



# Influence of Host Plants and Tending Ants on the Cuticular Hydrocarbon Profile of a Generalist Myrmecophilous Caterpillar

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## Abstract

In myrmecophilous organisms, which live in symbiosis with ants, cuticular hydrocarbons (CHCs) play a pivotal role in interspecific communication and defense against chemical-oriented predators. Although these interactions form complex information webs, little is known about the influence of biotic environmental factors on the CHC profiles of myrmecophiles. Here, we analyzed the effect of different host plants and tending ants on the larval CHC profile of *Synargis calyce* (Lepidoptera: Riodinidae), a polyphagous species with facultative myrmecophily. Groups of caterpillars were fed individually with three host plant species (without tending ants), and with two tending ant species. Through gas chromatography analysis, we compared the cuticular profiles of treatments and found a high similarity between plants and caterpillars (65–82%), but a low similarity between caterpillars and their tending ants (30–25%). Cluster analysis showed that caterpillars, ants, and plants form distinct groups, indicating that *S. calyce* caterpillars have their own chemical profile. These results are similar to those observed for Lycaenidae caterpillars indicating that there is functional convergence in the chemical strategies used by myrmecophilous caterpillar species with similar ecology. Also, the results suggest that the cuticular compounds of *S. calyce* are primarily influenced by their host plants rather than their tending ants. Thus, we propose that these caterpillars present a trade-off between camouflage and directly informing their presence to ants, maintaining their unique chemical profile, though slightly affected by biotic environmental factors.

**Keywords** Chemical compounds · Mutualistic interactions · Myrmecophilous butterflies · Riodinidae · *Synargis calyce*

## Introduction

Ants are predominant in many terrestrial ecosystems in terms of abundance and biomass (von Beeren et al. 2012). These eusocial insects live in complex societies where communication plays a crucial role in their functioning (Hölldobler and Wilson 1990; Yamaoka 1990; Lenoir et al. 1999; Akino 2008). In ants and other social insects, communication and particularly the recognition of nestmates is primarily based on chemical cues and signals (Yamaoka 1990; Lenoir et al. 1999; Akino 2008; Blomquist and Bagneres 2010; Nunes et al. 2014). Cuticular hydrocarbons (CHCs), the main class of cuticular compounds in ants, are colony-specific and actively participate in nestmate recognition (Howard and Blomquist 2005; Hefetz 2007; Ferguson et al. 2021). The ecological success, wide distribution, and social organization of ants have contributed to the evolution of diverse associations with organisms from various

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kingdoms, including plants, fungi, and animals, particularly other insects (Casacci et al. 2019). Organisms that interact with ants during at least part of their lifecycle are called myrmecophiles (Hölldobler and Wilson 1990; Kronauer and Pierce 2011; Hölldobler and Kwapich 2022). There are about 10,000 species of myrmecophiles across various insect orders (Thomas et al. 2005; Parker 2016; Hölldobler and Kwapich 2022).

Myrmecophily in Lepidoptera is primarily observed in the families Lycaenidae and Riodinidae, with 75% of the species in these families having immatures stages that interact with ants (Pierce et al. 2002; Casacci et al. 2019). These families commonly exhibit facultative and unspecific relationships, involving interactions with various ant taxa. However, there are butterfly species that establish obligatory and specific relationships with specific ant taxa (Fiedler 1994, 2021; Kaminski 2008; Pierce and Dankowicz 2022). Interactions between butterflies and ants can range from mutually beneficial outcomes, such as mutualism, to interactions where butterflies benefit without harming ants in commensalism, and to antagonist interactions where butterflies may be preyed by ants or where ants may be harmed by butterflies, as seen in social parasitism and competition for resources (Fiedler 1995, 1996; Pierce and Dankowicz 2022). Both lycaenid and riodinid species exhibit various adaptations resulting from the pressures exerted by their association with ants (Pierce et al. 2002). These adaptations include highly specialized ant-organs involved in chemical and acoustic deception (Cottrell 1984; Fiedler et al. 1996; Pierce et al. 2002; Elmes et al. 2002; Barbero et al. 2012; Hill et al. 2022). For instance, nectary organs such as the dorsal nectary organ (DNO) in Lycaenidae and tentacular nectary organs (TNOs) in Riodinidae liquid secretions rich in sugar and amino acids (Newcomer 1912; Malicky 1970; DeVries 1988). Caterpillars of these two families are also equipped with a pair tentacular organs (TOs) in Lycaenidae and anterior tentacular organs (ATOs) in Riodinidae, which emit chemical signals or vibroacoustic signals that modify the ant behavior (Henning 1983; DeVries et al. 1986; DeVries 1988; Gnatzy et al. 2017; Schönrogge et al. 2017).

Chemical strategies mediated by cuticular compounds enable myrmecophiles to overcome the barrier of chemical recognition employed by ants (von Beeren et al. 2012). One such strategy is chemical camouflage, where organisms resemble their background and avoid detection by chemically oriented predators (Silveira et al. 2010). In herbivorous organisms, achieving chemical camouflage with their host plants is possible through diet (Espelie et al. 1991; Barbero 2016; Lima et al. 2024). In Lepidoptera, this strategy has already been demonstrated in both non-myrmecophilous and myrmecophilous caterpillars (Akino et al. 2004; Portugal and Trigo 2005; Lima et al. 2021). One of the most extensively studied strategies is chemical mimicry, where

organisms possess chemical compounds that close resemble those of other organisms such as ants. This strategy has been observed in social parasitic species (Akino et al. 1999; Elmes et al. 2002; Schlick-Steiner et al. 2004; Schönrogge et al. 2004; Akino 2008). Additionally, some myrmecophilous caterpillars present low concentration of compounds on their surface, making their detection challenging - a strategy known as chemical insignificance (Inui et al. 2015; Barbero 2016). Recently, a new strategy has been proposed in myrmecophilous caterpillars called chemical conspicuousness. In this strategy, caterpillars that do not provide caloric rewards for ants exhibit a distinct cuticular profile compared to ants or host plants. However, their profile is similar to that of other caterpillars that offer caloric rewards to ants (Lima et al. 2021).

Although ant-plant-herbivore systems embrace complex communication networks involving multiple species (e.g., Lima et al. 2021, 2023), there is still a lack of information regarding the influence of phenotypic plasticity and biotic environmental factors on the cuticle compounds of generalist myrmecophilous species (Otte et al. 2018; Sprenger and Menzel 2020). Therefore, our aim was to investigate whether the CHC profile of a polyphagous caterpillar changes (1) according to their diet on different host plant species and (2) according to their interaction with different tending ant species. Considering that the caterpillar species used here were fed on their host plants, we hypothesized that their CHCs would be influenced primarily by their food source rather than the contact established with tending ants. Furthermore, due to the production of caloric rewards by caterpillars (trophobiosis) we predicted that caterpillars would exhibit a specific chemical profile distinct of both host plant and tending ants.

## Methods and Materials

**Study System** *Synargis calyce* C. Felder and R. Felder, 1862 (Lepidoptera: Riodinidae) (Fig. S1) is a Neotropical herbivorous and myrmecophilous butterfly whose caterpillars feed on several host plants in different families, including non-native species (Callaghan 1986; Beccaloni et al. 2008; Alves-Silva et al. 2018; Kaminski 2021). Female butterflies tend to lay their eggs on plants that are frequented by attendant ants and caterpillars are tended by ants during all instars (Callaghan 1986). Although it presents facultative myrmecophily, the caterpillars are almost always found with tending ants of several genera, but mainly *Camponotus* species (Callaghan 1986; Alves-Silva et al. 2018; Kaminski 2021). It is common to observe temporal turnover, with attendance by different species of ants during the day and night (LAK, personal observation). Due to the high degree of ecological plasticity of this butterfly in terms of both host plants

and tending ants, it is an excellent model of a generalist myrmecophile.

