SCIENTIA PLENA
www.scientiaplena.org.br
VOL. 20, NUM. 02 2024
doi: 10.14808/sci.plena.2024.027202

Chemical composition of the essential oil of *Eucalyptus* genotypes susceptible or resistant to the gall-forming wasp *Leptocybe invasa* Fisher & La-Salle, 2004 (Hymenoptera: Eulophidae)

Composição química do óleo essencial de genótipos de Eucalyptus suscetíveis e resistentes à vespa galhadora *Leptocybe invasa* Fisher & La-Salle, 2004 (Hymenoptera: Eulophidae)

J. O. Dantas[1]*; V. S. Pinto[2]; J. O. Melo[2]; A. F. Blank[2]; P. C. L. Nogueira[3]; G. T. Ribeiro[4]

[1]*Departamento de Ciências Biológicas/Laboratório de Biologia Animal/Instituto Federal de Sergipe/Campus São Cristóvão, 4910-000, São Cristóvão – Sergipe, Brasil*
[2]*Departamento de Agronomia/Núcleo de Excelência em Conservação, Caracterização e uso Sustentável de Recursos Genéticos de Plantas Aromáticas e Olerícolas/Universidade Federal de Sergipe, 49100-000, São Cristóvão – Sergipe, Brasil*
[3]*Departamento de Química/Laboratório de Pesquisa em Química Orgânica de Sergipe /Universidade Federal de Sergipe, 49100-000, São Cristóvão – Sergipe, Brasil*
[4]*Departamento de Ciências Florestais/Laboratório de Pragas Florestais/Universidade Federal de Sergipe, 49100-000, São Cristóvão – Sergipe, Brasil*

**jose.oliveira@ifs.edu.br*
(Recebido em 22 de novembro de 2023; aceito em 06 de fevereiro de 2024)

Eucalyptus are trees native to Australia and have been cultivated in various regions of the world. In its worldwide expansion, several native and exotic insects began to use these plants for their survival. Leptocybe invasa Fisher & La-Salle, 2004 (Hymenoptera: Eulophidae) is a gall wasp that lay eggs into leaves, young branches, and petioles causing galls; however, there are resistant and susceptible eucalyptus genotypes to gall formation. This study aimed to compare the chemical composition of eucalyptus genotypes and relate it to resistance and susceptibility to gall formation. Leaf oils were extracted by steam distillation and oil compositions were analyzed. The essential oils of eucalyptus genotypes resistant to gall formation had 12 constituents, which do not occur in susceptible genotypes, probably one of the factors that contributes to the resistance of these plants to gall formation. Thus, we suggest an early evaluation of resistant or susceptible eucalyptus genotypes, based on the chemical composition of the essential oils of these plants, avoiding economic losses of planting susceptible genotypes.
Keywords: gall wasp, essential oil, chemical composition.

Os eucaliptos são árvores nativas da Austrália e são cultivadas em várias regiões do mundo. Em sua expansão mundial, vários insetos nativos e exóticos passaram a usar essas plantas para sua sobrevivência. Leptocybe invasa Fisher & La-Salle, 2004 (Hymenoptera: Eulophidae) é uma vespa galhadora que põe ovos nas folhas, ramos jovens e pecíolos causando galhas. Este trabalho teve como objetivo comparar a composição química de genótipos de eucalipto e relacioná-la à resistência e suscetibilidade à formação de galhas. Os óleos das folhas foram extraídos por destilação a vapor e as composições dos óleos foram analisados. Os óleos essenciais de genótipos resistentes à formação de galhas apresentaram 12 constituintes, que não ocorrem em genótipos suscetíveis, provavelmente um dos fatores que contribuem para resistência dessas plantas à formação de galhas. Assim, sugere-se uma avaliação precoce de genótipos de eucalipto resistente ou suscetíveis, com base na composição química dos óleos essenciais dessas plantas, evitando perdas econômicas de plantio de genótipos suscetíveis.
Palavras-chave: vespa-da-galha, óleo essencial, composição química.

1. INTRODUCTION

The genus *Eucalyptus* L'Heritier 1789 originates from Australia, Indonesia, and New Guinea. It has a prominent place in the forestry sector due to its great adaptability to various soils and climates, as well as its high potential as a raw material for the timber industry and production of pulp, paper, and essential oils [1]. These factors were determinant for the expansion of areas

cultivated with the species worldwide. However, monoculture favored the spread of some native and exotic insects, which started to cause economic damage [2].

Native insects such as leaf-cutting ants, beetles, and leaf-eating caterpillars became *eucalyptus* pests. Other insects such as *eucalyptus* weevil (*Gonipterus scutellatus*), *eucalyptus* borer (*Phoracantha semipunctata*), blue gum psyllid (*Ctenarytaina eucalypti*), *eucalyptus* bronze bug (*Thaumastocoris peregrinus*), and gall wasp (*Leptocybe invasa*) were introduced causing damage in Brazil [3].

The gall wasp (*Leptocybe invasa*) is an Australian *eucalyptus* pest that was first reported in Israel in 2000 and has since spread to several continents [4]. In Brazil, it was first recorded in 2008, and now is established in several states [5]. Adults are 1.2 mm long with bright dark brown color and, although males have been reported, wasps are thelytokous parthenogenetic and produce diploid female offspring [6].

Gall wasps lay eggs in leaf midribs, petioles, and young branches of the plant and induce gall formation for the protection and nutrition of the immature insects. Gall formation causes the growing shoot tips to dry out and outgrowth of axillary buds, preventing normal plant growth [7]. Several control methods have been used to manage the pest in commercial crops worldwide. Broad spectrum systemic insecticides (neonicotinoids) can help reduce the insect population in seedling nurseries and field plantation. However, despite good results, the continued use of synthetic insecticides may favor the development of insect resistance to these compounds and make long-term control difficult [8].

Varietal resistance is the ideal method of controlling insects by developing and planting of resistant trees. It is a tool applied to Integrated Pest Management (IPM), which brings economic and ecological benefits to agriculture. Nevertheless, it takes a long time to develop *eucalyptus* species and hybrids and launch them into the market. Moreover, due to their allogamy, *eucalyptus* trees may show high variability in the resistance levels of the hybrids, with susceptible and resistant materials derived from the same genetic material [9].

This method is probably the most appropriate strategy for planting *eucalyptus* in areas of gall wasp occurrence, since efficient techniques to identify insect-resistant plants are a primary concern of breeding programs [10]. Despite the damage caused by the gall wasp, no methodology has been described for early identification of resistant and susceptible genotypes, which makes it difficult to select promising genetic materials. Because tree cultivation is a time-consuming and resource-demanding process, a method for shortening the crop cycle or advancing the selection can significantly contribute to the success of the *eucalyptus* breeding program, with the early selection as one of the options [11]. Resistance of plants to pests and diseases is attributed to physical and chemical mechanisms, including essential oil constituents [12].

