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# Volatile organic compounds of hoary stock are responsible for suppressing downy mildew of grape in an intercropping system

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**Abstract**

Grape downy mildew (*Plasmopara viticola*) is an air-borne disease and difficult to control. It has been observed that intercropping grapevines (*Vitis vinifera*) with aromatic plants can effectively suppress the airborne disease and volatile organic compounds (VOCs) from the aromatic plants are believed to have antimicrobial activities. In this study, a two-year field trial was established by intercropping grapevine and hoary stock (*Matthiola incana*) to evaluate the control of grape downy mildew. The field results showed that intercropping effectively suppressed grape downy mildew, particularly during the blooming stage of hoary stock. VOCs from hoary stock plants exhibited a dosage-dependent antimicrobial activity against grape downy mildew. To examine the role of VOCs, hoary stock plants were grown in an enclosed chamber, and VOCs were collected at the time points before and post blooming. The collected VOCs were subjected to gas chromatography-mass spectrometry (GC–MS) analysis and in vitro bioassays. Twenty-four VOCs found from pre-blooming and 36 VOCs from post-blooming hoary stock plants were identified. Seventeen VOCs demonstrated consistent inhibitory activities against *P. viticola*, including seven terpenoids, five benzenoids, and five aliphatics. Among the 17 VOCs, five were unique to post-blooming hoary stock, while 12 were common to both pre- and post-blooming hoary stock. The antimicrobial VOCs offers a potential eco-friendly alternative to managing downy mildew.

**Keywords** Grapevine downy mildew, Hoary stock, Volatile organic compounds, Intercropping, GC–MS analysis, Antimicrobial activities

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## Background

Downy mildew, caused by the biotrophic oomycete *Plasmopara viticola*, is one of the most devastating diseases of grapevine (*Vitis vinifera*), often resulting in severe economic losses in grape production (Koledenkova et al. 2022). *P. viticola* can infect grape leaves and young fruits through stomata, affecting the growth of the grapevine and causing severe reductions in grape yield and quality. This disease particularly occurs in areas with frequent rains, high humidity, and mild temperatures (Du et al. 2015; Koledenkova et al. 2022). The sporangia of *P. viticola* can spread widely through wind and raindrops, granting the pathogen robust transmission ability that contribute to the challenging of disease control (Gessler et al. 2011). Currently, downy mildew management mainly relies on the application of chemical fungicides. However, the appearance of fungicide-resistant strains and environmental pollution caused by frequent fungicide applications are driving research towards alternative or complementary strategies (Vezzulli et al. 2019).

It has been recognized that increasing plant diversity by using intercropping or mixed cropping is effective in boosting yields and decreasing diseases (Zhu et al. 2000; Zhu et al. 2022). Intercropping can reduce airborne diseases compared to monocrops, such as rice blast (*Magnaporthe grisea*) (Zhu et al. 2000), cereal powdery mildew (*Erysiphe graminis*) (Wolfe et al. 1985; Newton et al. 2009), and faba bean chocolate spot disease (*Botrytis fabae*) (Guo et al. 2019), when associated crops that have antimicrobial activities are correctly chosen (Boudreau 2013; Zhu et al. 2019, 2022).

Aromatic plants, as a companion crops, have also been intercropped in tea gardens, orchards, and fields to promote crop growth and reduce pests and diseases (Zhang et al. 2016; Brilli et al. 2019; Greff et al. 2023). For example, the yield of arecanut (*Areca catechu* L.) improved by intercropping aromatic plants of basil (*Ocimum basilicum*), davana (*Artemisia pallens*), and patchouli (*Pogostemon patchouli* Pellet.) in arecanut plantations (Sujatha et al. 2011). Intercropping marigolds (*Tagetes* spp.) in an apple orchard could mitigate nematodes disease and enhance the growth and fruit yield of apple trees (Yim et al. 2017). The pepper blight (*Phytophthora capsici*) was effectively mitigated by intercropping pepper with fennel (*Foeniculum vulgare*) (Li et al. 2022).

Intercropping may have multiple mechanisms for suppressing plant diseases, such as inoculum dilution, spore dispersal interfering, microenvironment modification, and allelopathy (Zhu et al. 2000; Zhu et al. 2019). Allelopathy, by using allelochemicals as mediators, plays an important role in suppressing disease in intercropping systems (Ding et al. 2015; Zhu et al. 2019). Volatile organic compounds (VOCs) are common allelochemicals, diffusing between the soil particles and spreading into the atmosphere over short or long distances (Sharifi and Ryu 2018; Weisskopf 2021), have potential advantages in controlling soil-borne or air-borne diseases without direct or physical contact between the VOCs producer and the target pathogens (Rice 1984; Weisskopf et al. 2021; Minerdi et al. 2021; Almeida et al. 2023). For example, plant VOCs, released naturally or in response to various stimuli, can protect the VOC-releasing plants or neighboring plants against diseases through direct inhibition or inducing disease resistance in plants (Neri et al. 2007; Yi et al. 2009; Holopainen et al. 2010). (E)-β-caryophyllene from *Arabidopsis thaliana* flowers serves as an antimicrobial substance to protect flowers against *Pseudomonas syringae* (Huang et al. 2012). VOCs from a common bean (*Phaseolus vulgaris*) cultivar induces the resistance of a susceptible cultivar to the fungus *Colletotrichum lindemuthianum* (Quintana-Rodriguez 2014). Aromatic plants are rich in aromatic VOCs, which are potential bioactive substances that can affect phytopathogens directly through inhibition or indirectly by inducing systemic resistance of plants (Kanchiswamy et al. 2015; Sharifi-Rad et al. 2017; Misra et al. 2019).

In our previous study, hoary stock (*Matthiola incana*) intercropped with grapevines in the field promoted grapevine growth and fruit quality due to some hoary stock VOCs (Yao et al. 2022; Deng et al. 2023). While it is unclear whether hoary stock and grapevines intercropping can alleviate grape downy mildew, and what the underlying mechanism if it works. In this study, we used hoary stock as an intercropping plant in the grape production system to (i) assess the impact of the intercropping on grape downy mildew in the field; (ii) determine the role of hoary stock VOCs for suppressing grape downy mildew; and (iii) identify antimicrobial compounds in the VOCs (Fig. 1). We hope to reveal the chemical mechanism involved in grapevine and hoary stock intercropping system for grape downy mildew management. The findings can help exploit an eco-friendly grapevine disease control method for sustainable viticulture and develop novel botanical fungicides or lead compounds for synthesizing new fungicides against crop downy mildew.

