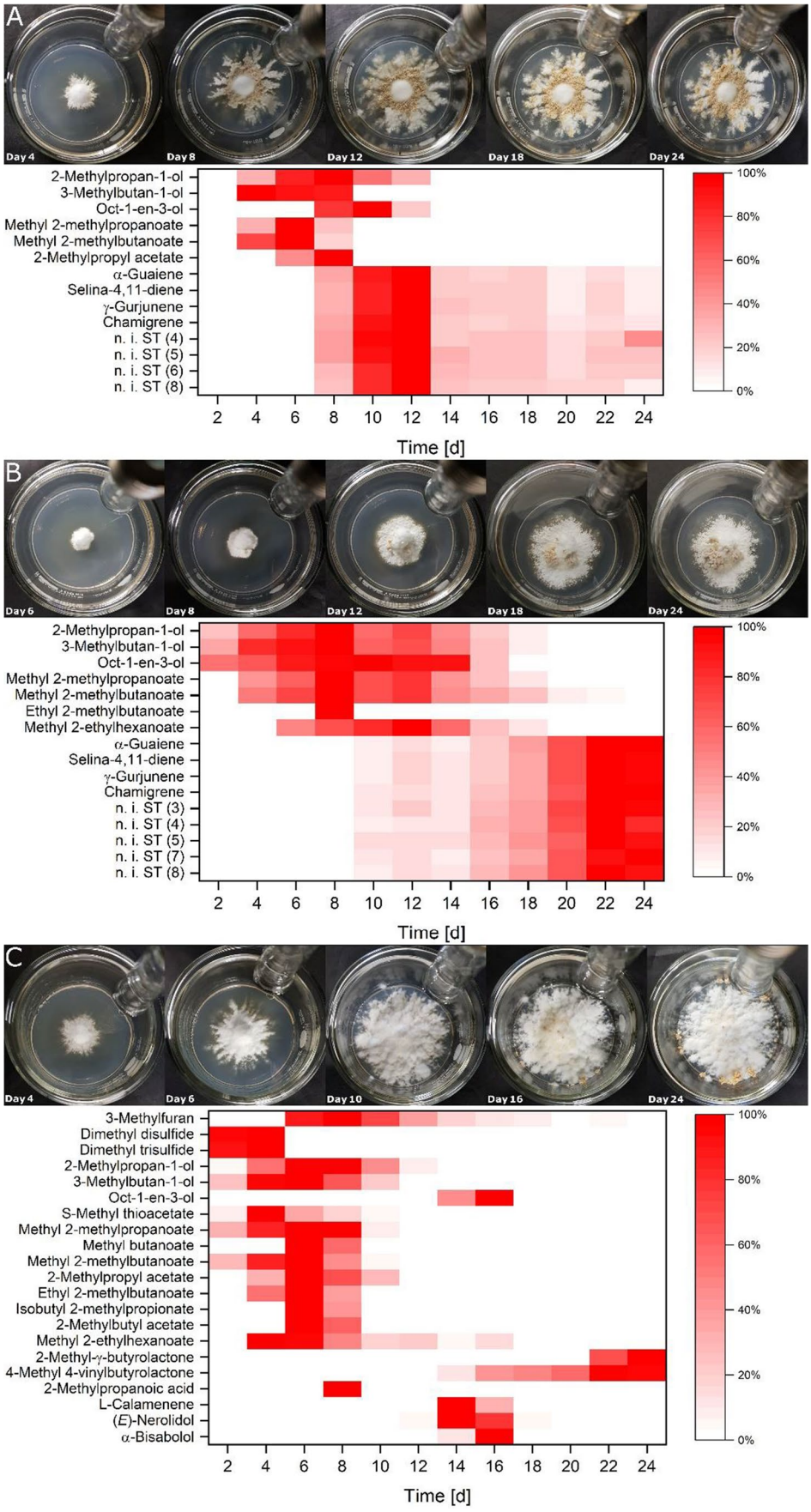
To further highlight the differences in the volatilomes, a principal component analysis (PCA) was carried out for the VOC composition of the different *S. commune* (Fig. 5). The first two principal components covered ~ 62% of the data's original variation and clustered roughly into two main groups consisting of strains able to form fruiting bodies and the ones unable to do so (Fig. 5). Despite being able to develop fruiting body like structures, the Δ*fst4*Δ*fst4* dikaryon samples clustered with the Δ*tea1*Δ*tea1*, Δ*wc-2*Δ*wc-2* and Δ*hom2*Δ*hom2* samples that only form mycelia, reflecting the lack of Δ*fst4*Δ*fst4* to form fruiting-related sesquiterpenes. Overall, the ability to develop fruiting bodies seems to have a tremendous effect on the volatilomes of *S. commune*.

