

Non-consumptive effects of ant mitigate fruit damage

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1 Title: Non-consumptive effects of ant mitigate fruit damage

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5 **Abstract** *Bactrocera dorsalis* (Hendel) is a major global pest of fruits and vegetables.
6 Interestingly, field studies revealed that the presence of the red imported fire ant,
7 *Solenopsis invicta* Buren, reduces the visiting frequency of *B. dorsalis* and fruit
8 damage. However, the underlying mechanisms remain unknown. In this study, we
9 hypothesized that *S. invicta* can produce semiochemicals that non-consumptively
10 repel *B. dorsalis*, thereby reducing damage to fruits caused by feeding and oviposition.
11 We investigated how *S. invicta* poop, saliva, and footprints influence the behavior of
12 *B. dorsalis*. Subsequently, we analyzed the chemical composition of *S. invicta*
13 footprints and conducted field experiments to evaluate their efficacy as repellents
14 against *B. dorsalis*. The results demonstrated that *S. invicta* footprints effectively repel
15 the visiting behavior and decrease the number of eggs laid by *B. dorsalis*. Among the
16 nine compounds identified, d-limonene, acetic acid, and a mixture of seven
17 compounds showed strong repellent effects on the visiting frequency and egg-laying
18 of *B. dorsalis*. Field experiments confirmed the effectiveness of *S. invicta* footprint
19 compounds in controlling *B. dorsalis*, resulting in reduced fruit damage in mango,
20 guava, and wax apple trees. These studies provide valuable insights into the
21 non-consumptive effects of *S. invicta* footprints on *B. dorsalis* behavior, unraveling
22 the chemical communication between these two invasive species, and offer new pest
23 control methods using invasive predators when direct release is limited.

24 **Keywords** Invasive species · the oriental fruit fly · predation risks · predator
25 kairomones · pest managements

26 **Introduction**

27 The invasive species, *Bactrocera dorsalis* (Hendel), poses a significant risk to
28 fruit production when introduced to new areas (Fletcher 1987; Schutze et al. 2015;
29 Zeng et al. 2019; Mutamiswa et al. 2021). This pest is widely distributed in Asia,
30 Australia, and the Hawaiian Islands, and is capable of attacking over 400 plant species
31 through egg-laying and larval feeding, leading to tissue breakdown and internal
32 rotting (Zeng et al. 2019). Traditional control methods, such as synthetic pesticides
33 and male lures, have been used to reduce the population of *B. dorsalis*, but these
34 methods have negative impacts on non-target organisms and can contribute to
35 insecticide resistance in the pest (Liu et al. 2019). Additionally, male lures only target
36 male *B. dorsalis*, leaving female populations largely unaffected. Therefore, there is an
37 urgent need for environmentally friendly control methods that can target both male
38 and female *B. dorsalis* populations, such as biomaterials derived from natural animals.

39 Animal-derived biomaterials, such as sex and aggregation pheromones, as well
40 as predator kairomones, have been increasingly used in pest control (Witzgall et al.
41 2010; Yang et al. 2022; Kim et al. 2023; Okosun et al. 2013). For example, the
42 cuticular hydrocarbon from predator backswimmer deterred female mosquitoes from
43 ovipositing in natural pools (Silberbush et al. 2010); while chemicals from abdominal
44 gland secretions reduced small brown planthopper populations in rice paddies (Wen
45 and Ueno 2021). These studies demonstrate the potential of animal-derived
46 biomaterials, particularly those derived from predators, as effective and eco-friendly
47 solutions for pest control.

48 In a previous study where we observed that carambola fruits in orchards with
49 active nests of red imported fire ants (*Solenopsis invicta*) had lower infection rates
50 from *B. dorsalis* and a reduced number of visits compared to neighboring orchards
51 without such nests (unpublished data). Previous research has also demonstrated that
52 the fire ant species can prey on the mature larvae or pupae of *B. dorsalis*, resulting in

53 a reduction in the population of *B. dorsalis* and a decrease in fruit damage rates
54 (Stibick 2004; Cao et al. 2012). However, the consumptive effects of *S. invicta* can
55 only have an impact within the specific orchard where they are present, as adult *B.*
56 *dorsalis* from adjacent orchards can still reach and cause damage to the fruits.
57 Therefore, we assumed that there must be non-consumptive effects from *S. invicta*
58 that contribute to the lower fruit damage rates and reduced number of visits by *B.*
59 *dorsalis*.

60 Predator non-consumptive effects have been well studied in many insect pests,
61 and studies have shown these effects can be mediated by predator semiochemicals
62 (Culshaw-Maurer et al. 2020). For example, studies have demonstrated that
63 predator-released semiochemicals, such as spider silk-treated plant leaves reducing
64 herbivory (Rypstra and Buddle 2013), or ladybeetle footprints deterring aphids from
65 visiting and colonizing (Ninkovic et al. 2013). Therefore, it is possible that the
66 observed decrease in both *B. dorsalis* infection rates and visits is caused by
67 non-consumptive effects mediated by the semiochemicals left by the fire ants.

68 The red imported fire ant is a highly invasive predator of many lepidopteran and
69 dipteran pests (Vogt et al. 2001; Rashid et al. 2013), including trypetid flies. As a
70 social insect, it uses multiple semiochemicals for communication with conspecifics
71 and other species (Vander Meer 1983; Vander Meer et al. 2002). Alarm pheromones
72 elicit aggression and attack in conspecifics, promote increased movement, gaped
73 mandibles, sting extrusion, trail laying, and aggressive postures (Morgan 2008;
74 Vander Meer et al. 2010). Recruitment and trail pheromones from Dufour's gland
75 organize large food retrieval, coordinate foraging and mediate mutualism with aphids
76 (Vander Meer et al. 1988, 1990; Xu et al. 2022). Cuticular hydrocarbons facilitate
77 nestmate and caste recognition (Monnin 2006), and venom defends against intruders
78 and prey (Greenberg et al. 2008). Semiochemicals are crucial for social
79 communication in ant colonies and may also serve as predator-prey communication
80 signals (Adams et al. 2020). It is widely recognized that some prey can use

81 predator-released cues to detect their predators and evade them (Culshaw-Maurer et al.
82 2020).