## Results

### Disease development under different cropping systems

Two years of field experiments demonstrated that intercropping hoary stock with grapevine effectively suppressed the development of grape downy mildew by decreasing the disease index and slowing down disease growth (Fig. 2). In 2020, the average disease index in the intercropping was 35.75, demonstrating a reduction compared to monoculture (50.54), particularly during full bloom (July 23–August 2), resulting in a significant decrease of 50.19% compared to monoculture ($P<0.05$) (Fig. 2a). As shown in Fig. 2c, the logistic model fitted well to the disease indexes of grapevine downy mildew in the two cropping patterns ($0.969 \le R^2 \le 0.975$, $P<0.05$). In intercropping, the maximum disease index (a) (Fig. 2a), the initial growth rate of the disease index (k), and the maximum instantaneous growth rate of the disease index ($I_{max}$) (Fig. 2c) were $80.93 \pm 6.23$, $0.10 \pm 0.02$ (/d), $2.10 \pm 0.08$ (/d), respectively, which were all significantly lower than those of monoculture ($P<0.01$). And the time to reach the maximum growth rate of the disease index ($t_{50}$) was $39.29 \pm 2.59$ (d), which was about 11 days

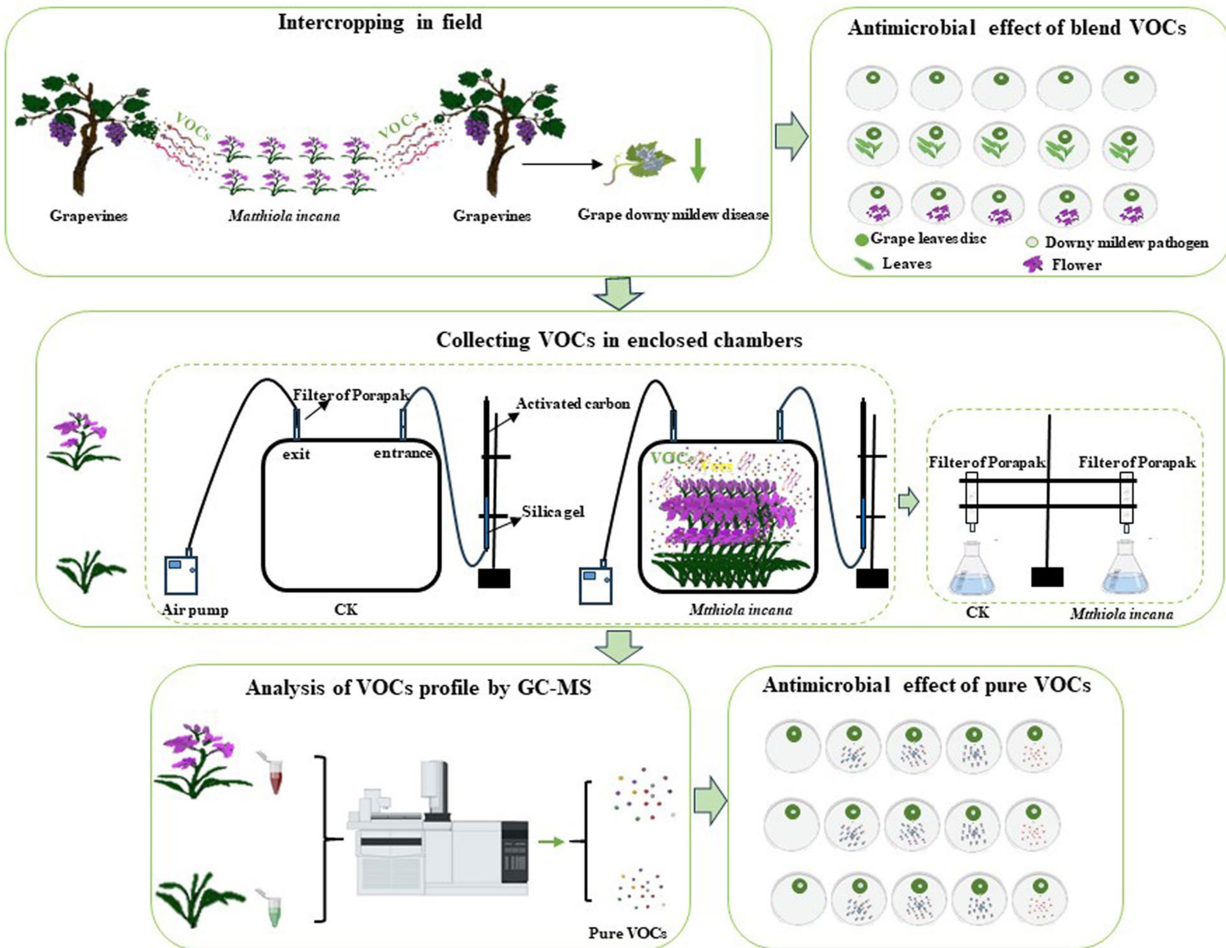


**Fig. 1** Flow chart of an experiment for assessing the impact of the intercropping hoary stock with grapevines on the control of grape downy mildew in the field and the antimicrobial effect of volatile organic compounds (VOCs) of hoary stock on *Plasmopara viticola*

later than that of monoculture ($P < 0.001$) (Fig. 2c). This implies that grapevine intercropped with hoary stock could delay the onset and slow down the development of grape downy mildew compared that in monoculture. In 2021 field trial, the progression of disease indexes in intercropping exhibited a comparable trend to those observed in the first year trial (Fig. 2b, d).

### VOCs from hoary stock showed dosage-dependent antimicrobial activity against *P. viticola*

The natural VOCs blends from the aerial parts of hoary stock at pre- or post-blooming showed a dosage-dependent antimicrobial ability to suppress grape downy mildew (Fig. 3). In 2020, the disease indexes of downy mildew on leaf discs decreased from 23.83 ± 0.73 to 14.00 ± 0.76 and 17.00 ± 1.00 to 10.00 ± 1.00, respectively, when leaf discs were exposed to pre- and post-blooming hoary stock (1–5 g), which were significantly lower than the control disease index of 46.00 ± 2.00 ($P < 0.05$) (Fig. 3a). At equal mass, the inhibitory effects of VOCs from post-blooming hoary stock were markedly stronger than those from pre-blooming hoary stock (Fig. 3c). The trend observed in 2021 trial closely paralleled that of the 2020 trial (Fig. 3b, d).

### A cluster of VOCs identified from the aerial parts of hoary stock

More components of VOCs were found from post-blooming than pre-blooming hoary stock. A total of 36 VOCs with mass spectra similarity scores of 70% or higher, comprising over 0.05% relative content and distinct from the control, were identified as the main components in the VOCs from the aerial part of post-blooming hoary stock (Fig. 4a). Based on the distinctions in the primary functional groups, these compounds were classified into seven groups, i.e., 17 esters, eight terpenoids, three alcohols, three alkanes, two ketones, two phenols, and one aldehyde. The highest percentage

