Insecticidal and oviposition deterrent effects of essential oils of Baccharis spp. and histological assessment against Drosophila suzukii (Diptera: Drosophilidae)

Michele Trombin de Souza¹, Mireli Trombin de Souza¹, Daniel Bernardi²*, Douglas José de Melo³, Paulo Henrique Gorgatti Zarbin¹ & Maria Aparecida Cassilha Zawadneak¹

The diverse flora of the Atlantic Forest is fertile ground for discovering new chemical structures with insecticidal activity. The presence of species belonging to the genus Baccharis is of particular interest, as these species have shown promise in pest management applications. The objective of this study is to chemically identify the constituents expressed in the leaves of seven species of Baccharis (B. anomal DC., B. calvescens DC., B. mesoneura DC., B. milleflora DC., B. oblongifolia Pers., B. trimera (Less) DC. and B. uncinella DC.) and to evaluate the toxicological and morphological effects caused by essential oils (EOs) on the larvae and adults of Drosophila suzukii (Diptera: Drosophilidae). Chemical analysis using gas chromatography-mass spectrometry (GC–MS) indicated that limonene was the main common constituent in all Baccharis species. This constituent in isolation, as well as the EOs of B. calvescens, B. mesoneura, and B. oblongifolia, caused mortality in over 80% of adults of D. suzukii at a discriminatory concentration of 80 mg L⁻¹ in bioassays of ingestion and topical application. These results are similar to the effect of spinosyn-based synthetic insecticides (spinetoram 75 mg L⁻¹) 120 h after exposure. Limonene and EOs from all species had the lowest LC₅₀ and LC₉₀ values relative to spinosyn and azadirachtin (12 g L⁻¹) in both bioassays. However, they showed the same time toxicity over time as spinetoram when applied to adults of D. suzukii (LT₅₀ ranging from 4.6 to 8.7 h) in a topical application bioassay. In olfactometry tests, 92% of D. suzukii females showed repellent behavior when exposed to the EOs and limonene. Likewise, the EOs of B. calvescens, B. mesoneura, and B. oblongifolia significantly reduced the number of eggs in artificial fruits (≅ 7.6 eggs fruit⁻¹), differing from the control treatment with water (17.2 eggs fruit⁻¹) and acetone (17.6 eggs fruit⁻¹). According to histological analyses, the L₃ larvae of D. suzukii had morphological and physiological alterations and deformations after exposure to treatments containing EOs and limonene, which resulted in high larval, pupal, and adult mortality. In view of the results, Baccharis EOs and their isolated constituent, limonene, proved to be promising alternatives for developing bioinsecticides to manage of D. suzukii.

The genus Baccharis (Asteraceae) comprises 435 species found exclusively in the Americas, with records from the south of Canada to southern South America¹. In Brazil, 179 species have been described, most of which occur in the southern region of the country². Baccharis spp. are distributed throughout the Atlantic Forest biome, a global hotspot of biodiversity that contains more plant species than other Brazilian biomes, with over 19,000 species, of which 7,600 are endemic³. Despite the remarkable levels of endemism that make the Atlantic Forest one of the most distinct regions of the Neotropics⁴, little is known about the potential genetic resources of aromatic plants present in this biome. Studies have only been carried out to verify the potential for biological control against arthropod pests for 27 species of Baccharis⁴.

¹Department of Basic Pathology, Federal University of Parana, Mailbox 19031, Curitiba, PR 81531-980, Brazil. ²Department of Plant Health, Federal University of Pelotas, Mailbox 354, Capão-do-Leão, RS 96010-900, Brazil. ³Department of Chemistry, Federal University of Parana, Mailbox 19020, Curitiba, PR 81531-990, Brazil. *email: dbernardi2004@yahoo.com.br
One important characteristic of *Baccharis* is the presence of secondary metabolites, specifically essential oils (EOs), which have a rich composition of terpenes that includes monoterpene, sesquiterpene, diterpene, and triterpene. The EOs of *Baccharis* spp. have been used for centuries as therapeutic agents in traditional medicine due to their spasmylytic, diuretic, anti-inflammatory, antibacterial, and antifungal properties. In addition, these EOs have been recognized for their fumigant, larvicidal, toxic and repellent effects against arthropods. Similarly, certain individual constituents of the oils, such as limonene, can cause the dissociation of lipids present in the cuticle of the exoskeleton of insects, causing dehydration and death.

Several EOs have shown promise for agricultural applications, mainly against mites and insects, including *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), a major pest of thin-skinned fruit with a global distribution. These values were significantly higher than those obtained with the spinosyn- and azadirachtin-based insecticides after 120 h of exposure (Table 2). This is because EOs have multiple modes of action that can reduce or prevent the evolution of resistance. They can also be used in organic production systems due to their high volatility and absence of residues on fruits.

Therefore, this study aims to: (i) characterize and isolate the main common constituents present in the leaves of seven species of *Baccharis* (*B. anomal*la, *B. calvescens*, *B. mesoneura*, *B. millefolia*, *B. oblongifolia*, *B. trimera*, and *B. uncinella*); (ii) evaluate the lethal toxicity of the EOs and isolated constituents on adults and larvae of *D. suzukii*; (iii) to assess the repellent effect of dry EO residues on oviposition by *D. suzukii*; and (iv) to analyze the morphological damage caused by EOs to the target organs of *D. suzukii* larvae, such as the brain, fat body, and Malpighian tubules using a histological assessment.

**Results**

In total, 29 chemical constituents were identified in the EOs from the samples of *Baccharis* spp. (Table 1, Fig. 1). These constituents comprised monoterpene hydrocarbons (which represented 34.9%–100% of EO constituents), oxygenated monoterpene (5.3–25.1%), sesquiterpene hydrocarbons (3.6–8.0%), and oxygenated sesquiterpenes (29.2–9.8%) (Table 1). Limonene was the main common constituent present in all species (12.5%–88.8%; Table 1). Other chemical constituents with a high relative proportion (%) included α-pinene (15.7%), β-pinene (11.8%), spathulenol (21.3%), and thujopsan-2-α-ol (13.2%) in *B. calvescens*; carquejyl acetate (22.0%) and palustrol (13.1%) in *B. trimera*; β-pinene (67.5%) in *B. millefolia*; α-pinene (72.6%) and β-pinene (14.1%) in *B. mesoneura*; α-thujene (20.2%), α-pinene (22.1%), and β-pinene (10.8%) in *B. oblongifolia*; and β-pinene (18.3%), thujopsan-2-α-ol (17.7%), and globulol (10.9%) in *B. anomal*la (Table 1, Fig. 1).

After 120 h of exposure, discriminatory concentrations of the EOs of *B. calvescens*, *B. mesoneura*, and *B. oblongifolia* and spinosyn (75 mg L−1) showed high toxicity, with *D. suzukii* adult mortality exceeding 90% due to ingestion and/or topical application (Fig. 2). These values were significantly higher than those obtained with limonene, the EOs of *B. anomal*la, *B. millefolia* *B. trimera*, and *B. uncinella*, or azadirachtin-based biopesticide (topical application [F = 212.32; d.f. = 9, 45; P < 0.0001]) ingestion [F = 194.3; d.f. = 9, 36; P < 0.0001]), which caused mortality rates of between 65 and 81% in the ingestion and topical application bioassays (Fig. 2). All of the products tested resulted in significantly (P < 0.0001) higher levels of mortality than the untreated controls (Fig. 2).

Based on the concentration–response curves and the overlapping confidence intervals of the LC50 and LC90 values for the ingestion and topical application bioassays, we found that these values were lower for all *Baccharis* EOs and limonene than for the spinosyn- and azadirachtin-based insecticides after 120 h of exposure (Table 2). Topical application of the *Baccharis* EOs and spinosyn showed no difference in LT90 values, which ranged from 4.55 to 8.71 h (Table 3). Meanwhile, the spinosyn-based insecticide had the lowest LT90 value in the ingestion bioassay (17.95 h; Table 3).

