Management of Hylesia nanus (Lepidoptera: Saturniidae) on Eucalyptus (Myrtaceae) Plantations

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Management of *Hylesia nanus* (Lepidoptera: Saturniidae) on *Eucalyptus* (Myrtaceae) plantations

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Abstract

The management of *Hylesia nanus* Walker (Lepidoptera: Saturniidae), a defoliator of *Eucalyptus* species (Myrtales: Myrtaceae), was evaluated on the northern coast of Bahia State, Brazil, by performing 3 connected experiments: (1) monitoring of *H. nanus* moths by using light traps; (2) biological control of *H. nanus* pupae by releasing the endoparasitoid *Palmistichus elaeisis* Delvare & La Salle (Hymenoptera: Eulophidae); and (3) chemical control of *H. nanus* larvae with 1 spraying of bifenthrin + etilenoxi. The light traps effectively assisted the monitoring and control of *H. nanus* moths, with a mean population peak (± SE) of 801 ± 438 individuals collected per trap in a 23 d nocturnal period. *Palmistichus elaeisis* was recovered from the *H. nanus* pupae 96 h post release, with a parasitism rate of 23 ± 3%, indicating an ability to parasitize and contribute to insect suppression. Release of this parasitoid can be integrated with monitoring using light traps. Chemical control was highly effective against the last instar *H. nanus* larvae, with only 11 ± 3 larval survivors per tree trunk 24 h post spraying. Thus, the monitoring of moths by using light traps, control of pupae by releasing *P. elaeisis*, and chemical control of last instar larvae are methods that could be integrated into a management plan for *H. nanus*.

Key Words: chemical control; light trap; *Palmistichus elaeisis*; Tachinidae

Resumo

O manejo de *Hylesia nanus* Walker (Lepidoptera: Saturniidae), um desfolhador de espécies de *Eucalyptus* (Myrtales: Myrtaceae), foi avaliado no litoral norte do estado da Bahia, Brasil através da realização de três experimentos conectados: (1) monitoramento de mariposas de *H. nanus* utilizando armadilhas luminosas, (2) controle biológico de pupas de *H. nanus* liberando o endoparasitoide *Palmistichus elaeisis* Delvare & La Salle (Hymenoptera: Eulophidae) e (3) controle químico de larvas de *H. nanus* com uma pulverização de bifentrina + etilenoxi. As armadilhas luminosas assistiram de forma eficaz o monitoramento e controle de mariposas de *H. nanus*, com um pico populacional médio (± EP) de 801 ± 438 indivíduos coletados por armadilha em um período noturno de 23 d. *Palmistichus elaeisis* foi recuperado das pupas de *H. nanus* 96 h após a liberação com taxa de parasitismo de 23 ± 3%, indicando uma capacidade de parasitar e contribuir para o controle de insetos no campo. Além disso, este parasitoide pode ser integrado com o monitoramento com armadilhas luminosas. O controle químico foi altamente eficaz contra as larvas de último instar de *H. nanus*, com apenas 11 ± 3 larvas sobreviventes por caule 24 h após a pulverização. Assim, o monitoramento de mariposas utilizando armadilhas luminosas, controle de pupas utilizando *P. elaeisis* liberado e controle químico das larvas de último instar são métodos que podem ser integrados em um plano de manejo para *H. nanus*.

Palavras Chave: armadilha luminosa; controle químico; *Palmistichus elaeisis*; Tachinidae

Native plants of the family Myrtaceae are abundant in Brazil (Landrum & Kawasaki 1997). Larvae of *Hylesia* species (Lepidoptera: Saturniidae) defoliate exotic and native plants of this family including species of eucalyptus (*Eucalyptus*; Myrtales: Myrtaceae) cultivated in Brazil (Pereira et al. 2006, 2009a; Dall'Oglio et al. 2013). *Hylesia nanus* Walker larvae display trailing behavior on the trunk of host plants with the individuals following the path of the insect ahead (Santos et al. 1988). Normally, *Hylesia* larvae remain on the trunk during the day and move to the canopy, where they consume the foliage, at night (Santos et al. 1996).

