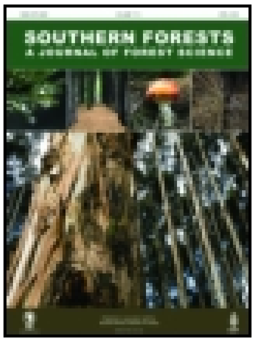

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*Research Note*

# Biology of *Litosermyle ocanae* in Colombian *Pinus patula* plantations

**Carlos A Rodas[1], Brett P Hurley[2]*, María D Bolaños[1], Ginna M Granados[1] and Michael J Wingfield[2]**

[1] *Forest Health Protection Programme, Smurfit Kappa, Valle, Colombia*
[2] *Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa*
* *Corresponding author, email: brett.hurley@fabi.up.ac.za*

**Plantations of *Pinus* spp. in Colombia are severely damaged by various phasmid (Phasmatodea: Diapheromeridae) insects. Of these, *Litosermyle ocanae* is one of the most serious defoliators. Since 1988, several outbreaks have been recorded in *Pinus patula* plantations, resulting in substantial economic losses. The aim of this study was to determine the life cycle of *Litosermyle ocanae* in *P. patula* plantations in Colombia. In addition, we assessed the population levels of *L. ocanae* and the presence of natural enemies of *L. ocanae* in these plantations. The development of *L. ocanae* was monitored under controlled conditions, and population fluctuations and the presence of natural enemies was assessed from field-collected eggs and field observations. The results showed a total life cycle of 225 d and 235 d for males and females, respectively, and a complete fourth instar nymph cycle of 121 d and 116 d for males and females, respectively. The sex ratio was 1:1.7 female to male. Females laid an average of 112 eggs. The number of eggs peaked in November and the most dominant natural enemy observed was a parasitoid wasp *Adelphe* sp. (Hymenoptera: Chrysididae). The results provide important information for the management of *L. ocanae* in *P. patula* plantations.**

**Keywords:** *Adelphe* sp., natural parasitism, phasmid, *Pinus patula*



From approximately 1950, commercial forestry plantations in Colombia have been negatively affected by a large group of defoliating insects (Rodas et al. 2014). The most important of these insects reside in the Lepidoptera (Geometridae) (Gallego 1959; Vélez 1972; Bustillo 1976; Rodas 1994), Hymenoptera (Formicidae) (Mackay and Mackay 1986) and Phasmatodea (Diapheromeridae) (Madrigal 1997). Since 1975, there have been heavy levels of infestation, particularly by phasmids that have caused extensive damage to plantations of *Pinus patula* in different geographic regions of the country (Madrigal and Abril 1994; Madrigal 1997, 2003).

Phasmatodea, commonly referred to as phasmids, phasmatids, stick-insects or walking stick insects, are well-known defoliators, including in forests (Gullan and Cranston 2005; Conle et al. 2011). The Phasmatodea are well known as a predominantly tropical order of more than 3 000 species that lack a phylogenetically based classification (Otte and Brock 2005; Conle et al. 2011). The Phasmatodea exhibit varied body shapes, including cylindrical and stick-like, flattened or leaf-like (Gullan and Cranston 2005), and have mandibulate mouthparts (Key 1991; Gullan and Cranston 2005). Members of the Phasmatodea do not have enlarged hind femora and are thus not capable of jumping (Triplehorn and Johnson 2005). They are slow-moving, phytophagous and mostly mimic various plant parts such as stems, sticks and leaves (Costa Lima 1938; Key 1970; Bedford 1978). In addition, phasmids have a nocturnal habit (Brock 1999) and display thanatosis, autotomy, diapause in eggs, and both sexual and asexual reproduction (Bedford 1978). When they are in very large numbers, they can cause serious damage to trees (Campbell and Hadlington 1967; Madrigal 2003; Triplehorn and Johnson 2005).

Between 1990 and 1993, Dr David Nickle of the National Museum of Natural History, Smithsonian Institution, USA, identified various species of Phasmatodea from Colombia. These included *Libethroidea inusitata* Hebard, *Litosermyle* sp. near *ocanae* Hebard (later confirmed as *Litosermyle ocanae*; Ramírez 2009; Conle et al. 2011), *Libethra strigiventrus* Hebard, *Libethra* sp., *Libethra spinicollis* Hebard, *Ceroys quadrispinosus* Hebard, *Heteronemia striatus*; Phasmatodea: Pseudophasmatidae: *Planudes cortex* Hebard; Phasmatodea: Bacillidae: *Acanthoclina* sp., *Acanthoclina* sp. near *hystrix* (D Nickle, National Museum of Natural History of the Smithsonian Institution, pers. comm., 1993). An additional 74 new species and four new genera of phasmids in Colombia were described by Conle et al. (2011), making up a total of 182 described species in Colombia. Conle et al. (2011) estimated that at least 300 species are present in Colombia.