83 In this study, we assumed that when *S. invicta* searches for food on the ground
84 and in trees, it may leave semiochemical residues such as footprints, poop, and saliva
85 on the surfaces of trees and fruits. These residues could potentially have repellent
86 effects on *B. dorsalis*, deterring them from visiting and laying eggs and consequently
87 reducing the rate of fruit damage. The study aimed to test this hypothesis by
88 investigating the repellent effects of *S. invicta* semiochemicals (including footprints,
89 poop, saliva, and residues containing these cues) on the behavior of *B. dorsalis*. We
90 then analyzed the chemical composition of *S. invicta* footprints using Gas
91 Chromatography-Mass Spectrometry (GC-MS) technology, and tested the repellent
92 effects of these footprint chemicals on *B. dorsalis* behavior. Finally, a field
93 experiment was conducted where we sprayed *S. invicta* footprint chemicals on foliage
94 and evaluated whether this decreased the rate of fruit damage caused by *B. dorsalis*.
95 The main objective of the study was to investigate whether the non-consumptive
96 effects mediated by ant semiochemicals, particularly footprints left by *S. invicta*,
97 could deter visits and oviposition by *B. dorsalis*. We sought to identify the underlying
98 mechanism and assess the potential of these semiochemicals for pest control in
99 real-world scenarios. The findings could contribute to a better understanding of the
100 role of predator non-consumptive effects in pest control programs, the development of
101 new methods for *B. dorsalis* control, and the utilization of invasive predators in a way
102 that minimizes adverse effects on humans and ecosystems.

103

104 **Methods and Materials**

105 Insect collection and rearing

106 *B. dorsalis* larvae were collected from rot fruits in carambola (*Averrhoa*
107 carambola) and mango orchards in Haidian Campus of Hainan University, Hainan
108 Province. The larvae were group-cultured in a mesh cage (1.0 × 1.0 × 1.0 m) under

109 laboratory conditions (27 ± 1 °C, L:D = 16:8 h, RH = 60-75%). Adult flies were reared
110 on a jelly-like food consisting of 90 g sugar, 30 g yeast, 15 g honey, 5 g agar, and
111 1000 mL water, while eggs were collected using an egg-collecting device made from
112 a 10-mL centrifuge tube. The tube wall had about 50-60 holes ($d = 1$ mm) for
113 ovipositor insertion, and 2-3 mL of fresh orange juice were added at the bottom to
114 stimulate oviposition. Eggs laid in the tube were collected and cultured on a
115 maize-based artificial diet containing 500 g of corn flour, 500 g of banana, 2 g of
116 sodium benzoate, 100 g of yeast, 100 g of sucrose, 100 g of paper towel, 4 mL of
117 hydrochloric acid, and 800 mL of water. The larvae hatched from eggs were
118 consistently raised on this diet until they reached the pupal stage. Additionally, *S.*
119 *invicta* workers were collected directly from carambola orchards and used in the
120 experiments without undergoing any additional manipulation or treatment.

121

122 The repellent effects of *S. invicta* semiochemicals on the visiting behavior of *B.*
123 *dorsalis*

124 Five different sources of semiochemical solutions were prepared: *S. invicta* poop:
125 Fecal matter were collected from 20 confined worker ants after 12 hours of
126 confinement in a 10 mL centrifuge tube; the collected fecal matter was then dissolved
127 in 1 mL of sterile water. *S. invicta* saliva: obtained by cutting bite points from 100
128 ants stimulated with a wooden stick and dissolved in 1 mL sterile water. *S. invicta*
129 footprints: Twenty starved worker ants were confined in 10 mL centrifuge tubes for
130 12 hours; before cleaning the tubes with water, any visible fecal matter was removed;
131 the tubes were then cleaned using 1 mL of sterile water, and the resulting clean water
132 was collected. *S. invicta* residues: Twenty worker ants were confined in a 10 mL
133 centrifuge tube for 12 hours, the tubes were then cleaned using 1 mL of sterile water,
134 and the resulting clean water was collected. Control: 1 mL sterile water without any *S.*
135 *invicta* semiochemicals.

136 A mesh cage (20 × 20 × 20 cm) was utilized and a cup (diameter = 2.5 cm,
137 height = 1.5 cm) was placed inside. A total of 1 mL fresh orange juice, mixed with
138 200 uL of one of the aforementioned solutions, was added to the cup. One *B. dorsalis*,
139 starved for 12 hours, was introduced into the cage and allowed to remain for 5
140 minutes. The observation period ended when the tested *B. dorsalis* either visited the
141 orange juice or did not visit within the allotted 5-minute period, at which point it was
142 removed and replaced with a new one until 10 *B. dorsalis* had been tested. Three
143 growth stages were used, namely 1-5 days-old, 6-8 days-old, and 10-15 days-old
144 (oviposited) females. Each semiochemical treatment or growth stage was repeated 20
145 times, and the number of visited *B. dorsalis* was recorded.

146

147 The repellent effects of *S. invicta* semiochemicals on *B. dorsalis* oviposition behavior

148 An egg collection device (as described previously) containing 2 mL fresh orange
149 juice was utilized. Ant poop, saliva, footprints, and residual materials were prepared
150 as outlined previously. Using a brush, 500 uL of each solution was evenly spread on
151 the internal wall of the egg collection device. The device was then placed in a mesh
152 cage (20 × 20 × 20 cm) and 10 female *B. dorsalis* (10-15 days old) were introduced
153 into the cage for egg laying. The experiment was conducted between 2:00 pm and
154 6:30 pm, during which time eggs in the device were collected and counted. Each
155 treatment was performed with 20 replicates.

156

157 Chemical composition of *S. invicta* footprints

158 The previous experiments revealed that only *S. invicta* footprints had a
159 significant repellent effect on the feeding and oviposition behavior of female *B.*
160 *dorsalis*. Therefore, we conducted a GC-MS analysis to investigate the chemical
161 composition of *S. invicta* footprints. One milliliter of *S. invicta* footprints was
162 obtained as previously described, but hexane was used as the solvent instead of water.
163 The resulting solution was concentrated into 200 uL under a gentle nitrogen stream

164 and stored at - 20 °C. For the control treatment, hexane was used to wash a clear
165 centrifuge tube, and the washing water was collected and concentrated into 200 μ L.
166 Each treatment had 3 replicates.