When the repellent effect of *D. suzukii* females was evaluated using olfactometers, it was observed that 92% of insects were repelled by treatments containing EOs, and 8% were repelled by the solvent (acetone; Fig. 3).

In addition, the dry residues of the EOs significantly reduced (F = 33.11; d.f.: 11, 28; P < 0.0001) oviposition by *D. suzukii* on artificial fruits treated with *B. calvescens* (7.5 eggs fruit−1), *B. mesoneura* (7.9 eggs fruit−1), and *B. oblongifolia* (7.2 eggs fruit−1) when compared to negative controls with water (17.2 eggs fruit−1) and acetone (17.6 eggs fruit−1) (Fig. 4).

All *Baccharis* spp. EOs and limonene caused greater larval mortality than controls with water or acetone (F = 22.14; d.f. = 9, 95; P < 0.0001), especially *B. anomal*la, *B. calvescens*, *B. mesoneura*, *B. millefolia*, and *B. oblongifolia*, which caused larval mortality of ≥94% (Table 4). A similar effect was also observed in the biological parameters of the pupation rate (F = 36.11; d.f. = 8, 95; P < 0.0001) and pupal mortality (F = 17.10; d.f. = 8, 95; P < 0.0001; Table 4). Also, these EOs and limonene caused macroscopic abnormalities on the surface of the cuticles of larvae, including diffuse pigmentation (Fig. 5I–f), darkening of the respiratory filaments (Fig. 5Ig), deformations and flaking (Fig. 5Ih,i), as well as decreased motility in *D. suzukii* L3 following a 2 h exposure to the treatments. Adult abnormalities were also observed, such as incomplete development (Figs. 4Ia, 5Ib), deformities in the abdomen (Fig. 5Ic–g), wings (Fig. 5Ic–g), legs (Fig. 5Ie), and pronotum (Fig. 5Ie). These effects were not observed in *D. suzukii* larvae and adults in treatments containing only water or acetone (Fig. 5).
Untreated *D. suzukii* larvae showed histological sections with well-defined morphology of the nervous system (Fig. 6Ia), the fat body (Fig. 6Iia), and the Malpighian tubules (Fig. 6Iia). Larvae treated with *Baccharis* EOs and limonene exhibited intense degeneration in the nervous system and the area of the neuropil (arrowheads; Fig. 6Ib–d), as well as irregular morphology of the cortical layer of the brain (arrow; Fig. 6Id). The fat body cells showed trophocytes with irregular morphology (arrows; Fig. 6Iib,c), changes related to nuclear chromatin condensation (dashed line; Fig. 6Iic), intense cytoplasmic vacuolization, and pycnotic nuclei (arrowhead; Fig. 6IId). The Malpighian tubules showed disintegration of the brush border (arrows; Fig. 6Iib,c), intense vacuolization, and nuclear chromatin condensation (pyknotic nuclei; arrowheads; Fig. 6IId).

### Discussion

This study provides the first verification that EOs extracted by hydrodistillation from the leaves of seven species of *Baccharis* and limonene, a constituent of these EOs, exhibit high toxicity against adults and larvae of *D. suzukii*. The EOs of *Baccharis* species are known for the predominance of monoterpenoids and sesquiterpenoids\(^1,4,5\), which have been reported to have the potential to cause mortality in different larval stages\(^31\), malformations in adults\(^9\), and to repel insects\(^7\). Of the different *Baccharis* species examined in this study, the only one whose oil has been reported in the literature as having insecticidal properties is *B. trimera*, which has been shown to be effective against pests of stored products\(^32\).

### Table 1. Essential oil composition (%) of samples fresh leaves of *Baccharis* spp. \(RI^\text{lit}\) Literature Retention Index, \(RI^\text{exp}\) Experimental Retention Index. *Species: 1 B. calvescens; 2 B. uncinella; 3 B. trimera; 4 B. milleflora; 5 B. mesoneura; 6 B. oblongifolia; and 7 B. anomala.* – Constituents not present.

| Constituents                  | \(RI^\text{lit}\) | \(RI^\text{exp}\) | % peak area |
|------------------------------|-------------------|-------------------|-------------|
|                              | 1     | 2     | 3     | 4     | 5     | 6     | 7     |
| α-thujene                    | 924   | 926   | 3.4   | -     | 0.3   | 4.2   | -    | 20.2 |
| a-pinene                     | 932   | 935   | 15.7  | -     | -     | -     | -    | 72.6 |
| sabine                      | 973   | 973   | -     | -     | -     | -     | -    | 9.9  |
| β-pinene                    | 974   | 974   | 11.8  | 7.6   | 2.0   | 67.5  | 14.1 | 10.8 |
| β-myrcene                    | 988   | 990   | -     | -     | 0.2   | -     | -    | 4.4  |
| α-cymene                     | 1022  | 1024  | 2.3   | -     | 0.3   | -     | -    | -    |
| limonene                     | 1024  | 1026  | 20.6  | 88.8  | 42.2  | 28.2  | 13.3 | 12.5 |
| β-phellandrene               | 1029  | 1033  | -     | -     | -     | -     | -    | 6.6  |
| β-phorene                    | 1043  | 1041  | -     | 0.4   | -     | -     | -    | -    |
| β-ocimene                    | 1044  | 1041  | -     | 0.3   | -     | -     | -    | -    |
| Monoterpane hydrocarbon      | 53.8  | 96.4  | 45.7  | 99.9  | 100.0 | 86.5  | 34.9 |
| terpene-4-ol                 | 1181  | 1177  | -     | -     | -     | -     | -    | 5.5  |
| a-terpineol                  | 1188  | 1894  | -     | -     | -     | -     | -    | 3.0  |
| myrtanol                     | 1194  | 1197  | -     | -     | -     | -     | -    | 2.3  |
| linalool                     | 1099  | 1095  | -     | -     | -     | -     | -    | -    |
| trans-pinocarveol            | 1135  | 1137  | -     | -     | -     | -     | -    | -    |
| carveol                      | 1216  | 1216  | -     | 3.1   | -     | -     | -    | -    |
| carqueyl acetate             | 1298  | 1299  | -     | 22.0  | -     | -     | -    | -    |
| Oxygenated monoterpane       | 0     | 0     | 25.1  | 0     | 0     | 5.5   | 5.3  |
| (E)-caryophyllene            | 1417  | 1418  | 4.9   | -     | -     | -     | -    | 1.7  |
| β-farnesene                  | 1442  | 1442  | 3.6   | -     | -     | -     | -    | -    |
| germacrene D                 | 1484  | 1485  | -     | -     | -     | -     | -    | 3.0  |
| bicyclogermacrene            | 1500  | 1500  | -     | -     | -     | -     | -    | 3.3  |
| Sesquiterpene hydrocarbon    | 4.9   | 3.6   | 0     | 0     | 0     | 8.0   | 0    |
| palustrol                    | 1577  | 1576  | -     | 13.1  | -     | -     | -    | -    |
| spathulenol                  | 1577  | 1576  | 21.3  | 2.6   | -     | -     | -    | 8.0  |
| thujopsan-2-α-ol             | 1587  | 1590  | 13.2  | -     | -     | -     | -    | 17.7 |
| globulol                     | 1590  | 1595  | -     | -     | -     | -     | -    | 10.9 |
| viridiflorol                 | 1592  | 1592  | 4.4   | 3.7   | -     | -     | -    | 7.2  |
| ledol                        | 1602  | 1602  | -     | 3.7   | -     | -     | -    | 3.7  |
| α-muurolol                   | 1644  | 1647  | -     | -     | -     | -     | -    | 6.3  |
| β-eudesmol                   | 1650  | 1653  | -     | 6.1   | -     | -     | -    | 6.0  |
| Oxygenated sesquiterpene     | 38.9  | 0     | 29.2  | 0     | 0     | 0     | 59.8 |
| Total chemical composition (%)| 97.6  | 100   | 100   | 99.9  | 100   | 100   | 100  |
Figure 1. GC/MS chromatogram of essential oil of species de Baccharis: (A) B. calvescens; (B) B. uncinella; (C) B. trimera; (D) B. milleflora; (E) B. mesoneura; (F) B. oblongifolia; (G) B. anomala. Chemical constituents: (1) α-thujene; (2) α-pinene; (3) β-pinene; (4) o-cymene; (5) limonene; (6) caryophyllene; (7) spathulenol; (8) thujopsan-2-α-ol; (9) viridiflorol; (10) farnesene; (11) sabinene; (12) β-myrcene; (13) β-phorone; (14) β-ocimene; (15) carveol; (16) carquejyl acetate; (17) palustrol; (18) Ledol; (19) β-eudesmol; (20) β-phellandrene; (21) terpinen-4-ol; (22) germacrene-D; (23) bicyclogermacrene; (24) linalool; (25) trans-pinocarveol; (26) α-terpineol; (27) myrtenol; (28) globulol; (29) α-muurolol.
The gas chromatography-mass spectrometry (GC–MS) analysis showed that limonene was the only major constituent found in all Baccharis species, the content of which varied between 12.5 and 88.8% in the studied species. In Brazil, limonene is a product marketed for use in treatments against fleas in domestic animals in the form of shampoos, sprays, and aerosols. However, previous studies have found that the compound exhibits toxic activity against several arthropods, such as Leptinotarsa decemlineata Say (Coleoptera: Chrysomelidae), Sitophilus zeamais Motschulsky, (Coleoptera: Curculionidae), Tribolium confusum du Val (Coleoptera: Tenebrionidae).

