DRYAS IULIA (LEPIDOPTERA, NYMPHALIDAE) LARVAL CHOICE AND PERFORMANCE ON FOUR PASSIFLORA SPECIES

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Abstract

The interaction among the Heliconiini butterflies and their Passiflora species is of great importance in ecology and coevolution studies. For the Heliconiini, the ability to choose a suitable host plant is determinant for the larval performance and conservation initiatives that target these species. Herbivorous insects may choose their food based on factors such as nitrogen and water content, as well as leaf digestibility and defences. In this study, we experimentally evaluated Dryas iulia (Nymphalidae) larval choice regarding four Passiflora (Passifloraceae) and the larval performance on the two most consumed species. We tested the hypothesis that D. iulia larvae would choose the Passiflora species with higher nutritional quality and lower physical defence. Dryas iulia larvae preferred P. misera (60.5% leaf consumption) over P. pohlii (28.9%), P. suberosa (15.5%), and P. edulis (not consumed). Passiflora misera presented the highest content of nitrogen and the third content of water, was ranked the second in non-glandular trichome density and did not present glandular trichomes. On P. misera, D. iulia presented the highest survival (23.1%), conversion efficiency (32.8%), relative growth rate (14.8%), heavier pupae (15.2%), and the lowest relative consumption rate (13.8%). Thus, in this experimental study, D. iulia larvae were able to choose the plant material that supported greater performance. Passiflora misera was the most suitable host species for conservation and management purposes of this Heliconiini butterfly.

Key words: Heliconiini, insect herbivory, larval performance, larval plant choice, leaf nutritional quality, leaf traits

INTRODUCTION

The relationship among plants and insects is one of the most important interactions in the maintenance of ecological communities. The study of plant traits (e.g. leaf nutrition, tissue hardness, and defences) is essential to evaluate their influence on herbivores, particularly herbivorous insects (Price et al. 1990; Pérez-Harguindeguy et al. 2003; Quintero & Bowers 2018). Because plant nutritional quality and defences (physical or chemical) vary widely among plant species and among individuals of the same species, (Awmack & Leather 2002) their importance in the selection and feeding by herbivorous insects is paramount (e.g., Price et al. 1990; Panizzi & Parra 2012).

The quality of plant material influences herbivorous insects’ behaviour, reproduction, development, and survival (Panizzi & Parra 2012; Quezada-Garcia et al. 2015). Among the traits associated with plant quality, leaf nitrogen content plays a central role in insects’ development (Mattson 1980; Uyi et al. 2018). Nitrogen is found in proteins, nucleic acids (DNA and RNA), polysaccharides such as chitin, and specialized secondary compounds (Mattson 1980; Samal et al. 2019). Higher nitrogen intake positively correlates with greater efficiency in converting consumed food into insect biomass (Scriber 1984; Schoonhoven et al. 2005). Other plant traits important in herbivores nutrition are the content of water and lipids (Nestel et al. 2016). In the absence or lower amount of these components, holometabolous insects may produce smaller adults with reduced reproductive capacity, altered behaviour, reduced immunity, and lower resistance to environmental stresses (Santos et al. 2008; Cahenzli et al. 2015; Nestel et al. 2016).

The quality of the plant material is also associated with physical and chemical defences such as trichomes and a myriad of secondary compounds. Trichomes are one of the most commonly found physical defence in plant species, preventing insect herbivores to move on plant tissues and reducing/disabling feeding. Trichomes are uni- or multicellular appendages of plant epidermal cells, which can be categorized into non-glandular and/or glandular...
type (Fernandes 1994; Stratmann & Bequette 2016). Non-glandular trichomes are non-secretive and act as mechanical barriers particularly efficient against small insects or early larval instars, while they may also prevent adult’s oviposition (Woodman & Fernandes 1991; Schoonhoven et al. 2005; Karabouniotis et al. 2020). Glandular trichomes are secretive, often releasing chemicals that are self-adhesive or toxic to herbivores (Scriber & Slansky 1981; Dasanayake & Hicks 1994; Schuurink & Tissier 2019).

Plants also developed large spectra of anti-herbivore defences through the production of compounds of low molecular weight such as alkaloids, terpenes, glucosinolates, or cyanogenic glucosides (van Ohlen et al. 2017). These chemical compounds may repel an enemy from a host plant or even harm the herbivore upon plant material ingestion (van Ohlen et al. 2017, for reviews see Fürstenberg-Hägg et al. 2013; War et al. 2018).

