Oviposition behavior and performance aspects of *Ascia monuste* (Godart, 1919) (Lepidoptera, Pieridae) on kale (*Brassica oleracea* var. *acephala*)

Patrícia Diniz Catta-Preta¹
Fernando Sérgio Zucoloto¹

**ABSTRACT.** Host part selection by ovipositing females of *Ascia monuste* (Godart, 1919) (Lepidoptera, Pieridae) on kale (*Brassica oleracea* var. *acephala*) was determined in greenhouse and field. Performance of offspring (larval period, efficiency of food utilization, number of eggs/female and others) was investigated under laboratory conditions. In the field, the number of *A. monuste* egg clutches on the apical and medium parts of kale leaves was greater than on the basal part. In greenhouse, *A. monuste* exhibited a strong preference for the apical part of kale leaves for ovipositing. The best results on food utilization indices, pupal mass and female wing size were obtained with the leaf apical part. This part of kale leaves exhibited the highest nitrogen and protein concentration and the smallest water content, when compared to the other leaf parts. However, the apical part of the leaves seems not to provide ovipositing females with enough protection against birds, making them easy preys in the field. We suggest that good relationship between oviposition preference and performance of offspring was hindered by predation in field conditions.

**KEYWORDS.** Brassicaceae; host parts selection; offspring performance; Pieridae; predation.

**INTRODUCTION**

Most hypotheses on the evolution of oviposition behavior agree that females choose plant species, individual plant or plant parts that maximize larval survival and growth. However, allelochemicals, nutritional chemistry, plant morphology, natural enemies and feeding modes (parasitism vs. grazing) have been pointed out as major determinants of host preference and specificity (Thompson & Pellmyr 1991).

Ehrlich & Murphy (1988), Quiring (1992) and Kause et al. (1999) discussed about the role of plant chemistry in plant-herbivore coevolution. However, Price et al. (1980) and Bernays & Graham (1988) believe that natural enemies of herbivore insects provide a major selection pressure for a restricted range of host plants.


Barros & Zucoloto (1999) demonstrated that *A. monuste* (Godart, 1919) butterflies preferred ovipositing in brassica species that presented a good performance of offspring and higher nitrogen and protein concentration, suggesting a different oviposition strategy of monophagous species. In previous studies, Fernandes-da-Silva & Zucoloto (1993) showed that nutritional differences (carbohydrate concentration) might exist among different parts of the same host, but it was not clear if *Ceratitis capitata* (Wiedmann, 1924) (Diptera) females prefer to oviposit in the part of the fruit which is more adequate for the larvae.

Field observations showed that there are three main oviposition sites of *A. monuste* on the kale leaves (*B. oleracea*): apical, medium and basal parts of the leaf (Figs. 1a, 1b, 1c, respectively).

The purpose of this study was (1) to determine the oviposition behavior pattern in the absence of their predators.

---

1. Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo. Av. Bandeirantes 3900, 14049-901 Ribeirão Preto-SP, Brazil. E-mail: zucoloto@ffclrp.usp.br
(in most cases, birds), and (2) to assess the nutritive value of these different parts of the same kale leaf for *A. monuste*.

The white butterflies (*A. monuste*) used in this experiment are common in the study area (São Paulo State, Brazil) and their eggs are only found on Brassicaceae family (group assembled by the presence of glucosinolates like sinigrin). The eggs are laid in clutches mainly on the leaf surfaces and normally hatch in five days. Caterpillars usually feed at initial instars on the part of the leaf where the eggs were laid (Diniz 1998). The mean larval period is ten days. The pupal phase takes place either on the soil or plants. The adults emerge in five days on average, and present mean life duration of five days (Shima & Gobbi 1981). In this work, the most important natural enemies of *A. monuste* butterflies and caterpillars were birds.

**MATERIAL AND METHODS**

1. **Oviposition behavior**

   The eggs were collected from an 86-plant vegetable garden kept at 90 m² of the Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras-USP experimental garden, at the outskirts of the laboratory. The kale (*B. oleracea*) leaves were obtained from an organically grown farm (Chácara Boa Esperança, Ribeirão Preto-SP, Brazil) nearby the university.

