

Determinants of Growth Rate on Chemically Heterogeneous Host Plants by Specialist Insects

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Key Word Index—*Passiflora*; *Heliconius*; Passifloraceae; Nymphalidae; cyanogenesis; alkaloids; tannins; nitrogen content; insect growth rates; phytochemical screening.

Abstract—Nine *Passiflora* species growing sympatrically in a Costa Rican rain forest were screened for the presence of alkaloids, tannins, cyanogenic compounds, saponins and cardenolides. The first three chemical classes were found to be present and the *Passiflora* species could be classified into five groups according to the presence/absence of each chemical class. In order to assess the effects of this chemical variability on specialist herbivores, larvae of *Heliconius ismenius* and *H. melpomene* were experimentally introduced to seven species of *Passiflora* host plants growing naturally in the rain forest. Survival and growth rates were subsequently monitored at 2 day intervals and were found to be independent of each other. Larval growth rate and larval survivorship were not significantly affected by cyanogenesis in the host plants, nor by the presence of alkaloids, tannins and non-tanning phenolic compounds. Larval growth rate was found to be correlated to percentage nitrogen in the host plants, but was independent of host water content and non-structural carbohydrate content. Although these insects would be classified as host-specific by most authors, the results indicate the presence of a sufficiently general detoxication apparatus to consume many different classes of potential toxins with no apparent ill effects. We conclude that *Passiflora* natural product diversity does not cause major deleterious effects in *Heliconius* growth characteristics and that these effects are not the major component maintaining the current pattern of larval feeding specialization in this plant-herbivore system.

Introduction

Successful insect feeding on host plants is influenced by several factors. These include plant secondary chemistry [1], plant nutritive value [2], plant-associated predators [3] and plant spatial distribution [4]. It is particularly interesting to investigate the relative influence of plant secondary chemicals on insect growth and fitness. Dethier [5] proposed that these chemicals exist primarily to defend plants against herbivores and parasites and that insects have become host specialists to be able to deal with these chemicals successfully. Although the view that chemicals act as defences has received considerable empirical support [1], the view that insects evolve host specificity to circumvent

host defensive chemicals is not as strongly supported by the evidence. For example, some insects which are highly host specific are capable of successfully exploiting a much wider range of host plants than they actually use, suggesting that specialization may evolve independently from host plant chemistry [6]. In addition, some insects appear to cope with a wide range of plant secondary products without ill-effects. For example, polyphagous insects are known which possess an enzyme system sufficiently general to neutralize a very wide range of types of plant secondary chemicals in the diet without a concomitant reduction in growth rate or feeding efficiency [2, 7]. It is generally presumed that specialist insects lack such a generalist physiological ability, but this has rarely been investigated empirically. The difficulty is that specialist insects refuse to feed on any but a few closely-related plant species, thus preventing the investigator from testing a wide range of chemical types.

Erikson and Feeny [8] circumvented this

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(Received 23 January 1984)

problem by infusing foreign compounds (sini-grin) into host plant leaves, and were able to show that chemical barriers do effectively prevent specialist *Papilio* larvae (Lepidoptera: Papilionidae) from feeding on non-host plants such as *Brassica* (Brassicaceae). An alternative to experimental infusion is to investigate a relatively 'specialist' insect (i.e. an insect species which only consumes a single genus or species of plant) which, nevertheless, can be induced to feed on a chemically diverse set of host plants. By observing feeding and growth performance it would then be possible to assess the ability of this specialist insect to effectively neutralize the effects of the chemicals present. If the insects were able to feed successfully on a wide range of compounds, it would suggest that specialist insects may possess a more generalist detoxication ability than has previously been assumed.