The relationship between insects’ host plant selection and offspring performance is historic and central in the study of insect-plant interactions. Overall, there is strong evidence for the preference–performance hypothesis (PPH) in which female adults select host plants of the greatest quality, where their larvae could attain higher fitness (Thompson 1988; Gripenberg et al. 2010). Nevertheless, this correlation is often not found (Refsnider & Janzen 2010; König et al. 2016), even among oligophagous species such as the Heliconiini butterflies (Castro et al. 2018). Many hypotheses have been created to explain this non-correlation (Thompson 1988; Gripenberg et al. 2010), such as the parasite/grazer hypothesis, the time hypothesis, and the patch dynamics hypothesis (Thompson 1988). In the parasite/grazer hypothesis, due to environmental or biotic reasons (e.g. small host plants, competition with other herbivores/ enemies, host plant mortality), a grazer-like behaviour may be favoured especially in insects with high mobility or in later larval instars. However, for specialists such as the Heliconiini butterflies, a non-correlation between oviposition and offspring performance or a disruption between the larva and its original host may be deadly (Castro et al. 2018). Therefore, when larvae are found under such unforeseeable circumstances, the ability to choose the most suitable plant material to feed is crucial (within larval mobility constraints) (Thompson 1988; Gripenberg et al. 2010).

Historically, the study of the Passiflora and the Heliconiini butterflies has been a model system for better understanding coevolution due to their inherent diversity of morphological and chemical aspects (Benson et al. 1975). Among the Heliconiini, Dryas iulia (Fabricius 1775; Nymphalidae) can be found throughout the Americas and feed on various Passiflora species (Benson et al. 1975; Beltrán & Brower 2008). The Passiflora is the most representative genus in the Passifloraceae containing a myriad of physical and chemical defenses against herbivores (Dhawan et al. 2004; Wosch et al. 2015). They exhibit a wide range of leaf morphological patterns that can vary intraspecifically or according to the leaf’s age (Braglia et al. 2013; Castro et al. 2018). Passiflora leaf variegation also seems to be very common, especially in species of the subgenus Decaloba (Gilbert 1971; Cardoso 2008). To discourage oviposition and herbivory, some Passiflora species present extraloral nectaries (Bentley 1977) and structures that mimic oviposited eggs and other arthropods on their stipules, tendrils, petioles, leaves, and flowers (Benson et al. 1975; Lev-Yadun 2009).

Among Passiflora physical defences, there are a few studies reporting the role of trichomes against herbivores (mainly non-glandular) (Castro et al. 2018). For instance, non-glandular trichomes in P. lobata and P. adenopoda from the Decaloba subgenus represent an efficient physical barrier against Heliconiini caterpillars (Gilbert 1971; Cardoso 2008). Glandular trichomes are reported in just a few Passiflora species: P. foetida (Durkee 1984), P. clathrata, P. lepidota, P. villosa (Dhawan et al. 2004), and P. suberosa (García et al. 2000). In addition, Passiflora species also stand out for producing an extensive range of secondary compounds, mainly alkaloids, saponins, phenols, glycosyl flavonoids, and cyanogenic compounds (Dhawan et al. 2004; Castro et al. 2018). Passiflora edulis and P. suberosa are some of the most studied Passiflora species concerning their phytochemicals (Dhawan et al. 2004), highlighting the wide variety of alkaloids and glycosides that these species possess (Spencer & Seigler 1987; Seigler et al. 2002; Dhawan et al. 2004; Cerqueira-Silva et al. 2018). However, some Heliconiini species can tolerate and even sequester certain cyanogenic glucosides and alkaloids found in their Passiflora hosts and allocate them into their chemical defence (Chauhan et al. 2013; Castro et al. 2018).

Studies that focus on the interaction between the Passiflora and the Heliconius open opportunities to broaden our coevolution knowledge and to support wildlife management and butterfly conservation initiatives. For instance, habitat construction is one of
the strategies used in Lepidoptera conservation efforts (Pegram & Melkonoff 2020). In these cases, the most suitable host species are evaluated and later used as guidelines for which plant species should be added/favoured in the Lepidoptera habitat (Pegram & Melkonoff 2020).

In this study, we evaluated *D. iulia* larval choice over four *Passiflora* species (*P. suberosa* L., *P. misera* Kunth, *P. pohlii* Mast. and *P. edulis* Sims f. *flavicarpa* Degener) (heretofore *P. edulis*) and their larval performance when reared with two of the most consumed species on the choice experiment. We tested the hypothesis that *D. iulia* larvae can choose the *Passiflora* plant that supports the greatest larval performance (Lee et al. 2012). Two experiments were performed to answer the following questions: i) do *D. iulia* larvae exhibit a preference for one of the four *Passiflora* hosts?; ii) are *D. iulia* larvae able to choose the host species that supports their best performance?; iii) which of the evaluated leaf traits could better explain larval food choice?; and finally, iv) which of the studied *Passiflora* species is the most suitable as a host for the management and conservation of *D. iulia*? We predict that the *Passiflora* leaf traits (i.e. nitrogen, and water content, trichome type, presence, and density) would affect *D. iulia* larval food choice. Furthermore, *D. iulia* larvae would choose the *Passiflora* host species with the greatest quality (i.e. high nitrogen and water content and lower trichome density) which would support the best larval performance (i.e. shorter development time, lower consumption rate, and higher conversion efficiency, growth rate, and survival). Finally, we predict that nitrogen content would be the leaf trait of greatest importance in larval food choice if trichomes are not present or at low density.