167 Two hundred microliters of each sample were added to a 20 mL headspace bottle,
168 and 10 μ L of 2-Octanol (10 mg/L stock in dH₂O) was added as an internal standard.
169 All samples were analyzed using GC-MS with a SPME cycle of PAL rail system. The
170 incubation temperature was 60 °C, the preheat time was 15 min, the incubation time
171 was 30 min, and the desorption time was 4 min. The analysis utilized an Agilent 7890
172 gas chromatograph system coupled with a 5977B mass spectrometer. The system used
173 a DB-Wax, injected in Splitless Mode, and helium as the carrier gas. The front inlet
174 purge flow was 3 mL min⁻¹, and the gas flow rate through the column was 1 mL
175 min⁻¹. The initial temperature was kept at 40 °C for 4 min, and then raised to 245 °C
176 at a rate of 5 °C min⁻¹, and kept for 5 min. The injection, transfer line, ion source, and
177 quad temperatures were 250, 250, 230, and 150 °C, respectively. The energy was -70
178 eV in electron impact mode, and the mass spectrometry data were acquired in scan
179 mode with the m/z range of 20-400, solvent delay of 0 min. Chroma TOF 4.3X
180 software of LECO Corporation and Nist database were used for raw peaks exacting,
181 the data baselines filtering and calibration of the baseline, peak alignment,
182 deconvolution analysis, peak identification, integration, and spectrum match of the
183 peak area. The target compounds were identified by comparing the GC and
184 fragmentation patterns between the footprints and control sample.

185

186 Biological assessment of *S. invicta* footprint compounds on the behavior of *B.*
187 *dorsalis*

188 We identified nine compounds, 7 of which were purchased from Hainan Hifly
189 Industrial Co. Ltd, including undecane, d-limonene, dodecane, acetic acid, dodecanoic
190 acid, tetradecanoic acid, and hexadecanoic acid. Because 2,6,10-trimethyltridecane
191 and 4,6-dimethyl-dodecane are difficult to purchase, so these chemicals were not

192 included in our experiments. Each compound was individually mixed with sterile
193 water to form a single emulsion (undecane, d-limonene, dodecane, dodecanoic acid,
194 tetradecanoic acid, or hexadecanoic acid emulsion) or solution (acetic acid solution),
195 with the concentration based on its relative abundance in the footprint extracts shown
196 in Table 1. Additionally, the 7 compounds were mixed together to create a mixture
197 emulsion with the concentration indicated in Table 1. In the feeding behavior assay, 1
198 mL of fresh orange juice was mixed with 200 uL of each emulsion/solution in a cup,
199 and the number of *B. dorsalis* visits was counted using the methods described earlier.
200 In the oviposition behavior assay, the emulsion/solution was applied to the internal
201 wall of a tube as described above, and the number of eggs laid by *B. dorsalis* in each
202 device was counted. Fifteen replicates were conducted for both feeding and
203 oviposition behavior assays.

204

205 Field evaluation of *S. invicta* footprint compounds for *B. dorsalis* control

206 A mixture emulsion with seven compounds (see above) was foliage sprayed at
207 three fruit orchards: mango (18°27'19.8"N, 109°42'30.5"E), guava (19°46'11.2"N,
208 110°37'35.7"E), and wax apple (19°54'52.1"N, 110°33'58.5"E), located in Baoting,
209 Haikou and Qionghai city, Hainan province. These orchards had abstained from
210 pesticide use for roughly 45 days prior to these experiments. The experiments were
211 conducted between April 12th to May 20th, 2023 in mango and wax apple orchards,
212 and between May 12th to June 2nd, 2023 in guava orchards. Each orchard area was
213 over 2 acres, containing more than 200 trees. Before the fruits matured, the *B. dorsalis*
214 population density was monitored using methyl eugenol traps, ensuring that the
215 population was abundant during the experiment period. Each orchard was divided into
216 12 plots, with an area of about 200 m² (roughly 20-25 trees per plot), and a distance of
217 more than 10 m between plots.

218 Each fruit species was treated with either a footprint emulsion spray or a water
219 spray (as a control) for 2 and 7 days. The five-point sampling method was used in

220 each plot, with each sample point comprising 1-2 trees and 50-150 fruits. To prevent
221 damage from *B. dorsalis*, the fruits were covered with paper bags before foliage
222 spraying.

223 For each fruit species, there were 12 plots in total, with 6 plots exposed to the
224 footprint emulsion spray and the other 6 plots treated with water spray. Fruits from 3
225 treated plots and 3 control plots were examined for damage 2 days after spraying.
226 Fruits in the remaining 3 plots (3 for treated plots and 3 for control plots) were
227 exposed to the treatments for an additional 5 days. Finally, all the fruits were checked
228 for damage rate between 5-7 days after being collected.

229

230 Data analysis

231 A generalized linear model (GLM) with a Poisson distribution was used to
232 compare the number of visits by *B. dorsalis*, considering *S. invicta* semiochemicals
233 and growth stages (*B. dorsalis*) as predictors. Kruskal-Wallis tests were used to
234 analyze the number of eggs laid when *B. dorsalis* was exposed to *S. invicta*
235 semiochemicals. The chemical composition of *S. invicta* footprints and control
236 samples was compared using PERMANOVA. The impact of *S. invicta* footprint
237 compounds on the number of visits and eggs laid by *B. dorsalis* was assessed with
238 Kruskal-Wallis tests. The fruit damage rate was analyzed using a binomial distributed
239 GLM, taking into account treatments (spraying of footprint compounds or water), host
240 types (mango, guava, and wax apple), and time intervals (2 and 7 days) as predictors.
241 Multiple comparisons were conducted using Tukey' s tests. The data analyses were
242 performed using R 4.2.2 software (R Core Team 2022).

243

244 **Results**

245 The repellent effects of *S. invicta* semiochemicals on the visiting behavior of *B.*
246 *dorsalis*

247 The type of *S. invicta* semiochemicals and *B. dorsalis* growth stages influenced
248 the number of visits to food sources (Table 2). *S. invicta* poop and saliva had no
249 impact on *B. dorsalis* visiting behavior, but *S. invicta* footprint and residue
250 (containing poop, footprint, and saliva) considerably decreased the number of visits
251 by *B. dorsalis*. Although the analysis of variance revealed that *B. dorsalis* growth
252 stages influenced the number of visits (Table 2), multiple comparisons showed no
253 significant differences (Fig. 1).