**Figure 2.** Drosophila suzukii mortality when submitted to various treatments in topical application and ingestion bioassays. Means followed by different letters on the columns (within each exposition bioassay) indicate significant differences between treatments (GLM with quasi-binomial distribution followed by post hoc Tukey test, \( P < 0.05 \)).

| Treatments | \( \text{Slope} \pm \text{SE} \) | \( \text{LC}_{50} \) (95% CI)a,b | \( \text{LC}_{90} \) (95% CI)a,b | \( \chi^{2} \) | df |
|------------|--------------------------|-------------------------------|-------------------------------|------------|----|
| **Ingestion bioassay** | | | | | |
| Limonene   | 2.90 ± 0.34               | 19.81 (18.54–22.15) b         | 25.11 (23.11–26.14) b         | 9.08       | 6  |
| B. anomala | 2.81 ± 0.42               | 11.64 (8.74–13.45) a          | 18.98 (17.10–20.05) a         | 8.13       | 6  |
| B. calvescens | 3.12 ± 0.31               | 8.89 (6.83–10.45) a           | 17.42 (16.08–20.15) a         | 7.12       | 6  |
| B. mesoneuera | 2.98 ± 0.24                | 6.71 (5.12–9.11) a            | 17.02 (16.04–19.78) a         | 8.11       | 6  |
| B. milleflora | 2.64 ± 0.32                | 6.44 (5.74–9.15) a            | 19.23 (18.78–20.07) a         | 8.45       | 6  |
| B. oblongifolia | 3.10 ± 0.43                | 6.52 (5.02–7.94) a            | 18.13 (16.17–20.19) a         | 7.12       | 6  |
| B. trimera | 2.67 ± 0.30               | 10.42 (8.16–11.11) a          | 21.04 (17.78–22.04) a         | 9.75       | 6  |
| B. uncincta | 3.14 ± 0.27               | 7.82 (6.57–10.14) a           | 17.20 (16.01–18.56) a         | 8.19       | 6  |
| Spinetoram | 2.79 ± 0.21               | 25.40 (21.50–27.17) b         | 51.89 (48.6–53.17) c           | 9.76       | 6  |
| **Topical application bioassay** | | | | | |
| Limonene   | 2.14 ± 0.13               | 11.52 (9.10–13.12) b          | 29.74 (27.11–30.05) b         | 7.13       | 6  |
| B. anomala | 3.11 ± 0.42               | 5.94 (3.72–7.10) a            | 18.74 (16.11–20.15) a         | 9.12       | 6  |
| B. calvescens | 2.75 ± 0.53                | 3.40 (2.75–5.10) a            | 19.45 (17.83–21.14) a         | 6.04       | 6  |
| B. mesoneuera | 2.89 ± 0.42                | 4.14 (3.74–5.01) a            | 18.11 (17.54–20.04) a         | 8.13       | 6  |
| B. milleflora | 3.12 ± 0.32                | 5.69 (3.15–7.97) a            | 19.75 (17.45–20.12) a         | 8.02       | 6  |
| B. oblongifolia | 2.98 ± 0.54                | 3.12 (2.66–3.89) a            | 22.15 (16.54–25.19) a         | 7.74       | 6  |
| B. trimera | 3.10 ± 0.64               | 5.83 (3.78–5.19) a            | 16.11 (13.07–22.78) a         | 6.82       | 6  |
| B. uncincta | 2.96 ± 0.89               | 7.76 (4.40–8.75) a            | 21.07 (16.01–23.98) a         | 7.02       | 6  |
| Spinetoram | 3.75 ± 0.11               | 10.55 (10.05–12.11) b         | 54.13 (49.54–58.11) c          | 8.10       | 6  |
| **Azadirachtin** | 2.18 ± 0.10               | 204.13 (199.66–206.11) c      | 416.84 (399.14–420.16) d       | 8.25       | 6  |

Table 2. Estimation of \( \text{LC}_{50} \) and \( \text{LC}_{90} \) (in mg L\(^{-1}\)) and confidence interval of Baccharis spp., limonene, spinosyn-based synthetic insecticide and azadirachtin on adults of Drosophila suzukii at 120 HAE in topical bioassays and ingestion. \( \text{df} \) degrees of freedom. \( ^{a} \text{LC}_{50} \) and \( ^{b} \text{LC}_{90} \) Insecticide concentrations (mg L\(^{-1}\)) required to kill 50% or 90% of D. suzukii adults, respectively (CI 95% confidence interval). \( ^{b} \text{LC}_{50} \) and \( ^{b} \text{LC}_{90} \) values designated by different letters within a column are significantly different from each other through nonoverlap of 95% Cis. \( ^{c} \text{P} > 0.05 \) in the goodness-of-fit test.
Table 3. Estimation of the median lethal time (LT₅₀, in h) and confidence interval of formulations with Baccharis spp., limonene, spinosyn-based synthetic insecticide and azadirachtin on Drosophila suzukii adults using the maximum concentration tested. df degrees of freedom. *LT₅₀: time required to kill 50% of D. suzukii adults following exposure to treatments (CI 95% confidence interval). **LT₅₀ values designated by different letters within a column are significantly different from each other through nonoverlap of 95% CIs. P > 0.05 in the goodness-of-fit test.