**Figure 1**: a) *Passiflora suberosa*, b) *P. misera*, c) *P. pohlii*, d) *P. edulis*. Photo credits: Nilton T.V. Junqueira.

### Material and Methods

**Dryas iulia-Passiflora system**

The *Passiflora* genus is native to Neotropical forests and widely distributed from Argentina to Mexico, while only 26 of its species are found in tropical and subtropical regions of Asia and Oceania (Castro et al. 2018). For this study, we selected four species of *Passiflora*: *P. suberosa, P. misera, P. pohlii*, and *P. edulis* (Fig. 1). In Brazil, these species occur in regions highly impacted by anthropic activities in the biomes of *Cerrado*, Atlantic Forest, and Pantanal (Bernacci et al. 2015). The occurrence of these *Passiflora* species in heavily impacted areas reinforces the need to better understand the *D. iulia-Passiflora* system to support conservation and management strategies for both genera.

Heliconini butterflies and their ecological relationship with the *Passiflora* was one of the coevolution model systems studied by Ehrlich and Raven (1964). Among them, *D. iulia* has been described to present high dispersion rates and can be found in various habitats, including anthropized areas (Paim et al. 2004). Although *D. iulia* larvae feed on various *Passiflora* species, they most commonly use species from the subgenus *Plectostemma* as host plants (including the four *Passiflora* in this study) (Benson et al. 1975; Paim et al. 2004). *Dryas iulia* eggs are yellow to opaque orange and are usually laid on the abaxial side of small to medium mature leaves (Benson et al. 1975; Paim et al. 2004). Caterpillars are solitary, short-lived (about 20 days) (Millan et al. 2013) and are known to be unpalatable to predators (Brown 1981).

**Larval and plant material**

For the larval choice experiment, five healthy individuals (firm and well-formed leaves with no indication of damage or illness) of each *Passiflora* species were selected (*P. suberosa, P. misera, P. pohlii, P. edulis*) from the Belo Horizonte Zoo and Botanical Foundation - BHZoo (S 19°51’; W 44°01’). Later, we used two criteria to select which of the four *Passiflora* species from the choice experiment would be used in the performance experiment. We considered plant material availability in the study region and *D. iulia* consumption in the choice test. It is true that under a no-choice test, larvae could have consumed all of the available *Passiflora* species. However, one of our motivations was to evaluate if larvae would be able to choose a plant material based on its quality.
Hence, using the most consumed *Passiflora* species to evaluate larval performance was the best option considering our goals.

*Dryas iulia* eggs used in the experiments were provided by the BHZoo butterfly nursery. Eggs were stored in sterile plastic pots and immediately transported to the laboratory where they were kept inside a Bio-Oxygen Demand Incubator (BOD; Ethiktechnology 411D) until larvae hatched. BOD conditions were 25 ± 2 °C, 55 ± 10% relative humidity (RH), and 12 hours photoperiod (PP). Egg collection took place on two occasions, one for each experiment (larval choice, and larval performance).

**Passiflora nutritional quality and physical defence**

We analysed *Passiflora* nutritional quality (i.e. leaf water and nitrogen concentration) and physical defences (i.e. presence/density of linear and/or glandular trichomes) to evaluate *D. iulia* larval plant choice among the four *Passiflora* species (*P. suberosa*, *P. misera*, *P. pohlii*, and *P. edulis*). For water content, one healthy and fully opened leaf was collected from each of the five individuals per *Passiflora* species (N = 5 leaves per species). Leaf fresh mass (LFM) was recorded using a precision scale and later oven-dried at 70 °C until its dry weight had stabilized for leaf dry mass (LDM) (72 - 96 h). The difference between LFM and LDM divided by LFM indicated leaf water content (LWC). For leaf nitrogen content, 10 leaves that were healthy and relatively of the same age were collected in each of the four individuals per *Passiflora* species. Subsequently, leaves were dried (47 °C for 96 hours), crushed and separated into samples (each containing 5g of dry mass). Leaf samples were sent to the Soil Department of the Federal University of Viçosa for nitrogen analysis (Kjeldahl method). *Passiflora* leaves water and nitrogen content did not follow a normal distribution. Hence, we used ANOVA on Ranks followed by a post hoc Student-Newman-Keuls test.

