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Ethological aspects of *Helicoverpa armigera* in the reproductive phase of cotton

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ABSTRACT
Pest behavior studies are important to inform the periods when the pest is more exposed to pesticide. This study aimed to evaluate the movement and feeding of *Helicoverpa armigera* larvae in the first three instars during the reproductive phase of cotton. First, larval behavior was evaluated with a completely randomized design in a 3 × 5 factorial design with eight replications. The treatments were the instars versus behaviors (crawling, resting, waving, eating and dropping-off). In addition, another 3 × 6 factorial design with eight replications was performed to evaluate the plant region most preferred by the caterpillars. The treatments were the instars versus six parts of the plant (abaxial surface, adaxial surface, on floral bud, internal surface of bract, petiole, and terminal growth). Then, the period of the day when caterpillars are more active was also evaluated by a 3 × 2 factorial essay. The treatments were the instars versus periods of the day. Eating and resting are the most frequent behaviors for all instars. The plant regions preferred by the caterpillars for crawling are on the floral bud and the adaxial face of the leaf. There is no preferred period of the day for eating, resting, waving or dropping-off. Caterpillars (until the third instar) prefer to walk in the morning and other behaviors can occur at any period. Therefore, sprays should preferably occur in the morning, and the insecticide drops/spray should reach the flower buds and the adaxial surface of the leaves to contaminate the larvae.

Keywords: *Gossypium hirsutum*; Heliothinae; behavior.

INTRODUCTION

The cotton crop is attacked by pests in all its phenological stages (SANTOS, 2015), and a significant reduction in production can occur when control is not carried out at the right time and in the correct way. *Helicoverpa armigera* (Lepidoptera: Noctuidae) is a pest of global economic importance (BEHERE et al., 2013; TAY et al., 2013), with high biotic potential (SILVA et al., 2018) and was detected in Brazil in 2013 in soybean and cotton crops (CZEPAK et al., 2013).

Heliothinae moths prefer to feed and lay eggs on plants that are flowering and have nectar (CUNNINGHAM; ZALUCKI, 2014). Their attack in cotton can occur in the vegetative (PASCUA; PASCUA, 2002; RODRIGUES et al., 2019) and reproductive (LUONG et al., 2016; GOMES et al., 2017) phases. The movement of *H. armigera* caterpillars on the plant is influenced by several factors such as microclimate, odors, wax, trichomes and the presence of flowers (CRIBB et al., 2010; PERKINS et al., 2010).

It is known that the efficiency of applying phytosanitary products during the reproductive phase of a crop is affected by factors such as droplet size, spray volume, uniformity in spraying distribution, weather conditions, or even the failure to reach the biological target (CAVALIERI et al., 2015; COSTA et al., 2019). Thus, knowing the habits of a pest is essential so that control strategies are designed and achieve satisfactory results. Caterpillars in the first instars are more vulnerable to natural enemies and insecticides, and therefore knowing the locations of the plant which they prefer to feed on and what time of day they are most active will provide information to assist in the decision of the appropriate time to perform control methods through either biological or chemical agents. In this context, the objective of this study was to analyze the behavior of caterpillars in 1st, 2nd and 3rd instars of *H. armigera* in the cotton crop reproductive phase.

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MATERIAL AND METHODS

The experiments were conducted at the Entomology Laboratory of Embrapa Agrosilvipastoril, Sinop, Mato Grosso, Brazil. The caterpillars used were obtained from existing breeding maintained in the laboratory and fed with an artificial diet proposed by PARRA (2005). BRS 369 RF (non-Bt) cotton cultivar seeds were sown in 5 L pots and kept in a greenhouse; the cotton plant received the necessary cultural treatments for its development. Plants that were between B2 and B3 according to the MARUR scale (MARUR; RUANO, 2001) were used to evaluate the behavior in the reproductive phase. The experiment was conducted following the methodology of RODRIGUES et al. (2019) who studied the behavior of *H. armigera* in the cotton vegetative phase.

