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The biology of *Antiteuchus innocens* (Hemiptera: Pentatomidae) under field conditions

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Abstract

Antiteuchus innocens Engleman & Rolston (Hemiptera: Pentatomidae) feeds on several species of pines in the highlands of Chiapas, Mexico. The biology and ecology of this species in pine ecosystems is unknown. In this paper, information concerning the life cycle of *A. innocens* is presented with the objective of providing information that elucidates its role as a pest of pines. Our field observations verified that the first adults of *A. innocens* typically appear by Apr of each year and reach the highest abundance in May. Afterwards, adults decrease gradually and are undetectable by Nov. Egg masses are laid on abaxial regions of primary branches, and on female strobili of pines. Following egg-hatching, the female remains with its brood, presumably for maternal care. We determined that *A. innocens* is an oligophagous species, feeding on *Pinus oocarpa* Shiede, *Pinus maximinoi* H.E. Moore, and *Pinus devoniana* Lindley (all Pinaceae). This species is univoltine under the climatic conditions of Chiapas, with a life cycle of about 310 d from egg to adult. Juvenile development of *A. innocens* is as follows (mean ± SE): 8 (0.66), 8 (0.92), 41 (7.5), 79 (8.1), 122 (5.9), and 52 (6.9) d for egg incubation, first, second, third, fourth, and fifth instar, respectively. The longevity of adults was estimated as 54 d. The longest duration (> 4 mo) of *A. innocens* occurred in the fourth instar, suggesting the possibility of diapause in this instar; however, this was not verified.

Key Words: Discocephalini; stink bugs; true bugs; life cycle; *Pinus*

Resumen

Antiteuchus innocens Engleman & Rolston (Hemiptera: Pentatomidae) es una chinche fitófaga que se alimenta de varias especies de pinos en las montañas de Chiapas, México. Hasta ahora se desconoce la biología y ecología de esta especie en los ecosistemas de pinos. En el presente trabajo se presenta información relacionada con el ciclo de vida de *A. innocens* con el objetivo de proporcionar información que permita esclarecer su papel como plaga de los pinos. Nuestras observaciones de campo verificaron que, los primeros adultos de *A. innocens*, típicamente aparecen en abril de cada año y alcanzan la abundancia más alta en mayo. Posteriormente, los adultos disminuyen gradualmente y para noviembre ya no son detectables. Las masas de huevos de esta chinche son puestas en la parte abaxial de las ramas primarias, y sobre los estróbilos femeninos de los pinos. Después de la eclosión de huevos, la hembra permanece con sus crías, presuntamente para brindarles cuidado maternal. Determinamos que *A. innocens* es una chinche oligófaga que se alimenta sobre *Pinus oocarpa* Shiede, *Pinus maximinoi* H.E. Moore, y *Pinus devoniana* Lindley (todos Pinaceae). Esta especie es univoltina bajo las condiciones climáticas de Chiapas, con un ciclo de vida de aproximadamente 310 d de huevo a adulto. Los tiempos para el desarrollo de inmaduros de *A. innocens* son como sigue (media ± ES): 8 (0.66), 8 (0.92), 41 (7.5), 79 (8.1), 122 (5.9), y 52 (6.9) días, para la incubación de huevos, primero, segundo, tercero, cuarto, y quinto instar, respectivamente. La longevidad de adultos fue estimada en 54 d. La duración más larga (> 4 meses) of *A. innocens* en el cuarto instar, indica la posibilidad de pasar por una diapausa, sin embargo, este hecho no fue verificado.

Palabras Clave: Discocephalini; chinche apestosa; ciclo de vida; *Pinos*

The genus *Antiteuchus* comprises 43 known species worldwide (Fernandes & Grazia 2006). Species in this genus are all phytophagous, feeding on the phloem of vascular plants (Grazia et al. 2015), such as annona, avocado, breadfruit, cacao, hibiscus, macadamia, and mango (Eberhard 1975; Umaña & Carballero 1995; Panizzi 1997; Ortega-León 2001; Villegas 2005). The biology and ecology of many of these species has never been studied in detail and information about host-plant records and damage are scarce. The species *Antiteuchus innocens* Engleman & Rolston (Hemiptera: Pentatomidae) is apparently of economic importance in avocado trees (*Persea americana* Miller) (Lauraceae) (Ortega-León 2001), and its presence has been reported in several countries of Central America, including Belize, Costa Rica, Guatemala, Honduras, and Mexico (Engleman & Rolston 1983; Ortega-León 2001; Grazia et al. 2015).

