Online Supplementary Material and Table S1 are available in the online version of this article (10.1111/j.1744-7429.2008.00438.x).
and survival in gaps and understory? We focused our experiments on one of the most abundant understory shrub species on BCI, Hybanthus prunifolius (Violaceae, Schultze) (Croat 1978) and its herbivore Zunacetha annulata (Lepidoptera: Dioptidae).

METHODS

STUDY SYSTEM.—This study took place in the lowland moist forest of BCI, at the field station operated by the Smithsonian Tropical Research Institute, in the late rainy season of 2003. BCI has a wet season from May to November, and an average annual rainfall of 2.6 m. Hybanthus prunifolius is common in both treefall gaps and in the understory on BCI. Although H. prunifolius is shade-tolerant, when a light gap is formed, the plant drops and replaces the shade leaves with sun leaves that have 2.5 times greater photosynthetic rates (Kursar & Coley 1999). Thus, there is potential for differences in leaf quality between sun and shade leaves that can affect herbivore rates (Kursar & Coley 1999). Thus, there is potential for differences in leaf quality between sun and shade leaves that can affect herbivore rates and by quantifying toughness, water and nitrogen content, and specific leaf area (SLA).

LEAF QUALITY.—We measured leaf quality through bioassays, field herbivory rates and by quantifying toughness, water and nitrogen content, and specific leaf area (SLA).

First instar Z. annulata larvae were collected from the field and fed recently expanded sun or shade H. prunifolius leaves. Hybanthus prunifolius leaves live for 1 yr both in the sun and in the shade (Kursar & Coley 1999). Thus, differences in larval response to sun and shade leaves were not due to differences in leaf age. The larvae came from one large egg cluster found on a plant in medium light conditions along the trail. We placed 25 first instar larvae on sun leaves and 25 on shade leaves. One larva and one leaf were placed in individual containers and kept in a screened porch under ambient conditions (27°C). Initial leaf areas and larval weight were recorded before they were placed in containers. Every other day larvae were removed, remaining leaf area was measured, and a new field collected leaf was added. New leaves were collected from at least five plants from each environment and the plants collected from varied every other day. We assigned leaves randomly to the larvae. Larvae were reweighed at 8 d, 14 d, and at pupation. From these bioassays, we calculated larval growth rates for the first 14 d, pupal weights, days to pupation, length of pupation, and leaf area consumed. We analyzed the data using an ANOVA or Mann--Whitney if the data did not pass the homogeneity of variance test.

Mature leaf herbivory rates were measured in the field in gaps and the understory. At the beginning of the rainy season, four of the most terminal leaves (recently matured) were tagged on 16 plants in gaps and 15 plants in the understory and previous herbivory was recorded. These plants were randomly selected and located throughout the old-growth forest of the island. We revisited the leaves at the end of the rainy season and recorded new herbivory. Herbivory was measured by tracing the leaves and holes on a piece of paper in the field and measuring areas in the lab with a leaf area meter (LI-COR Biosciences, Lincoln, Nebraska). We used a t-test to determine if there was a significant difference in herbivory rates between habitats.

Leaf toughness, SLA, water content, and nitrogen content were quantified for sun and shade leaves. We sampled five leaves from five randomly selected plants growing in treefall gaps and the understory. These plants were different than those used in the herbivory study. We measured leaf toughness by averaging the weight needed to push a metal rod through the leaf using a Chatillon®, 516–1000 MRP push gauge (Chatillon, Largo, Florida). After we recorded toughness, leaf area, and wet and dry weights, the dried samples were pooled into sun leaves and shade leaves and run through a Wiley® Mill (Thomas Scientific, Swedesboro, New Jersey). We analyzed three samples of the ground batches of sun and shade leaves for nitrogen content using a Total Kjeldahl Nitrogen (TKN) digestion (Hach Company, Loveland, Colorado). Due to the variability of the digestion, we analyzed nitrogen content using a two-tailed paired t-test, pairing gap, and understory samples that were digested in the same batch. We analyzed leaf toughness, SLA, and water content using t-tests.

