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Research Article

# *Arabidopsis thaliana* as Bioindicator of Fungal VOCs in Indoor Air

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**Abstract** In this paper, we demonstrate the ability of *Arabidopsis thaliana* to detect different mixtures of volatile organic compounds (VOCs) emitted by the common indoor fungus, *Aspergillus versicolor*, and demonstrate the potential usage of the plant as a bioindicator to monitor fungal VOCs in indoor air. We evaluated the volatile production of *Aspergillus versicolor* strains SRRC 108 (NRRL 3449) and SRRC 2559 (ATCC 32662) grown on nutrient rich fungal medium, and grown under conditions to mimic the substrate encountered in the built environment where fungi would typically grow indoors (moist wallboard and ceiling tiles). Using headspace solid phase microextraction/gas chromatography-mass spectrometry, we analyzed VOC profiles of the two strains. The most abundant compound produced by both strains on all three media was 1-octen-3-ol. Strain SRRC 2559 made several terpenes not detected from strain SRRC 108. Using a split-plate bioassay, we grew *Arabidopsis thaliana* in a shared atmosphere with VOCs from the two strains of *Aspergillus versicolor* grown on yeast extract sucrose medium. The VOCs emitted by SRRC 2559 had an adverse impact on seed germination and plant growth. Chemical standards of individual VOCs from the *Aspergillus versicolor* mixture (2-methyl-1-butanol, 3-methyl-1-butanol, 1-octen-3-ol, limonene, and β-farnesene), and β-caryophyllene were tested one by one in seed germination and vegetative plant growth assays. The most inhibitory compound to both seed germination and plant growth was 1-octen-3-ol. Our data suggest that *Arabidopsis* is a useful model for monitoring indoor air quality as it is sensitive to naturally emitted fungal volatile mixtures as well as to chemical standards of individual compounds, and it exhibits relatively quick concentration- and duration-dependent responses.

**Keywords** *Arabidopsis thaliana, Aspergillus versicolor*, Plant growth, Volatile organic compounds

Biogenic volatile organic compounds (VOCs) are numerous, diverse, and ubiquitous in our environment. Due to their distinct odorant properties, VOCs are extensively studied as food and flavoring agents, and as indicators of microbial contaminations in food. As low molecular weight compounds that easily become gases at room temperature, VOCs enable organisms to mediate important biological functions such as signaling, communication, antagonism, and inter- and intra-specific associations [1, 2]. Industrial processes also lead to the production of anthropogenic volatiles that are important as environmental pollutants, impacting the atmosphere and having harmful effects on biological systems. In recent years, increasing number of reports demonstrate that microbe-derived VOCs in the indoor environment are a major contributor to poor air quality, contamination, and adverse health effects [3-5]. Particularly, molds in damp indoor environment have been associated with negative impacts on air quality in the build environment and ineffective ventilation has been shown to cause significant accumulation of fungal VOCs [6]. Many species of fungi and their spores are known to cause allergies and mold-derived VOCs are often considered irritants to mucus membranes [6, 7]. Fungal VOCs readily diffuse through and accumulate within indoor air where inhalation and dermal uptake are important routes of exposure. Exposures to fungal VOCs have been associated with symptoms including headache, eye, nose, and throat irritation, dizziness, nausea and difficulty concentrating [8]. A growing number of studies demonstrate that low concentrations of certain fungal VOCs commonly found in indoor air can have potent physiological effects within and between species [2, 3].

Most existing assays to evaluate indoor air quality require considerable effort and expense. Moreover, adequate detection

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and assessment of fungal VOCs in air remain a challenge due to limitations in analytical methods and the instruments employed in these techniques. Therefore, the development of quick and inexpensive bioassays or bioindicators which can monitor fungal VOCs is desirable. A bioindicator is an organism or part of an organism that relays information on the quality of the environment [9]. Several organisms have been proposed as bioindicators including lichen, mosses, liverworts [10], higher plants (alfalfa, barley, beans, pines, tobacco, and squash) [9, 11], and insects for terrestrial ecosystems [12] and macroinvertebrates in aquatic ecosystems [13]. As bioindicators, plants provide information on the quality of the environment and the actual condition of the organism and ecosystem's health. Plants also absorb contaminants and subsequent analysis of their tissue can be used to quantify the impact of environmental levels [14]. Previously, we demonstrated the efficacy of using the plant model, *Arabidopsis thaliana* to test the phytotoxicity of synthetic standards of VOCs and selected air freshener products [15]. In this paper, we demonstrate *Arabidopsis*' ability to respond to VOCs emitted by common indoor mold, (*Aspergillus versicolor*) and demonstrate the potential of this plant for use as a bioindicator to monitor toxigenic mold VOCs in indoor air.