The first outbreaks of phasmids in forestry plantations in Colombia were recorded in the early 1980s. Between 1986 and 1993, occasional outbreaks caused alarm in the forestry sector. The stick insects involved included a complex of native species, such as *Planudes cortex*, *Heteronemia striatus*, *Libethroidea inusitata*, *Libethra* sp., *Paraceroys quadrispinosus* (previously *Ceroys quadrispinosus*) (Madrigal 1997, 2003; Conle et al. 2011) and *L. ocanae*. *Litosermyle ocanae* is considered one of the most important defoliators of *P. patula* in Colombia, with 86% defoliation and 15% mortality reported in Sombreros Farm, Colombia in 2016 (CAR unpublished data).

Little is known regarding the biology of the phasmids in Colombian plantation environments, although there have been a number of unpublished studies on the topic. The aim of the present study was, therefore, to determine the life cycle of *Litosermyle ocanae*. An additional aim was to assess the population levels of *L. ocanae* in Colombian *Pinus* plantations and to identify natural enemies of this insect present in these plantations.

The Sombreros farm (Salinas, Cauca Department) was used to evaluate the population of *L. ocanae* and to collect eggs for the life-cycle study. This site is located at 3°51′45″ N, 76°29′49″ W in plantations of 19-year-old *P. patula*. Climatic conditions included an annual average temperature of 16 °C, precipitation of 2 175 mm, and the site is located at 2 632 m above sea level (asl). The life-cycle studies were performed during 2007 and 2008 at the entomology laboratory of Smurfit Kappa (SK) in Restrepo, Valle del Cauca Department, located at 3°51′21″ N, 76°30′28″ W, with an annual average temperature of 20 °C, precipitation of 1 192 mm and at 1 500 masl (Figure 1).

Eggs of *L. ocanae* were used as a proxy for the abundance of the insect. Twenty-eight egg traps were randomly placed within the study site. The traps consisted of a 1 m × 1 m plastic cloth with a 10 cm × 10 cm mesh centre to allow water to pass through. These traps were erected 70 cm above the soil level and eggs were collected from each trap every 5 d between October 2007 and December 2014. At every collection date, the eggs for each trap were counted and placed in Petri dishes marked with the date, trap number and number of eggs. The number of eggs per collection period was pooled for each month and the mean monthly number of eggs was determined. Eggs were stored in laboratory conditions for further studies.

To obtain a laboratory population of stick insects, insects emerging from field-collected eggs were kept in glass bottles closed with mesh at the top, containing 500 g sterile sawdust to maintain the required moisture for egg emergence, and maintained at 76% relative humidity between 23 and 24 °C. Newly laid eggs from the laboratory-reared stick insects were removed daily and used for the study. The eggs were placed in separate petri dishes according to the date of oviposition and the duration of eclosion was determined. Emerged first instar nymphs were counted and transferred to 4 L glass bottles containing mature pine foliage as a food source. Each nymph was marked on the thorax with water paint to determine the change of nymphal instars. Nymphs were examined daily and newly moulted nymphs were transferred to separate containers, marked with the date of transfer. This process was continued until the adult stage, and the duration of the nymphal instars thus determined.

At the adult stage, male and female insects were counted to determine the sex ratio. Male and female insects were placed together for mating and adult longevity was determined. For the female insects, the pre- and post-oviposition periods and potential female fecundity were also determined. Data from insects that did not complete their life cycle were not used to determine the duration of the different life stages, but were used to determine the size of the different life stages.

In order to measure the impact of *L. ocanae* as a defoliator, a study was conducted between 2008 and 2010 on the Sombreros farm in three different areas. These study areas had no previous history of defoliation by the insect but there was a good possibility of their being affected by *L. ocanae*. In each of the study areas, 12 randomly selected groups of 25 trees were chosen to include trees that were dominant, co-dominant and suppressed. In all, there were 300 trees per area available for an annual evaluation in September of 2008, 2009 and 2010.

