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Production of fusel alcohols and fusel acetates by pathogenic fungi in the Ceratocystidaceae

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ABSTRACT

The family Ceratocystidaceae includes economically important plant pathogens that vary in host preference and lifestyle. These fungi are believed to attract insect vectors, for their dispersal through their floral and fruity scents. This study aimed to identify the volatiles produced by a subset of fungi within the Ceratocystidaceae using gas chromatography coupled with mass spectrometry. The primary volatiles produced by most genera in the family were fusel alcohols and fusel acetates, but their emission rates differed significantly between genera and isolates from a single species. *Ceratocystis albifundus* collected from *Protea cynaroides* produced higher levels of fusel acetates compared to isolates from *Terminalia sericea*. In addition, significant differences in volatile biosynthesis were observed between isolates grown under different temperatures. Results of this study demonstrate that Ceratocystidaceae exhibit varied volatile profiles, but further research is needed to understand the ecological and physiological mechanisms underlying this plasticity.

1. Introduction

The family Ceratocystidaceae (order: Microascales, class: Sordariomycetes) encompasses several economically important plant pathogens. Notably, species in the genera *Ceratocystis*, *Davidsoniella*, *Berkeleyomyces*, *Endoconidiophora*, *Bretziella* and *Thielaviopsis* have been identified as causative agents of plant diseases displaying symptoms such as stem cankers, blue staining of wood, wilting, root, and tuber rots (De Beer et al., 2014, 2017; Nel et al., 2018). For example, *Ceratocystis albifundus,* a pathogen indigenous to Southern Africa, causes wilting and death of indigenous *Protea* species, and invasive *Acacia mearnsii* trees (Barnes et al., 2005; Roux et al., 2007; Heath et al., 2009). On the opposite end of the diversity spectrum within the Ceratocystidaceae, species of *Huntiella*, *Ambrosiella* sensu lato, *Catunica* and *Solaloca* are non-pathogenic, causing superficial damage while utilizing available nutrients in wood or phloem sap from tree wounds (De Fine Licht and Biedermann, 2012; de Errasti et al., 2015; Mbenoun et al., 2016; Mayers et al., 2020).

Insect-mediated dispersal is a prevalent strategy in the Ceratocystidaceae, with various genera relying on different insect vectors (Harrington, 2009, 2013). *Endoconidiophora* species, for instance, are dispersed by conifer bark beetles (Coleoptera, Curculionidae), while *Ambrosiella* and some *Ceratocystis* species are associated with ambrosia beetles (Coleoptera, Curculionidae) (Moller and DeVay, 1968; Harrington and Wingfield, 1998; Six and Wingfield, 2011; Kandasamy et al., 2019). Nitidulid beetles (Coleoptera, Nitidulidae), are frequent carriers of fungi in the genera *Huntiella*, *Davidsoniella*, *Bretziella* and *Ceratocystis* (Wingfield and Van Wyk, 1993, Hayslett et al., 2008; Heath et al., 2009; De Beer et al., 2017). Fungi that rely on beetles for dispersion are often nutritional symbionts (Six, 2012; Mayers et al., 2020; Menocal et al., 2023; Six and Biedermann, 2023). In some cases, such as in ambrosia beetles, these nutritional associations are obligate with fungi being transported in specialized sac-like structures called mycangia (Mayers et al., 2022), whereas in the case of nitidulid and some bark beetles, fungus feeding is facultative (Harrington and Wingfield, 1998; Harrington, 2009; Six and Wingfield, 2011; Kandasamy et al., 2019).

Given their dependence on insect vectors, fungi in the Ceratocystidaceae have evolved distinct adaptations for efficient dispersal by their beetle vectors. During the sexual stage, they produce perithecia with extended ascomatal necks, which release adhesive masses of spores that readily attach to insect exoskeletons (Whitney and Blauel, 1972; Klepzig and Six, 2004; De Beer et al., 2017; Barnes et al., 2018). *Bretziella fagacearum,* formerly known as *C. fagacearum* (De Beer et al., 2017) and other *Ceratocystis* species such as *C. albifundus* and *C. manginecans* (M.J.

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Wingfield, pers comm) produce mycelial mats under the bark of infected trees resulting in cracks that provide access to insects that are attracted to the fruity volatiles produced by these fungi (Fergus and Stambaugh, 1957; Harrington, 2009; Heath et al., 2009).