This paper describes an investigation in which the growth performances of two host-specialist insects, *Heliconius ismenius* Doubleday and *H. melpomene* Bates (Lepidoptera: Nymphalidae), were assessed on a series of chemically diverse *Passiflora* L. (Passifloraceae) species. These insect species are host specific to Passifloraceae [9] and previous work at the field site has shown *H. melpomene* to be a specialist on *P. menispermifolia* HBK. while *H. ismenius* feeds on 3-4 species of *Passiflora* (Gilbert, L. E., Mallet, J. B. and Smiley, J. T., in preparation). Thus, both species may be considered specialist insects as defined by Dethier [5]. However, as reported in Smiley [6], these species can be induced to feed and grow on nearly any *Passiflora* species if the larvae are artificially placed on the plant.

Relatively few data are available as to presence/absence of major chemical classes in a single genus of plants. We define chemical classes as major groups of compounds, such as tannins or alkaloids. Great variability may exist among congeners with respect to a single class of compounds [10], or across chemical classes in a plant family or in an entire forest community [12]. In this project, we wished to determine (a) the variability in chemical classes present within the single genus *Passiflora*, and (b) the effects of this variability on growth rates and survival of specialist *Heliconius* insects which feed on these plants. We also wished to determine the effects,

if any, of differences in plant nutritive value to the insect, as measured by water content, non-structural carbohydrate content and total nitrogen content. All these leaf quality indices have been shown to affect insect growth rates in other organisms, but not in field populations of rain forest insects. All work was done in the native rain forest habitat to ensure natural levels of chemical variability and actual responses of field insects to that variation.

Results

Phytochemical screening of nine species of *Passiflora* at three different ages revealed a large degree of interspecific variation in chemical class constituents (Table 1). Although none of the species examined were found to contain saponins or cardenolides, three species contained tannins. These three species also contained alkaloids, but only two of the three species produced cyanide. Of the tanniferous *Passiflora*, *P. pittieri* Mast. produced tannins only in the older leaves while *P. vitifolia* HBK. and *P. alata* Dryand. contained tannins in the young leaves preferred by *Heliconius*. The non-tanniferous *Passiflora* included four species which were cyanogenic and contained alkaloids, with *P. ambigua* Hemsl. and *P. talaman-censis* Killip showing strong alkaloid precipitation in drop tests and *P. menispermifolia* and *P. coriacea* Juss. a weak reaction. The final two non-tanniferous *Passiflora* included *P. costaricensis* Killip, a cyanogenic species which lacked detectable alkaloids and *P. lobata* Killip, which gave an alkaloid precipitation but was not cyanogenic. Thus, the nine *Passiflora* could be classified into at least five distinct chemical profiles (Table 2).

Within a species, the phytochemical screening results taken from three individual plants were moderately consistent, especially for tannin precipitation (Table 1). Alkaloids and cyanogenesis appeared to be more variable, as for example in *P. ambigua*, where one set of new leaves gave a strong positive reaction for both alkaloids and cyanogenesis while another set of leaves gave a negative or slight reaction for both types of compounds. Only five out of 27 cases showed inconsistency in chemical presence/absence results (Table 1). Thus, little qualitative

TABLE 1. RESULTS OF SEMI-QUANTITATIVE CHEMICAL SCREENING ON THREE INDIVIDUALS OF EACH OF NINE SPECIES OF *PASSIFLORA* GROWING AT CORCOVADO NATIONAL PARK