VOCs emitted by members of the genus *Aspergillus* have been used as indirect biomarkers to detect fungi in the food supply, human disease, and the built environment [4, 16-18]. *Aspergillus* species are ubiquitous in terrestrial ecosystems and their spores are common in soil and air. Several species of *Aspergillus* including *A. versicolor*, *A. fumigatus*, *A. flavus*, and *A. terreus* are commonly found in urban ecosystems and their presence is considered an indicator for moisture problems in buildings [19]. Compounds such as 1-octen-3-ol, 3-methyl-1-butanol, and 2-methyl-1-propanol are often produced in large quantities by *Aspergillus* species [19, 20]. For Aspergilli and for other molds, the volatile profile is dependent on multiple parameters such as geography, time, and environmental conditions including nutrient content, microbial community composition, temperature, humidity, and pH [9, 21]. Some experts hypothesize that mold VOCs contribute to the adverse health effects associated with damp indoor environments [6, 22].

Different isolates and strains of a given species often produce different volatile profiles. We hypothesized that *Arabidopsis* is sensitive to the differences in fungal volatile production, leading to distinct plant responses. Our study had three goals. First, we sought to detect strain differences in the VOC profiles of two isolates of *Aspergillus versicolor* using solid phase microextraction (SPME)/gas chromatography-mass spectrometry (GC-MS). The second goal was to determine if the naturally occurring mixtures of VOCs emitted by growing cultures of the two *A. versicolor* strains could mediate changes in growth and seed germination in the plant model, *Arabidopsis thaliana*. Lastly, we measured plant responses to individual volatile standards to identify specific gas phase metabolites responsible for the observed effects on *Arabidopsis*.

## MATERIALS AND METHODS

***Aspergillus* strains and growth conditions.** *Aspergillus versicolor* strains SRRC 108 (NRRL 3449) and SRRC 2559 (ATCC 32662) were obtained from Dr. Geromy G. Moore at USDA-ARS-SRRC, New Orleans, LA. SRRC 2559 had come to our attention because it caused a student in close proximity, but having no direct physical contact with the strain, to experience unpleasant itching, *Aspergillus versicolor* strain SRRC 108 did not cause this effect.

For volatile analysis, an aqueous spore suspension was made from a two-week old culture of *Aspergillus versicolor* on potato dextrose agar. Five milliliters of substrates, yeast extract sucrose (YES) agar media, water saturated gypsum wallboard (WB), or water saturated ceiling tiles (CT) were placed into a 10-mL vial. YES was inoculated using a 50 µL spore suspension every 2 days for the duration of 14 days while building materials CT and WB were inoculated on the first and eighth day. At the end of the 14 days of fungal growth, all vials were sealed for 24 hr to build up VOCs in the headspace to be analyzed. Negative controls were fungal free substrates. For the plant exposure experiment, the fungi were grown in 35 × 10 mm Petri dishes on 4 mL of YES media, and incubated for 5 days at 27 ± 1°C in high humidity prior to the start of the volatile exposure experiments.

***Arabidopsis* growth conditions.** *Arabidopsis thaliana* seeds (ecotype Columbia-7) were obtained from the *Arabidopsis* Biological Resource Center (Columbus, OH, USA). The seeds were surface-sterilized in a 95% ethanol and 20% bleach solution. Surface-sterilized seeds were sown onto a 100 × 15 mm partitioned Petri dish (split or I-plate) or 60 × 15 mm Petri dish containing Murashige and Skoog medium with vitamins, 3% sucrose, and 0.03% phytagel (pH 5.7) (PhytoTechnology Laboratories, Overland Park, KS, USA). Seeds were stratified at 4°C for 3 days prior to volatile exposure.

**VOC analysis by headspace SPME/GC-MS.** SPME was employed as follows: a 1-cm fiber of Carboxen/DVM/PDMS (Supelco Inc., Bellefonte, PA, USA), stationary phase, was inserted into the headspace and exposed for 15 min. Samples were heated to 65°C for adsorption, injected into GC and desorbed at 270°C for 1 min. After removal, the fiber was heated at 270°C for 2.5 min. For analysis, a Combi-Pal autosampler (Leap Technologies, Carrboro, NC, USA) was used with an Agilent 6890 GC (Agilent Inc., Palo Alto, CA, USA) equipped with a 30-m DB-5 column with a 0.25-mm internal diameter, 1.0-µm film thickness, and phase thickness of 5% of cross-linked phenylmethylsilicate. Helium carrier gas was injected at 40 cm/sec at 25 psi, while the column temperature was ramped up from 50°C

for 1 min, to 100°C at 5°C/min, and then to 200°C at 10°C/min, then to 270°C at 25°C/min and held for 3 min for detection. Agilent 5973 MSD scan mode from 40 to 300 m/z; employing electron ionization was used for mass spectrum detection.

Compounds were initially identified by library match (Wiley Registry of Mass Spectral Data 7th edition with NIST98 Spectra; Palisdae Corporation, Newfield, NY, USA). Reference standards and extracts were analyzed under the same conditions to confirm the library identification. The chromatographic traces were compared to identify different headspace compositions and the identification of signature compounds.

**Plant exposure to VOCs of *Aspergillus versicolor*.** A double plate-within-a-plate system was used for plant exposure to *Aspergillus* VOCs according to previously described methods [23]. A small Petri plate (35 × 1 mm) containing *Aspergillus* grown on YES was placed into a larger partitioned Petri dish (100 × 15 mm) containing five stratified *Arabidopsis thaliana* seeds. Plants and fungi were grown together in a growth chamber at 23 ± 1°C with a 16-hr photoperiod for 14 days. For controls, plants were grown without exposure to fungi, i.e., the smaller Petri dish contained only medium. At the end of the exposure period, the plants were removed from the exposure conditions and photographed. Then the shoots were separated from the roots and weighed to obtain a fresh weight before the total chlorophyll concentration was determined.

