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Article

Chemical profiles of *Eucalyptus* essential oils associated with genotypic resistance to *Leptocybe invasa*

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Abstract - *Eucalyptus* species, essential to Brazil's forestry and renewable energy sectors, suffer significant losses caused by the gall wasp *Leptocybe invasa*, which impairs tree growth, productivity, and quality. In response to the need for sustainable strategies, this study investigated the chemical composition of essential oils (EOs) from eleven *Eucalyptus* genotypes previously classified as resistant or susceptible to the pest, aiming to identify compounds associated with these phenotypes. Using gas chromatography coupled with mass spectrometry (GC/MS), 92 compounds were identified, including monoterpenes and sesquiterpenes, some of which occurred exclusively in resistant or susceptible genotypes. Compounds such as 4-octene and eucalyptol were correlated with resistance and susceptibility, respectively. Although the direct effect of these compounds on *L. invasa* was not assessed, the results suggest that EO chemical profiles may serve as auxiliary markers in genetic selection programs. This study therefore offers a complementary approach within the framework of forest breeding for pest resistance and recommends future research to confirm the functional relationship between these compounds and plant responses to infestation.

Perfis químicos de óleos essenciais de *Eucalyptus* associados à resistência genotípica a *Leptocybe invasa*

Resumo - Espécies de *Eucalyptus*, essenciais para o setor florestal e de energia renovável do Brasil, enfrentam prejuízos significativos causados pela vespa-da-galha, *Leptocybe invasa*, que compromete o crescimento, a produtividade e a qualidade das árvores. Diante da necessidade de estratégias sustentáveis, este estudo investigou a composição química dos óleos essenciais (OE) de onze genótipos de Eucalyptus, previamente classificados como resistentes ou suscetíveis à praga, com o objetivo de identificar compostos químicos associados a esses fenótipos. Utilizando a técnica de cromatografia gasosa acoplada à espectrometria de massas (GC/MS), foram identificados 92 compostos, entre monoterpenos e sesquiterpenos, sendo que alguns ocorreram exclusivamente em genótipos resistentes ou suscetíveis. Compostos como 4-Octeno e Eucaliptol apresentaram correlações com resistência e suscetibilidade, respectivamente. Embora não se tenha avaliado diretamente o efeito desses compostos sobre *L. invasa*, os resultados sugerem que o perfil químico dos OE pode contribuir como marcador auxiliar em programas de seleção genética. Assim, esta pesquisa oferece uma abordagem complementar no contexto do melhoramento florestal voltado à resistência a pragas, recomendando-se estudos futuros que confirmem a relação funcional entre esses compostos e a resposta da planta à infestação.

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Introduction

The genus *Eucalyptus* comprises fast-growing tree species widely used in forestry and energy sectors. In Brazil, *Eucalyptus* plantations cover approximately 7.53 million hectares, accounting for 75.8% of the country's total planted forest area, particularly in the states of Minas Gerais, São Paulo, and Mato Grosso do Sul (Indústria Brasileira de Árvores, 2023). Beyond pulp, timber, and charcoal production, these species are used for bioenergy generation and essential oil extraction, playing a relevant role in Brazil's renewable energy matrix and contributing to the mitigation of greenhouse gas emissions (Ignacio et al., 2019; Empresa de Pesquisa Energética, 2023).

Despite their economic and ecological importance, *Eucalyptus* plantations face significant phytosanitary challenges. The eucalypt gall wasp (*Leptocybe invasa*) is an emerging and highly damaging pest that induces gall formation on young leaves and shoots, impairing growth, canopy architecture, and overall productivity (Wilcken et al., 2015). The severity of the damage demands the development of sustainable management strategies, especially considering the environmental and economic limitations of continued reliance on synthetic insecticides.

In this context, essential oils (EOs) produced in *Eucalyptus* leaves have drawn increasing attention as potential auxiliary tools for pest management. Compounds such as 1,8-cineole, α-terpineol, pinene, and various sesquiterpenes exhibit insecticidal, repellent, and behavioral-modulating properties, and are known to participate in the plant's chemical defense system (Dhakad et al., 2017; Zhou & Jander, 2021; Gupta et al., 2023). These EOs are synthesized and stored in specialized secretory glands distributed throughout the foliage, and their composition may vary significantly across species, populations, and genotypes (Keszei et al., 2008; Yong et al., 2019).

Recent studies have shown that EO chemical profiles may serve as markers of disease resistance, as demonstrated in *Eucalyptus* species challenged by myrtle rust (*Austropuccinia psidii*), where compounds discriminating among resistant, hypersensitive, and susceptible phenotypes have been identified (Hantao et al., 2013; Yong et al., 2019). Although the direct functional role of these compounds is not fully understood, the evidence supports their potential use as biochemical markers in forest breeding programs, either as functional indicators or traits genetically correlated with resistance (Silva et al., 2020). This approach offers promising advantages for accelerating genotype selection, reducing field-testing costs, and strengthening integrated pest management (IPM) strategies.

However, the use of EO profiles as biochemical markers remains underexplored in the context of insect pest resistance in *Eucalyptus*, particularly with regard to *L. invasa*. The scientific literature lacks studies examining the association between specific EO compounds and resistance phenotypes with direct applicability to breeding or marker-assisted selection.

To address this gap, the present study investigated the EO composition of eleven *Eucalyptus* genotypes previously classified as resistant or susceptible to *L. invasa*, aiming to identify chemical compounds potentially associated with resistance. The central hypothesis is that certain compounds, present exclusively or at higher concentrations in resistant genotypes, may serve as biochemical markers in breeding programs targeting gall wasp resistance. By proposing this approach, the research contributes an additional tool for pest management, grounded in plant genetics and chemical ecology.