First, a completely randomized design was implemented with eight replications to study the behavior of the caterpillar in the cotton reproductive phase. The treatments were assumed in a 3 × 5 factorial scheme with the ages (1st, 2nd and 3w instars) combined with five behaviors (crawling, resting, waving, eating and dropping-off). Then, another 3 × 6 factorial treatment was implemented to identify the plant region preferred by the caterpillars, in which the treatments were the three ages combined with six parts of the plant (adaxial leaf surface, abaxial leaf surface, on the floral bud, inner surface of bract, petiole and pointer). Next, further treatments in a 3 × 2 factorial design were applied combining the three instars with two periods of the day (morning and night) in order to assess the period of the day when the caterpillars are most active. The data were transformed using $\sqrt{x + 0.5}$ to correct the heterogeneity of variances and submitted to analysis of variance by applying the Fischer (F)-test. As the F-test rejected the hypothesis of equality, the Scott-Knott multiple comparison test was chosen to be applied as it presents greater control of error rates. The analyzes were performed with the Sisvar version 5.7 program (FERREIRA, 2019).

RESULTS AND DISCUSSION

Helicoverpa armigera larvae showed no difference in behavior between instars, however, the instar versus behavior interaction was significant (F = 2.417; p < 0.0195; CV = 24.69%). First and second instar caterpillars were seen more frequently feeding and this behavior differed from the others (Fig. 1).

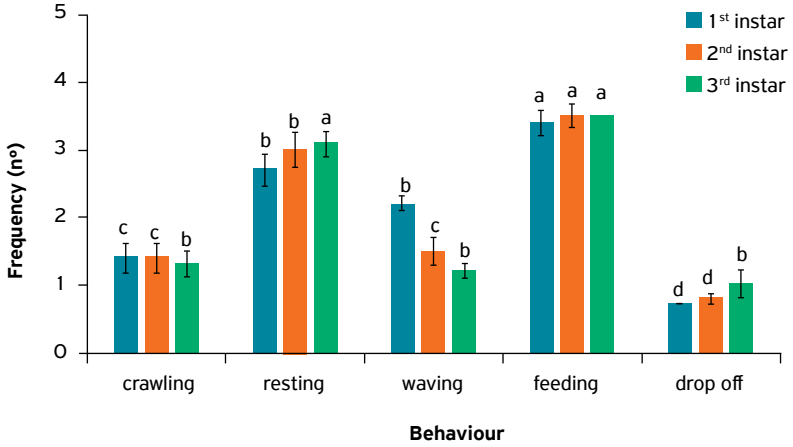

Figure 1. *Helicoverpa armigera* caterpillar behavior on cotton per instar. Average frequency followed by the same letter within each instar does not differ by the Scott–Knott test (p ≤ 0.05). The bar above each column is the standard error of the respective mean.

The resting and waving (head moving) behaviors were similar to each other for first-instar caterpillars, but differed from crawling and dropping-off. The resting frequency presented by the second-instar caterpillars differed from the other behaviors, but they crawled and waved in the same way. Third instar caterpillars fed and rested in a similar way, differing from crawling, waving and dropping-off, and these last three behaviors were similar to each other.

During data collection, it was found that the caterpillars in the first instar had the habit of eating and resting or eating and waving; while the second and third instar caterpillars had the habit of only eating and then resting. Resting is probably related to the digestive process of caterpillars. It appears that the two most frequent behaviors in all instars were eating and

resting, followed by crawling. JOHNSON; ZALUCKI (2005; 2007) and RODRIGUES et al. (2019) obtained similar results when they studied the behavior of *H. armigera* in the vegetative stage of mung bean (*Vigna radiata* L.) and cotton in the vegetative period (*Gossypium hirsutum* L.), respectively.

The analyzes carried out to identify the preferred region by *H. armigera* caterpillars within the crawling, resting and eating behaviors did not show significance between instars or in the interaction instar versus plant region. However, differences were detected between regions for these three behaviors (Fig. 2).