| Treatments | Concentration (mg L⁻¹) | Slope ± SE | LT₅₀ (95% CI)b | χ²c | df |
|------------|------------------------|------------|----------------|-----|----|
| Ingestion bioassay |  |  |  |  |  |
| Limonene | 80 | 2.95 ± 0.74 | 66.41 (50.10–70.32) | b | 4.12 | 27 |
| B. anomala | 80 | 2.89 ± 0.06 | 43.24 (40.11–50.12) | b | 7.11 | 27 |
| B. calvescens | 80 | 3.87 ± 0.15 | 48.05 (41.18–55.19) | b | 8.15 | 27 |
| B. mesoneura | 80 | 3.14 ± 0.21 | 43.16 (39.17–50.12) | b | 9.20 | 27 |
| B. milleflora | 80 | 3.00 ± 0.17 | 46.30 (40.14–53.12) | b | 7.14 | 27 |
| B. oblongifolia | 80 | 2.98 ± 0.23 | 55.42 (41.19–60.02) | b | 8.12 | 27 |
| B. trimera | 80 | 2.87 ± 0.10 | 42.76 (37.13–50.12) | b | 9.76 | 27 |
| B. uncincta | 80 | 2.99 ± 0.45 | 52.48 (42.19–59.13) | b | 8.30 | 27 |
| Spinetoram | 75 | 3.09 ± 0.41 | 17.95 (11.12–24.98) | a | 9.75 | 27 |
| Azadirachitin | 250 | 2.72 ± 0.22 | 60.10 (50.07–69.43) | b | 7.12 | 27 |
| Topical Application Bioassay |  |  |  |  |  |
| Limonene | 80 | 2.98 ± 0.45 | 11.78 (10.12–13.20) | b | 8.97 | 27 |
| B. anomala | 80 | 3.07 ± 0.31 | 7.76 (5.15–9.72) | a | 7.12 | 27 |
| B. calvescens | 80 | 2.17 ± 0.24 | 8.71 (6.45–9.32) | a | 5.23 | 27 |
| B. mesoneura | 80 | 2.15 ± 0.32 | 4.89 (3.73–7.11) | a | 6.10 | 27 |
| B. milleflora | 80 | 3.83 ± 0.43 | 4.76 (2.75–6.89) | a | 7.14 | 27 |
| B. oblongifolia | 80 | 3.67 ± 0.30 | 4.55 (4.00–6.45) | a | 8.12 | 27 |
| B. trimera | 80 | 3.94 ± 0.27 | 4.96 (4.32–5.12) | a | 7.94 | 27 |
| B. uncincta | 80 | 2.97 ± 0.31 | 7.10 (5.12–9.25) | a | 5.17 | 27 |
| Spinetoram | 75 | 3.11 ± 0.23 | 6.04 (4.13–8.19) | a | 9.32 | 27 |
| Azadirachitin | 250 | 3.80 ± 0.34 | 20.69 (19.13–22.74) | c | 9.45 | 27 |

Figure 3. Repellence of Drosophila suzukii adults in the in bioassays with two-way olfactometer. Asterisks indicate significant differences between treatments according to Student's t-test (P < 0.05).
These findings are corroborated by previous studies that found that Baccharis species contained large amounts of monoterpene hydrocarbons (α-thujene, α-pinene, β-pinene, and limonene), oxygenated monoterpenes (carquejyl acetate), and oxygenated sesquiterpenes (palustrol, spathulenol, and thujopsan-2-α-ol)\(^5\). However, for both bioassays performed in this study, we found that the substances contained in the EOs of B. calvescens, B. mesoneura, and B. oblongifolia had the greatest effect on adults of D. suzukii, with similar mortality rates (over 90%) to synthetic spinosyn-based insecticides. These species showed efficacy comparable to the organophosphates, pyrethroids and spinosyns used to management adults of D. suzukii\(^40–43\). It is known that the potential of EOs depends on the chemical constituents and their proportions found in the samples\(^44\). Likewise, the interactions of constituents contained in EOs have been reported to have synergistic action, providing a significant increase in the effectiveness of formulations\(^44,45\). The insecticide azadirachtin, meanwhile, showed the lowest toxicity on adults of D. suzukii. However, even though this product exhibits low toxicity for this pest, it may favor pest suppression by repelling the insects or reducing oviposition capacity, as verified in a previous study\(^40\).

In the topical application bioassays, we observed that adults of D. suzukii died more quickly (LT\(_{50}\) of 4.55–11.78 h) than during the ingestion bioassays (LT\(_{50}\) of 42.76–66.41 h). This difference in the toxicity of Baccharis spp. oils evaluated using the two bioassay methods can be attributed to the fact that topically applied EOs directly penetrate the insect hemolymph in a single dose. In contrast, ingested EOs are administered gradually and in small amounts over the feeding period (24 h). This also suggests that the higher toxicity by topical application results from damage to the nervous and/or respiratory systems of insects since these are the main routes of intoxication by substances absorbed by the cuticle\(^29\). Furthermore, during the ingestion period, treatments remain in the intestine of the insects for longer, requiring a longer time for metabolization and/or excretion of the chemical\(^29\). These results too may be related to the lipophilic constitution and the low molecular weight of

### Table 4. Larval mortality (LM), pupation rate (PR), pupal mortality (PM), and deformity of Drosophila suzukii adults exposed to different treatments. Columns followed by the same letter are not significantly different from one another (GLM with an almost binomial distribution followed by Tukey’s test: P > 0.05).

| Treatments       | LM (%)            | PR (%)            | PM (%)            |
|------------------|-------------------|-------------------|-------------------|
| Limonene         | 88.0 ± 4.06 ab    | 12.0 ± 4.06 b     | 95.3 ± 4.70 b     |
| B. anomala       | 94.0 ± 1.87 a     | 6.0 ± 1.65 bc     | 100.0 ± 0.00 b    |
| B. calvescens    | 97.0 ± 2.22 a     | 3.0 ± 2.22 c      | 100.0 ± 0.00 b    |
| B. mesoneura     | 99.1 ± 0.99 a     | 0.9 ± 0.97 c      | 100.0 ± 0.00 b    |
| B. milleflora    | 95.0 ± 2.73 a     | 5.0 ± 2.7 c       | 100.0 ± 0.00 b    |
| B. oblongifolia  | 100.0 ± 0.00 a    | –                 | –                 |
| B. trimera       | 86.0 ± 3.67 b     | 14.0 ± 3.67 b     | 96.3 ± 3.70 b     |
| B. uncinella     | 85.0 ± 4.74 b     | 15.0 ± 4.74 b     | 100.0 ± 0.00 b    |
| Acetone          | 0.00 ± 0.00 c     | 100.0 ± 0.00 a    | 0.0 ± 0.00 a      |
| Water            | 0.00 ± 0.00 c     | 100.0 ± 0.00 a    | 0.0 ± 0.00 a      |
| F                | 22.14             | 36.11             | 17.10             |
| d.f.             | 9.95              | 8.95              | 8.95              |
| P values         | < 0.0001          | < 0.0001          | < 0.0001          |

### Figure 4. Number of eggs of Drosophila suzukii in artificial fruits following immersion in treatments. Bars (± SE) with the same letter are not significantly different (GLM with a quasi-binomial distribution\(^47\) followed by Tukey post hoc test: P < 0.05).
the chemical constituents of these EOs\(^5\). These characteristics may enable diffusion through the cellular membrane, causing physiological disruptions in the insect membrane and leading to mortality\(^6\,7\). Likewise, they can trigger the inhibition of acetylcholinesterase (AChE) activity, which has been verified in adults of *S. zeamais*, *T. castaneum*\(^8\) and the spider mite, *Tetranychus urticae* Koch (*Acarina*)\(^9\).

In addition to their high toxicity, the EOs of *B. calvescens*, *B. mesoneura*, and *B. oblongifolia* reduced the oviposition capacity of *D. suzukii* by up to 43%. This fact corroborates the observations in the double-choice olfactometry repellency tests, in which females of *D. suzukii* avoided the olfactometry arm that contained a piece of filter paper containing 5 µL of EOs, preferring instead to move into arm containing the negative treatment (acetone).

