Isoprene and β-caryophyllene confer plant resistance via different plant internal signalling pathways

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Abstract
Isoprene and other terpenoids are important biogenic volatile organic compounds in terms of atmospheric chemistry. Isoprene can aid plant performance under abiotic stresses, but the fundamental biological reasons for the high emissions are not completely understood. Here, we provide evidence of a previously unrecognized ecological function for isoprene and for the sesquiterpene, β-caryophyllene. We show that isoprene induces a defence response in receiver plants that is similar to that elicited by monoterpens and depended on salicylic acid (SA) signalling. In contrast, the sesquiterpene, β-caryophyllene, induced resistance via jasmonic acid (JA)-signalling. The experiments in an open environment show that natural biological emissions are enough to induce resistance in neighbouring Arabidopsis plants. Our results show that both isoprene and β-caryophyllene function as allelochemical components in complex plant signalling networks. Knowledge of this system may be used to boost plant immunity against microbial pathogens in various crop management schemes.

KEYWORDS
Arabidopsis thaliana, β-caryophyllene, isoprene, jasmonic acid, plant resistance, Pseudomonas syringae, salicylic acid, terpenes, volatile organic compounds

1 INTRODUCTION
Isoprene, 2-methylbuta-1,3-diene, is the simplest terpene and most abundant biogenic volatile organic compound (VOC) on earth, providing an important role in atmospheric chemistry at regional and global scales (Claeys et al., 2004; Fuentes et al., 2000). More complex volatile terpenes, such as mono- and sesquiterpenes, also contribute to atmospheric reactions, enhancing, for example, secondary aerosol formation (Joutsensaari et al., 2005). Plants and other organisms emit a high diversity of various mono- and sesquiterpenes, compounds that are well known to function as signals in inter-specific interactions (Ninkovic, Rensing, Dahlin, & Markovic, 2019; Sharifi & Ryu, 2020). Isoprene, on the other hand, can protect plants from thermal and oxidative stresses, though the fundamental reasons for the large investments in isoprene emissions by several plant species are not yet completely elucidated (Monson, Weraduwage, Rosenkranz, Schnitzler, etc.)
& Sharkey, 2021; Sharkey & Monson, 2017). In addition to protection against abiotic stresses, isoprene has also been shown to affect flowering time (Terry, Stokes, Hewitt, & Mansfield, 1995), to speed up plant growth (Loi

\[ \text{\textit{JASMONATE RESISTANCE 1 (JAR1) protein (Nie et al., 2017). Systemic acquired resistance (SAR) is a systemic immune response in healthy tissues induced by local pathogen infection. The induction of SAR depends on SA-signalling via NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1; Cao, Glazebrook, Clarke, Volko, & Dong, 1997; Vlot, Dempsey, & Klessig, 2009) and on AGD2-LIKE DEFENSE RESPONSE PROTEIN 1 (ALD1; Song, Lu, McDowell, & Greenberg, 2004). ALD1 is an enzyme that produces the SAR-associated molecule piperocolic acid (Pip) and is required for SAR (Návarová, Berndorff, Döring, & Zeier, 2012; Song et al., 2004). We recently associated SAR with monoterpene emissions acting as cues for defence, both in systemic tissues during SAR and in neighbouring plants (Riedlmeier et al., 2017). The gene coding for LEGUME LECTIN LIKE PROTEIN 1 (LLP1) was, moreover, shown to be involved in SAR and monoterpine-induced resistance, acting downstream of both Pip and monoterpines in the establishment of immunity (Breitenbach et al., 2014; Wenig et al., 2019).}

Here, we tested the potential function of isoprene and the sesquiterpenes \( \beta \)-caryophyllene and \((\cdot)\)-thujopsene as volatile cues in inter-organismic signalling. We hypothesized that (a) exogenous isoprene and the sesquiterpenes \( \beta \)-caryophyllene and \((\cdot)\)-thujopsene can activate different signalling routes in \textit{Arabidopsis thaliana} plants and (b) that this activation leads into differentially induced resistance against the helminthothropic pathogen \textit{Pseudomonas syringae}. We aimed to first elucidate the functions of these volatile in experiments employing pure terpenes and various \textit{Arabidopsis} mutants defective either in JA-related (jar1 mutant) or SA-related (npr1, and ald1 mutants) signalling, or associated to these signalling pathways (llp1 mutant). In the second step, the function of the terpenes was tested in closed and open set-ups using biological terpene sources and wild type and mutant \textit{Arabidopsis} “receiver” plants. Based on our results, we suggest isoprene has a function in activating the SA-associated plant defence system, whereas \( \beta \)-caryophyllene, but not \((\cdot)\)-thujopsene, triggered plant resistance via JA-associated signalling.

2.1 | Plant and microbial materials and growth conditions

Wild-type \textit{Arabidopsis thaliana} plants (ecotype Columbia-0 (Col-0), transgenic \textit{Terpene synthase 21} (TPS21)-gene (At5g23960) over-expressing \textit{Arabidopsis} Col-0 (TPS21OE) and the mutant lines non-expressor of PR genes 1 (npr1) (Cao et al., 1997; Vlot et al., 2009), jasmonate resistant 1 (jar1) (Nie et al., 2017), \textit{legume lectin like protein 1} (llp1) (Breitenbach et al., 2014) and \textit{agd2-like defence response protein 1} (ald1) (Song et al., 2004) were used in the experiments. For construction of the TPS21OE line, the \textit{Arabidopsis} gene At5g23960 ([E]-\( \beta \)-caryophyllene synthase, EC 4.2.3.57), was cloned as described previously (Ting et al., 2015). The pBINPlus vector containing the gene was transfected in \textit{Agrobacterium tumefaciens} and the transformed \textit{Agrobacterium} strain was used for stable transformation of \textit{Arabidopsis} Col-0 through floral dipping (Liu et al., 2011)). TPS21OE
plants were selected in vitro on plates containing half-strength MS 1% (wt:vol) agar supplemented with 50 μg/ml kanamycin and selected transformed lines were made homozygous.

The Arabidopsis plants were grown in growth chambers in normal potting soil mixed with silica sand 5:1 and kept at 20/22 °C (night/day), 70% relative humidity and an incident photosynthetically active quantum flux density (PPFD) of 100 μmol photons m−2/s with a 10 hr photoperiod. The plants used for the experiments were 4.5 weeks old. Plant cultivation procedures have been described previously (Breitenbach et al., 2014; Riedlmeier et al., 2017; Vlot et al., 2008).