Material and methods

Study locations and sample collection

The study was carried out with samples from *Eucalyptus* plantations in Vila Nova dos Martírios, Maranhão State, Brazil. According to Correia Filho et al. (2011), Vila Nova dos Martírios is situated at an elevation of 307 meters with annual temperature variations from 21.9 °C to 32.1 °C. The Köppen climate classification is tropical (Aw), characterized by a wet season from November to May and a dry season from June to October, with annual rainfall averaging around 1,729 mm.

Essential oil extraction was conducted in Vila Nova dos Martírios, with all necessary materials, equipment, and glassware transported to the site for on-site extraction.

Eucalyptus genotypes analyzed

Eleven *Eucalyptus* genotypes resulting from specific crossbreeds were selected based on their contrasting phenotypes regarding resistance or susceptibility to *Leptocybe invasa*. This classification was carried out by trained professionals from the partner forestry company, based on long-term field monitoring and visual assessment of gall damage under natural infestation. The classification follows an internal rating scale established by the company, which integrates multiple phenotypic traits of commercial relevance, such as intensity and frequency of gall formation and overall plant vigor. While the scale itself is proprietary and not publicly disclosed, the selection in this study considered only genotypes with consistently distinct resistance levels, as previously validated under field conditions (Table 1).

Table 1. *Eucalyptus* genotypes used for essential oil extraction from leaves, Vila Nova dos Martírios, Maranhão State, Brazil.

Genotype	Wasp Phenotype	Crossing
C010	Resistant	*Eucalyptus grandis* x *Eucalyptus urophylla*
C011	Resistant	NA
C012	Resistant	NA
C013	Resistant	NA
C014	Resistant	*Eucalyptus urophylla*
C015	Resistant	*Eucalyptus brassiana* x *Eucalyptus grandis*
C016	Susceptible	*Eucalyptus urophylla* x *Eucalyptus grandis*
C017	Susceptible	NA
C018	Susceptible	*Eucalyptus grandis* x *Eucalyptus urophylla*
C019	Susceptible	*Eucalyptus grandis* x *Eucalyptus urophylla*
C020	Susceptible	NA

Genotype – genotype from which leaves were used for essential oil extraction. Wasp phenotype – indicates the classification of the genotype regarding resistance or susceptibility according to company criteria. Crossing – crossbreeding that originated the genotype. NA – not available.

Essential oil extraction from leaves

Leaves from three trees per selected genotype (Table 1) were harvested during the active flushing stage, which corresponds to young, expanding leaves and shoots. This is the phenological stage known to be most susceptible to gall induction by *Leptocybe invasa*, as the insect oviposits in apical meristems and induces gall formation in developing tissues (Wilcken et al., 2015). Felling, collection, and transportation of the material to the extraction facility were carried out by the partner company's technical team.

Leaves were transported in insulated containers with ice and, upon arrival, were finely sliced to facilitate oil release. Essential oil extraction was conducted by hydrodistillation using 2-liter round-bottom flasks for 1 to 2 hours per sample. The oils were stored in 2 mL screw-cap glass vials and kept refrigerated at 4 °C to ensure stability and prevent degradation of volatile compounds. The procedure followed established standards for essential oil extraction and conservation (Adams, 2017).

Chemical constituent identification in essential oil samples

Chemical analysis of the essential oil samples was performed using gas chromatography-mass spectrometry (GC/MS) on a Shimadzu GCMS-QP2020 system. The system was equipped with a DB-5MS capillary column (30 m x 0.25 mm x 0.25 µm). The operational conditions were as follows: interface temperature at 280 °C, ion source temperature at 280 °C, column initial temperature held at 50 °C for 1 minute, then ramped to 280 °C at 3 °C min^{-1}, and maintained for 20 minutes. Each 1 µL sample was injected in split mode, with helium as the carrier gas at a pressure of 15.6 psi, achieving a linear velocity of 48.9 cm s^{-1}, a purge flow of 3.0 mL min^{-1}, a total flow of 13.9 mL min^{-1}, and a column flow of 1.82 mL min^{-1}.

Identification of constituents was based on mass spectral comparison with the National Institute of Standards and Technology (NIST 14) database. Relative content calculations were performed by peak area integration, representing the percentage of each compound relative to the total identified compounds, focusing on the 20 most prominent peaks per sample.

R analysis for essential oil compound identification

Our analytical approach employed a comprehensive R script (R Core Team, 2023) to investigate the chemical profiles of essential oils from diverse *Eucalyptus* genotypes. We commenced our analysis by importing the dataset with the read.table() function, followed by a meticulous selection of relevant columns corresponding to compounds and genotypes, facilitated by the dplyr package. The tidyr package was instrumental in reshaping the data, which allowed us to pinpoint unique compounds inherent to each genotype. Through this data manipulation, we aimed to identify potential chemical markers indicative of either resistance or susceptibility to the gall wasp infestation.

Subsequently, for each genotype, the script pinpointed compounds exclusively present in resistant or susceptible genotypes, as well as those exhibiting differential abundance between the two groups. This selective identification is crucial for unraveling the biochemical underpinnings of the observed phenotypic resistance or susceptibility.

In our methodology, statistical tests were not employed, reflecting the preliminary nature of this investigation. Instead, the R script was meticulously configured to assess and juxtapose the average abundance of compounds prevalent in genotypes.

For the graphical representation of our findings, we utilized the ggplot2 package to create box plots that demonstrate the variability and mean distribution of compounds among genotypes. These visualizations offer a compelling narrative of the data, revealing discernible patterns that are crucial for subsequent interpretation and discussion.

In the concluding phase, our script compiled a collection of the most enlightening graphs, providing a unified perspective on the behavioral patterns of the compounds in relation to the resistance or susceptibility of genotypes to the gall wasp. This compilation of visual data serves as a comprehensive and integrated representation of our analytical insights, ready to steer future investigations into the innate pest resistance mechanisms within Eucalyptus genotypes.