<i>Passiflora</i> species	Foliage age	Chemical test for:					Composite score	Composite score
		HCN	Alkaloid	Tannin	Saponin	Cardenolide		
<i>pittieri</i>	NEW	1.5-2	1-1	0-0	nt	nt	2.5-3	5.25
	MAT	0.5	0	2	0	0	2.5	
	OLD	0	0	1	nt	nt	1	
<i>vitifolia</i>	NEW	0-0	0-2	1-2	nt	nt	1-4	5.5
	MAT	0	1	2	0	0	3	
	OLD	0	2	1	nt	nt	3	
<i>alata</i>	NEW	0.5-0.5	1-2	1-1	nt	nt	2.5-3.5	6.0
	MAT	0	1	2	0	0	3	
	OLD	0	2	1	nt	nt	3	
<i>ambigua</i>	NEW	0-1.5	1-3	0-0	nt	nt	1-4.5	6.0
	MAT	1	3	0	0	0	4	
	OLD	0	3	0	nt	nt	3	
<i>menispermifolia</i>	NEW	1-1.5	0-1	0-0	nt	nt	1.5-2	2.75
	MAT	1	0	0	0	0	1	
	OLD	0	1	0	nt	nt	1	
<i>coriacea</i>	NEW	0-1	0-1	0-0	nt	nt	0-2	2.00
	MAT	0	1	0	0	0	1	
	OLD	0	0	0	nt	nt	1	
<i>talamancensis</i>	NEW	1-1.5	2-2	0-0	nt	nt	3-3.5	5.75
	MAT	0.5	2	0	0	0	2.5	
	OLD	0.5	1	0	nt	nt	1.5	
<i>costaricensis</i>	NEW	0.5-2	0-0	0-0	nt	nt	0.5-2	1.25
	MAT	0	0	0	0	0	0	
	OLD	0	0	0	nt	nt	0	
<i>lobata</i>	NEW	0-0	1-2	0-0	nt	nt	1-2	3.5
	MAT	0	2	0	0	0	2	
	OLD	0	1	0	nt	nt	1	

For each species, new leaves (leaf nodes 1-5, counting from the tip) from two separate plants were tested independently (NEW), as were one series of mature-aged leaves (MAT nodes 5-10) and one sample of older senescing leaves (OLD), taken from a third individual plant. Scores range from zero (no reaction) to 3 (strongest reaction observed). The composite score was calculated by adding the HCN, alkaloid and tannin scores, (see test). The overall composite score was then calculated as (NEW + MAT), thus being a composite of young and mature-aged leaves, the food of most *Heliconius* larvae.

variability was observed between individual plants of the same species.

Figures 1(A)-1(C) illustrate the correlation between cyanogenesis, alkaloid precipitation, tannin precipitation and average growth rate for *Heliconius* larvae growing on the *Passiflora* species tested. No clear relationship was seen between any of these chemical constituents and growth rate, indicating that these two *Heliconius* species readily consume the chemicals with no apparent cost in growth rate. When the separate phytochemical screening scores were summed for new and medium-aged leaves (to obtain a composite allelochemicals score) and

then plotted with insect growth rate (Fig. 2), no relationship was seen. Thus, these *Passiflora*-feeding *Heliconius* were capable of consuming the observed range of potential allelochemicals with no apparent reduction in growth rate. An analysis of median survival time indicated that, like growth rate, survival was not significantly effected by allelochemicals (Fig. 1D-1F). Data reported elsewhere indicate that survival was most strongly effected by presence/absence of predaceous ants visiting extra-floral nectar glands on the plants (Smiley, J. T., in preparation).

The ability of *H. melpomene* to consume

TABLE 2. SUMMARY OF *PASSIFLORA* LEAF COMPOSITION AND MEAN LARVAL GROWTH RATE OF TWO *HELICONIUS* SPECIES ON EACH PLANT

<i>Passiflora</i> species	HCN	ALK	TAN	\overline{WC}	\overline{TNC}	\overline{N}	RGR		MST	
							ISM	MELP	ISM	MELP
<i>pittieri</i>	1.1 +	0.5 +	1.0 +	81				0.66		1.7
<i>vitifolia</i>	0.0 -	1 +	1.8 +	77	1.21	5.46	0.57	0.54	2.5	1.2
<i>alata</i>	0.25 +	1.3 +	1.5 +	81	1.99	4.49	0.58	0.27	1.9	1.2
<i>ambigua</i>	0.8 +	2.5 +	0.0 -	77				0.02	1.2	1.3
<i>menispermifolia</i>	1.1 +	0.25 +	0.0 -	79	1.26	3.6		0.62		1.4
<i>coriacea</i>	0.25 +	0.75 +	0.0 -	82	0.67	2.7		0.17		1.2
<i>talamancensis</i>	0.8 +	2.0 +	0.0 -	71						
<i>costaricensis</i>	0.6 +	0.0 -	0.0 -	79	1.05	1.88	0.49	0.43	2.0	1.8
<i>lobata*</i>	0.0 -	1.8 +	0.0 -	89	0.34				0	0

Average phytochemical screening score (New + Mature leaves) for HCN (cyanogenesis), ALK (alkaloids) and TAN (tannins); \overline{WC} indicates average percentage water in leaves, \overline{TNC} indicates average non-structural carbohydrate content in leaves, \overline{N} indicates average T crude protein in leaves; RGR indicates mass-specific growth rate of ISM (*H. ismenius*) and MELP (*H. melpomene*) larvae; MST indicates median survival time of ISM and MELP larvae.