To assess trichome type and density, six leaves were collected from three individuals of each studied species (N = 18 leaves per *Passiflora* species; total = 72). Soon after leaf collection, leaf epidermis was submitted to a dissociation technique (Jeffrey solution; details in Johansen 1940). Afterward, leaves were stained with 1% safranin and fixed on a slide. Trichomes on leaf surfaces were visualized through a Lambda optical microscope using a 5X objective containing a micrometric eye network. Trichomes were counted on both abaxial (N = 9 leaves per *Passiflora* species; total = 36) and adaxial (N = 9 leaves per *Passiflora* species; total = 36) leaf surfaces. The number of focal points for trichome counting per leaf depended on the known distribution of these foliar appendages per species. For *P. misera*, *P. pohlii*, and *P. edulis*, visualization was done in three focal points per leaf surface to obtain a more complete information on trichome density (N = 54 focal points per species - *P. misera*, *P. pohlii*, and *P. edulis*). For instance, these three species have a homogeneous trichome distribution between the border and middle leaf regions. On the other hand, trichomes of *P. suberosa* were heterogeneously distributed between the border and middle leaf regions. Thus, trichome counting in this species was performed using two focal points per leaf surface: one at the leaf border region and one at the leaf middle region (N = 36 focal points for *P. suberosa*). Trichome density was recorded by counting the number of trichomes and expressed in mm².

Tector trichome density (parametric) was compared among *Passiflora* species through Variance Analyses (ANOVA) followed by a post hoc Tukey test (5% significance). T-tests were used to compare glandular trichome density (parametric data) between foliar regions of *P. suberosa*. Statistical analyses and graphical demonstrations were done in the GraphPadPrism (GraphPad 5.0 Software, Inc., San Diego, CA, EUA).

**Dryas iulia larval food choice**

To evaluate the *D. iulia* larval choice among the four *Passiflora* species, twenty newly hatched larvae of uniform size were separated into individual Petri dishes and kept in a BOD under previously described conditions. To avoid food conditioning until the food choice experiment started, *D. iulia* larvae had the option to feed on leaf discs (ο = 1.0 cm) of all studied *Passiflora* species. This procedure continued until larvae reached the third instar as recommended for larval food choice evaluation (Malishev & Sanson 2015). Petri dishes (N = 20) were prepared with a wet filter paper to maintain leaf turgor and individually received two leaf discs (ο = 1.0 cm) per plant species (see details in Fig. 2). Leaf discs of all *Passiflora* species were always placed on Petri dishes with their adaxial side facing upwards to avoid any influence on the larval plant choice. Plant material of each *Passiflora* species was randomly collected from five leaves that were healthy and of the relatively same age. Third instar caterpillars fasted for 6 hours before the plant choice experiment began (Sagers 1992).
when they were gently placed at the centre of each Petri dish using a fine camel-hair brush. Petri dishes inside the BODs rotated every five hours to avoid any ambient influence on larval plant choice. Leaf remains were individually photographed to obtain the consumed surface area through the ImageJ software (Schneider et al. 2012). Consumption percentage was calculated subtracting the disc remaining area (after 20h assay) from the initial disc areas ($\varnothing = 1.0$ cm). Larval plant choice was ranked according to how much each *Passiflora* species was consumed.

Plant choice data (consumed leaf area) were parametric, thus, we used Analyses of Variance (ANOVA) to compare leaf consumption among the four *Passiflora* species and posteriorly a post hoc Tukey test with 5% significance. Statistical analysis and graphical demonstrations were done in the GraphPadPrism 5.0 software.

Finally, Planned Comparisons were performed using the general linear hypotheses for multiple comparisons in the “multcomp” package in R (Hothorn et al. 2008). This package enables us to perform simultaneous inferences to assess which of the evaluated leaf parameters (i.e. nitrogen, water, tector, and glandular trichome) and their respective concentration/density best explains the results for leaf consumption per *Passiflora* species. Each comparison (larval consumption x leaf parameter) was performed independently, followed by an evaluation of which results best fit the consumption data. These analyses were performed in the R software (2019).

**Dryas iulia performance**

Dryas iulia larvae performance in relation to its host plant was evaluated through the following parameters: larval survival, ingested food conversion efficiency (CE), relative growth rate (RGR), relative consumption rate (RCR), larval development time (days), pupal weight (g), and pupation time (days). These parameters are well established in herbivore performance literature and were calculated according to Panizzi and Parra (2012). Data analyses were performed only considering individuals that reached their adult stage, excluding those who appeared to be ill and/or deformed.