Only considering VOCs present in the HS of at least two samples of the triplicates, in total 54 VOCs were found in the HS of the tested strains during all 231 GC–MS measurements (Table 1). Based on the VOC data shown above, we classified these VOCs into three categories: (1) VOCs found in strains with and without the ability to form fruiting bodies, (2) VOCs only found in strains unable to form (proper) fruiting bodies and (3) VOCs only found in strains able to form fruiting bodies (Table 1). The first category mainly harbours esters and alcohols, which are as mentioned above typical for early mycelial stages of all samples but with only 2-methylpropan-1-ol, 3-methylbutan-1-ol, methyl 2-methylpropanoate and methyl 2-methylbutanoate being detected in the HS of all samples (Table 1). Additionally, some terpenes, namely, β-myrcene, δ-cadinene, L-calamene and α-bisabolol, were found in WT1, and certain *S. commune* samples unable to develop fruiting bodies showing that these terpenes do not require fruiting bodies for their formation (Table 1). The same applies to the terpenes α-terpineol, β-bisabolene and (*E*)-nerolidol, which could only be found in strains unable to form fruiting bodies (Table 1). Also some esters like methyl butanoate belong to this second category. Interestingly, certain C_8 volatiles such as oct-2-en-1-ol and sulphur containing VOCs, namely dimethyl disulphide and dimethyl trisulfide, could also be detected in strains unable to form fruiting bodies only. In contrast, nearly half of all VOCs could only be found in samples able to develop fruiting bodies being all terpenes, mainly sesquiterpenes (Table 1). The formation of these VOCs seems to require the presence of fruiting bodies. However, due to the fact that many of these were only found in WT1, it seems that the formation also requires the mycelium and/or fully developed fruiting bodies.

Discussion

We were able to show that developmental stages highly affect volatilomes of *S. commune* and that the presence of fruiting bodies have a remarkable influence on the VOCs emitted by the fungus. Previous studies analysed VOC formation in *S. commune* only at one time point. Pimenta et al. (2017) studied the VOC composition in the mycelia of three different *S. commune* wild-type strains grown for 6 days on PDA agar. The VOC composition of the strains slightly differed, but was comparable to our results, alcohols such as 2-methylpropan-1-ol and 3-methyl butan-1-ol, esters including methyl 2-methylbutanoate, ethyl 2-methylbutanoate and 2-methylbutyl acetate as well as sesquiterpenes such as α-bisabolol, α-curcumene and γ-himachalene were found. Interestingly, in the HS of mycelial samples of the *S. commune* wild-type strain 4–39, cultivated for 7 days on CYM agar, alcohols and esters but no sesquiterpenes were found (Wirth et al. 2018). However, our study shows that the VOC composition of *S. commune* depends on the cultivation approach and changes during different stages, which should be taken into account for the analysis of fungal VOCs especially regarding the potential of fungi to produce certain VOCs.

Numerous studies analysing fungal VOCs were carried out including research on *Calocybe gambosa* (Kleofas et al. 2015), *Clithocybe odora*, *Lentinellus cochleatus*, *Agaricus essettei* (Rapior et al. 2002), *Agaricus bisporus*, *Pleurotus florida*, *Calocybe indica* (Venkateshwarlu et al. 1999), *Pleurotus ostreatus* (Okamoto et al. 2002), *Tricholoma matsutake* (Cho et al. 2008), *Lentinula edodes* (Chen and Ho 1986), *Hygrophorus chrysodon* (Ouzouni et al. 2009), *Clitocybe fragrans*, *Hebeloma crustuliniforme*, *Lepista nuda*, *Tricholoma fracticum* and *Tricholoma terreum* (Malheiro et al. 2013). Notwithstanding the large number of studies on fungal VOCs, the connection between fungal development and VOC formation was not assessed in detail. Additionally, studies dealing with changes of volatilomes during fungal development mainly focus on the fruiting bodies of mushrooms, such as *A. bisporus* (Combet et al. 2009; Cruz et al. 1997; Zawirska-Wojtasiak et al. 2007), *Volvariella volvacea* (Mau et al. 1997; Zhang et al. 2008), *Tricholoma matsutake* (Cho et al. 2006; Li et al. 2016) and *Pleurotus ostreatus* (Tasaki et al. 2019; Zhang et al. 2008), analysing the aroma