Four different genotypes of grey poplar (Populus × canescens [Alton] Sm. INRA clone 7,171-B4) were used in the experiments: Two isoprene-emitting (IE) genotypes (WT and K12 [empty vector [pBinAR] line]) and two well-characterized isoprene non-emitting (NE), transgenic genotypes (35S::PcISPS-RNAi lines RA1 and RA2; see Behnke et al. (2007)). Plantlets were amplified by micropropagation under sterile conditions (Leplé, Brasilheiro, Michel, Delmotte, & Jouanin, 1992) and rooted plantlets (approx. Plant height 5 cm) were cultivated in the greenhouse in 2.2 L pots on a sandy soil (1:1 [vol:vol] silica sand and Frühstorfer Einheitserde). For optimum fertilization, the soil was initially complemented with a mixture of slow release fertilizers (Triabon (Compo, Münster, Germany) and Osmocote (Scotts Miracle-Gro, Marysville, USA); 1:1, 10 g/L of soil). Furthermore, liquid fertilizers (Triabon (Compo, Münster, Germany) and Osmocote (Scotts Miracle-Gro, Marysville, USA); 1:1, 10 g/L of soil). Furthermore, liquid fertilizer was applied every 2 weeks (0.1% [wt:vol] Hakaphos® Grün, Compo, Münster, Germany). Climate conditions in the greenhouse were maintained at 22/20 °C (day/night) and 50% RH) with a 16/8 hr photoperiod with supplemental lighting (200–240 μmol photons m−2/s PPFD at canopy level).

The Laccaria bicolor strain S238N (Maire P.D. Orton) was cultivated on Pachlewsie medium (PS) as described previously (Ditengou et al., 2015). To expose Arabidopsis to L. bicolor volatiles, two pieces of young mycelium (5 mm diameter) were transferred to the middle of a new Petri dish and cultivated at 20–25 °C in the darkness on PS medium for 12 days.

Pseudomonas syringae pv. tomato (Pst) DC3000 was used for infection assays and was maintained as described previously (Breitenbach et al., 2014). Pst carrying the bacterial type III secretion system effector protein AvrRpm1 (Pst AvrRpm1) was used to induce volatile emission from Arabidopsis in the plant-to-plant communication assay (Breitenbach et al., 2014; Riedlmeier et al., 2017).

2.2 | Fumigations of Arabidopsis with pure terpenes

For the treatment of wild-type Arabidopsis plants with different pure VOCs, four pots with three plants each were placed into 5 L gas-tight glass desiccators (Carl Roth GmbH, Germany) (Figure 1a). During exposure experiments with Arabidopsis mutants, 2–3 pots containing three Col-0 plants each and 2–3 pots with three mutant plants each were placed in the same desiccator. The plants were exposed for 72 hr to individual volatile compounds (Sigma-Aldrich) dissolved in hexane as previously described in detail (Riedlmeier et al., 2017). We used solutions of isoprene, α/β-pinene, β-caryophyllene and (-)-thujopsene, which were always freshly prepared before application. If the applied solutions evaporated in the headspace equally and no deposition took place, then following final concentrations could be reached: isoprene (43.6, 87.1, 435.6, 871.1 ppm), β-caryophyllene (0.2, 1.2, 1.7, 2.3 ppm), (-)-thujopsene (0.2, 1.2, 1.7, 2.3 ppm). A mixture of α/β-pinene (2.7 ppm) was used as a positive control, as this treatment has been shown to be highly efficient in inducing immunity in our previous work (Riedlmeier et al., 2017; Wenig et al., 2019). Hexane served as a negative control (Riedlmeier et al., 2017).

2.3 | Infection with the microbial pathogen Pseudomonas syringae

To verify whether the treatment with various VOCs induced resistance, two upper leaves of the Arabidopsis plants were infiltrated with 107 colony forming units (cfu) ml−1 (OD600 set to 0.0002) of Pst. Bacterial growth in the plant was monitored as described before (Breitenbach et al., 2014). To determine the duration of the induced resistance after the terpene exposure, plants were infiltrated with Pst on days 4, 7 and 10 (counted from the start of volatile exposure). The leaf discs were harvested 4 days after the infiltration (following the protocol in Riedlmeier et al., 2017).

2.4 | Toxicity assay

Hexane solution (200 μl) containing resistance-inducing concentrations of isoprene, α/β-pinene, β-caryophyllene or (-)-thujopsene were applied to Pst medium (NYGA: 5 g/L bactopeptone, 3 g/L yeast extract, 20 ml/L glycerol, 15 g/L agar). As a negative control, 200 μl pure hexane was used. Serial dilutions of a Pst suspension (105 cfu/ml, OD600mm set to 0.002) were spotted (20 μl per spot) on plates without or with 200 μl terpene/hexane solution. Pst colonies were counted after 2 days of cultivation at 25–28 °C. Bacterial growth on the treated plates was monitored as described before (Riedlmeier et al., 2017).

2.5 | Exposure of Arabidopsis to biological VOC sources in desiccators

To expose Arabidopsis to poplar volatiles in glass desiccators, mature poplar leaves (leaf # 7–12 below the apex) were cut from the greenhouse grown trees. The leaves were kept with the petioles submerged in tap water in glass beakers outside of the desiccators for 30 min to prevent interference with wounding related stress signals (Breitenbach et al., 2011). For the exposure of Arabidopsis, the poplar leaves in the beakers were placed in the middle of the desiccators next to the Arabidopsis “receiver” plants (Figure 1a). The experiments were conducted in the growth chambers with the same settings as described above. Isoprene solution of 200 μl (87.1 ppm in the headspace) and...
Different experimental set-ups used to expose Arabidopsis thaliana to VOCs. (a) Arabidopsis receiver plants were co-cultivated in a closed glass desiccator (5.5 L volume) with a filter paper containing different terpene solutions or a biological VOC source, such as Laccaria bicolor colonies or leaves of poplar (Populus × canescens). The desiccators were placed in a cultivation chamber for the exposure period (3 days). (b) Arabidopsis Col-0 receiver plants were co-cultivated with β-caryophyllene emitting transgenic Arabidopsis line (TPS21OE) in an open glass vase. (c) In an open space in a greenhouse chamber Arabidopsis receiver plants were co-cultivated with increasing distance (1, 20, 50 and 80 cm) to isoprene-emitting (IE) and non-emitting (NE) poplar trees (details of the lines can be found in Behnke et al., 2007). IE and NE poplars were separated by 4–5 m. Col-0 plants placed at a 2 m distance between the poplar lines served as a room control to verify the specificity of the poplar-derived signal. Enhanced resistance to Pseudomonas syringae pv. tomato in the receiver plants is indicated by red arrows.
200 μl pure hexane served as positive and negative controls, respectively.