The presence of Saturniidae adults is monitored with light traps on eucalyptus plantations to determine when the application of control methods becomes necessary to prevent the build-up of large pest populations (Dall'Oglio et al. 2013; Ribeiro et al. 2016; Zanuncio et al. 2016a). *Palmistichus elaeisis* Delvare & La Salle (Hymenoptera: Eulophidae) is a generalist endoparasitoid that is easily mass reared in the laboratory using pupae of alternative hosts such as mealworms, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). When released in the field, *P. elaeisis* has been reported to parasitize *Citioica anthonilis* (Herrich-Schäeffer) (Saturniidae: Ceratocampinae) on *Piptadenia gonoacantha* (Mart.) J. F. Macbr. (Fabales: Fabaceae) (Tavares et al. 2012) and *Dirphia moderata* Bouvier (Saturniidae: Hemileucinae) (Pereira et al. 2008) and *Hylesia* sp. pupae (Soares et al. 2009) on eucalyptus plantations. *Palmistichus elaeisis* is efficient at parasitizing *Hylesia* pupae

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during outbreaks of these pests on eucalyptus plantations (Soares et al. 2009). It is released in the field to control young Saturniidae pupae (Pereira et al. 2009b) though other stages are controlled by spraying insecticides.

The region at the northern coast of Bahia State produces the highest quality of cellulose, with 99% purity, from eucalyptus wood. Forest product companies should emphasize the use of monitoring and biological control and should use chemical control only when necessary to suppress pest populations using registered products (Zanuncio et al. 2016b). *Hylesia nanus* was the most severe lepidopteran defoliator of eucalyptus in the first half of 2015 in this region, and the severity and the time frame in which the defoliation happened stimulated this research. The objectives of this work were to monitor the adults of this species with light traps, to evaluate the pupal parasitism rate induced by release of *P. elaeisis*, and to assess the effectiveness of bifenthrin + etilenoxi treatment on larvae.

Materials and Methods

STUDY SITE

In Apr 2015, the monitoring system of the Copener Florestal Ltda. (Ltda. in Brazil is equivalent to LLC "Limited Liability Company" in the USA) in Alagoinhas, Bahia State, Brazil, reported the presence of last instar *H. nanus* larvae in a portion of stand 27 in the Encantado region, which has a total area of 34 ha (12.67°S, 38.40°W) and, in Mar and Apr 2015, the system indicated such larvae in a portion of stand 3 in the Água Santa region, which has a total area of 37 ha (12.50°S, 38.23°W). Young pupae were also reported in a portion of stand 3 in the Água Santa region in Mar and Apr 2015. Larvae of *H. nanus* had also been found in a portion of the Encantado region in Oct and Nov 2014. Trees on the eucalyptus plantations in the 2 regions were 3 m apart with 3 m between the rows. The infested clones (hybrids of the rose gums, *Eucalyptus urophylla* S. T. Blake, and *Eucalyptus grandis* W. Hill ex Maiden and *E. urophylla*; Myrtales: Myrtaceae) were about 5 yr old and had attained a height of about 25 m. Temperature and rainfall data were obtained from the nearest weather station, located approximately 10 km from the Encantado region and 8 km from the Água Santa region.

COLLECTION AND IDENTIFICATION OF THE ADULTS

About 200 larvae (100 larvae from both the Encantado and Água Santa regions) and 100 pupae from the Água Santa region were collected in the field, brought to the Entomology Laboratory of the Copener Florestal Ltda. in Alagoinhas, and transferred to wood and screen rearing cages (85 × 45 × 48 cm HWL) with branches of eucalyptus (hybrid of *E. urophylla*). The branches were replaced daily until the emergence of adults or natural enemies. This clone was chosen to feed the larvae because of its susceptibility to insect pests (Masson et al. 2009). The pupae developing from these larvae were placed in groups of 3 in 200 mL plastic containers with perforated plastic lids with mesh fabric hot glued over the openings. The pupae collected in the field were also placed in groups of 3 in similar 200 mL plastic containers. Three males and 3 females of the adults obtained from these pupae were killed with 70% ethanol, mounted, and sent to the Federal University of Paraná (UFPR) in Curitiba, Paraná State, Brazil, to be identified to species level.

BIOLOGICAL AND MORPHOLOGICAL OBSERVATIONS

This experiment was performed at 25 ± 2 °C, 70 ± 12% RH, and a 12:12 h L:D photoperiod. Eighty larvae were collected from both Encantado and Água Santa regions; the resulting total of 160 *H. nanus* adults, were placed in wood and screen rearing cages (85 × 45 × 48 cm HWL) with adults separated by region. These cages were lined with white paper as a substrate for oviposition, and a 50 mL vial of 10% sugar solution with the opening covered by a cotton swab was placed on the bottom of each cage. Eggs were removed from the cages and placed with a moistened cotton pad in Petri dishes (15 cm diameter, 1.5 cm height) until larvae hatched.