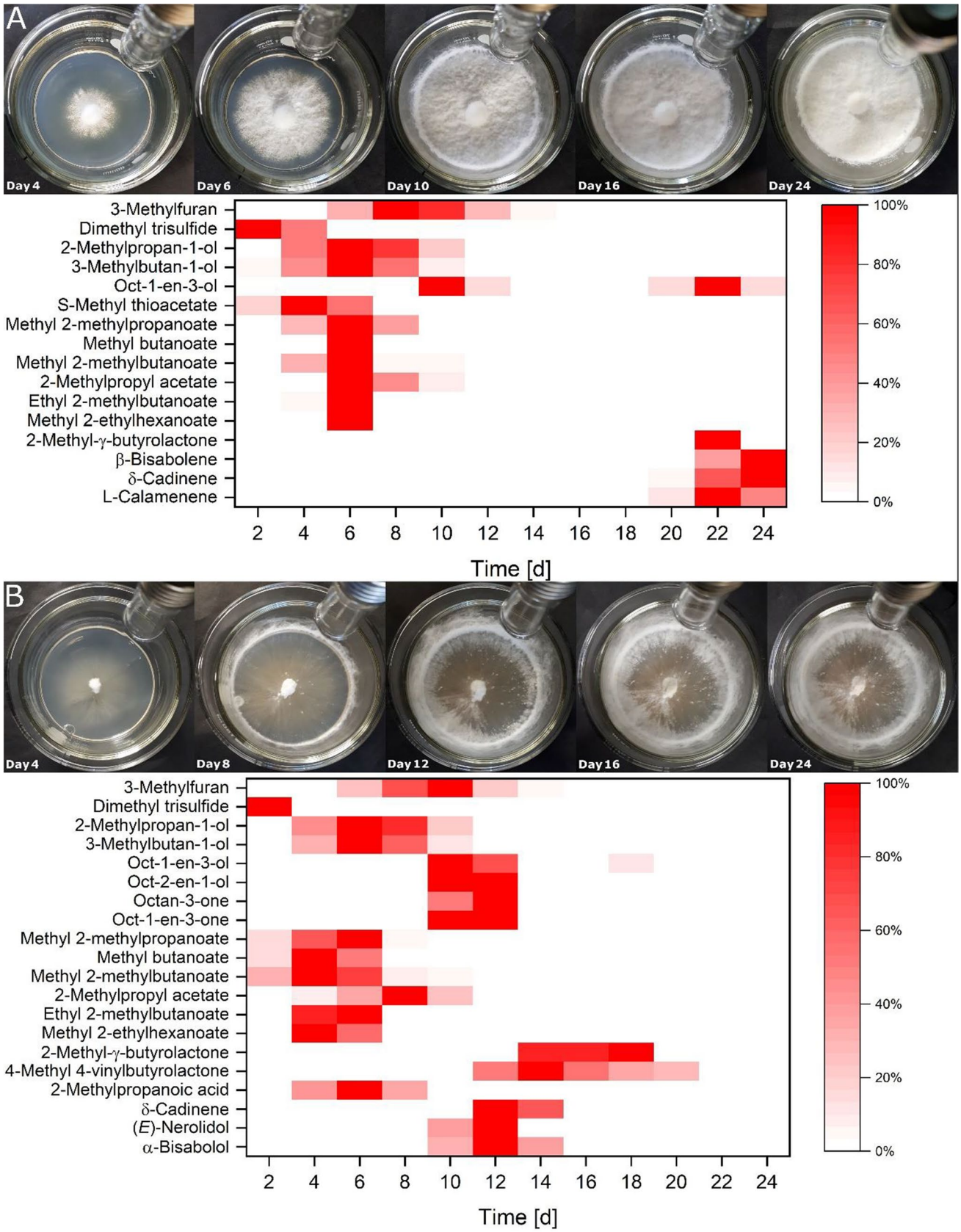

A
Day 4
Day 8
Day 12
Day 18
Day 24
2-Methylpropan-1-ol
3-Methylbutan-1-ol
Oct-1-en-3-ol
Methyl 2-methylpropanoate
Methyl 2-methylbutanoate
2-Methylpropyl acetate
α-Guaiene
Selina-4,11-diene
γ-Gurjunene
Chamigrene
n. i. ST (4)
n. i. ST (5)
n. i. ST (6)
n. i. ST (8)
100%
80%
60%
40%
20%
0%
2 4 6 8 10 12 14 16 18 20 22 24
Time [d]
B
Day 6
Day 8
Day 12
Day 18
Day 24
2-Methylpropan-1-ol
3-Methylbutan-1-ol
Oct-1-en-3-ol
Methyl 2-methylpropanoate
Methyl 2-methylbutanoate
Ethyl 2-methylbutanoate
Methyl 2-ethylhexanoate
α-Guaiene
Selina-4,11-diene
γ-Gurjunene
Chamigrene
n. i. ST (3)
n. i. ST (4)
n. i. ST (5)
n. i. ST (7)
n. i. ST (8)
100%
80%
60%
40%
20%
0%
2 4 6 8 10 12 14 16 18 20 22 24
Time [d]
C
Day 4
Day 6
Day 10
Day 16
Day 24
3-Methylfuran
Dimethyl disulfide
Dimethyl trisulfide
2-Methylpropan-1-ol
3-Methylbutan-1-ol
Oct-1-en-3-ol
S-Methyl thioacetate
Methyl 2-methylpropanoate
Methyl butanoate
Methyl 2-methylbutanoate
2-Methylpropyl acetate
Ethyl 2-methylbutanoate
Isobutyl 2-methylpropionate
2-Methylbutyl acetate
Methyl 2-ethylhexanoate
2-Methyl-γ-butyrolactone
4-Methyl 4-vinylbutyrolactone
2-Methylpropanoic acid
L-Calamenene
(E)-Nerolidol
α-Bisabolol
100%
80%
60%
40%
20%
0%
2 4 6 8 10 12 14 16 18 20 22 24
Time [d]