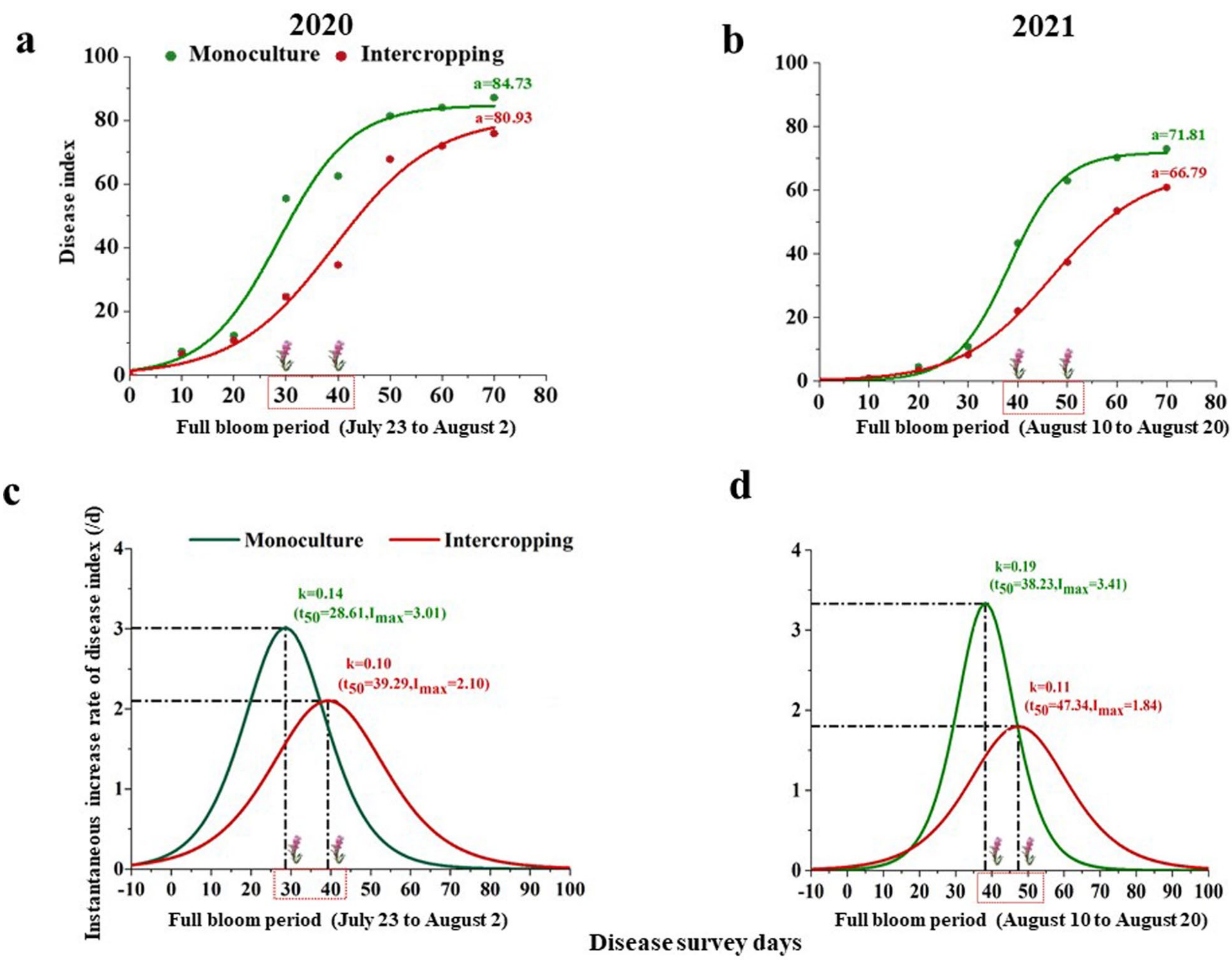


**Fig. 2** Disease progress of grape downy mildew in a grapevine-hoary stock intercropping and monoculture systems in fields. **a** Disease index in 2020. **b** Disease index in 2021. **c** Instantaneous increase rate of disease index estimated using Logistic model in 2020. **d** Instantaneous increase rate of disease index estimated using Logistic model in 2021. Data are presented as the means ± standard errors (SEs)

was recorded for terpenoids, accounting for 25.41%, followed by esters, accounting for 14.92% (Additional file 1: Table S1). Similarly, a total of 24 compounds were identified in the VOCs from the aerial part of pre-blooming hoary stock, consisting of nine esters, five alkanes, four terpenoids, two alcohols, two ketones, one phenol, and one aldehyde (Fig. 4b). Esters represented the highest percentage at 9.66%, followed by alkanes at 6.34% (Additional file 1: Table S2). Compared with the main volatile components, post-blooming hoary stock VOCs contained 21 unique compounds, with farnesene (α-farnesene and β-farnesene) exhibiting the highest relative content (Fig. 4c). There were 15 same compounds present in pre- and post-blooming hoary stock VOCs, which included five esters (ethyl benzoate, amyl acetate, ethyl valerate, (E)-ethyl-2-methyl-2-butenoate, ethyl-2-methylbutyrate), four terpenoids (limonene, 1,8-cineole, α-pinene, β-pinene), two alkanes (2,6-dimethylundecane, tridecane), one alcohol (phenethyl alcohol), one phenol (2,6-di-tert-butyl-4-methylphenol), one aldehyde (1,1-diethoxy-ethane), and one ketone (2-hexanone) (Fig. 4d).

### Antimicrobial activities of VOCs against *P. viticola*

The 15 VOCs shared by both pre- and post-blooming hoary stock and eight VOCs only found in post-blooming hoary stock, were examined for their inhibitory activities against *P. viticola*. Grape leaf discs were not affected by water or any of the test VOCs (Additional file 1: Table S3). The bioassay results revealed that the 23 VOCs had antimicrobial properties against *P. viticola* at different levels (Additional file 1: Table S3). The antimicrobial activities were dosage dependent. Tridecane, phenethyl alcohol, eugenol, limonene,