Results

We observed that certain compounds were consistently more abundant in genotypes identified as resistant, implying their potential role in conferring defense against the gall wasp. Conversely, a distinct set of compounds exhibited elevated mean abundance in genotypes deemed susceptible, shedding light on their potential association with increased vulnerability to the pest. These findings highlight the dualistic nature of chemical profiles in imparting either resistance or susceptibility traits to the *Eucalyptus* genotypes, thereby providing a clearer understanding of the chemical ecology involved in pest interactions.

Chemical composition of essential oil extracted from leaves

Gas chromatography-mass spectrometry (GC/MS) analysis revealed a variety of compounds in *Eucalyptus* genotypes resistant and susceptible to gall wasp. In total, 92 different compounds were identified. Of these, 24 compounds were present only in resistant genotypes, 18 only in susceptible genotypes. The majority, 26 compounds, are monoterpenes, with two sesquiterpenes and the rest belonging to other groups.

While most genotypes displayed all 20 detectable compounds, genotypes C015 and C017 showed between 11 and 13 compounds. Compounds “(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene”, “o-Cymene”, “D-Limonene”, and “Eucalyptol” were found in all 11 genotypes analyzed.

Although the less abundant compounds vary between genotypes, their significance for the study requires further investigation. Some of these compounds may have bioactive properties contributing to the resistance or susceptibility of the genotypes.

Exclusive compounds by genotype

It is presented in Table 2 unique compounds for each genotype. These exclusive compounds are particularly important for understanding the mechanisms of resistance and susceptibility, and therefore, deserve attention in future studies. It is noteworthy that genotypes C018 and C013 do not possess exclusive compounds. Conversely, genotype C016 has a remarkable eight exclusive compounds. The lineage of these genotypes could provide significant insights into the potential causes of such variations, offering clues to the genetic lineage and potential resistance traits against gall wasp.

Exclusive compounds by resistance phenotype

An analysis of exclusive compounds found in each of the two phenotypes, resistant and susceptible, to the gall wasp is presented in Table 3. The resistant group exhibited a greater diversity of exclusive compounds, with 24 identified monoterpenes suggesting a significant role in the genotypes' resistance to the gall wasp. On the other hand, the susceptible group had 18 exclusive monoterpenes, indicating potentially higher susceptibility to infestations and serving as a starting point for future research.

Notably, some compounds, such as "4-Octene, 2,2,3,7-tetramethyl-, [S-(E)]-" and "3-Ethyl-2-methyl-1-heptene," were exclusive to the resistant group as well as genotype C014, while "Bicyclo[2.2.1] heptane, 2,2-dimethyl-5-methylene-" was exclusive to genotype C019 and the susceptible group (Tables 2 and 3).

Table 2. Exclusive compounds of essential oil of each *Eucalyptus* genotype analyzed by gas chromatography-mass spectrometry technique.

Genotype	Wasp phenotype	Compound
C010	Resistant	3-Hexen-1-ol, acetate, (E)- Bicyclo[3.1.0]hex-2-ene, 4,4,6,6-tetramethyl-
C011	Resistant	1,4-Hexadiene, 5-methyl-3-(1-methylethylidene)- 2,4,6-Octatriene, 2,6-dimethyl- Butanoic acid, 3-hexenyl ester, (Z)- Butanoic acid, 3-hexenyl ester, (E)- (3R,6R)-3-Hydroperoxy-3-methyl-6-(prop-1-en-2-yl)cyclohex-1
C012	Resistant	1-Propanamine, 2-methyl-N-(2-methylpropylidene)- Ethanone, 1-cyclopropyl-2-(4-pyridinyl)- Carveol 2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate-23,9 (E)-.beta.-Famesene 5-Ethyl-2-furaldehyde
C013	Resistant	
C014	Resistant	4-Octene, 2,2,3,7-tetramethyl-, [S-(E)]- Spiro[4.5]dec-1-ene 3-Ethyl-2-methyl-1-heptene 2-Octen-4-one
C015	Resistant	Cyclopentane, 1,2,3,4,5-pentamethyl- L-.alpha.-Terpineol Propanoic acid, 2-methyl-, 2-methylbutyl ester
C016	Susceptible	2,6-Dodecadiene, 2,6-dimethyl- Cyclopentanone, 2-(3-methyl-2-buten-1-yl)- 2,6,10-Dodecatrienal, 3,7,11-trimethyl-, (E,E)- 2-Cyclohexen-1-one, 2-methyl-5-(1-methylethyl)-, (S)- Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-meth Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl- (1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6 Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyle
C017	Susceptible	2-Nonen-4-one
C018	Susceptible	
C019	Susceptible	Bicyclo[2.2.1]heptane, 2,2-dimethyl-5-methylene- Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, acetate, (1S-exo)-
C020	Susceptible	Cis-Linaloloxide-12,7 cis-p-mentha-1(7),8-dien-2-ol Benzene, (2-methyl-1-propenyl)- Pinocarvone

Table 3. Exclusive compounds present in the essential oil extracted from *Eucalyptus* leaves genotypes.