◂ **Fig. 3** VOCs of fruiting body forming *S. commune* strains cultivated accordingly to the second cultivation approach: wild type strain H4-8 (WT2, **A**), monokaryotic transformant *hom2*^{con} (**B**) and dikaryotic deletion strain Δ*fst4*Δ*fst4* (**C**). For WT2, at day 5, first primordia appeared developing into fruiting bodies around day 8, and first mature fruiting bodies developed about day 10. For *hom2*^{con}, primordia grew around day 7, developing into mature fruiting bodies around day 18. For Δ*fst4*Δ*fst4*, primordia appeared around day 14, developing into spherical, fruiting body like structures with ongoing cultivation. The heatmaps show the development of the relative amounts of VOCs between days 2 and 24 after inoculation. The per cent disclosure refers to each substance individually, meaning that 100% is related to the highest peak area of the compound measured during the experiment. Samples were grown in triplicates on MM at 24 °C in the dark in modified crystallizing dishes sealed with Parafilm. Two days after inoculation, the Parafilm was removed and the samples were transferred to a climate chamber (24 °C, 95% rH, 12/12 h day/night rhythm) and cultured for a further 22 days. VOCs were collected by SPME from day 2 onwards: volatiles were absorbed directly from the HS of the crystallizing dish for 1 h and analysed by GC–MS. n. i. ST: not identified sesquiterpene

profiles in context of food quality. In contrast, studies analysing fungal volatilomes under natural circumstances considering mycelia and fruiting bodies are rare. Recently, a comprehensive study on the volatilomes of *C. aegerita strains* during different stages showed that the release of VOCs is connected to fungal development (Orban et al. 2020). For *C. aegerita*, in the HS of early mycelial stages before primordia appeared alcohols and ketones, including oct-1en-3-ol, 2-methylbutan-1-ol and cyclopentanone, were the dominant substances. Here, we showed that in *S. commune* wild-type samples, the alcohols 2-methylpropan-1-ol and 3-methylbutan-1-ol as well as the structural related esters methyl 2-methylpropanoate and methyl 2-methylbutanoate were the main VOCs present in the HS during early developmental stages. Interestingly, Δ*fst4*Δ*fst4*, Δ*tea1*Δ*tea1*, Δ*wc-2*Δ*wc-2* and Δ*hom2*Δ*hom2* samples, all being unable to develop proper fruiting bodies, produced a remarkably higher variability of esters compared to *hom2*^{con} and the wild-type samples. Hence, for *S. commune*, these alcohols and esters are most likely produced in the mycelium rather in the fruiting bodies. This assumption is supported by the fact that in a previous study none of these VOCs was found in fruiting bodies of *S. commune* (Ziegenbein et al. 2006). Several fungal volatile alcohols, aldehydes and esters are derived from amino acids. In *Saccharomyces cerevisiae,* 2-methylpropan-1-ol is produced by the Ehrlich pathway with valine as precursor (Dickinson et al. 2003). Accordingly*, S. commune* seems to produce enzymes of the Ehrlich pathway as not only 2-methylpropan-1-ol was present in all analysed samples but also the related acid, 2-methylpropanoic acid, was detected in two *S. commune* mutant strains. Even an esterified product of 2-methylpropan-1-ol, methyl 2-methylpropanoate, was present in all analysed strains. Besides the Ehrlich pathway compounds derived from valine, products of leucine, such as 3-methylbutan-1-ol, and isoleucine, such as methyl 2-methylbutanoate, were present as well. The relation between isoleucine and methyl 2-methylbutanoate was proven by Zhang et al. (2018) in liquid cultures of *L. edodes* applying stable isotope labelled precursor-feeding studies. However, further research is necessary to reveal whether the formation of amino acid derived VOCs during early mycelial stages is a common feature in fungi and if these VOCs play a biological role during fructification.