In the highlands of Chiapas, Mexico, *A. innocens* has been observed in high densities feeding on pines (*Pinus* sp.). Its presence has been related to the decline of forest plantations (CONAFOR 2014). Previous reports of phytophagous Hemiptera feeding on pines include *Lygus lineolaris* (Palisot de Beauvois) (Miridae), *Gastrodess grossipes* De Geer (Lygaeidae), *Leptoglossus occidentalis* Heidemann (Coreidae), *Priapismus pini* Rolston (Pentatomidae), and *Antiteuchus rukesi* Rolston (Pentatomidae) (Rolston 1991; Feci et al. 2003; Dixon & Fasulo 2015). *Antiteuchus innocens* is considered an emergent pest of pines in the highlands of Chiapas (CONAFOR 2014).

The purpose of this study was to determine the life cycle of *A. innocens* in pine ecosystems of Chiapas, Mexico. Observations on the biology and ecology of *A. innocens* will increase our knowledge of this species and assist in the development of pest management strategies.

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Table 1. Number of adults and immature stages of *Antiteuchus innocens* collected from pines during 2 yr in 3 localities of Chiapas, Mexico.

		Instars					
Site	Egg masses	I	II	III	IV	V	Adults
Chalota	451	1,490	3,745	2,265	1,770	475	1,184
Pamala	248	652	3,246	1,967	2,028	858	1,008
Naranjo	222	520	1,998	1,347	1,520	648	872

Materials and Methods

The study was conducted in the municipality of Altamirano, located in the central highlands of Chiapas. The climate in this area is semi-warm, wet, with rain almost uniformly distributed throughout the year (García 2004). There were 3 experimental sites: Paraje Chalota (16.725833°N, 92.030833°W, 1,264 masl); Pamala (16.883055°N, 91.998611°W, 1,267 masl), and Naranjo (16.715555°N, 92.037222°W, 1,298 masl). The region is important for conifer diversity, and the studied localities are naturally populated with *Pinus oocarpa* and *P. maximinoi* (both Pinaceae).

To determine the host-plant range of *A. innocens*, in each site we observed the different species of pines and surrounding vegetation to detect the presence or damage by *A. innocens*. In the case of pines, 10 *P. oocarpa* that exhibited damage by *A. innocens* (subapical part of the bud) were selected in each site. We took sample branches (40–50 cm long) that were cut with scissors and taken to the laboratory in plastic bags. Samples were taken every 3 wk during 2 yr (2012 and 2013). In the laboratory, samples were processed, and the developmental stages of *A. innocens* were identified by comparison with those described by Engleman & Rolston (1983), and Ortega-León (2001). We tabulated the frequency of biological stages of *A. innocens* collected over time, and with this information we obtained the life cycle of *A. innocens*. Specimens were preserved in vials with 70% ethanol.

Finally, 30 gravid females were collected in the field and taken to the laboratory. They were kept individually in 14.5 × 10.5 cm plastic containers and offered pieces (2–4 cm long) of *P. oocarpa* as oviposition substrate. The containers were maintained at 25 °C and a 12:12 h (L:D) photoperiod. The following variables were measured: number of egg masses per female, number of eggs, egg-hatching time, and development time from first to second instar. Pine branch tips were offered as food, and replaced when needed. Because the rearing of this insect beyond the second instar was unsuccessful, we used the insect stage frequencies of our field samplings to estimate the insect development through instars 2 to 5 following the Manly model (Manly 1974). This model assumes an insect population containing individuals developing through several stages, with a normal distribution for entry times to each biological stage, and a constant daily survival rate in all stages, which allows estimation of the duration of instars. The statistical analyses were performed using the R version 3.4.4 (R Development Core Team 2018).