Essential oils are liquid lipid substances with complex combinations of chemical compounds, mostly mono and low molecular weight sesquiterpenes volatile on exposure to the air [13]. The volatile compounds emitted by *eucalyptus* have insecticidal action against agricultural and forest pests, which trigger defense action against biotic stresses. Studies have demonstrated the biological potential of *eucalyptus* essential oil against these organisms. Farashiani et al. (2016) [14] found that 53 species of the genus *Eucalyptus* have fumigation toxicity against stored product pests such as *Sitophilus oryzae* (L.) at LC50 between 22.87 and 59.12 $\mu L.L^{-1}$. Bett et al. (2017) [15] investigated the residual contact and repellency of *Eucalyptus saligna* Smith essential oil and proved that it has insecticidal and repellent effect against *Tribolium castaneum*, *Aconthoscelides obtectus,* and *Sitophilus zeamais*, with capacity to eliminate up to 60% of the population by the action of volatile compounds.

Although chemical defense mechanisms exist in *eucalyptus,* there are reports of pest attacks to genetic materials used in several plantations. Susceptible genotypes were identified by Dittrich-Schroder et al. (2012) [16] in a study on resistance and susceptibility to gall wasp of 50 *eucalyptus* genotypes and hybrids. The authors verified that 30 out of 50 genotypes showed symptoms of pest attack. In addition, hybrids from *Eucalyptus grandis*, *Eucalyptus urophylla*, and *Eucalyptus camaldulensis* showed the greatest variability in infestation severity (0-52.34%). However, as far we know, no report has been found on factors that favor the resistance and susceptibility of hybrids to pests, as well as the relationship between secondary metabolism and incidence of *L. invasa* in commercial plantations.

Considering the above, our study, when comparing the chemical composition of essential oils from resistant and susceptible *eucalyptus* genotypes to gall formation, induced by the oviposition of *Leptocybe invasa*, proposes that the constituents of *eucalyptus* essential oils can be used to identify resistant plants to attack and damage caused by this insect pest.

2. MATERIALS E METHODS

2.1. Plant material

Ten hybrid *Eucalyptus* genotypes (1404, 1249, 1250, 0321, 5341, 1724, 1277, 1262, 1275, 0292) (Table 1) were provided by Bracell Ltda (Copener Florestal). Plants were grown in 5-L plastic containers with substrate containing black soil, washed sand, and cattle manure (3: 1: 1), in the nursery of the Federal University of Sergipe (UFS), São Cristóvão Campus.

Table 1. Parents and susceptibility of Eucalyptus to Leptocybe invasa, genotypes provided by Bracell Ltda / Copener Florestal.

Genotypes	Parental		Origin
	Mother species	Father species	
1404	***E. urophylla***	***E.* spp.**	**Inhambupe /**
1249	***E. grandis***	***E. urophylla***	**Inhambupe /**
1250	***E. grandis***	***E. urophylla***	**Inhambupe /**
0321	***E. grandis***	***E. urophylla***	**Entre Rios/Copener**
5341	***E.* spp**	***E.* spp**	**Aracruz/Aracruz**
1724	***E. urophylla***	***E.* spp**	**- -**
1277	***E. grandis***	***E. camaldulensis***	**Sátiro Dias/Copener**
1262	***E. grandis***	***E. urophylla***	**Inhambupe /**
1275	***E. camaldulensis***	***E.* spp**	**Sátiro Dias/Copener**
0292	***E. grandis***	***E. urophylla***	**Entre Rios/Copener**

2.2. Extraction of essential oils (EO) and analysis of chemical composition

Leaves of the *eucalyptus* genotypes were dried in a forced-air oven at 40 °C for five days. Essential oil (EO) was extracted in triplicate by hydrodistillation in a modified Clevenger apparatus using 50 g samples of dried leaves in 2000 mL distilled water for 140 minutes. EOs were stored in amber vials at -20 °C until analysis.

The essential oils were analyzed in a GC/MS/FID detection (GCMSQP2010 Ultra, Shimadzu Corporation, Kyoto, Japan) equipped with an AOC-20i autosampler (Shimadzu). Separations were performed on a Rtx®-5MS Restek (5%-diphenyl-95%-dimethylpolysiloxane) fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 μm film thickness), Helium as carrier gas at a constant flow of 1.0 mL min^{-1} [17].

The injection port temperature was set at 280 °C, injection volume of 1.0 μL (10 mg mL^{-1}), and split ratio of 1:30. Oven temperature was programmed to rise from 50 °C (isotherm for 1.5 min) to 200 °C at 4 °C min^{-1}, then up to 300 °C at 10 °C min^{-1}, and hold for 5 min. Molecules in the GC/MS were ionized by electron ionization at 70 eV. Fragments were analyzed by a quadrupole system programmed to filter fragments/ions at *m/z* from 40 to 500 Da and detected by an electron multiplier [17].

Data was processed using the GCMS software Postrun Analysis (Labsolutions-Shimadzu). Ionization in GC/FID occurred by the flame created by the combustion of hydrogen 5.0 (30 mL min^{-1}) and synthetic air (300 mL min^{-1}). The ions collected and the generated electric current was amplified and processed. Data was processed using the CG software Postrun Analysis (Labsolutions-Shimadzu).

The identification of EO constituents was performed by comparison with the retention rates in the literature [18]. The retention index was determined by the equation given by Van den Dool and Kratz (1963) [19] for a homologous n-alkane series (*n*C9 - *n*C18). In addition, we used three MS libraries (Wiley8, Nist107 and Nist21) that allowed the comparison of the spectrum data with those of the libraries using a similarity index of 80% [17].

2.3. Susceptibility and resistance of *eucalyptus* genotypes

Ten plants of each *eucalyptus* genotype, totaling one hundred plants, were randomly distributed and kept in contact with the gall wasp. After the three-month period, the plants were evaluated, and plants that did not develop galls were considered resistant. The susceptibility level of *eucalyptus* genotypes was determined by adapting the Fournier's Index (1975) [20] for studying plant phenology, which allows estimating the percentage of intensity of a given event in plants. Using a semi-quantitative scale with 25% intervals, the method of Fournier assigns the genotypes into one of the five categories (0 to 4), as follows: absent = 0; 1-25% = 1; 26-50% = 2; 51-75% = 3; and 76-100% = 4. In this study, we evaluated the incidence of galls in plants, considering 0 for resistant genotypes, 1 for low susceptibility, 2 for medium/moderate susceptibility, 3 for high susceptibility, and 4 for very high susceptibility.

2.4. Test of the majority constituents of essential oils

The constituents α-terpinyl acetate and γ-terpineol were applied alone to plants with gall wasp oviposition to verify the action of the constituents in inhibiting gall development.

2.5. Statistical analysis

Content and chemical composition data of the eucalyptus essential oils were subjected to analysis of variance (ANOVA), and the means were compared by Scott-Knott test ($p < 0.05$) using the software Sisvar®.