Products that reduce oviposition or repel *D. suzukii* females can reduce the incidence of epidermal rupture by oviposition, which consequently reduces phytopathogen infestation\(^10\), while also avoiding the attraction of other drosophilids such as *Zaprionus indianus* Gupta (*Diptera: Drosophilidae*), which can accelerate damage to crops, as seen in strawberry\(^11\) and persimmons\(^12\). In addition, it helps decrease pest population density in crops\(^13,14\).

In addition to their repellent effects and, consequently, their ability to reduce oviposition, *B. anomala*, *B. calvescens*, *B. mesoneura*, *B. milleflora*, *B. oblongifolia*, and limonene had a major impact on the L3 larvae of *D. suzukii*. Specifically, they were able to affect the species' pupation rate and pupal mortality negatively. The larvicidal effect of these materials may be related to the polarity of the EOs (lipophilic substances), which allows *D. suzukii*. Specifically, they were able to affect the species' pupation rate and pupal mortality negatively. The higher mortality during the juvenile phase\(^15\). Besides, impact on females' fertility, as well as, emerged larvae have less vitality as a result of insufficient food intake and development following treatment with *B. anomala* and limonene. (II) (c–g) deformities in the abdomen and wings treated with *B. mesoneura*, *B. milleflora* and *B. oblongifolia*, and limonene. (II) (c–e); (g) leg deformities treated with *B. anomala*, *B. mesoneura*, *B. uncinella*, and limonene. (I) (d) deformities in the pronotum treated with *B. oblongifolia*. All larvae and adults were assessed at a discriminatory concentration of 8% of EOs.

**Figure 5.** Macroscopic damage to larvae and adults of *Drosophila suzukii* after treatment with essential oils (EOs) from leaves of *Baccharis* spp. and limonene (40×). (I,II) No color change or deformity was observed in *D. suzukii* larvae and adults 2 h after the treatments (control group). (I) (a,b) swelling of L3 epithelial cells treated with limonene and *Baccharis trimera*, respectively. (I) (c,d) darkening in the respiratory filaments of L3 treated with *B. calvescens* and *Baccharis oblongifolia*, respectively. (I) (e–h) diffuse pigmentation in the cuticle of L3 treated with *B. anomala*, *B. mesoneura*, *B. milleflora*, and *B. uncinella*. (I) (h,i) deformations and skin flakes of L3 treated with *B. calvescens* and *B. oblongifolia*. (II) (a,b) emergence and incomplete development following treatment with *B. anomala* and limonene. (II) (c–g) deformities in the abdomen and wings treated with *B. mesoneura*, *B. milleflora* and *B. oblongifolia*, and limonene. (II) (c–e); (g) leg deformities treated with *B. anomala*, *B. mesoneura*, *B. uncinella*, and limonene. (I) (d) deformities in the pronotum treated with *B. oblongifolia*. All larvae and adults were assessed at a discriminatory concentration of 8% of EOs.
In this study, *B. calvescens*, *B. mesoneura*, and *B. oblongifolia* were shown to have neurotoxic mechanisms, including the neurodegeneration and alteration of the morphology of the cortical layer and neuropils. Similar observations were reported with larvae of *Cochliomyia macellaria* (Diptera: Calliphoridae) after being treated with the oil of *Curcuma longa* L.52. In that study, the authors demonstrated the occurrence of vacuolar degeneration and alteration of the hypnotic profile of the brain. Also, *D. suzukii* larvae exposed to EOs showed damage to the adipose body, including cytoplasmic vacuolization and irregular morphology of trophocytes with hypnotic nuclei, signaling a possible mechanism of excretion of EOs. This process of vacuolization may indicate that these cells are in the process of dying, as has been demonstrated in larvae of *C. quinquefasciatus*60. Besides, we observed that the fat body nucleus of *D. suzukii* larvae was divided into smaller fragments with the presence of nuclear chromatin and pyknotic nuclei (np) when exposed to the constituent limonene (arrowheads). These results corroborate those described for *Apis mellifera* (Hymenoptera: Apidae)61 and *C. macellaria*52. These physiological disturbances caused by EOs and limonene in *D. suzukii* larvae are typical of cells submitted to classical apoptosis62, consisting of self-destruction of cells into smaller, highly condensed fragments.

The results found in the study of larval and adult *D. suzukii* clearly demonstrate the toxic activity and sublethal effects of the EOs of *Baccharis* spp. and limonene, an isolate of these EOs. Furthermore, this study is the first to verify the histological effects of EOs on *D. suzukii* larvae. This can help to determine the action sites of these compounds on insects. However, considering that these findings has not been fully explained, we are aware that new tests, focused especially on the selectivity of these botanists over natural enemies intentionally released63,64 and naturally present in the environment65,66, may in the future subsidize methods for integrating natural enemies and the development of EO-based biopesticides. Despite this, the use of these substances as such has limitations due to flammability, low dispersion in water, phytotoxicity67–69. In this sense, the development of formulations based on stable EO reduces these negative aspects and, at the same time, improves the effectiveness against pests and reduces the side effects on the beneficial ones. We are currently conducting work to investigate
| Treatments       | Description                                          | Geographic coordinates of origin | Discriminatory concentration tested | Origin/manufacturer                      |
|------------------|------------------------------------------------------|----------------------------------|-------------------------------------|-----------------------------------------|
| Limonene EO      | Essential oil extracted from the leaves of *B. anomalisa DC. (pre-commercial) | 25° 29' 45.04" S 48° 59' 56.58" W | 80                                  | Sigma Aldrich (São Paulo, SP, Brazil)   |
| Baccharis anomalisa EO | Essential oil extracted from the leaves of *B. anomalisa DC. (pre-commercial) | 25° 30' 18.44" S 49° 1' 14.47" W | 80                                  | Laboratory extraction and formulation (Curitiba, Paraná, Brazil) |
| Baccharis calvescens EO | Essential oil extracted from the leaves of *B. calvescens DC. (pre-commercial) | 25° 29' 33.95" S 49° 0' 41.05" W | 80                                  | Laboratory extraction and formulation (Curitiba, Paraná, Brazil) |
| Baccharis mesoneura EO | Essential oil extracted from the leaves of *B. mesoneura DC. (pre-commercial) | 25° 30' 38.67" S 49° 0' 24.12" W | 80                                  | Laboratory extraction and formulation (Curitiba, Paraná, Brazil) |
| Baccharis milleflora EO | Essential oil extracted from the leaves of *B. milleflora DC. (pre-commercial) | 25° 28' 37.90" S 48° 59' 35.40" W | 80                                  | Laboratory extraction and formulation (Curitiba, Paraná, Brazil) |
| Baccharis oblongifolia EO | Essential oil extracted from the leaves of *B. oblongifolia Pers. (pre-commercial) | 25° 30' 38.67" S 49° 0' 51.23" W | 80                                  | Laboratory extraction and formulation (Curitiba, Paraná, Brazil) |
| Baccharis trimera EO | Essential oil extracted from the leaves of *B. trimera (Less) DC. (pre-commercial) | 25° 31' 4.29" S 48° 59' 57.55" W | 80                                  | Laboratory extraction and formulation (Curitiba, Paraná, Brazil) |
| Baccharis uncinella EO | Essential oil extracted from the leaves of *B. uncinella DC. (pre-commercial) | 25° 31' 4.29" S 48° 59' 57.55" W | 80                                  | Laboratory extraction and formulation (Curitiba, Paraná, Brazil) |
| Delegate         | Spinetoram (250 g kg⁻¹)                              |                                  | 75                                  | Corteva Agriscience (São Paulo, São Paulo, Brazil) |
| Azamax           | Azadirachtin (12 g L⁻¹)                              | 250                              |                                     | UPL Brazil, Ltda (Campinas, São Paulo, Brazil) |

Table 5. Insecticides evaluated for the management of *Drosophila suzukii*. *a*Laboratory of Ecophysiology, Federal University of Paraná (Extraction) and Laboratory of Semi-chemistry, Federal University of Paraná (Formulation), Curitiba, Paraná State, Brazil. *b*Concentration: 75 mg of commercial product per L of water (Delegate); 250 mL of commercial product per 100 L of water (Azamax); 80 mg L⁻¹ (0.16 µL) of essential oils per 2 mL of acetone.