Forty neonate caterpillars of uniform size were separated into individual plastic containers and fed with each *Passiflora* species (N = 20 per species; total = 40). Each container had a wet filter paper covering its bottom and was closed by a perforated plastic lid covered with nylon fabric to ensure aeration. Plastic containers were cleaned, and filter papers were replaced daily, as well as the addition of two fresh leaf discs ($\varnothing = 1.0$ cm) per container. Leaf discs were always from healthy and fully opened leaves from the five individuals of each *Passiflora* species (*P. misera* and *P. suberosa*). Caterpillars designated to a treatment were exclusively fed from the experiment beginning with its respective *Passiflora* species. Larval performance experiment began when larvae were still neonates, thus, more susceptible to host plant quality (Malishev & Sanson 2015; Quintero & Bowers 2018). Caterpillar survival was daily recorded, and leaf remains, fresh plant material, and larvae were all weighed. Consumption was obtained with the difference between the fresh leaf weight recorded on the previous day and the weight of the foliar remains on the next day. Cephalic capsule and/or larval exoskeleton presence was used as an indication of instar change. Larvae were kept in a BOD for the experiment duration (25 ± 2 °C; 55 ± 10% RH; 12 h PP) and plastic containers rotated daily inside the BODs to avoid any ambient influence on larval consumption. On the second day of pupation, pupae were weighed and fixed in a wooden support. Finally, adults’ eclosion date from their chrysalis was recorded.

Larvae survival was calculated using the chi-square test. The performance indexes (CE, RGR, and

**Figure 2:** Layout position in the larval plant choice test. Each dish contained two fresh leaf discs per *Passiflora* species made available to a third instar *D. iulia* larvae, which was placed in the dish centre.
RCR), the development time (total and of each instar between Passiflora species), pupal weight, and pupation time were compared by Student’s T-tests. The Mann-Whitney Rank Sum statistical test was performed on final development time (non-parametric) to compare performance responses between the two species. We compared the correlation between the last caterpillar instar and pupae weight using the Pearson correlation test. Analyses and graphical illustrations were done in the GraphPadPrism 5.0 software.

**RESULTS**

**Passiflora analyses and D. iulia larvae plant choice**

Leaf water content varied significantly among the four studied Passiflora species (P. suberosa, P. misera, P. pohlii, and P. edulis) (P < 0.0001; Fig. 3a; Table S1). The highest amount of water content was found in P. suberosa (84%), followed by P. edulis (73%), P. misera (68%), and P. pohlii (59%). Leaf nitrogen (N) content in P. misera was, on average, 15.6%, 29.9%, and 39.3% higher compared to P. edulis, P. pohlii, and P. suberosa, respectively. Therefore, P. misera presented the highest nitrogen content (P = 0.0017; Fig. 3b; Table S1) while there was no statistical difference in N content among the other three Passiflora species. The highest trichome density was found in P. pohlii (1.2 trichomes/mm²), followed by P. misera and P. suberosa (respectively, 0.6 and 0.2 trichomes/mm²) (P < 0.0001; Fig. 3c; Table S1). Thus, tector trichomes were detected in P. misera, P. pohlii, and P. suberosa (Fig. 4), while glandular trichomes were found only in P. suberosa. The distribution of glandular trichomes differed significantly between the middle and border leaf regions of P. suberosa: border regions (0.073 ± 0.006) had

![Figure 3: Average (± SE) water content (a), nitrogen content (b), and tector trichomes density (c) found in leaves of the species: P. suberosa, P. misera, P. pohlii, and P. edulis. Different lowercase letters indicate a statistically significant difference among the groups.](image-url)

### Table 1: Water and nitrogen content, tector trichomes density, and leaf consumption percentage of the Passiflora species: P. suberosa, P. misera, P. pohlii, and P. edulis. Different lowercase letters indicate a statistically significant difference among the groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P. suberosa (X ± SE)</th>
<th>P. misera (X ± SE)</th>
<th>P. pohlii (X ± SE)</th>
<th>P. edulis (X ± SE)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf water content (%)</td>
<td>82.001 ± 0.545</td>
<td>68.187 ± 0.922</td>
<td>59.180 ± 0.222</td>
<td>72.720 ± 0.259</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Leaf nitrogen content (dag/Kg)</td>
<td>3.373 b ± 0.199</td>
<td>4.529 a ± 0.106</td>
<td>3.605 b ± 0.111</td>
<td>4.076 b ± 0.282</td>
<td>= 0.0017</td>
</tr>
<tr>
<td>Number of tector trichome/mm²</td>
<td>0.051 c ± 0.007</td>
<td>0.491 b ± 0.075</td>
<td>1.194 a ± 0.157</td>
<td>0.000 ± 0.000</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Leaf consumption (%)</td>
<td>20.540 b ± 3.422</td>
<td>66.094 a ± 4.987</td>
<td>22.038 b ± 1.588</td>
<td>0.000 ± 0.000</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
55.2% greater density of glandular trichomes than the leaf middle regions (0.029 ± 0.005) (P = 0.0095).

Among the four studied plant species, *P. misera* was the most consumed by *D. iulia* larvae, with an average leaf consumption of 61% whereas *P. edulis* was not consumed (P < 0.001; Fig. 5). Of the evaluated leaf traits (i.e. water and nitrogen content; tector and glandular trichomes presence/density), nitrogen best explained the consumption of the preferred *Passiflora* species (P < 0.0001).