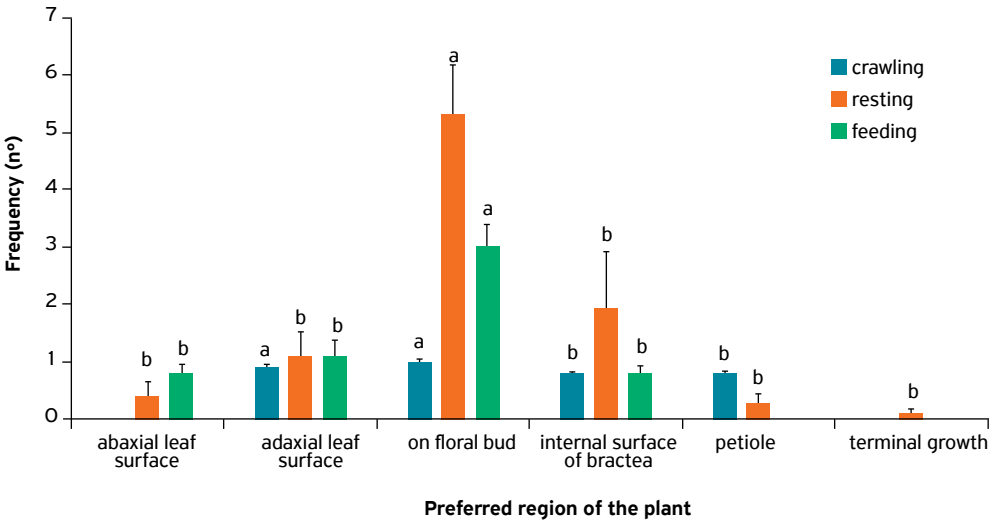

Figure 2. Preferred regions by *Helicoverpa armigera* caterpillars for crawling, resting and eating on cotton plants in the reproductive stage. Average frequency followed by the same lowercase letter within each behavior does not differ by the Scott–Knott test ($p \leq 0.05$). The bar above each column is the standard error of the respective mean.

The plant regions most preferred by the caterpillars for crawling were on the flower bud and on the adaxial face of the leaf ($F = 3.01$; $p < 0.03$; CV = 38.05%) (Fig. 2). Therefore, it can be inferred that the caterpillars are more exposed to the action of natural enemies and this exposure can be used for insecticides to reach them. These results coincide with those of RODRIGUES et al. (2019) who observed caterpillars of the first three instars crawling more frequently on the adaxial face of cotton leaves in the vegetative phase.

Regardless of the instar, the caterpillars prefer to rest ($F = 16.38$; d.f. = 126; $p < 0.0001$; CV = 57.9%) and eat ($F = 47.25$; d.f. = 84; $p < 0.001$; CV = 51.44%) more on the floral bud than on the other regions of the plant (Fig. 2). Flower buds were probably chosen for feeding because they are more nutritious, soft and do not have trichomes. PASCUA; PASCUA (2002) observed that small cotton flower buds were preferred by *H. armigera*, and YANG et al. (2008) found that first instar caterpillars present in flower buds on transgenic cotton were heavier than those located on leaflets. PERKINS et al. (2010) report that first-instar caterpillars were more frequently found in reproductive structures of pea plants.

The choice of flower bud in these behaviors may also be associated with the absence of trichomes. It is known that the presence of trichomes affects the feeding and movement of *H. armigera* caterpillars, since the caterpillars will spend more time and energy removing them and then start biting; a fact confirmed by SHELOMI et al. (2010) in neonate larvae of *H. armigera* which cut trichomes when feeding on tobacco and tomato plants.

Significant differences regarding waving behavior were detected in the instar versus region interaction for the first and second instars ($F = 2.08$; d.f. = 105; $p < 0.04$; CV = 39.01%) (Fig. 3).

The region preferred by first-instar caterpillars for waving was over the floral bud ($F = 9.20$; d.f. = 105; $p < 0.0001$). Meanwhile, the second instar caterpillars chose the adaxial face of the leaf and on the floral bud ($F = 2.98$; d.f. = 105; $p < 0.02$) (Fig. 3). The waving movement refers to searching and locating a suitable place for feeding, and the place where the caterpillars preferred to wave coincided with those chosen to eat. This corroborates what JONHSON; ZALUCKI (2007) reported for caterpillars first waving and looking for signs that indicate a nutritionally adequate place, and then starting to feed.