Results

Adults and immature stages of *A. innocens* frequently were found in branches of the pines *Pinus oocarpa*, *P. maximinoi*, and *P. devoniana* (all Pinaceae). In total we collected 921 egg masses, 24,539 nymphs, and 3,064 adults, in a 2 yr period that correspond to 2 generations of *A. innocens* (Table 1). It was observed that the insect was more abundant in tall pines (> 5 m), and did not attack trees less than 3 m high. Even though we sampled other plants such as *Myrica cerifera* L. (Myricaceae), *Eupatorium* sp. (Asteraceae), *Miconia argentea* (Sw.) DC. (Melastomataceae), *Psidium guajava* L. (Myrtaceae), and *Vernonia* sp. (Asteraceae), among others, only pines were found to be damaged by *A. innocens*.

We determined that *A. innocens* has a univoltine life cycle. The life cycle from egg to adult was completed in about 310 d (Fig. 1). The juvenile development of this insect was: mean number of d (± SE): 8 (0.66), 8 (0.92), 41 (7.5), 79 (8.1), 122 (5.9), and 52 (6.9) d for egg incubation, and first, second, third, fourth, and fifth instar, respectively (Table 2). The sex ratio (males:females) was calculated to be 1.3:1.7. Longevity of adults was estimated to be about 54 d.

Adults of *A. innocens* do not exhibit sexual dimorphism. Generally, they gather in small groups up to 10 individuals in the subapical region of secondary branches of pines, and up to 20 individuals on the abaxial region of primary branches. They feed on pine needles in the subapical part of shoots (Fig. 2a). Copulation is typically undertaken at sunset (Fig. 2b), and can last several hours, sometimes until dawn. Adults disperse to other pines in short flights, generally late in the afternoon. Adults of *A. innocens* commonly gather in bark crevices of pines (Fig. 2d).

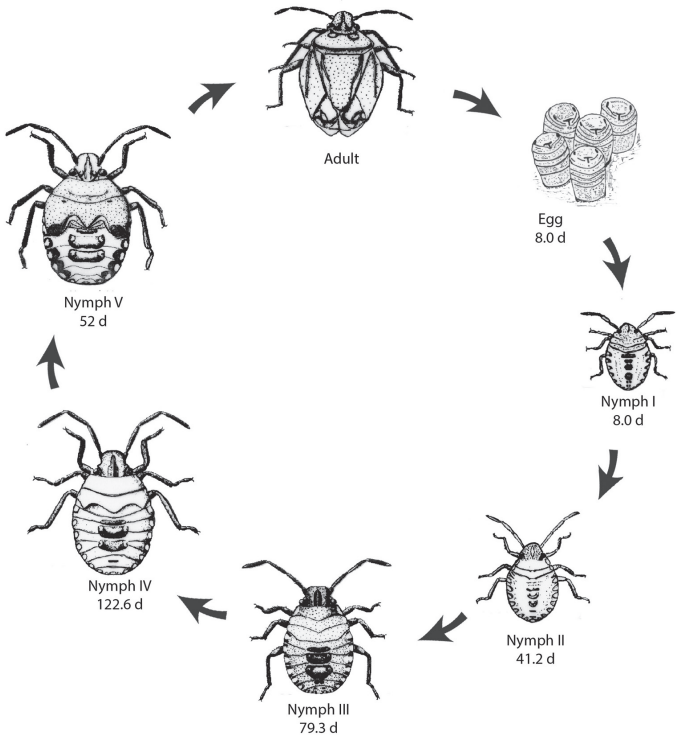

Fig. 1. Typical life cycle (egg to adult) of the bug *Antiteuchus innocens* in the highlands of Chiapas, Mexico, showing the developmental stages and mean duration of each.

Table 2. Analysis of stage frequency data from field samples to estimate the instar duration of the bug *Antiteuchus innocens* following the Manly model (Manly 1974).