254

255 The repellent effects of *S. invicta* semiochemicals on *B. dorsalis* oviposition behavior

256 The number of eggs laid by *B. dorsalis* was significantly affected by *S. invicta*
257 semiochemicals ($\chi^2 = 72.32$, $df = 4$, $p < 0.001$), with *S. invicta* footprint and residue
258 significantly reducing the number of eggs deposited by *B. dorsalis* (Fig. 2).

259

260 Chemical composition of *S. invicta* footprints

261 We identified 9 major chemical compounds in *S. invicta* footprint with mass
262 abundance by comparing the GC and fragmentation patterns between the footprints
263 and control sample (Fig. 3; Table 1), including undecane (4.87 % relative abundance),
264 d-limonene (4.18 %), dodecane (2.24 %), 2,6,10-trimethyltridecane (0.66 %),
265 4,6-dimethyl-dodecane (1.54 %), acetic acid (4.69 %), dodecanoic acid (11.74 %),
266 tetradecanoic acid (1.24 %), and hexadecanoic acid (0.79 %). Our results showed that
267 the chemical composition of *S. invicta* footprint was significantly different from the
268 control (PERMANOVA, $F_{1,5} = 9.04$, $p < 0.033$).

269

270 Biological assessment of *S. invicta* footprint compounds on the behavior of *B.*
271 *dorsalis*

272 The chemical compounds present in *S. invicta* footprint had a significant impact
273 on the number of visits to the food source ($\chi^2 = 77.81$, $df = 8$, $p < 0.001$). Specifically,
274 d-limonene and acetic acid as well as a mixture of 7 compounds were found to

275 significantly reduce the number of visits, with the mixture being the most efficient in
276 repelling *B. dorsalis* compared to any individual compound (Fig. 3).

277 *S. invicta* footprint were also found to significantly affect the number of eggs laid
278 by *B. dorsalis* ($\chi^2 = 70.17$, $df = 8$, $p < 0.001$). All of these compounds, as well as their
279 mixture, were effective in reducing the number of eggs laid by *B. dorsalis*, with the
280 mixture being the most efficient in repelling *B. dorsalis* compared to any other single
281 compound (excluding d-limonene).

282

283 Field evaluation of *S. invicta* footprint compounds for *B. dorsalis* control

284 The results from field experiments demonstrated that the experimental treatments,
285 hosts, and time all had significant effects on the fruit damage rates caused by *B.*
286 *dorsalis* (Table 2). The *S. invicta* footprint was found to significantly reduce the fruit
287 damage rates across all hosts 2 days after spraying (Fig. 5a, b, c). However, after 7
288 days of spraying, the *S. invicta* footprint did not show a significant reduction in fruit
289 damage rates.

290

291 **Discussion**

292 Our study revealed that semiochemicals found in *S. invicta* footprint have the
293 ability to reduce the visit frequency of *B. dorsalis* to food sources and decrease the
294 number of eggs laid. This indicates the presence of non-consumptive effects of *S.*
295 *invicta* on *B. dorsalis*. The GC-MS analysis identified 9 chemical compounds in *S.*
296 *invicta* footprint, with 7 of them, along with their mixture, found to repel *B. dorsalis*.
297 This affected their visiting and egg-laying behaviors, confirming that
298 non-consumptive effects were mediated by *S. invicta* footprint. Furthermore, a field
299 experiment demonstrated that the application of *S. invicta* footprint compounds
300 significantly reduced the fruit damage rates in mango, wax apple, and guava orchards.
301 This highlights the potential of these compounds in managing *B. dorsalis*.

302

303 *S. invicta* footprints repel the visiting and egg laying behavior in *B. dorsalis*

304 Adult *B. dorsalis* commonly damages fruits through their egg-laying behavior,
305 which leads to rotting as the larvae feed inside the fruit. Consequently, reducing the
306 population of *B. dorsalis* or the number of eggs laid by them is crucial. Previous
307 studies indicate *S. invicta* can directly prey on immature stages of *B. dorsalis* in the
308 ground (Vogt et al. 2001; Stibick 2004; Cao et al. 2012; Rashid et al. 2013),
309 potentially reducing fruit damage rates. However, since *B. dorsalis* is a pest with high
310 flight ability, it can migrate from adjacent orchards and continue to feed and lay eggs
311 in the fruits. Therefore, non-consumptive effects from *S. invicta* play important roles
312 in reducing fruit damage rates in the orchard.

313 Our results indicate that *S. invicta* footprints have repellent effects on *B. dorsalis*,
314 discouraging both their visiting and egg-laying behavior. This suggests the presence
315 of non-consumptive effects between these two invasive species. However, it also
316 highlights a tradeoff, where a reduction in the frequency of food visits by *B. dorsalis*
317 could decrease their food intake, subsequently impacting the fecundity of female *B.*
318 *dorsalis*. This tradeoff may have confounding negative effects on the *B. dorsalis*
319 population and has important implications for the persistent control of *B. dorsalis*.

320 This study also indicated that adult *B. dorsalis* can detect the footprints left by *S.*
321 *invicta* and respond with avoiding behavior. This ability of *B. dorsalis* to detect and
322 respond to risk is crucial for their survival and for protecting their offspring from
323 predators (Fischer and Frommen 2019), but may incur developmental and oviposition
324 costs (Magnhagen 1991; Culshaw-Maurer et al. 2020), as demonstrated above.

325 The repellent effects of *S. invicta* footprints on *B. dorsalis* are evident in present
326 studies; however, the chemical composition of the *S. invicta* footprint remains
327 unknown. Unraveling the chemical nature of this *footprint* is crucial for gaining a
328 deeper comprehension of the chemical communication between *B. dorsalis* and *S.*
329 *invicta*, as well as for the development of environmentally friendly strategies to
330 control *B. dorsalis*.

