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CROP PROTECTION

Validation of the sampling methodology for *Opsiphanes invirae* caterpillars on oil palm plantations in the Brazilian Amazon

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ABSTRACT: *This study validated the use of leaf 17 on the sampling of Opsiphanes invirae Hübner (Lepidoptera: Nymphalidae) through the quantification of caterpillar abundance on the different spirals leaves, in comparison to leaf 17, and, on the apical, intermediate and basal leaf regions. This study was performed in the state of Pará, between March-2014 and March-2015. Results confirmed that leaf 17 is the most adequate method for monitoring this defoliator pest.*
Key words: *Defoliator insects, Elaeis guineensis Jacquin (Arecaceae), monitoring, oil palm.*

Validação da metodologia de amostragem para lagartas de *Opsiphanes invirae* em plantios de palma de óleo na Amazônia brasileira

RESUMO: *Esta pesquisa validou a utilização da folha 17, nas amostragens de Opsiphanes invirae* Hübner (*Lepidoptera: Nymphalidae), através da quantificação da abundância de lagartas nas diferentes folhas que compõem os espirais em comparação com a folha 17, e, em diferentes regiões da folha (apical, intermediária e basal). O estudo foi realizado no Pará entre março de 2014 e março de 2015. Os resultados confirmam que a folha 17 é a mais adequada para ser utilizada no monitoramento desse desfolhador.*
Palavras-chave: *Desfolhadores, Elaeis guineensis Jacquin (Arecaceae), monitoramento, palma de óleo d.*

The state of Pará is the largest producer of oil palm (*Elaeis guineensis* Jacquin, *Arecaceae*) in Brazil (IBGE-SIDRA, 2017). Phytosanitary problems are still issues to be resolved, such as lepidopteran defoliating pests *Opsiphanes invirae* Hübner and *Brassolis sophorae* L. (*Lepidoptera*: *Nymphalidae*). Monitoring of *O. invirae* is a strategic tool for the Integrated Pest Management (IPM) of this insect and, due to common outbreaks, control measures are adopted based on the economic threshold (ET) of the caterpillars on leaf 17, i.e., 10 caterpillars/leaf.

Use of palm leaves in the monitoring of defoliating pests is a recurrent practice (GENTY et al., 1978; GONZÁLEZ et al., 2011). In oil palm, leaf emergence is regular and constant, in a spiral arrangement, with 8 leaves in a specific direction and 13 leaves in the opposite direction. In this way, it is possible to locate and classify the oil palm leaves, numbering them sequentially. For this, the youngest leaf, with recently opened leaflets, is located; the next leaf, in the next spiral, is the leaf 9 and the next, in the other spiral, the leaf 17 (Figure 1) (MÜLLER & ANDRADE, 2010).

Although the use of leaf 17 in the monitoring of *O. invirae* is a recurrent practice, there is no scientific evidence of the efficiency of this method since it was implemented, based on empiric use, on the monitoring of *O. cassina* (L.) (*Lepidoptera*: *Nymphalidae*) in Colombia. Thus, the current study proposed to validate the use of leaf 17 in the sampling of *O. invirae* in Brazil. We hypothesized that the abundance of *O. invirae* are higher in leaves located in the medium spiral - leaves 9-17 (Figure 1), independently of the leaf

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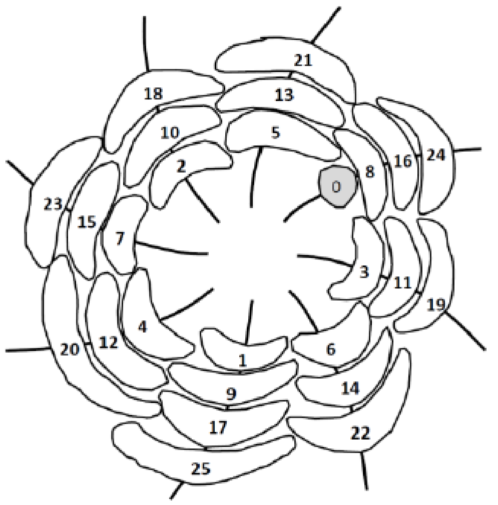

Figure 1 - Phyllotaxy of the palm tree, *Elaeis guineenses*. Source: FAIRHURST & HADFTER (2003).

sampled. Abundance of caterpillars on the leaves and on the apical, intermediate and basal regions was compared to leaf 17.