To expose Arabidopsis to L. bicolor volatiles, Petri dishes containing 12-days old L. bicolor colonies were placed in the desiccators for 3 days. β-caryophyllene solution of 200 μl (0.2 ppm in the headspace) and 200 μl pure hexane served as positive and negative controls, respectively.

When living organisms were used as VOC sources, the desiccators were opened on the third day of co-cultivation to avoid CO2 depletion and high humidity.

2.6 | Exposure of Arabidopsis to biological VOC sources in open systems

Plant-to-plant communication between TPS21OE and Col-0 was analysed in open glass vessels without lid (Figure 1b). Four pots containing three Col-0 sender plants each were sprayed with 0.01% Tween-20 (vol:vol) solution containing either 10^8 cfu/ml of Pst AvrRpm1 (to induce defence response) or 10 mM MgCl2 (mock control treatment) and left to dry for 30 min. TPS21OE sender plants were also mock treated. Four treated pots with three sender plants and four pots containing two Col-0 receiver plants each were placed in each vase. The plants were incubated for 3 days after which the receiver plants were challenged with Pst as described above.

To investigate isoprene signalling between poplar genotypes and Arabidopsis in an open space, communication experiments were carried out in a separate greenhouse cabin (22/20 °C (day/night), 16/8 photoperiod at a PPFD of 500 μmol photons m^{-2}/s; 40% RH). Wild-type Arabidopsis plants (4.5 weeks old) were fixed underneath a mature poplar leaf (1 cm) or placed in 20, 50 or 80 cm distance from the trees (Figure 1c). Care was taken that there was no shading on any of the Arabidopsis plants due to the poplar. Isoprene-emitting (IE: WT and K12) and non-emitting plants (NE: RA1 and RA2) were placed on opposite sides of the room in 4.5 m distance from each other to prevent cross communication. Control Arabidopsis plants were placed in the middle of the cabin in ca. 2 m distance from IE and from NE plants (Figure 1c). After 3 days of co-cultivation, Arabidopsis plants were challenged with Pst as described above.

2.7 | VOC analyses

For isoprene measurements over the whole poplar tree, leaf disks were cut by a hole punch (diameter 13 mm) from all the mature leaves of three poplar plants grown in green house. From each leaf, three disks were cut and used as technical replicates. After 30 min of stabilization time, each leaf disk was incubated in 200 μl of carbonated mineral water in 2 ml transparent glass vials for 2 hr under 32 °C and light intensity of 1,000 μmol m^{-2} s^{-1} PPFD, similar to as described earlier in Loivamäki et al. (2007). Isoprene was measured from the headspace of the vial by proton transfer reaction-time of flight-mass spectrometer (PTR-ToF-MS, Ionicon Analytic, Innsbruck, Austria). The headspace of the samples was injected into the PTR-ToF-MS by using N2 as carrier gas at a flow rate of 100 ml/min. As standard, 10.9 ppm isoprene in N2 was used (BASI Schöberl GmbH, Germany).

To quantify isoprene concentration from poplar leaves in the desiccators, an aliquot of the headspace was sampled after incubating one leaf per desiccator for 6 hr in the growth chamber (22°C, 70% relative humidity, PPFD of 50 μmol photons m^{-2}/s inside of the desiccators). Volatiles were trapped on glass tubes filled with adsorbents (Tiiva et al., 2009) at an airflow rate of 20 ml/min for 5 min.

To quantify VOC emission of the TPS21OE lines, dynamic headspace sampling was carried out in glass cuvettes as described in Riedlmeier et al. (2017). Inside the cuvettes, the temperature was 23.7 ± 0.7 °C and light intensity 135 ± 15 μmol photons m^{-2}/s photosynthetic active radiation. VOCs were collected at a flow rate of 0.1 L/min for 120 min from TPS21OE plants and 180 min from wild-type plants into glass cartridges filled with adsorbents (for details please see Riedlmeier et al., 2017).

The VOCs were analysed with gas chromatography–mass spectrometer (GC–MS) following established procedures (Ghirardo et al., 2020; Ghirardo, Heller, Fladung, Schnitzler, & Schröder, 2012).

2.8 | Statistics and reproducibility

For the statistical analyses, IBM SPSS Statistics version 21 (Armonk, NY, USA) was used. All data were tested using Kruskal–Wallis test followed by Mann–Whitney U test. Each statistical test is specified within the legend of a corresponding figure. Exact p values are given in Table S1. The raw data are given in Table S2.

3 | RESULTS

3.1 | Exogenous isoprene and β-caryophyllene improve plant resistance

Arabidopsis plants were exposed to selected terpenes in the surrounding gas phase to test if these compounds affect the “receiver” plant’s resistance to the bacterial pathogen Pseudomonas syringae (for experimental set-up please see Figures 1a and 2a). When Arabidopsis was exposed to isoprene, the resistance to Pseudomonas syringae pv. tomato (Pst) was induced in a manner similar to that for a mixture of the monoterpenes α/β-pinene serving as the positive control (Figure 2b; Riedlmeier et al., 2017). The sesquiterpene β-caryophyllene also induced plant resistance, while the other sesquiterpene (−)-thujopsene was ineffective at the test concentration (Figure 2b). The effect of the single volatile terpenes was dependent on the applied concentration (Figures S1 and 2b). We observed that the effective dose decreased in concentration according to an increase in the structural complexity of the terpenes; from the simple C5 hemiterpene isoprene to C10 monoterpenes to the C15 sesquiterpene β-caryophyllene (Figure S1).

A 3-day exposure of Arabidopsis to gaseous isoprene, α/β-pinene or β-caryophyllene led to persistent state of priming, lasting up to 4 days post-exposure. Isoprene-induced resistance lasted even up to 6 days.
post-exposure (Figure 2c). The sesquiterpene (-)-thujopsene was ineffective at the concentration of 0.2 ppm at all tested time points (Figure 2c). This compound, however, slightly induced the resistance at the concentration of 1.2 ppm, whereas no effect was observed when higher concentrations were tested (Figure S1). Control experiments showed that the applied concentrations of terpenes had no direct antibiotic effect and did not directly influence the growth of Pst (Figure S2).