**Collection and Rearing of Study Species** Insects were collected at the Universidade de São Paulo (21.1637° S, 47.8592° W), Ribeirão Preto Campus, SP, Brazil, between January 2021 and April 2022. To conduct our chemical analysis, we collected ~90 eggs of *S. calyce* for rearing in the laboratory. Initially, ~20 field observations were conducted to identify plant species used by butterflies for oviposition and seven species were identified serving as host plants. Subsequently, eggs were collected from three of these host plants (*Senegalia polyphylla* (DC.) Britton and Rose (Fabaceae), *Inga laurina* (Sw.) Willd. (Fabaceae), and *Terminalia catappa* Linnaeus (Combretaceae)) at various study locations by harvesting branches where oviposition had been observed. In the field, three ant nests of *Camponotus crassus* Mayr, 1862 (Formicidae: Formicinae) were collected and transferred to the laboratory. Each nest was placed in two connected boxes measuring 9 × 26.6 × 26.6 cm. One box served as the nesting area and contained test tubes (15 cm long) filled with water, plugged with hydrophilic cotton. The other box served as the foraging area. The ant colonies were provided with a diet of *Tenebrio molitor* Linnaeus, 1758 larvae (Coleoptera: Tenebrionidae), diluted sugar solution (10%), and water *ad libitum*. Each colony consisted of approximately 150 workers, a queen, and some brood (eggs, larvae and pupae). Additionally, approximately, 600 workers of *Paratrechina longicornis* Latreille 1802 (Formicidae: Formicinae) were collected from three established colonies near the laboratory buildings. The insects were kept under controlled conditions at a temperature of 25 °C and a photoperiod of 12 h of light and 12 h of darkness.

**Does the Chemical Composition of Caterpillars Change According to Their Food Sources?** As the caterpillars studied here are polyphagous, we selected three host plant species on which the caterpillars feed in the study area: two native, *S. polyphylla*, and *I. laurina*, and one non-native, *T. catappa*. Our aim was to investigate whether the CHCs of the caterpillar change according to its diet in the absence of tending ants. To conduct the experiment, we placed individually each egg in a plastic container (250 ml). Once the eggs hatched, we provided the caterpillars with shoots containing young leaves and extrafloral nectaries from the host plants. The shoots were replaced daily and kept in contact with moistened cotton to prevent them from drying. The *S. calyce* caterpillars were reared separately in plastic containers on three host plant species: caterpillar-*S. polyphylla* ( $n=10$ ), caterpillar-*I. laurina* ( $n=10$ ), caterpillar-*T. catappa* ( $n=6$ ). After reaching the fifth instar, we killed the caterpillars by freezing and kept them at -20 °C until CHC extractions were performed. Additionally, we collected leaves of *S. polyphylla*

( $n=10$ ), *I. laurina* ( $n=10$ ), and *T. catappa* ( $n=10$ ) for CHC extractions (See Table S1).

**Does the Chemical Composition of Caterpillars Change According to Their Tending Ants?** In our field site, the caterpillars are attended by four different ant species: *C. crassus*, *P. longicornis*, *Camponotus renggeri* Emery, 1894, and *Wasmannia auropunctata* Roger, 1863 (AVCG, personal observation). Thus, to assess whether the caterpillar CHCs change according to their tending ants, we individually reared *S. calyce* caterpillars in a plastic container as previously described, along with a group of associated ants. Specifically, we reared the caterpillars with two experimental groups: (caterpillar-*C. crassus*) ( $n=10$ ), and (caterpillar-*P. longicornis*) ( $n=10$ ). These caterpillars were fed with the host plant *S. polyphylla*. Each caterpillar was placed together with 10 workers of *C. crassus* or 30 workers of *P. longicornis*. The number of ants was based on the average amount observed in the field. The ant workers were replaced every day until the caterpillars were frozen for chemical extraction, as previously mentioned. For chemical analysis, we also collected worker ants from colonies reared in the laboratory for *C. crassus* ( $n=8$  colonies;  $n=20$  ants for chemical analyses) and from colonies established near the laboratory for *P. longicornis* ( $n=10$  colonies;  $n=300$  ants for chemical analyses) (See Table S1).

**Chemical Analyses** To perform the chemical analyses, we placed insects or plant shoots in glass vials (1.5 ml) and covered them with *n*-hexane (Macron Fine Chemicals, 95% *n*-hexane, USA) for 1 min (Lima et al. 2023). For each sample, a fifth-instar larva of *S. calyce*, two workers of *C. crassus*, 30 workers of *P. longicornis*, and one young shoot with two leaves from each plant species were used individually. External standards were exclusively employed and the samples were not weighed. Subsequently, we left each vial at room temperature in a flow chamber to allow for drying. Once completely dried, we resuspended the contents in 5 µl of hexane, of which 2 µl were manually injected. The samples were analyzed with gas chromatography coupled to a mass spectrometer (GC/MS; Shimadzu, model QP2010 Plus), using a 30 m Rxi-1ms column, with helium gas flow rate set at 1 ml/min. The oven temperature was initially set to 40 °C and then increased by 3 °C min<sup>-1</sup> until reaching 310 °C (held for 15min), following da Silva et al. (2021). The injector temperature was set to 250 °C. Data were analyzed by GC/MS Solutions for Windows (Shimadzu Corporation), and compounds were identified based on their mass spectra, including diagnostic and molecular ions (Carlson et al. 1998). Additionally, a retention index was calculated for each identified peak using a standard solution of different synthetic linear hydrocarbons (*n*-C<sub>21</sub> to *n*-C<sub>40</sub>). We also consulted the Registry of Mass Spectral Data (Wiley) and

National Institute of Standards and Technology (NIST) mass spectra search program (version 2.2) Libraries database for identification (Lima et al. 2023).

**Statistical Analyses** We used Morisita's Similarity Index (SI) which ranges from 0% (indicating no similarity) to 100% (representing complete similarity) (Krebs 1999) to compare CHC profiles of different groups, following the methodology of Lima et al. (2021). This analysis was carried out using PAST software (Version 4.13) (Hammer et al. 2001). Furthermore, to assess the overall chemical similarity or dissimilarity between groups, we performed a permutation analysis (PERMANOVA). This analysis was performed using the *adonis* function from the *vegan* package (Oksanen et al. 2013) with 9999 permutations. In order to represent the multivariate chemical dataset and check for the cluster formation, we next performed a Principal Component Analysis (PCA). For this, we used the *prcomp* function of the *stats* package (R Core Team 2019). We also ran a multivariate similarity analysis (SIMPER) using the Bray-Curtis distance and adopting 999 permutations. The SIMPER analysis allowed us to determine the contribution of each chemical variable to the existing variation among samples. For this analysis, we used the *simper* function from the *vegan* package (Oksanen et al. 2013). For all tests, we determined the relative abundance percentages of each compound present in the cuticular extracts, treating the compounds as 100% and then analyzed the data. All these analyses were conducted using R version 4.0.2 (R Core Team 2019).

## Results

**Overall Chemical Information** A total of 78 peaks were identified in the cuticular extracts from the different groups studied (Table 1). *Senegalia polyphylla* exhibited 22 peaks, *I. laurina* had 29 peaks, *T. catappa*, *C. crassus* had 28 peaks each, *P. longicornis* had 22 peaks, and *S. calyce* caterpillars had 25–30 peaks. These peaks corresponded to various chemical compounds, including branched hydrocarbons (mono-, di-, and trimethylated), linear alkanes, alkenes, alcohols, and aldehydes. The carbon lengths of the identified compounds ranged from 18 to 36.