FIELD PREDATOR EXPERIMENTS.—We ran an enclosure experiment in the late rainy season of 2003 (29 October–12 November). We located nine paired gap and understory H. prunifolius plants, with members of each pair < 100 m apart. Enclosure cages were placed around one branch of each plant. The cages consisted of bridal veil 1 mm2 diameter mesh suspended above a branch from a PVC pipe staked to the ground and a stiff wire loop (30 cm diameter) attached at the top of the pipe. This method held the material off the branch. We traced all the leaves on the branch to be enclosed for herbivory damage and then we placed eight third instar Z. annulata larvae on the terminal leaf of the branch. We sewed the material closed around the branch with larvae and applied Tanglefoot® to all access points to the branch to prevent ants from chewing through the bridal veil. We also placed eight more third instar larvae on the terminal leaf of an opposing uncaged branch on the same plant. We returned after 24 and 48 h and searched the plants for remaining larvae in and outside the enclosure. At the end of the experiment when we took down the cage, we traced the leaves to quantify new herbivory. We analyzed the leaf area consumed per larva per day using a paired t-test. We used an ANOVA to compare percent of larvae remaining per day in and outside enclosures (treatment effect) and between plants in gaps and in the understory (habitat effect). In the analysis we included enclosure pairs as a random factor and the values were not transformed. Predation rates between habitats were calculated as 100 minus the difference in the percent of the larvae remaining in the enclosures versus outside of the enclosures.

ESTIMATED LARVAL SURVIVAL.—Using the results from the field experiment and bioassays we calculated an estimate for larval survival. These calculations incorporated percent predation per day, the effects of food quality on the number of days to pupation, and survivorship to adulthood.
LARVAL BEHAVIOR.—We recorded time budgets and distance traveled by the larvae in the field predation experiments. We observed the larvae for 30 min and recorded larval activity every minute. Activities included eating, resting, traveling to other leaves, and searching (in which the larva was walking and moving its head back and forth). We recorded time budgets for the larvae placed on the open branch at the beginning of the experiments and for the remaining larvae after 24 h. All of the time budgets measurements were made at the same time of day at 0900–1200. We recorded time budgets in this manner for all pairs of plants in gaps and the understory within 1.5 h and in random order. Time budgets of larvae that were molting were excluded from the analysis. We tested if larvae behaved differently between gaps and understory using a MANOVA. The dependent variables tested were percent of time spent eating, searching, and traveling. We excluded the time spent resting from the analysis to increase independence between behavior categories.

In addition, we measured the distance traveled by the larvae everyday during the experiments. We measured the distance of each larva from the initial placement or previous known location to the current location at the time of census. The measurements followed the branches in the shortest most likely path the larva could have taken. We recorded the distance traveled after the 30-min time budgets, the next day (24 h) and on the second day (48 h). We used a Kruskal–Wallis test and analyzed each day separately.

RESULTS

LEAF QUALITY AND CATERPILLAR PERFORMANCE.—Zunacetha annulata larvae that were fed gap leaves had higher growth rates and ate less than larvae feeding on understory leaves (Table 1; $F_{1,39} = 151.2$ and Mann–Whitney $U, Z = −3.80, P < 0.001$, respectively). Larvae on gap leaves also had higher pupal weights, took fewer days to reach pupation than larvae on understory leaves (Mann–Whitney $U, Z = −4.32$ and $−4.49, P < 0.001$, respectively). Mortality was higher in larvae that feed on understory leaves (64% vs. 44%), although not significant ($\chi^2 = 1.087, df = 1, P = 0.30$). The main difference in mortality of larvae occurred during the pupation stage. There was 20 percent mortality of larvae before pupation in both treatments.