Five hundred 1st instar *H. nanus* larvae obtained from insects collected in the Encantado and Água Santa regions (250 larvae per region) were individually placed in 200 mL plastic containers with the opening covered using mesh fabric affixed with hot glue. The larvae were fed ad libitum with mature eucalyptus leaves from a hybrid of *E. urophylla*. The leaves were taken from the middle-third canopy of 5 yr old trees and changed twice daily until the insects reached pre-pupa stage. Resulting pupae were transferred to plastic containers as previously described until adults emerged. The containers were cleaned as necessary.

The average duration of the pre-pupal and pupal stages and the length of female and male pupae from each region were evaluated. The sex of pupae was identified based on the external morphological characteristics of Lepidoptera (Tavares et al. 2013a). The emergence rate, sex ratio, and adult wing span were also determined.

MONITORING THE ADULTS IN THE ÁGUA SANTA REGION

In the Água Santa region, 5 "Luiz de Queiroz" model light traps, each with a 12 V Moura® battery, a 20 W white fluorescent Philips® lamp, and a 20 L capacity nylon bag to hold the insects collected, were installed 2 m above the ground in the interior of the plantation, where the outbreak was severe (Dall'Oglio et al. 2013). This light trap is effective for collecting moths of this group as demonstrated in the collection of *Hylesia nigricans* Berg (Lepidoptera: Saturniidae) in Rio Grande do Sul State, Brazil (Iserhard et al. 2007). These traps were installed 1 d after collecting the larvae and pupae in the field and were maintained until the number of adults collected fell below the damage level determined by the Copener Florestal Ltda.'s monitoring system: an average of 125 *H. nanus* adults collected from 7 p.m. to 5 a.m. in 5 light traps. The traps were left in the field for 10 h per day, and insects were removed from the bags daily. The total weight of 100 adults captured per trap was used to calculate the mean weight; mean weight of moths is routinely used by the company to estimate the number of adult moths captured by the light traps per day.

BIOLOGICAL CONTROL AND NATURAL PARASITISM OF THE PUPAE IN THE ÁGUA SANTA REGION

About 1,500 females of the *H. nanus* pupae endoparasitoid, *P. elaeisis*, mated previously for 48 h and fed ad libitum with pure honey, were reared using up to 2-d-old *T. molitor* pupae as hosts in the laboratory at 25 ± 2 °C, 70 ± 12% RH and 12:12 h L:D photoperiod. On 18 Mar 2015 (date corresponding to the outbreak peak), these females were released near the trunk and 1.5 m above the base of 100 trees divided in 4 plots (25 trees per plot) infested by young pupae of *H. nanus*. About 5 females per pupa were released in the Água Santa region. No parasitoids were released in 4 plots (control), each with 25 similarly infested trees, 200 m away from the treated plots. We sampled 60 pupae (15 pupae per plot) from both the treated and untreated plots 96 h after the release to study the parasitism rate by *P. elaeisis*.

We also recorded the number of pupae collected as larvae and of pupae collected from the field in the Água Santa region with emergence of chalcidid and tachinid parasitoids. From these numbers, we calculated the percentage of natural parasitism.

CHEMICAL CONTROL AND NATURAL PARASITISM OF THE LARVAE IN THE ENCANTADO REGION

We designed the chemical control experiment based on results of our initial field observations. In the Encantado region, the insecticide bifenthrin (Talstar 100 EC; Food Machinery Corporation Química do Brasil Ltda., Campinas, Brazil) + spreader-sticker etilenoxi (Agral; Syngenta Proteção de Cultivos Ltda., Paulínia, Brazil) was applied between 8 and 10 a.m. at a rate of 30 mL per 100 L water by backpack sprayer with a fan-type nozzle, model XR110-02 (TeeJet Technologies, São Paulo, Brazil), to 4 plots of 25 trees until the trunks appeared wet. At 24 h after spraying, the numbers of surviving larvae found per trunk were recorded (Treatment 1). The control also had 4 plots with 25 trees per plot, but no insecticide treatment was applied (Treatment 2). The untreated plots were located 200 m from the insecticide-treated plots. A second replication was conducted in a similarly infested area of the Encantado region. The insecticide was applied to the trunk, on larvae located from base to around 2.5 m high; larvae located above this height were not sprayed because the backpack sprayer jet could not reach beyond 2.5 m. We compared the number of remaining larvae found per trunk in the test plots with the number found in the control plots 24 h after the experiment started by using 1-way ANOVA (R Development Core Team 2011). The effectiveness of the insecticide solution was calculated using Abbott's correction (Abbott 1925).