**Dryas iulia** larval performance between *P. misera* and *P. suberosa*

Survival at the end of the larval stage was 23.1% higher in *D. iulia* caterpillars fed with *P. misera* (16) in comparison with *P. suberosa* (13) (not plotted; x² = 5.17; P < 0.05). The conversion efficiency (CE) was 32.8% higher on *P. misera* than on *P. suberosa* (P = 0.0001; Fig. 6a; Table 2), specifically on the second (P = 0.0235), third (P = 0.0004), fourth (P = 0.0006), and fifth (P = 0.0055) instars (Fig. 6b; Table 2). Likewise, larval RGR was 14.8% greater on *P. misera* than on *P. suberosa* (P = 0.0070; Fig. 6c; Table 2), particularly in the third (P = 0.0182) and fourth instars (P = 0.0008; Fig. 6d; Table 2). On the other hand, larval RCR was 13.8% higher among caterpillars fed with *P. suberosa* (P = 0.048; Fig. 6e; Table 2), even though there was no statistical difference when the comparison was made among each instar (Fig. 6f; Table 2). *Dryas iulia* larvae development time until pupation was significantly lower (16.3%) on *P.
Figure 6: Average (± SE) of the following D. iulia larvae performance parameters when treated with P. suberosa and P. misera: conversion efficiency (CE) during full larval development (a) and at each instar (b); relative growth rate (RGR) during full larval development (c) and at each instar (d); relative consumption rate (RCR) during full larval development (e) and at each instar (f); necessary time to complete development (g) and to change instar (h). Different lowercase letters indicate a statistically significant difference among the groups.
Table 2: *D. iulia* larvae performance parameters when treated with *P. suberosa* and *P. misera*: conversion efficiency (CE) during full larval development and at each instar; relative growth rate (RGR) during full larval development and at each instar; relative consumption rate (RCR) during full larval development and at each instar; necessary time to complete development and to change instar. Different lowercase letters indicate a statistically significant difference among the groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>P. suberosa</em> (X ± SE)</th>
<th><em>P. misera</em> (X ± SE)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conversion efficiency (CE) (g/g/days)</td>
<td>18.754 b ± 0.614</td>
<td>24.570 a ± 0.502</td>
<td>= 0.0001</td>
</tr>
<tr>
<td>II instar</td>
<td>22.340 b ± 2.744</td>
<td>31.517 a ± 2.262</td>
<td>= 0.0235</td>
</tr>
<tr>
<td>III instar</td>
<td>27.255 b ± 1.330</td>
<td>44.829 a ± 2.734</td>
<td>= 0.0004</td>
</tr>
<tr>
<td>IV instar</td>
<td>30.216 b ± 2.411</td>
<td>48.564 a ± 2.200</td>
<td>= 0.0006</td>
</tr>
<tr>
<td>V instar</td>
<td>15.005 b ± 0.886</td>
<td>20.036 a ± 0.813</td>
<td>= 0.0055</td>
</tr>
<tr>
<td>Relative growth rate (RGR) (g/g/days)</td>
<td>0.054 b ± 0.001</td>
<td>0.062 a ± 0.001</td>
<td>= 0.0070</td>
</tr>
<tr>
<td>II instar</td>
<td>0.290 a ± 0.019</td>
<td>0.280 a ± 0.013</td>
<td>&gt; 0.005</td>
</tr>
<tr>
<td>III instar</td>
<td>0.2709 b ± 0.018</td>
<td>0.365 a ± 0.024</td>
<td>= 0.0182</td>
</tr>
<tr>
<td>IV instar</td>
<td>0.224 b ± 0.015</td>
<td>0.327 a ± 0.024</td>
<td>= 0.0008</td>
</tr>
<tr>
<td>V instar</td>
<td>0.146 a ± 0.006</td>
<td>0.183 a ± 0.006</td>
<td>&gt; 0.005</td>
</tr>
<tr>
<td>Relative consumption rate (RCR) (g/g/days)</td>
<td>0.289 a ± 0.014</td>
<td>0.252 b ± 0.005</td>
<td>= 0.048</td>
</tr>
<tr>
<td>II instar</td>
<td>1.753 a ± 0.188</td>
<td>1.277 a ± 0.081</td>
<td>&gt; 0.005</td>
</tr>
<tr>
<td>III instar</td>
<td>1.362 a ± 0.111</td>
<td>1.500 a ± 0.269</td>
<td>&gt; 0.005</td>
</tr>
<tr>
<td>IV instar</td>
<td>1.077 a ± 0.115</td>
<td>0.964 a ± 0.116</td>
<td>&gt; 0.005</td>
</tr>
<tr>
<td>V instar</td>
<td>1.711 a ± 0.215</td>
<td>1.293 a ± 0.095</td>
<td>&gt; 0.133</td>
</tr>
<tr>
<td>Time development (days)</td>
<td>19.916 a ± 0.570</td>
<td>17.250 b ± 0.462</td>
<td>= 0.0312</td>
</tr>
<tr>
<td>II instar</td>
<td>4.470 a ± 0.363</td>
<td>3.789 a ± 0.163</td>
<td>&gt; 0.005</td>
</tr>
<tr>
<td>III instar</td>
<td>4.333 a ± 0.361</td>
<td>2.921 b ± 0.159</td>
<td>= 0.0006</td>
</tr>
<tr>
<td>IV instar</td>
<td>4.307 a ± 0.183</td>
<td>3.529 b ± 0.212</td>
<td>= 0.0036</td>
</tr>
<tr>
<td>V instar</td>
<td>5.538 a ± 0.192</td>
<td>4.812 a ± 0.208</td>
<td>&gt; 0.005</td>
</tr>
<tr>
<td>Weight pupae (g)</td>
<td>0.392 b ± 0.016</td>
<td>0.459 a ± 0.015</td>
<td>= 0.0155</td>
</tr>
</tbody>
</table>
misera (avg. 17 days) when compared to P. suberosa (avg. 20 days, \( P = 0.0312 \); Fig. 6g; Table 2), specifically in the third \( (P = 0.0006) \) and fourth instars \( (P = 0.0036) \) (Fig. 6h; Table 2). Dryas iulia pupae from caterpillars fed with P. misera were, on average, 15.2% heavier than those fed with P. suberosa \( (P = 0.0155) \); Table 2). Finally, pupal duration did not differ statistically between treatments, regardless of the larval performance.