Comp_Phenotype_Wasp_R	Comp_Phenotype_Wasp_S
4-Octene, 2,2,3,7-tetramethyl-, [S-(E)]-	Bicyclo[2.2.1]heptane, 2,2-dimethyl-5-methylene-
3-Ethyl-2-methyl-1-heptene	(+)-4-Carene-9,4
1-Propanamine, 2-methyl-N-(2-methylpropylidene)-	Cis-Linaloloxide-12,7
Cyclopentane, 1,2,3,4,5-pentamethyl-	Benzene, (2-methyl-1-propenyl)-
Bicyclo[2.2.1]heptane, 7,7-dimethyl-2-methylene-	Pinocarvone
Spiro[4.5]dec-1-ene	2-Nonen-4-one
Ethanone, 1-cyclopropyl-2-(4-pyridinyl)-	2,6-Dodecadiene, 2,6-dimethyl-
4-Hexen-1-ol, acetate	Cyclopentanone, 2-(3-methyl-2-buten-1-yl)-
3-Hexen-1-ol, acetate, (E)-	2,6,10-Dodecatrienal, 3,7,11-trimethyl-, (E,E)-
Propanoic acid, 2-methyl-, 2-methylbutyl ester	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, acetate, (1S-exo)-
1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	cis-p-mentha-1(7),8-dien-2-ol
1,4-Hexadiene, 5-methyl-3-(1-methylethylidene)-	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethyl)-, (S)-
Bicyclo[3.1.0]hex-2-ene, 4,4,6,6-tetramethyl-	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-
2,4,6-Octatriene, 2,6-dimethyl-	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-meth
endo-Borneol	Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl-
2-Octen-4-one	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R
Butanoic acid, 3-hexenyl ester, (Z)-	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6
Butanoic acid, 3-hexenyl ester, (E)-	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyle
(3R,6R)-3-Hydroperoxy-3-methyl-6-(prop-1-en-2-yl) cyclohex-1	
L-.alpha.-Terpineol	
Carveol	
2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate-23,9	
(E)-.beta.-Famesene	
5-Ethyl-2-furaldehyde	

Comp_Phenotype_Wasp_R = resistant group, Comp_Phenotype_Wasp_S = susceptible group.

Given the company's robust forest genetic improvement program, it is reasonable to assume that the process of selecting genotypes, directly or indirectly, already favors resistance, explaining the higher number of exclusive compounds in the resistant group.

However, it's important to exercise caution as some compounds were detected in only a single plant, requiring further analysis to either corroborate these findings or disregard them. Since compounds with attractant or repellent/toxic properties to the insect might be present in very low doses in the plant, their presence should not be dismissed without certainty of their significance. All compounds were selected regardless of their presence in one or more replications due to the preliminary nature of this study and its considerable relevance to the environmental sustainability of silviculture.

While numerous studies investigate the use of essential oils from various plant species, including *Eucalyptus*, for agricultural pest control, relatively few have focused on the use of leaf-derived essential oils for pest management within *Eucalyptus* plantations. Moreover, only a limited number of studies have explored the potential application of essential oil composition as a phenotypic or biochemical marker for genetic selection in forest breeding programs, particularly in relation to insect resistance. Moreover, as the presence in only one plant may be an environmental effect, the absence in other plants may also result from environmental factors. Therefore, future research may determine whether such differences are due to genetic effects, plant-pest interactions, or environmental factors. However, with caution, certain compounds will be highlighted, considering the criterion "presence in more than one plant and/or genotype". Thus, compounds such as "Bicyclo[2.2.1]heptane, 7,7-dimethyl-2-methylene-" (C010, C012), "4-Hexen-1-ol, acetate" (C011, C015), "Propanoic acid, 2-methyl-, 2-methylbutyl ester" (C015), "1,3,6-Octatriene, 3,7-dimethyl-, (Z)-" (C010, C011), "endo-Borneol" (C014, C013, C010, C012), and "2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate-23,9" (C012) warrant special attention (Figure 1). Applying the same criterion as above, compounds such as "(+)-4-Carene-9,4" (C018, C019), "Cis-Linaloloxide-12,7" (C020), "2-Nonen-4-one" (C017), "cis-p-mentha-1(7),8-dien-2-ol" (C020), "2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-" (C016, C020), and "Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R" (C019, C020) are highlighted (Figure 2).