The cuticular profile of the three host plant species revealed a shared class of compounds, including linear alkanes, alcohols, and aldehydes. However, variations existed among them, particularly in the proportion and presence or absence of specific alcohols and aldehydes. For instance, 1-hexadecanol, 1-hexacosanol, and hexacosanal were exclusively present in *I. laurina*, while 1-triacontanol acetate was found only in *T. catappa* (Table 1). The SIs of the cuticular compounds of the plants varied according to

the pairs of compared species. *Terminalia catappa* with *S. polyphylla* showed the highest SI of up to 77%, followed by *T. catappa* with *I. laurina*, which exhibited a SI of up to 65%, and *I. laurina* with *S. polyphylla*, which showed a SI of up to 62%. *Senegalia polyphylla* and *I. laurina* shared 20 compounds, representing 69% and 91% of their respective cuticles. *Senegalia polyphylla* shared 15 compounds with *T. catappa*, representing 52% and 68.2% of their respective cuticles. *Terminalia catappa* shared 18 compounds with *I. laurina*, representing 60% and 62.06% of their respective cuticles. Although post hoc pairwise comparisons did not reveal a significant difference based on relative abundance or chemical composition (Table 2), the PCA revealed that the three species form separate groups, in which the first and the second principal component explained 30% and 13.77%, respectively. (Fig. 1). In terms of major compounds, *S. polyphylla* had the *n*-C29, 1-triacontanol and triacontanal; *I. laurina* had the *n*-C29, Hexacosanol and *n*-C31, and *T. catappa* had the *n*-C29 and *n*-C31.

### Does the Chemical Composition of Caterpillars Change According to Their Food Sources?

When comparing the compounds found in caterpillars reared on three different host plants, we found the following similarity indices: The cuticular profiles of caterpillars reared on *T. catappa* showed a SI of up to 82% with *T. catappa*, up to 74% with *I. laurina*, and up to 61% with *S. polyphylla*. The cuticular profiles of caterpillars reared on *I. laurina* showed a SI of up to 76% with *I. laurina* and *T. catappa*, and up to 60% with *S. polyphylla*. Finally, caterpillars reared on *S. polyphylla* exhibited a SI up to 65% with *S. polyphylla*, and up to 64% with *T. catappa*, and *I. laurina*. Moreover, certain compounds were exclusively found in groups of caterpillars reared on specific plant species. For example, 1-hexadecanol was found only in the cuticular profile of caterpillars reared on *I. laurina*, and this particular compound was also identified in the chemical profile of this plant. Similarly, 1-docosanol, identified in *T. catappa*, was also detected in caterpillars reared on this plant but was absent in those reared on *S. polyphylla* (Table 1). The qualitative similarity varied according to the host plant. Specifically, caterpillars shared 19 compounds with *S. polyphylla*, representing 70.37% and 86.36% of their respective cuticles. Caterpillars and *I. laurina* shared 23 compounds, representing 76.66% and 79.31% of their respective cuticles, while caterpillars and *T. catappa* shared 17 compounds, representing 58.6% and 60.7% of their respective cuticles. However, there was a low degree of similarity in the relative abundance of compounds between caterpillars and their host plants. The post hoc pairwise comparisons revealed significant differences in relative abundance and in the chemical composition among caterpillars reared on different plants (Table 2). However, most of the compounds were shared in the three groups and we found an overlap among caterpillars

Table 1 Cuticular compounds found on different groups of the *S. calyce* caterpillars, attendant ants and host plants [mean relative abundance  $\pm$  Standard Deviation (SD)]

Compounds	Host plants					Caterpillars with host plants (without ants)					Ants					Caterpillars with ants		
	RT	RI	MDI	Sp	II	Tc	CS	CI	CT	Cc	PI	CC	CP	CC	CP			
4meC17	14.874	1756	211	-	-	-	-	-	-	-	0.76 $\pm$ 0.65	-	-	-	-			