**Discussion**

Insects possess chemoreceptors by which they can assess and choose which plant material provides the highest nutritional value for the lowest feeding cost due to physical and chemical defences (Jaenicke 1978; Spencer 1988; Barbehenn et al. 2015). A suitable host species chosen by the adult Lepidoptera is frequently associated with higher survival and performance of caterpillars (Périco & Araújo 1991; Scriber et al. 1991; Friberg & Wiklund 2008; Lee et al. 2012). In our study, it is possible that some components of nutritional quality (highest nitrogen content), and the absence of glandular trichomes in P. misera may have contributed to D. iulia larval choice over the other three Passiflora species (P. suberosa, P. pohlii, and P. edulis). This is expected since N is a limiting nutrient for herbivorous insects and, therefore, fundamental for their development and fecundity (Mattson 1980; Panizzi & Parra 2012). In the absence of determinant antifeedant barriers, insects such as Julia coenia (Hübner) butterflies, select food plants that present the highest nitrogen content (Mattson 1980; Prudic et al. 2005). Despite leaf water content (LWC) relevance to leaf digestibility and survival (Scriber & Slansky 1981; Agosta et al. 2017), LWC differences among the four Passiflora species may not have been sufficiently determinant to host choice. It is likely that none of the plant species presented a LWC lower than a minimum requirement for D. iulia larvae. In fact, even though P. pohlii had the lowest amount of LWC (25% lower than the first ranked species), it was still the second most consumed Passiflora, alongside P. suberosa. It has also been suggested that the choice of Passiflora species is associated with leaf age (see Rodrigues & Moreira 1999). Younger leaves are softer and possess higher nitrogen content (Coley et al. 2006) favouring the development of caterpillars, as observed for Heliconius erato phylidis (Rodrigues & Moreira 1999).

Concerning plant physical defences, tector trichome (TT) density found in P. suberosa, P. misera, and P. pohlii apparently did not represent a barrier sufficiently high (less than 2 trichomes/mm²) to discourage D. iulia leaf consumption. Other Passiflora such as P. lobata not only are more pubescent (15.8 ± 2.40 trichomes/mm²) but their trichomes present a hook-like shape (uncinate), which makes them more effectively in deterring Heliconini larvae leaf consumption (Cardoso 2008). At lower densities, such as those found in this studied species, trichomes may not be determinant to larval plant choice. And even when trichomes are found at high densities, there are reports that D. iulia larvae can cut off these foliar appendages (i.e., P. lobata) while they move and feed (Cardoso 2008).

Furthermore, the absence of glandular trichomes in P. misera in relation to P. suberosa may have influenced the larval choice for P. misera. Glandular trichomes are known to secrete substances that discourage, hinder, or even prevent the interaction between caterpillars and their host plant species (Fernandes 1994; Ambrósio et al. 2008). As P. suberosa leaves possess glandular trichomes, it is possible that these physical defences and the chemical compounds stored in them may have contributed to P. suberosa not being chosen and the poorer performance of D. iulia larvae reared on this plant. Additionally, the higher density of glandular trichomes at the leaf border of P. suberosa is an aggravating factor as Lepidoptera in general and other Nymphalidae usually start feeding from the leaf borders (Young & Muyschondt 1975).