**Plant exposure to chemical standards of individual VOCs.** The major compounds identified through SPME analysis were selected for further study. Chemical standards of 2-methyl-1-butanol, 3-methyl-1-butanol, 1-octen-3-ol, limonene, and β-farnesene were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). For comparison, we also included β-caryophyllene, a natural bicyclic sesquiterpene. We exposed seeds and plants to a 0.5 μL/L (vol/vol) concentration of each individual compound. These germination assays and vegetative exposure assays were performed with minor modification as described previously [15].

A Petri dish containing 50 surface sterilized *Arabidopsis thaliana* seeds was placed into a glass tissue culture jar. An appropriate aliquot of each compound was added, volatilized, and the jar was sealed with a translucent polypropylene screw cap. The seeds were exposed to the compound for 72 hr in a growth chamber at 23 ± 1°C with a 16-hr photoperiod. The control seeds were exposed to the same conditions without the addition of VOCs. At the end of the exposure, the seeds were removed and examined visually using light microscopy. The seeds were scored into three categories, no germination, germinated (presence of radical), and seedling formation (presence of radical, hypocotyls, and cotyledons). For the vegetative exposure assay, five plants were grown in a growth chamber at 23 ± 1°C with a 16-hr photoperiod for 14 days following stratification. A Petri plate containing 14-day-old plants was placed into a glass tissue culture jar and then the aliquot of each respective compound was added, volatilized, the jar was sealed, and then placed in the growth chamber for 72 hr. At the end of the 72-hr exposure, the plants were removed from experimental conditions and observed for leaf size, color, and other morphological features. The individual plants were then weighed and assayed for total chlorophyll concentration.

**Chlorophyll quantification.** Total chlorophyll concentration of plants exposed to *Aspergillus* VOCs was determined by submerging the shoot overnight in 1 mL of 80% acetone in the dark at 4°C. The total chlorophyll concentration (chlorophyll *a* and *b*) was calculated from the equation $[(8.02)(A663) + (20.2)(A645)]V/1{,}000 \times W$, where A is absorbance at specific wavelength, $V$ is final volume of chlorophyll extract in 80% acetone, $W$ is plant fresh weight, and extinction coefficient for chlorophyll. The chlorophyll data were expressed in relation to the fresh weight of the plant shoot.

Three replicates were used per treatment condition, and the experiments were repeated three times. Quantitative results were expressed as standard error of the mean and analyzed using Excel software (Microsoft, Redmond, WA, USA) and SigmaPlot (SPSS Inc., Chicago, IL, USA). Student's *t* test and/or one way analysis of variance (ANOVA) between groups were performed for all quantitative data.

## RESULTS

**Volatile analysis.** The volatile compounds were identified from headspace analysis of *A. versicolor* strains SRRC 2559 and SRRC 108 grown on YES media, CT, or WB (Table 1). Both strains grew best on YES, where SRRC 2559 emitted 14 compounds and SRRC 108 emitted 12 compounds. The 14 VOCs detected by SPME analysis included three alcohols, one ketone, one alkene, two heterocyclic aromatics, and seven terpenes and terpene derivatives. Two unidentified diterpenes were found only from strain 2559 on YES and CT. With the exception of limonene, Strain 108 did not produce any detectable terpenes on either CT or WB. Five VOCs (2-methyl-1-butanol, 3-methyl-butanol, 1-octen-3-ol, dimethoxy benzene, and limonene) were produced by both strains on all three substrates. However, relative volatile concentrations varied with the substrate. For both strains, the dominant peak observed on CT and WB was 1-octen-3-ol; whereas 2-methyl-1-butanol, 3-methyl-butanol, and limonene were observed at only trace levels on these substrates.

The amount of each compound produced changed over time. On YES, the highest levels of 3-methyl-1-butanol were observed at day 4 and steadily decreased through day 14 (Fig. 1). Similar results were observed for 2-methyl-1-butanol, 3-hexanone, and 1-octen-3-ol (data not shown). The emission of terpenes, sesquiterpenes, and diterpenes

**Table 1.** Headspace analysis (SPME/GC-MS) of volatiles collected from *Aspergillus versicolor* SRRC 2559 and SRRC 108 grown on YES media, CT, and WB

