

## HELICONIUS CATERPILLAR MORTALITY DURING ESTABLISHMENT ON PLANTS WITH AND WITHOUT ATTENDING ANTS<sup>1</sup>

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**Abstract.** Rates of disappearance (mortality) in *Heliconius* caterpillars of various sizes were investigated by experimental placement on *Passiflora* host plants growing in their native rain forest habitat. Survivorship of experimental larvae was similar to that of *Heliconius* larvae in natural populations: 60% mortality during the initial 2 d, followed by 30% mortality during each 2-d period thereafter. Survivorship was not dependent on larval size or instar, nor was it related to larval growth rate or host plant isolation from surrounding foliage. However, survivorship was appreciably lower on host plants on which ants were attending extrafloral nectaries (70% vs. 45% mortality in first 2 d), indicating that ants may be a primary source of high mortality. I conclude that for these *Heliconius*, high first-instar mortality is not due to the small size of the caterpillars but rather is a consequence of ant attendance to some host plants; most caterpillars on plants with ants are killed in the first instar, while caterpillars on ant-free plants have much lower mortality and make up the majority of the cohort in later instars.

**Key words:** ant-plant mutualism; establishment mortality; extrafloral nectar glands; *Heliconius*; host plant heterogeneity; insect herbivore; insect survivorship; *Passiflora*; size-related mortality; tropical forest.

### INTRODUCTION

In many insect species that eat foliage there is higher mortality in early stages of development than in later stages (Price 1975; see also Watanabe 1976, Cheke 1978, Chapman and Page 1979, Jordan 1981), although for some species in urban or agricultural environments this may not be so (Harcourt 1963, Ito and Miyashita 1968). Although few supporting data are available, it may be that this higher mortality is due to small size per se; vulnerability to numerous invertebrate predators (such as ants and spiders), and to environmental stresses (such as desiccation or drowning in surface water on plant leaves) may be greater for smaller than for larger insects (Price 1975). Alternatively, higher mortality of early stages may be the result of risks suffered during establishment on the plant, risks that are independent of size. Distinguishing between these two alternatives is necessary for understanding insect survivorship. This paper reports a study in which these two alternatives were experimentally tested.

Higher mortality during establishment may result from high variability in plant quality. The risk during establishment is high when a substantial fraction of the plants on which larvae are found are unsuitable, because of predators localized on the plant, defensive chemicals, or other localized factors. Such a situation isolates survivors on high-quality host plants, so that mortality rates decline with age.

In species of *Heliconius* butterflies with higher dis-

appearance rates in the first larval instar, the caterpillars are sedentary and do not leave the host plant before the final instar (Smiley 1978b; see also Fig. 1). The host plants of many *Heliconius* caterpillars are known to be heterogeneous with respect to nutritive value to larvae and the presence of predaceous ants at extrafloral nectar glands (Benson et al. 1976, Smiley 1978a, b). In this study, I measured *Heliconius* mortality as a function of instar, plant nutritive quality, and presence/absence of ants attending the host plants. I also determined the effects on mortality of plant isolation from surrounding foliage.

### METHODS

Eggs of *H. ismenius* and *H. melpomene* were obtained from a greenhouse colony at the University of California, Irvine, that had been established 1 yr previously with field-caught individuals from a field site at La Sirena Park Headquarters, Corcovado National Park, Costa Rica (8°30' N, 83°35' W). The greenhouse butterflies had been maintained under semi-natural conditions on host plants from the La Sirena site for ≈ 1 yr (six generations). Eggs were transported to the field site; upon hatching, larvae of the two species were fed in cups on leaves of their preferred host plants, *Passiflora alata* and *P. menispermifolia*, respectively. After feeding for ½ d (first instar) to 6 d (third instar), the larvae were carefully placed on wild *Passiflora* vines of various species between 11 and 16 June 1981. Larvae of *H. ismenius* and *H. melpomene* are known to feed successfully on all *Passiflora* species tested (Waage et al. 1981), and positive growth rates were obtained in the majority of cases.