3.2 | Isoprene induces resistance through SA, β-caryophyllene through JA

To decipher the activated plant internal signalling routes upon perceiving a volatile terpene, we employed various Arabidopsis mutants that were deficient either in JA or SA signalling. Our results indicate a role of SA in the isoprene-triggered resistance: In SAR-deficient npr1
Arabidopsis mutants, gaseous isoprene or α/β-pinene did not induce resistance against Pst (Figure 3a). In contrast, β-caryophyllene induced resistance in npr1 mutants, suggesting that this sesquiterpene functions independently of the SA-related npr1-gene in Arabidopsis (Figure 3a).

The function of ALD1, an enzyme that produces the SAR-associated molecule Pip and is required for SAR (Návarová et al., 2012; Song et al., 2004), was tested using ald1 mutants. The mutant was insensitive to isoprene or α/β-pinene, resulting in no change in plant resistance (Figure 3b). LLP1 acts downstream of both Pip and monoterpenes in the establishment of plant immunity (Breitenbach et al., 2014; Wenig et al., 2019). In the present study, the exposure of the Arabidopsis llp1 mutant to α/β-pinene did not lead to an increased resistance (Figure 3c). The presence of the hemiterpene isoprene in the headspace of the llp1 mutants, however, triggered resistance to Pst (Figure 3c and 4).

Knowing that the JA-signalling pathway depends on JASMONATE-RESISTANT 1 (JAR1), we also tested the effect of volatile
terpenes on the jar1 mutant (Nie et al., 2017; Staswick, Su, & Howell, 1992). Both isoprene and α/β-pinene were observed to induce resistance in jar1 plants, whereas the exposure of jar1 mutants to β-caryophyllene showed no change in resistance to Pst (Figure 3d).

3.3 Biological terpene emitters activate specific signalling routes in receiver plants

To investigate the ecological relevance of our findings, we asked whether a natural isoprene emitter can induce a similar response as volatilized pure terpene compounds. To this end, we tested the resistance-inducing ability of natural terpene emitters, such as the ectomycorrhizal fungus Laccaria bicolor (Müller et al., 2013) or isoprene-emitting (IE) and non-emitting (NE) gravy poplar trees (Populus × canescens) (Behnke et al., 2007, Figure S3).

In a first step, we exposed Arabidopsis plants to the emissions of IE and NE poplar leaves (Figure S3). The results demonstrate that IE poplar leaves triggered resistance to Pst, whereas NE leaves were not able to induce plant resistance (Figure 4). The induction of defence was similar to that obtained with non-biogenic isoprene fumigation (Figure 3a–d) and was dependent on gas-phase concentration: one or two leaves of IE plants sharing the headspace with Arabidopsis plants triggered resistance, whereas a higher number of leaves did not. Young leaves of poplar emit monoterpenes, which in elderly mature leaves become replaced by isoprene emission (Brilli et al., 2009). Even in mature leaves, however, some monoterpenes are released, and this also occurs from NE poplars that have suppressed isoprene emission (Figure S3). The specificity of the isoprene effect, compared to the naturally co-occurring monoterpene emissions of poplar leaves, was therefore tested with lp1 mutants, in which mono- and sesquiterpene-induced resistance was compromised (Figure 3c). Resistance induced by exposure of plants to IE poplar leaves developed normally in the lp1 mutant (Figure 4), consistent with the results obtained with synthetic isoprene (Figure 3c).

The relevance of sesquiterpenes from a natural source for initiation of resistance was tested by cultivating Arabidopsis plants in a common headspace with axenic cultures of L. bicolor, an ectomycorrhizal fungus, which is known to emit sesquiterpenes such as β-caryophyllene, α-muurolene, β/γ-selinene and γ-cadinene (Ditengou et al., 2015). The exposure of Arabidopsis to the L. bicolor volatile blend led to an induction of plant resistance (Figure 5) comparable to that induced by exposure to synthetic β-caryophyllene (Figure 3a–d). We observed that one to two fungal colonies were most effective in inducing the resistance. Similar to the exposure to pure compounds (Figure 3d), the resistance was not induced by fungal colonies in the jar1 mutant, which is deficient in JA-signalling. Comparable to the results with pure β-caryophyllene (Figure 3a), the L. bicolor...
resistance under ambient air (Figure 5), which is deficient in SA-signalling.

To test the function of terpenes as volatile cues in plant-to-plant communication under natural, ambient air conditions, we conducted two assays using open-air experimental systems. First, we co-cultivated wild-type Arabidopsis “receiver” plants together with β-caryophyllene synthase overexpressing Arabidopsis “sender” plants (TPS21OE, Figure S4, Alquézar et al., 2017) in open-top glass containers (Figure 1b, the same set-up was previously used by Wenig et al., 2019). TPS21OE-plants constitutively emit β-caryophyllene and two minor sesquiterpenes, cis-caryophyllene and α-humulene (2 and 21% of the three most abundant, novel sesquiterpenes, respectively, Figure S4). These compounds were not detected in the emission profiles of wild-type Arabidopsis plants. Similar to the application of synthetic β-caryophyllene using closed desiccators, the “receiver” plants in the open system showed induced defence responses after exposure to constitutive β-caryophyllene emissions from nearby TPS21OE “sender” plants (Figures 1b and 6).

Proximity to IE poplar trees also induced resistance to Pst in Arabidopsis when positioned close together in the greenhouse. Arabidopsis "receiver" plants perceived isoprene from IE poplar trees across distances up to 80 cm and developed improved resistance against Pst (Figures 1c and 7 and). The induction of resistance was stronger in plants growing next to the emission source, compared to plants growing farther away (Figure 7). Arabidopsis plants placed 200 cm distance between IE and NE plants served as control (for details Figure 1c). Data shown are normalized mean of [IE 1 cm distance]. Dots indicate the results obtained from three-to-nine independent experiments, each experiment containing three-to-six replicates. The median is represented by the horizontal line in the box blot. The top and the bottom of the box represent 75th and 25th percentile, and the boxes together with whiskers represent the 90th and 10th percentile of the data, respectively. Asterisks indicate significant differences between treatments and controls (Kruskal–Wallis and Mann–Whitney U test; ***p ≤ .001) [Colour figure can be viewed at wileyonlinelibrary.com]