n-C18	15.159	1800	254	0.40 $\pm$ 0.72	0.34 $\pm$ 0.30	-	0.08 $\pm$ 0.05	0.11 $\pm$ 0.08	0.04 $\pm$ 0.02	-	-	-	-	-	-			
1-hexadecanol	16.074	1881	-	-	1.59 $\pm$ 0.87	-	-	0.424 $\pm$ 0.26	-	-	-	-	-	-	-			
n-C19	16.282	1900	268	0.21 $\pm$ 0.33	0.19 $\pm$ 0.13	-	0.10 $\pm$ 0.05	0.09 $\pm$ 0.04	0.042 $\pm$ 0.02	2.53 $\pm$ 2.17	-	0.11 $\pm$ 0.09	0.12 $\pm$ 0.04	-	-			
4-meC19	16.834	1928	71/238	-	-	-	-	-	-	-	0.91 $\pm$ 0.75	-	-	-	-			
n-C20	17.425	2000	282	0.42 $\pm$ 0.59	0.57 $\pm$ 0.40	-	0.25 $\pm$ 0.15	0.216 $\pm$ 0.11	0.08 $\pm$ 0.06	0.90 $\pm$ 0.61	-	0.14 $\pm$ 0.07	0.22 $\pm$ 0.08	-	-			
1-octadecanol	17.702	2024	-	-	-	0.15 $\pm$ 0.30	-	-	-	-	-	-	-	-	-			
1-octadecanol	18.405	2084	-	1.02 $\pm$ 0.65	2.60 $\pm$ 1.06	0.24 $\pm$ 0.13	0.32 $\pm$ 0.37	0.68 $\pm$ 0.32	0.43 $\pm$ 0.14	-	-	0.10 $\pm$ 0.16	0.7 $\pm$ 0.66	-	-			
n-C21	18.572	2100	296	0.23 $\pm$ 0.27	0.33 $\pm$ 0.21	-	0.23 $\pm$ 0.15	0.17 $\pm$ 0.10	0.14 $\pm$ 0.10	14.34 $\pm$ 3.54	-	1.068 $\pm$ 1.37	0.30 $\pm$ 0.18	-	-			
n-C22	19.718	2200	310	0.47 $\pm$ 0.38	0.77 $\pm$ 0.36	0.004 $\pm$ 0.01	0.33 $\pm$ 0.18	0.284 $\pm$ 0.13	0.22 $\pm$ 0.14	0.46 $\pm$ 0.32	-	0.17 $\pm$ 0.087	0.25 $\pm$ 0.10	-	-			
1-icosanol	20.733	2289	-	0.01 $\pm$ 0.03	1.88 $\pm$ 1.02	0.07 $\pm$ 0.14	0.14 $\pm$ 0.24	0.08 $\pm$ 0.07	0.10 $\pm$ 0.09	-	-	0.008 $\pm$ 0.01	0.06 $\pm$ 0.05	-	-			
C23:1	20.844	2276	322	-	-	-	-	-	-	1.00 $\pm$ 1.22	-	-	-	-	-			
n-C23	20.863	2300	324	1.91 $\pm$ 0.98	1.36 $\pm$ 0.72	0.25 $\pm$ 0.35	0.86 $\pm$ 0.48	1.20 $\pm$ 0.57	0.41 $\pm$ 0.31	3.70 $\pm$ 1.05	-	0.58 $\pm$ 0.59	1.96 $\pm$ 0.83	-	-			
n-C24	21.993	2400	338	0.55 $\pm$ 0.51	0.66 $\pm$ 0.34	0.18 $\pm$ 0.24	0.48 $\pm$ 0.41	0.34 $\pm$ 0.21	0.22 $\pm$ 0.23	-	-	0.19 $\pm$ 0.09	0.34 $\pm$ 0.12	-	-			
1-docosanol	23.060	2492	-	-	0.93 $\pm$ 0.59	-	-	0.27 $\pm$ 0.40	0.20 $\pm$ 0.17	-	-	-	-	-	-			
ZC25	22.864	2476	352	-	-	0.08 $\pm$ 0.17	-	-	-	-	-	-	-	-	-			
n-C25	23.140	2500	352	2.55 $\pm$ 1.03	1.94 $\pm$ 0.89	1.37 $\pm$ 2.78	0.83 $\pm$ 0.60	1.625 $\pm$ 1.88	0.80 $\pm$ 0.43	-	1.46 $\pm$ 0.33	0.52 $\pm$ 0.24	0.55 $\pm$ 0.50	-	-			
3-Mec25	24.004	2573	57/337	-	-	0.08 $\pm$ 0.13	-	-	-	-	-	-	-	-	-			
n-C26	24.319	2600	366	0.59 $\pm$ 0.33	1.42 $\pm$ 0.51	0.44 $\pm$ 0.44	1.04 $\pm$ 0.85	0.85 $\pm$ 0.47	0.61 $\pm$ 0.46	-	-	0.27 $\pm$ 0.21	0.33 $\pm$ 0.12	-	-			
ZC27	25.256	2678	380	-	-	0.41 $\pm$ 0.86	-	-	-	-	-	-	-	-	-			
n-C27	25.524	2700	380	4.69 $\pm$ 1.15	6.40 $\pm$ 1.08	4.48 $\pm$ 2.28	4.83 $\pm$ 0.92	8.598 $\pm$ 3.38	5.80 $\pm$ 2.31	1.17 $\pm$ 1.19	6.43 $\pm$ 1	5.53 $\pm$ 1.97	4.87 $\pm$ 2.34	-	-			
13-;11-MeC27	26.259	2732	225/197/169/253	-	-	-	-	-	-	-	0.60 $\pm$ 0.33	-	-	-	-			
9-MeC27	26.305	2736	141/281	-	-	-	-	-	-	-	0.08 $\pm$ 0.03	-	-	-	-			
Unknown	26.314	2766	-	-	-	0.11 $\pm$ 0.13	-	-	-	-	-	-	-	-	-			
7-MeC27	26.369	2742	112/309	-	-	-	-	-	-	-	0.52 $\pm$ 0.12	-	-	-	-			
5-MeC27	26.482	2751	85/337	-	-	-	-	-	-	-	0.17 $\pm$ 0.06	-	-	-	-			
3-MeC27	26.753	2773	57/365	-	-	-	-	-	-	-	1.28 $\pm$ 0.36	-	-	-	-			
n-C28	26.714	2800	394	3.56 $\pm$ 0.60	3.43 $\pm$ 0.69	1.72 $\pm$ 0.54	2.78 $\pm$ 1.39	2.57 $\pm$ 1.17	2.12 $\pm$ 0.53	4.99 $\pm$ 2.08	-	1.71 $\pm$ 0.55	1.47 $\pm$ 0.31	-	-			
hexacosanol	27.202	2838	-	-	4.74 $\pm$ 1.58	-	-	-	-	-	-	-	-	-	-			
ZC29	27.674	2876	408	-	-	0.17 $\pm$ 0.40	-	-	-	-	-	-	-	-	-			
C29:1	27.806	2879	406	-	-	-	-	-	-	0.43 $\pm$ 0.39	-	-	-	-	-			
1-hexacosanol	27.964	2898	-	-	11.56 $\pm$ 3.49	-	-	-	-	-	-	-	-	-	-			
n-C29	27.978	2900	408	40.32 $\pm$ 6.33	20.31 $\pm$ 2.70	39.57 $\pm$ 3.50	13.35 $\pm$ 1.58	16.01 $\pm$ 2.12	16.75 $\pm$ 2.88	5.67 $\pm$ 1.65	14.16 $\pm$ 2.59	17.70 $\pm$ 2.24	16.47 $\pm$ 1.35	-	-			
15-;13-;11-;9-MeC29	28.759	2936	225 / 197 / 253 / 169 / 281 / 141 / 309	-	-	-	-	-	-	-	22.30 $\pm$ 1.73	-	-	-	-			
15-;13-;11-MeC29	28.670	2929	225/197/253/169/281	-	-	-	-	-	-	0.51 $\pm$ 0.40	-	-	-	-	-			
7-MeC29	28.801	2940	112/337	-	-	-	-	-	-	0.053 $\pm$ 0.06	-	-	-	-	-			
4-MeC29	28.671	2960	71/379	-	-	-	-	0.11 $\pm$ 0.08	0.082 $\pm$ 0.05	-	-	-	-	-	-			
3-MeC29	28.809	2973	57/393	-	-	-	-	0.07 $\pm$ 0.12	0.04 $\pm$ 0.03	2.03 $\pm$ 1.20	-	0.03 $\pm$ 0.06	0.025 $\pm$ 0.04	-	-			