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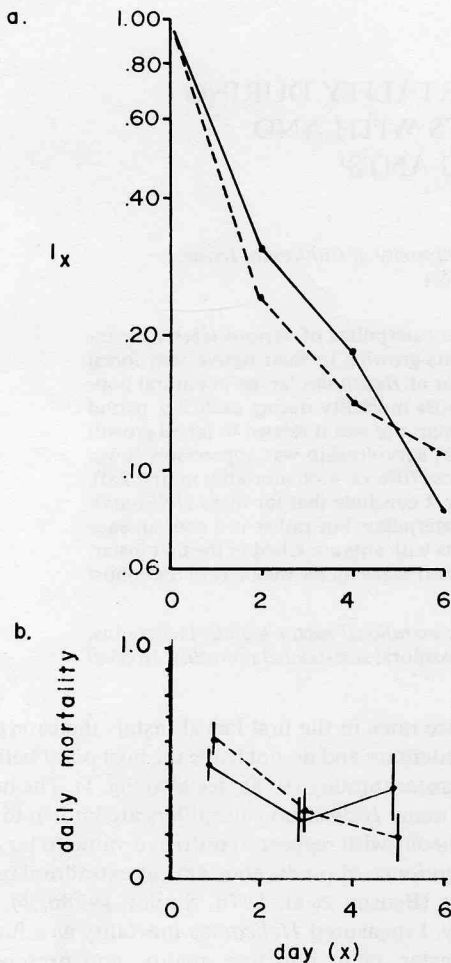


FIG. 1. Survival (a) and daily mortality rate (b) in naturally-occurring (—) vs. experimental (----) *Heliconius* from the beginning of the first instar.  $I_x$  is the fraction surviving to day  $x$ , and daily mortality rate is the number of deaths during an interval divided by the mean number of individuals alive during that interval. Note the high risk of mortality in the initial 2-d period.

To test experimental techniques, three first-instar larvae of *H. melpomene* were carefully placed on each of seven *Passiflora* species in the greenhouse. All *Passiflora* species at La Sirena were included but two: *P. pittieri* and *P. lobata*. After 2 d, 100% of the larvae were alive, feeding, and growing. *P. pittieri* was later shown to support growth and establishment under field conditions (J. Smiley and C. Wisdom, *personal observation*), and *P. lobata* was excluded from the present study owing to its unsuitability as a *Heliconius* host. An additional check revealed that the experimental techniques did not cause undue mortality: the overall survivorship of the experimental first-instar larvae at La Sirena was similar to that of naturally occurring first-instar larvae of the closely related species *H. hecale* and *H. cydno* in a similar habitat at La Selva, Costa Rica ( $P > .05$ ; see below and Fig. 1).

Growth rates and survivorship were estimated from observations of the larvae that had been placed in the field. Although in each case the entire plant was searched, surviving larvae were usually found on the part of the host plant where they had been placed. They were photographed at 1:1 magnification, and body size was estimated from the photographs; this technique estimates body mass to  $\approx 10\%$  accuracy (Smiley and Wisdom 1982). Size measurements were used to calculate mass-specific (relative) growth rates. Growth rate is one assay of plant nutritive quality in these rapidly growing insects (larval development time: 9–12 d). Larvae that could not be found were assumed dead. However, empty host plants were still checked every 2 d, and in only one case out of 150 was a larva subsequently found that had previously been scored as missing. Observation on a plant was terminated after 8 d.

On 20–24 June, I recorded data on other host plant characteristics. These included (1) ant presence/absence (Bentley 1977), (2) plant isolation from surrounding foliage, i.e., whether or not the plant was emergent from, congruent with, or buried inside the vegetation canopy, and (3) plant water content (percent water) of the branch on which the larvae were feeding (Scriber and Slansky 1981). Ant presence/absence tends to be very constant for each individual *Passiflora* vine (J. Smiley, *personal observation*, C. Wolin, *personal communication*), so that these censuses, taken 1 wk after the experiments were completed, provided accurate data on ant presence during the experiments.

Daily mortality (with standard error) was calculated for each interval, using algorithms of Elandt-Johnson and Johnson (1980). The computer programs of SPSS-8 were used to calculate a rank-order statistic for determining whether or not the two survivorship curves in Fig. 1 were significantly different (Lee and Desu 1972, Hull and Nie 1981). This statistic does not assume that mortality rates are constant with each population, and is thus applicable to these data. In addition, I identified significant sources of establishment mortality (=dis-

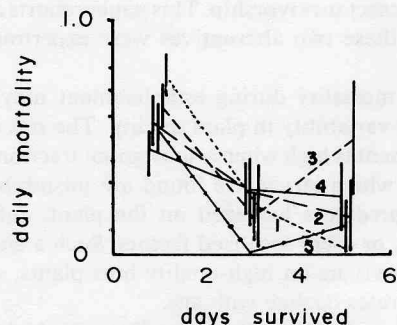


FIG. 2. Daily proportional mortality in experimental larvae of two *Heliconius* species placed on field *Passiflora*. (1) *H. melpomene* first instar, (2) second instar, (3) third instar; (4) *H. ismenius* first instar, (5) second instar.

TABLE 1. Sources of mortality in *Heliconius*, analyzed by log-linear contingency analysis. Higher order interaction terms were nonsignificant.