3.4 Isoprene and β-caryophyllene emitters induce resistance under ambient air

4 DISCUSSION

4.1 Isoprene confers plant resistance similar to monoterpenes, via salicylic acid signalling

Monoterpenes and sesquiterpenes have various signalling functions in inter-specific interactions (Ninkovic et al., 2019; Sharifi & Ryu, 2020). High biogenic isoprene emissions by the leaves of some plant species (Sharkey & Monson, 2017), has, however, been proposed to serve as protection against abiotic stresses, such as high temperatures, drought or excessive light intensities (Behnke et al., 2007; Loreto et al., 2001;
Previously, volatile-triggered changes in JA-associated signalling networks have been shown more often than those triggering SA networks. For example, GLVs (Arimura et al., 2001; Frost et al., 2008), indole (Erb et al., 2015) or the rare volatile (E,S)-conophthorin (Helms et al., 2017) were all shown to induce or adjust JA-dependent signalling. Moreover, in the present study, the sesquiterpene β-caryophyllene induced resistance through LLP1 and JAR1, but acted independently of NPR1, suggesting that the sesquiterpene cue was processed in a JA-dependent manner. This result is consistent with the recent finding that β-caryophyllene can bind to the TPL-like protein, co-repressor of JA-mediated signalling (Nagashima et al., 2019). TPL, together with adaptor proteins, blocks default activity of the transcription factor MYC2 in the absence of the JA-derivative, jasmonoyl-isoleucine (JA-ile [Perez & Goossens, 2013; Li, Uhrig, Thurow, Huang, & Gatz, 2019]). This function of β-caryophyllene is similar to the action of plant phytohormones, such as JA or auxin, which also function through transcription co-repressors (Nagashima et al., 2019; Perez & Goossens, 2013). Release of TPL may enable activation of MYC2 target genes, which play a central role in JA-mediated signalling (Nagashima et al., 2019). Our data thus stress the importance of β-caryophyllene in inter-specific interactions. It does not only function as a signal emitted by several plant and microbial species to interact with their environment (Huang et al., 2011; Hung, Lee, & Bennett, 2013; Rasmann et al., 2005), but this specific sesquiterpene can also be perceived by plants leading to changes in JA-signalling and induced resistance. In this context, the high number of isomeric sesquiterpenes is very interesting, if they can transmit distinct inter-specific signals. Moreover, not only plants, but also microbes emit a high variety of different terpenoids (Lemfack et al., 2017) that may be perceived by plants. When exploring other possible VOC signals, we found that (−)-thujopsene, a sesquiterpene released among others by the ectomycorrhizal fungi L. bicolor (Ditengou et al., 2015), did not induce plant resistance above-ground when applied in similar concentration as β-caryophyllene. This compound might, however, be effective at other concentrations: Our results suggest that at a specific concentration also (−)-thujopsene may be able to induce resistance. On the other hand, (−)-thujopsene can alter plant performance by other means than by inducing resistance. Interestingly, (−)-thujopsene was previously shown to impact Arabidopsis root architecture belowground, while β-caryophyllene did not (Ditengou et al., 2015). Together, these observations indicate that the response of (−)-thujopsene and β-caryophyllene may be tissue-specific and they reveal the possibility that distinct functions and activities occur among different isomers in the family of sesquiterpenes.

4.3 Distinct signalling cues and specific plant responses?

We propose a scheme whereby the interactions of genes and phytohormones are required for volatile terpene-induced resistance (Figure 8). Isoprene requires two SA-signalling related proteins, ALD1 and NPR1 to induce resistance, while monoterpene-induced resistance additionally depends on LLP1. β-Caryophyllene induces resistance through LLP1 and JAR1, but acts independently of NPR1, suggesting involvement of JA-signalling. The activation of specific signalling routes suggests that natural selection has favoured the
isoprene, or the common monoterpens α- and β-pinene. Erb (2018) suggested recently, however, that such rather common volatile cues from different sources, such as microbes and neighbouring plants, might be integrated in a receiver plant for a more specific defence response. Integration of various cues, together with plant internal signalling cross talk, might create a complex network of signals that plants use for reliable resolution of responses to external biotic and abiotic stresses (Erb, 2018; Junker et al., 2018).

Similar to other volatile signals, which are often more powerful as a bouquet than as individual molecules (Simpraga, Takabayashi, & Holopainen, 2016), isoprene, although a relatively abundant VOC at least in tree canopies of isoprene emitters, may be part of a more complex volatile blend forming specific signals in different ratios with other compounds. Often isoprene-emitting trees facing biotic stress induce the emission of mono- and sesquiterpenes, which appears to compensate for reductions in the constitutive emission of isoprene (Brilli et al., 2009). Thus, reduced rather than increased isoprene concentrations in the surrounding atmosphere might, along with increases in induced VOCs, be associated with the likelihood of increased stress.

4.4 | Distance and VOC concentration matter

According to previous research, the concentration of the volatile compound seems to play an important role in perceiving and recognizing the cue (Baldwin, Halitschke, Paschold, von Dahl, & Preston, 2006; Riedmeier et al., 2017). Although the applied concentrations of pure solutions were relatively high in the present study, it is likely that these, as point source applied solutions, in fact resulted (due to possible deposition on surfaces and/or chemical reactions) in only short transient concentration peaks in the headspace of the exposure chamber. Thus, the actual effective concentration of individual terpenes cannot be identified from these “proof-of-principle” data. The biological effectiveness of isoprene emitted from young poplars in a free atmosphere and the perception of the low β-caryophyllene emission from transgenic Arabidopsis plants in open systems, however, imply that low rather than very high volatile concentrations may be effective cues. Ultimately, concentration gradients in the atmosphere regulate the VOC exposure of a receiver plant (Baldwin et al., 2006). The present data also show that isoprene-induced resistance depends on the physical distance of the receiver from the sender in an open space. The concentration and spatial distribution of volatile terpenes within plant canopies can indeed vary greatly, for example, due to their high volatility, rapid atmospheric dilution and chemical reactivity (Holopainen & Blande, 2013; Pinto, Blande, Souza, Nerg, & Holopainen, 2010; Mofikoya et al., 2019). Within the blend of volatiles, isoprene is unique; in that, it possesses an extremely low boiling point (Mofikoya et al., 2019) and diffusivity, causing it to disperse rapidly by passive diffusion (Baldwin et al., 2006). Previously, however, also other highly volatile compounds, such as methacrolein, were shown to be an important part of VOC signalling between sagebrush (Artemisia tridentata) and tobacco (Nicotiana attenuata) plants within 15 cm distance (Kessler, Halitschke, Diezel, & Baldwin, 2006). This is a distance that would also permit isoprene to effectively transmit information (present study).