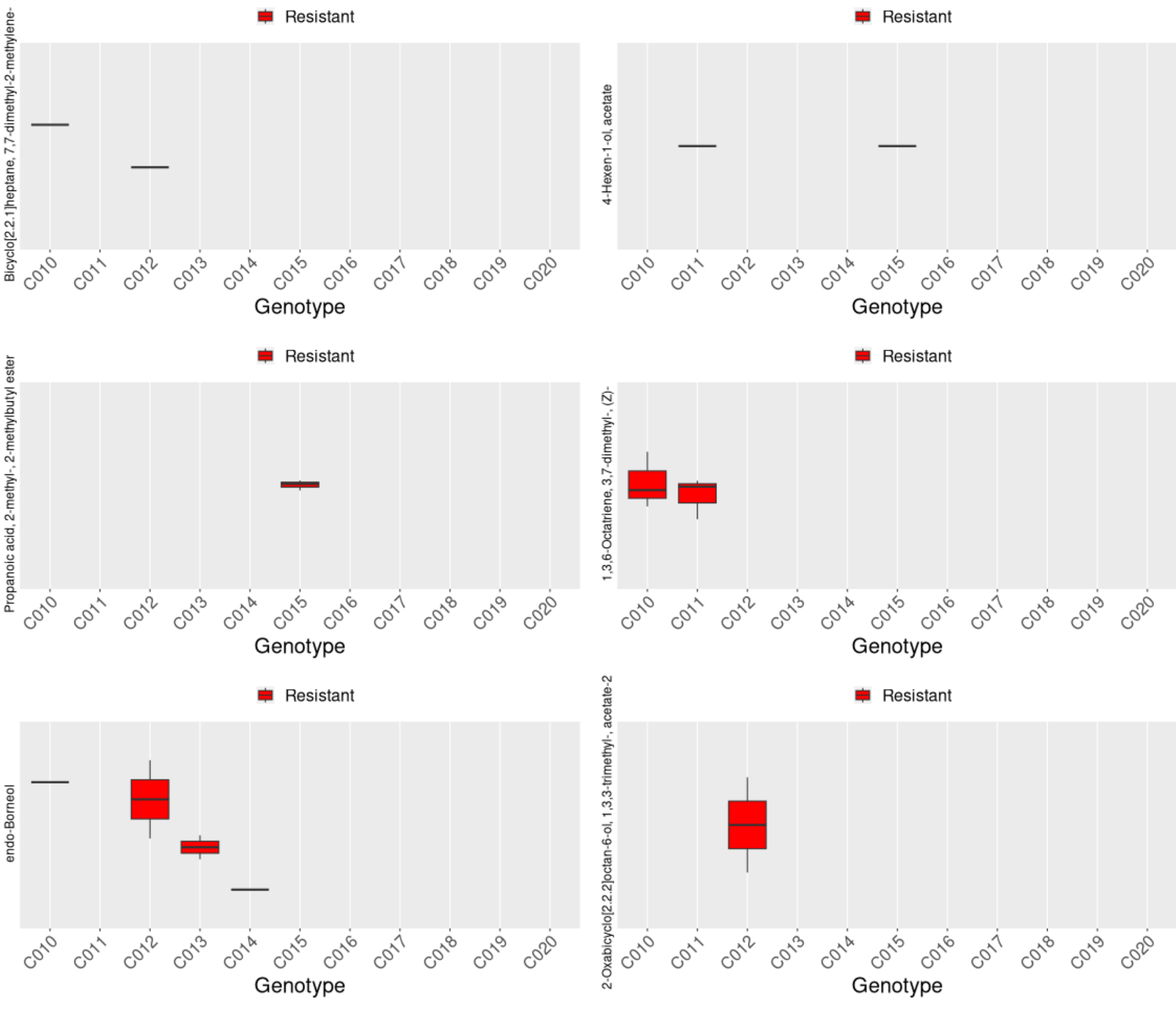

Figure 1. Relative abundance (based on gas chromatography-mass spectrometry area percentage) of six volatile organic compounds identified exclusively in resistant *Eucalyptus* genotypes to *Leptocybe invasa*. Boxplots represent the distribution of compound abundance across different genotypes, based on measurements from three individual trees per genotype. The y-axis indicates the relative abundance (%) of each compound, and the x-axis denotes genotype identity. Variation in box size reflects differences among individual trees; when all individuals share the same value, the boxplot appears as a horizontal line. Resistance status is color-coded (red = resistant). From top to bottom and left to right, the following compounds are shown: "Bicyclo[2.2.1]heptane, 7,7-dimethyl-2-methylene-", "4-Hexen-1-ol, acetate", "Propanoic acid, 2-methyl-, 2-methylbutyl ester", "1,3,6-Octatriene, 3,7-dimethyl-, (Z)-", "endo-Borneol" and "2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate-23,9".