Another important aspect that may have influenced the food choice of D. iulia is possibly associated with the composition and concentration of secondary compounds produced by the studied Passiflora. Passiflora species are known to produce, among other compounds, a variety of alkaloids and cyanogenic glycosides (CG) that are present in different concentrations in their tissues (Benson et al. 1975; Castro et al. 2018). Studies carried out with 91 species of Passiflora by Abourashed et al. (2003) detected the presence of harman alkaloids in P. suberosa (harmine) and P. edulis (harmane, harmaline, and harmine) from HPLC analysis. However, they found no harman alkaloids in P. misera. Among alkaloids, harmine has anti-herbivory properties against caterpillars (Rizwan-ul-Haq et al. 2009). Apparently, the absence of alkaloids in P. misera may have influenced D. iulia plant choice. On the other hand, several Heliconini can sequester harman alkaloids from their host Passiflora (Cavin & Bradley 1988).

Other studies indicated that P. suberosa possess-
es two types of cyclopentenoid CGs (i.e., passisuberosin and epipassisuberosin) (Spencer & Seigler 1987), while *P. edulis* possess more than ten types of glycosides (Dhawan et al. 2004). The CGs were also detected in *P. misera* (Hay-Roe & Nation 2007), but no information was found on the presence of these compounds in *P. pohlii*. In addition, Hay-Roe & Nation (2007) reported that cyanide liberated from mature leaves of *P. misera* was around 300 to 400 µg of CG/g of dry tissue. Although all Neotropical Heliconini species can detect and deal with the CG’s toxicity (Benson et al. 1975; Nahrstedt & Davis 1983), Arthur (2009) reported that *D. iulia* caterpillars seem to prefer *P. biflora* leaves with low CG concentration. Thus, it is also possible that *P. misera* presents a low CG concentration, which may have contributed to *D. iulia* larval choice among the *Passiflora* species. Future studies on cyanogenic glycoside content among *Passiflora* species are suggested to better elucidate the choice and performance of *D. iulia* caterpillars.

In this context, the best performance indexes (higher CE and RGR and lowest RCR) of *D. iulia* larvae were found in *P. misera*. These parameters suggest a better digestibility and efficiency on leaf conversion into biomass by *D. iulia* caterpillars reared with *P. misera*. In accordance with our results, Kerpel and Moreira (2005) studying another Heliconini species (*Heliconius erato*) also observed that *P. misera* leaves are overall easier to digest and converted into biomass than *P. suberosa*. For instance, earlier instars are especially vulnerable to host plant leaf quality - nutritionally and defensively (Malishev & Sanson 2015; Quintero & Bowers 2018). However, later larval instars possess improved morpho-physiological capabilities which may aid them in overcoming plant physical defences and even compensate for potential digestibility issues (Nestel et al. 2016; Quintero & Bowers 2018). This was not different in our study, while significant performance differences (i.e. CE, RGR, and development time) were found in earlier instars (i.e. third and fourth instars), this overall disappeared in the last larval instar.

The results concerning larval higher RCR and longer developmental time in *P. suberosa* may indicate a compensatory behaviour (Nestel et al. 2016). Specifically, larvae fed with *P. suberosa* presented a greater leaf intake as a potential strategy to compensate for the poorer leaf quality and obtain an equivalent nutritional amount (Nestel et al. 2016). In addition, the longer developmental time may be an additional strategy or consequence to prolong the larval/feeding stage until a certain amount of nutrients are consumed. Regardless, these strategies were not successful as *D. iulia* on *P. suberosa* still performed worse, ultimately reflecting in the lower larval survivability. Also, by prolonging the larval duration, they not only increased the exposure time to possible predators and parasites (Coley et al. 2006), but also reduced their number of generations in a given time period (Chew 1975). Furthermore, pupal weight (PW) was greater in larvae reared with *P. misera*; this may positively correlate with greater adult performance, including their fecundity (Schoonhoven et al. 2005; Uyi et al. 2018).

This study identified quantitative and qualitative knowledge gaps on the secondary metabolites (e.g., cyanogenic glycosides) of the studied *Passiflora* species. These data would greatly assist in the interpretation of our results on *D. iulia* plant choice and performance, given the importance that chemical compounds represent in the *Passiflora* interactions with herbivores. Nevertheless, our results showed that *D. iulia* larvae have chosen *P. misera* over the other three *Passiflora* species (*P. suberosa*, *P. pohlii*, *P. edulis*). Among the evaluated leaf parameters (nitrogen and water content, glandular and non-glandular trichome presence and density), leaf nitrogen seems to be important in *D. iulia* larval plant choice and performance. Even in their immature stages, *D. iulia* larvae were able to choose the plant material that supported the best larval performance. Hence, among the four studied *Passiflora* species, *P. misera* is the most suitable plant for initiatives (e.g., habitat construction) that aim the management and conservation of *D. iulia* populations. We hope that the knowledge generated in this study also brings contributions to improve the understanding of coevolution between *Passiflora* and Heliconiinae.

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