In addition, microbes can also emit isoprene (Kuzma, Nemecek-Marshall, Pollock, & Fall, 1995), which can be sensed by plants. Because microbes often grow in close association with plants, the signalling distance may be shorter than in the plant–plant interactions described here. Recently, it has been shown that root-internal isoprene can lead to changes in root architecture and redox status in certain root cells (Miloradovic van Doorn et al., 2020). For example, if epiphytic or endophytic bacteria release isoprene, the compound could lead to sensitive adjustments in plant redox balance and alter plant performance. The potential ecological significance of isoprene in microbe–plant interactions should be elucidated in the future.

4.5 | Volatile terpenes as signalling cues in plant communities

A potential function between isoprene emitters and non-emitters was recently proposed for complex plant communities (Ormeño et al., 2020). Taken the relatively short distance of the effective cue, isoprene might function especially in dense ecosystems, or even as a rapid, within-plant signalling cue. Baldwin et al. (2006) have suggested that for highly volatile substances, signalling may be most beneficial for protecting the foliage of the emitter tree, and other neighbouring trees with intertwined canopies. In natural populations of plants, there is a high likelihood that neighbours are genetically related, and therefore, that they share genetic contributions to fitness that are carried forward to future generations. Isoprene is emitted, for example, by bryophytes (Hanson, Swanson, Graham, & Sharkey, 1999) and by many arctic species (Ghirardo et al., 2020; Tiiva et al., 2007; Tiiva et al., 2009), which grow in small scale and species-rich, dense ecosystems. The present data stress the need to investigate whether isoprene plays a role in such ecosystems, for example, in the Arctic and/or in the tropics, where “eavesdropping” to isoprene-emitting neighbours may be advantageous for boosting plant immunity.

In a past commentary, Lerdau (2002) made the suggestion that while it is clear that plants emit complex mixtures of VOCs to the atmosphere, it is not clear that they possess mechanisms to respond to those compounds when received in a gaseous state. We have shown that there do appear to be mechanisms that allow plants to “listen” to the messages sent by their neighbouring organisms. Some
of the most commonly emitted VOCs, such as isoprene and several monoterpenes and sesquiterpenes, appear to sensitize complex signaling pathways in plant cells that, in turn, trigger cellular responses that enhance plant fitness. Understanding the communication traits, and their effects to harden plants against the detrimental influences of microbial infections and abiotic stresses, will yield new strategies for the effective management of agriculture and forestry.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Jörg-Peter Schnitzler conceived and designed the experiments with Lena Frank, Maaria Rosenkranz and A. Corina Vlot. Lena Frank and Maaria Rosenkranz prepared the manuscript with contributions from Jörg-Peter Schnitzler and A. Corina Vlot. Lena Frank and Marion Wenig performed VOC exposure assays and analysed data. Lena Frank, Maaria Rosenkranz, Andrea Ghirardo and Alexander von der Krol performed VOC analyses. Alexander van der Krol provided TPS21OE plants. A. Corina Vlot provided Arabidopsis mutants and edited the manuscript. Jörg-Peter Schnitzler and Maaria Rosenkranz supervised the study and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Alquézar, B., Volpe, H. X. L., Magnani, R. F., de Miranda, M. P., Santos, M. A., Wulff, N. A., ... Peña, L. (2017). β-Caryophyllene emitted from a transgenic Arabidopsis or chemical dispenser repels Diaphorina citri, vector of Candidatus liberibacter. Scientific Reports, 17, 5639.
Arimura, G., Ozawa, R., Horuchi, J., Nishioha, T., & Takabayashi, J. (2001). Plant–plant interactions mediated by volatiles emitted from plants infested by spider mites. Biochemical Systematics and Ecology, 29, 1049–1061.
Baldwin, I. T., Hallitschke, R., Paschold, A., von Dahl, C. C., & Preston, A. (2006). Volatile signaling in plant-plant interactions: “Talking trees” in the genomics era. Science, 10, 812–815.
Behnke, K., Ehlting, B., Teuber, M., Bauerfeind, M., Louis, S., Hänisch, R., ... Schnitzler, J. P. (2007). Transgenic, non-isoprene emitting poplars don’t like it hot. Plant Journal, 51, 485–499.
Behnke, K., Kaiser, A., Zimmer, I., Brüggemann, N., Janz, D., Polle, A., ... Schnitzler, J. P. (2010). RNAI-mediated suppression of isoprene emission in poplar transiently impacts phenolic metabolism under high temperature and high light intensities: A transcriptomic and metabolomics analysis. Plant Molecular Biology, 74, 61–75.
Breitenbach, H. H., Wenig, M., Wittek, F., Jordá, L., Maldonado-Alconada, A.-M., Sarioglu, H., ... Vlot, A. C. (2014). Contrasting roles of the apoplastic aspartyl protease apoplastic, enhanced disease SUSCEPTIBILITY1-DEPENDENT1 and legume lectin-like PROTEIN1 in Arabidopsis systemic acquired resistance. Plant Physiology, 165, 791–809.
Brilli, F., Ciccioli, P., Frattoni, M., Prestininzi, M., Spanedda, A. F., & Loreto, F. (2009). Constitutive and herbivore-induced monoterpenes emitted by Populus x euroamerica leaves are key volatiles that orient Chrysomela populi beetles. Plant, Cell & Environment, 32, 545–552.
Brilli, F., Ruuskkanen, T. M., Schnitzhofer, R., Müller, M., Breitenlechner, M., Bittner, V., ... Hansel, A. (2011). Detection of plant volatiles after leaf wounding and darkening by proton transfer reaction “time-of-flight” mass spectrometry (PTR-TOF), PLoS One, 6, e20419.
Cao, H., Glazebrook, J., Clarke, J. D., Volko, S., & Dong, X. (1997). The Arabidopsis NPR-1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell, 88, 57–63.
Caparrotta, S., Boni, S., Taiti, C., Palm, E., Mancuso, S., & Pandolfi, C. (2018). Induction of priming by salt stress in neighboring plants. Environmetal and Experimental Botany, 147, 261–270.
Claeys, M., Graham, B., Vas, G., Wang, W., Vermeulen, R., Pashynska, V., ... Maenhaut, W. (2004). Formation of secondary organic aerosols through photooxidation of isoprene. Science, 303, 1173–1176.
Ditengou, F. A., Müller, A., Rosenkranz, M., Felten, J., Lasok, H., Miloradovic van Doorn, M., ... Polle, A. (2015). Volatile signalling by sesquiterpenes from ectomycorrhizal fungi reprogrammes root architecture. Nature Communications, 6, 6279.
Erb, M. (2018). Volatiles as inducers and suppressors of plant defense and immunity–origins, specificity, perception and signaling. Current Opinion in Plant Biology, 44, 117–121.
Erb, M., Veyrat, N., Robert, C. A., Xu, H., Frey, M., Ton, J., & Turlings, T. C. (2015). Indole is an essential herbivore-induced volatile priming signal in maize. Nature Communications, 6, 6273.
Frost, C. J., Mescher, M. C., Dervinis, C., Davis, J. M., Volko, S., & Dong, X. (1997). The NPR-1 gene that controls systemic acquired resistance in Arabidopsis systemic acquired resistance. Plant Molecular Biology, 35, 2192–2207.
Ghirardo, A., Lindstein, F., Koch, K., Buegger, F., Schloter, M., Albert, A., ... Rinnan, R. (2020). Origin of volatile organic compound emissions from subarctic tundra under global warming. Global Change Biology, 26, 1908–1925.
Hanson, D. T., Swanson, S., Graham, L. E., & Sharkey, T. D. (1999). Evolutionary significance of isoprene emission from mosses. American Journal of Botany, 86, 634–639.
Harvey, C. M., & Sharkey, T. D. (2016). Exogenous isoprene modulates gene expression in unstressed Arabidopsis thaliana plants. Plant, Cell & Environment, 39, 1251–1263.
Helms, A. M., De Moraes, C. M., Tröger, A., Albom, H. T., Francke, W., Tooker, J. F., & Mescher, M. C. (2017). Identification of an insect-
produced olfactory cue that primes plant defenses. *Nature Communications*, 8, 337.