The two multivariate methods, cluster analysis and principal component analysis (PCA), were performed using the software Statistical 7.0. Then, a dissimilarity matrix was constructed based on the chemical constitution of the essential oils of each genotype using Euclidean distances. The dissimilarity matrix was simplified with dendrograms using the Ward's clustering method. Mean chemical constituents and their standard errors of means were obtained with the software Graph Pad Prism®.

3. RESULTS E DISCUSSION

The *Eucalyptus* genotypes 1404, 1249, 1250, 0321, and 5341 were classified as resistant to gall formation, even with the occurrence of oviposition in some plants [21] (Figure 1). Applying the Fournier Index (1975) [20], genotypes 1277 and 1275 as lowly susceptible, 1724 as moderately susceptible, and 1262 and 0292 as very highly susceptible.

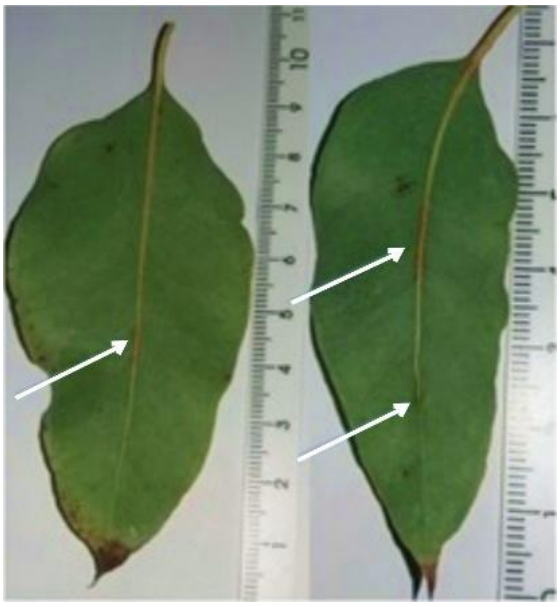

Figure 1: Presence of wasp oviposition on eucalyptus leaves.

The essential oil content of the *eucalyptus* genotypes ranged from 2.60% to 1.20%. In resistant genotypes the variation was from 1.20% to 1.80% and in susceptible genotypes the variation ranged from 1.73% to 2.60%. Although some susceptible genotypes were statistically similar to some resistant genotypes, we found that the susceptible genotypes had higher oil levels. The mean among resistant genotypes was 1.49% and among susceptible genotypes was 2.09% (Table 2).

The chemical analysis identified 35 compounds in the essential oil of the *Eucalyptus* genotypes. The main constituents were 1,8-cineol, α-pinene, *p*-cymene, α-perpineol, γ-terpinene, α-terpinyl acetate, *trans*-pinocarveol, and borneol (Table 2).

The compounds α-phellandrene, (*Z*)-linalool oxide, linalool, allo-ocimene, γ-terpineol, (*Z*)-carveol, (*E*)-linalool oxide acetate, α-terpinyl acetate, bicyclogermacrene, (*Z*)-calamenene, viridiflorol, and oxygenated sesquiterpene have been identified as constituents of essential oils of the gall-resistant genotypes (1404, 1249, 1250, 0321, and 5341), which have not been found in the EOs of susceptible genotypes (Table 2).

In the principal component analysis, the first principal component explained 42.72% of the total variance and was negatively related to the compounds α-campholenal (r = -0.98), α-terpenil acetate (r = -0.91), viridiflorol (r = -0.89), borneol (r = -0.89), camphene (r = -0.88), linalool (r = -0.86), *trans*-pinocarveol (r = -0.84)), endo-fenchol (r = -0.83), globulol (r = -0.81), oxygenated sesquiterpene (r = -0.78), α-pinene (r = -0.77), terpinolene (r = -0.76), and α-phellandrene (r = -0.72) (Figures 2 and 3).

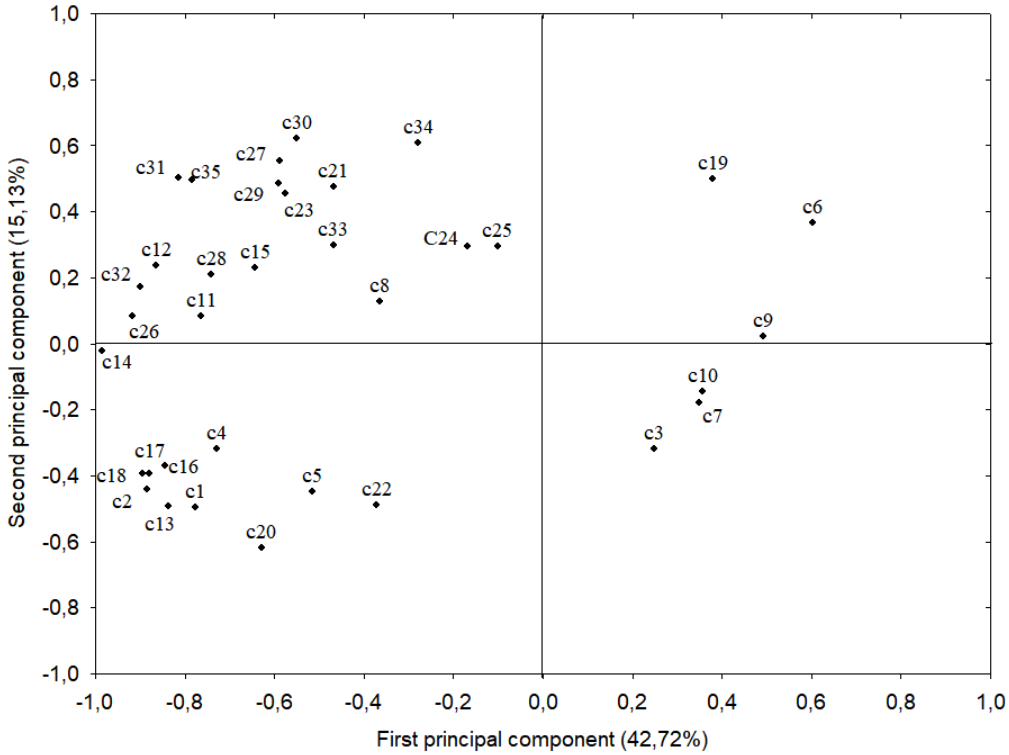

Figure 2. Distribution of the chemical constituents of the essential oil of Eucalyptus spp. resistant (R) and susceptible (S) to Leptocybe invasa (Hymenoptera: Eulophidae) in relation to the two principal components of the principal component analysis (PCA). Compounds: (C_1) α-pinene, (C_2) camphene, (C_3) β-pinene, (C_4) α-phellandrene, (C_5) isoamyl isobutyrate, (C_6) p-cymene, (C_7) 1,8-cineole, (C_8) (Z)-β-ocimene, (C_9) γ-terpinene, (C_{10}) (Z)-linalool oxide, (C_{11}) terpinolene, (C_{12}) linalool, (C_{13}) endo-fenchol, (C_{14}), α-campholenal, (C_{15}) allo-ocimene, (C_{16}) trans-pinocarveol, (C_{17}) pinocarvone, (C_{18}) borneol, (C_{19}) terpinen-4-ol, (C_{20}) α-terpineol, (C_{21}) myrtenol, (C_{22}) γ-terpineol, (C_{23}) (Z)-carveol, (C_{24}) p-mentha-1(7),8-dien-2-ol, (C_{25}) (E)-linalool oxide acetate, (C_{26}) α -terpinyl acetate, (C_{27}) (E)-caryophyllene, (C_{28}) bicyclogermacrene, (C_{29}) (Z)-calamenene, (C_{30}) spathulenol, (C_{31}) globulol, (C_{32}) viridiflorol, (C_{33}) isoleptospermone, (C_{34}) leptospermone, (C_{35}) oxygenated sesquiterpene.

