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Building of Leaf Shelters by *Stenoma scitiorella* Walker (Lepidoptera: Elachistidae): Manipulation of Host Plant Quality?

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Construção de Abrigos Foliares por *Stenoma scitiorella* Walker (Lepidoptera: Elachistidae): Manipulação da Qualidade da Planta Hospedeira?

RESUMO - A construção de abrigos foliares é um comportamento comum entre lagartas de microlepidópteros e, ao que parece, tais abrigos proporcionam um refúgio contra as intempéries e o ataque de predadores e parasitóides. Estudos recentes sobre a ecologia nutricional de insetos têm sugerido que tal comportamento seria, na realidade, uma estratégia evolutivamente selecionada para maximizar a qualidade da planta hospedeira que consomem. As lagartas de *Stenoma scitiorella* Walker unem as folhas jovens de *Xylopia aromatica* (Lam.) Mart. (Annonaceae) para a construção de um abrigo no qual se refugiam alimentando-se exclusivamente das folhas internas. Por proporcionar as melhores condições para o desenvolvimento larval, a maioria dos abrigos foliares (53,4%) é ocupada por apenas uma lagarta. Os resultados revelaram que, apesar de alterar os teores de água e carboidratos solúveis, a construção dos abrigos foliares não afeta a concentração de taninos nem a preferência das lagartas por folhas externas ou internas. Assim, tal comportamento não representa uma estratégia adaptativa do inseto para superar as defesas químicas da planta hospedeira. Dessa forma, é provável que os abrigos foliares de *S. scitiorella* sejam construídos para outros fins como, por exemplo, proteção das lagartas contra dessecação ou predação.

PALAVRAS-CHAVE: Cerrado, herbivoria, lagarta, tanino, *Xylopia aromatica*

ABSTRACT - Building of leaf shelters is a widespread behavior among microlepidopteran larvae. Apparently, these shelters provide refuge to caterpillars against bad weather and reduce the risks of predation and parasitism. Recent studies about nutritional ecology of insects have suggested that such behavior would actually be an strategy evolutionarily selected to maximize the quality of the host plant consumed by them. *Stenoma scitiorella* Walker caterpillars tie together young *Xylopia aromatica* (Lam.) Mart. (Annonaceae) leaves to build a shelter in which they retreat, feeding exclusively on inner leaves. The accumulation of damage and excrement in leaf shelters with several caterpillars may lead to a decline in larval development and survivorship. For providing the best conditions for larval development most leaf shelters (53,4%) are occupied by only one caterpillar. Although altering the water and soluble carbohydrates contents, the building of shelters did not affect either the tannins concentrations or the preference of caterpillars for external or internal leaves. Hence, probably this behavior does not denote an adaptative strategy of the insect to overcome the host plant chemical defenses. Thus, it is possible that the leaf shelters of *S. scitiorella* caterpillars are built for other purposes as, for example, to protect caterpillars from desiccation and predation.

KEY WORDS: Cerrado, herbivory, caterpillar, tannin, *Xylopia aromatica*

Although plants are apparently available and accessible to all or any life forms, several of their defense mechanisms were selected in response to selective pressures of herbivores (Coley & Barone 1996). Among the different defense mechanisms, stand out the chemical defenses that are represented by metabolites present in the plant tissues and known as allelochemicals. Allelochemicals differently from amino acids, carbohydrates, proteins and lipids, are produced by secondary metabolic paths and for this reason are known as secondary substances (Whittaker & Feeny 1971).

Therefore, in order to specialize on a given host plant, an insect needs to physiologically and behaviorally surpass the chemical barriers imposed to herbivory by that plant species (Barbehenn *et al*. 2001).

Based on the fact that alterations on the primary metabolism can affect the secondary metabolism, recent studies on nutritional ecology of insects have suggested that the young-leaf rolling behavior, shown by caterpillars of some species of Lepidoptera, would actually be an evolutionary strategy selected to reduce solar incidence on the inner leaves, consequently inducing a decrease in the phenolic substances such as tannins and lignin, without modifying the nutritional quality of these leaves (Sagers 1992).

Xylopia aromatica (Lam.) Mart. (Annonaceae) is an arboreal species with a maximum height ranging from 4 m to 6 m and its distribution widens from Central America to the north of the Paraná State, South Brazil, being very common in the "cerrado" or savanna areas (Oliveira-Filho & Ratter 1995).