Holopainen, J. K., & Blande, J. D. (2013). Where do herbivore-induced plant volatiles go? *Frontiers in Plant Science*, 11, 185.

Huang, M., Sanchez-Moreiras, A. M., Abel, C., Sohrabi, R., Lee, S., Gershenzon, J., & Tholl, D. (2011). The major volatile organic compound emitted from *Arabidopsis thaliana* flowers, the sesquiterpene (E)-β-caryophyllene, is a defense against a bacterial pathogen. *New Phytologist*, 193, 97–1008.

Hung, R., Lee, S., & Bennett, J. W. (2013). *Arabidopsis thaliana* as a model system for testing the effect of *Trichoderma* volatile organic compounds. *Fungal Ecology*, 6, 19–26.

Joutenssaari, J., Loivamaki, M., Vuorinen, T., Miettinen, P., Nerg, A. M., Holopainen, J. K., & Laaksonen, A. (2005). Nanoparticle formation by ozonolysis of inducible plant volatiles. *Atmospheric Chemistry and Physics*, 5, 1489–1495.

Junker, R. R., Kupper, J., Amo, L., Blande, J. D., Borges, R. M., van Dam, N. M., et al. (2016). Covariation and phenotypic integration in chemical communication displays: Biosynthetic constraints and eco-evolutionary implications. *New Phytologist*, 220, 739–749.

Kessler, A., Hallatschke, R., Diezel, C., & Baldwin, I. T. (2006). Priming of plant defense responses in nature by airborne signaling between Artemisia tridentata and *Nicotiana attenuata*. *Oecologia*, 148, 280–292.

Kuzma, J., Nemecek-Marshall, M., Pollock, W. H., & Fall, R. (1995). Bacteria produce the volatile hydrocarbon isoprene. *Current Microbiology*, 30, 97–103.

Laathawornkitkul, J., Paul, N. D., Vickers, C. E., Possell, M., Taylor, J. E., Leplé, J. C., Brasileiro, A. C., Michel, M. F., Delmotte, F., & Jouanin, L. Lemfack, M. C., Gohlke, B.-O., Toguem, S. M. T., Preissner, S., Mofikoya, A. O., Bui, T. N. T., Kivimäenpää, M., Holopainen, J. K., & Velikova, V. (2001). Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone photolysis atmospheres: The ecological effects. *Oecologia*, 343, 34–34.

Miloradovic van Doorn, M., Meri-Pham, J., Ghiarred, A., Fink, S., Polle, A., Schnitzler, J. P., & Rosenkranz, M. (2020). Root isoprene formation alters lateral root development. *Plant Cell & Environment*, 43, 2207–2223. https://doi.org/10.1111/pce.13814.

Mofikoya, A. O., Bui, T. N. T., Kivimäenpää, M., Holopainen, J. K., Himanen, S. J., & Blande, J. D. (2019). Foliar behaviour of biogenic semi-volatiles: Potential applications in sustainable pest management. *Arthropod-Plant Interactions*, 13, 193–212.

Monson, R. K., Weraduwage, S. M., Rosenkranz, M., Schnitzler, J. P., & Sharkey, T. D. (2021). Leaf isoprene emission as a trait that mediates the growth-defense tradeoff in the face of climate stress. *Oecologia*. https://doi.org/10.1007/s00442-020-04813-7

Müller, A., Faubert, P., Hagen, M. Zu, C. W., Polle, A., Schnitzler, J. P., & Rosenkranz, M. (2013). Volatile profiles of fungi-chemotyping of species and ecological functions. *Fungal Genetics and Biology*, 54, 25–33.

Nagashima, A., Hijiki, T., Koeduka, T., Ishigami, K., Hosokawa, S., Watanabe, H., ... Touhara, K. (2019). Transcriptional regulators involved in responses to volatile organic compounds in plants. *Journal of Biological Chemistry*, 15, 2256–2266.

Návarová, H., Bernsdorf, F., Döring, A. C., & Zeier, J. (2012). Picrocolic acid, an endogenous mediator of defense amplification and priming, is a critical regulator of inducible plant immunity. *Plant Cell*, 24, 5123–5141.

Naznin, H. A., Kiyohara, D., Kimura, M., Miyazawa, M., Shizunia, M., & Hyakumachi, M. (2014). Systemic resistance induced by volatile organic compounds emitted by plant growth-promoting fungi in *Arabidopsis thaliana*. *Phytochemistry*, 97, 1489–1497.