In later stages around day 18, we observed that in *S. commune* wild-type samples, especially counting for WT1, most of the sesquiterpenes appeared in a short period during fruiting body maturation. Simultaneously with the development of fruiting bodies, sesquiterpenes were also detected in the HS of the *hom2*^{con} strain. These results are in good agreement with previous observations on *C. aegerita*, where especially during sporulation, sesquiterpenes were the main VOCs in the HS (Orban et al. 2020). Interestingly, also for the bracket fungi *Fomitopsis pinicola* and *Fomes fomentarius* (Fäldt et al. 1999) as well as for *C. cinerea* (Chaisaena 2009; Thakeow 2008), an obvious increase of sesquiterpenes was observed during sporulation. Hence, sesquiterpene release closely connected to the development of fruiting bodies could be a common feature in higher fungi of the phylum *Basidiomycota*. Sesquiterpenes can exhibit antimicrobial activities and may therefore protect fungi during critical developmental stages against other parasitic fungi or bacteria (Ishikawa et al. 2001; Solís et al. 2004). VOCs from *S. commune*, especially β-bisabolol and bisabolene, inhibit mycelial growth of *P. ostreatus*, *Ganoderma lucidum*, *Flammulina velutipes* and *Kuehneromyces mutabilis*, all fungi competing with *S. commune* for natural resources (Wirth et al. 2021). Additionally, the composition of sesquiterpenes differs remarkably between fungal species (Chaisaena 2009; Fäldt et al. 1999; Orban et al. 2020) predestining these VOCs, along with C_8 oxylipins, for a role as species-dependent infochemicals that could be involved in the communication between different fungal species (El Ariebi et al. 2016; Hiscox and Boddy 2017; Hynes et al. 2007), between fungi and animals (Drilling and Dettner 2009; Holighaus et al. 2014; Kües et al. 2018), between fungi and bacteria (Orban et al. 2023; Wirth et al. 2021) and/or fungi and plants (Ezediokpu et al. 2022).

Regardless of the function of sesquiterpenes, the question remains, which part of the fungus, mycelium or fruiting body, is responsible for the formation of these VOCs. The deletion mutants, Δ*fst4*Δ*fst4*, Δ*tea1*Δ*tea1*, Δ*wc-2*Δ*wc-2* and Δ*hom2*Δ*hom2*, showed remarkably less sesquiterpenes in the HS than strains able to form fruiting bodies. However, the sesquiterpenes found in the HS of these samples, namely (*E*)-nerolidol, δ-cadinene, L-calamenene, α-bisabolol and β-bisabolene, were not present in *hom2*^{con} or WT2 that mainly formed fruiting bodies and barely mycelium.


A
Day 4
Day 6
Day 10
Day 16
Day 24
3-Methylfuran
Dimethyl trisulfide
2-Methylpropan-1-ol
3-Methylbutan-1-ol
Oct-1-en-3-ol
S-Methyl thioacetate
Methyl 2-methylpropanoate
Methyl butanoate
Methyl 2-methylbutanoate
2-Methylpropyl acetate
Ethyl 2-methylbutanoate
Methyl 2-ethylhexanoate
2-Methyl-γ-butyrolactone
β-Bisabolene
δ-Cadinene
L-Calamenene
2 4 6 8 10 12 14 16 18 20 22 24
Time [d]
100%
80%
60%
40%
20%
0%
B
Day 4
Day 8
Day 12
Day 16
Day 24
3-Methylfuran
Dimethyl trisulfide
2-Methylpropan-1-ol
3-Methylbutan-1-ol
Oct-1-en-3-ol
Oct-2-en-1-ol
Octan-3-one
Oct-1-en-3-one
Methyl 2-methylpropanoate
Methyl butanoate
Methyl 2-methylbutanoate
2-Methylpropyl acetate
Ethyl 2-methylbutanoate
Methyl 2-ethylhexanoate
2-Methyl-γ-butyrolactone
4-Methyl 4-vinylbutyrolactone
2-Methylpropanoic acid
δ-Cadinene
(E)-Nerolidol
α-Bisabolol
2 4 6 8 10 12 14 16 18 20 22 24
Time [d]
100%
80%
60%
40%
20%
0%

◂**Fig. 4** VOCs of *S. commune* strains unable to form fruiting bodies and cultivated accordingly to the second cultivation approach: dikaryotic deletion strain Δ*wc-2*Δ*wc-2* (**A**) and dikaryotic deletion strain Δ*hom2*Δ*hom2* (**B**). Shown is the development of the relative amounts of VOCs between days 2 and 24 after inoculation. The per cent disclosure refers to each substance individually, meaning that 100% is related to the highest peak area of the compound measured during the experiment. Samples were grown in triplicates on MM at 24 °C in the dark in modified crystallizing dishes sealed with Parafilm. Two days after inoculation, the Parafilm was removed and the samples were transferred to a climate chamber (24 °C, 95% rH, 12/12 h day/night rhythm) and cultured for a further 22 days. VOCs were collected by SPME from day 2 onwards: volatiles were absorbed directly from the HS of the crystallizing dish for 1 h and analysed by GC–MS. n. i. ST: not identified sesquiterpene