331

332 The chemical profile and repelling of *S. invicta* footprints on *B. dorsalis* behavior

333 We studied the impact of seven chemical compounds on *B. dorsalis* behavior and
334 discovered that d-limonene, acetic acid, and a compound mixture significantly
335 reduced *B. dorsalis* visits. This confirms the non-consumptive effects of *S. invicta*
336 footprints on *B. dorsalis*. D-limonene, a volatile compound known for influencing
337 insects, acts as a deterrent or repellent (Mursiti et al. 2019). It is commonly used by
338 plants to defend against herbivores and keep mosquitoes and flies away
339 (Hollingsworth 2005; Theochari et al. 2020). Acetic acid, found in ant secretions,
340 serves as an alarm pheromone within the colony (Pasteels et al. 1989; Mizunami et al.
341 2010; Tragust et al. 2013). *B. dorsalis* likely detects d-limonene and/or acetic acid and
342 avoids food with such cues. However, fruit flies are attracted to acetic acid, using it as
343 a cue to find fermenting fruits and vegetables (Cha et al. 2014; Ishii et al., 2015).

344 Contrary to their visiting behavior, our findings show that undecane, d-limonene,
345 dodecane, acetic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, and
346 their mixture significantly reduce *B. dorsalis* egg-laying. Notably, d-limonene
347 exhibits a repellent effect comparable to the mixture, suggesting its importance in the
348 repellent action of *S. invicta* footprints. Undecane and dodecane are cuticular
349 hydrocarbons (Yusuf et al. 2010) or gland secretions (Fujiwara-Tsujii et al. 2006;
350 Mekonnen et al. 2021) with various functions, including insect pheromonal or
351 repellent activities (Walter et al. 1993; Fujiwara-Tsujii et al. 2006). Dodecanoic acid,
352 tetradecanoic acid, and hexadecanoic acid serve as primary trail pheromones in ant
353 communication (Huwyler et al. 1975; Fauziah and Fatmahjihan 2004; Igwe and
354 Offiong 2015). Our studies suggested, these chemicals not only aid intra-colony
355 communication but also act as kairomones, repelling *B. dorsalis* from laying eggs.
356 However, it is worth noting that certain saturated fatty acids, like dodecanoic acid,
357 have been observed to attract oviposition in *Aedes aegypti* and *Culex quinquefasciatus*

358 (Sivakumar et al., 2011). This finding suggests the multifunctionality of these
359 chemicals in environmental signal communication.

360 Furthermore, our findings show that the mixture is the most effective in repelling
361 *B. dorsalis*, both in terms of visiting behavior and oviposition. This suggests that the
362 mixture may provide more precise and complete signals of risk for *B. dorsalis*,
363 leading to a stronger response. This study enhances our understanding of the chemical
364 signals that *B. dorsalis* use to communicate with their predators, allowing us to gain
365 insight into their behavior and potentially develop a novel methods to use invasive
366 predator for pest control.

367

368 The inclusion of *S. invicta* footprint chemical compounds in pest control

369 In our field experiment, we observed a significant reduction in fruit damage to
370 mango, wax apple, and guava within just two days of applying a mixture of *S. invicta*
371 footprint chemicals. These results provide strong evidence for the effectiveness of
372 these chemicals in real-world field settings against *B. dorsalis*. The reduction in fruit
373 damage is due to the repellent effect of the footprint chemicals, deterring *B. dorsalis*
374 from laying eggs on the fruit, consistent with previous lab findings. Similar repellent
375 effects have been observed in studies involving lady beetles and aphids (Ninkovic et
376 al., 2013), as well as rove beetles and small brown planthoppers in rice fields (Wen
377 and Ueno, 2022; Wen et al., 2023). These findings highlight the potential of
378 non-consumptive pest control strategies in reducing pest populations and impacting
379 population dynamics.

380 Several repellent chemicals have been assessed for their effectiveness against *B.*
381 *dorsalis*, such as botanicals from *Seriphidium brevifolium*, *Piper nigrum*, *Azadirachta*
382 *indica*, and quercetin, as well as various oils. These compounds have shown
383 promising results in repelling *B. dorsalis* and reducing crop damage (Liu et al. 2019;
384 Jaleel et al. 2020; Jaffar et al. 2022). However, their efficacy in natural environments
385 remains uncertain.

386 In our studies, we have tested the repellent effects and control efficiency of *S.*
387 *invicta* footprint chemicals in real-world conditions. We have found that these
388 chemicals have a high potential for controlling *B. dorsalis*. Furthermore, these
389 compounds are different from other plant-derived or naturally occurring repellents,
390 which presents an opportunity to explore predator-derived biomaterials for pest
391 control.

392 As a repellent, *S. invicta* footprint chemicals can be used in conjunction with
393 other pest management methods, such as the “push-pull” strategy, to achieve additive
394 or synergistic effects in controlling *B. dorsalis* (Culshaw-Maurer et al. 2020). Current
395 studies also suggest the use of indirect methods, such as utilizing predator cues, to
396 harness the impact of invasive predators, as direct release may be limited due to
397 potential negative effects on humans and the environment.

398 However, the control efficacy of the *S. invicta* footprint chemical mixture was
399 found to be equivalent to the control treatment after 7 days of spraying. This suggests
400 that the mixture lacks a long-lasting repellent effect on *B. dorsalis*. Analysis of the
401 chemical composition identified volatile and unstable compounds like d-limonene,
402 acetic acid, tetradecanoic acid, and hexadecanoic acid in the mixture, which may
403 contribute to its short duration as a repellent. Therefore, further research is needed to
404 explore methods such as sustained release technology to extend the repellent duration,
405 enhancing the practicality of this mixture in field applications.

406

407 **Conclusion**

408 This study explored the reduction of fruit damage caused by *B. dorsalis* in
409 orchards with live *S. invicta* nests. Both lab and field experiments demonstrated that
410 the *S. invicta* footprint can effectively repel *B. dorsalis* feeding and egg-laying,
411 leading to decreased fruit damage. These findings highlight the potential of *S. invicta*
412 footprint chemicals as an efficient and environmentally-friendly control method for *B.*
413 *dorsalis* in the field. We also emphasize the importance of integrating

414 non-consumptive predator effects into integrated pest management strategies,
415 including the use of lure traps to establish a “pull-push” system. Additionally, our
416 study proposes an alternative pest control approach, utilizing kairomones from
417 invasive predators instead of the predators themselves.