	Instars				
Parameters	II	III	IV	V	Adult
Total number of insects entering the stage (M)	915	691	866	558	423
Standard error (M)	97.2	48.9	123	84.4	123.3
$P < 0.001$	$6.12\ e^{-14}$	$< 2.0\ e^{-16}$	$3.9\ e^{-09}$	$5.9\ e^{-08}$	$1.7\ e^{-03}$
Mean entry time μ	71.9	113	192	315	367
Standard error (μ)	6.4	2.9	6.91	4.25	4.21
$P < 0.001$	$< 2.0\ e^{-10}$	$< 2.0\ e^{-16}$	$< 2.0\ e^{-16}$	$< 2.0\ e^{-16}$	$< 2.0\ e^{-16}$
Age specific death rate θ	0.006	0.005	0.01	0.019	0.037
Standard error (θ)	0.0008	0.0005	0.001	0.003	0.012
$P < 0.001$	$< 2.5\ e^{-10}$	$1.77\ e^{-14}$	$8.4\ e^{-08}$	$7.64\ e^{-07}$	$4.0\ e^{-03}$
Standard deviation of this mean σ	37.64	13.6	48.1	29.2	14.1
Standard error (σ)	8.24	3.4	7.29	4.14	3.37
$P < 0.001$	$< 2.1\ e^{-5}$	$1.0\ e^{-04}$	$1.8\ e^{-08}$	$1.43\ e^{-08}$	$2.0\ e^{-04}$
Instar duration (d)	41.2	79.3	122.6	52.0	54.0

The stage specific survival for stage 1 is then estimated by *M2/M1* and the duration of stage 1 can be estimated by μ2 to μ1.