| Compound | *A. versicolor* SRRC 2559 | | | | *A. versicolor* SRRC 108 | | |
|---|---|---|---|---|---|---|---|
| | RT | YES | CT | WB | YES | CT | WB |
| Alcohols | | | | | | | |
| 2-Methyl-1-butanol | 6.64 | + | + | + | + | + | + |
| 3-Methyl-1-butanol | 6.52 | + | + | + | + | + | + |
| 1-Octen-3-ol | 14.06 | + | + | + | + | + | + |
| Ketone | | | | | | | |
| 3-Hexanone | 8.11 | + | − | − | + | − | − |
| Alkene | | | | | | | |
| (5Z)-Octa-1,5-dien-3-ol | 13.96 | + | + | − | + | − | − |
| Heterocyclic aromatic | | | | | | | |
| Tetramethyl-pyrazine | 16.60 | + | − | − | + | − | − |
| Dimethoxy benzene | 18.18 | + | + | + | + | + | + |
| Terpenes and terpene derivatives | | | | | | | |
| Limonene | 15.47 | + | + | + | + | + | + |
| Terpinolelene | 18.69 | + | + | − | + | − | − |
| 2-Norpinene | 22.28 | + | + | − | + | − | − |
| β-Farnesene | 22.82 | + | + | − | + | − | − |
| Sesquiterpene 2 | 22.93 | + | + | − | + | − | − |
| Diterpene 1 | 26.18 | + | + | − | − | − | − |
| Diterpene 2 | 26.66 | + | + | − | − | − | − |

Retention time (RT) is given in minutes.

SPME, solid phase microextraction; GC-MS, gas chromatography mass spectrometry; YES, yeast extract sucrose; CT, ceiling tiles; WB, wallboard; +, presence of compound; −, none detected.

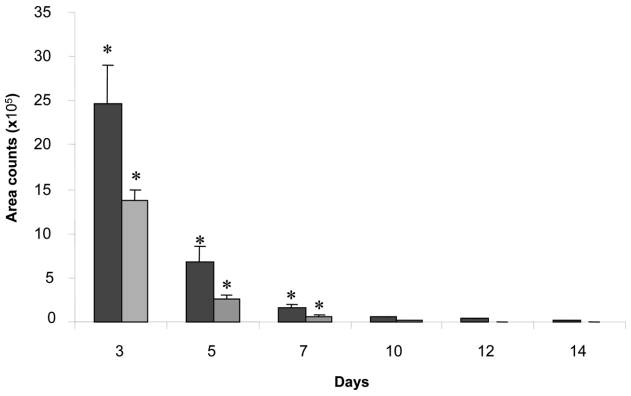


**Fig. 1.** Comparison of 3-methyl-1-butanol production from *Aspergillus versicolor* SRRC 2559 and SRRC 108 grown on yeast extract sucrose media from day 3~14. Black and gray indicate SSRC 108 and SSRC 2559, respectively. *Significance.

also varied with time. We observed a steady production of limonene in *A. versicolor* SRRC 2559 while SRRC 108 exhibited an initial increase followed by a steady decline after 5 days of growth (Fig. 2). Similarly, β-farnesene {7,11-dimethyl-3-methylene-dodeca-1,(E)6,10-triene} increased over time in SRRC 2559 and decreased in SRRC 108. Two diterpenes were produced exclusively by SRRC 2559 (Fig. 3).

**Differential plant responses to VOCs from growing cultures of *Aspergillus versicolor*.** *Arabidopsis thaliana* seeds were grown together with *Aspergillus versicolor* SRRC 2559 or SRRC 108 for 14 days. The split plate assay (Fig. 4) provides physical separation while allowing gas exchange to occur between the organisms. In general, plants exposed to VOCs emitted by SRRC 2559 exhibited reduction in plant size, fewer leaves, and decrease in root growth (Fig. 4A and 4B). Plants exposed to SRRC 108 were similar to control plants both in size and in number of leaves present. These plant growth and developmental stages were in accordance with established phenotypic characteristics for *Arabidopsis thaliana* [24]. However, compared to controls, we saw a reduction in root growth among plants exposed to SRRC 108. We also observed accumulation of reddish-purple pigment in the underside of plant leaves (Fig. 4B). Although the pigment was observed in plants exposed to both SRRC 2559 and SRRC 108, more plants exposed to a shared atmosphere with VOCs from SRRC 2559 displayed pigment production.

Average shoot fresh weight and total chlorophyll concentration of plants exposed to naturally occurring mixtures of *A. versicolor* volatiles are shown in Fig. 5. Plants were exposed to VOCs emitted by SRRC 259 and SRRC 108 grown on YES media for 14 days. The average chlorophyll concentration of control plants was 0.76 ± 0.13 mg per gram of fresh tissue with a total shoot weight of 25 ± 2 mg. Although plants exposed to SRRC 2559 had comparable shoot fresh weight (24 ± 3 mg), there was a significance reduction in total chlorophyll, a 51% decrease. Plants exposed to SRRC 108 had slightly higher fresh weight while the total chlorophyll did not differ significantly compared to