Mortality contingent upon:	Degrees of freedom	G statistic of partial association	Trend
Ant presence	1	2.43 ( $P = .12$ )	higher mortality with ants
Larval instar	2	0.06	
Plant isolation	2	0.02	
Plant water content	1	0.00	
<i>Heliconius</i> species	1	3.85 ( $P = .05$ )	higher mortality in <i>H. melpomene</i>

appearance during the first 2 d) with BMDP log-linear contingency analysis (Dixon 1983).

### RESULTS

A larger fraction of the 57 experimental first-instar larvae of *H. ismenius* and *H. melpomene* died during the first 2 d of life than in any of the subsequent 2-d periods (Fig. 1). The pattern was similar for naturally occurring first-instar larvae of *H. cydno* and *H. hecale* (Fig. 1). Although mortality for *H. melpomene* was somewhat greater than for the other species, there was an overall similarity (Lee-Desu statistic = 2.7,  $P = .10$ ) between experimental and naturally occurring survivorship curves, which suggests that the procedures involved in the experimental placing of larvae on host plants did not in themselves cause significant levels of mortality.

To test the hypothesis that larval body size affected mortality, survivorship was compared for experimental *H. melpomene* and *H. ismenius* placed on host plants as first- ( $N = 26, 28$ , respectively), second- ( $N = 23, 19$ ), and third-instar individuals ( $N = 31, 5$ ). If the high first-instar mortality shown in Fig. 1 were caused by small size per se, then survivorship among second- and third-instar larvae should be significantly greater than that of first-instar larvae. However, the data (Fig. 2) do not support this interpretation. High levels of mortality were observed in all instars of both species during the first 2-d period, indicating that larger size contributed little advantage in *Heliconius* during establishment on host plants. These results indicate that most of the improved survivorship seen in older naturally occurring and experimental larvae was not due to larger

size or greater maturity but rather to their having successfully passed through a risky phase of establishment on the host plant.

Log-linear contingency analysis confirmed that larval instar had little direct effect on mortality ( $G = 0.06$ ,  $P = .97$ ; Table 1) and indicated that ant presence did cause a substantial amount of mortality ( $G = 2.43$ ,  $P = .12$ ). Plant water content and plant isolation from surrounding foliage had no measurable effects ( $G = 0.02$  and  $0.00$ , respectively), and there were no significant higher order interaction terms. *Heliconius* species had a significant effect on survivorship ( $G = 3.85$ ,  $P = .05$ ) and interacted with other variables. I therefore re-analyzed each *Heliconius* species separately, omitting plant water and plant isolation as variables (Table 2). In *H. melpomene*, ant presence had a strong effect on mortality ( $G = 5.27$ ,  $P = .02$ ), while larval instar had little effect ( $G = 0.19$ ). All instars died with similar frequency on plants with ants, as indicated by a nonsignificant interaction term ( $G = 2.56$ ,  $P = .28$ ). Fig. 3 illustrates these trends. In *H. ismenius*, sample sizes were smaller, but there was a significant interaction term ( $G = 6.84$ ,  $P = .03$ ), with other interactions nonsignificant. Apparently, first-instar *H. ismenius* are less affected by ant presence than second-instar larvae.

To determine whether host plant nutritive or chemical quality affected mortality, I compared the survivorship of 18 larvae with relative growth rates higher than the median ( $>0.557 \text{ d}^{-1}$ ) with the survivorship of 17 larvae with low growth rates ( $\leq 0.556 \text{ d}^{-1}$ ). As average growth rate was approximately the same in both species, the data for *H. ismenius* and *H. melpomene* were lumped together (Fig. 4). No effect of growth rate on survivorship was seen. Since low growth rate

TABLE 2. Sources of mortality in *Heliconius*, each species analyzed separately, plant isolation and water content excluded.

Mortality contingent upon:	Degrees of freedom	G statistic of partial association	Trend
<i>H. melpomene</i>			
Ant presence	1	5.27 ( $P = .02$ )	higher mortality with ants
Instar	2	0.19	
Ant presence $\times$ instar	2	2.56 ( $P = .27$ )	
<i>H. ismenius</i>			
Ant presence	1	1.84 ( $P = .17$ )	
Larval instar	2	1.01	
Ant presence $\times$ instar	2	6.84 ( $P = .03$ )	ant-related mortality higher in second instar

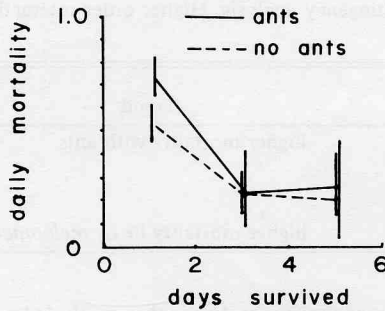


FIG. 3. Daily mortality rate for *H. melpomene* larvae placed singly on plants with ( $N = 37$ ) and without ( $N = 48$ ) attending ants. Note the decline in rates with time.

was not a cause of high postestablishment mortality, it is unlikely that it had an effect during the establishment period (only larvae that survived at least 2 d had their growth rates measured). Thus, host plant nutritive or chemical differences were probably not responsible for establishment mortality. The possibility that plant repellency prevented larvae from consuming any plant material at all can be discounted, since  $>5 \text{ mm}^2$  of feeding damage was observed on nearly every host plant.