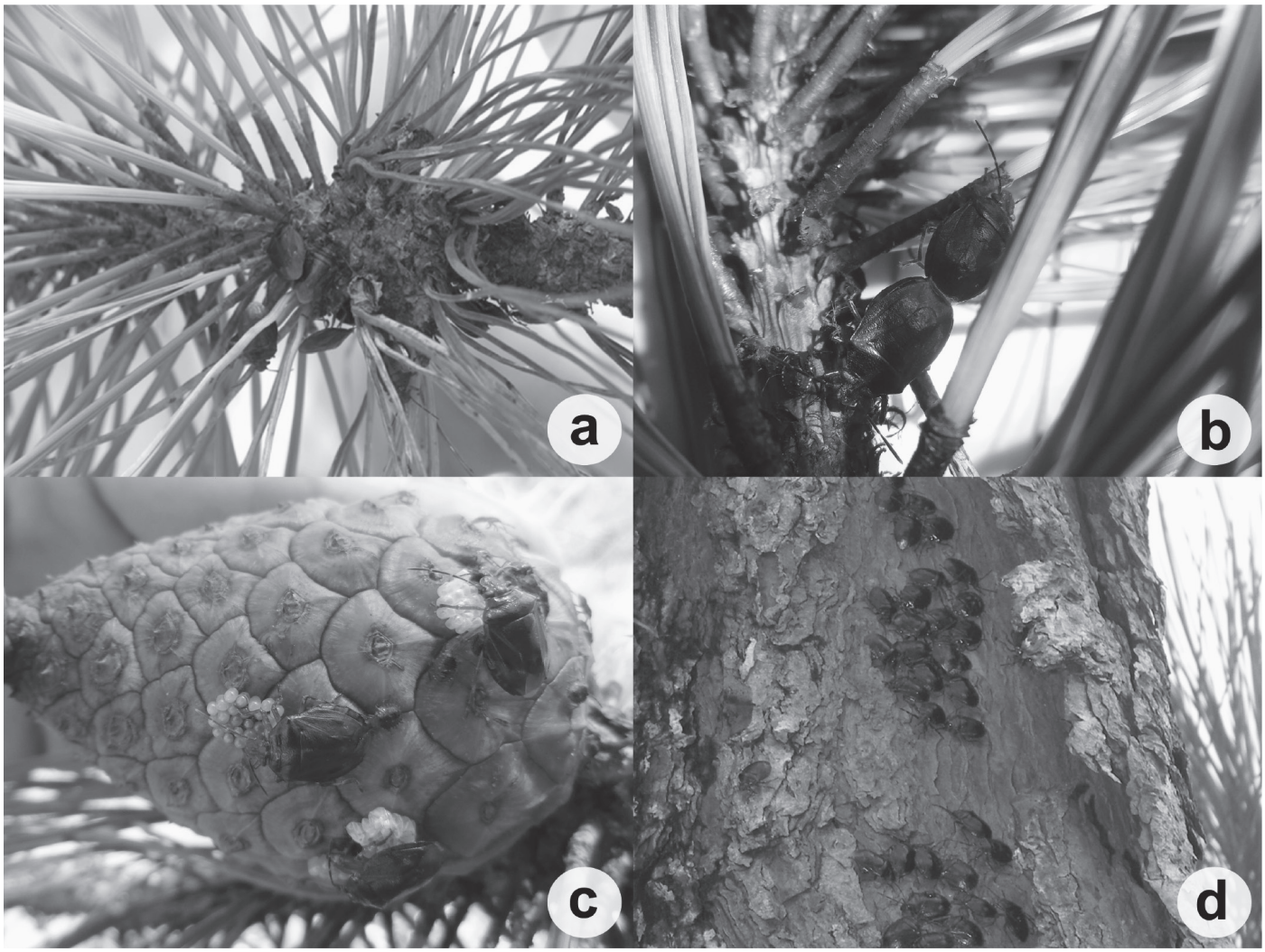

Fig. 2. *Antiteuchus innocens* on the pine *Pinus oocarpa*, in the highlands of Chiapas, Mexico: (a) adults feeding on needles, (b) adults mating, (c) female protecting eggs deposited on a female strobilus, (d) adults gathering on a stem crevice.

Females lay eggs at sunset, during the night or at an early hr in the morning. Egg masses are placed on abaxial stems of primary branches, on female strobili, and less frequently on small stems of secondary branches of pines. As with other species of hemipterans, eggs are barrel-shaped with a ring of small appendages. Initially they are white, changing to pink as incubation progresses. Females of *A. innocens* lay only a single mass of eggs, and abandon maternal care at the end of the first instar (Fig. 2c). Using their bodies, they protect egg masses to resist the attack of parasitoids and predators. The mean number (± SE) of eggs per mass was 28 (± 1), with 92.5% of eggs hatching.

Adults appeared in the field by Apr and reached their greatest abundance in May. Afterwards, adult abundance decreased gradually until Nov, when they were not recorded in the samples (Fig. 3). Oviposition occurred from Jun to Aug. After egg-hatching, the young nymphs remained clustered together near the remains of eggshells, and apparently do not feed during the first days of life. Instar I was detected in the field from Jun to Aug. Nymphs of instar II disperse from the oviposition sites (abaxial side of primary branches) to subapical parts of secondary branches of pines, presumably seeking food. Nymphs of instar II were present predominantly from Jun to Oct. From Oct onwards, most of the 5 instars of *A. innocens* were present in samples. Instars IV and V were more abundant in winter, during a period of 5 to 6 mo.

Discussion

This is the first study on the biology of *A. innocens* in pine ecosystems. This species is univoltine under the climatic conditions of Chiapas. Given that feeding of *A. innocens* was restricted to 3 species of pine, it was categorized as an oligophagous species. Previous reports mentioned avocado as a host of this bug (Ortega-León 2001; Saulich & Musolin 2014). However, our observations did not verify this fact and there are no reports of *A. innocens* feeding on avocado in Chiapas. Considering that most pentatomids are polyphagous (Panizzi 1997), the feeding of *A. innocens* on other species of plants in Chiapas cannot be discounted.

The reproduction of adults coincides with the hottest months of the year. Similar behavior also was observed with the bug *Antiteuchus tripterus* (Hemiptera: Pentatomdae) in Brazil (Panizzi & Grazia 2001). Oviposition by *A. innocens* females on the abaxial parts of primary branches may protect eggs from extreme abiotic factors, such as rain and sunlight. However, when pines produce female strobili (Jul and Aug), the bugs oviposit on it, perhaps because strobili are a rich source of nutrients that assures development of the progeny (Thompson & Pellmyr 1991). The maternal care exhibited by *A. innocens* is a common behavior of stink bugs belonging to subfamily Discocephalinae, and particularly for species of *Antiteuchus* (Eberhard 1975; Santos & Albuquerque 2001a, b; Taylor 1988). Maternal care in the genus *Antiteuchus* is more effective for predators than for parasitoids. However, it is also important to resist parasitoids, because to some degree it prevents egg parasitization by wasps of the genera *Phanuropsis* and *Trissolcus* (Hymenoptera: Platygastridae) (Eberhard 1975; Santos & Albuquerque 2001a; Matthews & Matthews 2010).