The number of field-collected larvae prior to spraying parasitized by chalcid and tachinid parasitoids (natural parasitism) that emerged from pupae was recorded in the laboratory after rearing. The parasitoids were identified to genus level using keys and taxonomic descriptions (Tavares et al. 2013b, 2014).

Results

FIELD OBSERVATIONS: FEATURES OF LARVAL SHELTERS AND FORAGING

We observed that *H. nanus* larvae build 2 types of shelters, the first comprised of leaf clusters and silk and located at the end of the branches (conical shape) and the other consisting of tree bark and silk and located on the tree trunks, particularly from the tree base to a height of 2.5 m. Although *Hylesia* larvae normally remain on trunks during the day and move to the canopy at night to consume foliage, at the high densities we found in the 2 studied regions, the larvae were feeding during the diurnal period. The drop of larval fecal material from the trees produced noticeable noise during the day.

BIOLOGICAL AND MORPHOLOGICAL PARAMETERS

The duration of the pre-pupal stadium in *H. nanus* was about 24 h (n = 100). Mean pupal length (± SE) was 22.6 ± 1.5 mm for males (n = 100) and 27.8 ± 7.9 mm for females (n = 100). The duration of the pupal stadium was 18.9 ± 1.5 d for males (n = 100) and 19.6 ± 1.5 d for females (n = 100). The emergence rate was 58% (n = 100) and 45% (n = 100) in the Encantado and Água Santa regions, respectively, with a female to male sex ratio of 45:55 (n = 100). The wingspan of the female insect was 46.6 ± 0.9 mm (n = 50) whereas for the male it was 37.3 ± 0.6 mm (n = 50).

MONITORING OF ADULTS AND BIOLOGICAL CONTROL OF THE PUPAE

The numbers of adults collected by the light traps (N = 5) remained high for 2 wk. After that, it decreased dramatically, ending with an average of only 124 insects per trap in the last collection (Table 1). This last value is below the damage threshold according to the company's monitoring system. Rains were absent during the collection period, which corresponded to the month before the beginning of the first rains in the regions; the average temperature in the regions during the collection period was 30 °C. The rains in the regions began in the second half of Apr, so the insects were not observed for at least 3 mo; they were, however, observed prior to the rainy season. *Hylesia nanus* may occur during short periods (up to 1 mo) in periods throughout the year if not effectively controlled. The number of insects collected dropped off despite suitable weather for their survival.

In total, 120 *H. nanus* pupae (60 from both treated and control plots) were recovered from the field 96 h after the parasitoid release. The percentage of parasitism (± SE) in plots with parasitoid releases was 23 ± 3%, whereas no parasitism by *P. elaeisis* was detected in control plots.

Two species of flies of the genus *Belvosia* (Diptera: Tachinidae) emerged from the *H. nanus* pupae collected from the field. One species of *Brachymeria* (Hymenoptera: Chalcididae), a hyperparasitoid, emerged from the puparia of Tachinidae parasitizing *H. nanus* pupae. Emergence of *Belvosia* sp. 1 and *Belvosia* sp. 2 was 22 and 10%, respectively, of pupae from larvae collected in the Encantado region, 15 and 2%, respectively, of pupae from larvae collected in the Água Santa region, and 35 and 2%, respectively, of pupae collected in the Água Santa region. Emergence of *Brachymeria* sp. was 8% of pupae from larvae collected in the Encantado region, and 8 and 12% of pupae from larvae collected and pupae collected in the Água Santa region, respectively. Emergence of *H. nanus* was 58% of pupae from larvae collected in the Encantado region, and 72 and 45% of pupae from larvae collected and pupae collected in the Água Santa region, respectively.

CHEMICAL CONTROL OF THE LARVAE

Chemical control with a single application of bifenthrin + etilenoxi was highly effective against the last instar of *H. nanus*. In both replications, larval numbers were reduced by 99%. The number of larvae alive per trunk was significantly different between the treated and non-treated trees after 24 h (F = 3.79; df = 7; P < 0.001). The presence of larvae, even in small numbers, in the Encantado region 24 h post spraying with the insecticide could be due to the descent of larvae from unsprayed upper parts of the trunk to the sprayed bottom part of the trunk (below 2.5 m).

Discussion

The eucalyptus clones infested by *H. nanus* facilitate the construction of the shelters on their trunks by enabling the bark to become

Table 1. Mean mass of *Hylesia nanus* (Lepidoptera: Saturniidae) moths (± SE), total mass of moths captured, and estimated total number of moths captured by light traps in *Eucalyptus* (Myrtaceae) plantations.

