Biological aspects of *Periga circumstans* Walker, 1855 (Lepidoptera: Saturniidae: Hemileucinae) with larvae reared on khaki and mate-plant leaves

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Abstract

The goal of the present study was to investigate biological aspects of *Periga circumstans* Walker, 1855 (Lepidoptera: Saturniidae: Hemileucinae) whose larvae were fed on leaves of khaki-plant (*Diospyros khaki* Linnaeus – Ebenaceae) and Mate-plant (*Ilex paraguariensis* Saint Hilaire – Aquifoliaceae) leaves. The biological parameters were obtained from specimens kept under controlled conditions: temperature of 25 ± 1 °C, relative humidity of 70 ± 10%, and photoperiod of 12 hours. For each developmental stage, morphological and ethological parameters are described. The larvae passed through six instars with a growth average rate of 1.4 for each instar. The host plants influenced significantly only the total duration of the larval phase, which was prolonged for larvae fed on khaki-plant leaves. Several aspects related to the morphology and the ethology of *P. circumstans* are similar to those described for *Lonomia obliqua* Walker, 1855.

Keywords: erucism, life cycle, morphology, medical importance.

Aspectos biológicos de *Periga circumstans* Walker, 1855 (Lepidoptera: Saturniidae: Hemileucinae) com larvas criadas em folhas de caquizeiro e erva-mate

Resumo

O presente estudo objetivou investigar aspectos biológicos de *Periga circumstans* Walker, 1855 (Lepidoptera: Saturniidae: Hemileucinae) cujas larvas foram alimentadas com folhas de caquizeiro (*Diospyros khaki* Linnaeus - Ebenaceae) e de erva-mate (*Ilex paraguariensis* Saint Hilaire - Aquifoliaceae). Os parâmetros biológicos foram obtidos de exemplares mantidos em condições controladas: temperatura de 25 ± 1 °C, umidade relativa de 70 ± 10%, e fotoperíodo de 12 horas. Para cada estágio de desenvolvimento são descritos parâmetros morfológicos e etológicos. As larvas passaram por seis instares larvais e apresentaram uma razão média de crescimento de 1,4. As plantas hospedeiras influenciaram significativamente apenas o período larval, que foi mais prolongado nas larvas que se alimentaram de folhas de caquizeiro. Diversos aspectos relacionados com a morfologia e etologia de *P. circumstans* são similares aos descritos para *Lonomia obliqua* Walker, 1855.

Palavras-chave: erucismo, ciclo de vida, morfologia, importância médica.

1. Introduction

Periga Walker, 1855, composed of 12 species, is phylogenetically close to *Lonomia* Walker, 1855 (Lepidoptera: Saturniidae: Hemileucinae). This taxonomic proximity has led some authors to consider *Periga* to be a subgenus of *Lonomia* (e.g. Gallo et al., 2002; Lima and Racca Filho, 1996). Larvae of *Lonomia* are important health hazards because they can inoculate hemorrhagic substances that are fatal to humans, besides being urticating (Lemaire, 2002).

According to Lemaire (2002), *Periga circumstans* Walker, 1855 occurs in southwestern and southern Brazil and adjacent areas in Paraguay, having been recorded for the Brazilian states of Mato Grosso, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, and Santa Catarina. There are also some records for the state of Rio Grande do Sul, Brazil (Biezanko, 1986; Corseuil et al., 2002; Nunes et al., 2003; Specht et al., 2005) and one for Uruguay (Biezanko et al., 1957).

Although not considered to be a Lepidopteran species of medical importance in Brazil (Moraes, 2003), *P. circumstans* commonly causes erucism in workmen during the coffee harvest. Larvae find their way on to the skin of workmen by adhering to the harvesting cloth, falling into the sieves during fanning, or by holding on to falling coffee beans (Gallo et al., 2002).