Other studies confirmed the presence of α-bisabolol and β-bisabolene in mycelial samples of *S. commune* (Schalchli et al. 2011; Pimenta et al. 2017), which make the mycelium likely to be the main origin of these VOCs. However, (*E*)-nerolidol, δ-cadinene and α-bisabolol were also found in extracts of *S. commune* fruiting bodies obtained by hydrodestillation, showing that these sesquiterpenes can also be formed in at least mature fruiting bodies (Ziegenbein et al. 2006). Certain sesquiterpenes, including α-guaiene, chamigrene and γ-gurjunene, were only found in the presence of fruiting bodies and a previous study confirmed the occurrence of α-guaiene in fruiting bodies of *S. commune* (Ziegenbein et al. 2006) leading to the assumption that these sesquiterpenes originate from fruiting bodies. Many sesquiterpenes, *inter alia* α-cubebene, α-copaene, β-cubebene, viridiflorene, α-himachalene and germacrene D, were only detected in the HS of WT1 samples and not in WT2 or *hom2*^{con} samples. The different cultivation approaches for the *S. commune* wild-type strain H4-8 resulted in a more pronounced mycelial growth and less but bigger fruiting bodies in case of WT1, whereas WT2 samples developed many but smaller fruiting bodies and produced less mycelium. These alternations in the phenotype are most likely the reason for the differences in the volatilomes. Recently, combined volatilomic and transcriptomic data of *C. aegerita* during different developmental stages showed that, despite their release during sporulation, the origin of most sesquiterpenes in *C. aegerita* is probably the mycelium (Orban et al. 2020, 2021). Taking the less pronounced mycelial growth of WT2 samples into account, we can speculate that the origin of sesquiterpenes in the WT1 samples mentioned above may be the mycelium rather than the fruiting body. Also in other fungi, differences in VOC composition of different morphological structures were observed. In stipes and in the pileus of the pine-mushroom *Tricholoma matsutake*, VOCs like hexanal, linalool and octan-3-ol contribute differently to the overall odour impression (Cho et al. 2008). Formation of sesquiterpenes or other VOCs in different parts of the fungus with changing patterns during development could be an additional aspect of VOCs functioning as infochemicals. In case of the monokaryotic *hom2*^{con} strain, the absence of certain sesquiterpenes, despite the ability to grow fruiting bodies and having pronounced mycelial growth, might be connected to an incomplete development of fruiting bodies. For *C. aegerita*, it was shown that monokaryotic strains, even if they can form fruiting bodies, produce much less sesquiterpenes than the dikaryon during sporulation (Orban et al. 2020). Formation of sesquiterpenes in mycelium triggered by certain developmental stages of the fruiting body, like sporulation, might be a common feature of fungi and should be considered for future studies.

As discussed above, we could show that VOC biosynthesis in *S. commune* depends on the developmental stage and is intertwined with fruiting body formation. However, the formation processes of fungal VOCs are in many cases still barely understood (Dickschat 2017). Even for the ubiquitously present C_8 VOCs, including oct-1-en-3-ol, knowledge is still missing. It is widely accepted that C_8 VOCs are derived from linoleic acid. Nonetheless, a resilient connection of further steps in the formation process involving lipoxygenases (LOXs), dioxygenases (DOXs), hydroperoxide lyases (HPLs), alcohol dehydrogenases (ADHs) and ene-reductases representing the complete C_8 pathways is still missing (Chen and Wu 1984; Karrer et al. 2021; Orban et al. 2021; Tasaki et al. 2019; Teshima et al. 2022; Wanner and Tressl 1998; Wurzenberger and Grosch 1984a, b, c). The biosynthesis of fungal