However, 44 percent of the larvae on understory leaves died during pupation compared to 24 percent of the larvae on gap leaves. There was no difference in pupal weights between those that eclosed or died during pupation ($F_{1,23} = 1.36, P = 0.25$).

Hybanthus prunifolius leaves in gaps and the understory had different characteristics that affect food quality (Table 2). Leaves growing in gaps had a higher SLA ($t_{6.2} = 2.81, P = 0.03$) and lower nitrogen content than understory leaves ($t_2 = −4.04, P = 0.04$). Sun and shade leaves did not differ in toughness and water content ($t_{4.9} = −2.17$ and $t_{6.8} = −0.647, P > 0.05$, respectively). In the field, herbivory rates in gaps were significantly higher than in the understory ($t_{24.5} = 2.04, P = 0.05$, Table 2).

FIELD PREDATION EXPERIMENTS.—Larvae survival rates were higher in the enclosures. There was a significant treatment effect on the percentage of larvae remaining on the plants ($F_{1,8} = 16.6, P = 0.004$; Fig. 1). There were significantly fewer larvae remaining on gap plants than on understory plants ($F_{1,8} = 5.73, P = 0.04$; Fig. 1). However, there was no interaction between habitat and treatment on larvae remaining ($F_{1,8} = 1.11, P = 0.32$). The average percent predation per day was 26.5 in gaps and 13.8 in the understory (95% CIs of 14.2–30.8% and 3.8–20.4%, respectively).

Most larvae were removed without leaving any evidence; nonetheless, we found visual signs of predation on larvae outside and inside of the enclosure. These included finding the remains of a predator attack. We found three times as many larvae showing evidence of attack in gaps as compared to the understory outside the enclosures (8.8% vs. 2.7%). In addition, of the 12 larvae that died in enclosures on gap plants, three of them were from predator attack. The predators either attained access by chewing through the veil or in one case the larva was attacked through the veil and pieces were pulled though the mesh. The other causes of death of larvae in the enclosures were disease and drowning. Drowning occurred when the larvae crawled between the mesh and the stem or wire loop, water from heavy rains collected in these areas and the larvae were trapped. None of the four larvae that died in the understory enclosures were from predation. These direct observations of predation support our results (Fig. 1) of higher rates in gaps.

### Table 1. Bioassay results from Z. annulata larvae feeding on gap leaves and understory leaves of H. prunifolius in Panama.

<table>
<thead>
<tr>
<th></th>
<th>Gap leaves</th>
<th>Understory leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth rates during first 2 weeks (mg/d)**</td>
<td>0.123 ± 0.005</td>
<td>0.054 ± 0.003</td>
</tr>
<tr>
<td>Leaf area consumed (cm²)*</td>
<td>136 ± 5</td>
<td>211 ± 12</td>
</tr>
<tr>
<td>Days to pupation*</td>
<td>18.9 ± 0.4</td>
<td>25.0 ± 1.0</td>
</tr>
<tr>
<td>Pupal weight (g)*</td>
<td>0.084 ± 0.004</td>
<td>0.054 ± 0.002</td>
</tr>
<tr>
<td>Length of pupation (d)</td>
<td>8.38 ± 0.40</td>
<td>9.33 ± 0.24</td>
</tr>
<tr>
<td>Percent survival to adult</td>
<td>56%</td>
<td>36%</td>
</tr>
</tbody>
</table>

Asterisk indicates a significant difference between larvae feeding on gap and understory leaves (**ANOVA; *Mann–Whitney, P < 0.01**).