*Possesses hooked trichomes (see text).

tannins was particularly interesting. This species is a specialist on *P. menispermifolia* at the field site, a species lacking tannins. Since *H. melpomene* grew rapidly on *P. vitifolia*, a tanniferous plant, the species can evidently cope with these chemicals at naturally occurring concentrations. A quantitative analysis on one greenhouse-grown *P. vitifolia* has revealed that the tannin dry weight concentrations ranged from near zero at the shoot tip to 15% in full-sized leaves, with concentrations ranging from 7% to 15% in the leaves being consumed by the *H. melpomene* larvae (Fig. 3). Thus, *H. melpomene*, a specialist which does not normally encounter tannins, was able to consume high concentrations of tannins without apparent physiological effects. Preliminary tests, in which the tannin from *P. alata* was fed to the generalist insect *Heliothis virescens*,

indicated that *Passiflora* tannins are very effective antifeedants at low concentrations (Fig. 4).

Among three nutritional quality indices, only the percentage nitrogen content showed a significant correlation with growth rate (Fig. 5; $r^2=0.38$, $P<0.05$). The other two nutritional indices, leaf water and non-structural carbohydrates, showed virtually no correlation to growth rate (Figs 6 and 7; $r^2=0.07$ and 0.0, respectively). It thus appears that, as found for many other herbivores [2], *Heliconius* growth rates are dependent on the nitrogen content of their food.

Ambient light levels ranging from 0% to 50% full sunlight did not affect larval growth rate ($r^2=0.00$), indicating that photoactivation of the *Passiflora* alkaloids did not cause toxicity to *Heliconius*.