The longest period of development (> 4 mo) occurs in the fourth instar, possibly indicating the presence of diapause (Saulich & Musolin 2014). However, the presence of diapause was not verified, and the long developmental period is possibly due to the suboptimal climatic conditions, including reduction in photophase by about 2 h and decrease in temperature to about 9 °C in winter (Serrano et al. 2006). Even though members in the genus *Antiteuchus* are characterized by having long biological cycles that last several mo (and by its association with perennial plants), the life cycle of *A. innocens* was longer than those reported for other species. For instance, *A. variolosus* (Westwood) has a life cycle of 79 to 83 d at 22 °C (Barrera 1973); for *A. tripterus* (F.), it is 94 to 108 d at 24 °C (Umaña & Carballo 1995); and for *A. sepulcralis* (F.), it is 96 d at 25 °C (Santos & Albuquerque 2001). The long life cycle is one of the principal difficulties in establishing a laboratory colony of this species (Ortega-León 2001).

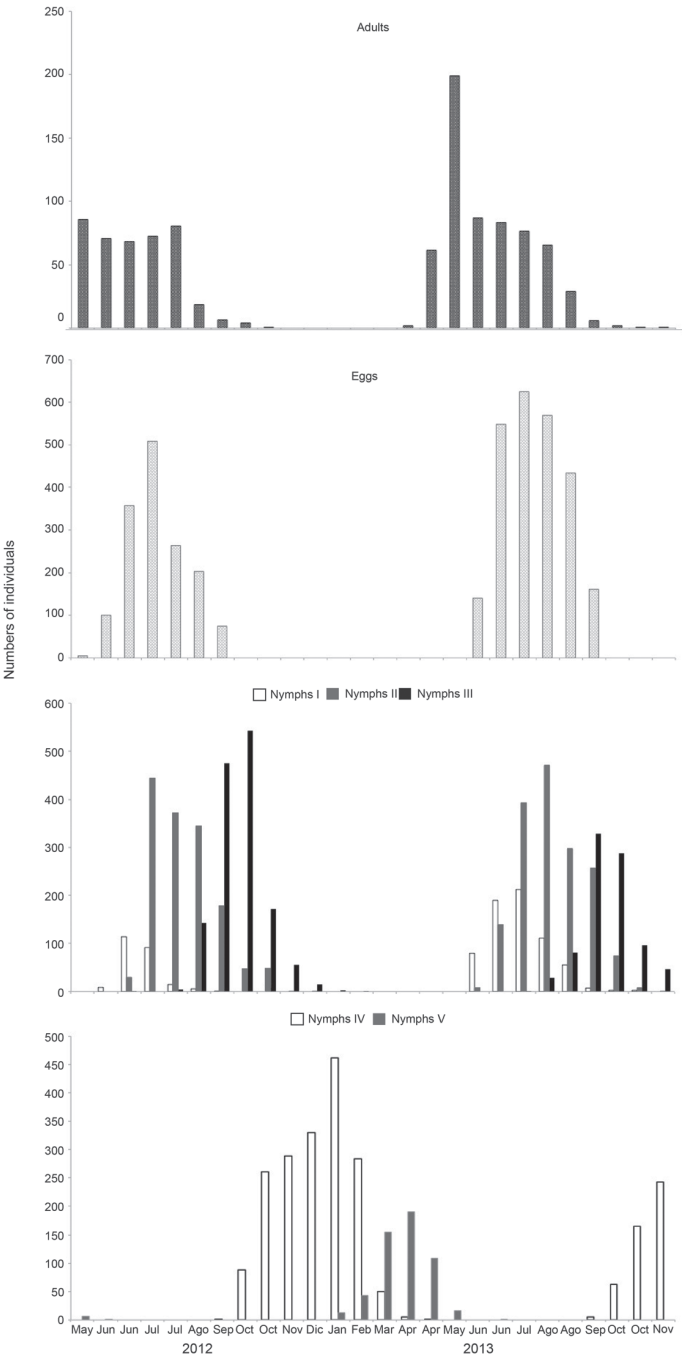

Fig. 3. Relative frequencies per branch of the biological stages of *Antiteuchus innocens* collected on pine ecosystems of Chiapas, Mexico, during 2 consecutive yr.

Although our study was not designed to assess the level of damage by *A. innocens* to pines, it was clear that adults and nymphs attack pine cones for nourishment. Feeding on the cones can limit the proper growth of pines, and may cause damage to the plant, as mentioned for other species of bugs (Bates et al. 2000, 2002; Bracalini et al. 2013). Experiments designed to determine the economic injury level for this species should be performed in the near future.

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