The intense scents containing sweet, fruity, and floral notes such as peach, banana, pear, rose, or citrus emitted by fungi in the Ceratocystidaceae attract insects (Lanza et al., 1976; Christen et al., 1994, 1997; Bramorski et al., 1998; Soares et al., 2000; Ranger et al., 2021; Tobin et al., 2024). These scents are blends of volatile secondary metabolites, predominantly terpenes and fusel acetates (Brakhage, 2013; Kandasamy et al., 2016). Although volatiles are believed to play a significant role in the dispersal of fungi in the Ceratocystidaceae family, there is limited knowledge regarding the specific compounds and blends produced by the majority of species within this family. A study by Kandasamy et al. (2019) demonstrated that *Endoconidiophora polonica* produced fusel alcohols and acetates, which were found to be attractive to its vector, the Eurasian spruce bark beetle, *Ips typographus*. Despite the significance of these findings, comprehensive volatile profiles for many other fungi in the Ceratocystidaceae have not yet been described. Species such as *Ceratocystis fimbriata*, *Ceratocystis platani*, *Ceratocystis albifundus*, *Ceratocystis cacaofunesta* and *Huntiella monilliformis* are known to produce similar floral and fruity scents (Christen et al., 1994; Sanchez et al., 2002; Bürki et al., 2003; Gao et al., 2022; Araújo et al., 2024), suggesting they may also emit fusel alcohols and acetates. However, detailed analyses of the volatile blends produced by these and other species within the family are lacking. Understanding these profiles is crucial for elucidating the ecological roles of volatiles in fungal dispersal and for potentially harnessing these compounds for industrial applications.

Fusel alcohols and acetates are valuable compounds with significant applications across various industries, including pharmacology, food processing, hygiene, and perfumery. These compounds are currently produced in large quantities - thousands of tons annually - through synthetic processes that are often environmentally harmful (Etschmann et al., 2002; Goettmann et al., 2006; Hua and Xu, 2011; Yang et al., 2016). The biosynthesis of fusel alcohols and acetates with branched aliphatic or aromatic carbon skeletons, has been extensively studied in brewer's yeast (*Saccharomyces cerevisiae*) (Ehrlich, 1907; Dickinson et al., 2003; Schoondermark-Stolk et al., 2005; Hazelwood et al., 2008; Koonthongkaew, 2022). These short-chain alcohols and acetates are derived from the catabolism of amino acids and are by-products of alcoholic fermentation. While they are major contaminants of alcoholic beverages they are important for attracting fruit flies for the dispersal of wild yeast strains (Sentheshanmuganathan and Elsden, 1958; Walker and Stewart, 2016; Becher et al., 2018). Given the increasing demand for sustainable production methods, there is a growing interest in finding natural sources for these metabolites. Fungi in the Ceratocystidaceae proliferating on cheap woody substrates, including waste products such as bark and saw dust, could potentially be a more cost-effective alternative source of commercially important volatiles compared to genetically engineered model fungi (Wang et al., 2019).

The aim of this study was to analyse the volatile blends produced by a wide range of fungi in the Ceratocystidaceae family, which could serve as a natural source for fusel alcohols and acetates, potentially reducing the environmental impact associated with traditional synthetic production methods. For this purpose, we included important pathogens such as *Ceratocystis fimbriata* and *Ceratocystis platani*, and non-pathogens such as *Huntiella moniliformis* and *Huntiella omanensis*. In addition, to determine the variation in volatile biosynthesis within a single species, we analysed the volatiles from multiple strains of *Ceratocystis albifundus* isolated from two different hosts, *Protea cynaroides* and *Terminalia sericea*. We also compared the volatile profiles of these strains grown under different abiotic conditions. The results of this study will shed some light on the volatiles produced by this group of fungi, as well as enhance our understanding of the chemical ecology of these fungi and their interactions with insect vectors, providing insights that could inform both biological control strategies and biotechnological applications.

2. Methods and materials

2.1. Fungal strains and culturing

In total, 36 strains from different species in the family Ceratocystidaceae were included in this study. This included one strain of *Bretziella* spp., three strains of *Berkeleyomyces* (*Be. basicola*), 19 strains of *Ceratocystis* (four *C. albifundus,* four *C. eucalypticola,* four *C. fimbriata,* one *C. harringtonii* and six *C. manginecans),* two strains of *Davidsoniella* (*D. eucalypti* and *D. virescens*), four strains of *Endoconidiophora* (three strains of *E. laricicola* and one strain of *E. polonica*), four strains of non-pathogenic *Huntiella* (one strain of *H. bhutanensis*, *H. decipiens, H. moniliformis* and *H. omanensis*) and one strain of *Thielaviopsis* (*T. punctulata*) (Table S1).