Material and methods

Collection of plant material for essential oil extraction. Table 5 summarizes information on the selected species of *Baccharis* (*B. anomalisa, B. calvescens, B. mesoneura, B. milleflora, B. oblongifolia, B. trimera, and B. uncinella*) used in the treatments and control. The species were identified by the specialist Osmar dos Santos Ribas and vouchers were deposited at the Municipal Botanical Museum (MBM Herbarium) in Curitiba, Paraná, Brazil (25° 28’ 37.90 S, 49° 59’ 34.50 W and 960 m altitude). The collected leaves were cut into segments of approximately 2 cm, and EOs were hydrodistilled in a Clevenger-type apparatus (Vidrolabor, São Paulo, Brazil) for 4 h and 30 min. Subsequently, the hydrodistillate was separated using anhydrous sodium sulfate. The samples were kept in a freezer at −20 °C until chemical analysis was carried out. We decided to include limonene on its own in the bioassays because it is the only major constituent (≥10%) present in all species studied. Samples of D-limonene (CAS: 5989-27-5) were obtained from Sigma-Aldrich Brazil (São Paulo, Brazil) with ≥99% purity.

Chemical analysis of essential oils: identification and quantification. We performed GC–MS using a Shimadzu 2030 gas chromatograph coupled to a Shimadzu TQ8040 sequential mass detector (GC–MS/MS). The GC was equipped with a fused HP-5MS capillary column (film thickness 30 m x 0.25 mm x 0.25 µm) coated with a stationary phase of 5% phenyl-95% dimethylpolysiloxane. Helium was used as a drag gas at a flow rate of 1.0 mL min⁻¹. The temperature setting was set to increase from 60 to 240 °C at a rate of 3 °C min⁻¹ and held at 240 °C for 10 min. The injector temperature was maintained at 250 °C. The essential oil samples were diluted into a 1% hexane solution, and 1.0 µL of the solution was injected with a partition ratio of 1:30. The mass detector was operated in electron impact mode (70 eV). The transfer line was kept at 240 °C for 10 min. The injector temperature was maintained at 250 °C. The essential oil samples were treated with the same experimental conditions, and compared with data in the literature. The structure of limonene was confirmed by injecting a commercial standard solution (Sigma-Aldrich Brazil).
Breeding and maintenance of *Drosophila suzukii*. The adults of *D. suzukii* used in bioassays were in their tenth generation. Breeding was performed using insects collected in the strawberry fields (*Fragaria × ananassa* Duchesne) in January 2018 in Curitiba, Paraná, Brazil (31° 38’ 20” S, 52° 30’ 43” W). In the laboratory, the infested strawberries were placed individually in plastic jars (150 mL) with a perforated lid (2 cm in diameter) and covered with cheesecloth containing a thin layer of vermiculite (1 cm). The fruits were kept in an air-conditioned room (25 ± 2 °C, 70 ± 10% RH, and 12-h photoperiod) until the emergence of adults. Following emergence, the adults (males and females) were transferred to glass bottles (300 mL) containing an artificial diet (12 mL)³. Seven-day-old adults were used in all bioassays, which were deprived of food for 8 h, though they were supplied with water in hydrophilic cotton.

Bioassays. All bioassays were conducted under controlled conditions (25 ± 2 °C, 70 ± 10% RH, and 12-h photoperiod) using a completely randomized design. The treatments and discriminatory concentrations used are listed in Table 5. Concentrations (solutions) of 2.5, 5.0, 7.5, 10, 20, and 40 mg L⁻¹ of the intact EOs of *Baccharis* spp. (*B. anomala*, *B. calvescens*, *B. mesoneura*, *B. milleflora*, *B. oblongifolia*, *B. trimera*, and *B. uncinella*) and limonene were prepared by diluting all treatments in acetone (PanReac-UV-IR-HPLC-GPC PAI-ACS, 99.9% purity). A spinosyn-based insecticide (Spinetoram –7.5 mg a.i. L⁻¹; Delegate 250WG, Dow AgroSciences, Santo Amaro, São Paulo, Brazil) and an azadirachtin-based bioinsecticide (azadirachtin+3-tigloyl-azadiractol, 1.2 mL a.i. L⁻¹; Azamax 1.2 EC, UPL Brazil, Campinas, São Paulo, Brazil) were used as positive controls (Table 5). The solvent (water or acetone) used in the solubilization of the respective treatments were used as negative controls.

Discriminatory bioassays (initial experiment). In order to evaluate the lethal toxicity of *Baccharis* spp. EOs and limonene, initial tests were performed using ingestion bioassays and topical application using discriminatory concentrations on adults of *D. suzukii* (Table 5). For the ingestion bioassays, 16 adults of *D. suzukii* (eight couples) were grouped in transparent plastic cages (1 L) inverted in plastic Petri dishes (25 cm diameter). The top side of the cages (i.e., the bottom of the containers) was sealed with a cheesecloth-type fabric to allow gas exchange. Once the solutions (treatment) were prepared, the products were supplied to the flies by capillarity in hydrophilic cotton rolls inside a 10 mL glass bottle. After 24 h of exposure, the treatments were removed and replaced with an artificial diet and distilled water until the end of the evaluation period.

In the topical application bioassay, adults of *D. suzukii* (ten couples) were separated and placed in transparent glass tubes (1.3 cm in diameter × 10 cm in length), which were closed at the top with hydrophilic cotton. Subsequently, the flies were transferred to a petri dish (9 cm in diameter) lined with filter paper and sedated in ethyl ether for 40 to 60 s to apply the treatments. The solutions (2 mL) were then sprayed using a Potter Tower (Burkard Scientific, Uxbridge, UK) at a working pressure of 0.049 MPa, resulting in an average residue deposition of 1.0 mg cm⁻². After spraying, the insects were placed in transparent plastic cages (1 L) as described above and fed an artificial diet and distilled water throughout the evaluation period.

In both tests, the experimental design was entirely randomized, with 10 treatments containing five repetitions (cages) with 16 adults (eight couples) in the ingestion bioassay and four repetitions (cages) with 20 adults (ten couples) in the topical bioassay. Mortality in each treatment was evaluated at 1 h intervals for the first 24 h after exposure to treatments (HAET) and every 24 h between 24 and 120 HAET. Insects that did not react to the touch of a fine-tip brush were considered dead. The corrected mortality was calculated using Abbott’s formula⁴.

Concentration–response curves and average lethal time of the most promising treatments against *Drosophila suzukii*. Based on the initial bioassays, the most promising treatments were selected and submitted to a new bioassay to estimate the lethal concentrations that would result in mortality of 50% or 90% mortality among the flies (LC₅₀ and LC₉₀, respectively). Seven concentration ranges were defined for each treatment and exposure type in the bioassay: 25–80 mg L⁻¹ for the EOs of *Baccharis* spp. (*B. anomala*, *B. calvescens*, *B. mesoneura*, *B. milleflora*, *B. oblongifolia*, *B. trimera*, and *B. uncinella*) and limonene; 5–75 mg L⁻¹ for spinetoram; and 25–250 mg L⁻¹ for commercial azadirachtin-based bioinsecticide⁵. The exposure and assessment procedures, as well as the mortality criteria, were identical to the initial tests. Four replicates were used in the ingestion bioassays, each containing 20 flies (n = 80) for each insecticide concentration. In the topical bioassays, five replicates were performed with 16 flies per replicate (n = 80) per concentration of each insecticide tested. For the determination of LC₅₀ values (mean time required to kill 50% of the population) of the treatments on *D. suzukii* adults, the maximum concentration tested in the bioassays of ingestion and the topical application was used (Table 5). The experimental design and bioassay procedures were identical to those used in the initial experiments.