A preliminary analysis of the guild of arthropods associated to leaves of *X. aromatica* has revealed that among the 179 morphospecies that use this feeding resource, the caterpillar *Stenoma scitiorella* Walker (Lepidoptera: Elachistidae) is predominant (Barosela 1999). *S. scitiorella* is a species of microlepidopteran from the subfamily Stenomatinae whose females oviposit preferentially on *X. aromatica* primordial leaves. After eclosion the caterpillars tie together the adjacent young leaves with a silk thread and retreat inside this shelter feeding exclusively on inner leaves. *S. scitiorella* caterpillars occur from October to June, which correspond to the maximum leaf production period for *X. aromatica,* and are responsible for more than 50% of the damages caused to this plant species by herbivores.

The objective of this study was to evaluate the frequency of occupation of leaf shelters built on *X. aramatica* by *S. scitiorella* caterpillars and use the relations between these two species to test the hypothesis that the leaf-rolling behavior represents an adaptation strategy of the caterpillars against chemical defenses of the host plant.

Material and Methods

The present study was carried out on a *sensu stricto* "cerrado" area of the "Pé-de-Gigante" (Giant's Foot) clod, which constitutes one of the six discontinuous natural vegetation clods that compose the Parque Estadual de Vassununga (Vassununga State Park), located in Santa Rita do Passa Quatro County, state of São Paulo, Brazil.

In order to verify how the leaf shelters built by *S. scitiorella* caterpillars are occupied, 457 attacked sprouts were randomly colleted on *X. aromatica* plants during January and February 2001.

The preference of caterpillars was evaluated in relation to two leaf categories: exposed and non-exposed to sunlight. To simulate the low luminosity conditions to which the inner leaves of the shelters were submitted, one primordial leaf of each of 20 different *X. aromatica* individuals were protected with an opaque-brown paper bag until full foliar expansion.

For the feeding preference bioassays, two 0.5-cm disks of a sunlight exposed leaf blade as well as of a protected one were collected. The disks were then place into Petri dishes together with one *S. scitiorella* caterpillar. This procedure was performed for 20 caterpillars and repeated three times starting from disks collected at different regions of the leaf blade. The caterpillars were kept under these conditions for 24h. The experiments were carried out under controlled environmental conditions in the laboratory (25ºC temperature; 60 ± 10% RH; and 14h photophase).

The preference was considered when the consumption of the leaf- disk area reached more than 50%. If the caterpillar had consumed both leaf disks or did not feed, no preference was recorded.

In order to determine the physical and chemical characteristics of the *X. aromatica* leaves, which could influence the choice by the caterpillars, samples composed of sunlight-exposed leaves and sunlight-protected ones were collected for analysis of specific foliar mass as well as of water, nitrogen, soluble sugars and tannins contents. To obtain the specific foliar mass, 10 known-area disks were cut from some leaves at each development stage, which were then dehydrated and weighed. The specific foliar mass represents the amount of dry mass per area of a given leaf and was used as an estimate of the amount of fibers, i.e., the degree of leaves sclerophylly. In the computation of the specific foliar mass the following formula was used: SFM = Dm/A, where SFM = specific foliar mass, Dm = dry mass (mg), and A = foliar area (cm^2). The remaining collected leaves were immediately weighed and then brought to the laboratory where they were maintained in an Owen at 50ºC for 48h to interrupt enzymatic activity and reach full dehydration. The percent water content was computed by the formula: [(Fm – Dm). 100] / Fm, where Fm = fresh mass and Dm = dry mass. For quantification of nitrogen, soluble sugars and tannins contents the Kjeldahl (Allen *et al.* 1974), Buysse & Merckx (1993), and Hagerman & Butler (1978) methods were respectively used.

Results and Discussion

From the 457 leaf shelters built on *X. aromatica* by *S. scitiorella*, 34.6% had no caterpillars inside them, more than half (53.4%) had only one caterpillar and inside 12.0% of shelters there were two or more caterpillars.

The high frequency of leaf shelters containing only one caterpillar and the low occurrence of leaf shelters with two or more caterpillars is an indication of the disadvantage of co-habitation. The use of the same foliar sprout by more than one caterpillar promotes a reduction in the feeding quality of the leaf as a consequence of the increased herbivory and accumulation of feces and may affect larval development as well (Cappuccino 1993). Insects found under these conditions were occupying different regions within the leaf shelter. Besides, the majority of those caterpillars were in the initial instars and only one or two of them were already in more advanced instars. Hence, results suggest that the presence of only one caterpillar per shelter provides the best conditions for *S. scitiorella* larval development on leaves of *X. aromatica*.

The analysis of the sunlight-exposed and sunlight-protected leaves as well as the results obtained on the feeding preference bioassays have shown that except for water contents, which were higher (t = 3.23; df = 10.0; P < 0.001) in the protected leaves and for soluble carbohydrates contents, which were higher (t = 3.37; df = 10.0; P < 0.001) in the sunlight-exposed ones, the differences for the remaining factors evaluated were not statistically significant (Table 1). Thus, although the building of shelters had induced reduction in the concentration of soluble carbohydrates and increase in the water content in the inner leaves, these differences were not sufficient to influence the choice by the caterpillars.