No difference was detected between the periods when analyzing the time of day preferred by *H. armigera* caterpillars to perform the five behaviors, except for the crawling behavior ($F = 6.14$; d.f. = 42; $p < 0.01$; CV = 41.58%), in which the caterpillars moved more in the morning (Fig. 4).

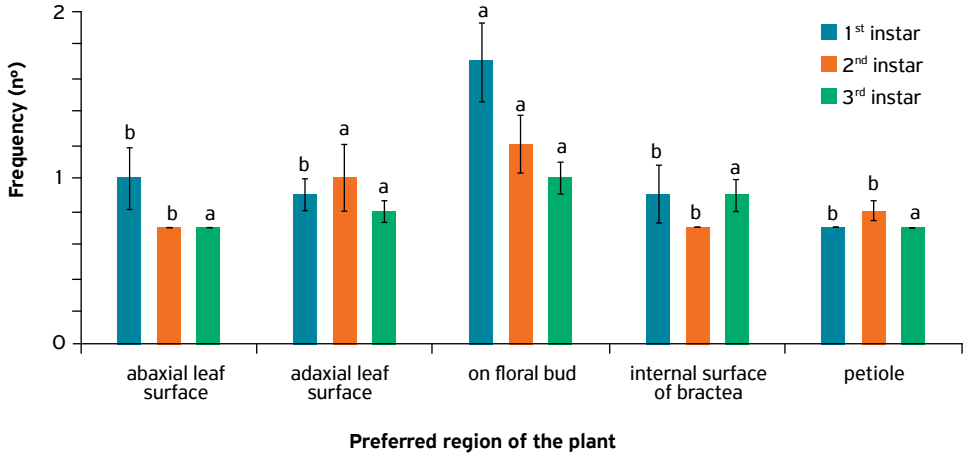

Figure 3. Plant regions chosen by *Helicoverpa armigera* caterpillars for waving on cotton plants in the reproductive stage. Average frequency followed by the same lowercase letter within the behavior does not differ by the Scott–Knott test ($p \leq 0.05$). The bar above each column is the standard error of the respective mean.

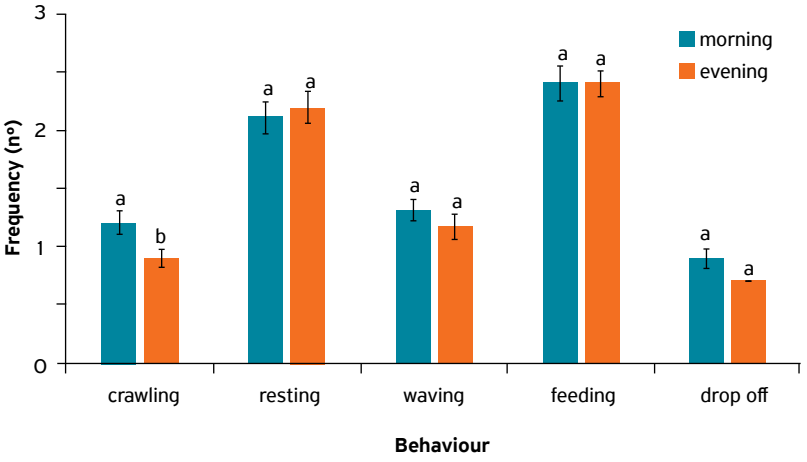

Figure 4. Time of day that *Helicoverpa armigera* caterpillars prefer to crawl, rest, wave, eat and drop-off on/from cotton plants in the reproductive phase. Average frequency followed by the same lowercase letter within the behavior does not differ by the Scott–Knott test ($p \leq 0.05$). The bar above each column is the standard error of the respective mean.