In addition, 17 strains of *Ceratocystis albifundus* were used in this study, which were isolated from geographically diverse regions and two host species (Table S2), *Protea cynaroides* in Western Cape and *Terminalia serecia* in Limpopo and Mpumalanga. Cultures of all the isolates used in this study were obtained from the CMW Culture Collection at the Forestry and Agricultural Biotechnology Institute at the University of Pretoria. Fungal cultures were grown in triplicate on half strength potato dextrose agar (PDA; Merck, USA) with a final concentration of 50 μg/ml streptomycin (Sigma-Aldrich, USA) for 7 days at room temperature (approximately 22 °C).

In addition, an assay at two different temperatures was conducted using four isolates of *C. albifundus* (CMW42477, CMW23839, CMW42408 and CMW42417). Isolates CMW 42477, CMW 42408 and CMW 23839 were isolated from *Protea cynaroides* in Western Cape while isolate CMW 42477 was isolated from *Terminalia sericea* in Limpopo (Table S2) and grown on half strength PDA (Merck, USA) for seven days. Three replicate plates of each isolate were incubated at 20 °C and three replicate plates of each isolate were incubated at 26 °C for seven days.

2.2. Gas chromatography mass spectrometry

The headspace volatiles of all the fungi in this study were analysed using an Agilent 7890B gas chromatograph coupled to an Agilent 5977 mass spectrometer (GC-MS). A 5 mm diameter plug of fungal mycelium was removed with a cork borer from the edge of a 7-day-old culture. The disks of mycelium were inserted into a sterile 1.5 ml glass vial (Separations, SA) with mycelium facing upwards and allowed to equilibrate for 15 min. Prior to this, a negative control was analysed with an agar plug alone to identify volatiles from the PDA. A solid phase micro extraction fibre (SPME) was used to sample the volatiles from the air above the fungal plug through the septum of the vial for 20 min. The SPME fibre was coated with 50/3 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (Supelco, USA) and was directly injected into the GC injection port and volatiles were desorbed from the SPME fibre at a temperature of 300 °C.

A HP-5 MS 19015-h33μI (30m long, 0.25 mm inner diameter, and 0.25 μm film thickness) GC column (Chemetrix, SA) was used for compound separation. The column was held at an initial temperature of 40 °C for 20 s, and the temperature was increased to 300 °C using a gradient of 20 °C/min and held at 300 °C for 5 min before reconditioning at 50 °C. The flow rate of the helium carrier gas was 1 L/min. The following parameters of the mass spectrometer were set: Electron ionization was at 70 eV, ion source temperature was 230 °C, and the interface temperature was 250 °C. Compounds detected by the mass analyser were identified by comparing them in all the samples with spectra from the NIST library version 14 compiled by the National Institute for Standards and Technology (Boulder, USA) and integrating the identified peaks using Mass Hunter qualitative analysis B.08.00 (Agilent). Pure standards were analysed for 15 compounds detected (Supplemental Fig. 1) and their retention times and mass spectra were compared with spectra from fungal volatiles (Supplemental Figs. 2 and 3). Integrated peaks were exported in csv format and imported into

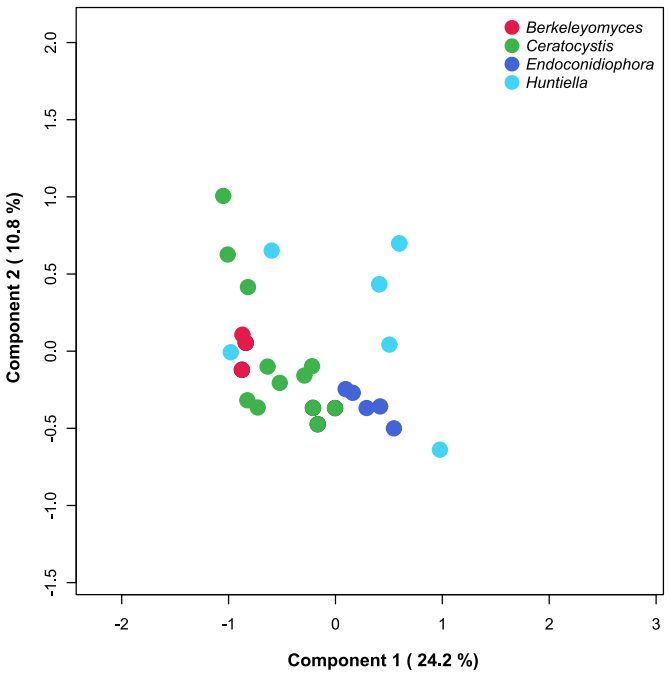


Fig. 1. Principal component analysis of total volatile profiles of selected genera in the Ceratocystidaceae. Cultures of different isolates in the Ceratocystidaceae were grown for 7 days at 22 °C on half-strength PDA. Volatiles were sampled by exposing a SPME fibre to the headspace of a 5 mm diameter mycelial plug in a 1.5 mL glass vial and were analysed by direct injection into a GC-MS. Relative peak areas of fusel alcohols and acetates, sesquiterpenes, alkanes, alkenes and long-chain fatty alcohols were included in the data. The PCA plot was generated using Metaboanalyst v. 6.0.