The differences found for water content may be attributed to the fact that leaves under shading conditions transpire less than those exposed to light. Concerning nutritional quality of leaves, however, such differences are not important since there are experimental evidences that the performance of herbivorous caterpillars is only affected by water concentrations lower than 75% (Scriber & Slansky 1981).

Studying herbivory of some species of lepidopteran caterpillars of the Pyralidae and Ctenuchidae families on the sub-shrub *Psychotria horizontalis* Sw. in the forests of Barro Colorado, in Panama, Sagers (1992) observed that the shading caused by shelters on expanding young internal leaves cause decrease in the hardness and tannins concentrations, which determine the preference of caterpillars for those leaves.

Leaf hardness is generally caused by the arrangement of lignin in the secondary walls of plant cells. The lignin and tannins are phenolic compounds that function as quantitative defenses (Feeny 1976). The lignin links to cellulose and some given proteins and due to its physical resistance and durability turns plant tissues relatively indigestible by herbivores. The tannins are substances widely distributed among plant families (Mole 1993). They are involved in protecting the plant against dehydration, rotting and damages caused by microorganisms and animals, acting in the later ones by the association with soluble proteins and consequent formation of insoluble complexes that reduce the digestibility of attacked tissues (Bernays *et al.* 1989).

The majority of phenolic compounds such as lignins and tannins are synthesized through the shikimic acid metabolic pathway. This pathway starts from simple carbohydrates derived from photosynthesis and trough several reactions promote production of the aromatic amino-acids tryptophan, tyrosine and phenylalanine. Phenylalanine is converted to cynamic acid, which is the precursor of complex phenolic compounds (Haslam 1974). Therefore, as the chikimic acid pathway is directly related to photosynthetic paths, the high intensity of visible solar light is capable of inducing production of phenolic substances by increasing photosynthetic activities (Anderson & Kasperbauer 1973). However, according to Mann (1987) some paths of synthesis of secondary metabolites are activated during some particular growth and development stages or in periods of stresses caused by nutritional limitations or yet in response to attack of plant tissues by fungi, bacteria or herbivorous animals.

Thus, as in the majority of the tropical plants in which the expanding young leaves are more intensely attacked by leaf-eaters than the mature ones (Coley & Kursar 1996), *S. scitiorella* caterpillars build their shelters using only young leaves that are starting to expand.

The expanding young leaves use all the available resources and photosynthetic products in the processes of cellular multiplication and differentiation in such a way that during growth the majority of alternative pathways, including the chikimic acid pathway, present low activity.

Therefore, although the restriction of solar incidence on the inner leaves of the shelters might affect photosynthetic activity, causing reduction on the synthesis of soluble carbohydrates, this limitation does not alter the activity of the paths of metabolic synthesis of secondary substances, which were not yet fully activated.

Thus, the building of leaf shelters by *S. scitiorella* caterpillars does not represent an adaptation strategy of the insect to surpass chemical defenses without altering the nutritional quality of the *X. aromatica* leaves.

Nevertheless, as the herbivores demand more than a feeding resource from their host plants (Lawton 1983) the leaf shelter-building behavior in this species must have been selected in response to other selective pressures as for example desiccation and protection against predators and parasitoids.

Table 1. Means (± SE) of the physical and chemical characteristics of young leaves of *X. aromatica* sunlight-exposed and sunlight-protected by opaque-brown paper bags under field conditions and feeding preference of *S. scitiorella* for these two types of leaves, obtained from results of experiments carried out in the laboratory under environmental controlled conditions (25ºC temperature, 60 ± 10% RH, 14h photophase).

Physical and chemical characteristics and feeding preference	Sunlight-protected leaves (Bagged)	Sunlight-exposed leaves (Not bagged)
Specific leaf mass (mg/cm^2)	4.4 ± 0.61 a	4.8 ± 0.45 a
Water content (%)	76.7 ± 1.74 b	73.3 ± 2.00 c
Soluble carbohydrates (% dm)	4.0 ± 0.93 d	6.5 ± 1.61 e
Nitrogen (% dm)	2.4 ± 0.13 f	2.3 ± 0.09 f
Tannins (% dm)	2.7 ± 0.39 f	2.6 ± 0.29 f
Preference (# of caterpillars)	7.3 ± 2.08 g	8.0 ± 2.00 g

Values followed by the same letter in the columns are not statistically different by the t-Student test (P £ 0.05).
dm = dry mass

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