Our study was performed using a methodology similar to that used in Colombia, to select the lines and quantity of plants sampled, but with small adaptations used by the technicians of the studied plantations. The study was carried out between March 2014 and March 2015 in commercial plantations in the municipality of Tailândia-PA. Samplings were done in the months of pest incidence: 1st sampling 03/2014, 2nd 04/2014, 3rd 06/2014, 4th 07/2014, 5th 09/2014, 6th 11/2014, 7th 01/2015 and 8th 03/2015.

Oil palm trees were randomly selected, with the Excel® 97-2003 software, always excluding the first and last palms. Twenty-five leaves (leaves 1-25) were cut from each palm and the number of caterpillars on them was counted. The choice for such leaves occurred because the technicians reported the occurrence of *O. invirae* on all these leaves. After locating the "leaf arrow", all the other leaves were counted, following the phyllotaxy of the palm tree (Figure 1). Leaves were divided in three regions: apical (leaflet pairs from 1 to 50), intermediate (from 51 to 101), and basal (from 102 and onward) (RHAINDS et al., 1996).

Data was submitted to a variance analysis with the Scott & Knott test ($p<0.05$). Initially, all data was analyzed (whole sampling period) to determine the leaf with highest caterpillar abundance. Finally, caterpillar abundance was compared within leaf regions (apical, intermediate and basal). All analyses were run on the statistical program AgroEstat.

Opsiphanes invirae caterpillars varied on the different leaves ($F=38.17$; $df=24$; $P<0.0001$), from 2.48±1.70 caterpillars on leaf 01, to 32.44±1.59 on leaf 17. The highest numbers of caterpillars were reported in leaves located in the middle and upper spirals (leaves 9-25), disagreeing with the hypothesis that the highest numbers would occur only in leaves located in the median spiral (leaves 9-17). It was also verified that the caterpillars were not abundant in the new leaves from the inferior spiral (1-8) (Figure 1 and Table 1). Caterpillars of *Acharia* spp. showed

Table 1 - Number of *O. invirae* (Lepidoptera: Nymphalidae) caterpillars per leaf of *Elaeis guineenses*, between March 2014 and March 2015, in commercial plantations of Pará state.

Leaves	Mean	Variance	SD	MSE	
L_1	2.48	926.72	30.44	1.70	g
L_2	7.67	506.29	22.50	1.26	f
L_3	11.74	370.89	19.26	1.08	e
L_4	12.47	339.24	18.42	1.03	e
L_5	14.39	283.85	16.85	0.94	e
L_6	15.96	237.27	15.40	0.86	d
L_7	17.77	418.39	20.45	1.14	d
L_8	20.38	430.07	20.74	1.16	c
L_9	21.49	250.23	15.82	0.88	c
L_{10}	22.96	176.01	13.27	0.74	c
L_{23}	23.26	414.13	20.35	1.14	c
L_{24}	23.46	330.78	18.19	1.02	c
L_{25}	24.16	275.81	16.61	0.93	c
L_{21}	24.88	467.69	21.63	1.21	c
L_{12}	25.70	330.44	18.18	1.02	b
L_{18}	26.23	313.44	17.70	0.99	b
L_{22}	26.49	356.03	18.87	1.05	b
L_{20}	26.73	270.31	16.44	0.92	b
L_{11}	27.02	542.48	23.29	1.30	b
L_{19}	27.27	368.63	19.20	1.07	b
L_{13}	27.48	618.89	24.88	1.39	b
L_{14}	27.99	413.74	20.34	1.14	b
L_{16}	30.93	821.87	28.67	1.60	a
L_{15}	31.17	1304.92	36.12	2.02	a
L_{17}	32.44	807.21	28.41	1.59	a

Means followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability. Where, SD= standard deviation; MSE= mean standard error.