Metaboanalyst v. 5.0 for statistical analysis (Pang et al., 2021). In Metaboanalyst, the data was log transformed with range-scaling. The default settings were used to generate the PCA plots and heat maps. T-tests with an alpha value of $p < 0.05$ were used to compare volatiles produced by isolates from different hosts and isolates grown at different temperatures. Figures were rendered using Metaboanalyst and Adobe illustrator CS5.

3. Results

3.1. Different genera in the Ceratocystidaceae produce different volatile profiles

All the fungi from the family Ceratocystidaceae that were analysed in this study produced volatile secondary metabolites. The majority of the compounds detected were fusel alcohols and fusel acetates. The genera *Berkeleyomyces, Ceratocystis, Endoconidiophora* and *Huntiella* varied in their production of volatiles (Fig. 1; Table S1). *Ceratocystis, Berkeleyomyces* and *Endoconidiophora* produced mostly fusel alcohols and fusel acetates. However, *Huntiella* also produced high levels of sulcatol and sulcatone, while some *Ceratocystis* isolates also produced monoterpenes such as citronellol and geraniol acetate (Supplemental Table S3).

Species of *Ceratocystis* and *Berkeleyomyces* produced mainly branched chain fusel alcohols and fusel acetates such as isoamyl alcohol, isoamyl acetate and isobutyl acetate (Fig. 2). Species of *Endoconidiophora, Davidsoniella* and *Thielaviopsis*, on the other hand, mainly produced the aromatic fusel acetate, *2*-phenylethyl acetate, and low levels of isobutyl acetate (Fig. 2). Furthermore, there were no major differences in production of fusel alcohols and fusel acetates on genus level in *Ceratocystis* and *Berkeleyomyces* (Fig. 2). *Huntiella* and *Davidsoniella* species differed significantly in their production of fusel acetates (Fig. 2). For instance, *D. eucalypti* produced mainly isoamyl acetate and isobutyl acetate while *D. virescens* primarily produced *2*-phenylethyl acetate (Table S1). Similarly, *Huntiella moniliformis* mostly produced the branched chain volatiles, isoamyl alcohol and isoamyl acetate, whereas *H. bhutanensis* did not produce high levels of these two compounds (Fig. 2). There was also a slight variation in the production of fusel alcohols and fusel acetates between species in the genus *Thielaviopsis*, but these species generally produced lower levels of branched-chain volatiles (Table S1; Fig. 2).

3.2. High variation in fusel alcohol biosynthesis in C. albifundus isolates under different environmental conditions

Different isolates of the same species, *C. albifundus* differed in their production of fusel alcohols and fusel acetates (Fig. 3). Isolate CMW 42477 produced more ethyl acetate (Fig. 3A) and isoamyl acetate (Fig. 3B) and did not produce *2*-phenylethyl acetate (Fig. 3C). Furthermore, production of these compounds varied at two different temperatures. Isolate CMW 42408 and CMW 42417 produced marginally more