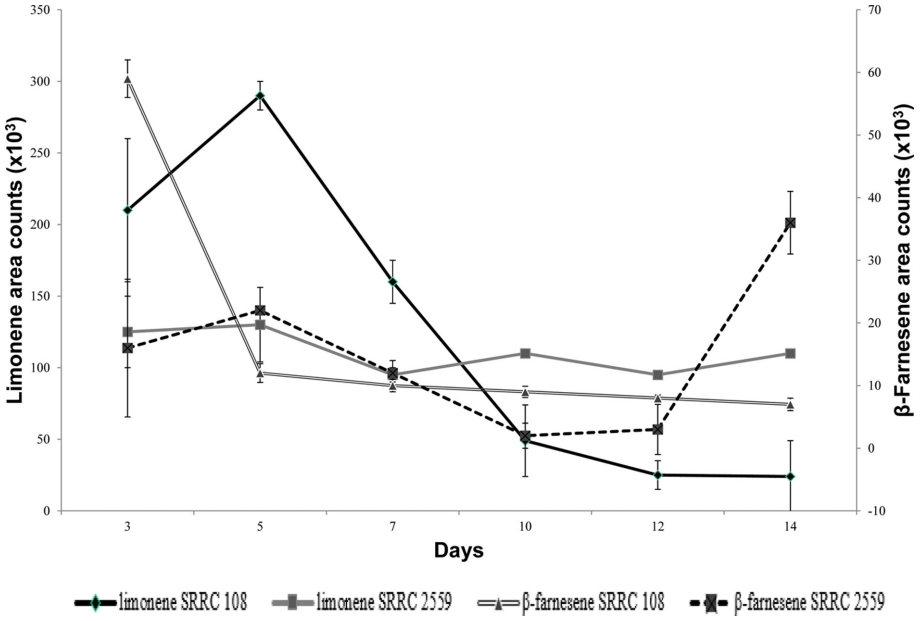


**Fig. 2.** Production of limonene and β-farnesene from *Aspergillus versicolor* SRRC 2559 and SRRC 108 grown on yeast extract sucrose media over 14-day observation period.

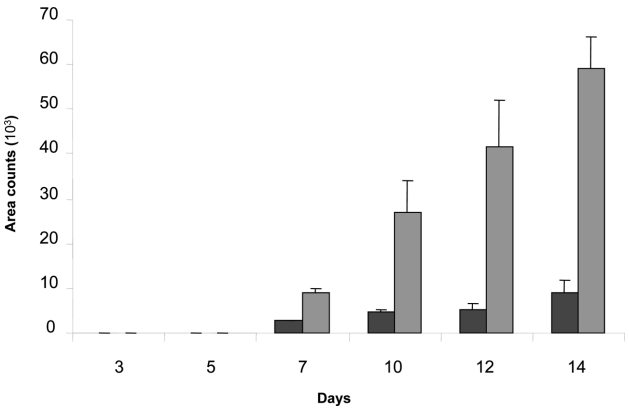


**Fig. 3.** Production of diterpenes by *Aspergillus versicolor* SRRC 2559 grown on yeast extract sucrose media over 14-day observation period. Black and gray indicate diterpene 1 and diterpene 2, respectively.

controls (ANOVA, $p = 0.01$). When we monitored $CO_2$ levels in our experimental setup, we found similar levels comparable to the background levels of $CO_2$ (data not shown).

**Effects of individual VOCs on seed germination and plant growth.** Seed germination stages were classified into three groups: no germination, germination without seedling formation (Fig. 6C), and seedling formation (Fig. 6A and 6B). The percentage of seeds that germinated and developed into seedlings in the presence of individual VOCs is summarized in Fig. 6D. In the control, an average of 82% of seeds had undergone successful germination and progressed into the seedling stage. Of these, 7% exhibited various stages of seedling development (i.e., emergence of hook and cotyledons). The three terpenes tested (limonene, β-farnesene, and β-caryophyllene) showed seedling formation rates from 76~79 ± 5%, with a significant increase in the 'germinated' rate and significant decrease in "no germination" percentage compared to control seeds. Exposure to the alcohols 2-methyl-1-butanol and 3-methyl-1-butanol produced inhibitory effects and decreased seedling formation by 29% and 23% respectively, a significant amount. Seeds exposed to these compounds had a higher germination rate than controls but many seeds only exhibited radical protrusion with no indication of hypocotyl elongation. The most inhibitory compound tested was 1-octen-3-ol. The percentage of non-germinated seeds was approximately 50%. Those seeds that did germinate (the remaining 50%) all had radicles less than 1 mm in length (ANOVA, $p = 0.01$). At the end of the exposure period, seeds were removed from the exposure conditions and placed into clean, sterile plant media for an additional 72 hr without VOC exposure. Subsequent recovery and seedling formation were approximately 90% for all treatments (data not shown).

Fourteen-day-old vegetative *Arabidopsis* plants were exposed to 0.5 μL/L of standard individual fungal VOC for 72 hr. The average fresh shoot weight and total chlorophyll concentration of controls and plants exposed to VOCs are summarized in Fig. 7. Control plants were healthy with fully expanded green leaves with an average fresh shoot weight of 56.56 ± 3.72 mg. Plants exposed to each of the VOCs except 2-methyl-1-butanol exhibited either a significant increase or decrease in plant growth. Plants exposed to 1-octen-3-ol and β-farnesene exhibited inhibitory effects with smaller plant size and reduced total chlorophyll concentrations. Plants exposed to 1-octen-3-ol also had