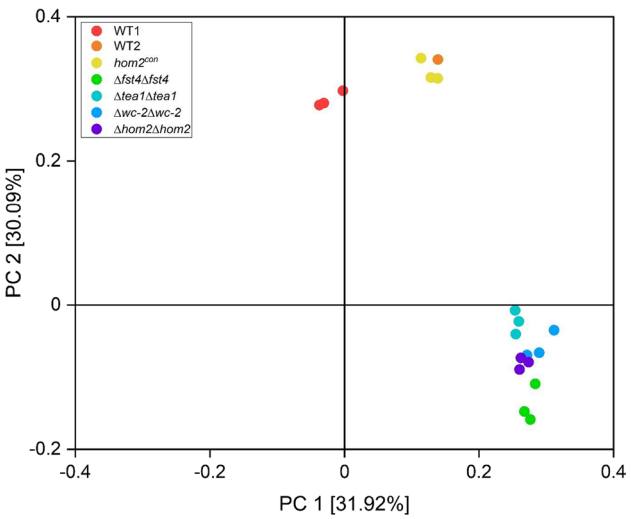


Fig. 5 Principal component analysis (PCA) for volatilome data of *S. commune* strains. A dot represents the data of one sample of the triplicates. Each sample was checked for the presence of a specific VOC at each sample time point, and the data was summarized showing if a certain VOC was present during any of the stages or not. This means that the PCA data for a sample is not limited to one time point but represents the volatilomes of all stages. WT1: wild-type samples cultivated accordingly to the first cultivation approach (for details see Material and Method section). WT2: wild-type samples cultivated accordingly to the second cultivation approach

Table 1 Total number of VOCs found in the HS of the *S. commune* strains

VOCs	H4-8 WT1	H4-8 WT2	hom2^{con}	Δfst4	Δtea1	Δwc-2	Δhom2
3-Methylfuran	+	-	-	+	+	+	+
2-Methylpropan-1-ol	+	+	+	+	+	+	+
3-Methylbutan-1-ol	+	+	+	+	+	+	+
Oct-1-en-3-ol	+	+	+	+	-	+	+
S-Methyl thioacetate	+	-	-	+	+	+	-
Methyl 2-methylpropanoate	+	+	+	+	+	+	+
Methyl 2-methylbutanoate	+	+	+	+	+	+	+
2-Methylpropyl acetate	+	+	-	+	+	+	+
Ethyl 2-methylbutanoate	-	-	+	+	+	+	+
Methyl 2-ethylhexanoate	-	-	+	+	+	+	+
2-Methyl-γ-butyrolactone	+	-	-	+	-	+	+
β-Myrcene	+	-	-	-	+	-	-
δ-Cadinene	+	-	-	-	-	+	+
L-Calamenene	+	-	-	+	-	+	-
α-Bisabolol	+	-	-	+	-	-	+
Dimethyl disulfide	-	-	-	+	-	-	-
Dimethyl trisulfide	-	-	-	+	-	+	+
Oct-2-en-1-ol	-	-	-	-	-	-	+
Octan-3-one	-	-	-	-	-	-	+
Oct-1-en-3-one	-	-	-	-	-	-	+
Methyl propanoate	-	-	-	-	+	-	-
Methyl butanoate	-	-	-	+	+	+	+
Isobutyl 2-methylpropionate	-	-	-	+	-	-	-
2-Methylbutyl acetate	-	-	-	+	+	-	-
4-Methyl 4-vinylbutyrolactone	-	-	-	+	-	-	+
2-Methylpropanoic acid	-	-	-	+	-	-	+
α-Terpineol	-	-	-	-	+	-	-
β-Bisabolene	-	-	-	-	-	+	-
(*E*)-Nerolidol	-	-	-	+	-	-	+
α-Pinene	+	-	-	-	-	-	-
β-Pinene	+	-	-	-	-	-	-
α-Cubebene	+	-	-	-	-	-	-
α-Copaene	+	-	-	-	-	-	-
Sativene	+	-	-	-	-	-	-
β-Cubebene	+	-	-	-	-	-	-
α-Santalene	+	-	-	-	-	-	-
α-Guaiene	+	+	+	-	-	-	-
Calarene	+	-	-	-	-	-	-
α-Himachalene	+	-	-	-	-	-	-
Selina-4,11-diene	+	+	+	-	-	-	-
γ-Gurjunene	+	+	+	-	-	-	-
Viridiflorene	+	-	-	-	-	-	-
Chamigrene	+	+	+	-	-	-	-
Germacrene D	+	-	-	-	-	-	-
Cadina-1,4-diene	+	-	-	-	-	-	-
n. i. ST (1)	+	-	-	-	-	-	-
n. i. ST (2)	+	-	-	-	-	-	-
n. i. ST (3)	+	-	+	-	-	-	-
n. i. ST (4)	+	+	+	-	-	-	-
n. i. ST (5)	+	+	+	-	-	-	-
n. i. ST (6)	+	+	-	-	-	-	-
n. i. ST (7)	+	-	+	-	-	-	-
n. i. ST (8)	+	+	+	-	-	-	-
n. i. ST (9)	+	-	-	-	-	-	-

Table 1 (continued)

+: VOC detected in at least two samples of the triplicates. -: VOC detected only in one or none of the samples. *WT1*, wild-type *S. commune* dikaryon cultivated accordingly to the first cultivation approach (for details see the Material and Method section). *WT2*, wild-type *S. commune* dikaryon cultivated accordingly to the second cultivation approach. *n. i. ST*, not identified sesquiterpene