Table 2: Contents (%) (means and standard deviation) of chemical constituents of essential oils of Eucalyptus genotypes resistant (R) and susceptible (S) to gall resulted from the oviposition of Leptocybe invasa (Hymenoptera: Eulophidae). RIl: Retention index literature; RIc: Retention index calculated.

Constituents	RIl	RIc	1404R	1249R	1250R	0321R	5341R	1724S	1277S	1262S	1275S	0292S
α-Pinene	932	934	43.97 (0.24)	22.26 (0.06)	42.37 (0.18)	37.74 (0.12)	34.36 (0.06)	5.73 (0.03)	39.42 (0.04)	32.59 (0.17)	15.31 (0.08)	1.60 (0.01)
Camphene	946	949	1.37 (0.11)	0.62 (0.10)	1.06 (0.05)	1.13 (0.04)	0.82 (0.06)	0.25 (0.03)	0.75 (0.01)	0.43 (0.05)	0.34 (0.01)	-
α -Pinene	974	978	0.41 -	0.12 (0.01)	0.23 -	0.21 (0.01)	0.36 -	1.50 (0.01)	2.60 (0.01)	0.23 (0.01)	-	-
α-Phellandrene	1002	1006	0.66 (0.01)	0.18 -	0.17 (0.01)	0.26 -	0.27 -	-	-	-	-	-
Isoamyl isobutyrate	1007	1012	0.92 (0.02)	0.30 (0.01)	0.12 (0.01)	0.16 -	0.17 -	-	-	0.08 -	-	-
p-Cymene	1020	1027	2.79 (0.03)	-	0.33 (0.01)	0.40 (0.01)	0.87 -	7.73 (0.03)	0.37 -	2.18 (0.01)	6.51 (0.01)	32.17 (0.19)
1,8-Cineole	1026	1035	29.93 (0.14)	56.37 (0.41)	31.14 (0.15)	34.49 (0.07)	36.41 (0.33)	60.53 (0.12)	47.12 (0.03)	53.75 (0.18)	66.64 (0.17)	14.18 (0.01)
(*Z*)-β-Ocimene	1032	1038	0.98 (0.04)	0.47 (0.13)	1.57 (0.05)	0.90 (0.02)	0.78(0.05)	1.66 (0.20)	-	-	-	0.43 (0.13)
γ-Terpinene	1054	1060	0.32 (0.01)	0.30 -	-	-	0.17 -	17.27 (0.05)	0.27 -	1.86 (0.02)	3.55 (0.02)	42.22 (0.13)
(*Z*)-Linalool oxide	1067	1073	-	0.24 -	-	0.16 -	0.25 -	-	-	-	-	-
Terpinolene	1086	1090	0.69 (0.02)	0.66 (0.02)	0.49 (0.01)	0.39 (0.04)	0.60 (0.08)	0.24 (0.02)	0.33 (0.01)	0.23 (0.01)	-	0.26 (0.01)
Linalool	1095	1101	0.23 (0.01)	0.37 (0.01)	0.17 -	0.35 -	0.31 (0.13)	-	-	-	-	-
endo-Fenchol	1114	1116	0.92 (0.01)	0.43 (0.01)	0.89 (0.01)	0.89 (0.01)	0.58 (0.14)	0.25 (0.01)	0.66 (0.01)	0.37 (0.01)	0.33 (0.01)	-
α -Campholenal	1122	1128	0.37 (0.01)	0.28 -	0.35 (0.01)	0.46 (0.01)	0.43 (0.17)	-	0.13 -	0.20 -	-	-
allo-Ocimene	1128	1130	-	-	0.39 (0.02)	0.14 (0.02)	0.18 (0.09)	-	-	-	-	-
trans-Pinocarveol	1135	1143	2.43 (0.03)	1.55 (0.02)	2.68 (0.01)	2.72 (0.02)	2.26 (1.53)	-	1.98 (0.01)	1.81 (0.02)	1.44 (0.01)	-
Pinocarvone	1160	1166	0.97 (0.02)	0.55 (0.05)	0.81 -	1.10 (0.03)	0.76 (0.48)	-	0.61 -	0.58 (0.01	0.44 -	-
Borneol	1165	1169	1.99 (0.05)	0.92 (0.03)	2.03 (0.01)	1.97 (0.02)	1.34 (0.15)	0.42 (0.01)	0.98 (0.01)	0.82 (0.03)	0.75 (0.01)	-
Terpinen-4-ol	1174	1180	0.59 (0.02)	0.90 -	0.27 (0.02)	0.26 (0.01)	0.83 (0.44)	1.46 (0.02)	0.53 (0.02)	0.39 (0.03)	0.96 (0.02)	3.07 (0.01)
α-Terpineol	1186	1194	4.29 (0.08)	1.91 -	4.02 (0.10)	4.85 (0.02)	1.74 (1.09)	1.82 (0.03)	2.40 (0.02)	3.62 (0.02)	2.19 (0.13)	0.94 (0.02)
Myrtenol	1194	1201	-	-	-	0.16 (0.01)	0.92 (1.05)	-	0.17 (0.02)	-	-	-
γ-Terpineol	1194	1201	4.29 (0.08)	1.91 -	-	0.16 -	0.40 (0.09)	-	-	-	-	-
(*Z*)-Carveol	1226	1223	-	0.59 (0.03)	0.24 -	0.29 (0.01)	0.24 (0.07)	-	-	-	-	-
p-Mentha-1(7),8-dien-2-ol	1227	1231	-	1.60 (0.01)	0.26 (0.03)	0.29 -	0.31 (0.02)	-	0.26 (0.03)	-	0.55 (0.07)	-
(*E*)-Linalool oxide acetate	1287	1289	-	0.17 (0.01)	0.13 -	0.35 -	-	-	-	-	-	-
α-Terpinyl acetate	1346	1353	2.83 (0.06)	2.23 (0.03)	5.57 (0.01)	4.74(0.01)	3.38 (2.35)	-	-	-	-	-
(*E*)-Caryophyllene	1417	1426	0.41 (0.01)	0.37 -	0.54 (0.01)	0.58 -	2.50 (1.85)	0.47 (0.01)	-	0.08 -	-	0.16 -
Bicyclogermacrene	1500	1504	-	-	0.23 (0.02)	0.39 -	0.21 (0.02)	-	-	-	-	-
(*Z*)-Calamenene	1528	1530	-	-	-	0.17 (0.01)	0.35 -	-	-	-	-	-
Spathulenol	1577	1586	0.29 (0.01)	0.38 (0.01)	0.15 (0.01)	0.23 -	1.00 (0.01)	0.17 -	-	0.06 -	-	0.24 (0.01)
Globulol	1590	1593	0.39 (0.01)	0.49 (0.01)	0.69 (0.01)	0.79 (0.01)	1.44 (0.01)	0.22 (0.01)	-	0.15 (0.01)	-	0.16 (0.01)
Viridiflorol	1592	1601	0.23 -	0.28 (0.02)	0.53 (0.02)	0.74 (0.01)	0.44 -	-	-	-	-	-
Iso-leptospermone+(?)	1621	1625	-	1.41 (0.01)	1.43 (0.01)	0.58 (0.02)	0.29 -	0.29 (0.01)	-	0.27 -	-	1.49 -
Leptospermone	1629	1633	-	0.38 -	0.32 (0.02)	0.37 (0.01)	0.23 (0.03)	-	-	0.08 -	-	0.43 -
Oxygenated sesquiterpene	1637	1637	0.18 (0.01)	0.27 (0.01)	0.39 (0.03)	0.32 (0.01)	0.94 (0.06)	-	-	-	-	-
Oil content (%)	-	-	1.20C (0.20)	1.73B (0.31)	1.80B (0.20)	1.66B (0.12)	1.26C (0.12)	2.13A (0.76)	1.73B (0.12)	2.60A (0.20)	2.26A (0,12)	1.73B (0.23)

