The last decades have witnessed an enormous growth in studies dealing with patterns of host plant selection by herbivorous insects. Many of these studies have addressed the relationship between female oviposition preference and offspring performance in an attempt to shed light in the evolution of interactions between insect herbivores and their host plants (see Thompson & Pellmyr 1991). Although several hypotheses were raised to explain the described patterns of attack by herbivorous insects at several ecological scales (Price 1997), a consensus on the likely mechanisms and processes involved may be premature; perhaps owing to the enormous diversity of taxa and feeding modes of herbivores and their derived results. The Plant Vigor Hypothesis (PVH; Price 1991) predicts that insect herbivores will choose preferentially large, more vigorously growing plants or plant modules (preference prediction) and that offspring performance will be greater on these more vigorous plants or plant modules (performance prediction). Several studies support both PVH predictions (Price et al. 1987a,b; Craig et al. 1989; Kimberling et al. 1990; Price & Ohgushi 1995; Stein & Price 1995; Woods et al. 1996; Inbar et al. 2001; Bruyn et al. 2002), while other studies have partially supported the preference prediction (Cornelissen et al. 1997; Prado & Vieira 1999; Fritz et al. 2000; Cornelissen & Fernandes 2001; Ferrier & Price 2004; Cunningham & Floyd 2006; Cornelissen et al. 2008) or even refuted both PVH predictions (Bruyn 1995; Faria & Fernandes 2001; Rehill & Schultz 2001).
Amongst the few opposing evidences against the PVH, Faria & Fernandes (2001) argued that the amount of resources has been underestimated in shoots belonging to larger size classes (see Price 1991 for details). In their study, the higher attack rates of females of Baucharopelma dracunculifolii (Burckhardt (Hemiptera, Psyllidae) on the longest shoots of Baccharis dracunculifolia DC. (Asteraceae) were due to the higher availability of resources (leaves) on longer shoots compared to shorter shoots. When they incorporated the amount of resources on the shoot to estimate the random probability of attack, the pattern of attack on vigorous shoots disappeared. Here we tested the PVH using two methods based in shoot (Price 1991) and leaf distribution (Faria & Fernandes 2001) to find the estimated distribution of attack.

For three consecutive years the attack by the leaf galling species, Asphondylia microcapillata Maia (Diptera, Cecidomyiidae) on Bauhinia brevipes Vogel (Fabaceae) was studied. The genus Asphondylia comprises 82 neotropical univoltine or multivoltine species inducing galls only when young undifferentiated plant tissue is available; generally these galls are induced on flowers and fruits (Gagné 1994).

Some gall midges on this genus represent exceptions to the general rule of gall feeding on the nutritious gall tissues. These insects feed upon the spores of symbiotic fungi which are probably introduced by the egg-laying female (Stone & Schönhöge 2003). Asphondylia microcapillata is univoltine and induces galls only when immature expanding or unfolded B. brevipes leaves are available. Asphondylia microcapillata is the second most abundant gall-former species on B. brevipes, whereas another leaf-galling midge, Schizomyia macrocapillata Maia (Diptera, Cecidomyiidae) is the dominant species (Cornelissen & Fernandes 1998, 2001; Cornelissen et al. 2002; Santos et al. 2008).

Previous studies on these two galling herbivores showed that S. macrocapillata has a strong preference for longer shoots (Cornelissen et al. 1997; Fernandes 1998; Cornelissen & Fernandes 2001, Santos et al. 2008) while A. microcapillata lacks a pattern of attack on shoots (Cornelissen et al. 1997). In this 3-yr study we tested the preference-performance predictions of PVH that gall induction and survivorship of A. microcapillata are significantly greater on larger shoots and we analyzed the effects of top-down mortality factors on the performance of A. microcapillata. Here, we enlarged the sampled variables and added a temporal effect, thereby generating a wider scenario to describe insect-plant interactions. Three questions were asked: a) what are the effects of top-down mortality factors on the survivorship of A. microcapillata?; b) what are the effects of shoot size in the oviposition preference of A. microcapillata on B. brevipes?; and c) what is the effect of B. brevipes shoot size in A. microcapillata offspring survival?

MATERIAL AND METHODS

Study area.