Table 1 (continued)

Compounds	RT	RI	MDI	Host plants						Caterpillars with host plants (without ants)						Caterpillars with ants		
				Sp	II	Tc	CS	CI	CT	Cc	PI	CC	CP					
<b>9,17-; 9,19-diMeC29</b>	<b>29.118</b>	<b>2966</b>	<b>141/267/197/323/169/295</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>C30:1</b>	29.312	2982	420	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>n-C30</b>	<b>29.124</b>	<b>3000</b>	<b>422</b>	<b>3.02 ± 0.23</b>	<b>3.35 ± 1.44</b>	<b>2.89 ± 0.46</b>	<b>4.44 ± 1.55</b>	<b>3.45 ± 1.17</b>	<b>3.84 ± 0.52</b>	<b>11.02 ± 3.99</b>	<b>3.13 ± 0.52</b>	<b>3.006 ± 0.43</b>	-	-	-	-	-	-
1-heptacosanol	29.286	3014		-	7.20 ± 0.98	-	-	-	-	-	-	-	-	-	-	-	-	-
octacosanal	29.643	3043		0.35 ± 0.14	2.93 ± 1.99	1.90 ± 0.58	0.05 ± 0.14	0.28 ± 0.34	0.20 ± 0.11	-	-	0.32 ± 0.24	0.52 ± 0.52	-	-	-	-	-
2-MeC30	29.902	3065	43/421	-	-	0.04 ± 0.05	2.91 ± 1.27	2.48 ± 1.85	2.38 ± 0.88	-	-	0.03 ± 0.07	0.18 ± 0.25	-	-	-	-	-
x,y-diMeC30	29.98	3062		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ZC31	30.078	3079	436	-	-	0.34 ± 0.10	-	-	-	-	-	-	-	-	-	-	-	-
C31:1	30.480	3080	434	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
n-C31	30.317	3100	436	7.78 ± 3.02	9.75 ± 6.88	32.44 ± 4.06	22.48 ± 2.56	23.23 ± 2.55	25.97 ± 2.13	5.14 ± 1.54	4.41 ± 1.11	24.34 ± 1.7	24.45 ± 1.42	-	-	-	-	-
1-octacosanol	30.376	3105		1.85 ± 1.12	6.30 ± 4.35	-	-	-	-	-	-	-	-	-	-	-	-	-
15-;13-;11-9-MeC31	31.098	3132	225/239/197/281/169/309/141/337	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2-MeC31	31.064	3165	43/435	-	-	-	0.23 ± 0.22	0.08 ± 0.13	0.12 ± 0.09	-	-	-	-	-	-	-	-	-
3-MeC31	31.203	3176	57/421	-	-	-	0.08 ± 0.11	-	0.03 ± 0.03	-	-	0.98 ± 1.91	0.86 ± 1.62	-	-	-	-	-
C32:1-1	31.630	3177	448	-	-	-	-	-	-	-	-	1.16 ± 0.80	-	-	-	-	-	-
C32:1-2	31.717	3185	448	-	-	-	-	-	-	-	-	4.04 ± 1.70	-	-	-	-	-	-
n-C32	31.47	3200	450	0.60 ± 0.29	1.51 ± 0.60	0.90 ± 0.23	6.37 ± 0.78	4.88 ± 0.69	5.46 ± 0.91	3.97 ± 2.09	-	5.31 ± 0.92	5.79 ± 0.65	-	-	-	-	-
Unknown	31.566	3208		0.53 ± 0.28	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-octacosanol acetate	31.66	3216		-	3.38 ± 0.96	2.91 ± 0.95	-	-	-	-	-	-	-	-	-	-	-	-
X-MeC32	32.315	3235		-	-	-	-	-	-	-	-	2.20 ± 0.96	-	-	-	-	-	-
14-16MeC32	32.585	3258	281/253	-	-	-	-	-	-	-	-	2.99 ± 0.88	-	-	-	-	-	-
triacontanal	32.038	3250		9.97 ± 2.28	1.17 ± 1.11	2.11 ± 0.57	0.39 ± 0.25	0.19 ± 0.13	0.24 ± 0.14	-	-	0.267 ± 0.38	0.59 ± 0.36	-	-	-	-	-
2-MeC32	32.195	3262	43/448	-	-	-	0.58 ± 0.28	0.37 ± 0.51	0.58 ± 0.47	-	-	0.265 ± 0.4	0.14 ± 0.29	-	-	-	-	-
triMethyl-C32	32.235	3266		-	-	-	-	-	-	-	-	2.49 ± 1.21	-	-	-	-	-	-
<b>C33:1</b>	<b>32.855</b>	<b>3283</b>	<b>462</b>	-	-	-	-	-	-	-	-	<b>13.71 ± 2.86</b>	-	-	-	-	-	-
<b>n-C33</b>	<b>32.633</b>	<b>3300</b>	<b>464</b>	-	<b>1.05 ± 0.34</b>	<b>1.80 ± 0.35</b>	<b>27.79 ± 3.57</b>	<b>25.19 ± 4.89</b>	<b>26.43 ± 2.54</b>	<b>1.58 ± 0.56</b>	-	<b>29.73 ± 2.6</b>	<b>29.23 ± 3.64</b>	-	-	-	-	-
<b>1-triacontanol</b>	<b>32.741</b>	<b>3309</b>	<b>18.97 ± 7.47</b>	<b>1.66 ± 2.03</b>	<b>2.18 ± 0.89</b>	-	-	-	-	-	-	-	-	-	-	-	-	-
17-;15-;13-MeC33	33.410	3329	253/225/281/197/30	-	-	-	-	-	-	-	-	3.18 ± 0.53	-	-	-	-	-	-
17,21-diMeC33	33.698	3356	197/253/267/323	-	-	-	-	-	-	-	-	4.13 ± 0.81	-	-	-	-	-	-
C34:1-1	33.954	3380	476	-	-	-	-	-	-	-	-	1.08 ± 0.28	-	-	-	-	-	-
C34:1-2	34.032	3386	476	-	-	-	-	-	-	-	-	2.50 ± 1.67	-	-	-	-	-	-
n-C34	33.743	3400	478	-	0.7 ± 2.29	-	3.12 ± 0.53	1.69 ± 0.46	2.065 ± 0.79	0.67 ± 0.46	-	1.91 ± 0.65	2.13 ± 0.48	-	-	-	-	-
1-triacontanol acetate	33.935	3417		-	-	2.56 ± 0.70	-	-	-	-	-	-	-	-	-	-	-	-
Unknown 2	34.329	3452		-	-	0.65 ± 0.22	-	-	-	-	-	-	-	-	-	-	-	-
2-MeC34	34.434	3461	43/477	-	-	-	-	0.05 ± 0.08	-	-	-	-	-	-	-	-	-	-
C35:1	35.109	3482	490	-	-	-	-	-	-	-	-	3.19 ± 0.74	-	-	-	-	-	-
n-C35	34.875	3500	492	-	-	-	5.61 ± 0.80	4.30 ± 1.00	4.51 ± 1.27	-	-	4.44 ± 1.82	5.15 ± 1.38	-	-	-	-	-
17-;15-;13-MeC35	35.627	3528	239/281/225/309/197/337	-	-	-	-	-	-	-	-	6.14 ± 1.25	-	-	-	-	-	-

Table 1 (continued)

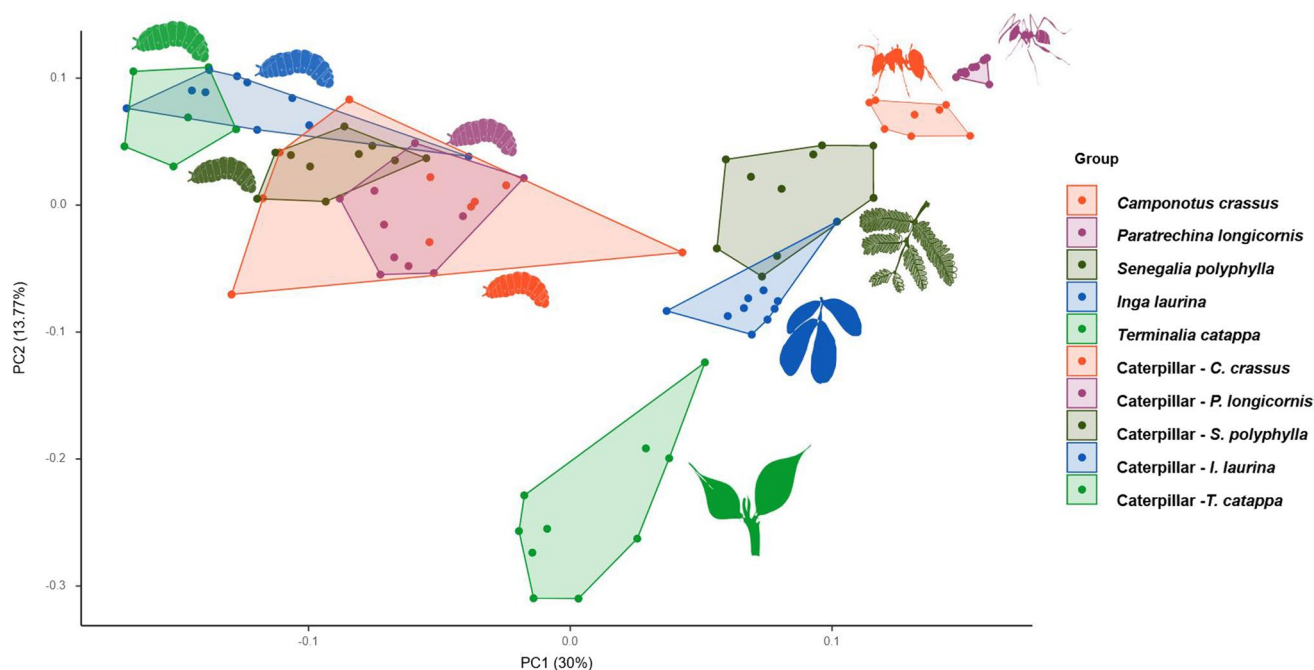
Compounds	Host plants			Caterpillars with host plants (without ants)				Ants			Caterpillars with ants		
	RT	RI	MDI	Sp	II	Tc	CS	CI	CT	Cc	PI	CC	CP
15.19-; 15.21-dimC35	35.627	3554	225/323/253/295	-	-	-	-	-	-	-	3.95 ± 1.1	-	-
<i>n</i> -C36	35.945	3600	506	-	-	-	0.37 ± 0.35	0.13 ± 0.14	0.15 ± 0.23	-	-	-	-

Major compounds in bold. (-) = compound not detected. A total of 10 samples per group was used for the chemical analysis, with the exception of *C. crassus* (8 samples) and *S. calyce-Terminalia catappa* (6 samples). RT (retention time), RI (retention index), MDI (molecular diagnostic ions), Sp (*Senegalia polyphylla*), II (*Inga laurina*), Tc (*Terminalia catappa*), CS (caterpillars raised on *S. polyphylla*), CI (caterpillars raised on *I. laurina*), and CT (caterpillar raised on *T. catappa*). Ants were also mentioned as Cc (*Camponotus crassus*) and PI (*Paratrechina longicornis*), along with caterpillars raised with them, like CC (caterpillars raised with *C. crassus*) and CP (caterpillars raised with *P. longicornis*)