   The couples of butterflies obtained from eggs at the vegetable garden and reared in the laboratory were placed in iron-made cages covered with tulle (1.0 x 1.5 x 0.5 m). Each cage was placed over two kale plants at the greenhouse, at 28 ± 5 °C and about 75% relative air humidity. The photoperiod was approximately 12h/day in the summer. Cages also were used for excluding birds (Sipura 1999).

   A sucrose solution was prepared by dissolving 20 gr of reagent-grade sucrose (Merck, Darmstadt, Germany) in 200 ml of drinking water and 2 gr of food-grade yellow or blue dye, and offered daily on a Petri dish (50 x 16 mm) to the butterflies (Barros & Zucoloto 1999). After five days, the leaves were removed and the egg clutches counted. The number of egg clutches in each part of the leaf (basal, medium and apical – increasing distance to the peduncle), characteristics of the kale leaf and of the egg clutch were noted.

2. **Performance of offspring**

   The following parameters were employed to assess the nutritional value of different parts of the host chosen by *A. monuste*: larval period (days), indices of food consumption and utilization (AD, ECI and ECD), pupal mass (mg), percentage of adult emergence, wing size (mm) and number of eggs/female.

   Fresh parts of the leaf to be tested (apical, medium or basal) were placed on slightly moistened filter paper in acrylic boxes (11 x 11 x 3 cm) with 1/3 of the newly hatched caterpillars from one single clutch. One single source of food was supplied to each set of test caterpillars until they reached the target growth stage.

   In the fifth instar, each caterpillar was transferred to a cylindrical glass vial (10 cm high x 3 cm diameter) covered with gauze, where each was allowed to feed from the same food source during 24 h (Felipe & Zucoloto 1993). The boxes and the vials were placed in incubators at 29±1°C, about 75% relative air humidity, and illuminated for 10h/day from a 400 lx fluorescent light bulb. The fifth instar is representative of the immature phase (Felipe & Zucoloto 1993).

   The amount of food consumed, fecal matter produced and weight gained by individual fifth-instar caterpillars were determined on a dry weight basis using methods described by Slansky & Scriber (1985) and Felipe & Zucoloto (1993). The dry weight of ingested food was calculated as the difference between the dry weights of leaves left over by the caterpillar and those of the initial food. The weight gained by the insect was calculated as the difference between the dry weights of caterpillars (n=10) before and after feeding for 24 h (Corbett et al. 1996). The samples were dried in oven at 80 °C for 24 h. Indices of consumption, digestion and utilization of food were described by Slansky & Scriber (1985) and Corbett et al. (1996).

   The amount of ingested food that is digested (AD) was calculated as:

   $$AD = \frac{\text{Dry weight of food ingested (g)} - \text{Dry weight of feces (g)}}{\text{Dry weight of food ingested (g)}} \times 100$$

   The efficiency with which ingested food is converted to body matter (ECI) which is a measure of the overall efficiency of an insect to grow on a given food, was calculated as:

   $$ECI = \frac{\text{Dry weight gained by insect (g)}}{\text{Dry weight of food ingested (g)}} \times 100$$

   The efficiency with which digested food is converted to body matter (ECD) was calculated as:

   $$ECD = \frac{\text{Dry weight gained by insect (g)}}{\text{Dry weight of food ingested (g)} - \text{Dry weight of feces (g)}} \times 100$$

   For other parameters that require living insects, the caterpillars were kept in the boxes (n=15) until the target-stage. After adult’s emergence, couples of butterflies were placed in cages and each cage was placed over two kale plants at the greenhouse. The wing size was estimated by measuring the right wing M-Cubital nervure (M₅ to Cu₁a) in 10 frozen females (Zucoloto 1991).

3. **Chemical composition of the kale leaves**

   Twenty fresh kale (*B. oleracea var. acephala*) leaves were split into the three studied parts and weighed immediately after sampling. The samples were dried in oven at 100 °C for 72 h. Percent water content was calculated as

   $$\left(1 - \frac{\text{Dry weight}}{\text{Fresh weight}}\right) \times 100$$

   (Quiring 1992). The leaves, dried as previously described, were
ground and the total organic nitrogen content was determined by the Kjeldhal method (FLECK & MUNRO 1965; BREMNER & MULVANEY 1982 apud QUIRING 1992).