This study was performed in the Estação Ecológica de Pirapitinga - IBAMA (EEP) in Três Marias, Minas Gerais, southeastern Brazil. The EEP is a 1.100ha man-made island, built in 1965 in the Três Marias reservoir (18°23’S, 45°20’W), at an altitude of 560m a.s.l. (Azevedo et al. 1987). The average annual temperature varies from 21 to 25°C and the average annual precipitation is 1.200mm, with rainy summers and dry winters. The vegetation is primarily cerrado (Brazilian savanna) with sandy, shallow and nutrient-poor soils, with high aluminum saturation (Gonçalves-Alvim & Fernandes 2001).

The system.

Bauhinia brevipes is a deciduous shrub, growing up to 3m high and abundant in the cerrado vegetation (Vaz & Tozzi 2003). Blooming occurs between June and September whereas fructification peaks between September and October. Leaf flushes starts at the onset of the rainy season in October and lasts until the end of the rainy season in March. Leaf fall takes place during the dry season, beginning in May and ending up in August; during September all individuals are leafless (Silveira F. A. O. unpublished data).

Bauhinia brevipes is attacked by seven galling species (three leaf gall midges and four stem gallers - one Cecidomyiidae, one Lepidoptera, and two Curculionidae (Coleoptera) (Cornelissen et al. 1997; Cornelissen & Fernandes 1998, 2001). The three gall midges, A. microcapillata, S. macrocapillata and one undetermined species induce leaf galls. Gall induction and formation were observed in October when leaves are flushed synchronously. The spheroid leaf galls of A. microcapillata are covered with short, whitish trichomes. The gall is positioned between the adaxial leaf lamina and has a single chamber where a single galling larva is found. The two polar regions of the spheroid galls collapse the two-lobed leaves of B. brevipes. At the beginning of gall formation, the trichomes are whitish but may change color as the galls mature, and when exposed to direct sunlight present reddish tones at the distal portion of the trichomes (Maia & Fernandes 2005).

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of shoots</th>
<th>Number of leaves</th>
<th>Galled shoots</th>
<th>Galled leaves</th>
<th>Gall abundance</th>
<th>Galls/shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>1,947</td>
<td>12,344</td>
<td>55 (2.82%)</td>
<td>59 (0.48%)</td>
<td>122</td>
<td>0.06</td>
</tr>
<tr>
<td>2002</td>
<td>1,996</td>
<td>14,192</td>
<td>41 (2.05%)</td>
<td>48 (0.34%)</td>
<td>114</td>
<td>0.06</td>
</tr>
<tr>
<td>2003</td>
<td>1,848</td>
<td>12,291</td>
<td>111 (6.01%)</td>
<td>147 (1.20%)</td>
<td>359</td>
<td>0.19</td>
</tr>
<tr>
<td>Total</td>
<td>5,791</td>
<td>38,827</td>
<td>207 (3.57%)</td>
<td>254 (0.65%)</td>
<td>595</td>
<td>0.10*</td>
</tr>
</tbody>
</table>

* mean for all years

Table I. Asphondylia microcapillata (Diptera, Cecidomyiidae) abundance and density on the host plant Bauhinia brevipes (Fabaceae) during three consecutive years (2001, 2002, and 2003) in a cerrado vegetation (Três Marias, Minas Gerais). Numbers in parentheses represent the amount of galled shoots or galled leaves in relationship to the respective abundance of shoot or leaves in each year.
Where $G$ was defined as galled shoots, galled leaves or galls and $R$ was defined as resource, shoots or leaves. Thus, the ratio between $G$ and total of $R$ in a sample is taken as a measure of the probability of attack ($P$). If a size class $i$ is made of $M$ shoots, then $MP$ shoots will be galled. Subsequently, the preference prediction was tested by comparing the expected and observed distribution along shoot length classes using a Chi-square distribution (see Faria & Fernandes 2001). Shoot length classes with smaller sample sizes were combined to form classes containing at least five shoots per class in the expected distribution (Zar 1996). Proportion of larvae survival and gall diameter at each shoot length classes and their distribution along shoot length classes were used to estimate

$$P = \frac{\text{Total sum of } G \text{ in the sample}}{\text{Total sum of } R \text{ in the sample}}$$
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Fig. 3. Observed and expected distributions of total number of galled shoots (left column), galled leaves (middle column) and total number (right column) of *Asphondylia microcapillata* (Diptera, Cecidomyiidae) galls on *Bauhinia brevipes* (Fabaceae) during three consecutive years (2001, 2002, and 2003) in a cerrado vegetation (Três Marias, Minas Gerais). a) Preference estimated using shoots, see *Method a* by Price (1991) and b) Preference estimated using leaves abundance per shoot length classes (probability of attack though of abundance of resource, *Method b*).
the performance prediction. Differences in the proportion of larvae survival and gall diameter among shoot size classes of *B. brevipes* were analyzed by Kruskal-Wallis test or Mann-Whitney Test. We used non-parametric tests because the data did not present normal distribution (Zar 1996; STATISTICA 6.0 - StatSoft 2001).