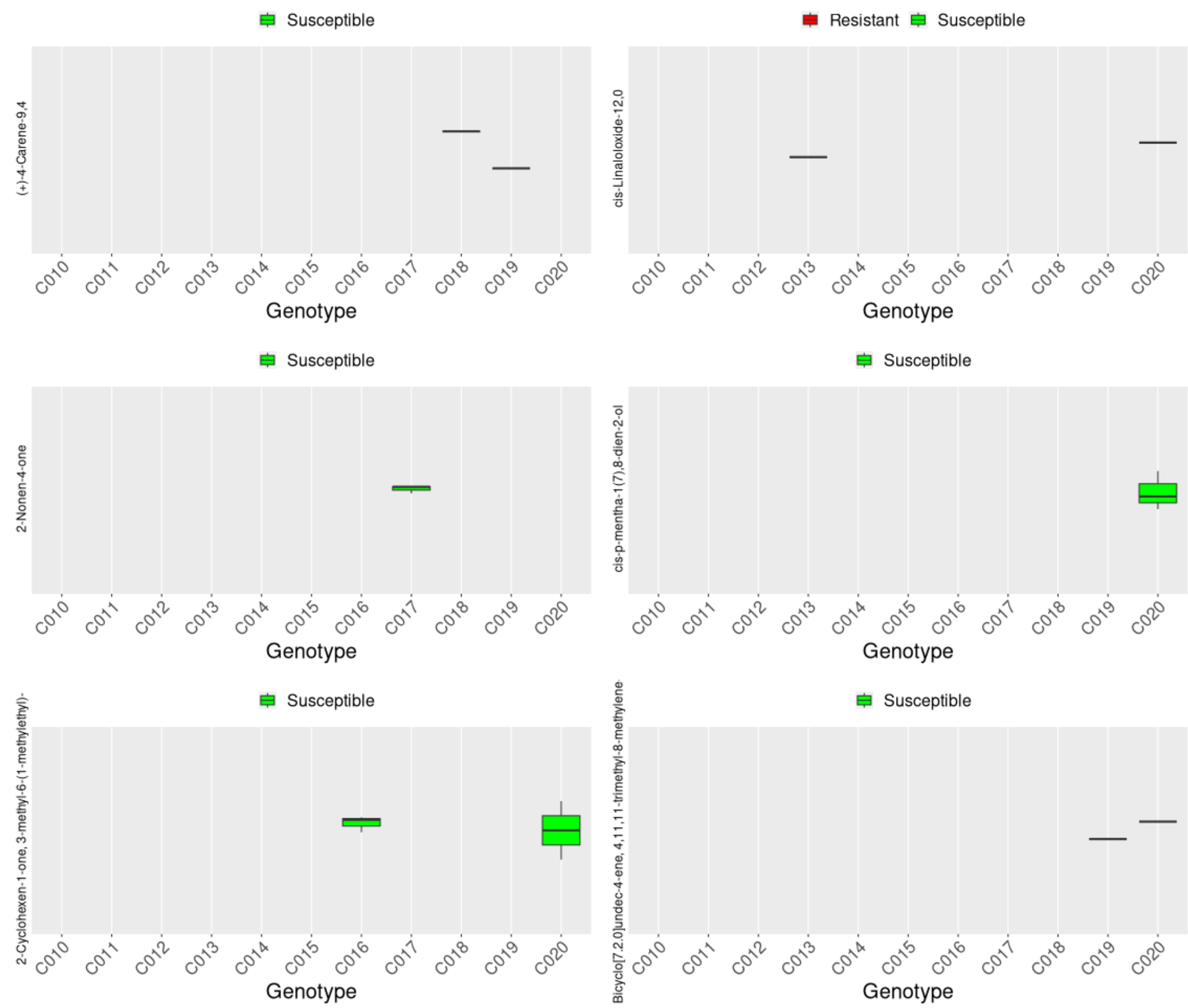

Figure 2. Relative abundance (based on gas chromatography-mass spectrometry area percentage) of six volatile organic compounds identified exclusively in susceptible *Eucalyptus* genotypes to *Leptocybe invasa*. Boxplots represent the distribution of compound abundance across different genotypes, based on measurements from three individual trees per genotype. The y-axis indicates the relative abundance (%) of each compound, and the x-axis denotes genotype identity. Variation in box size reflects differences among individual trees; when all individuals share the same value, the boxplot appears as a horizontal line. Resistance status is color-coded (green = susceptible). From top to bottom and left to right, the following compounds are shown: “(+)-4-Carene-9,4”, “Cis-Linaloloxide-12,7”, “2-Nonen-4-one”, “cis-p-mentha-1(7),8-dien-2-ol”, “2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-” and “Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R”.

This analysis of exclusive compounds can offer valuable insights into the biochemical mechanisms underlying the resistance and susceptibility of *Eucalyptus* genotypes to this pest. These findings should be considered in future studies to develop more effective management strategies.

The cross-referencing of data from Tables 2 and 3 reveals that two compounds exclusive to genotype C014, namely “4-Octene, 2,2,3,7-tetramethyl-, [S-(E)]-” and “3-Ethyl-2-methyl-1-heptene”, are also exclusive to the resistant group. In contrast, the compound “Bicyclo[2.2.1]heptane, 2,2-dimethyl-5-methylene-”, exclusive to genotype C019, is also exclusive to the susceptible group. No other compounds with these characteristics were found.

Relative abundance of compounds by resistance phenotype

This analysis revealed 32 compounds with higher mean relative abundance in resistant *Eucalyptus* genotypes compared to susceptible ones (Table 4), and 17 compounds showing the opposite pattern, with greater abundance in susceptible genotypes (Table 5).

Particularly noteworthy is the compound "(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene," which is not only present in all 11 genotypes but also shows greater abundance in resistant genotypes. This compound may be a potential indicator of resistance and warrants further investigation.

Among the most abundant compounds in susceptible genotypes, "Eucalyptol" stands out. Although this compound is present in all genotypes analyzed, its relative abundance is significantly higher in susceptible genotypes, which may suggest an important role in their susceptibility to gall wasp.

It is crucial to note that the relative abundance of a compound does not necessarily indicate its efficacy in conferring resistance or susceptibility. However, the presence in high concentrations may imply a greater biological relevance, justifying future studies to elucidate its role in defense mechanisms or vulnerability to pests.

It is listed in Tables 4 and 5 the most abundant compounds in each phenotypic group, highlighting those with a difference in abundance between resistant and susceptible genotypes.

Table 4. Volatile organic compounds with higher mean relative abundance in resistant Eucalyptus genotypes to *Leptocybe invasa*. Values are expressed as mean percentages of gas chromatography-mass spectrometry peak area (relative abundance).

Compound	Resistant	Susceptible
Propanoic acid, 2-methyl-, 2-methylpropyl ester	0.1875	0.0700
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	20.9578	15.3747
Camphene	0.5318	0.4020
Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S)-	0.5267	0.3000
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	0.0950	0.0600
Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	8.2411	0.2567
.beta.-Myrcene	0.5733	0.1550
3-Hexen-1-ol, acetate, (Z)-	0.6600	0.3700
trans-.beta.-Ocimene-10,6	1.7173	0.2715
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	0.7350	0.1300
(+)-4-Carene-12,6	0.2567	0.2100
3-Oxatricyclo[4.1.1.0(2,4)]octane, 2,7,7-trimethyl-	0.1125	0.0700
Fenchol	1.5367	0.6814
(1,2,2-trimethyl-3-cyclopenten-1-yl)acetaldehyde	0.1300	0.1267
.alpha.-Campholenal	0.4775	0.3733
2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	0.6125	0.0800
Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1.al	0.6709	0.5100
Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-	0.2200	0.1767
Bicyclo[2.2.1]heptan-2-ol, 2,3,3-trimethyl-	0.2460	0.1600
Cyclohexene, 3-methyl-6-(1-methylethylidene)-	0.7500	0.2533
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	2.1900	1.3000
2-Isopropenyl-5-methylhex-4-enal	0.7525	0.5550
Citral	0.7250	0.7167
Terpinen-4-ol	0.6150	0.2567
.alpha.-Terpineol	6.1065	3.2360
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	0.4250	0.1650
Bornyl acetate	0.9150	0.3000
.alpha.-Terpinyl acetate	5.3215	3.0031
Caryophyllene	2.1700	1.6500
Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-	1.5833	0.8433
5-Hydroxy-2,2,6,6-tetramethyl-4-propionylcyclohex-4-ene-1,3-d-28,7	0.6700	0.1300
Humulene	0.4450	0.1600

Table 5. Volatile organic compounds with higher mean relative abundance in susceptible *Eucalyptus* genotypes to *Leptocybe invasa*. Values are expressed as mean percentages of gas chromatography-mass spectrometry peak area (relative abundance).

Compound	Resistant	Susceptible
3-Pentanone, 2,4-dimethyl-	0.0467	0.0900
Propanoic acid, 2-methyl-, 3-methylbutyl ester	0.1750	0.3756
.alpha.-Phellandrene	0.4100	1.2060
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	0.1750	0.3700
o-Cymene	4.0233	9.3673
D-Limonene	10.7572	12.7307
Eucalyptol	40.1050	47.0020
.gamma.-Terpinene	5.0217	6.1670
cis-Linaloloxide-12,0	0.1200	0.2100
trans-Linalool oxide (furanoid)	0.1400	0.2400
Linalool	0.1300	0.1982
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	0.6973	1.1192
trans-p-mentha-1(7),8-dien-2-ol	0.2900	0.7725
p-Mentha-1(7),8-dien-2-ol	0.0700	0.0900
trans-Pinocarvyl acetate	0.1200	0.2800
1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-meth	0.1900	0.2500
(-)-Spathulenol	0.1100	0.2300

This set of compounds may provide valuable clues to the understanding of the biochemical mechanisms underlying resistance and susceptibility to gall wasp attacks and should be the target of future investigations.