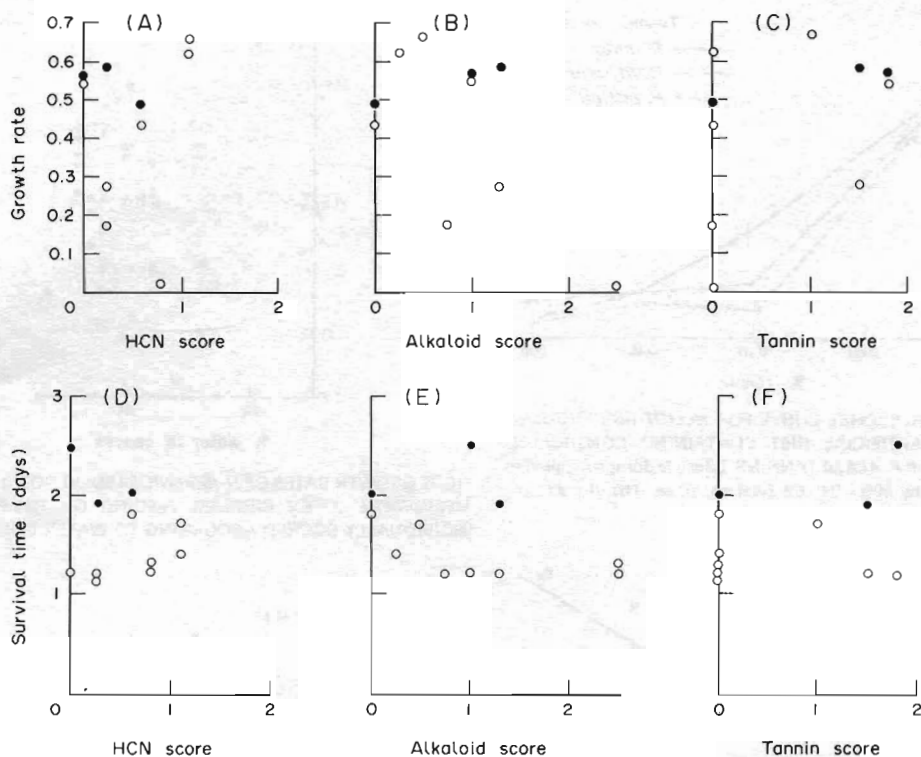


FIG. 1. (A-C) MASS-SPECIFIC GROWTH RATE OF *H. ISMENIUS* (SOLID DOTS) AND *H. MELPOMENE* (OPEN CIRCLES) LARVAE RAISED ON *PASSIFLORA* SPECIES CONTAINING DIFFERENT QUANTITIES OF THREE CLASSES OF ALLELOCHEMICS. Note that no significant effect was observed by any single allelochemical. (D-F) Median survival time of larvae of *H. ismenius* (solid dots) and *H. melpomene* (open circles), raised on the same *Passiflora* as in (A-C).

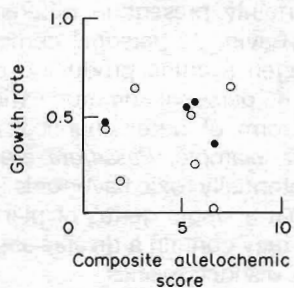


FIG. 2. GROWTH RATES OF *H. ISMENIUS* (SOLID DOTS) AND *H. MELPOMENE* (OPEN CIRCLES) RAISED ON *PASSIFLORA* SPECIES SCORED ACCORDING TO THE SPECIES COMPOSITE ALLELOCHEMIC SCORE. Note that no significant effect was observed (see text for further explanation).

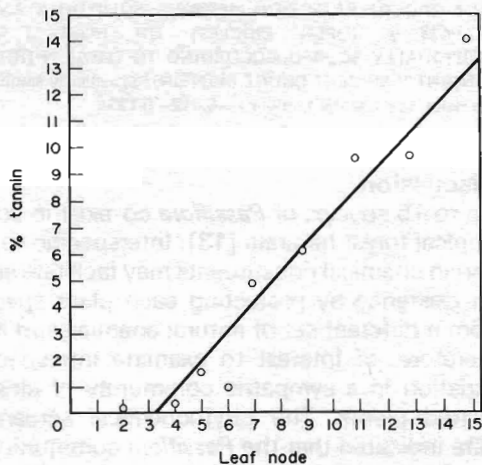


FIG. 3. TANNIN CONTENT IN LEAVES OF *P. VITIFOLIA* ARRANGED ACCORDING TO POSITION ON BRANCH. Leaves 7-15 are nearly fully expanded and were used for feeding sites by *H. melpomene*.

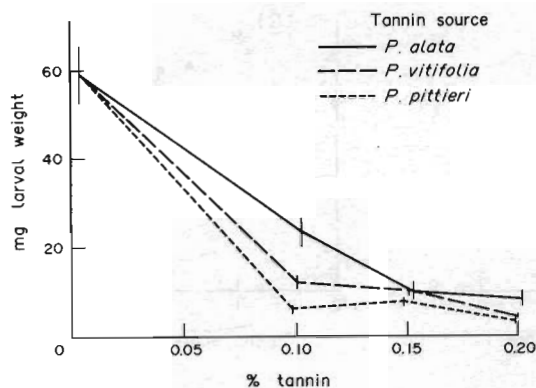


FIG. 4. DOSE-RESPONSE CURVE FOR *HELIOTHIS VIRESCENS* RAISED ON ARTIFICIAL DIET CONTAINING CONTROLLED QUANTITIES OF *P. ALATA* TANNINS. Effective dosage required to reduce growth by 50% = 0.016% (wet wt) or ca. 0.6% dry wt.