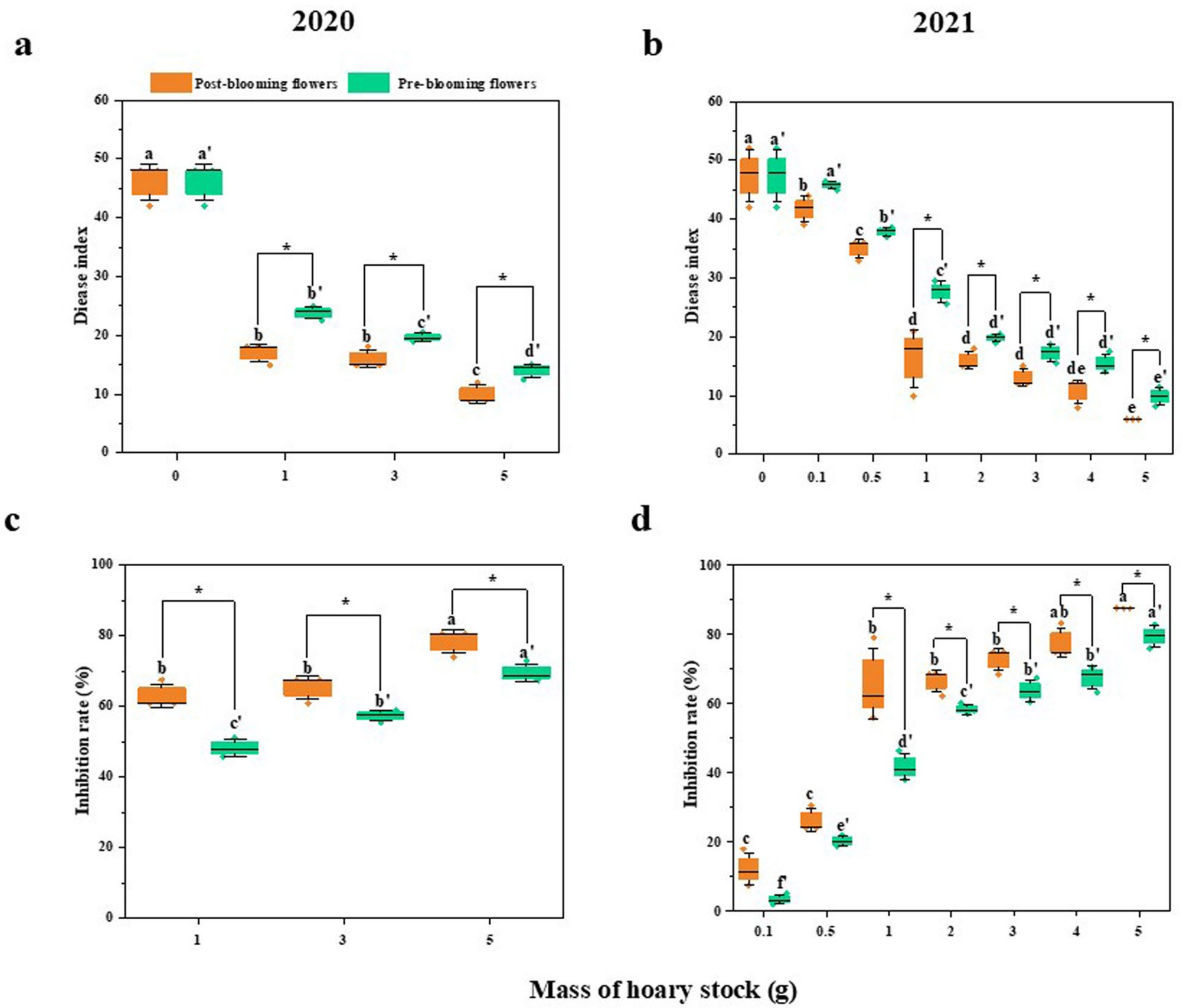


**Fig. 3** Effect of volatile organic compounds (VOCs) from the aerial part of hoary stock collected at pre- or post-blooming on the development of grape downy mildew on leaf discs in the first (2020) and second (2021) years. **a**, **b** Disease index. **c**, **d** Inhibition rate. Means are separated or compared in three ways: (1) Effects of VOCs from post-blooming flowers, which are marked by lower-case letters a–e; (2) Effects of VOCs from pre-blooming flowers, which are marked by lower-case letters a'–f'; and (3) Comparisons between pre- and post-blooming flowers, which are indicated by an asterisk. Within each group, different letters indicate significant differences of the measurements (Tukey's post hoc ANOVA; $P<0.05$). Asterisks indicate statistically a significant difference between two data points (independent-samples *t* test; $P<0.05$). Bars represent standard errors (SEs)

ethyl-2-methylbutyrate, 1,8-cineole, anethol, 2,6-di-tert-butyl-4-methylphenol, 1,1-diethoxy-ethane, methyl phenylacetate, and β-caryophyllene were effective at concentrations greater than 0.5 mg/L (Fig. 5a), while (E)-ethyl-2-methyl-2-butenoate, ethyl benzoate, amyl acetate, α-pinene, β-pinene, and β-farnesene were effective at concentrations greater than 4 mg/L (Fig. 5b). The rest six compounds including 2-hexanone, 2,6-dimethyl-undecane, ethyl valerate, 1-octene-3-ol, isoamyl acetate, and α-farnesene demonstrated inconsistent and unstable antimicrobial activities, which were not dosage dependent. For example, 2,6-dimethylundecane, ethyl valerate, and isoamyl acetate showed antimicrobial activities at the concentration of 6 mg/L and 20 mg/L, while no effects at other test concentrations (Fig. 5c). Among the 17 effective VOCs, 12 compounds, namely tridecane, phenethyl alcohol, eugenol, limonene, ethyl-2-methylbutyrate, 1,8-cineole, anethol, 2,6-di-tert-butyl-4-methylphenol, 1,1-diethoxy-ethane, methyl phenylacetate, (E)-ethyl-2-methyl-2-butenoate, and ethyl benzoate exhibited significant antimicrobial activity with $EC_{50}$ values ranging from $0.54 \pm 0.12$ to $4.79 \pm 0.00$ mg/L. Conversely, the other five compounds including amyl acetate, β-caryophyllene, α-pinene, β-pinene, and β-farnesene demonstrated lower

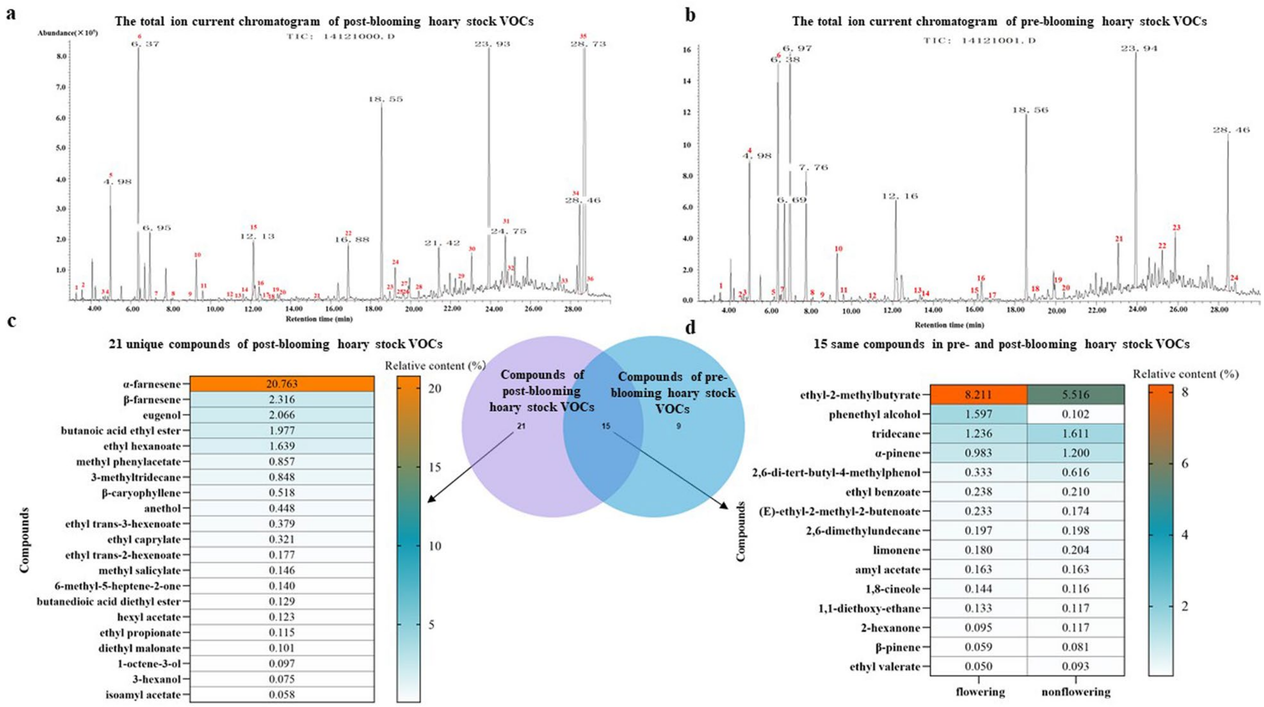


**Fig. 4** Comparative analysis of volatile organic compounds (VOCs) from hoary stock, collected at either pre- or post-blooming stages. **a** Chromatograms of total ion current of VOCs from post-blooming hoary stock. **b** Chromatograms of total ion current of VOCs from pre-blooming hoary stock. **c** Identified 21 unique VOCs from post-blooming hoary stock. **d** Identified 15 same VOCs from pre-and post-blooming hoary stock

antimicrobial activity with $EC_{50}$ values from 5.72 ± 0.09 to 126.92 ± 54.14 mg/L (Table 1). Notably, five out of the 17 effective VOCs (β-farnesene, eugenol, methyl phenylacetate, β-caryophyllene, and anethol) only found in post-blooming hoary stock, while the remaining 12 shared by pre- and post-blooming hoary stock. The total relative content of the 12 compounds in post-blooming hoary stock was 13.51%, higher than that in pre-blooming hoary stock (10.11%) (Table 2).