### Table 2. Leaf quality characteristics and field herbivory rates of gap leaves and understory leaves.

<table>
<thead>
<tr>
<th></th>
<th>Gap leaves</th>
<th>Understory leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>N content (%/dry mass)*</td>
<td>3.26 ± 0.11</td>
<td>4.04 ± 0.08</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>79.3 ± 0.5</td>
<td>78.8 ± 0.3</td>
</tr>
<tr>
<td>Toughness (g)</td>
<td>44.7 ± 5.9</td>
<td>17.4 ± 2.8</td>
</tr>
<tr>
<td>SLA (cm²/mg)*</td>
<td>292 ± 8</td>
<td>343 ± 10</td>
</tr>
<tr>
<td>Herbivory-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In the field (%/day)*</td>
<td>0.170 ± 0.04</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>In enclosures (cm²/day/larva)</td>
<td>3.24 ± 0.45</td>
<td>3.33 ± 0.37</td>
</tr>
</tbody>
</table>

Asterisk indicates a significant difference (t-test, $P < 0.05$).

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Herbivory in enclosures did not differ between gaps and understory ($t_{8} = -0.19, P = 0.85$; Table 2). However, there was a marginal difference in the number of leaves with new herbivory between gap and understory ($7.7 \pm 0.8$ and $9.9 \pm 1.4$ leaves respectively, $t_{8} = -2.05, P = 0.07$). Herbivores sampled more leaves in the understory than in gaps.

**LARVAL BEHAVIOR.**—There were significant main effects of habitat (gap vs. understory) and time (first 30 min vs. after 24 h) on larval behavior (Wilks’ Lambda $0.745$, $P = 0.034$ and $0.46$, $P < 0.001$, respectively; Fig. 2). While there was no habitat effect on time spent eating ($F_{1,31} = 0.049, P > 0.05$), larvae in the understory spent more time searching and traveling ($F_{1,31} = 5.68$ and $6.01, P < 0.05$, respectively). In the first 30 min time budget, the larvae spent more time eating and searching than in the time budget 24 h later ($F_{1,31} = 8.79$ and $35.1, P < 0.05$, respectively). There was no habitat by time interaction (habitat $\times$ time Wilks’ Lambda $0.993, P > 0.05$).

Larvae in the understory traveled greater distances than those in gaps (Fig. 3). There was no significant difference in the distance traveled in the first 30 min (Kruskal–Wallis, $\chi^{2} = 2.76$, df = 1, $P = 0.10$). However, larvae traveled significantly greater distances in the following 2 d (Day 1, $\chi^{2} = 22.4$, df = 1, $P < 0.001$; day 2, $\chi^{2} = 10.5$, df = 1, $P = 0.001$).

**ESTIMATED LARVAL SURVIVAL.**—We estimated that the combined effects of food quality and predation rates should lead to lower larval survival in gaps than in the understory. We calculated the cumulative predation for larvae in gaps and the understory based on the duration of the larval stage (Table 1, days to pupation). When this predation measure was combined with the length of the larval stage and survivorship to adult determined in the feeding bioassays (Table 1), overall survivorship was calculated to be 0.22 percent in gaps and 1.02 percent in the understory.

**DISCUSSION**

Larvae that fed on *H. prunifolius* in gaps and in the understory experienced differences in potential predation and food quality. In gaps, where food quality and predation were higher, larvae were less active. In comparison, where food quality and predation were lower in the understory, larvae were more active.
Predation experiments using enclosures can be difficult to interpret because manipulations made to limit predator access to the larvae will also limit larvae from migrating off focal plants. However, combining the predation experiment with behavioral data helps clarify whether the missing larvae are from migration, predation, or both. We found multiple lines of evidence demonstrating that larvae in the understory migrate more than those in gaps. Behavioral observations showed that larvae in the understory spent more time traveling and went longer distances than those in the sun. Sipura and Tahvanainen (2000) also found significantly more migration in Chrysomelidae in the shade than in the understory. Therefore, we suggest that missing larvae in the understory are from a combination of predation and migration. In comparison, larvae in gaps did not show the same tendency for migration and we found more signs of predation. Thus, we suggest that missing larvae in gaps are primarily due to predation. Significantly more larvae missing in gaps suggest higher predation in gaps.