418

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Figures

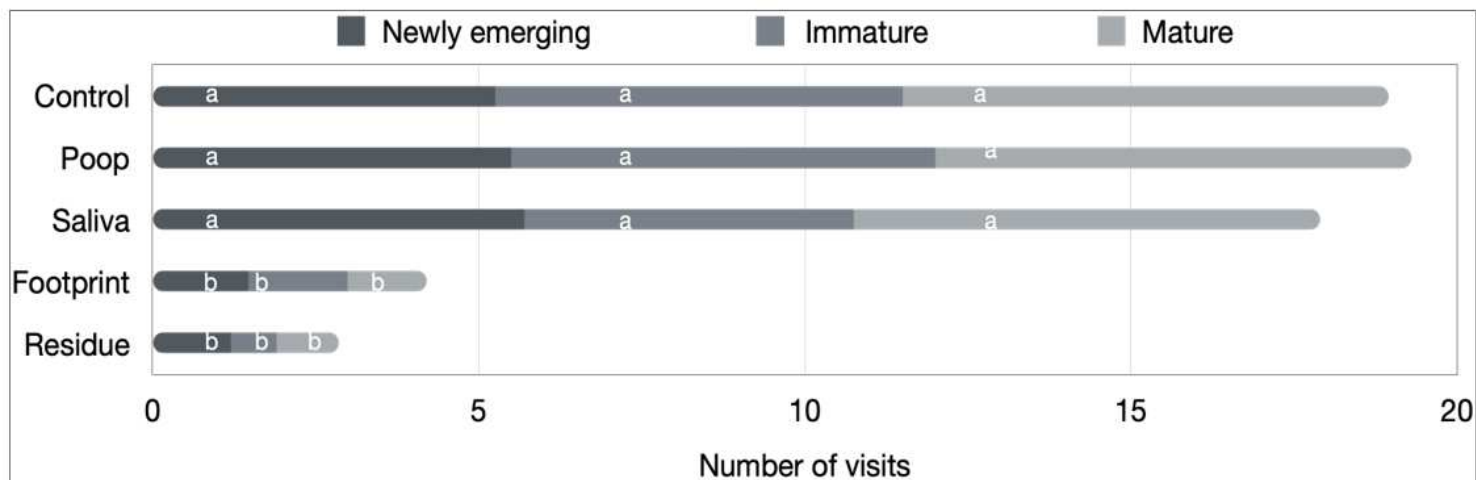


Figure 1

The number of visits by different growth stages of *B. dorsalis* was examined when they were exposed to different types of *S. invicta* semiochemicals (poop, saliva, footprint, residue, and control). Letters in lowercase within the bars indicate significant differences among treatments within the same growth stage (Tukey's test, $p < 0.05$)

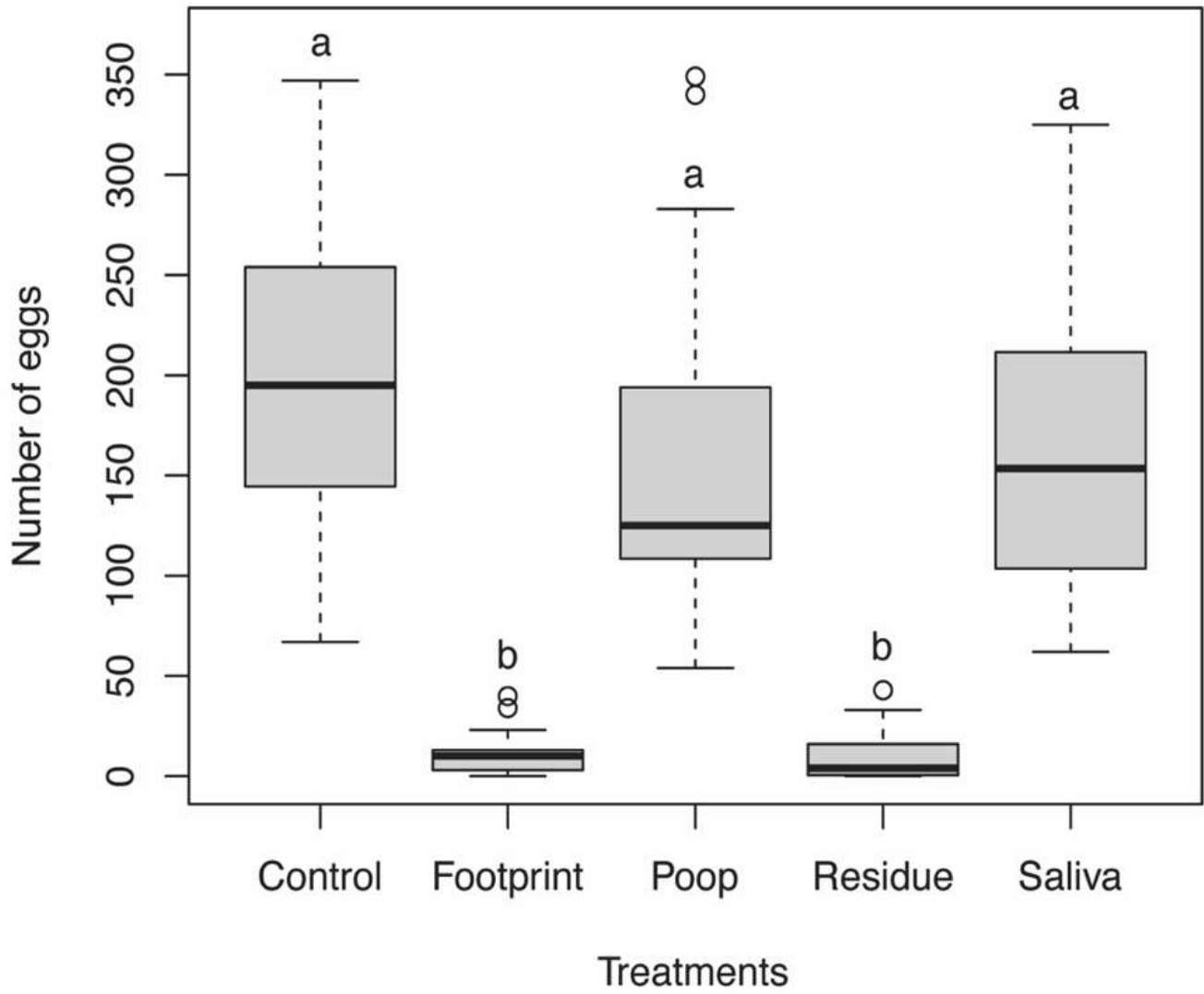


Figure 2

The number of eggs laid by *B. dorsalis* when exposed to different types of *S. invicta* semiochemicals (poop, saliva, footprint, residue, and control). Lowercase letters above the bars indicate significant differences among treatments (Tukey's test, $p < 0.05$)