## Discussion

We have explored the possible way for controlling grape downy mildew by intercropping hoary stock with grapevines. The intercropping system effectively suppressed the disease. This disease suppression was due to the VOCs released from hoary stock, which were most abundant during the blooming period.

We have found that hoary stock VOCs effectively inhibit downy mildew development in the field, with the heightened inhibitory effect observed during the blooming period. Further analysis indicated that direct antimicrobial compounds against *P. viticola* contained in post-flowering hoary stock VOCs are more abundant than that in pre-flowering hoary stock VOCs. The mechanism is due to the composition and relative content of VOCs emitted dynamically at different growth stages of hoary stock. Post-flowering hoary stock have 36 main compounds with a high percentage of terpenoids, whereas pre-flowering hoary stock contain 24 main compounds with a high percentage of esters. This was consistent with other plant VOCs regulated by plant growth development are dynamic at different growth stages of plants (Li et al. 2022). For example, the main compounds of *Acer truncatum* VOCs fluctuate seasonally, with higher concentration of the total volatile compound during its flowering season compared to other seasons (Song et al. 2014). The VOCs profile of *Michelia alba* varied between the flowering and wilting stages, with significantly higher levels of monoterpenoids and sesquiterpenoids observed during the flowering stage compared to the post-wilting stage (Shang et al. 2002). The inhibitory effects of both blended hoary stock VOCs and individual compounds on *P. viticola* were concentration dependent, as shown in many previous studies (Groenhagen et al. 2013; Lazazzara et al. 2018; Garbeva and Weisskopf 2020). This suggested that coinciding the bloom period of hoary stock with the onset of grape downy mildew, along with increasing the intercropped hoary stock with grapevine, are essential for achieving optimal control effects in the field, given the feasibility and rationality in farm operation.

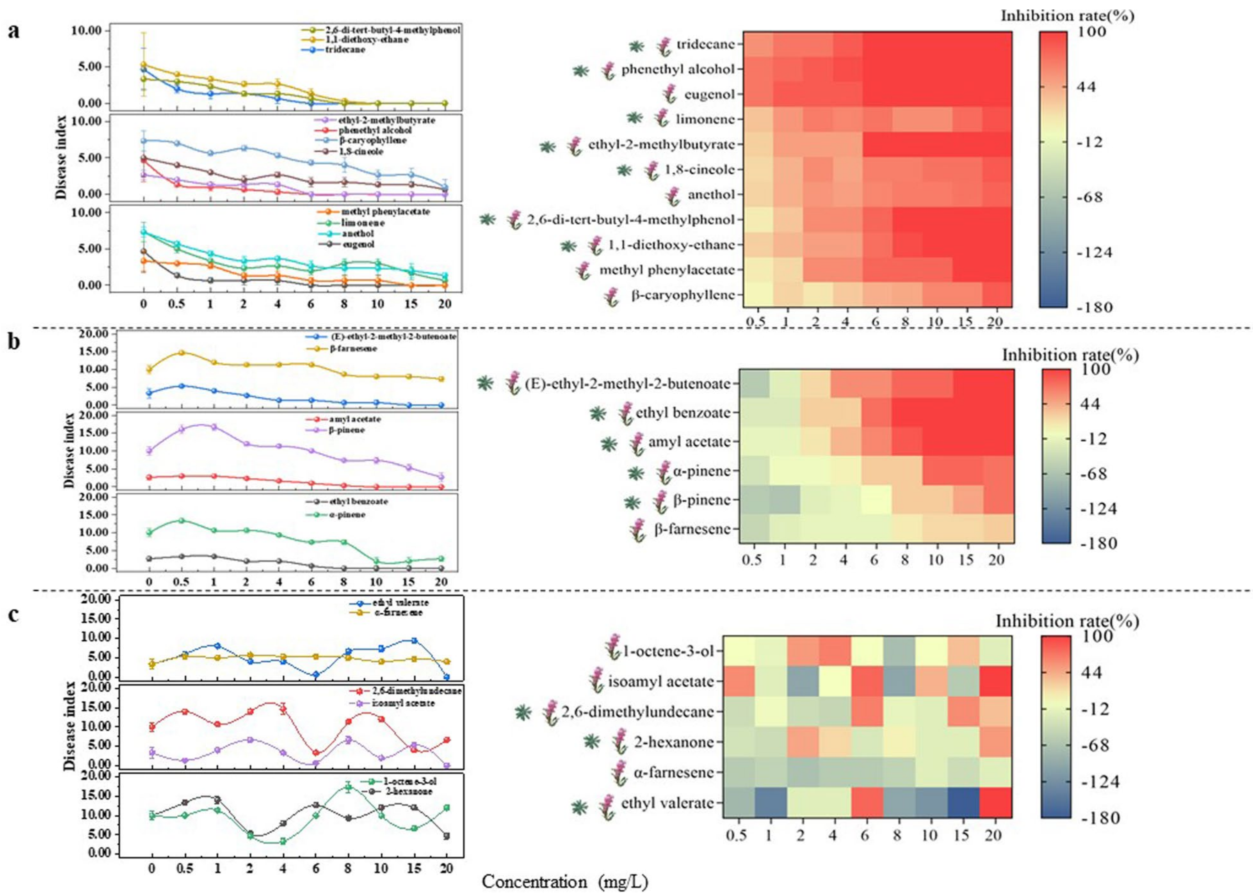

**Fig. 5** Effects of volatile organic compounds (VOCs) from hoary stock, collected at either pre- or post-blooming stages, on the inhibition of *Plasmopara viticola* inoculated on grape leaf discs, measured by disease index. Bars represent standard errors

**Table 1** Effective concentration for 50% inhibition ($EC_{50}$) of volatile organic compounds on *Plasmopara viticola*

| Compounds | $EC_{50}$ (mg/L) | Compounds | $EC_{50}$ (mg/L) |
|---|---|---|---|
| Tridecane | 0.54 ± 0.12 | Methyl phenylacetate | 3.67 ± 1.20 |
| Phenethyl alcohol | 0.63 ± 0.18 | (E)-ethyl-2-methyl-2-butenoate | 4.37 ± 0.38 |
| Eugenol | 0.82 ± 0.24 | Ethyl benzoate | 4.79 ± 0.0 |
| Limonene | 1.38 ± 0.62 | Amyl acetate | 5.72 ± 0.09 |
| Ethyl-2-methylbutyrate | 1.85 ± 0.47 | β-caryophyllene | 9.33 ± 1.92 |
| 1,8-cineole | 2.37 ± 0.12 | α-pinene | 10.71 ± 0.74 |
| Anethol | 2.56 ± 0.64 | β-pinene | 14.49 ± 0.83 |
| 2,6-di-tert-butyl-4-methylphenol | 2.93 ± 0.86 | β-farnesene | 126.92 ± 54.14 |
| 1,1-diethoxy-ethane | 3.03 ± 0.64 | | |

Plant VOCs can be divided into groups like terpenoids, fatty acid derivatives, and benzenoids according to biosynthetic pathways (Dudareva et al. 2013), most of which have direct antimicrobial activities (Scala 2013; Kanchiswamy et al. 2015; Schiestl 2015). In our study, 17 compounds presented in both post- and pre-flowering hoary stock VOCs had direct antimicrobial ability for *P. viticola*, which were seven terpenoids, five benzenoids, and five aliphatics. These active compounds exhibited different antimicrobial properties for *P. viticola* in vitro. One aliphatic tridecane showed the strongest inhibitory effect in suppressing downy mildew on grape leaf discs.