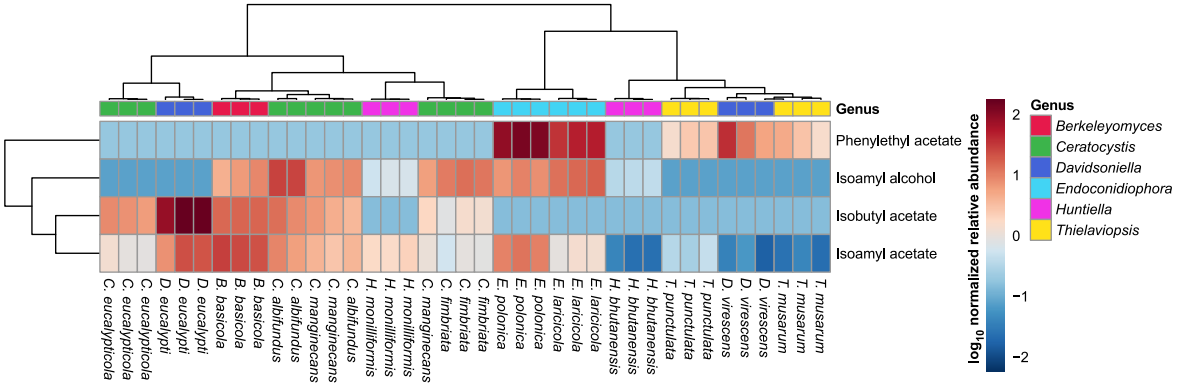
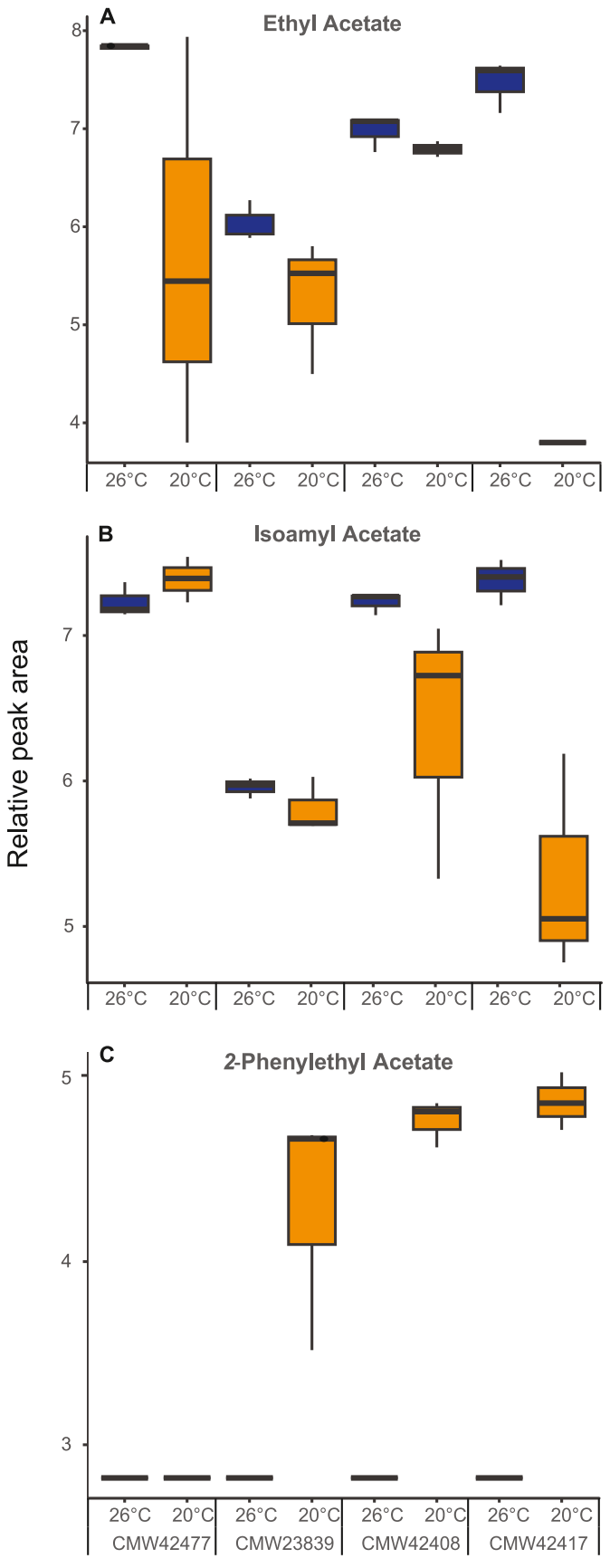


Fig. 2. Heat map showing the normalized peak areas of fusel alcohols and fusel acetates that were produced by selected fungi belonging to the family Ceratocystidaceae. Brown indicates the highest production of volatiles, while blue indicates the lowest. The heat map tree displays species that clustered comparable volatiles together. There is variation in volatile production within species of *Ceratocystis, Davidsoniella Huntiella* and *Thielaviopsis.* Cultures of fungal isolates grown for 7 days at 22 °C on half-strength PDA were analysed in triplicate. Volatiles were sampled by exposing a SPME fibre to the headspace of a 5 mm diameter mycelial plug in a 1.5 mL glass vial and were analysed by direct injection into a GC-MS. The heat map was generated using the default settings in Metaboanayst v. 6.0 from integrated peak areas. The hierarchical clustering reveals that *Endoconidiophora*, *Ceratocystis* and *Thielaviopsis* species produced similar volatiles and that emission patterns of isoamyl and isobutyl acetate were most similar. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



(caption on next column)