Table 2 Morisita's similarity index (mean ± standard error), F-value, R2 and P-value of the shared cuticular hydrocarbons of pairs of groups (caterpillars, host plants and attendant ants)

	Caterpillars vs. plants			Caterpillars vs. ants			Plants vs. plants			Caterpillars vs. caterpillars					
	C-Sp vs. Sp	C-II vs. II	C-Tc vs. Tc	C-Cc vs. Cc	C-PI vs. PI	C-Pl vs. Pl	Sp vs. II	Sp vs. Tc	Tc vs. II	C-Sp vs. C-II	C-Sp vs. C-Tp	C-II vs. C-Tc	C-Cc vs. C-PI	C-PI vs. C-Sp	C-Cc vs. C-Sp
Morisita's similarity index (mean ± standard error)	0.58 ± 0.05	0.63 ± 0.11	0.78 ± 0.06	0.30 ± 0.05	0.25 ± 0.05	0.62 ± 0.07	0.62 ± 0.07	0.77 ± 0.05	0.7 ± 0.10	0.96 ± 0.02	0.95 ± 0.02	0.93 ± 0.04	0.99 ± 0.01	0.98 ± 0.02	0.99 ± 0.01
F-value	629.58	116.40	110.61	37.441	457.22	1.814	1.814	3.47	0.97	42.323	49.329	62.603	1.390	1.786	1.786
Coefficient of determination (R2)	0.9	0.89	0.86	0.675	0.962	0.091	0.091	0.167	0.051	0.70	0.89	0.68	0.071	0.090	0.090
P-value	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	> 0.05	> 0.05	> 0.05	> 0.05	< 0.05*	< 0.05*	< 0.05*	> 0.05	> 0.05	> 0.05

*S. calyce* caterpillars: C-Sp = caterpillars reared on *S. polyphylla*; C-II = caterpillar reared on *I. laurina*; C-Tc = caterpillars reared on *T. catappa*. C-Cc = caterpillars reared with *C. crassus*; C-PI = caterpillars reared with *P. longicornis*; Plants: Sp = *S. polyphylla*; II = *I. laurina*; Tc = *T. catappa*; Ants: Cc = *C. crassus*; PI = *P. longicornis*



**Fig. 1** Principal component analysis (PCA) of chemical compounds in *S. calyce* caterpillars (three groups reared on three plants species without ants and two groups reared with two ants species), the atten-

dant ants (*C. crassus* and *P. longicornis*) and the host plants (*S. polyphylla*, *I. laurina*, and *T. catappa*)

in the PCA (Fig. 1). Specifically, caterpillar–*T. catappa* and caterpillar–*I. laurina* shared 27 compounds, representing 93.1% and 90% of their respective cuticles. Caterpillar–*T. catappa* and caterpillar–*S. polyphylla* shared 26 compounds, representing 89.7% and 92% of their respective cuticles. Caterpillar–*I. laurina* and caterpillar–*S. polyphylla* shared 25 compounds, representing 83.3% and 92.6% of their respective cuticles. In general, all caterpillar groups had *n*-alkanes (C29, C31, and C33) as their major compounds and they also showed a few methylated compounds, alcohols, and aldehydes, these last two also present in all plants.

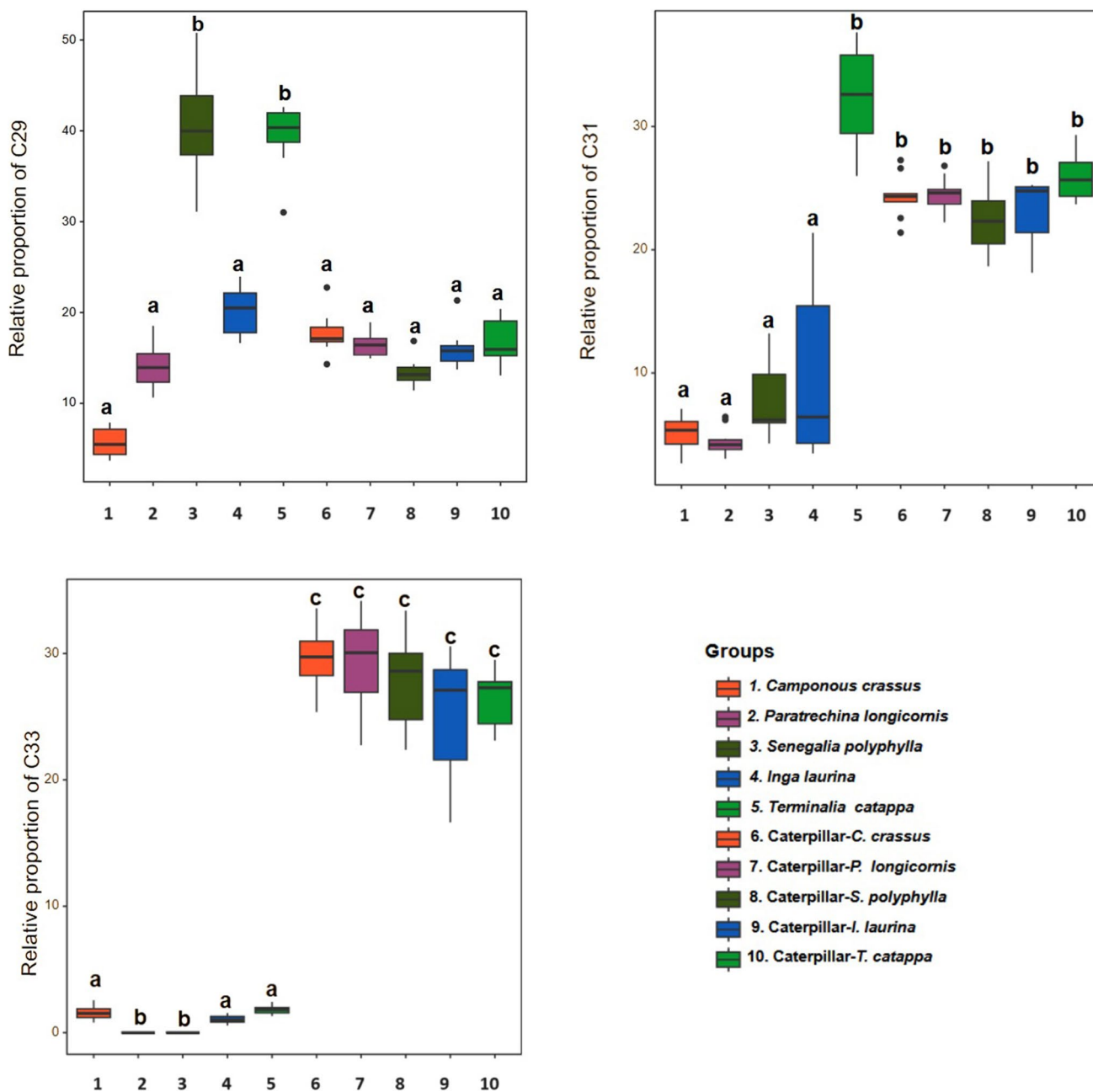
**Does the Chemical Composition of Caterpillars Change According to Their Tending Ants?** The cuticular profiles of caterpillars reared with *C. crassus*, or *P. longicornis* ants, as well as caterpillars reared without attendant ants, had a high SI (> 90%). Moreover, the cuticular profiles of all caterpillar groups were qualitatively similar, with 24 shared compounds, representing 96% of their respective cuticles. Hence, we found an overlap among samples of caterpillars in the PCA (Fig. 1). The post hoc pairwise comparisons did not show differentiation based on chemical composition among caterpillars reared with different species of attendant ants (Table 2).