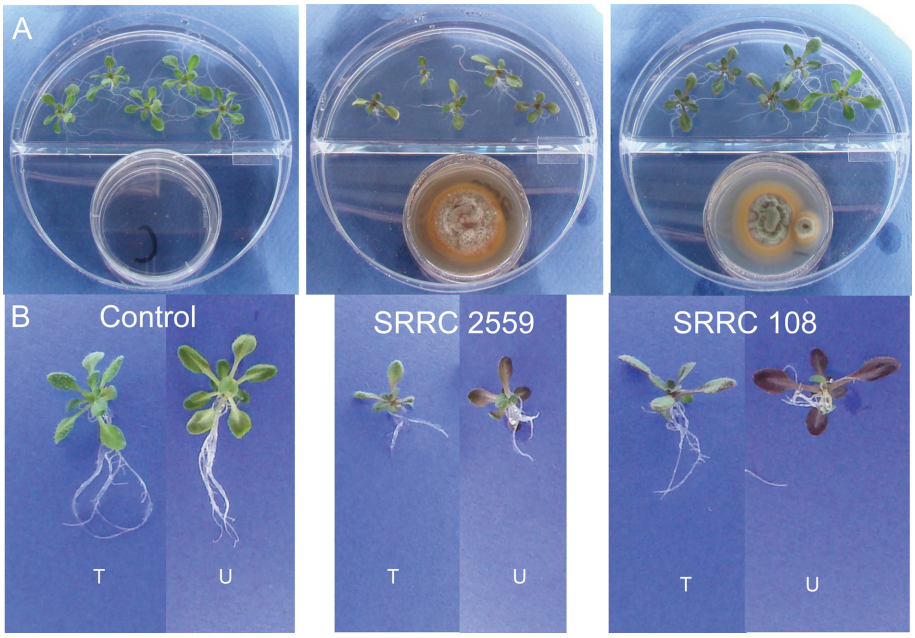


**Fig. 4.** *Arabidopsis thaliana* exposed to *Aspergillus versicolor* SRRC 2559 and SRRC 108 for 14 days. A, Split plates showing controls and *Aspergillus versicolor* exposed plants; B, *Arabidopsis* plants removed from growth medium (top [T] and underside [U] of plants exposed to volatile organic compounds).

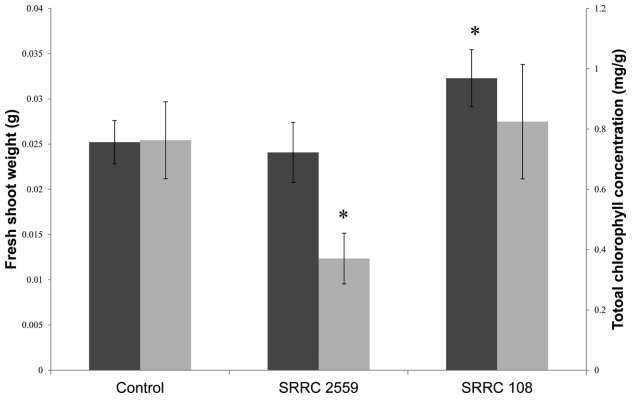


**Fig. 5.** Average fresh weight and total chlorophyll concentration of *Arabidopsis thaliana* exposed to *Aspergillus* SRRC 2559 and SRRC 108 grown on yeast extract sucrose media for 14 days. Black and gray indicate fresh shoot weight and total chlorophyll concentration, respectively. *Significance.

necrotic lesions on a few areas of the leaf, as well as yellowing of cotyledons and leaves. Plants exposed to 3-methyl-1-butanol, limonene, and β-caryophyllene showed significant increases in both plant biomass and total chlorophyll concentrations. The increase in fresh shoot weight ranged from 10% to 20% while total chlorophyll concentration increased 14% to 29% (ANOVA, $p = 0.01$). Root weights were similar in each testing condition (data not shown). Little or no purple pigment was observed in the underside of leaves when individual VOCs were tested. We also performed additional tests to determine if chirality of compounds, 1-octen-3-ol and limonene, would affect seed germination and vegetative growth in plants. We did not find significant differences in plant responses to the enantiomers of these compounds (data not shown).

## DISCUSSION

The usage of plants as bioindicators and as part of ecological risk assessment of various pollutants has a long history [25, 26]. The purpose of our study was to determine if *Arabidopsis thaliana* was sensitive enough to detect subtle differences in the volatile production of two *Aspergillus* strains and to understand the volatile specific responses of the fungal VOCs. Our results demonstrate that *Arabidopsis thaliana* has the potential to be used a bioindicator to assess potentially toxigenic fungal VOCs in indoor air.

We evaluated the volatile production of *A. versicolor* strains SRRC 108 and SRRC 2559 grown on nutrient rich fungal medium (YES) and compared to built environment conditions where fungi would typically grow in the built environment (WB and CT). As expected, both strains grew best and produced the largest number of detectable volatile compounds on YES. Both strains produced 2-methyl-1-butanol, 3-methyl-1-butanol, and 1-octen-3-ol on all three substrates, supporting the findings of others who have studied the VOCs of this species [19, 20]. For example, in buildings with moisture and microbial problems, the reported levels of these individual compound ranged from 0.1 to 10 $\mu g/m^3$. In some cases, 1-octen-3-ol was found at high concentration of up to 900 $\mu g/m^3$ and 3-methyl-1-butanol at 270 $\mu g/m^3$ [3]. Comparison of the volatile