a preference for leaves 1-12 (youngest, upper layer), while *Euprosterna eleasa* Dyar and *Euclea* sp. (*Lepidoptera: Limacodidae*) preferred leaves 13-26 (VERA, 2000). The preference of oil palm defoliators for old leaves has been reported in other studies (HOWARD, 2001). Low abundance of caterpillars in young leaves is likely related to the nutritional variation between young and old palm leaves (RODRIGUES et al., 2006). In addition, it is believed that younger leaves are less attractive to the caterpillars due to the synthesis of defensive secondary metabolites. These metabolites can be produced in higher amounts in younger leaves, preventing the attack of these herbivores (HARTMANN, 1996). Since the current study did not aim to evaluate the production of primary and secondary metabolites by oil palm trees, we suggested that further studies be carried out to test the above hypothesis.

Although, different leaves (leaves 9-25) harbor high abundance of caterpillars, the efficiency of leaf 17 in monitoring *O. invirae* was confirmed since that leaf showed the highest abundance of caterpillars (Table 1). Use of different leaves to monitor palm tree defoliators is a recurring practice. There are recommendations for using leaves 9, 17, and 25 for monitoring *O. cassina* (GENTY et al., 1978; GONZÁLEZ et al., 2011); leaf 25 for *Euclea diversa* Druce, and *Talima straminea* Schaus (*Lepidoptera: Limacodidae)*; and leaves 17 and 33 for *Acharia* spp. Hübner and *E. elaeasa* (*Lepidoptera: Limacodidae*) (GENTY et al., 1978).

Our results showed that the abundance of *O. invirae* caterpillars varied depending on the leaf region sampled (F=24.28; df=959; P<0.0001), from 4.52 caterpillars on the apical region of leaf 9 to 15.74 on the intermediate region of leaf 16. The highest abundance was on leaflets 51 to 101, which corresponds to the intermediate region of the leaves. However, starting from leaf 21, the presence of caterpillars on the apical region was similar to what was observed on the intermediate region (Table 2), revealing that, as the leaves grow older, the caterpillars migrate from the intermediate region of the leaf towards the apical region, and then, both regions start to hold high numbers of caterpillars. *Automeris liberia* Cramer (*Lepidoptera: Saturniidae*), another defoliator observed in the plantations studied, was detected in higher abundance in leaflets closer to the leaf apex (ALDANA et al., 2010). The highest number of caterpillars of *O. cassina* has been reported for leaflet pairs 41 to 80, due to the lower levels of tanines and high levels of nitrogen in these leaflets (GONZÁLEZ et al., 2011). This difference in the number of *O. invirae* caterpillars between the different regions of the leaf reinforces the need to evaluate the primary and secondary metabolites produced in the palm trees in different phenological stages.

In conclusion, leaf 17 is an acceptable methodology for the sampling of *O. invirae* caterpillars, which occur in higher numbers in the intermediate region of the leaf. Although, other leaves present similar numbers of caterpillars, we recommend that leaf 17 continues to be used for monitoring this pest, due to the expertise of the field technicians (pest monitors) in locating this leaf on site.

Table 2 - Mean number of *O. invirae* (Lepidoptera: Nymphalidae) caterpillars in different regions of the oil palm leaves (*Elaeis guineenses*), in commercial plantations.

Leaflet regions	Leaves																
	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Apical	4.52b	5.39b	6.74b	7.07b	7.63b	7.8b	0.60b	9.02b	10.41b	9.23b	9.50b	9.59b	9.11a	10.19a	9.63a	9.82a	9.66b
Intermediate	11.83a	12.14a	14.13a	12.54a	13.61a	13.42a	15.33a	15.74a	15.63a	11.66a	13.23a	12.50a	11.43a	11.74a	10.12a	10.17a	11.23a
Basal	5.48b	5.36b	6.16b	5.87b	6.17b	6.80b	6.61b	5.98b	6.34c	4.89c	4.80c	4.71c	4.41b	4.55b	3.51b	3.53b	3.65c

Means followed by the same letter, in the column, do not differ from each other by the Scott-Knott test at 5% probability.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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