Fig. 3. Relative peak areas of A) ethyl acetate, B) isoamyl acetate, and C) 2-phenylethyl acetate in four *C. albifundus* isolates that were either incubated at 26 °C (blue) or at 20 °C (orange) for 7 days before sampling. Whole cultures of the isolates grown on half-strength PDA were sampled by exposing a SPME fibre to the headspace of a 5 mm diameter mycelial plug in a 1.5 mL glass vial. Volatiles were analysed by direct injection into a GC-MS. Reported volatile concentrations are relative peak areas. Statistical differences at the two different temperatures were analysed using a Student's t-test. Statistics are reported in the text. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

ethyl acetate ($p > 0.05$) and significantly more isoamyl acetate ($p = 0.025$) when the isolates were incubated at a 26 °C compared to when the isolates were incubated at 20 °C (Fig. 3A and B). Isolates CMW 23839, CMW 42408 and CMW 42417 produced *2*-phenylethyl acetate when incubated at 20 °C but not at 26 °C (Fig. 3C). Isolate CMW 42417 was the largest producer of *2*-phenylethyl acetate while isolate CMW 23839 was the lowest producer under these conditions.

3.3. *Ceratocystis albifundus isolates from two different hosts emitted different quantities of volatiles*

Isolates of *C. albifundus* that infect *P. cynaroides* differed in their production of fusel acetates from isolates infecting *T. sericea* (Fig. 4A), two indigenous hosts of this fungus in South Africa. Overall, isolates from *P. cynaroides* produced higher levels of fusel acetates compared to isolates from *T. sericea* ($p < 0.001$, ethyl acetate; $p < 0.001$, isobutyl acetate; $p < 0.001$, isoamyl acetate). Isolates infecting *T. sericea* produced very low levels of ethyl acetate (Fig. 4B), as well as lower levels of isobutyl acetate (Fig. 4C) and isoamyl acetate (Fig. 4D) compared to isolates from *P. cynaroides*.

4. Discussion

We analysed the volatiles produced by a wide variety of genera and fungal species in the family Ceratocystidaceae. Most species included in this study produced high levels of fusel alcohols and acetates, except for *Huntiella*, a saprophytic genus, which produced low levels of fusel acetates, but high levels of other volatile classes such as aliphatic alcohols that is part of the chemical communication systems in certain moths and beetles to attract mates (Meier et al., 2019). Among the species and genera producing mainly fusel alcohols and acetates, large interspecific and intraspecific qualitative and quantitative differences were noted.

Overall, the volatile emissions from fungi in the Ceratocystidaceae closely resembled that of *Saccharomyces cerevisiae*, brewer's yeast; however, several fusel alcohols and their acetates are also produced by other filamentous fungi, including *Aspergillus parasiticus* and *Neurospora crassa* (Roze et al., 2010; Huberman et al., 2021; Dickinson et al., 2003). Brewer's yeast produces branched chain and aromatic fusel alcohols and acetates during amino acid catabolism (Dickinson and Norte, 1993; Iraqui et al., 1998; Dickinson et al., 2003) and even produces the oxygenated monoterpenes e.g. citronellol and derivatives (Takahashi et al., 2007). Citronellol and its derivatives have been reported to possess antimicrobial activities, making them valuable in combating bacterial infections (Gochev et al., 2008). It is therefore possible that the oxygenated monoterpenes detected in our *Ceratocystis* and *Endoconidiophora* strains may be a chemical defence against other microbes.

Similar to species in the Ceratocystidaceae, significant intraspecific variation in the emissions of these volatiles exists in different *S. cerevisiae* strains (Arguello et al., 2013). It is possible that the variation in production of fusel alcohols and their acetates within single species is brought about by differences in genes encoding enzymes involved in the volatile biosynthesis pathways or their regulatory elements among different isolates. Species in the Ceratocystidaceae are known to contain high levels of transposable elements, which can alter specific genetic characters (Fourie et al., 2020). Genome comparisons, genome-wide