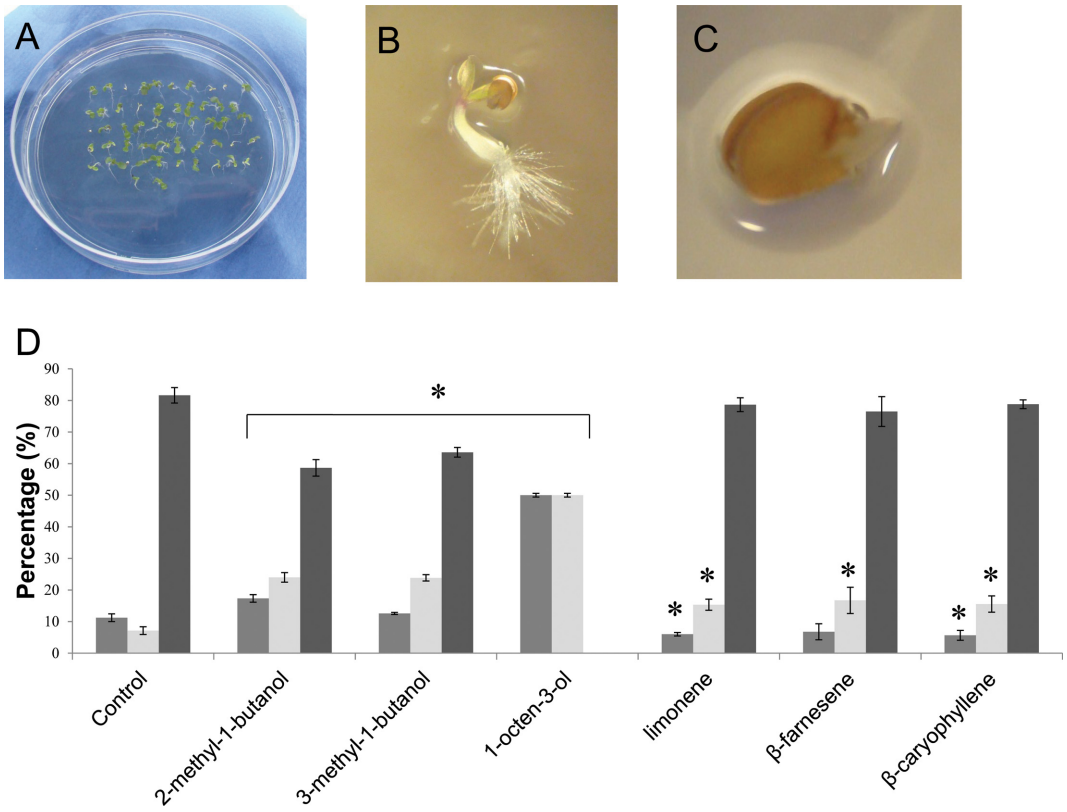


**Fig. 6.** *Arabidopsis thaliana* seeds exposed to chemical standards, individual volatile organic compounds at 0.5 µL/L for 72 hr. A, Seedlings; B, Emerging seedling; C, Germinated seed; D, Average percentage of seed germination and seedling development. Gray, light gray, and black indicate no germination, germinated seed, and seedling, respectively. *Significance.

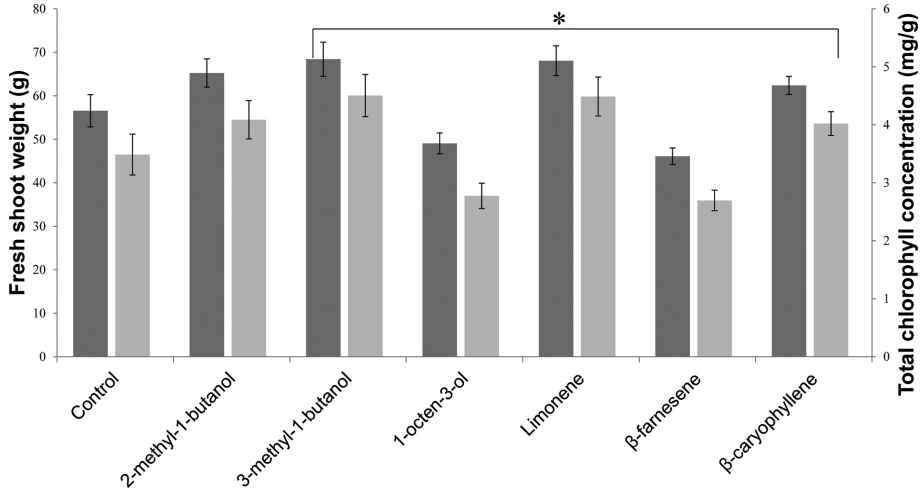


**Fig. 7.** Average shoot fresh weight and total chlorophyll concentrations of *Arabidopsis thaliana* exposed to chemical standards of volatile organic compounds at 0.5 µL/L for 72 hr. Black and gray indicate fresh shoot weight and total chlorophyll concentration, respectively. *Significance.

profiles of the two *Aspergillus versicolor* strains showed an increased production of terpenes, sesquiterpenes, and diterpenes in SRRC 2559 relative to SRRC 108. Other studies have also detected the production of terpenes and sesquiterpenes by *Aspergillus* species [3].