Nie, P., Li, X., Wang, S., Guo, J., Zhao, H., & Niu, D. (2017). Induced systemic resistance against Botrytis cinerea by Bacillus cereus AR156 through a JA/ET- and NPR1-dependent signaling pathway and activates PAMP-triggered immunity in *Arabidopsis*. *Frontiers in Plant Science*, 8, 238.

Ninkovic, V., Rensing, M., Dahlin, I., & Markovic, D. (2019). Who is my neighbor? Volatile cues in plant interactions. *Plant Signaling & Behaviour*, 14, 1634993.

Ormeño, E., Viros, J., Mévy, J. P., Tonetto, A., Saunier, A., Bousquet-Méluo, A., & Fernandez, C. (2020). Exogenous isoprene confers physiological benefits in a negligible isoprene emitter (Acer monspessulanum L) under water deficit. *Plants*, 9, 159.

Perez, A. C., & Goossens, A. (2013). Jasmonate signalling: A copycat of auxin signalling? *Plant, Cell & Environment*, 36, 2071–2084.

Pinto, D. M., Blande, J. D., Souza, S. R., Nerg, A., & Holopainen, J. K. (2010). Plant volatile organic compounds (VOCs) in ozone (O₃) polluted atmospheres: The ecological effects. *Journal of Chemical Ecology*, 36, 22–34.

Rasmann, S., Källner, T. G., Degenhardt, J., Hiltpold, I., Töpfer, S., Kuhlmann, U., ... Turings, T. C. (2005). Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature*, 434, 732–737.

Riedlmeier, M., Ghiarred, A., Wenig, M., Knappe, C., Koch, K., Georgii, E., ... Vlot, A. C. (2017). Monoterpenes support systemic acquired resistance within and between plants. *Plant Cell*, 29, 1440–1459.

Sharifi R., & Ryu C.-M. (2021). Social networking in crop plants: Wired and wireless cross-plant communications. *Plant Cell Environ*, 44, 1095–1110. https://doi.org/10.1111/pce.13966.

Sharkey, T. D., & Monson, R. K. (2017). Isoprene research–60 years later, the biology is still enigmatic. *Plant, Cell & Environment*, 40, 1671–1678.

Simpraga, M., Takabayashi, J., & Holopainen, J. K. (2016). Language of plants: Where is the word? *Journal of Integrative Plant Biology*, 58, 343–349.

Song, J. T., Lu, H., McDowell, J. M., & Greenberg, J. T. (2004). A key role for ALD1 in activation of local and systemic defenses in *Arabidopsis*. *Plant Journal*, 40, 200–212.

Stasz, P. E., Su, W., & Howell, S. H. (1992). Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis* neighbor? Volatile cues in plant interactions. *Proceeding of the National Academy of Sciences USA*, 105, 17430–17435.

Loreto, F., Mannonzzi, M., Maris, C., Nascetti, P., Ferranti, F., & Pasqualini, S. (2001). Ozone quenching properties of isoprene and its antioxidant role in leaves. *Plant Physiology*, 126, 993–1000.

Loreto, F., & Schnitzler, J. P. (2008). Isoprene interferes with the attraction of bodyguards by herbaceous plants. *Proceeding of the National Academy of Sciences USA*, 105, 17430–17435.

Loreto, F., & Velikova, V. (2001). Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiology*, 127, 1781–1787.

Miloradovic van Doorn, M., Meri-Pham, J., Ghiarred, A., Fink, S., Polle, A., Schnitzler, J. P., & Rosenkranz, M. (2020). Root isoprene formation alters lateral root development. *Plant Cell & Environment*, 43, 2207–2223. https://doi.org/10.1111/pce.13814.
Tiiva, P., Faubert, P., Räty, S., Holopainen, J. K., Holopainen, T., & Rinnan, R. (2009). Contribution of vegetation and water table on isoprene emission from boreal peatland microcosms. *Atmospheric Environment*, 43, 5469–5475.

Tiiva, P., Rinnan, R., Faubert, P., Räsänen, J., Holopainen, T., Kyrö, E., & Holopainen, J. K. (2007). Isoprene emission from a subarctic peatland under enhanced UV-B radiation. *New Phytologist*, 176, 346–355.

Ting, H. M., Delatte, T. L., Kolkman, P., Misas-Villamil, J. C., van der Hoorn, R. A. L., Bouwmeester, H. J., & van der Krol, A. R. (2015). SNARE-RNAi results in higher terpene emission from ectopically expressed caryophyllene synthase in *Nicotiana benthamiana*. *Molecular Plant*, 8, 454–466.

Van Loon, L. C., Bakker, P. A. H. M., & Pieterse, C. M. J. (1998). Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology*, 36, 453–483.

Vlot, A. C., Dempsey, D. A., & Klessig, D. F. (2009). Salicylic acid, a multifaceted hormone to combat disease. *Annual Review of Phytopathology*, 47, 177–206.

Vlot, A. C., Liu, P. P., Cameron, R. K., Park, S. W., Yang, Y., Kumar, D., ... Klessig, D. F. (2008). Identification of likely orthologs of tobacco salicylic acid-binding protein 2 and their role in systemic acquired resistance in *Arabidopsis thaliana*. *Plant Journal*, 56, 445–456.

Wenig, M., Ghirardo, A., Sales, J. H., Pabst, E. S., Breitenbach, H. H., Antritter, F., ... Vlot, A. C. (2019). Systemic acquired resistance networks amplify air-borne defense cues. *Nature Communications*, 10, 3813.

Yamagiwa, Y., Inagaki, Y., Ichinose, Y., Toyoda, K., Hyakumachi, M., & Shiraishi, T. (2011). *Talaromyces wortmannii*, FS2 emits β-caryophyllene, which promotes plant growth and induces resistance. *Journal of General Plant Pathology*, 77, 336–341.

Yi, H. S., Heil, M., Adame-Alvarez, R. M., Ballhorn, D. J., & Ryu, C. M. (2009). Airborne induction and priming of plant defenses against a bacterial pathogen. *Plant Physiology*, 151, 2152–2161.

Zuo, Z., Weraduwage, S. M., Lantz, A. T., Sanchez, L. M., Weise, S. E., Wang, J., ... Sharkey, T. D. (2019). Isoprene acts as a signaling molecule in gene networks important for stress responses and plant growth. *Plant Physiology*, 180, 124–152.

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