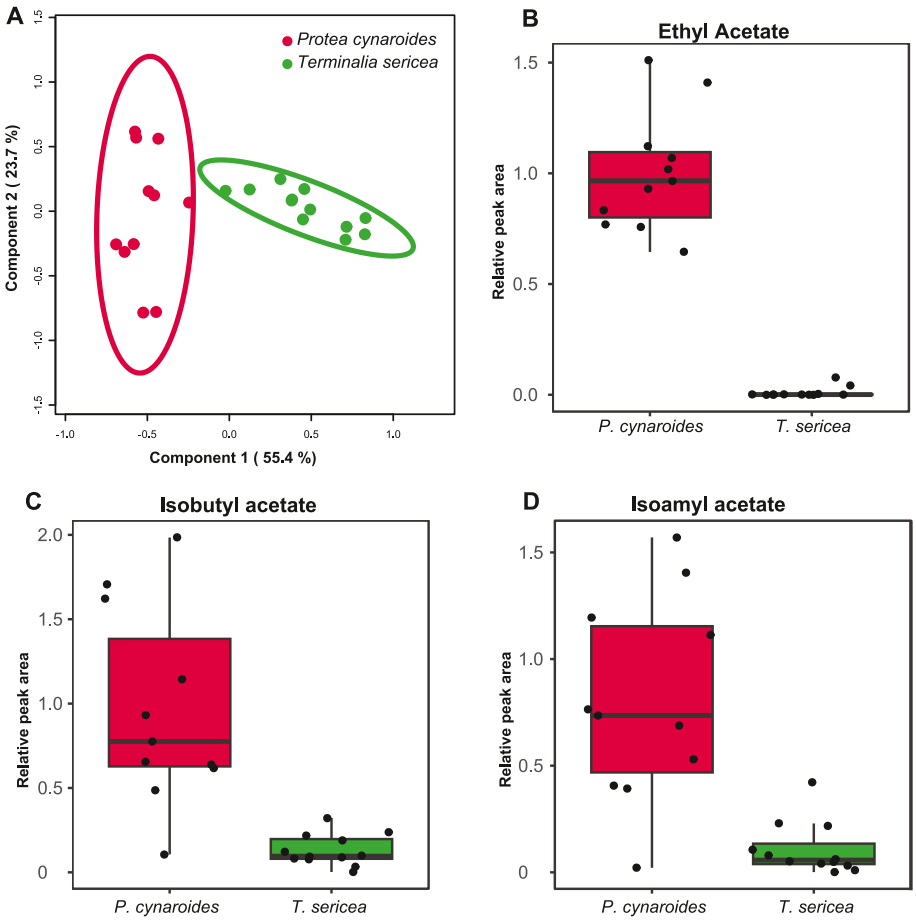


Fig. 4. Isolates of *C. albifundus* from two different hosts, *Protea cynaroides* and *Terminalia sericea* differ in their production of fusel alcohols and fusel acetates (A). The most significant differences were in the concentrations of ethyl acetate (B), isobutyl acetate (C) and isoamyl acetate (D). Whole cultures of fungal isolates grown for 7 days at 22 °C on half-strength PDA were analysed in triplicate. Volatiles were sampled by exposing a SPME fibre to the headspace of a 5 mm diameter mycelial plug in a 1.5 mL glass vial and were analysed by direct injection into a GC-MS. Graphs were generated using the default settings in Metaboanayst v. 5.0 from integrated peak areas.

association studies and differential gene expression studies using a wide range of species and strains might shed more light on the cause for the variation in volatile production rates of these fungi (van der Nest et al., 2014, 2019).

In addition to genetic factors, incubation temperature, fungal growth rate and the age of fungal cultures play a significant role in the dynamics of volatile production. In *S. cerevisiae*, shorter fermentation times typically yielded fusel alcohols, with a linear increase in fusel acetate production with increasing fermentation time (Landaud et al., 2001; Quilter et al., 2003; Singh et al., 2008). More studies are needed on the timing of production of fusel alcohols or acetates in the Ceratocystidaceae in both liquid and solid cultures, as we measured volatiles only from seven-day-old solid cultures and consequently observed high emissions of fusel acetates.

Volatile emission by fungi is also influenced by growth rate. Some isolates of *C. albifundus* grew slower than others and this might have had an impact on the production rate of fusel alcohols and fusel acetates. This is supported by the fact that cell density in yeast cultures is positively correlated with the production rate of fusel acetates (Ito et al., 1990; Sanchez et al., 2002). However, the most dramatic changes in volatile emissions were noted in this study when the same isolates of *C. albifundus* were incubated at two different temperatures. Cultures grown at higher temperatures produced more branched-chain fusel acetates whereas cultures grown at a lower temperature produced the aromatic fusel acetate, *2*-phenylethyl acetate, which was absent in the cultures incubated at the higher temperature. This might be a stress-induced phenotype, but further study is required to understand the effect of temperature in differentially regulating different volatile biosynthesis pathways.