Green highlighted rows indicate VOCs found in strains with and without the ability to form fruiting bodies, orange highlighted rows indicate VOCs only found in strains unable to form (proper) fruiting bodies and blue highlighted rows indicate VOCs only found in strains able to form fruiting bodies

terpenes is comparatively well understood. The large diversity of terpenes is derived from only two precursors, dimethylallyl diphosphate and isopentenyl diphosphate, which in fungi are produced from acetyl-CoA by the mevalonate pathway (Miziorko 2011). Condensation of these two isomers results in linear molecules with different chain length: C_{10} geranyl diphosphate, C_{15} farnesyl diphosphate, C_{20} geranylgeranyl diphosphate and C_{25} geranylfarnesyl diphosphate. Dephosphorylation and cyclization reactions of these hydrocarbons, catalysed by terpene synthases, are the origin of the wide range of terpenes including sesquiterpenes (Christianson 2006). Additionally, modifications of terpenes catalysed by cytochrome P450 monooxygenases, oxidoreductases and different transferases contribute also to the high diversity of terpenes in fungi (Quin et al. 2014).

In recent years, the interest in fungal sesquiterpene synthases increased remarkably (Agger et al. 2009; Engels et al. 2011; Ichinose and Kitaoka 2018; Lopez-Gallego et al. 2010; Mischko et al. 2018; Ntana et al. 2021; Zhang et al. 2020), but the regulation of the formation of fungal sesquiterpenes and other VOCs is barely understood. In *S. commune*, Thn1 is a regulator of G-protein signalling, and its deletion resulted in reduced formation of aerial mycelium with dikaryotic Δ*thn1*Δ*thn1* and Δ*thn1* mutants unable to form fruiting bodies (Wirth et al. 2018). Sesquiterpenes, including β-bisabolol, β-bisabolene and α-bisabolol, were found in the HS of *S. commune* Δ*thn1* samples but not in the HS of wild-type samples. The authors therefore proposed that Thn1 negatively regulates sesquiterpene synthesis in *S. commune* (Wirth et al. 2018). However, the study analysed VOCs and gene expression patterns only at day 7 after inoculation. Our results show that the release of sesquiterpenes in *S. commune* is highly connected to the cultivation conditions and developmental stage. Hence, the observed absence of sesquiterpenes in the *S. commune* wild-type strain could be due to the sampling process rather than negative regulation of sesquiterpene formation by Thn1. Alternatively, alternations of the VOC patterns between wild-type strain and Δ*thn1* mutant may be related to the growth differences of the mycelia and, thus, are only indirectly caused by Thn1.

Here, we analysed the effect of transcription factors on volatilomes in *S. commune*. To the best of our knowledge, this is the first study investigating volatilomes of basidiomycete deletion strains during different developmental stages. Deletion of the FRGs *wc-2* (coding for a transcription factor involved along with WC-1 in blue light sensing (Ohm et al. 2013)), the homeodomain gene *hom2* (Ohm et al. 2011; Pelkmans et al. 2017) and the TEA/ATTS transcription factor gene *tea1* (Pelkmans et al. 2017) led to the formation of mycelia unable to produce fruiting bodies. In case of the zinc finger transcription factor gene *fst4* (Ohm et al. 2011; Pelkmans et al. 2017), inactivation resulted in the formation of only rudimentary fruiting body structures. Accordingly, Δ*wc-2*Δ*wc-2*, Δ*hom2*Δ*hom2*, Δ*fst4*Δ*fst4* and Δ*tea1*Δ*tea1* samples showed comparable VOC compositions in the HS (Table 1, Fig. 5). These results are in good agreement with previous transcriptome data showing similarities in the RNA profiles of Δ*wc-2*Δ*wc-2*, Δ*hom2*Δ*hom2* and Δ*fst4*Δ*fst4* 12 days after inoculation (Pelkmans et al. 2017). Expression of a constitutive active Hom2 protein, $Hom2^{con}$ (Pelkmans et al. 2017), as well as the wild type showed comparable VOCs in the HS but at different times. However, the release of VOCs, especially sesquiterpenes, was tightly connected to fruiting body development. Taking the strong connection between developmental stage and VOC release observed for all samples into account, it seems likely that WC-2, Hom2, Fst4 and Tea1 affect the volatilomes of *S. commune* indirectly by influencing fruiting rather than being direct regulators of sesquiterpene synthesis.

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Author contribution All authors contributed to the study conception and design. Lisa Janaina Schwab and Axel Orban carried out experiments and analysed data. The first draft of the manuscript was written by Axel Orban and Martin Rühl. All authors commented on the manuscript and approved the final version.

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Data availability All data are included in this article and in the supporting file.

Declarations

Conflict of interest The authors declare no competing interests.

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