Values with the same letters do not differ statistically, for essential oil content. The means were compared by the Scott-Knott ($p < 0$).

The *Eucalyptus* genotypes were classified by the high variability of constituents in the essential oils. This high variability was found in susceptible and resistant genotypes, so that the genotypes were clustered into resistant and susceptible clusters, except for cluster 2, which clustered the susceptible genotypes 1724 and 1275 with the resistant genotype 1249.

The cluster analysis classified the essential oil constituents into three chemical clusters. Cluster I comprised the genotype 0292 (S) with the following major constituents: γ-terpinene (42.22%), p-cymene (32.17%), and 1.8 cineole (14.18%). Then, Cluster II with genotypes 1249 (R), 1724 (S), and 1275 (S) with 1.8 cineole (56.37% to 66.64%), α-pinene (5.73% to 22.26%), γ-terpinene (0.3% to 17.24%), and α-terpineol (1.82% to 2.29%); and Cluster III with genotypes 1277 (S) and 1262 (S) with 1,8-cineole (47.12% to 53.75%), α-pinene (32.59% to 39.42%), α-terpineol (2.4% to 3.62%), and *trans*-pinocarveol (1.81% to 1.983%); 1404 (R), 1250 (R), 0321 (R), and 5341 (R) with 1,8-cineole (36.41% to 29.93%), α-pinene (34.36% to 43.97%), α-terpineol (1.74% to 4.85%), and α-terpinyl acetate (2.83% to 5.57%) as major constituents (Figures 3 and 4).

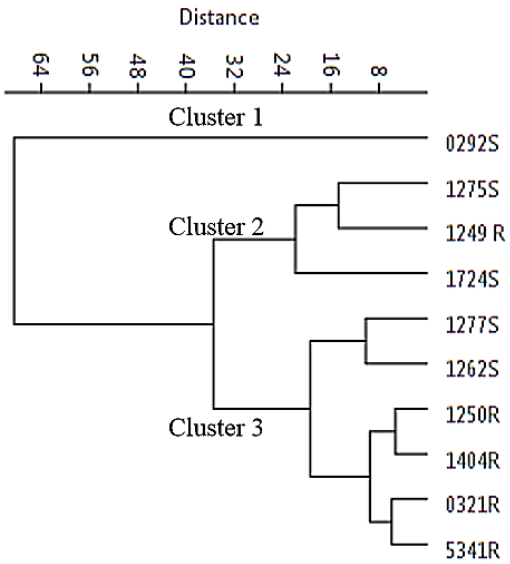

Figure 3: Two-dimensional dendrogram obtained in the cluster analysis of the chemical composition of essential oils showing the similarity between Eucalyptus genotypes resistant (R) and susceptible (S) to Leptocybe invasa (Hymenoptera: Eulophidae).

The chemical constituents α-phellandrene, (*Z*)-linalool oxide, linalool, allo-ocimene, γ-terpineol, (*Z*)-carveol, (*E*)-linalool oxide acetate, α-terpinyl acetate, bicyclogermacrene, (*Z*)-calamenene, viridiflorol, and oxygenated sesqueterpene were identified only in the gall-resistant genotypes 1404, 1249, 1250, 0321, and 5341 (Table 2).

The correlation between α-phellandrene and isoamyl isobutyrate (r = 0.94), α-terpinyl acetate and bicyclogermacrene with viridiflorol (0.95 and 0.92 respectively) was positive. The compounds α-phellandrene and γ-terpineol (r= 0.88), α-campholenal, (*Z*)-carveol and viridiflorol with linalool (0.85, 0.83, and 0.83), allo-ocimene and α-terpinyl acetate allo-ocimene and α-terpinyl acetate (0.82), (*Z*)-carveol and isoleptospermone (0.83), α-terpinyl acetate and bicyclogermacrene (0.82), spathulenol, globulol and oxygenated sesquiterpene with (*Z*)-calamenene (0.84, 0.88 and 0.87), and spathulenol and myrtenol with oxygenated sesquiterpene (0.89 and 0.85) are strongly and positively correlated with each other (Table 2).