On host plants with ants, the mortality rate for *H. melpomene* was higher during the establishment period than after (Fig. 3); however, high mortality during establishment also occurred on plants without ants, which indicates that some factor other than ant presence was influencing the risk of establishment. Sample sizes were too small to determine if mortality declines in *H. ismenius* after the first 2 d, although the trends suggest that this is so on plants with ants. For example, 6 out of 15 first-instar larvae survived the first 2 d while 5 out of 6 survived the second 2-d period.

#### DISCUSSION

The experimental data argue against the hypothesis that small size per se is the primary cause of enhanced first-instar mortality in these insects. Rather, the data indicate that larvae ranging in mass from 1 to 25 mg are subject to about the same probability of disappearance. Since predation is the probable agent of mortality, these results suggest that the number of predators that take small prey does not differ substantially from the number that take larger prey. These results differ from those of Valiela (1969), who found that the smallest dipteran larvae were more susceptible to predation than larger larvae. However, the data of Valiela (1969) were obtained from insects living in simplified artificial habitats. In general, few other data are available for insects of different sizes growing under natural conditions, subject to a wide range of predators.

Larvae of all sizes suffered similarly high levels of mortality after being placed on the host plants. The result that ant presence on 22 June was a strong indicator of larval mortality during 11–17 June argues

strongly that between-plant heterogeneity in ant attendance is one likely causative agent for high establishment mortality. Overall, only 9 of 60 (15%) larvae survived the first 2 d on ant-plants, while 22 of 66 (33%) survived on plants without ants in constant attendance. This latter rate is close to the 52% survival rate observed for larvae already established on those same plants (13/25). Demonstration of ant-induced mortality in insect herbivores is not new (Bentley 1977), although these are the first published quantitative data for the *Heliconius/Passiflora* system (Benson et al. 1976, Brown 1981, Turner 1981).

The spatial patchiness caused by ant mortality (Majer 1972, Leston 1978) deserves further discussion. Sedentary herbivores are exposed to the same ant species repeatedly, but species of ants vary in their ability to harm herbivores, with the result that there is herbivore heterogeneity among host plants. Because of this heterogeneity, a cohort of herbivores may suffer greater mortality in the early life stages, while many individuals are on high-risk plants. After insects on risky plants have died, cohort mortality among the survivors on favorable host plants will be low. For this pattern to occur, the mortality risk on a given plant must be temporally stable: low-risk plants must remain so throughout the insect's residence. This condition is probably satisfied by *Passiflora* attended by ants, and some of our observed decline in mortality rates during the 2–6 d after establishment on the host plants was probably caused by heterogeneity in ant presence.

Distinguishing between mortality related to size and that related to host heterogeneity is important to theories of insect/plant interactions. Coevolution of plant defenses and insect counteradaptations depends on selective pressures between insects and plants. Heterogeneity in host suitability provides a mechanism for both types of selection: the insects on less suitable plants are disproportionately killed, and plants that support too many insect herbivores are killed or at least have their fecundity reduced (Feeny 1976). Distinguishing the sources of early-stage mortality also is important for modeling insect/plant population dynamics. If her-

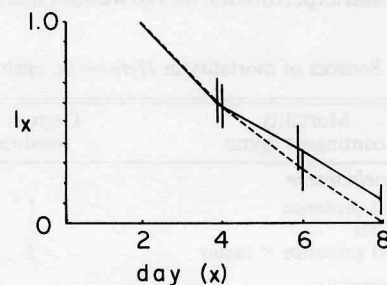


FIG. 4. Postestablishment survival on host plants which supported high ( $>0.557 \text{ d}^{-1}$ ; ----) vs. low ( $<0.557 \text{ d}^{-1}$ ; —) mass-specific growth rates (rgr), both species lumped together.  $0.557 \text{ d}^{-1}$  = median growth rate.

bivory occurs on host plants with low mortality rates, then these plants will tend to suffer more from herbivory, forcing the insects to use less suitable hosts (at a higher rate of mortality). The increased mortality will then tend to retard further increases in the insect herbivore population. In this way herbivore numbers may be regulated by the availability of highly suitable hosts.

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