Date	Mean moth mass (g)	Total moth mass (g)	Estimated number of moths
11 Mar 2015	0.22 ± 0.06	532	2,415
12 Mar 2015	0.10 ± 0.05	304	3,136
13 Mar 2015	0.16 ± 0.04	953	5,956
17 Mar 2015	0.07 ± 0.01	518	7,393
18 Mar 2015	0.12 ± 0.03	523	4,480
19 Mar 2015	0.09 ± 0.01	476	5,284
20 Mar 2015	0.19 ± 0.04	524	2,757
02 Apr 2015	0.12 ± 0.02	77	619

fragmented into pieces in vertical lines and to detach from the trunk. Clones with smooth trunks and those that release less bark could present a physical barrier, limiting shelter construction. The shelters were also observed in the trees above 2.5 m height although they were less numerous. These shelters appear to completely isolate the pre-pupae and pupae from the action of natural enemies. Tachinids were observed near the groups of unprotected larvae but not near the shelters. Conical-shaped shelters at the end of the branches seem to facilitate rainwater and insecticide solution run-off, which could affect insect mortality. The shelter types described for *H. nanus* were also reported in many other plants of the families Anacardiaceae (Sapindales), Aquifoliaceae (Aquifoliales), Euphorbiaceae (Malpighiales), Fabaceae (Fabales), Combretaceae, Lythraceae, Myrtaceae (Myrtales), and Rubiaceae (Gentianales) (Veloso 1951; Silva et al. 1968; Gonçalves & Gonçalves 1973; Santos et al. 1988, 1996).

The feeding of the *H. nanus* larvae on eucalyptus plants during the diurnal period suggests the extension of the foraging period; normally, their feeding is nocturnal (Santos et al. 1988). The duration of the life stages and the size parameters of *H. nanus* that feed on eucalyptus are similar to what was previously reported for *H. nanus* that feed on *Joannesia princeps* Vell. (Malpighiales: Euphorbiaceae) (Santos et al. 1988).

The ability to cause over 20% parasitism following release of parasitoids suggests that parasitoids could contribute to population reduction of the pest. Parasitoid release can be used in combination with light trap population monitoring and, if timed properly, might reduce the need for application of insecticides.

The high natural parasitism rate of *H. nanus* by *Belvosia* spp. suggests that these parasitoids may contribute to population reduction of this species, an effect that was reported for the parasitism of this pest by *Belvosia bicincta* Robineau-Desvoidy and *Belvosia potens* (Wiedemann) (Diptera: Tachinidae) (Gonçalves & Gonçalves 1973, 1974; Guimarães 1977). The presence of *Brachymeria* sp. hyperparasitizing the pupae of Tachinidae parasitizing *H. nanus* may be unfavorable because it could reduce the efficiency of biological control by the tachinid parasitoid. A similar effect was observed for *Brachymeria koehleri* Blanchard (Hymenoptera: Chalcididae) as a hyperparasitoid of *Lespesia melloi* **sp. nov.** Gil-Santana, Nihei & Nunez (Diptera: Tachinidae) pupae in the larvae of snow white, *Thagona tibialis* (Walker) (Lepidoptera: Erebidae) defoliating tropical almond, *Terminalia catappa* L. (Myrtales: Combretaceae) in Brazil, a shade tree grown across the world (Tavares et al. 2013b). In Mapire, Venezuela, the parasitism rate on ashen moth, *Hylesia metabus* (Cramer) (Lepidoptera: Saturniidae) increased from 33.0 to 91.1% in the third cycle studied, with *Belvosia* spp., *Sarcodexia lambens* (Wiedemann) (Diptera: Sarcophagidae), and species of Ichneumonidae, Chalcididae, Perilampidae, and Eulophidae being the most common (Hernandéz et al. 2009). In Pedernales, Venezuela, the parasitism rate decreased from 29.5 to 16.1% and *Belvosia* spp. were the most abundant parasitoids, reaching levels of parasitism between 70.7 and 96.2% (Hernandéz et al. 2009).

In summary, we presented some of the biological and behavioral aspects of *H. nanus*, including the extension of the foraging larval period for the diurnal duration and the construction of peculiarly shaped shelters, because this information may help in the management of this species. Light traps can be used in the monitoring and capture of adults. This tool can be integrated with the release of natural enemies like *P. elaeisis*, which was recovered from the field parasitizing the young pupae of the pest. Chemical control should not be ignored but used to control larvae and old pupae in severe infestations, especially on the late instar larvae, with a single spray treatment of bifenthrin + etilenoxi being sufficient to cause 99% mortality. The monitoring and control methods studied for *H. nanus* can be included in an integrated management plan for this pest on eucalyptus forest plantations.

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