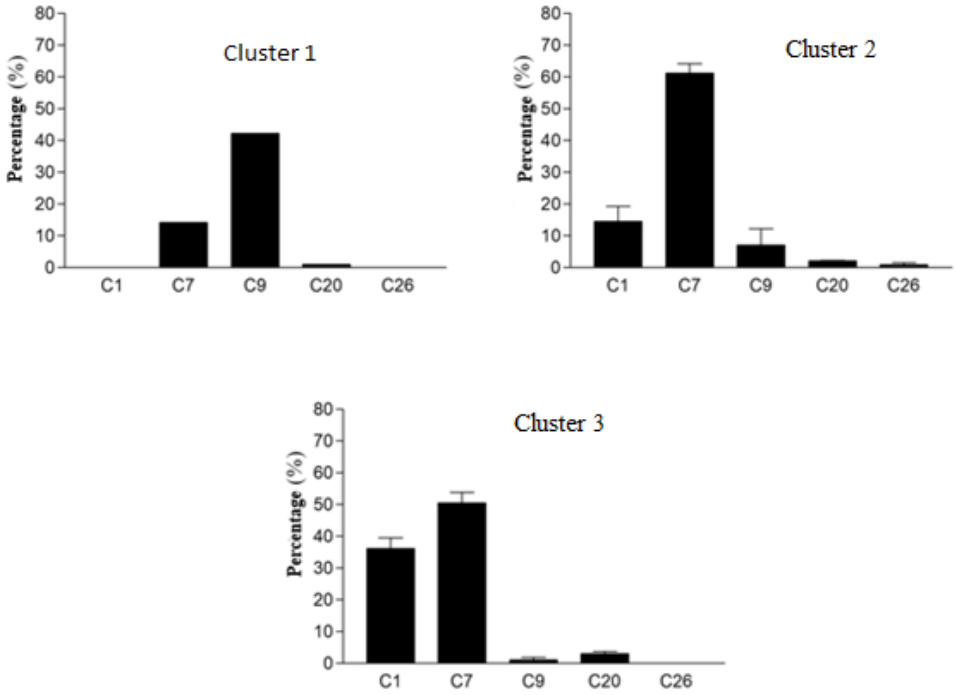

Figure 4: Means of the essential oil chemical constituents of Eucalyptus genotypes, clusters 1-4. (C_1) α-pinene, (C_7) 1,8-cineole, (C_9) γ-terpinene, (C_{20}) α-terpineol, (C_{26}).

In this study, EO contents were relatively higher in *eucalyptus* genotypes susceptible to galls induced by *L. invasa*. Because essential oils are secondary metabolites produced for plant's defense [13], genotypes attacked by wasps with gall formation are likely to produce more essential oil as defense strategy [22]. These results corroborate with Morais and Castanha (2012) [23], who found higher concentrations of monoterpenes in the essential oil of basil attacked by mealybug, and Queiroz-Voltan et al. (1995) [24] in *Hyptis suaveolens* populations attacked by herbivores, which produced larger amounts of essential oil.

Variation in the chemical composition of essential oils is reported by several studies [25-27]. It may be associated with abiotic factors such as luminosity, temperature, rainfall, nutrition, harvest time, collection time, as well as harvest and postharvest techniques [28]. It may also be associated with leaf age and the phenological phase [29, 30]. Since the plants in our study were subjected to the same environmental conditions and management, it is likely that the genetics and the insect-plant interaction influenced the diversity of the EO chemical constituents of the *eucalyptus* genotypes [29].

This study found that plants with the same parents (1249, 1250, 0321, 1262, 0292) varied in EO chemical composition and in resistance and susceptibility levels (Tables 1 and 2). Interspecific crosses between pure species form hybrid genotypes that can synthesize new compounds differing from their parents. The hybrid's genetic structure is the result of the combination of parental genes, in addition to the cytoplasm of the female parent, with mitochondrial and chloroplast DNA [17], where the set of inherited genes may or may not be expressed in certain plants.

According to Sun et al. 2004 [31], even if all parental genes are inherited by the hybrid, their performance or phenotype may differ from their parents, which demonstrates heterosis. With rare exceptions, the cells of an organism have the same genes, but during development, their metabolic requirements are different and the control mechanisms turn on and off genes expressing a set of specific metabolites. Thereby, and the phenotype evolves through changes in spatial and temporal patterns of gene expression [32].

The compounds α-phellandrene, (*Z*)-linalool oxide, linalool, allo-ocimene, γ-terpineol, (*Z*)-carveol, (*E*)-linalool oxide acetate, α-terpinyl acetate, bicyclogermacrene, (*Z*)-calamenene, viridiflorol, and oxygenated sesquiterpene found in eucalyptus genotypes resistant to gall formation (1404, 1249, 1250, 0321 and 5341) are mainly monoterpenes. Most of these compounds

are cited as responsible for the insecticidal activity of essential oils: The compounds α-phellandrene, (*Z*)-linalool oxide, linalool, allo-ocimene, γ-terpineol, (*Z*)-carveol, (*E*)-linalool oxide acetate, α-terpinyl acetate, bicyclogermacrene, (*Z*)-calamenene, viridiflorol, and oxygenated sesquiterpene found in *eucalyptus* genotypes resistant to gall formation (1404, 1249, 1250, 0321 and 5341) are mainly monoterpenes. Most of these compounds are cited as responsible for the insecticidal activity of essential oils: α-phellandrene [33-35], (*Z*)-linalool oxide [36], linalool [37, 38], allo-ocimene, γ-terpineol [39], (*Z*)-carveol, (*E*)-linalool oxide acetate [39], α-terpinyl acetate [39, 40], bicyclogermacrene [39], (*Z*)-calamenene [41], viridiflorol [34] and oxygenated sesquiterpene [41]. Therefore, these compounds probably contribute to the resistance of plants to the formation of galls.

Genotypes that express resistance have physical or biochemical traits that modify behavioral responses (xenobiosis) or adversely affect the development or survival of pest insect species via metabolic anomalies (antibiosis) [42]. However, this is not the case with the eucalyptus genotypes in this study, given that gall wasps lay eggs on gall-resistant plants [21]. The constituents α-terpinyl acetate and γ-terpineol when applied alone did not inhibit the formation of galls, thus, the synergism of the EO compounds (Figure 2) is the probable cause of resistance in the eucalyptus genotypes evaluated in this study, since even with the occurrence of wasps' egg laying there was no gall formation.

Therefore, this study proposes a rapid method for selection of eucalyptus genotypes susceptible or resistant to *L. invasa*-induced gall based on the occurrence of these essential oil constituents (Figure 2), leading to reduction of costs with planting and maintenance operations and saving time, as there is no need to wait long to establish whether or not galls were formed.

Additionally, varietal resistance is a useful tool for Integrated Pest Management, bringing economic and ecological benefits to agriculture [43]. Early selection studies have been conducted with several forest species such as *Tachigali vulgaris* [44], *Hevea brasiliensis* [45], *Pinus banksiana* [46], *Eucalyptus* sp. for various purposes [47], and *Larix decidua* [48].

4. CONCLUSIONS

The analyzes performed allow us to conclude that:

- Five of the ten eucalyptus genotypes are resistant to gall formation induced by wasp oviposition.
- Constituents of essential oils were identified as relevant markers and possibly contribute for the resistance of eucalyptus genotypes to gall formation.
- The resistant genotypes were successfully grouped according to their essential oil constituents in the early stages of development.

5. ACKNOWLEDGMENT

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Finep.