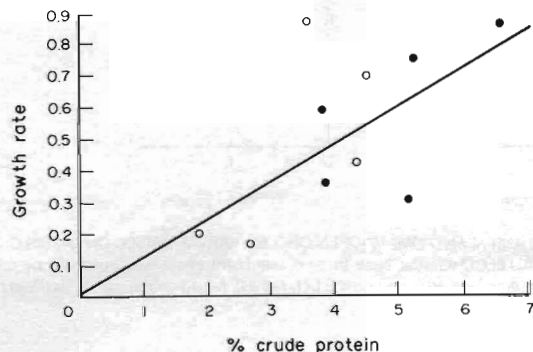


FIG. 5. GROWTH RATES OF *H. ISMENIUS* (SOLID DOTS) AND *H. MELPOMENE* (OPEN CIRCLES) ON HOST PLANTS INDIVIDUALLY SCORED ACCORDING TO TOTAL NITROGEN CONTENT (PER CENT CRUDE PROTEIN). Statistically significant regression line has the formula $Y = 0.012 + 0.123X$.

Discussion

Up to 15 species of *Passiflora* co-exist in some tropical forest habitats [13]. Interspecific variation in chemical constituents may facilitate such co-existence by protecting each plant species from a different set of natural enemies and it is, therefore, of interest to examine interspecific variation in a sympatric community of closely related plants. The phytochemical screening data indicated that the *Passiflora* community at La Sirena contains at least five distinct chemical profiles characterized by the presence/absence of three major classes of potentially toxic compounds. Within each profile, additional inter-

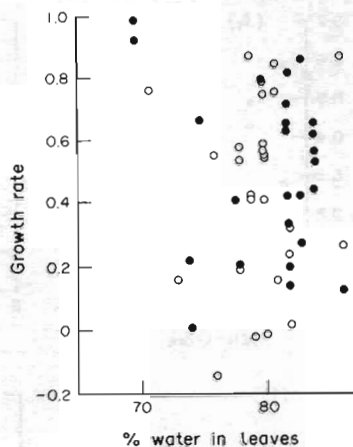


FIG. 6. GROWTH RATES OF *H. ISMENIUS* (SOLID DOTS) AND *H. MELPOMENE* (OPEN CIRCLES) FEEDING ON *PASSIFLORA* INDIVIDUALLY SCORED ACCORDING TO WATER CONTENT.

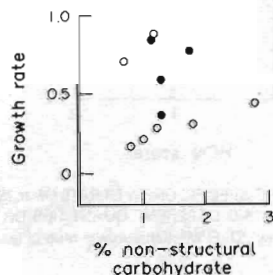


FIG. 7. GROWTH RATES OF *H. ISMENIUS* (SOLID DOTS) AND *H. MELPOMENE* (OPEN CIRCLES) FEEDING ON *PASSIFLORA* INDIVIDUALLY SCORED ACCORDING TO NON-STRUCTURAL CARBOHYDRATE CONTENT.

specific variation was seen. Additional variation is also certainly present in different types of alkaloids (Cavin, J., personal communication) and hydrogen cyanide producing compounds (Spencer, K., personal communication), as well as in the form of other chemical classes not tested. For example, *Passiflora* are known to contain potentially toxic flavonoids [14]. It thus appears that a single genus of plants, such as *Passiflora*, may contain a diverse assemblage of chemically distinct species.

Little comparable data appear to be available from other plant genera. Gage and Strong [15] found that most classes of allelochemicals were lacking in species of *Heliconia* (wild banana, Musaceae). However, other plant genera and

single species are known to contain a diversity of chemical classes [16] and interspecific variation in a single chemical class is commonly present [10]. If so, it implies that even plant-genus specific insects, which would be classed as monophagous by most authors [5, 17], must cope with a relatively wide range of potential allelochemicals in their diet. Thus, an efficient, relatively generalized detoxication system may be of selective advantage to genus- or family-specific insects as well as to generalists. The fact that *Heliconius ismenius* and *H. melpomene* feed and grow equally well, whether or not the three classes of defensive compounds are present, indicates that specialist insects may indeed possess such a system.