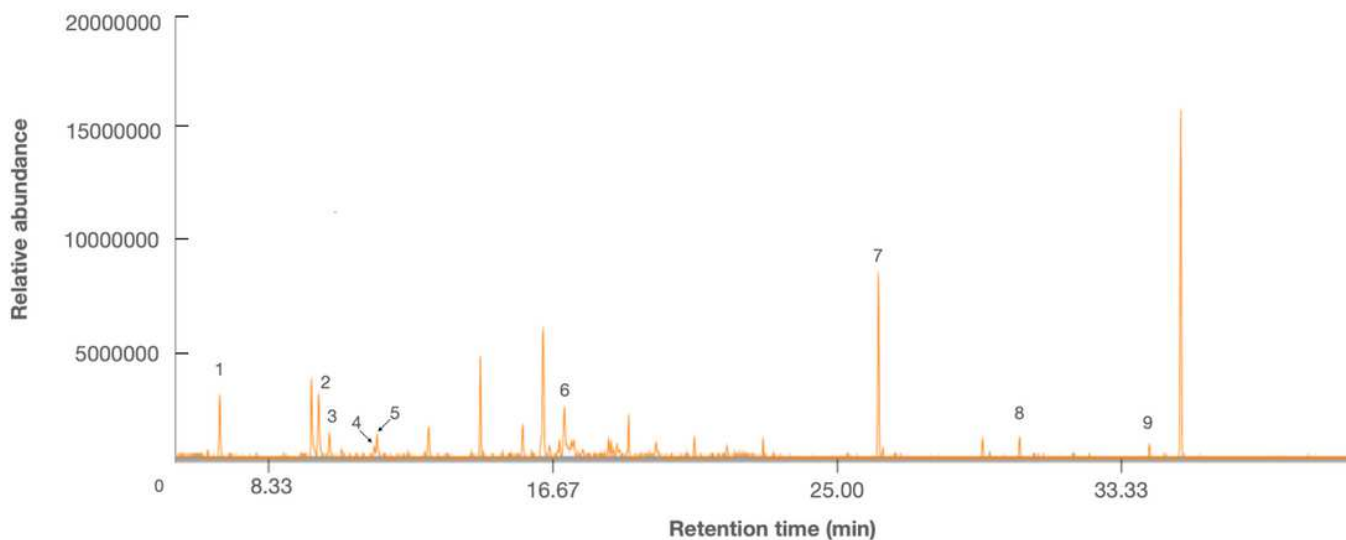


Figure 3

Gas chromatograms (GC) of *S. invicta* footprints (a) and control (b). The target compounds were identified by comparing the GC and fragmentation patterns between the footprints and control sample. Peak numbers correlate to the chemicals listed in Table 1

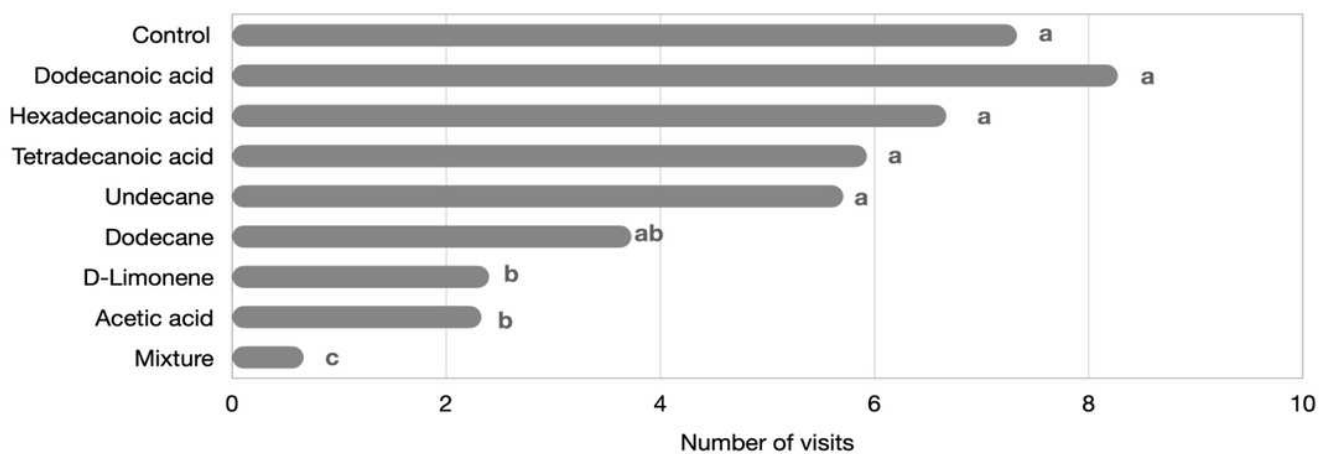


Figure 4

The number of visits by *B. dorsalis* when they were exposed to either a single compound or a mixture of *S. invicta* footprint compounds. Lowercase letters on the right side of the bars indicate significant differences among treatments (determined by Tukey's test, $p < 0.05$)