In contrast, the cuticular profiles of caterpillars and attendant ants showed low similarity indexes and varied according to the attendant ant (SI < 30% for *C. crassus* and SI < 25% for

*P. longicornis*). Caterpillars and *C. crassus* shared 14 compounds, representing 54% and 50% of their respective cuticles. Caterpillars and *P. longicornis* shared four compounds, representing 16% and 18.2% of their respective cuticles. The post hoc pairwise comparisons showed a significant differentiation based on chemical composition between all groups of caterpillars and their respective attendant ants (Table 2). Moreover, the PCA revealed that ants and caterpillars form separate groups. In terms of major compounds, *C. crassus* had the *n*-C21, *n*-C30 and C33:1, and *P. longicornis* had the *n*-C29, 15-;13-;11-;9-MeC29, 15-;13-;11-MeC29 and 9.17-;9.19-diMeC29 (Table 1).

In general, caterpillars, host plants, and attendant ants shared the *n*-C27, *n*-C29, and *n*-C31 compounds (Fig. 3). Only caterpillars and plants showed alcohols and aldehydes in their chemical profiles (hexadecanol, octadecanol, eicosanol, octacosanal, docosanol, and triacontanal). The most significant differentiating compounds among the groups, according to SIMPER analysis, were the *n*-C33, *n*-C31 and, *n*-C29, which were major compounds in caterpillars (Fig. 2; Table S2). Overall, we observed that all groups of caterpillars presented high similarity in composition and proportion of their cuticular compounds. Host plants and caterpillars had a higher number of compounds in common, with *n*-alkanes (*n*-C29 and *n*-C31) as their major compounds. Host plants also had various alcohols and aldehydes, which are also present in caterpillars, but in smaller proportions. The three plants exhibited qualitative





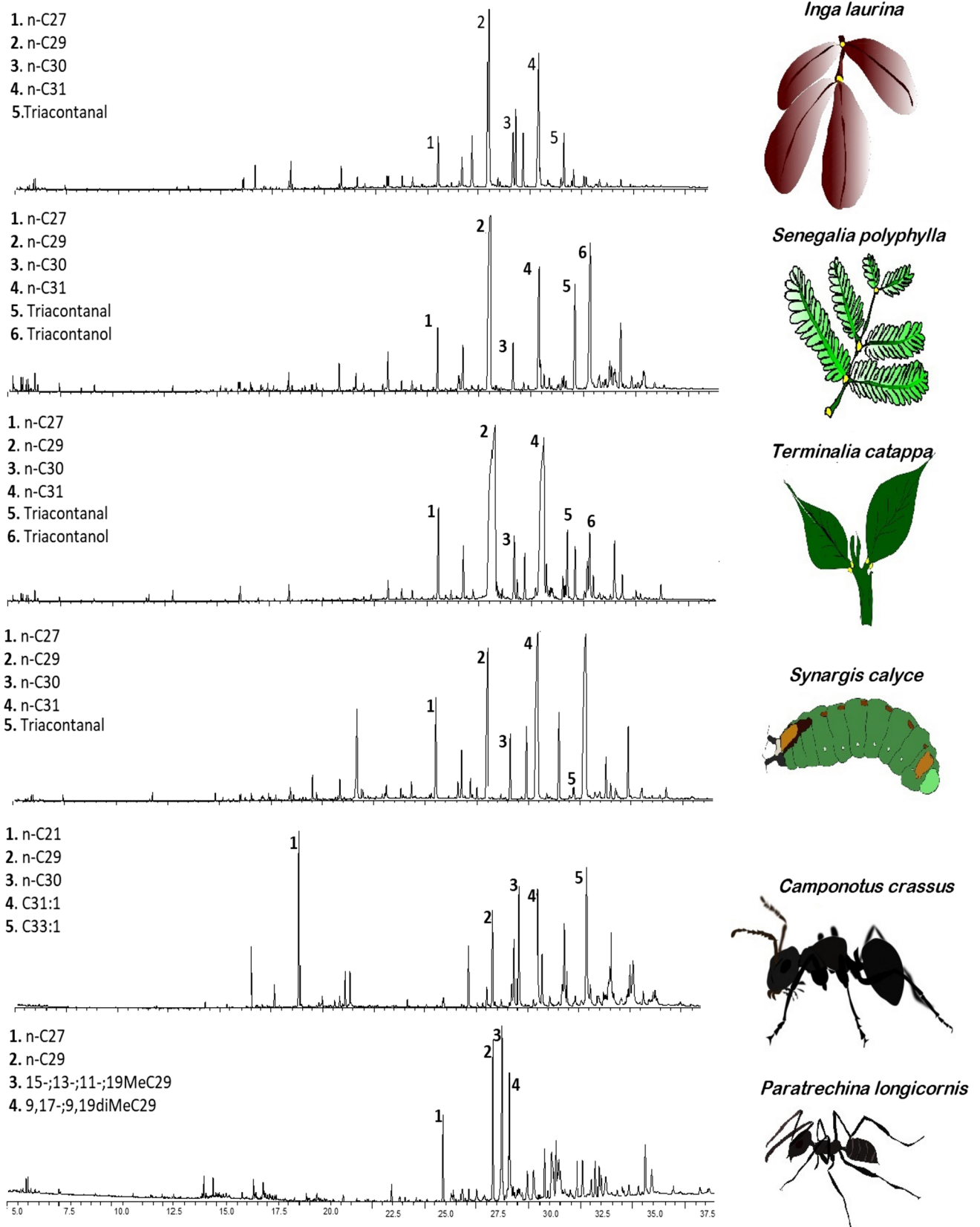
**Fig. 2** Box plots of the relative abundance of the most important chemical compounds (*n*-C29, *n*-C31 and *n*-C33) that contributed to differentiating the groups according to SIMPER. Groups 1 and 2

include attendant ants; 3–5 include host plants and 6–10 include caterpillars exposed to different conditions. Different letters represent  $p < 0.05$

similarities in their linear alkanes, with some variations in proportion. Additionally, there were qualitative differences in the alcohols and aldehydes among the three species. Thus, the cuticular profile of caterpillars was not influenced by attendant ants. Ant species showed a greater diversity of compounds compared to caterpillars, including various branched alkanes, alkanes, and alkenes (Fig. 3).

## Discussion

We found that the cuticular profile of caterpillars are more similar to those of the host plants rather than to their tending ants. Moreover, caterpillars reared on different species of plants, without ants or in close contact with ants also had similar cuticular composition and proportion, meaning that in an overall perspective caterpillar cuticular composition is weakly affected by exogenous factors. However, some of



**Fig. 3** Comparison of CHC profiles among host plant, myrmecophilous caterpillar, and attendant ants. All compound identities can be found in Table 1

their compounds varied depending on the host plants where they were reared, which suggests that they acquired part of these compounds through their diet. Thus, *S. calyce* caterpillars have a chemical profile that it is slightly altered by their food. Consequently, our hypothesis that the caterpillar cuticular composition is influenced by their food source was partially corroborated. Even though the caterpillars are not chemically identical to their host plants, there is a higher similarity between caterpillar-plant when compared to caterpillar-ant species. Given that some compounds, such as 1-hectacontanol, are present in one of the host plants and in caterpillars reared on that plant, but not in the other groups of caterpillars, this may suggest that at least part of the caterpillar chemical composition should derive from their food source. In this way, we suggest that the chemical composition of caterpillars is mainly genetically derived and slightly influenced by the environment. Acquiring compounds from host plants through diet and the usage of them when interacting with ants stands out as a promising strategy and it has also been observed in other plant-herbivorous insect systems (e.g., Silveira et al. 2010; Lima et al. 2021, 2024).