When a caterpillar moves, the possibility of finding food with higher nutritional quality, encountering natural enemies (JOHNSON; ZALUCKI, 2005), or being hit by insecticide drops increases. According to CAVALIERI et al. (2015) and COSTA et al. (2019), failure to reach the biological target is one of the factors which influences the efficiency of the insecticide applied in the reproductive phase of a crop. Therefore, identifying the time of day preferred by caterpillars to perform these behaviors is important when thinking about the ideal time to reach the target (caterpillar) via spraying. As they crawl more in the morning, this is a good indication that a good time to spray to reduce their population is in the morning. A similar result was obtained by RODRIGUES et al. (2019) when they studied the behavior of up to third instar *H. armigera* on cotton in the vegetative phase. Therefore, spraying to control *H. armigera* in the vegetative and reproductive phases of cotton should be done in the morning.

CONCLUSIONS

With the results obtained, it appears that first, second and third instar *H. armigera* caterpillars have eating and resting as the most frequent habits during the reproductive phase of cotton.

The cotton plant regions preferred by caterpillars for crawling are the adaxial face of the leaf and above the flower bud. There is no preferred time of day for *H. armigera* caterpillars to eat, rest, wave or drop-off.

Also, it is observed that *H. armigera* caterpillars up until the third instar prefer to crawl in the morning and the other behaviors can occur at any time. Thus, cotton producers will be able to manage these caterpillars with more precision using the information about the preferred regions and the period of the day in which they move.

AUTHORS' CONTRIBUTIONS

Conceptualization: Rodrigues, S.M.M.; Pitta, R.M. **Data curation:** Rodrigues, S.M.M. **Formal analysis:** Rodrigues, S.M.M.; Pitta, R.M. **Writing – original draft:** Rodrigues, S.M.M. **Writing – review & editing:** Rodrigues, S.M.M.; Pitta, R.M.

AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this study are included in this published article.

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CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest.

ETHICAL APPROVAL

Not applicable.

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REFERENCES

BEHERE, G.T.; TAY, W.T.; RUSSELL, D.A.; KRANTHI, K.R.; BATTERHAM, P. Population genetic structure of the cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in India as inferred from EPIC-PCR DNA Markers. *PLoS One*, San Francisco, v.8, n.1, e53448, 2013. https://doi.org/10.1371/journal.pone.0053448

CAVALIERI, J.D.; RAETANO, C.G.; MADUREIRA, R.P.; MOREIRA, L.Q. Spraying systems and traveling speed in the deposit and spectrum of droplets in cotton plant. *Revista Engenharia Agrícola*, Jaboticabal, v.35, n.6, p.1042-1052, 2015. https://doi.org/10.1590/1809-4430-Eng.Agric.v35n6p1042-1052/2015

COSTA, A.G.F.; MACIEL, C.D. de G.; ROGOSKI, T.; LIRA, A.J.S.; HELVIG, E.O. Spraying deposits using different nozzles and application volumes for pest management of cotton at reproductive stage. *Australian Journal of Crop Science*, Sidney, v.13, n.11, p.1770-1776, 2019. https://doi.org/10.21475/ajcs.19.13.11.p1549

CRIBB, B.W.; HANANN, J.; ZALUCKI, M.P.; PERKINS, L.E. Effects of plant microenvironment on movement of *Helicoverpa armigera* (Hübner) larvae and the relationship to a hierarchy of stimuli. *Arthropod-Plant Interactions*, Basel, v.4, p.165-173, 2010. https://doi.org/10.1007/s11829-010-9097-0

CUNNINGHAM, J.P.; ZALUCKI, M.P. Understanding Heliothine (Lepidoptera: Heliothinae) pests: what is a host plant? *Journal of Economic Entomology*, Oxford, v.107, n.3, p.881-896, 2014. https://doi.org/10.1603/EC14036

CZEPAK, C.; ALBERNAZ, K.C.; VIVAN, L.M.; GUIMARÃES, H.O.; CARVALHAIS, T. Primeiro registro de ocorrência de *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) no Brasil. *Pesquisa Agropecuária Tropica*l, Goiânia, v.43, n.1, p.110-113, 2013.