Scriber and Slansky [2] reported that leaf water content and nitrogen content could profoundly affect insect growth rates. However, they found that under conditions of high humidity, where water conservation is presumably less important, leaf water content did not affect growth. Therefore, it was not surprising that the *Heliconius* grew faster in response to elevated leaf nitrogen but not to elevated leaf water content, since they were living in an extremely humid rain forest environment.

The results of this investigation suggest that specialist insects may be subject to a wide variety of potential allelochemicals in their diet. These herbivores apparently counteract the potential deleterious effects of these allelochemicals very efficiently, as presence/absence of the allelochemicals in host plants had no detectable effect on growth rate. We conclude that potential adverse effects of *Passiflora* natural product diversity cannot be responsible for maintaining the patterns of host specificity seen in *Passiflora* specialist *Heliconius*.

Experimental

Heliconius and *Passiflora* were investigated at the La Sirena Headquarters of Corcovado National Park, Puntarenas Province, Costa Rica (8°30'N, 83°35'W.). The techniques consisted of phytochemical screening procedures, N, carbohydrate and H₂O content analysis, ambient light intensity measurements, and *Heliconius* growth and survival rates, as described below.

Phytochemical screening. In the phytochemical screening procedure, three plants in each of nine *Passiflora* species were tested, belonging to three leaf age classes: new (nodes 1–5, counting down from the shoot tip), mature (nodes

6–10), and old, senescing leaves (Table 1). The screening procedure was modified from Farnsworth [18] and consisted of a cyanogenesis test (picric acid test strips), an alkaloid precipitation test (Mayer's reagent), a saponin test (froth/Lieberman-Burchard/Salkowski tests), a cardenolide test (dinitrobenzoic acid test) and a tannin test (gelatin precipitation/FeCl₃ reagent). Previous work with *Passiflora* has shown that precipitation of acid H₂O extract with Mayer's reagent to be a sensitive assay of *Passiflora* alkaloids (Cavin, J., personal communication).

Passiflora leaf water content was estimated gravimetrically by weighing samples of fresh leaves and subsequent weighing after desiccation in a plant dryer. Total N and non-structural carbohydrate content was measured at the Palmer Soils Laboratory, Palmer, Alaska. Plants (ca. 100) belonging to nine species were assayed for leaf water content, but only 10 plants belonging to five species were assayed for total N and non-structural carbohydrates. In all cases, new and medium-aged leaves were sampled, corresponding to the foliage classes normally consumed by *Heliconius* larvae.

Ambient light intensity was measured on a sunny day for a total of 23 plants in six *Passiflora* species. Light intensity was integrated over a 24 h period using vials containing a solution of anthracene and the results expressed as a percentage of full sunlight as determined by spectrophotometric comparison with dark and full sunlight controls [19]. The primary purpose of these measurements was to determine if high or low light levels influenced insect feeding performance. This is of particular interest in *Passiflora* investigations because the harmaline alkaloids of *Passiflora* are known to be phototoxic, i.e. their toxicity to micro-organisms increased after exposure to light [20].

Insect measurements. Experimental larvae (instars 1–3) of *H. ismenius* and *H. melpomene* were obtained by hatching eggs collected from the University of California at greenhouse populations, the original source of which was the Sirena field site. After hatching, larvae were raised on their preferred hosts, *P. alata* and *P. menispermifolia*, respectively, until field placement. After placement, larvae were checked every 2 days by searching the host plant and photographing the larva at 1:1 magnification, if present. Missing larvae were scored as dead, a reasonable assumption for these highly sedentary insects which do not voluntarily leave the host plant prior to pupation (Smiley, J. T., personal observation). Larval weights were estimated by taking accurate measurements from the photographs [21]. These estimates were then used to calculate mass-specific growth rates using the formula $r = \ln(W_t/W_0)/t$, where W_t is the weight t days after placement on the plant at time 0, and W_0 is the weight at time of placement. Survivorship was analysed using SPSS version 9, which calculates a median survival time. This is defined as the time required for 50% of the larvae to have disappeared [22].

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