**Table 2** Relative contents (%) of antimicrobial compounds in post-and pre-flowering hoary stock volatile organic compounds (VOCs)

| Identical compounds | Post-flowering | Pre-flowering | Unique compounds | Post-flowering | Pre-flowering |
|---|---|---|---|---|---|
| Ethyl-2-methylbutyrate | 8.211 | 5.516 | β-farnesene | 2.316 | 0 |
| Phenethyl alcohol | 1.597 | 0.102 | Eugenol | 2.066 | 0 |
| Tridecane | 1.236 | 1.611 | Methyl phenylacetate | 0.857 | 0 |
| α-pinene | 0.983 | 1.200 | β-caryophyllene | 0.518 | 0 |
| 2,6-di-tert-butyl-4-methylphenol | 0.333 | 0.616 | Anethol | 0.448 | 0 |
| Ethyl benzoate | 0.238 | 0.210 | | | |
| (E)-ethyl-2-methyl-2-butenoate | 0.233 | 0.174 | | | |
| Limonene | 0.180 | 0.204 | | | |
| Amyl acetate | 0.163 | 0.163 | | | |
| 1,8-cineole | 0.144 | 0.116 | | | |
| 1,1-diethoxy-ethane | 0.133 | 0.117 | | | |
| β-pinene | 0.059 | 0.081 | | | |
| Total relative content (%) | 13.51 | 10.11 | | 6.205 | 0 |

Interestingly, tridecane is often reported as a scent gland secretion of insects, such as *Oebalus pugnax* (F.) and *Halyomorpha halys,* and acted as a kairomone to attract other pests, such as *Orius insidiosus* (Fraga et al. 2016). Its antimicrobial activity had not been reported previously. Two benzenoids, phenethyl alcohol and eugenol, displayed strong inhibitory efficacy against *P. viticola.* In fact, phenethyl alcohol, often found in plant essential oils, has a wide antimicrobial spectrum and shows antimicrobial activity against bacteria, fungi, and oomycetes, such as *Escherichia coli*, *Rhizoctonia solanacearum*, *P. digitatum*, and *P. viticola* (Lazazzara et al. 2018). In a previous study, eugenol inhibited ascomycetes, oomycetes, and deuteromycetes (Schiestl 2015). Among the remaining 14 compounds, six terpenoids, including limonene, 1,8-cineole, anethol, β-caryophyllene, α-pinene, and β-pinene showed inhibitory activity, with limonene having the strongest effect. Limonene has been previously demonstrated to have a broad spectrum of antimicrobial activity (Zahi et al. 2017), and can directly inhibit pathogenic fungi *Botrytis cinerea* and *Penicillium chrysogenum*, food-borne bacteria *E. coli* and *Listeria monocytogenes* (Umagiliyage et al. 2017), and the germination of *Colletotrichum lindemuthianum* conidia (Quintana-Rodriguez 2014). It can be concluded that these substances with different antimicrobial activities in hoary stock VOCs played a key role in inhibiting grape downy mildew. Perhaps because allelopathic substances usually act in synergistic, adjunctive or antagonistic forms in suppressing diseases in natural ecosystems (De Vrieze, et al. 2015), the inhibitory effect of the natural VOCs blends from hoary stock on grape downy mildew was positively correlated with hoary stock biomass. To the best of our knowledge, this is the first report to profile hoary stock VOCs and examine their antimicrobial activities against *P. viticola*.

Due to the obligate biotrophic lifestyle of *P. viticola*, the inhibitory effects of VOCs against downy mildew could only be tested on host tissues. Since many individual plant VOCs, such as eugenol, limonene, α-pinene, β-pinene, and β-caryophyllene, can inhibit pathogens directly and also induce defense responses in recipient plants to prevent disease (Schiestl 2015; Riedlmeier et al. 2017; Frank et al. 2021), it is possible that the studied VOCs may induce plant resistance against downy mildew. However, this requires further investigation.

## Conclusions

Grape downy mildew was effectively suppressed in a grapevine-hoary stock intercropping system, showcasing an exemplary instance of intercropping for disease control. The aromatic VOCs produced by hoary stock plants played a crucial role in inhibiting grape downy mildew. These active compounds hold the potential to serve as natural alternatives to synthetic fungicides or as leading compounds for creating novel fungicides.

## Methods

### Field trials

A field experiment was carried out in a vineyard at the Daheqiao farm, Xundian County, Yunnan, China (25.56° N, 103.25° E; altitude of 1885 m) for a two-year period of 2020 and 2021. The mean annual temperature in Xundian was 14.5°C, and the mean annual rainfall was 1045 mm, with the majority of this rainfall occurring between May and October each year. The vineyard had been cultivated with the cultivar Red Globe for nine years. Grapevines were spaced with 1.0 m vines within the row and 2.2 m between rows. The Y-type trellis was adopted in the vineyard.

The experiment included two treatments: a monoculture of grape 'Red Globe' (Fig. 6a) and an intercropping system of grape 'Red Globe' and hoary stock 'Stock Yume no Uta' (Fig. 6b). The experimental design was a completely randomized plot design with three replicates. Each plot included two rows of grapevines, approximately 50 grapevines per plot, each plot measuring 22.5 m in length and 2.2 m in width. To avoid disturbance of the VOCs, the monoculture was separated from the intercropping system by two plots planted with 'Red Globe' as a buffer zone (Fig. 6b). Hoary stock seeds were purchased from Lincheng Flowers Co., Ltd., Yunnan, China and grown in a greenhouse at Yunnan Agricultural University. For the intercropping treatment, approximately 180 hoary stock seedlings at the eight-to-ten leaf stage were transplanted into four rows between the grapevine rows in April. The spacing between the grapevines and hoary stock rows was set at 58 cm, with the hoary stock spaced 35 cm apart both between rows and within rows. The hoary stock plants bloomed from July to August, coinciding with the grape maturity in the local region. Vineyard management with respect to pruning, fertilizers, weed control, and insect pest control was uniformly performed to all vines using local standard practices. Each treatment comprised three plots.

**Disease assessment**

The incidence of grape downy mildew in the field was recorded when downy mildew was first observed in the vineyard every season, and the observation was repeated every ten days until the end of the season. The disease was assessed on ten plants per row, and a total of 20 plants per plot. Two new shoots of each plant were randomly selected, and ten leaves of each shoot were investigated from top to bottom (Deng et al. 2017).