FERREIRA, D.F. SISVAR: a computer analysis system to fixed effects split plot type designs: Sisvar. *Brazilian Journal of Biometrics*, Lavras, v.37, n.4, p.529-535, 2019. https://doi.org/10.28951/rbb.v37i4.450

GOMES, E.S.; SANTOS, V.; ÁVILA, C.J. Biology and fertility life table of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in different hosts. *Entomological Science*, v. 20, p. 419-426, 2017. https://doi.org/10.1111/ens.12267

JOHNSON, M.L.; ZALUCKI, M.P. Feeding and foraging behaviour of a generalist caterpillar: are third instars just bigger versions of firsts? *Bulletin of Entomological Research*, Cambridge, v.97, n.1, p.81-88, 2007. https://doi.org/10.1017/S0007485307004750

JOHNSON, M.L.; ZALUCKI, M.P. Foraging behavior of *Helicoverpa armigera* first instar larvae on crop plants of different developmental stages. *Journal of Applied Entomology*, Berlin, v.129, n.5, p.239-245, 2005. https://doi.org/10.1111/j.1439-0418.2005.00958.x

LUONG, T.T.A.; DOWNES, S.J.; CRIBB, B.W.; PERKINS, L.E.; ZALUCKI, M.P. Oviposition site selection and survival of susceptible and resistant larvae of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on Bt and non-Bt cotton. *Bulletin of Entomological Research*, Cambridge, v.106, n.6, p.710-717, 2016. https://doi.org/10.1017/S0007485316000328

MARUR, C.J.; RUANO, O. A reference system for determination of developmental stages of upland cotton. *Revista de Oleaginosas e Fibrosas*, Campina Grande, v.5, n.2, p.313-317, 2001.

PARRA, J.R.P. *Técnicas de criação de insetos para programas de controle biológico*. Piracicaba: FEALQ, 2005. 134p.

PASCUA, L.T.; PASCUA, E.M. The distribution and movement of cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) larvae on cotton. *Philippine Journal of Science*, Manila, v.131, n.2, p.91-98, 2002.

PERKINS, L.E.; CRIBB, B.W.; HANAN, J.; ZALUCKI, M.P. The movement and distribution of *Helicoverpa armigera* (Hübner) larvae on pea plants is affected by egg placement and flowering. *Bulletin of Entomological Research*. Cambridge, v. 100, p. 591-598, 2010. https://doi.org/10.1017/S0007485309990654

RODRIGUES, S.M.M.; SANTOS, N.C.; PITTA, R.M.; CORASSA, J.N. Behavioral aspects of *Helicoverpa armigera* in the cotton vegetative phase. P*esquisa Agropecuária Tropical*, v.49, e55628, 2019. https://doi.org/10.1590/1983-40632019v4955628

SANTOS, W.J. Manejo das pragas do algodão com destaque para o cerrado brasileiro. *In:* FREIRE, E.C. (Ed.). *Algodão no Cerrado do Brasil*. 3. ed. Brasília: Positiva, 2015. chap.9, p.267-347.

SHELOMI, M.; PERKINS, L.E.; CRIBB, B.W.; ZALUCKI, M.P. Effects of leaf surfaces on first-instar *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) behavior. *Australian Journal of Entomology*, v.49, p.289-295, 2010. https://doi.org/10.1111/j.1440-6055.2010.00766.x

SILVA, I.F.; BALDIN, E.L. L.; SPECHT, A.; SOSA-GÓMEZ, D.R.; ROQUE-SPECHT, V.F.; MORANDO, R.; PAULA-MORAES, S.V. Biotic potential and life table of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) from three Brazilian regions. *Neotropical Entomology,* Goiânia, v.47, n.3, p.344-351, 2018. https://doi.org/10.1007/s13744-017-0529-8

TAY, W.T.; SORIA, M.F.; WALSH, T.; THOMAZONI, D.; SILVIE, P.; BEHERE, G.T.; ANDERSON, C.; DOWNES, S. A brave new world for an old world pest: *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Brazil. *PLoS One*, San Francisco, v.8, n.11, e80134, 2013. https://doi.org/10.1371/journal.pone.0080134

YANG, Y.; JONHSON, M.-L.; ZALUCKI, M.P. Possible effect of genetically modified cotton on foraging habits of early instar *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae. *Australian Journal of Entomology*, v.47, n.2, p.137-141, 2008. https://doi.org/10.1111/j.1440-6055.2008.00640.x

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