Periga circumstans is considered a secondary pest of coffee (Gallo et al., 2002; Cavalcante, 1983; Parra et al., 1992; Zucchi et al., 1993; Lima and Racca Filho, 1996; AGROFIT, 2009), and have also been found on two other hosts, khaki-plant and ligustro (Specht et al., 2005). The only information in the literature about this species refers to systematic aspects, occurrence and characterisation of the adults (Lemaire, 2002), as well as the record of a gynandromorph (Moraes, 2005). Even though we are aware of D'Antonio's dissertation (1983, unpublished data), we were not able to compare his results with ours, because his experiment was conducted under different conditions, and on a different host plant (Coffee-plant).

In this work we describe the biology of *P. circumstans* reared on khaki and mate-plants. Additionally, we describe morphological features of the immature stages, which can be used to broaden our understanding of the taxonomy of the group.

2. Material and Methods

The duration and survival of the developmental stages, width of the larval cephalic capsule and the biotic potential of *P. circumstans* were obtained from individuals reared at the Laboratório de Biologia, Campus Universitário da Região dos Vinhedos, Universidade de Caxias do Sul, Rio Grande do Sul, Brazil. The environmental room was maintained at 25 ± 1 °C, 70 ± 10% of RH and 12 hours photophase. Observations were carried out daily. We started the rearing experiment in November, 2006, when we collected a total of 236 second-instar larvae from a branch of a khaki-plant in Bento Gonçalves (also in the state of Rio Grande do Sul, Brazil). Data were obtained from the second and third generations. Second-generation larvae were reared on khaki-plant leaves. Given our observation that several wild larvae were feeding on mate-plants, we offered this host plant to the third generation. The mate-plant was promptly accepted by the newly born larvae.

Morphological measurements were obtained with a digital caliper and a stereoscopic microscope with micrometric ocular with a precision of a hundredth of a millimeter.

Egg phase. Each egg mass was individualised on a Petri dish layered with filter-paper moistened in distilled water, where it remained until the larvae hatched. We measured the largest diameter and height of each egg and used this information to describe the external morphology.

2.1. Larval phase

Larvae from each batch were kept in screened cages until they pupated. The cages were 80 cm high, 50 cm wide and 42 cm deep. Larval food, provided daily, consisted of branches of the host plants which we maintained fresh and turgid by immersing their bases in 50 ml Erlenmeyers with distilled water. Daily, during cage maintenance activities, the cephalic capsules were collected and later measured. As observed for most Hemileucinae (e.g. Lemaire, 2002; Specht et al., 2006a, 2006b, 2007a, 2007b, 2009, 2010), larvae of *P. circumstans*, particularly in the first instar, have a gregarious habit. For this reason, it was not possible to rear each larva individually to estimate the duration of the instars separately. Consequently, the duration of the larval phase was analysed by individualising each larva at the beginning of the pre-pupal period.

We considered the pre-pupal period as starting when the larva stops feeding and seeks shelter among the leaves and other dry plant structures at the bottom of the cage. At this point, each individual was transferred to a 500 ml glass recipient layered in filter-paper moistened with distilled water at the bottom, and covered with PVC wrap at the top.

2.2. Pupal phase

On the second day after metamorphosis, when the skin was harder, we removed the pupae for measurement and sexing, following Butt and Cantu (1962).

Adult phase. After the emergence, the adults were sacrificed in a freezer at –17 °C, stretched with appropriate extensors (Winter Junior, 2000) and incorporated as vouchers in the collection of the Biology Laboratory (CUCS), Campus Universitário da Região dos Vinhedos, Universidade de Caxias do Sul, state of Rio Grande do Sul, Brazil (CARVI-UCS).

2.3. Data analysis

The morphometric parameters were analysed using descriptive statistics with the calculation of means and standard deviations. The means were compared using the *t*-test, with the confidence level set at 95%. The biotic potential (BP) was estimated to determine the intrinsic capacity of the species to generate descendants under different rearing conditions, with environmental resistance assumed to be absent (Silveira Neto et al., 1976). The biotic potential, as defined by the latter authors, corresponds to the sexual proportion [nº of females/(number of females + number of males)] multiplied by the number of descendants and elevated to the number of generations per year.