Certain species in the Ceratocystidaceae that depend on insect vectors for their dispersal may produce volatiles to facilitate these interactions. For instance, *S. cerevisiae* produces volatiles that attract fruit flies from the genus *Drosophila*. These fruit flies feed on yeasts and disperse them to new substrates (Becher et al., 2018). Similarly, many Ceratocystidaceae species also rely on insect vectors for dispersal and might produce comparable volatiles to support these relationships. For example, *E. polonica* produces high levels of *2*-phenylethyl acetate to attract the Eurasian spruce bark beetle, *I. typographus*, that has dedicated olfactory sensory neurons to detect this volatile (Kandasamy et al., 2019). Since insect-mediated dispersal offers significant advantages to fungi, it is plausible that high production of 2-phenylethyl acetate evolved in this genus under selection pressure. Similar evolutionary scenarios might apply to other Ceratocystidaceae species, emphasizing the need for further research on volatile-mediated interactions between these fungi and their insect vectors.

Ceratocystis albifundus is genetically highly diverse within its native geographic range in Africa (Barnes et al., 2005). However, Lee et al. (2016) showed that the population on *P. cynaroides* in the Western Cape Province originated from a recent introduction event and had very low genetic diversity. In addition, Danki et al. (2024) showed that isolates from *P. cynaroides* created longer lesions in pathogenicity tests on *Acacia mearnsii* (non-native) than isolates from other native woody hosts. It is

therefore possible that the founder strains of the population from *P. cynaroides*, by chance, produced higher levels of volatiles and were more virulent than conspecifics from native hosts. Further studies are needed to understand the link between fungal virulence and volatile production, but it is interesting to note, that *Huntiella* species, which are non-pathogenic, similarly produced significantly lower levels of fusel acetates compared to the pathogenic genera included in our study.

Analyses of the volatile profiles from *C. albifundus* strains isolated from two different native hosts, revealed that strains isolated from *P. cynaroides* produced significantly higher levels of volatiles compared to strains isolated from *T. sericea*. As was expected strains isolated from native *T. sericea* were shown to be non-pathogenic to the tree (Roux et al., 2007). Although *C. albifundus* is generally not a pathogen on native hosts, the fungus is highly pathogenic on *P. cynaroides* and causes a vascular wilt disease, which results in plant mortality (Roux et al., 2007). It is therefore possible that *C. albifundus* strains that the pathogenic strains produce more volatiles compared to the non-pathogenic strains.

It is not clear if fusel alcohol and acetate production in the Ceratocystidaceae influence pathogenicity. Ethyl acetate and ethanol are very prevalent in most fungi of the Ceratocystidaceae. These volatiles are toxic and may cause insects that don't normally feed on fungi to graze less, leaving the mycelium available for vectors like nitidulids, which can normally withstand high mycotoxin concentrations (Harrington, 2009). Furthermore, fusel alcohols produced by several fungi of the Ceratocystidaceae are phytotoxic (Tabachnik and DeVay, 1980). Phytotoxins have been linked to several diseases caused by *Ceratocystis* species. Many of these diseases, such as oak wilt and canker stain display vivid coloration patterns on the leaves of infected branches which may be linked to the production of phytotoxins. However, further research is required to link the production of fusel alcohols and acetates to fungal pathogenicity.

5. Conclusion

The biosynthesis of fusel alcohols and fusel acetates in the Ceratocystidaceae is complex, as fungal emission rates show large environmental plasticity as well as inter- and intraspecific differences. The reasons for the complexity of the biosynthesis of these volatiles might be linked to their ecological or physiological functions, but further research is required to gain a deeper understanding of their roles. This includes optimizing methods for quantitative analysis, studying volatile emissions at different fungal developmental stages and on natural substrates. This might also help in understanding ways to control and manage the spread of fungi in the Ceratocystidaceae.

Fusel alcohols and acetates have important applications in the pharmacological, food processing, hygiene and perfume industries and thousands of tons of these compounds are produced annually using environmentally harmful synthetic processes (Etschmann et al., 2002; Goettmann et al., 2006; Hua and Xu, 2011; Yang et al., 2016). Fungi in the family Ceratocystidaceae could be used as a natural source of these metabolites, due to their high emissions and their effective utilization of woody substrates, which are available in large quantities as waste products from other industrial processes. However, in order to utilize these fungi as a natural source of fusel alcohols and acetates, further studies are required to understand their biosynthesis and their regulation at a finer scale.

CRediT authorship contribution statement

Dineo M. Mailula: Writing – original draft, Investigation, Formal analysis, Data curation. **Brenda D. Wingfield:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Magrieta A. van der Nest:** Writing – review & editing, Project administration, Investigation, Funding acquisition, Conceptualization. **Almuth Hammerbacher:** Writing – review & editing, Visualization, Validation, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Conflict of interest

The authors declare no competing interests and the manuscript has not been submitted elsewhere for review.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2025.101427.

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