These findings reinforce a dualistic chemical pattern, in which certain compounds may be associated with resistance while others may contribute to susceptibility. The contrasting abundance of specific compounds between groups offers relevant biochemical markers for further investigation.

Among these compounds, "Bicyclo[3.1.1] heptane,6,6-dimethyl-2-methylene-,(1S)-", ".beta.-Myrcene", "trans-.beta.-Ocimene-10,6", and ".alpha.-Terpineol" deserve to be highlighted (Figure 3), which had a higher mean in the resistant group, and the compounds "Propanoicacid,2-methyl-,3-methylbutylester" and "Linalool" which had a lower mean in the susceptible group (Figure 4).

The results indicate a significant diversity in the chemical composition of essential oils among the analyzed genotypes. Genotype C016 stands out for its richness in exclusive compounds, while genotypes C018 and C013 did not present exclusive compounds. Moreover, cross-analysis of Tables 2 and 3 revealed that certain compounds exclusive to individual genotypes are also exclusive to phenotypic groups of resistance or susceptibility. Specifically, two compounds from genotype C014 are exclusive to the resistant group, and one compound from genotype C019 is exclusive to the susceptible group. These findings may have direct implications for future studies focused on understanding the mechanisms of resistance or susceptibility to gall wasp.

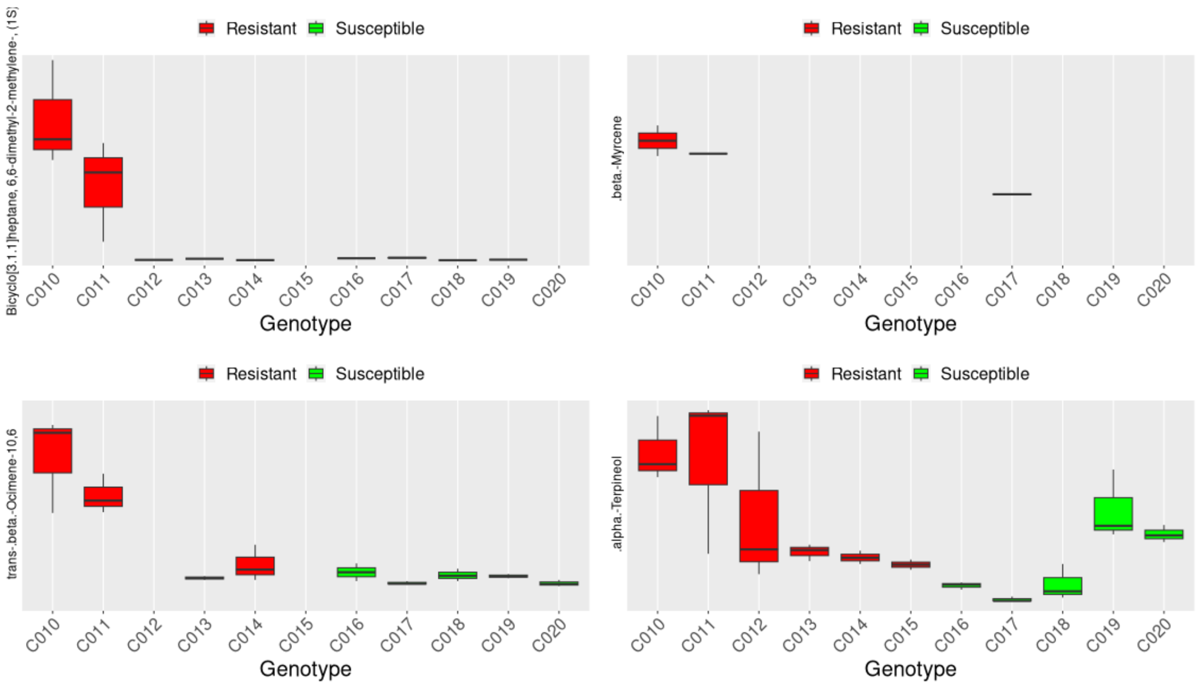

Figure 3. Relative abundance (based on gas chromatography-mass spectrometry area percentage) of four volatile organic compounds with a higher average in resistant genotypes to gall wasp. Boxplots represent the distribution of compound abundance across different genotypes, based on measurements from three individual trees per genotype. The y-axis indicates the relative abundance (%) of each compound, and the x-axis denotes genotype identity. Variation in box size reflects differences among individual trees; when all individuals share the same value, the boxplot appears as a horizontal line. Resistance status is color-coded (red = resistant; green = susceptible). From top to bottom and left to right, the following compounds are shown: “Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene-,(1S)-”, “.beta.-Myrcene”, “trans-.beta.-Ocimene-10,6” and “.alpha.-Terpineol”