**RESULTS**

Temporal variation of attack, survivorship and mortality factors.

A total of 595 galls were found on 38,827 leaves and 5,791 *B. brevipes* shoots collected during the 3-yrs study period (Table I). Approximately three galls were found per galled leaf ($\bar{X} = 2.87 \pm 2.00$; range: one to 13 galls/leaf; n = 207). Overall, only 3.57% and 0.65% of total amount of shoots and leaves sampled were galled, respectively. Gall abundance, galled shoots, and galled leaves were similar in 2001 and 2002, but increased in 2003. In 2003, the number of galled shoots and number of galls per shoot increased almost 3-fold in relation to 2001 (Table I).

Nearly one quarter of *A. microcapillata* larvae that successfully induced galls survived. Survival rates of *A. microcapillata* were 23.0%, 25.4%, and 19.5% in 2001, 2002, and 2003, respectively (Fig. 1). In 2001, parasitism was the most important mortality factor acting upon *A. microcapillata* larvae, accounting for 44.3% of killed larvae; followed by predation which killed 21.3% of total larvae. An increase in predation rate was observed in subsequent years (43.0% in 2002 and 62.7% in 2003). Unidentified parasitoid wasps killed 16.7% and 14.5% of larvae in 2002 and 2003, respectively. Mortality due to unknown factors reached its peak in 2002 (13.2%) but decreased in the following year to 3%. Few pathogenic fungi hyphae were found inside galls indicating that pathogens did not represent a strong mortality factor against *A. microcapillata* during the studied period (Fig. 1).

Resource distribution and oviposition preference.

Smaller shoots of *B. brevipes* were always more abundant whereas longer shoots were rare (Fig. 2). The percentage of galled shoots and galled leaves in each shoot length class increased with decreasing shoot length until the medium-size shoots (9.6-24.5 cm) in all years (Fig. 2). Larger shoots were rarely attacked, except in 2003 (Fig. 2). This trend was not expected by the PVH, which predicts higher herbivore attack rates on larger shoots classes.

The analysis of oviposition preference based in Method a (shoot abundance) on the pattern of attack of *Asphondylia* showed that the observed number of galled shoots in each shoot length class differed from the expected value in all years (2001 - $\chi^2 = 45.25$ df = 4 $p < 0.0001$; 2002 - $\chi^2 = 35.43$ df = 4 $p < 0.0001$ and 2003 - $\chi^2 = 45.44$ df = 5 $p < 0.0001$; Fig. 3a). This same trend was observed for the number of galled leaves (2001 - $\chi^2 = 44.94$ df = 4 $p < 0.0001$; 2002 - $\chi^2 = 41.19$ df = 5 $p < 0.0001$ and 2003 - $\chi^2 = 59.35$ df = 6 $p < 0.0001$; Fig. 3a) and gall abundance (2001 - $\chi^2 = 67.53$ df = 5 $p < 0.0001$; 2002 - $\chi^2 = 126.10$ df = 6 $p < 0.0001$ and 2003 - $\chi^2 = 198.21$ df = 8 $p < 0.0001$; Fig. 3a).

When Method b (resource availability) was used, the number of galled shoots in each shoot length class differed from the expected value in 2001 ($\chi^2 = 16.74$ df = 4 $p < 0.002$) and 2002 ($\chi^2 = 15.77$ df = 4 $p < 0.003$), but not in 2003 ($\chi^2 = 8.44$ df = 6 $p = 0.208$; Fig. 3b). The same trend was observed for the number of galled leaves galls in 2001 ($\chi^2 = 16.88$ df = 4 $p < 0.002$), 2002 ($\chi^2 = 17.22$ df = 4 $p < 0.002$) and 2003 ($\chi^2 = 12.18$ df = 6 $p = 0.058$; Fig. 3b). The observed number of galls differed from the expected value in all years (2001 - $\chi^2 = 21.09$ df = 6 $p < 0.002$; 2002 - $\chi^2 = 67.71$ df = 7 $p < 0.001$, and 2003 - $\chi^2 = 68.71$ df = 9 $p < 0.001$; Fig. 3b).