We tested the impact of the VOCs produced by the two strains on seed germination and plant growth. Using standardized protocols developed in our laboratory for exposing *Arabidopsis thaliana* to fungal VOCs [15, 23], seeds and vegetative plants were exposed to volatile emissions

from the two *Aspergillus versicolor* strains grown on YES. Seeds and plants exhibited differential responses between the two strains and the production of $CO_2$ was determined not to be a major contributor to these growth responses. While plants exposed to volatiles emitted by SRRC 108 were larger, the total chlorophyll content remained similar to the control. Plants exposed to VOCs emitted by SRRC 2559 were similar in average fresh shoot weight relative to controls; however, the plants had a significant reduction in chlorophyll content, indicating a stress response to the presence of VOCs.

Plants exposed to VOCs from both strains exhibited a reddish-purple coloration on the underside of the plant leaves, most likely anthocyanin pigments. Anthocyanin production is a known plant response to unfavorable environmental conditions, hypothesized to allow plants to survive under occasional periods of harmful irradiation through modulation of light absorption [27]. Exposure to fungal VOCs and subsequent accumulation of anthocyanin in *Arabidopsis* have been documented [28]. In summary, the decrease in total chlorophyll content and presence of pigment in the shoot indicates a stress response to the VOCs emitted by both strains of *Aspergillus versicolor*; however, the stress response was stronger with strain 2559.

In an attempt to determine the sensitivity of plants to individual compounds emitted by the *Aspergillus* strains we evaluated plant responses to acute exposures of six chemical standards at seed and vegetative growth stages. We tested to determine if the chirality of the compounds, such as 1-octen-3-ol and limonene, could affect physiological responses observed. Since we saw a minimal impact of enantiomers, we used standards with racemic mixtures in subsequent experiments. We observed compound-dependent plant responses using three alcohols (2-methyl-1-propanol, 3-methyl-1-butanol, and 1-octen-3-ol) and two terpenes (limonene and β-farnesene) identified through our gas analysis. We also tested β-caryophyllene, a common microbe and plant volatile, as a representative sesquiterpene for comparison. In our study, the alcohols were more inhibitory to seed germination and seedling formation than terpenes. Of the alcohols tested, 1-octen-3-ol was the most inhibitory. It caused seeds to arrest at germination, preventing further growth and development. It also was phytotoxic to vegetative plants causing localized death in plant tissue and overall reduction in chlorophyll. The antagonistic activities of 1-octen-3-ol have been reported previously [29]. It is important to note that the inhibitory effects observed on seed germination were not lethal; once the seeds were removed from the volatile treatment conditions they went on to complete germination and form seedlings. In contrast, at relatively low concentrations, exposing seeds to terpenes improved seed germination and seedling rate.

In vegetative plant exposure studies, differences were observed in plant size, leaf size and numbers, root growth, and chlorophyll concentration. Exposure to volatile phase 3-methyl-1-butanol, limonene, and β-caryophyllene improved plant growth, leading to larger, more robust plants with increased chlorophyll concentration. Exposure to volatile phase β-farnesene and 1-octen-3-ol reduced plant sizes and total chlorophyll concentration; plants exposed to 1-octen-3-ol exhibited additional symptoms of phytotoxicity such as curling of the leaf and presence of necrotic lesions. Localized cell death in the plant tissue is indicative of oxidative burst caused by the overabundance of reactive oxygen species [30]. Little or no purple pigment was observed in plants exposed to these individual compounds.

Most of the prior work on the effects of biogenic volatile phase compounds on plant growth has been conducted using mostly bacteria and few select fungi from the rhizosphere. The focus previously has been put on improving agricultural yields by increasing plant growth through direct stimulation of the plant and/or reductions in the incidence of plant disease [31]. Volatile detection has been used as an indirect marker to indicate the presence of *Aspergillus* species and other molds in indoor environments of stored agricultural crops [4]. To our knowledge, this report is the first to show that VOCs from *A. versicolor* can have an impact on plant growth and seed germination. We also demonstrate that there is a potential for developing plants to monitor indoor air quality. *Arabidopsis* is a good candidate for use as a plant bioindicator. It is fast growing and compact; the plant responds relatively quickly to volatile exposure; and the cost associated with analyzing plant tissue is relatively inexpensive, making this an ideal organism for quantitative approaches as well. Furthermore, we have identified several *Arabidopsis* genes involved in volatile responses including growth and stress related genes. It may be possible to create a transgenic *Arabidopsis* that can report on the presence and concentration of specific fungal VOCs and thereby serve as an even more sensitive biomonitor for toxigenic fungal VOCs in indoor environments.

In summary, we have compared the VOC profiles of two strains of *Aspergillus versicolor,* assessed plant responses to the presence of their VOCs, and tested some individual VOCs from the mixture emitted by the two molds. Plants and their growth stages were differentially affected by the VOC mixtures emitted by the two strains with the VOCs from SRRC 2559 causing a strongly negative impact on *A. thaliana*. Anecdotally, it is of interest that this strain came to our attention because it caused an irritant reaction in a student taking an *Aspergillus* identification workshop while the student was in close proximity, but not in physical contact, to the strain.

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