The three plant species used to feed the caterpillars exhibit qualitative similarities in their linear alkanes, as well as in certain alcohols and aldehydes. This suggests that *S. calyce* females likely tend to lay eggs on plants with similar chemical profiles, a similar pattern was observed in lycaenids (Lima et al. 2021). There is evidence that alkanes and alcohols can serve as signals for host plant selection (Li and Ishikawa 2006; Barbero 2016; Berteza et al. 2020). In a recent study involving *S. calyce*, it was observed that butterflies sometimes mistakenly lay eggs directly on treehoppers because the treehoppers have a cuticular profile similar to that of the host plant (Lima et al. 2023). The compounds found on the surface of leaves have been described to play a role in the chemical defense of plants (aliphatic hydrocarbons, fatty and phenolic acids derivatives) (Martemyanov et al. 2015; Berteza et al. 2020). Hence, there is a possibility that an evolutionary arms race between caterpillars and plants has driven caterpillars to develop mechanisms countering the chemical defenses of plants. This may involve detoxification of compounds through enzymes or the sequestration of such compounds. Consequently, caterpillars may exhibit a preference for specific chemical compounds present in various host plants (Zu et al. 2020).

Through chemical analyses, we found that the chemical profile of caterpillars were not influenced by their attendant ants thus confirming our second hypothesis that *S. calyce* CHCs are not affected by the interactions that they establish with different ant species. The two ant species studied showed distinct chemical profiles, while the groups of *S. calyce* caterpillars reared with different ant species or without ants had similar cuticular profiles composition and proportion. This indicates that *S. calyce* caterpillars have cuticular

profiles that are independent of their attendant ants, indicating that they do not use chemical mimicry as a strategy when interacting with them. This finding is supported by the dissimilarity in chemical composition between caterpillars and ants, compared to the similarity between caterpillars and plants. Additionally, *S. calyce* caterpillars have facultative association with several species of ants, and they do not exploit ant nests (Callaghan 1986; Alves-Silva et al. 2018; Kaminski 2021). Previous studies demonstrating chemical mimicry between caterpillars and ants have typically involved obligate interactions with a few specific ant species, where the CHCs of caterpillars closely resemble those of the ants (Henning 1983; Elmes et al. 1991, 2002; Dettner and Liepert 1994; Akino et al. 1999; Schönrogge et al. 2004; Hojo et al. 2009, 2014; Thomas et al. 2013; Witek et al. 2013; Barbero 2016; Casacci et al. 2019). Chemical mimicry with attendant ants is an effective strategy for social parasitic caterpillars, as they typically inhabit ant nests and benefit from being perceived as members of the colony, allowing them to exploit valuable resources within the nest such as ant larvae or trophallaxis (Fiedler 1991; Barbero 2016; Casacci et al. 2019). We also ruled out the possibility of a chemical insignificance strategy in *S. calyce* caterpillars as in previous studies where caterpillars and pupae used chemical insignificance, their cuticular profile consisted of only a few hydrocarbons in very small proportions (Lohman et al. 2006; Inui et al. 2015). In contrast, *S. calyce* caterpillars showed 27–30 cuticular compounds, with some of them in high proportions.

Thus, it seems that this species maintains its own chemical profile in a chemical conspicuousness strategy (*sensu* Lima et al. 2021). This strategy is the most likely since the chemical profiles of caterpillars exposed to various conditions remained mainly unchanged, with all groups exhibiting a high degree of similarity. Chemical conspicuousness becomes advantageous when interacting with attendant ants, increasing the likelihood that ants will associate the reward with specific cuticular profiles (Hojo et al. 2014). Studies conducted with Neotropical Lycaenidae species have demonstrated that these species possess conspicuous chemical profiles (Lima et al. 2021). Additionally, there are studies showing that caterpillars or pupae of facultative lycaenid butterflies from other regions have unique chemical profiles recognized by ants, which helps maintain their attending behavior (Ômura et al. 2009, 2012; Hojo et al. 2014; Mizuno et al. 2018). However, to our knowledge, this is the first study to explore cuticular hydrocarbons (CHCs) and investigate chemical strategies in a facultative species of the Riodinidae family.

In this study, caterpillars and plants shared several compounds when compared to ants. Notably, linear alkanes such as C29 and C31 are present in significant proportions across all groups, along with some alcohols and aldehydes.

Therefore, we cannot rule out the possibility that in certain instances, caterpillars may employ chemical camouflage. Moreover, caterpillars reared on one host plant species exhibited a similarity exceeding 80%, which has been previously demonstrated as sufficient to serve as chemical camouflage strategy in other insect groups (Silveira et al. 2010). Host plants play a crucial role in providing an effective background for herbivorous organisms, allowing them to avoid detection by visually or chemically resembling their surroundings. This strategy is observed in various organisms that have close relationships with ants, enabling them to interact with ants without being attacked (von Beeren et al. 2012; Barbero 2016; Lima et al. 2024). Consequently, we propose that these caterpillars may employ a trade-off strategy between camouflage and informing their presence to ants, which could vary depending on the presence of predators or mutualist ants. Given the presence of numerous non-attendant ant species that visit the host plants, many of which have extrafloral nectaries, it is highly likely that chemical camouflage has been selected as an efficient strategy for *S. calyce* caterpillars to avoid attacks from different ants (Akino et al. 2004). On the other hand, informing their presence to attendant ants also is efficient for the caterpillars. The complexity of CHCs profiles is well-known, and it is likely that each chemical trait serves a distinct function (Sprenger and Menzel 2020). There may even be conflicts or trade-offs among the various functions of the chemical profile (Steiger and Stöckl 2014; Ingleby 2015). Camouflage might be the protagonist during encounters with non-attendant ants, while directly informing their presence becomes more prominent with attendant ants, as it aims to induce ants to associate caterpillars' chemical profile with the chemical reward, consequently securing ant protection (Hojo et al. 2014).

We suggest that specific compounds such as some aldehydes present in both plants and caterpillars or methylated alkanes present in caterpillars, play a role in making the caterpillars blend with the background or informing their presence to attendant ants respectively. For instance, in studies conducted on *Lycaeides argyrognomon* (Bergsträsser, 1779) (Lepidoptera: Lycaenidae), pupal cuticular lipids were found to contain various long-chain aliphatics aldehydes, including 1-octacosanal and 1-triacontanal, which were found to suppress ant aggression (Mizuno et al. 2018). Interestingly, these two compounds were found in all groups of *S. calyce* caterpillars and host plants, suggesting their potential importance in the interaction between *S. calyce* caterpillars and ants.

Studying tri-trophic relationships can present challenges when analyzing the results. For instance, in the PCA results, we observed a low percentage for PC1 and PC2. We suspect that these low percentages values may be linked to the number of groups included in the analysis, comprising five

caterpillars, three plants, and two ant groups. The low existent variation within each main group (e.g. ant, caterpillar, and plant) may have contributed to an overall lower dissimilarity percentage when comparing all the groups at once. Thus, to elucidate our findings, we employed more than one type of analysis. Using multiple approaches to analyze the data stands out as a useful strategy when working with complex systems.

Ants exert strong selection pressure on myrmecophilous caterpillars, leading to the development of multimodal adaptations (Pierce and Dankowicz 2022; Marquis and Koptur 2022). These adaptations include morphophysiological, behavioral, chemical, and acoustic traits that caterpillars utilize to deceive, attract, alarm, or appease attending ants (Fiedler et al. 1996; Casacci et al. 2019). *Synargis calyce* caterpillars possess functional TNOs, which have been shown to contribute to the association with ants in other riodinids (DeVries 1988; Kaminski and Carvalho-Filho 2012; Kaminski et al. 2013; Mota et al. 2020; Kaminski et al. 2021). To further enhance our understanding of the multimodal signaling in myrmecophile systems, future studies should conduct behavioral assays to experimentally confirm the chemical strategy employed and investigate the products and effects of caterpillars' ant-organs on attending ants, as well as compare them with the products of extrafloral nectaries from plants. This research will contribute to unraveling the specific role of these chemical strategies and organs in the complex interactions between myrmecophilous caterpillars and ants.

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**Data Availability** Data was deposited in the supplementary section.

## Declarations

**Competing Interests** The authors declare no competing interests.

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