6. REFERENCES

1. Silva DEM, Azevedo LAS, Poltronieri TPS. Avaliação da resistência de espécies de eucalipto à ferrugem (*Puccinia psidii* Winter). Summa Phytopathol. 2014 Mar;40(1):54-62. doi: 10.1590/S0100-54052014000100008
2. Vechi A, Magalhães Júnior CAO. Aspectos positivos e negativos da cultura do eucalipto e os efeitos ambientais do seu cultivo. Rev Valore. 2018 Jan/Jun;3(1):495-507. doi: 10.22408/reva312018101495-507
3. Ribeiro GT, Zanuncio JC, Tavares WS, Ramalho FS, Serrão JE. Cosntancy, distribution and frequency of Lepidoptera Defoliators of Eucalyptus grandis and *Eucalyptus urophylla* (Myrtaceae) in Four Brazilian Regions. Neotrop Entomol. 2016 Jun;45(6):629-36. doi: 10.1007/s13744-016-0410-1

4. Silva PHM, Junqueira LR, Araujo MJ, Wilcken CF, Moraes MLT, Paula RC. Susceptibity of eucalypt taxa to a natural infestation by *Leptocybe invasa*. New Forest. 2019 Oct;55(1):753-783. doi: 10.1007/s11056-019-09758-1
5. Costa VA, Berti-Filho E, Wicken CF, Stape JL, La Salle J, Teixeira LD. Eucalyptus gall wasp, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) in Brazil: New forest pest reachers the new world. Rev Agric. 2008 Set;83(2):136-9. doi: 10.37856/bja.v83i2.1479
6. Mendel Z, Protasav A, Fisher N, LaSalle J. The taxonomy and natural history of *Leptocybe invasa* (Hymenoptera: Eulophidae) gen & sp. nov., an invasive gall inducer on *Eucalyptus*. Australian J Entomol. 2004 May;43(2):101-13. doi: 10.1111/j.1440-6055.2003.00393.x
7. Garlet J, Costa EC, Boscardini J, Deponti G, Shwengber CR, Machado LM. *Leptocybe invasa* em *Eucalyptus* sp. no estado do Rio Grande do Sul, Brasil. Ciênc Rural. 2013 Dez;43(12):2175-7. doi: 10.1590/S0103-84782013001200009
8. Rinaldi DAMF, Barbosa LR, Wilcken CF, Zaché B, Araújo MM, Carvalho RCZ. Ocorrência de *Leptocybe invasa* (Hymenoptera: Eulophidae) em mudas de eucalipto no estado do Paraná. Pesqui Florest Bras. 2013 Set;33(75):327-30. doi: 10.4336/2013.pfb.33.75.400
9. Gebremdein MB. Varietal screening for resistance against field and storage crop pests: An implication for Ethiopian crop variety development. J Plant Breed Crop Sci. 2018 Aug;10(8):203-9. doi: 10.5897/JPBCS2018.0728
10. Porto NA, Roque JV, Wartha CA, Cardoso W, Peternelli LA, Barbosa MHP, et al. Early prediction of sugarcane genotypes susceptible and resistant to *Diatraea saccharalis* using spectroscopies and classification techniques. Spectrochim Acta Part A: Mol Biomol Spectrosc. 2019 Jul;218:69-75. doi: 10.1016/j.saa.2019.03.114
11. Corrêa TR, Picoli EAT, Souza GAS, Condé AS, Silva NM, Lopes-Mattos KLB, et al. Phenotypic markers in early seletion for tolerance to dieback in *Eucalyptus*. Ind Crops Prod. 2017 Nov;107:130-8. doi: 10.1016/j.indcrop.2017.05.032
12. War AR, Paulrai MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S, et al. Mechanisms of plant defense against insect herbivores. Plant Signal Behav. 2012 Oct;7(10):1306-20. doi: 10.4161/psb.21663
13. Zahran HEDM, Abou-Taleb HK, Abdelgaleil SAM. Adulticidal, larvicidal and biochemical properties of essential oils against *Culex pipiens* L. J Asia-Pac Entomol. 2017 Mar;20(1):133-9. doi: 10.1016/j.aspen.2016.12.006
14. Farashiani ME, Awang RM, Assareh MH, Omar D, Rahmani M. Fumigant toxicity of 53 Iranian *Eucalyptus* essential oils against stored product insect, *Sitophilus oryzae* L. Iranian J For Range Prot Res. 2016 Jul;13(2):132-9. doi: 10.22092/ijfrpr.2016.106020
15. Bett PK, Deng AL, Ogendo JO, Kariuki ST, Kamatenesi-Mugisha M, Mihale JM, et al. Residual contact toxicity and repellence of *Cupressus lusitanica* Miller and *Eucalyptus saligna* Smith essential oils against major stored product insect pests. Ind Crops Prod. 2017 Dec;110(30):65-74. doi: 10.1016/j.indcrop.2017.09.046
16. Dittrich-Schoder G, Wingfied M, Hurley B, Slippers B. Diversity in *Eucalyptus* susceptibility to the gall-forming wasp *Leptocybe invasa*. Agric Forest Entomol. 2012 Nov;14(4):419-27. doi: 10.1111/j.1461-9563.2012.00583.x
17. Melo JO, Blank AF, Nunes RS, Alves PB, Arrigoni-Blank MF, Gagliardi PR, Nascimento Júnior AF, Sampaio TS, Lima AD, Nizio DAC. Esential oils of *Lippia gracilis* and *Lippia sidoides* chemotypes and their major compounds carvacrol and thymol: nanoemulsions and antifungal activity against *Lasiodiplodia theobromae*. Res, Soc Dev. 2022 Feb;11(3):e36511326715. doi: 10.33448/rsd-v11i3.26715
18. Adams RP. Identification of essential oil components by gas chromatograpy/mass spectroscopy. 4th ed. Illinois (US): Allured Publishing Corporation, Carol Stream; 2007.
19. Van den Dool H, Kratz PD. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr A. 1963 Aug;11:463-71. doi: 10.1016/s0021-9673(01)80947-x
20. Fournier LA, Charpantier C. El tamaño de la muestra y la frecuencia de observaciones en el estudio de las características fenológicas de los árboles tropicales. Turrialba. 1975 Dez;25(1):45-8.
21. Dantas JO, Aragão JRV, Lisi CS, Silva EC, Anchieta RL, Ribeiro, GT. Oviposition of the gall wasp *Leptocybe invasa* (Hymenoptera: Eulophidae) and morphological changes in *Eucalyptus* spp. Genotypes susceptible. Rev Floresta. 2021 Mai;51(3):668-76. doi: 10.5380/rf.v51i3.72040
22. Yactayo-Chang JP, Tang HV, Mendoza J, Christensen AS, Block AK. Plant defense chemicals against insect pests. Agronomy. 2020 Jul;10(1156):1-14. doi: 10.3390/agronomy10081156
23. Morais LAS, Castanha RF. Composição química do óleo essencial de manjericão naturalmente submetido ao ataque de cochonilhas. Hortic Bras. 2012 Jul;30(2):2178-82.