Repellent effect against *Drosophila suzukii* in olfactometer bioassay. To verify the effectiveness of EOs at repelling females of *D. suzukii* relative to acetone treatments, we began by placing individual females aged up to 24 h into glass tubes (1.3 cm in diameter × 10 cm in length). In the test, the glass tube containing the female was connected to a double-choice glass olfactometer with a diameter of 8.0 cm and an initial compartment of 20 cm on each side, under fluorescent light (60 W, luminance 290 lx). At the end of one of the olfactometer arms, we placed a filter paper measuring 4 × 10 cm and bent into an accordion shape, which contained 5 µL of an EO of *Baccharis* spp. (*B. anomala*, *B. calvescens*, *B. mesoneura*, *B. milleflora*, *B. oblongifolia*, *B. trimera*, or *B. uncinella*) or limonene at the discriminatory concentration (80 mg L⁻¹ of oil). Another filter paper was placed at the end of the other arm (4 × 10 cm), which contained 5 µL of acetone (control). Airflow in the system was supplied at a rate of 0.8 L min⁻¹ from a previously filtered source with active carbon and humidified in distilled water. The olfactometer was washed with neutral soap and hexane after every fourth repetition and then dried in a sterilization oven at 150 °C. After this process, the substances were replaced, and the evaluation
continued. Each treatment consisted of 40 replicates, each of which consisted of a female of D. suzukii (n = 40). The responses were considered positive (EOs, limonene, or acetone) when D. suzukii females reached the odor source or traveled at least 10 cm inside the olfactory arms and remained there for at least 1 min. Flies that did not move to either of the olfactory arms after one minute of release were discarded.

**Deterrence of oviposition by Drosophila suzukii.** Artificial fruits prepared with agar (19 g), raspberry gelatin (10 g), methylparaben (8 mL), consisting of 0.8 g dissolved in 8 mL of 99.9% ethyl alcohol; Nipagin, Vetec, Química Finz, Duque de Caxias, Rio de Janeiro, Brazil), and distilled water (reflux; 850 mL) were used as a substrate for oviposition. Using a Potter Tower (working pressure 0.049 MPa (Berkud Scientific, Uxbridge, United Kingdom)) 1 mL treatments of B. anomala, B. calvescens, B. mesoneura, B. milleflora, B. oblongifolia, B. trimera, and B. uncinella EOs and limonene were sprayed to a mean deposition residue of 0.4 mg cm⁻². The artificial fruits were then placed in an air-conditioned room (25 ± 2 °C, 70% ± 10% RH, and 12-h photoperiod) for three hours to let the excess moisture evaporate and, in turn, for residue deposition to occur. The fruits were placed individually in a plastic container (250 mL), covered on top with cheesecloth to allow gas exchange with the internal and external environment of the container. Five couples of D. suzukii (≥ 7 days old) that had previously mated were then released. After 24 h, the adults were removed, and the eggs in each fruit were counted using a Stemi 2000-C stereoscopic microscope (Carl Zeiss, Germany; × 40 magnification). The experimental design was in random blocks, with 30 replicates (fruits) per treatment.

**Lethal and sublethal effect on Drosophila suzukii larvae.** To evaluate the larvicidal effect of Baccharis spp. EOs, (B. anomala, B. calvescens, B. mesoneura, B. milleflora, B. oblongifolia, B. trimera, and B. uncinella) and limonene, groups of 20 D. suzukii larvae in stage L3 were placed in transparent glass tubes (2.5 cm diameter × 8 cm length) containing a filter paper (2 × 4 cm) impregnated with 0.2 mL of EO solutions solubilized in acetone (PanReac-UV-IR-HPLC-GPC PAI-ACS, 99.9% purity). For each treatment, a discriminatory concentration of 80 mg L⁻¹ of oils was used. Acetone and distilled water were used as negative control. Following EO application, the glass tubes were sealed at the top with cheesecloth to facilitate aeration and transferred to controlled conditions (25 ± 2 °C, 70 ± 10% RH, and 12-h photoperiod). The macroscopic damage to the larvae of D. suzukii was recorded with a Stemi 2000-C stereoscopic microscope (Carl Zeiss, Germany; × 40 magnification). The experimental design was entirely randomized with five replicates (20 larvae per replicate) for each concentration (n = 100). Larval mortality was assessed at 0, 24, and 48 h after the larvae and treatments were placed in the tubes. Total mortality (TM), pupation rate (PR), pupal mortality (PM), and adult deformity (AD) were calculated. Statistical analysis. All bioassays were conducted using a completely randomized design. Generalized linear models (GLM) of the quasi-binomial distributions were used to analyze mortality rate data. In all cases, the fit of the GLM was determined by using the half-normal probability plot with a simulation envelope. When significant differences were found among treatments, multiple comparisons (Tukey test, P < 0.05) via the ght function in the multcomp package with adjusted p values was performed. For comparisons of the average of two treatments in the repellency bioassay, we used the Student’s t-test. All of these analyses were carried out using R statistical software, version 2.15.1. A binomial model with a complementary log–log link function (gompit model) was used to estimate the lethal concentrations (LC₅₀ and LC₉₀), using the Probit Procedure in the software SAS version 9.2. A likelihood ratio test was used to test the hypothesis that the LCP or LTP values (lethal concentration or lethal time at which a percent mortality P is attained) were equal. If the hypothesis was rejected, pairwise comparisons were performed and significance was stated if CIs did not overlap. Finally, the mean lethal time (LT₅₀) was estimated for Probit analysis of correlated data. The percentage repellence (PR) was calculated using the formula: PR (%) = [(Nc – Nt)/(Nc + Nt)] × 100, where Nc was the number of insects present in the negative control (acetone) and Nt was the number of insects present in the treatment (EOs).

**Data availability**
This article does not report new empirical data or software. Received: 25 October 2020; Accepted: 4 February 2021 Published online: 17 February 2021
References