Additional information was gathered about the host plants from observations carried out in the native woods of the Campus Universitário in Bento Gonçalves, and from larvae collected by students and the general public and sent to the Entomology Laboratory of the Universidade de Passo Fundo. These larvae were also reared in the laboratory, and were fed the leaves of the host plant on which they had been collected. Throughout this paper, plant names and nomenclature follow Backes and Nardino (2001), and common, scientific and family names, as well as references, are given.

3. Results and Discussion

The duration of the life cycle of *P. circumstans* populations reared on different hosts differed by approximately five days. This difference was most significant ($p > 0.05$) in the larval phase (excluding the pre-pupae period). However, the percent duration of each developmental phase did not differ between the two the treatments (Table 1).

The eggs (Figure 1) have an ellipsoid, almost round shape, with greatest diameter of 1.487 ± 0.032 mm and a slightly flattened micropilar region. They can be found grouped on several lines, or isolated, attached to the substrate by their inferior pole. After being laid, the eggs have a light-green uniform colour. After a few days, the corium of the fertile eggs acquires a vitreous and translucent aspect, allowing the observation of embryonic development. The sterile eggs, on the other hand, take on a yellowish colour and dry out. In all aspects listed above, the eggs of *P. circumstans* are similar to those of of *L. obliqua,* which are slightly larger and longer (Lorini, 1999, 2008; Lorini and Corseuil, 2001). The embryonic development lasted about 17 days at 22 °C and 13 days at 25 °C (Table 1), similar to what has been observed for other Hemileucinae (Lorini, 1999, 2008; Specht et al., 2006b, 2007).

The relatively high viability of the eggs of *P. circumstans* (Table 2) has also been described for several representatives of Hemileucinae (Lemaire, 2002), including *L. obliqua* (Lorini et al., 2004).

The larvae have accentuated gregarism. In our study, they were always seen together, preferably on the dorsal face of the leaves, and always moved on a single row. They were active and fed at night; during the day, they took shelter in dark places beneath old leaves, in ground debris, among the branches made available for nourishment or behind the glass where these branches were being kept. These cryptic and nocturnal habits, observed in the laboratory, are consistent with reports of larvae observed in coffee plantations. In the latter, the larvae remain still and together amongst old leaves and debris, at the base of the coffee-plant during the day, feeding at night. Larvae of *P. circumstans* have been observed attacking the yerba mate plant and consuming the leaves of the pointer completely, in a similar way as described for coffee crop infestations (Gallo et al., 2002).

The larvae are sensitive to external stimuli, especially variations in light, acoustic, and tactile stimuli. Larvae in the same cage displayed a defensive behaviour in response to any minor movement or alteration in luminosity during maintenance, when food items were changed. On these occasions, several individuals would loosen up from the host plant and contort their bodies aggressively, probably to intimidate the aggressor and to facilitate inoculation of urticating substances present in their urticating setae. Because individuals in the aggregations are usually very close to one another, larvae sometimes hurt themselves by rubbing against the bristles on the integument of neighbouring larvae, causing their haemolymph to spill. In addition, after the behavioural reaction is triggered, the larvae regurgitate a greenish substance and disperse throughout the cage, impairing their ability to return to their original state of aggregation. This aggressive behaviour, followed by dispersion, could be responsible for the low larval viability (Table 2) in comparison with previous reports for *A. illustris* (91.6 %; Specht et al., 2006a), *A. naranja* (97.3%) (Specht et al., 2007) and *Molippa cruenta* (Walker, 1855) (91,6%) (Specht et al., 2010). We strived to interfere as little as possible with the larvae during the maintenance activities, at least until the pre-pupal period. Aggressive behaviour in the face of several

Table 1. Mean duration (days) plus standard errors and proportional percentage of the total duration of development stages of *P. circumstans* reared with khaki-plant and mate-plant leaves (25 ± 1 °C; 70 ± 10% UR; 12 hours photophase).