The higher survival in our understory treatments is consistent with results from previous studies done on BCI during the rainy season (Harrison 1987, Richards & Coley 2007). Harrison (1987) found 65 percent daily survival of Z. annulata larvae in gaps compared to 78 percent in the understory. These results are similar to our survival rates recorded for larvae outside the exclosures, 64 and 81 percent, respectively (Fig. 1). In addition, we estimated daily predation rates from the differences in survival of caged and uncaged larvae. These rates were 26 percent in gaps and 14 percent in the understory, which is consistent with predation rates found on artificial caterpillars, 18 percent in gaps and 12 percent in the understory (Richards & Coley 2007). Thus, independent studies with real and artificial caterpillars have found higher predation rates in the sun.

Although understory leaves had higher nitrogen content, larvae preformed poorly on understory leaves. These data and Harrison’s (1987) data show that larvae grew 25 percent faster on gap leaves. Generally, herbivore growth rates are positively correlated with leaf nitrogen content (Mattson 1980). Therefore, other traits that we did not measure, such as chemical defenses, must be driving these differences in performance.

The differences in larval behavior between gaps and the understory reflect the differences in predation pressure and food quality. In gaps, leaves are of adequate quality for growth, which reduces the need for larvae to travel and search for higher quality leaves. In addition, higher predation pressure in gaps may also result in a reduction in larval activity as a means to decrease visibility to natural enemies. In comparison, poor food quality in the understory caused the larvae to be more active and sample more leaves in search of better quality food. Thus, the need to find better food apparently outweighed the increased visibility to natural enemies.

Wolda and Foster (1978) estimated larval survivorship for Z. annulata at 0.4 percent, which is similar to our estimates (gaps: 0.22%; understory: 1.02%). Our estimate for larval survivorship did not differ between habitats due to balancing effects of food quality and predation. Our estimates, however, do not account for predation on eggs and pupae. In addition, predation rates were measured for third instar larvae, however, predation rates often change through development with higher rates on earlier instars. Our estimates of survivorship are considerably lower than the results of Hawkins et al. (1997), in which they found 86 percent survivorship to pupation for tropical larvae. Our study examined a single species while Hawkins et al. (1997) summarized patterns in 11 tropical/subtropical studies including several holometabolous herbivore insects.

Adult Z. annulata prefer to lay eggs in gaps (Wolda & Foster 1978) where predation rates are higher. Similarly, Sipura and Tahvanainen (2000) found that the herbivores Galerucella lineola and Phratora vitellinae (Coleoptera: Chrysomelidae) preferred to oviposit on sun leaves in the field, even though predation was higher and sun leaves were a poorer food source than shade leaves. The authors suggested that ambient temperatures dictated adult preference. Abiotic differences between gaps and understory may contribute to the differences in oviposition preference of adults. However, Harrison (1987) found no temperature effects on Z. annulata growth rates. Thus, we suggest that food quality is driving oviposition preferences. There are likely other factors affecting survivorship not included in our estimates that can alter the magnitude of the differences between gaps and understory.

Light-gaps created by treefalls are reliable sources of habitat heterogeneity on the tropical forest floor. Many plant species have evolved traits to take advantage of these patches of high light (Denslow et al. 1990, Hubbell et al. 1999, Dalling et al. 2001), including H. prunifolius (Kursar & Coley 1999). Hybanthus prunifolius in gaps may be less defended because not only can they quickly replace biomass lost to herbivores, but also natural enemies help keep herbivores in check. We found evidence that predators limited larvae in the high light habitats of gaps, whereas the quality of food resources limited larvae in the shaded understory. These results support the hypothesis that herbivores experience greater predator limitations in gaps and greater resource limitations in the understory (Richards & Coley 2007). In addition, our findings are consistent with the prediction that predators have the greatest impact on herbivores in patches of high productivity (Hochberg & van Baalen 1998).

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LITERATURE CITED