1. Budel, J. et al. Essential oils of five Baccharis species: Investigations on the chemical composition and biological activities. Molecules 23, 1–19 (2018).
2. Heiden, G. & Schneider, A. Baccharis in Lista de Espécies da Flora do Brasil, Jardim Botânico do Rio de Janeiro (2015). http://flora.dobrasil.b/jbrj/gb/floradobrasil/FB255. Accessed 10 September 2020.
3. Forzza, R. C. et al. New Brazilian floristic list highlights conservation challenges. Bioscience 62, 39–45 (2012).
4. Ramos Campos, F., Bressan, J. & Jasinski, V. C. G. Baccharis (Asteraceae): Chemical constituents and biological activities. Chem. Biodivers. 13, 1–17 (2016).
5. Trombin-Souza, M. et al. Chemical composition of the essential oils of Baccharis species from southern Brazil: A comparative study using multivariate statistical analysis. J. Essent. Oil Res. 29, 400–406 (2017).
6. Alves, K. F. et al. Baccharis dracunculifolia (Asteraceae) essential oil toxicity to Culex quinquefasciatus (Culicidae). Environ. Sci. Pollut. Res. 25, 31718–31726 (2018).
7. García, M., Donadel, O. J., Aranda, C. E., Tonon, C. E. & Sosa, M. E. Toxic and repellent effects of Baccharis salicifolia essential oil on Tribolium castaneum. Pest Manage. Sci. 61, 612–618 (2005).
8. Buss, E. A. & Park-Brown, S. G. Natural Products for Insect Pest Management. Preprint at https://edis.ifas.ufl.edu/in197 (2002).
9. Park, C. G., Jang, M., Yoon, K. A. & Kim, J. Insecticidal and acetylcholinesterase inhibitory activities of Lamiaeae plant essential oils and their major components against Drosophila suzukii (Diptera: Drosophilidae). Ind. Crop. Prod. 89, 507–513 (2016).
10. Asplén, M. K. et al. Invasion biology of Spotted Wing Drosophila (Drosophila suzukii): A global perspective and future priorities. J. Pest Sci. 88, 469–494 (2015).
11. De La Veja, G. J., Corley, J. C. & Soliani, C. Genetic assessment of the invasion history of Drosophila suzukii in Argentina. J. Pest Sci. 93, 63–75 (2020).
12. Rota-Stabelli, O. et al. Distinct genotypes and phenotypes in European and American strains of Drosophila suzukii: Implications for biology and management of an invasive organism. J. Pest Sci. 93, 77–89 (2020).
13. Bernardi, D. et al. Potential use of Annona by products to control Drosophila suzukii and toxicity to its parasitoid Trichopria anastrephae. Ind. Crop. Prod. 110, 30–35 (2017).
14. Kienzle, R., Groß, L. B., Caughman, S. & Rohlfs, M. Resource use by individual Drosophila suzukii reveals a flexible preference for oviposition into healthy fruits. Sci. Rep. 10, 3132 (2020).
15. Souza, M. T. et al. Physicochemical characteristics and superficial damage module persimmon infestation by Drosophila suzukii and Zapirios indianaus (Diptera: Drosophilidae). Environ. Gilead. Entomol. 49, 1290–1299 (2020).
16. Hamby, K. A. et al. Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of Drosophila suzukii. J. Pest Sci. 89, 605–619 (2016).
17. Sánchez-Ramos, I., Gómez-Casado, E., Fernández, C. E. & González-Núñez, M. Reproductive potential and population increase of Drosophila suzukii at constant temperatures. Entomol. Gen. 39, 103–115 (2019).
18. Spitaler, U. et al. Yeast species affects feeding and fitness of Drosophila suzukii adults. J. Pest Sci. 93, 1295–1309 (2020).
19. Santoëmme, G. et al. Habitat preference of Drosophila suzukii across heterogeneous landscapes. J. Pest Sci. 92, 485–494 (2019).
20. Tait, G. et al. Drosophila suzukii daily dispersal between distinctly different habitats. Entomol. Gen. 40, 25–37 (2020).
21. Delbac, L., Rusch, A. & Thiéry, D. Temporal dynamics of Drosophila suzukii in vineyard landscapes. Entomol. Gen. 40, 285–295 (2020).
22. Rendema, J. M., Wright, D., Buitenhuis, R. & Hallett, R. H. Plant essential oils and potassium metabisulﬁte as repellents for Drosophila suzukii (Diptera: Drosophilidae). Sci. Rep. 6, 21432 (2016).
23. Wiman, N. G. et al. Drosophila suzukii and population response to environment and management strategies. J. Pest Sci. 89, 653–665 (2016).
24. Santoëmme, G. et al. Integrated management of Drosophila suzukii in sweet cherry orchards. Entomol. Gen. 40, 297–305 (2020).
25. Mermez, S. et al. Timing and order of different insecticide classes drive control of Drosophila suzukii: a modeling approach. J. Pest Sci. https://doi.org/10.1007/s10340-020-01292-w (2020).
26. Gress, B. E. & Zalom, F. G. Identiﬁcation and risk assessment of spinosad resistance in a California population of Drosophila suzukii. Pest Manage. Sci. 75, 1270–1276 (2018).
27. Van Timmeren, S., Sial, A. A., Lank, S. K., Spaulding, N. R. & Isaacs, R. Development of a rapid assessment method for detecting insecticide resistance in Spotted Wing Drosophila (Drosophila suzukii Matzdorfa). Pest Manage. Sci. 75, 1782–1793 (2019).
28. Zanardi, O. Z. et al. Bioactivity of a matrine-based biopesticide against four pest species of agricultural importance. Crop Prot. 67, 160–167 (2015).
29. Souza, M. T. et al. Chemical composition of essential oils of selected species of Piper and their insecticidal activity against Drosophila suzukii and Trichopria anastrephae. Environ. Sci. Pollut. Res. 27, 13056–13065 (2020).
30. Kostyukovsky, M., Rafael, A., Gileadi, C., Demchenko, N. & Shaaya, E. Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: Possible mode of action against insect pests. Pest Manage. Sci. 58, 1101–1106 (2002).
31. Chaban, A. et al. Insecticidal activity of Baccharis dracunculifolia essential oil against Coccidiomyia macellaria (Diptera: calliphoridae). Nat. Prod. Res. 32, 2854–2958 (2017).
32. Charlie-Silva, L., Souza, L. M., Pereira, C. C., Mazzoneto, F. & Belo, M. A. A. Insecticidal efﬁcacy of aqueous extracts of Ricinus communis, Baccharis trimera and Chenopodium ambrosioides on adults of Alphitobius diaperinus. Arq. Vet. 35, 7–11 (2019).
33. Khorrarm, M. S., Nasabi, N. T., Jafarian, S. & Khosroshahi, S. The toxicity of selected monoterpine hydrocarbons as single compounds and mixtures against different developmental stages of Colorado potato beetle, Leptinotarsa decemlineata Say (Coleoptera: Chrysomelidae). J. Entomol. 8, 404–416 (2011).
34. Fang, R. et al. Insecticidal activity of essential oil of carum carvi fruits from China and its main components against two grain storage insects. Molecules 15, 9391–9402 (2010).
35. Malacrino, A., Campolo, O. & Laudani, A. Fumigant and repellent activity of limonene enantiomers against Tribolium confusum du Val. Neotrop. Entomol. 45, 597–603 (2016).
36. Macchioni, F. et al. Acaricidal activity of pine essential oils and their main components against Tyrophagus putrescentiae, a stored food mite. J. Agric. Food Chem. 50, 4586–4588 (2002).
37. Vignieri, R. et al. The role of the monoterpene composition in Pinus spp. needles, in host selection by the pine processionary caterpillar, Thaumetopoea pityocampa. Phytoparas. 27, 263–272 (1999).
38. Schuster, D. J., Thompson, S., Ortega, L. D. & Polston, J. E. Laboratory evaluation of products to reduce settling of sweet potato whitefly adults. J. Econ. Entomol. 102, 1482–1489 (2009).
39. Raina, A. et al. Effects of orange oil extract on the Formosan Subterranean Termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 100, 880–883 (2010).
40. Andreazza, F. et al. Toxicities and effects of insecticidal toxic baits to control Drosophila suzukii and Zapirios indianaus (Diptera: Drosophilidae). Pest Manage. Sci. 73, 146–152 (2017).
41. Bruck, D. J. et al. Laboratory and field comparisons of insecticides to reduce infestation of Drosophila suzukii in berry crops. Pest Manage. Sci. 67, 1375–1385 (2011).
42. Beers, E. H. et al. Developing Drosophila suzukii management programs for sweet cherry in the western United States. Pest Manage. Sci. 67, 1386–1395 (2011).
We thank the Brazilian Federal Agency for the Support and Evaluation of Graduate Education (CAPES) and the Brazilian National Council for Scientific and Technological Development (CNPQ) for financial support and scholarships.
Author contributions
M.T.S., M.T.S., D.B., P.H.G.Z. and M.A.C.Z. conceived and designed the research. M.T.S., M.T.S., D.B. and D.J.M. conducted experiments. M.T.S., M.T.S., D.B., D.J.M. and M.A.C.Z. analyzed the data. M.T.S., M.T.S., D.B., D.J.M., P.H.G.Z. and M.A.C.Z. wrote the manuscript. All authors reviewed and approved the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to D.B.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access
This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2021