Stages	Khaki-plant			Mate-plant		
	N	Mean ± error	%	N	Mean ± error	%
Egg	182	13.46 ± 0.54	12.71	56	13.23 ± 0.92	13.19
Larvae	132	57.53 ± 10.35	54.38	40	53.80 ± 13.56	53.66
Pre-pupae	111	3.48 ± 1.11	3.29	32	3.29 ± 2.46	3.28
Pupae	94	25.79 ± 2.08	24.37	31	24.46 ± 4.80	24.40
Female	10	5.43 ± 1.89		7	5.23 ± 2.03	
Male	10	5.67 ± 1.36		8	5.74 ± 2.54	
Longevity		5.56 ± 1.26	5.25		5.49 ± 1.88	5.47
Total		105.82	100.00		100.27	100.00

Figures 1-8. *Periga circumstans* Walker: 1) eggs batch; 2-3) last instar larvae; 4) lateral view of pupae; 5) dorsal view of pupae; 6) ventral view of pupae; 7) female; 8) male.

Table 2. Survival (%) in each development stage of *P. circumstans* reared with leaves of khaki-plant and mate-plant (25 ± 1 °C; 70 ± 10% UR; 12 hours photophase).

Phase	**Khaki-plant**		**Mate-plant**	
	N	**%**	**N**	**%**
Egg	204	89.22	61	91.80
Larvae	182	51.65	56	55.36
Pupae	94	86.17	31	80.65
Total		39.70		40.99

stimuli has already been described for other Hemileucinae in *Hylesia* (e.g. Hodge, 1972; Specht et al., 2006a, 2007a) and *Automeris* (Specht et al., 2006b, 2007b) and is probably also responsible for the difficulties encountered by researchers who have tried to breed *L. obliqua* in the laboratory (Lorini, 1999; Lorini et al., 2007).

The larvae go through six instars, with a growth average rate of approximately 1.4 (Table 3) for the cephalic capsules gathered after ecdysis, which is in agreement with the predictions of the Dyar (1890) rule. The widths of the head capsules (Table 3) of larvae fed on different host plants did not differ significantly from one another, indicating that the host plant and temperature did not influence the final size of the individuals, even though it interfered with the duration of the life cycle (Table 1).

From the first instar on, the larvae have a smooth cephalic capsule with a typically white front. During development, the light-hazelnut uniform colour of the integument, observed right after eclosion, becomes progressively darker; the mid-dorsal, sub-dorsal, lateral and subspiracular lines become conspicuous, especially after the fourth instar.

In the last instar (Figures 2 and 4), the larvae are about 45 mm long; they have a dark-hazelnut head, with a clear, almost white front; dark-hazelnut integument, lightly grayed by numerous small white dotted spots. The dorsal scolus of the prothorax, mesothorax, and the ninth abdominal segment are longer than the others, a larval feature very common among Hemileucinae (Lemaire, 2002); the scoli are slightly clearer than the integument, but their urticating setae are darker. The lateral scoli of *P. circumstans* are longer than the dorsal and sub-dorsal ones, especially on the abdomen, a distinctive characteristic of this species when compared with other representatives of the subfamily (Lemaire, 2002). The medium-dorsal line is dark, continuous, with the borders a little lighter than the rest of the integument; part of the mesothorax extends continuously up to the ninth abdominal segment. Likewise, the sub-dorsal line, limited to the base of the dorsal scolus**,** is also dark, interrupted at each segment; in some specimens the sub-dorsal line is quite evident, while in others it can pass unnoticed. The lateral and subspiracular lines are thin, yellow-orange in colour, uninterrupted, and situated very close to the lateral and subspiracular scoli**,** respectively. The spiracles are white and relatively small. Below the subspiracular line, the colouration of the integument becomes clearer with rosy shades. The thoracic legs and the larvopods are hazelnut in colour.

Seven host plants have been listed for *P. circumstans*, all in different botanical families (Table 4). As with other Hemileucinae, larvae of this species can feed on a wide variety of hosts, indicating that the species has the capacity to adapt to different plant groups (Lemaire, 2002).