24. Queiroz-Voltan RB, Stubblebine WH, Shepherd G. Variação de terpenos em *Hyptis suaveolens* e seu papel na defesa contra herbívoros. Bragantia. 1995 Aug;54(2):217-35. doi: 10.1590/S0006-87051995000200001
25. Huang HC, Ho YC, Lim JM, Chang TY, Ho CL, Chang TM. Investigation of the anti-melanogenic and antioxidant characteristics of *Eucalyptus camaldulensis* flower essential oil and determination of its chemical composition. Int J Mol Sci. 2015 May;16(5):10470-90. doi: 10.3390/ijms160510470
26. Sewanu SO, Bongekile MC, Folusho OO, Adejumobi LO, Rowland OA. Antimicrobial and efflux pumps inhibitory activities of *Eucalyptus grandis* essential oil against respiratory tract infectious bacteria. J Med Plants Res. 2015 Mar;9(10):343-8. doi: 10.5897/JMPR2015. 5652
27. Knezevic P, Aleksic V, Simin N, Svircev E, Petrovic A, Mimica-Dukic N. Antimicrobial activity of *Eucalyptus camaldulensis* essential oils and their interactions with conventional antimicrobial agents against multi-drug resistant *Acinetobacter baumannii*. J Ethnopharmacol. 2016 Dec;178:125-36. doi: 10.1016/j.jep.2015.12.008
28. Bapista EB, Zimmermann-Franco DC, Lataliza AAB, Raposo NRB. Chemical composition and antifungal activity of essential oil from *Eucalyptus smithii* against dermatophytes. Rev Soc Bras Med Trop. 2015 Nov-Dez;48(6):746-52. doi: 10.1590/0037-8682-0188-2015
29. Salem N, Kefi S, Tabben O, Ayed A, Jallouli S, Feres N, et al. Variation in chemical composition of *Eucalyptus globulus* essential oil under phenological stages and evidence synergism with antimicrobial standards. Ind Crops Prod. 2018 Nov;124:115-25. doi: 10.1016/j.indcrop.2018.07.051
30. Porres-Martínez M, González-Burgos E, Carretero ME, Gómez-Serranillos MP. Influence of phenological stage on chemical composition and antioxidant activity of *Salvia lavandulifolia* Vakl. Essential oils. Ind Crops Prod. 2014 Feb;53:71-7. doi: 10.1016/j.indcrop.2013.12.024
31. Sun Q, Wu L, Ni Z, Meng F, Wang Z, Lin Z. Differential gene expression patterns in leaves between hybrids and their parental inbreds are correlated with heterosis in a wheat diallel cross. Plant Sci. 2004 Mar;166(3):651-7. doi: 10.1016/j.plantcsi.2003.10.033
32. Kelly M. Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. Philos Trans R Soc B. 2019 Jan;374 (2018176):1-14. doi: 10.1098/rstb.2018.0176
33. Chaaban A, Richardi VS, Carrer AR, Brum JS, Cipriano RR, Martins CEN, et al. Insecticide activity of *Curcuma longa* (leaves) essential oil and its major compound *α*-phellandrene against *Lucilia cuprina* larvae (Diptera: Calliphoridae): Histological and ultrastructural biomarkers assessment. Pestic Biochem Phys. 2019 Jan;153:17-27. doi: 10.1016/j.pestbp.2018.10.002
34. Park IK, Lee SG, Choi DH, Park JD, Ahn YJ. Inseticidal activities of constituints identified in the essential oil from leaves of *Chamaecyparis obtuse* against *Callosobruchus chinensis* (L.) and *Sitophilus oryzae* (L.). J Stored Prod Res. 2003 Mar,39(4):375-84. doi: 10.1016/S0022-474X(02)00030-9
35. Kiran S, Kujur A, Patel L, Ramalakshmi K, Prakash, B. Assessment of toxicity and biochemical mecanisms underlying the insecticidal activity of chemically characterized Boswellia carterii essential oil against insect pest of legume seeds. Pestic Biochem Phys. 2017 Apr,139:17-23. doi: 10.1016/j.pestbp.2017.04.004
36. Nyasembe VO, Tchouassi DP, Mbogo CM, Sole CL, Pirk C, Torto B. Linalool oxide: generalist plant based lure for mosquito disease vectors. Parasit & Vectors. 2015 Nov,8(581):1-8. doi: 10.1186/s13071-015-1184-8
37. Quan M, Liu, QZ, Liu, ZL. Identification of insecticidal constituints from the essential oil from the aerial parts *Stachys riederi* var. *japonica*. Molecules. 2018 May;23(5):1200. doi: 10.3390/molecules23051200
38. Wu P, Tang X, Jian R, Li J, Lin M, Dai H, et al. Chemical composition, antimicrobial and insecticidal activities of essential oils of discarded perfume lemon and leaves (*Citrus limon* (L.) Burm. F.) as possible sources of functional botanical agents. Front Chem. 2021 May;9:679116. doi: 10.3389/fchem.2021.679116
39. Govindarajan M, Benelli G. Eco-friendly larvicides from indian plants: Effectiveness of lavandulyl acetate and bicyclogermacrene on malaria, dengue and japonese encephalitis mosquito vectors. Ecotoxicol Environ Saf. 2016 Nov;133:395-402. doi: 10.1016/j.ecoenv.2016.07.035
40. Song HJ, Yong SH, Kim HG, Kim DH, Park KB, Shin KC, et al. Insecticidal activity agains *Myzus persicae* of terpenyl acetate and bornyl acetate in *Thuja occidentalis* essencial oil. Horticulture. 2022 Sep;8(10):969. doi: 10.3390/horticulturae8100969
41. Murillo MC, Suarez LEC, Salamanca JAC. Chemical composition and insecticidal properties of essential oils of *Piper septuplinervium* and *P. subtomentosum* (Piperaceae). Nat Prod Commun. 2014 Aug;9(10):1527-30. doi: 10.1177/1934578X1400901031
42. Throne JE, Baker JE, Messina FJ, Kramer KJ, Howard JA. Varietal resistence. In: Subramanyam B, Hagstrum DW, editors. Alternatives to Pesticides in Stored-Product IPM. Boston (MA): Springer; 2000. p.165-95.

43. Smith CM, Clement SL. Molecular bases of plant resistance to arthropods. Annu Rev Entomol. 2012 Jan;57:309-28. doi: 10.1146/annurev-ento-120710-100642
44. Farias Neto JT, Castro AWV, Bianchetti A. Aplicação da seleção precoce em famílias de meio - irmãos de taxi-branco. Acta Amazôn. 2003 Jan-Mar;33(1):85-91. doi: 10.1590/1809-4392200331091
45. Gonçalves PS, Bortoletto N, Fonseca FS, Bataglia OC, Ortolani AA. Early selection for growth vigor in rubber tree genotypes in northwestern São Paulo State (Brazil). Genet Mol Biol. 1998 Dec;21(4):1-6. doi: 10.1590/S1415-47571998000400018
46. Weng YH, Tosh KJ, Park YS, Fullarton MS. Age-related trends in genetic parameters for jack pine and their implications for early selection. Silvae Genet. 2007 Dec;56(5):242-52. doi: 10.1515/sg-2007-0035
47. Sampaio TF, Dalcin TE, Bogiani JC, Mori ES, Guerrini IA. Selection of *Eucalyptus* clones and adjustment of potassium doses for extended drought in Bahia savanna. Rev Árvore. 2016 Nov-Dez;40(6):1031-9. doi: 10.1590/0100-67622016000600008
48. Mihai G, Alexandru A, Mirancea I. Genetic variation and early selection in *Larix decidua* Mill. from progeny test in Romania. Ann For Sci. 2019 Aug;76(81):1-11. doi: 10.1007/s13595-019-0864-5