Disease severity was classified using a 0 to 5 scale (0 = no visible disease symptoms; 1 = <5% infected leaf area; 2 = 5 to 25%; 3 = 26 to 50%; 4 = 51 to 75%; and 5 > 75%) (Zang et al. 2014). Disease index = [Σ(Number of diseased leaves at certain scale × Disease scale)/

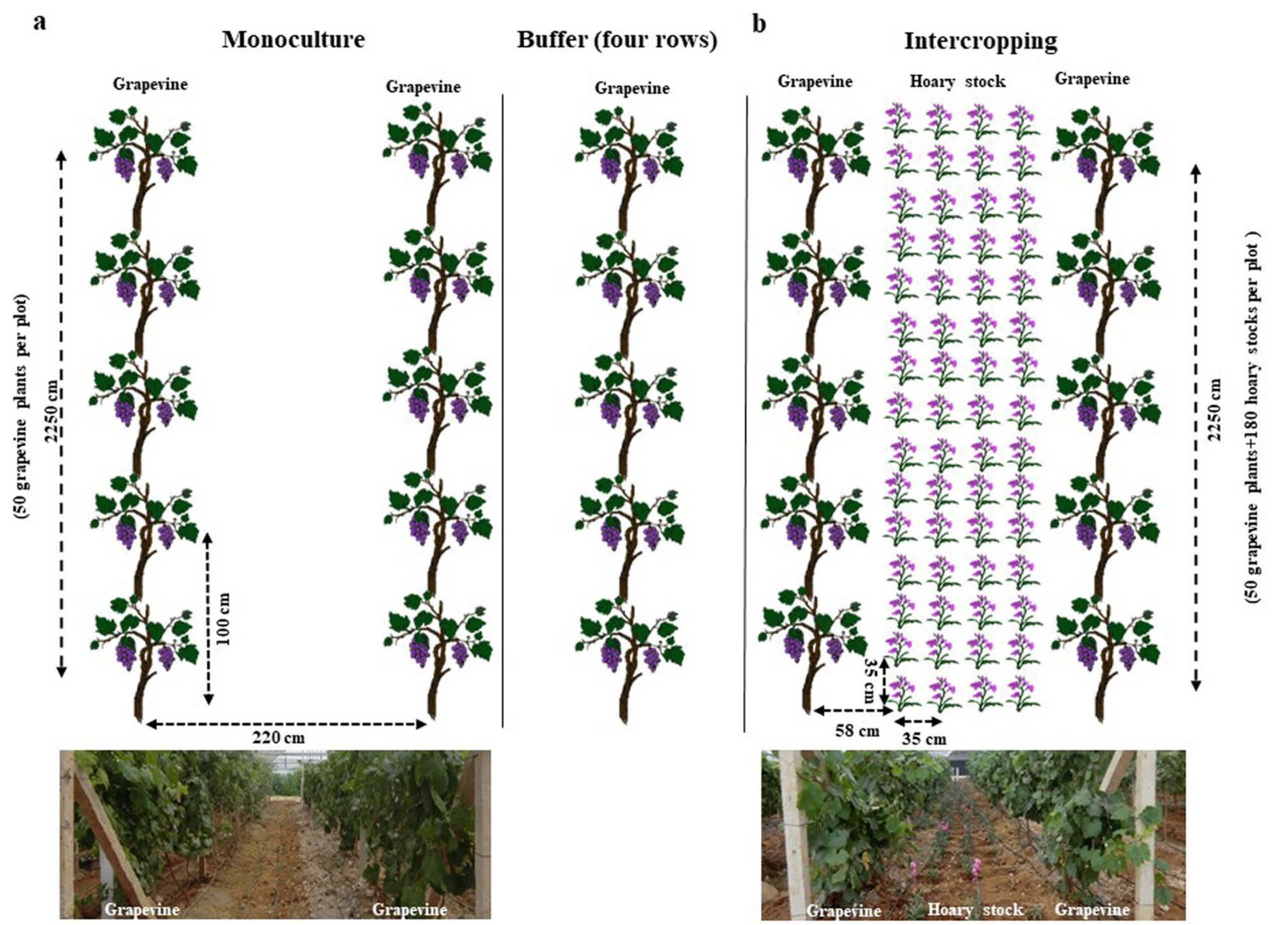


**Fig. 6** **a** Field experimental design of monoculture of grapevine and **b** intercropping system using grape 'Red Globe' and Hoary stock 'Stock Yume no Uta'

(Total number of evaluated leaves × Highest disease scale)] × 100. Logistic model was used to evaluate the dynamic changes of grape downy mildew in monoculture and intercropping base on the maximum disease index (a), the initial growth rate (k), the maximum instantaneous growth rate ($I_{max}$), and the time to reach the maximum growth rate ($t_{max}$). The specific methods were as follows: the disease indexes of the two cropping patterns were collated and fitted to a logistic equation using the least squares method (Formula 1), respectively (Andersen et al. 2007; Trinder et al. 2012). And the disease indexes growth curve was obtained according to Formula 1, the instantaneous growth rates were based on Formula 2 derived from Formula 1. In the Formula 1, Dt was the disease index on the t day during the entire growing season in the two cropping patterns, a was the maximum disease index, k was the initial growth rate, and $x_c$ was the time to reach the maximum instantaneous rate. According to the Formula 2, the instantaneous growth rate reached its peak at $D_t = a/2$, therefore, the maximum instantaneous growth rate ($I_{max}$) occurring at time $x_c$ ($t_{max} = x_c$) was k × a/4. All these parameters were estimated using the OriginPro 2021 software (OriginLab Corporation, Inc. Northampton, MA, USA) with the Slogistic 1 program.

$$D_t = \frac{a}{1 + e^{k*(x_c - t)}} \tag{1}$$

$$\frac{dD_t}{dt} = k * D_t * \left(1 - \frac{D_t}{a}\right) \tag{2}$$

#### Effect of VOCs from the aerial parts of hoary stock against grape downy mildew in the leaf disc assay

To determine whether hoary stock VOCs played a role in the development of grape downy mildew, a leaf disc inoculation test was designed in vitro. Healthy grape leaves (the fourth-sixth leaf starting from the apex of each shoot) were collected from Red Globe grapevines without using fungicides under rain-sheltered cultivation in other vineyards at Daheqiao farm, Xundian County, Yunnan. The leaves whose petioles were moisturized with absorbent cotton were cultivated in an incubator (20 ± 1°C, relative humidity ≥ 95%, a 12 h/12 h day/night cycle) for five days, and those without downy mildew symptoms were selected for subsequent leaf disc tests. As described by Lazazzara et al. (2018), leaf disc assay were performed in vitro in both years. In 2020, the grape leaves were punched to obtain leaf discs (25 mm diameter), and a total of 20 leaf discs were placed onto wet sterilized filter paper in each Petri dish (15 cm diameter), with the abaxial surface facing up. Aerial tissues from pre-and post-flowering hoary stock at 0, 1, 3, and 5 g were placed in tin-foil bowls and placed into a Petri dish. Simultaneously, leaf disc of grape in the Petri dishes were inoculated with a 20 μL sporangia suspension of *P. viticola* at $1.0 \times 10^6$ sporangia/mL. In 2021, the same test was performed, but the biomasses of hoary stock tissues were adjusted to 0.1, 0.5, 1, 2, 3, 4 and 5 g. All dishes were sealed with Parafilm and incubated at 20 ± 1°C under a 12 h/12 h day/night cycle. Three replicates were arranged for each treatment. Disease severity was assessed at six days post-inoculation using a 0–5 scale, and disease indexes and inhibition rates were calculated. Inhibition rate (%) = [(Disease index of control−Disease index of treatment)/Disease index of control] × 100. The inhibitory effects of pre- and post-blooming hoary stock tissues were compared.