In the pre-pupal period, when the larvae stop feeding and seek a place to transform into pupa, they become darker and smaller. In our study, the larvae sought places amongst the foliage and soil to form a cell without using silk, in a manner similar to the representatives of *Lonomia* and some *Dirphia* Hübner, [1819] (Saturniidae) (Lemaire, 2002).

The prolonged larval period, which lasts more than 50% of the entire development (Table 1), is characteristic of these Lepidopterans, which count on several strategies for protection against predators, such as mimetic colouring, cryptic habits, gregarious behaviour and structures that produce and inoculate urticating substances (Bernays and Janzen, 1988).

The pupae are very sclerotised and show a dark-hazelnut colour, with clearer regions especially on the abdominal segments (Figures 4-6). The latter have small sclerotised projections arranged in crowns that allow the pupae to bury

Table 3. Mean plus standard errors of the largest width of cephalic capsules of *P. circumstans*, in each instar, fed with leaves of khaki-plant and mate-plant (25 ± 1 °C; 70 ± 10% UR; 12 hours photophase).

Instar	Khaki-plant (n = 15)		Mate-plant (n = 15)	
	Width of cephalic capsule	Ratio	Width of cephalic capsule	Ratio
I	0.823 ± 0.044	-	0.818 ± 0.041	-
II	1.147 ± 0.082	1.393	1.126 ± 0.071	1.376
III	1.593 ± 0.090	1.389	1.599 ± 0.096	1.420
IV	2.255 ± 0.132	1.415	2.275 ± 0.140	1.423
V	3.217 ± 0.189	1.426	3.237 ± 0.180	1.423
VI	4.497 ± 0.161	1.398	4.537 ± 0.137	1.402
Mean		1.404		1.409

Table 4. Host plants of *P. circumstans* larvae, from previous references and this work.

Common name	Scientific name	Botanical family	Refs.
Japanese prunes	*Eriobotrya japonica* Lindl.	Rosaceae	3
Coffee-plant	*Coffea arabica* Linnaeus	Rubiaceae	1
Khaki-plant	*Diospyros khaki* Linnaeus	Ebenaceae	2
Mate-plant	*Ilex paraguariensis* Saint Hilaire	Aquifoliaceae	3
Guava	*Psidium guajava* Linnaeus	Myrtaceae	3
“Ipê-roxo”	*Tabebuia heptaphylla* (Velll.) Toledo	Bignoniaceae	3
Ligustro	*Ligustrum vulgare* Linnaeus	Oleaceae	2

1) Gallo et al. (2002); 2) Specht et al. (2005); 3) Observed in the present work.

Table 5. Mean plus standard errors of the length and width of pupae of *P. circumstans* reared with leaves of khaki-plant and mate-plant (25 ± 1 °C; 70 ± 10% UR; 12 hours photophase).

		N	Khaki-plant	Mate-plant	Hosts
Length	Female	12	25.023 ± 1.170	25.041 ± 0.997	ns
	Male	16	22.215 ± 0.987	22.195 ± 0.781	ns
Width	Female	11	8.585 ± 0.553	8.458 ± 0.579	ns
	Male	14	7.790 ± 0.289	7.813 ± 0.419	ns
			*	*	

The differences between the sizes of the pupae, according to the host plant, are expressed in the last column and between the genders on the line. *Indicates significant difference by the *t*-test at 95% significance, considering the different variances, ns – not significant.

Table 6. Comparative sex ratio, fecundity, number of survivals by female, number of annual generations and biotic potential estimated for *P. circumstans* reared with leaves of khaki-plant and mate-plant (25 ± 1 °C; 70 ± 10% UR; 12 hours photophase).

	Khaki-plant	Mate-plant
Sex ratio	0.47	0.45
Fecundity	132.56	143.87
Numbers of survivals	52.60	58.97
Number of annual generations	3.45	3.64
Biotic potential	63,840.82	152,417.59

themselves in debris or soft sand. The cremaster is long and pointed. The great mobility of the abdomen and the absence of hooks on the cremaster indicate that the pupae of this Lepidoptera are not restricted to the place chosen for metamorphosis, as is the case in other Hemileucinae (Lemaire, 2002).