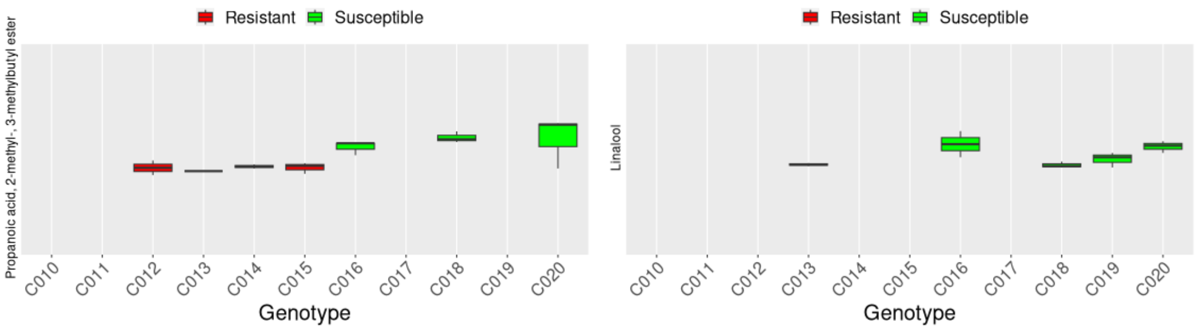

Figure 4. Relative abundance (based on gas chromatography-mass spectrometry area percentage) of two volatile organic compounds with a higher average in susceptible genotypes to gall wasp. Boxplots represent the distribution of compound abundance across different genotypes, based on measurements from three individual trees per genotype. The y-axis indicates the relative abundance (%) of each compound, and the x-axis denotes genotype identity. Variation in box size reflects differences among individual trees; when all individuals share the same value, the boxplot appears as a horizontal line. Resistance status is color-coded (red = resistant; green = susceptible). From to left to right, the following compounds are shown: “Propanoicacid,2-methyl-,3-methylbutylester” and “Linalool”.

Discussion

The interplay between genetic resistance and chemical ecology of *Eucalyptus* genotypes is pivotal in their response to *Leptocybe invasa* infestations. Essential oils derived from these trees serve not only as bioactive pest repellents but also as potential genetic markers for breeding resistant cultivars. This study enhances our understanding of the chemical profiles of essential oils from resistant and susceptible genotypes, revealing specific compounds that may indicate resistance. Our findings support and extend current knowledge on the critical bioactive compounds in essential oils for sustainable pest management strategies (Avila et al., 2022).

Some of the exclusive or more abundant compounds in resistant genotypes, such as "4-Octene, 3-Ethyl-2-methyl-1-heptene", "Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-", and "Camphene", are known for their bioactivity and could serve as candidate markers for resistance selection in breeding programs. Their consistent presence in resistant genotypes strengthens their potential as biochemical indicators rather than direct insecticidal agents.

The use of biochemical traits, including volatile compounds, as phenotypic markers for resistance selection has been explored in several crops and forest species. In *Eucalyptus*, Silva et al. (2020) demonstrated that limonene content was associated with resistance to *Austropuccinia psidii*, suggesting a role of essential oil composition in disease resistance. Similarly, Yong et al. (2019) showed that specific foliar terpene profiles could discriminate rust resistance phenotypes in *E. globulus* and *E. obliqua*. These findings support the hypothesis that the consistent presence and relative abundance of certain compounds may serve as biochemical indicators in breeding strategies focused on pest resistance.

Conversely, compounds prevalent or exclusive in susceptible genotypes, such as "Bicyclo[2.2.1] heptane, 2,2-dimethyl-5-methylene-" and "(+)-4-Carene-9,4", may be exploited to develop adult wasp attractants. The attractant effect of *E. globulus* essential oils demonstrated by Tampe et al. (2020) substantiates the potential of these compounds in integrated pest management (IPM) strategies.

Resistant genotypes exhibited a higher concentration of bioactive compounds, with some being exclusive, such as "Propanoicacid, 2-methyl-, 2-methylpropylester", "Endo-Borneol", and "4-Hexen-1-ol, acetate". These compounds could serve as chemical markers of resistance, warranting further investigation (Aouadi et al., 2020). Some authors (e.g., Ebadollahi et al., 2022) argue that the persistence and abundance of certain compounds can influence their ecological effectiveness, either through prolonged repellent effect or systemic action. However, in the context of genetic resistance, the focus lies in their role as consistent biochemical markers associated with phenotypic expression. Additionally, "Caryophyllene" and "Humulene", prevalent in resistant genotypes and known for their anti-inflammatory and antimicrobial properties, imply a multifunctional role in plant defense (Benelli et al., 2018).

The chemical composition and bioactivity of *Eucalyptus* essential oils have significant implications for the management of major insect pests. Such compounds align with the objectives of IPM, aiming to mitigate the environmental and health impacts of synthetic pesticides (Dhakad et al., 2017).

Our results contribute to understanding the ecological chemistry of *Eucalyptus* and its interaction with the gall wasp, emphasizing the need for approaches that consider genotypic and environmental variations. Future research should confirm the efficacy of these compounds in laboratory and field trials and their commercial applications in pest and disease management.

Conclusions

This study highlights the potential of essential oil chemical profiling to support genetic improvement strategies for pest resistance in *Eucalyptus*. Specific compounds associated with resistance or susceptibility to *Leptocybe invasa* may serve as biochemical markers for early selection of resistant genotypes and as inputs for developing eco-friendly pest management approaches. This dual application promotes the sustainability of *Eucalyptus* cultivation, ensuring both environmental integrity and economic viability. The direction outlined here for methodological and indicator use contributes to a more sophisticated and effective paradigm in pest management. Nonetheless, these results are preliminary and should be further validated through rigorous field trials and feasibility assessments to confirm their applicability in commercial forestry programs.

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Authors' Contributions

Jéssica Santos: formal analysis; investigation; writing – original draft; writing – review & editing. **Paulo Spinola Filho**: formal analysis; investigation. **Tiago Neves**: formal analysis; investigation. **Ariadne Marques**: formal analysis; investigation. **Any Rodrigues**: writing – review & editing. **Cristiane Grael**: methodology; writing – review & editing. **Lílian Pantoja**: methodology; writing – review & editing. **Alexandre Santos**: methodology; writing – review & editing. **Janaína Gonçalves**: writing – original draft; writing – review & editing. **Edival Zauza**: conceptualization; investigation; methodology. **Everton Soliman**: conceptualization; investigation; methodology. **Rafael Carvalho**: investigation; writing – original draft. **Emerson Lopes**: writing – original draft; writing – review & editing. **Marcelo Luiz de Laia**: conceptualization; formal analysis; methodology; writing – review & editing.

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