4. Data analysis

Data were tested using Kolmogorov-Smirnov test to verify normality. One-way Analysis of Variance (ANOVA) and Student-Newman-Keuls test were used for Matched Data with Gaussian distribution of means. Friedman Repeated Measures ANOVA on Ranks and Student-Newman-Keuls test were used for Matched Data with not Gaussian distribution of means. Both were used with the level of significance set at α=5% (SigmaStat for Windows, 1994- Jandel Corporation; Manugistics Inc, 1993).

RESULTS

1. Oviposition behavior

In the field, the mean values of number of egg clutches on the apical part of the leaf (1.60 ± 0.84) (mean ± SD) was not statistically different from the medium part (2.90 ± 0.99) and higher than on the basal part (0.50 ± 0.71) (P=0.0024). However, in greenhouse the number of egg clutches (Fig. 1) on the apical part of the leaf (3.10 ± 1.29) was statistically different from the medium (1.30 ± 1.34) and the basal part (0.60 ± 0.70) (P=0.0029) (Fig. 2).

2. Performance of offspring

Some parameters demonstrate that the apical part of the leaf is a better food source than the medium and basal parts. Caterpillars reared on the apical part had a shorter larval period, lower values of AD and higher values of ECD. Caterpillars reared on the medium part had a short larval period, but high values of AD for low values of ECD. The basal part provided a longer larval period, lower values of AD and lower values of ECD (Table I). Pupae and adults of A. monuste whose caterpillars were reared on the apical and medium parts grew well, but pupae and adults from the apical part were bigger than from medium part.

3. Chemical composition of kale leaves

The basal part was a poor food source, promoting lower values of pupal mass and of percentage of emergence (Table II).

DISCUSSION

In a previous study, A. monuste butterfly chose to oviposit their eggs on adult leaves (with the greatest development and size), on the dorsal face of the leaf (where there is no direct sunlight incidence) and on the limb region (few or no clutches on the central nervures) (as also founded by BASTOS et al. 1997). The symmetry of the egg clutches was partially kept (the distance between eggs and its distribution was not the same for all eggs), even though the number of asymmetrical clutches was small (DINIZ 1998). This suggests that the greenhouse conditions used in this study were adequate to mimic the natural environment.

Most parameters measured in the greenhouse experiments were comparable to the ones measured in the field, except the distance of the egg clutches to the leaf peduncle. In the field, the number of egg clutches on the apical part of the leaf was not statistically different from the medium part, but was bigger...
than on the basal part. However, in greenhouse the number of egg clutches on apical part of the leaf was statistically different from both the medium and basal parts. Oviposition was more frequent on the apical part. Why do females choose these parts and why do they change their choice in greenhouse?

Many authors discuss about the preference/performance subject. The lower values of pupal mass and percentage of adult emergence, and the longer larval period on the basal leaf part indicate that this part is nutritionally poorer for this species, when compared to the apical and medium parts. Fiber content could have made the digestion more difficult and impacted digestive performance of the caterpillars on the leaf basal part. The long larval period when caterpillars are fed with the basal part of the leaves and consequently long feeding time could result in an equal number of egg/females for basal, medium and apical parts. But, it also would result in a higher exposure of the caterpillars to natural enemies. The best results on food consumption and utilization indices for the apical over the other leaf parts demonstrate that the apical part of the kale leaves is a better food, as it is readily digested and better utilized. This can be due to the lower fiber content of the apical part, where there are few and thin secondary nervures, which makes the digestion easier. Furthermore, pupal mass and wing size are higher on the apical part than on the medium part.

ZUCOLOTO (1991) and FERNANDES-DA-SILVA & ZUCOLOTO (1993) suggested that nutritional differences might exist among different parts of the same host. Their results suggest that the lower part of the tested fruit may have higher nutritive value to Ceratitis capitata due to the greater concentration of sugars in that mature part. The opposite may be the case with kale leaf parts.

Total protein and nitrogen are statistically more concentrated in the older part (apical) of the leaf than in the younger ones (medium and basal). The opposite happens with water and fiber content, due to the nervure caliber. The apical part of the kale leaf grows first, contains nervures with smaller caliber, and

Table I. Performance of Ascia monuste caterpillars fed with different kale (Brassica oleracea var. acephala) leaf parts.