In all treatments, the pupal stage corresponded to little more than 24% of the ontogenetic cycle (Table 1), although the average duration of this phase was 24.46 days for larvae fed with mate and 25.79 days for those larvae fed with khaki-plant. The female pupae were larger than the males (Table 5). This difference, previously reported for *P. circumstans* (Lemaire, 2002), is common among Hemileucinae and is part of the pronounced sexual dimorphism displayed by representatives of this group. However, there were no significant differences between the pupae of individuals fed on khaki and yerba mate plants (Table 5). These results demonstrate that the host plant can change the duration of the development, especially in the larval phase, when the insects are growing; however, the final size of the individuals is the same.

Likewise, the pupae showed reduced viability (Table 2) in comparison with previous reports for *A. illustris* (98.7%) (Specht et al., 2006a), *A. naranja* (95.1%) (Specht et al., 2007) and *M. cruenta* (100%) (Specht et al., 2010), but superior to the viability of *L. obliqua* (71.4%) (Lorini et al., 2007).

Adult females (Figure 7) displayed a significantly greater mean length for the costal margin of the front wing (37.487 ± 0.983 mm; n = 15) when compared with the males (Figure 8) (28.132 ± 0.879 mm; n = 15). The size of individuals bred in the laboratory is within the interval mentioned by Lemaire (2002), with a wingspan of 33-42 mm for females and 25-33 mm for males.

In our data, adult males lived longer than adult females ($p < 0.05$); however, adults of *P. circumstans*, as in the case of other Hemileucinae, have a lifespan of less than one week, which represents about 5% of the total cycle (Table 1). This short duration is presumably related to the fact that Hemileucinae do not feed during the adult phase (Lemaire, 2002).

The fecundity of *P. circumstans*, when compared with that of some representatives of *Hylesia* (Santos et al., 1988, 1996; Specht et al., 2006a) and *Automeris* (Specht et al., 2006b), was low in all treatments (Table 6). It was, however, similar to the fecundity of *L. obliqua* (Lorini et al., 2004), a species that *P. circumstans* shares several biological, morphological and ethologic aspects with. It is important to point out that the aggregation that was used in the laboratorial breeding was composed of 236 larvae that most likely originated from a single posture, which corresponds to practically twice the number of eggs obtained in the laboratory for each female.

For a more accurate analysis of fertility, in future studies, we suggest the counting of the number of eggs present inside the abdomen of the females, as described for *H. nanus* (Santos et al., 1988, 1996), because adults do not feed and have a very short life span, and females already emerge with a pre-determined number of eggs to be laid (Lemaire, 2002).

The completion of the life cycle at 25 °C (Table 1) took approximately 100 days, which corresponds to nearly 3.5 annual generations, characterising the species as multivoltine, especially in warmer climates. It is clear that *P. circumstans* showed reduced overall survival (Table 2) when compared with other Hemileucinae bred in similar conditions. The survival rates we found, which varied from 39.70 to 40.99%, represent nearly half of those found for *A. illustris* (79.87%) (Specht et al., 2006), *A. naranja* (83.94 %) (Specht et al., 2007) and *M. cruenta* (90,5%) (Specht et al., 2010). This reduced viability is related mainly to the difficulties in rearing the larvae, which respond aggressively to several stimuli, similarly to what has been observed for *L. obliqua* in the laboratory (Lorini, 1999; Lorini et al., 2007). Thus, because *P. circumstans* is considered a secondary pest of the coffee-plant (Gallo et al., 2002; Cavalcante, 1983; Parra et al., 1992; Zucchi et al., 1993; Lima and Racca Filho, 1996; Brasil, 2009) we believe that the values regarding the biotic potential (Table 6) are underestimated, due particularly to the the low viability of the larval stage and underestimation of the true fertility.

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