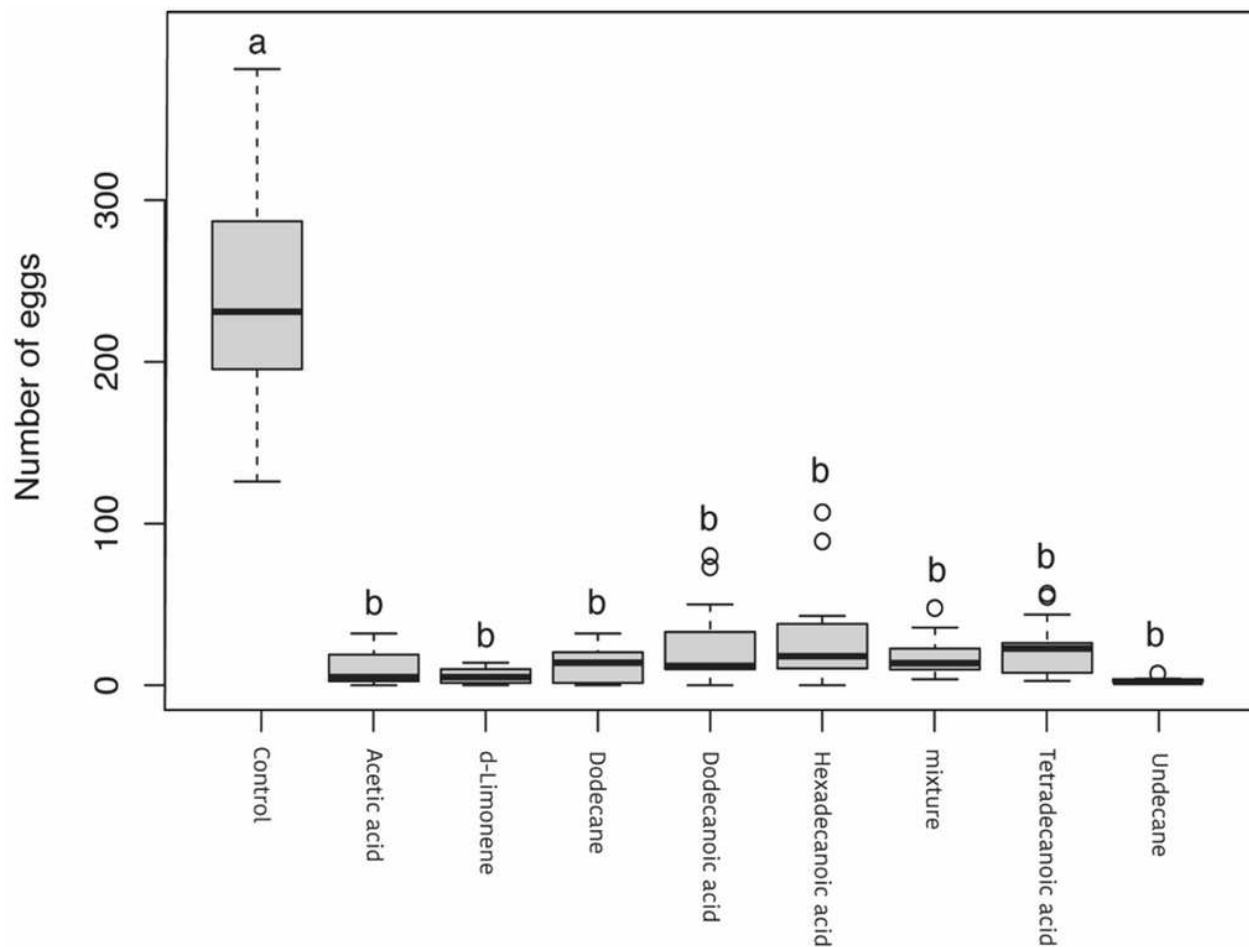


Figure 5

The number of eggs laid by *B. dorsalis* when exposed to either a single compound or a mixture of *S. invicta* footprint compounds. Lowercase letters above the bars indicate significant differences among treatments (determined by Tukey's test, $p < 0.05$)

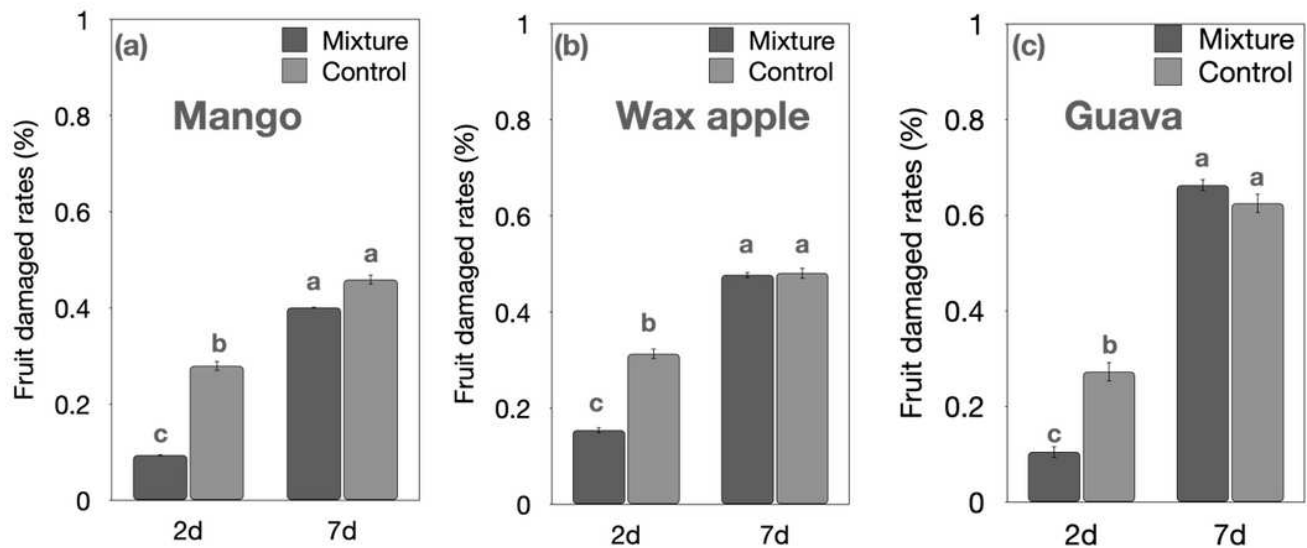


Figure 6

The effects of spraying *S. invicta* footprint or water (control) on the fruit damage rate in mango (a), wax apple (b), and guava (c) trees in the field. Lowercase letters above the bars indicate significant differences among treatments (determined by Tukey's test, $p < 0.05$)

Supplementary Files

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- [Tables.docx](#)