Results shown by both methods indicate that the number of observed galled shoots and leaves and the number of galls

![Graph](image-url)
were always smaller than expected on the smaller shoot classes. However, this pattern did not hold from the 9cm shoot length class upward. The observed gall abundance and galled shoots were always greater than expected on intermediate shoot length classes. Therefore, high levels of attack were observed on medium-sized shoots (9.5-24.5 cm).

These results also suggest a lack of preference for smaller shoots because the attack rate on these shoot length classes was smaller than expected.

**Attack on medium-sized shoots.**

Due to the higher preference for intermediate shoot length classes (9.5 cm-24.5 cm), the data were further analyzed by separating the shoot length classes into three main classes: small (0.5-9.5 cm), medium (9.6-24.5 cm), and large (24.6-48.5 cm) (Table II). More than 50% of the attacked shoots and galled leaves were concentrated on medium-sized class shoots. Medium-sized shoots supported from 46% to 70% of the total galls sampled during the three years of study. When the effect of resource availability was taken into account by dividing the number of galls per number of leaves in each shoot class, medium-sized shoots supported from 47% to 57% of all galls.

Furthermore, during two consecutive years *A. microcapillata* survival was greater on medium-sized shoots (2002 - Kruskal-Wallis test: H(2, N=17) = 9.492, p = 0.009 and 2003 - Kruskal-Wallis test: H(2, N=17) = 9.659, p = 0.008; Fig. 4-5, Table II). In 2001, higher survival was achieved on the smaller shoots (Fig. 4-5, Table II) (Kruskal-Wallis test: H(2, N=17) = 12.117, p = 0.002). The percentage of larvae killed by natural enemies varied widely among shoot length classes, without a consistent pattern (Fig. 5, Table II). However, gall diameter, another indicator of larval performance, did not differ among shoot size classes in 2001 (U = 326.5, p = 0.532), 2002 (Kruskal-Wallis test: H(2, N=17) = 2.237, p = 0.327) or 2003 (Kruskal-Wallis test: H(2, N=17) = 2.2, p = 0.333).

**DISCUSSION**

The relative importance of the mortality factors acting upon *Asphondylia* galls varied during the study period. In 2001, the attack by parasitoid wasps represented the strongest mortality factor acting upon *A. microcapillata*, while in the following years, predators killed most of larvae. In different plant-herbivore-parasitoid systems the attack of parasitoid wasps are regarded as the main mortality factor of galling insects (Weis et al. 1985; Price & Clancy 1986; Espírito-Santo et al. 2004). The consumption of gall tissue by Lepidoptera larvae, adult Coleoptera and Orthoptera may kill the galling larva or lead to a desiccation process (Fernandes & Price 1992). Interestingly, the mortality factors acting upon the other gall midge, *S. macrocapillata*, differed significantly from that acting upon *A. microcapillata* on the same host plant. On *S. macrocapillata*, an induced resistance mechanism, hypersensitive reaction (a bottom-up effect) accounted for more than 90% of the mortality, making few galls available to be found and killed by natural enemies (Fernandes 1990, 1998; Fernandes et al. 2000; Santos et al. 2008). Pathogenic fungi accounted for no more than 3% as predicted by Fernandes & Price (1992) in xeric environments. In general, we observed that the *A. microcapillata* mortality ratio by natural enemies is in agreement with that found for many other galling species (e.g., Weis et al. 1985; Price & Clancy 1986; Abrahamson & Weis 1987; Espírito-Santo et al. 2004). Regardless of the strength of each individual mortality factor, the population dynamics of *A. microcapillata* was not affected because survival ratio was relatively constant. Our results also indicate an important role of temporal variability of natural enemies on the performance of *A. microcapillata*. *Asphondylia microcapillata* oviposited and survived preferentially on medium-sized shoots (9.6-24.5 cm). This result does not corroborate the predictions of the PVH, which predicts a strong female preference and increased larval survival on the largest plant modules (Price 1991). However, these results are similar to those reported by Craig et al. (1999).
The relationship between oviposition preference and offspring performance is crucial to understand the evolution of interactions between herbivorous insects and their host plants (Thompson & Pellmyr 1991). A strong link between female preference and larval performance of *A. microcapillata* was found on medium-sized shoots on *B. brevipes*. Higher survival rates resulted from the female preferential attack on medium-sized shoots can provide an evolutionary mechanism for the maintenance of preference on intermediate shoots. Otherwise, this pattern should be further studied in relation to the other herbivorous insects in the community that feed on similar resources.

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