<table>
<thead>
<tr>
<th>Kale leaf parts</th>
<th>Larval period (days) (P&lt;0.001)</th>
<th>AD (%) (P=0.001)</th>
<th>ECI (%) (P=0.12)</th>
<th>ECD (%) (P=0.02)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APICAL</td>
<td>10.3 ± 0.5 a</td>
<td>44.3 ± 7.6 a</td>
<td>26.4 ± 5.1 a</td>
<td>60.5 ± 15.2 a</td>
</tr>
<tr>
<td>MEDIUM</td>
<td>10.2 ± 0.4 a</td>
<td>50.4 ± 9.9 b</td>
<td>25.8 ± 4.9 a</td>
<td>46.9 ± 14.6 b</td>
</tr>
<tr>
<td>BASAL</td>
<td>10.8 ± 0.4 b</td>
<td>41.8 ± 6.7 a</td>
<td>23.7 ± 4.1 a</td>
<td>56.4 ± 14.5 ab</td>
</tr>
</tbody>
</table>

The results represent the mean ± SD of 20 experiments. Mean values followed by the same letters in the same column are not statistically different (Student-Newman-Keuls method).
thus ages faster than the medium and basal parts. According to Scriber & Fenny (1979) and Quiring (1992), plants accumulate many minerals (including nitrogen) and dissipate deterrent substances as they grow, promoting a better performance of animals feeding on them.

In studies with Tyria jacobaeae (Lepidoptera, Arctiidae), the larval development time was negatively correlated to plant nitrogen content, whereas the larval weight, growth rate and pupal weight were positively correlated to plant nitrogen content (Soldaat 1991 apud Tinney et al. 1998). Low concentrations of water and nitrogen of proteins were the main factors reducing pupal mass of Epirrita autumnata (Lepidoptera, Geometridae) (Kause et al. 1999).

Barros & Zucolo (1999) also found better performance of Ascia monuste fed on kale (with more nitrogen) than on mustard. Furthermore, ovipositing attractive substances (sinigrin, alkane and glucosinolate) are found in higher concentrations in large plants (Spencer 1996; Spencer et al. 1997), and consequently in older leaves. These results corroborate with the conducted leaf part assay and the butterfly choice (apical part).

Ovipositing on the dorsal face of the medium part of the leaves, butterflies are covered by the leaf where they oviposit, making them invisible to birds. When a female oviposits on the apical part of the leaf, its wings are partially visible, becoming easy preys to birds.

Therefore, part of the females ovipositing on the apical leaf part could have been predated, decreasing the number of egg clutches on that leaf part. In greenhouse, predation does not occur and oviposition on the apical leaf part is more frequent than on the medium part.

Thus, the highest-value best part of the leaf (nutritionally) seems not to provide ovipositing females with enough protection against birds, making them more visible in the field. The “enemy-free space hypothesis” (Gilbert & Singer 1975; Lawton 1978 apud Thompson 1988) suggests that performance on plant species may be influenced as much by enemies, including predators. As some examples we have Ogyris amaryllis (Lepidoptera, Lycaneidae) females that oviposit on the nutritionally inferior host Amyema maidenni (Loranthaceae) without ants rather than on the nutritionally superior A. prestiti with ants (Thompson 1988).

Pyralid caterpillars preferred the older leaves (more suitable for rolling and producing a shelter) than younger leaves (better for growth), showing that protection was dominant over nutritional factors (Damman 1987). Several authors have discussed that the predation or parasitism factories could impact (promoting lower values of larval growth and development) more than plants’ lower nutritional value (Price et al. 1980; Bernays & Graham 1988; Yamaga & Ohgushi 1999). Ohsaki & Sato (1994), working with Pieridae, infer that specialist parasitoids may be one of the most important ecological factors affecting the evolution of food plant preferences. Thus, we ask if Ascia monuste is adapting its nutritional ecology (physiology and behavior) to escape from predator, as it is suggested by Scriber & Slansky (1981).

In conclusion, we propose that good relationship between Ascia monuste oviposition preference and performance of offspring has been hindered by predation in field conditions.

Acknowledgments. We thank L. Mazzocato and A. P. dos Santos for technical assistance (Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, USP). Thanks to H. Vanuchi (Faculdade de Medicina de Ribeirão Preto,USP) for analyses of foliage nutrient contents. CNPq financially supported this study.

REFERENCES


Received in 19.IV.2002; accepted in 30.XI.2002