#### VOCs profile from hoary stock aerial parts

To determine the active compounds in hoary stock VOCs, spontaneously emitted VOCs from the aerial parts of pre- and post-blooming hoary stock plants were collected according to a "push–pull" system previously described by Berhal et al. (2017). The constituents of the VOCs were identified using gas chromatography–mass spectrometry (GC–MS). Hoary stock roots were wrapped with tin foil, and the entire plants were placed in closed glass desiccators (40 cm in diameter, 25 cm in height) (Additional file 2: Figure S1a). Air was pulled through charcoal active powder (AR, Suzhou Xinqing Technology Co., Ltd., Suzhou, China) and silica gel (AR, Tianjin Kermel Chemical Reagent CO., Ltd., Tianjin) with an atmospheric sampling pump (QC-1B, Beijing Municipal Institute of Labor Protection, Beijing, China) at a constant airflow of 400 ~ 600 mL/min. A trap filter made of a glass cartridge filled with 100 mg of adsorbent material (Porapak™ Q 80–100 mesh, Waters, USA) was placed at the exit of the glass desiccator to catch the volatile compounds. The filters were previously washed with acetone (purity of 99.9%, Thermo Fisher Technology Co., Ltd. in China, CG-MS grade) followed by n-hexane (purity of 99.9%, Thermo Fisher Technology Co., Ltd. in China, CG-MS grade) until no substances were detected with GC (Additional file 2: Figure S1b). All parts were connected by a soft Teflon tube. Three replicates were conducted simultaneously, and a desiccator containing only tinfoil was used as a control for the background correction of the VOCs. The trap filters were removed after three days, desorbed by 5 mL n-hexane and concentrated to 2 mL under a nitrogen stream (purity of 97%). The collected VOC samples were sent to the Kunming Institute of Botany, Chinese Academy of Sciences, for analysis by gas chromatography–mass spectrometry (GC–MS) (HP6890GC/5973MS, Agilent, USA). Mass spectra of the components were compared with those in the Wiley 7n.1

Mass Spectrometry Library. Components that appeared simultaneously in three replicates of collection with a confidence rate greater than 70% were regarded as the main volatile monomers, and the monomers were compared to find the same and unique components in pre- and post-blooming hoary stock plants.

### Inhibitory effects of VOCs on *P. viticola* infection on grape leaf discs

The same and unique components identified above were selected to test the individual activity against *P. viticola* symptom on leaf discs at different dosages in air by using corresponding pure standards (Shanghai Aladdin Bio-Chem Technology Co., Ltd., Shanghai, China). The component selection was based on the availability of standards, and the relative contents covered the highest, medium, and lowest content gradients in their respective groups.

Healthy grape leaves were punched to obtain leaf discs (25 mm diameter), and a total of ten leaf discs were placed onto wet sterilized filter paper in Petri dishes (9 cm diameter), with the abaxial surfaces uppermost. Each leaf disc was inoculated with a 20 μL sporangia suspension of *P. viticola* ($1.0 \times 10^6$ sporangia/mL). The gradient concentrations (0.5, 1, 2, 4, 6, 8, 10, 15, and 20 mg/L) of VOCs were set based on the estimated accumulation concentrations of hoary stock VOCs during intercropping of grapevine and hoary stock in the field. Then, a sterilized centrifuge tube lid with the respective pure compounds was placed in the Petri dishes, corresponding to concentrations of 0, 0.5, 1, 2, 4, 6, 8, 10, 15, and 20 mg/L in air (mass of pure standard/volume of Petri dish), calculated assuming complete VOC evaporation from the lid. To exclude the toxic effect of VOCs on grape leaves, leaf discs that were treated with the same amounts of the pure compounds in the sterilized centrifuge tube lid were inoculated with 20 μL sterilized water. All dishes were sealed with Parafilm and incubated at 20 ± 1°C under a 12 h/12 h day/night cycle. Three replicates (ten disks of each replicate) were assessed for each treatment, and disease severity was assessed at six days post inoculation using a 0–5 scale as described earlier. The disease indexes and inhibition rates were calculated according to the above method. The effective concentration 50% ($EC_{50}$), at which 50% of the maximum effect was achieved, was used to determine the overall inhibitory activity of the pure VOCs. The $EC_{50}$ value was calculated as follows. The inhibition rates were converted to probability values, and the concentrations of the individual compounds were converted to log base 10 (lg). Taking the probability values of the inhibition rate as the Y-axis and the log base 10 of the concentration as the X-axis, the toxicity regression equation was established, and the $EC_{50}$ was calculated according to the regression equation. The inhibitory activity of the compounds against grape downy mildew was classified into four levels as follows: $EC_{50} \le 1$ mg/L; 1 mg/L $< EC_{50} \le 5$ mg/L; 5 mg/L $< EC_{50} \le 50$ mg/L, and $EC_{50} > 50$ mg/L (Avenot et al. 2020).

### Statistical analysis

Data were analyzed with SPSS version 18.0 software (SPSS Inc., Chicago, Illinois, USA). An independent-samples *t* test was used to determine if the grape downy mildew indexes differed significantly ($P < 0.05$) between intercropping and monoculture field designs and if the disease indexes and inhibition rates differed significantly ($P < 0.05$) between pre- and post-blooming hoary stock VOCs in vitro. Mean separation of treatments was analyzed by one-way analysis of variance (ANOVA) and Tukey's test ($P < 0.05$). Heatmaps were drawn with GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA).

#### Abbreviations

| | |
|---|---|
| VOCs | Volatile organic compounds |
| GC–MS | Gas chromatography-mass spectrometry |
| $EC_{50}$ | Effective concentration for 50% inhibition |
| lg | Log base 10 |
| SEs | Standard errors |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-024-00291-4.

**Additional file 1: Table S1.** Main chemical compounds identified in volatile organic compounds of post-flowering hoary stock. **Table S2.** Main chemical compounds identified in volatile organic compounds of pre-flowering hoary stock. **Table S3.** Inhibitory effect of 23 compounds in volatile organic compounds of hoary stock on *Plasmopara viticola*in grapevine leaf discs.

**Additional file 2: Figure S1.** Collection and elution of volatile organic compounds (VOCs) of hoary stock by a "push-pull" system. **a** Collecting hoary stock VOCs in closed glass desiccators. **b** Eluting hoary stock VOCs from filters with n-hexane.

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#### Author contributions

WD and FD performed the experiments, analyzed the data and wrote the manuscript. SZ conceived the study, coordinated the experiments and revised the manuscript. RY and HY analyzed the data. MY and XM contributed to the chemical analysis. CY supervised the experiments. SL drew the field experiment diagram. YL and HH revised the manuscript. All authors read and approved the paper before submission.

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**Availability of data and materials**
All data generated or analysed during this study are included in this published article [and its supplementary information files].

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

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