Eggs collected from the litter in the field from eight egg traps were used to determine whether naturally occurring egg parasitoids were present. Parasitoid wasps emerging

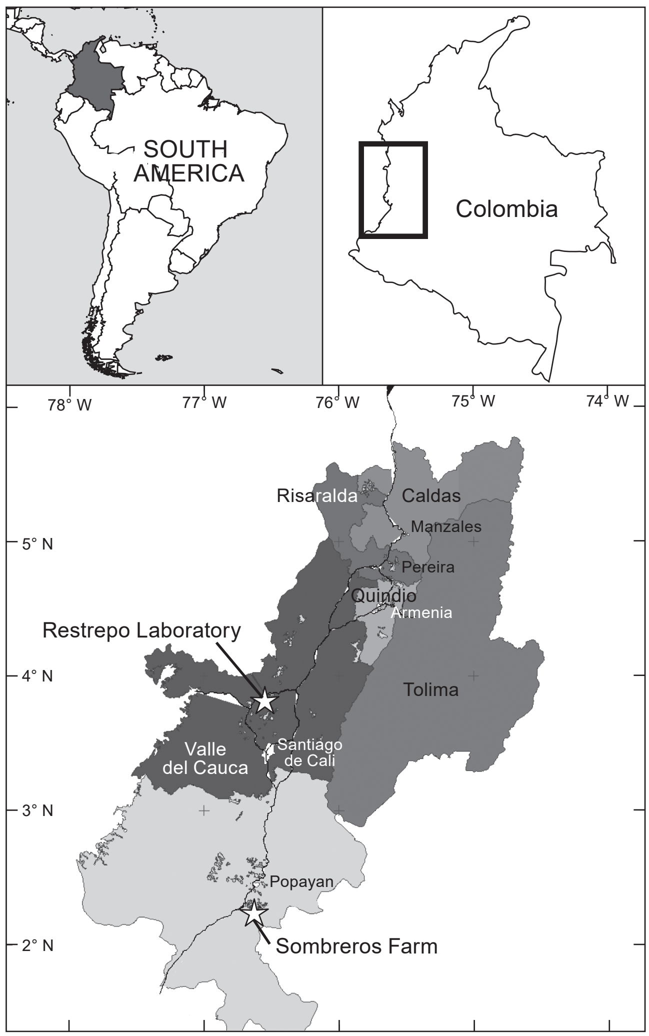


**Figure 1:** Localities where research was conducted in Colombia: Sombreros Farm in Cauca Department and the entomology laboratory in Restrepo (Valle del Cauca Department)

from eggs were counted and identified and the percentage parasitism determined. In addition, observations were made in the field of natural enemies of the nymph and adult stage.

Data were analysed using descriptive analysis in SAS version 9.2 (SAS Institute, Cary, NC, USA, 2009).

Over the period that the eggs were collected, between October 2007 and December 2014, the greatest number of eggs was consistently collected from October to December (Figure 2). This was consistent with the period of highest defoliation. The egg production decreased gradually from December to May and from May to August the egg production remained at a low level, suggesting a high presence of nymphs. During the evaluation period, most nymphs became adults by August.

In 2009, it was found that the greatest number of eggs was 25 338, followed by 2011 with 22 605 eggs, 20 827 eggs produced in 2008, and a similar egg production in 2012 with 20 422 eggs collected. During October and December 2007, 8 948 eggs were collected. In 2013, the egg production was reduced by half compared with 2009 with 12 762 eggs collected. In 2014 the total number of eggs collected was only 3 298, with the decrease in egg numbers attributed to management intervention. Data from July to December 2010 were not collected due to traps having been destroyed.

Development of *L. ocanae* from eggs to adult death required an average of 225.8 d (200–242 d, SD = 9.8, $n$ = 23) for adult males, and 235.5 d (187–257 d, SD = 14.2, $n$ = 55) for adult females. Egg incubation under laboratory conditions required an average of 65.8 d (65–68 d, SD = 1.4) for males and 69.8 d (56–94 d, SD = 10.7) for females. The nymphal stage was 121.6 d (103–135 d, SD = 9.7) for the males and 116.7 d (99–136 d, SD = 12.4) for females. The adult stage was an average of 38.3 d (16–53 d, SD = 11.2) for males and 49.5 d (26–70 d, SD = 9) in the case of females. The adult females (Figure 3a) displayed a pre-oviposition period of 7.8 d (2–17 d, SD = 3.5, $n$ = 55); oviposited for an average of 37.5 d (18–51 d, SD = 9), had an average post-oviposition period of 4.2 d (1–11 d, SD = 3.3), and produced an average of 111.7 eggs female$^{-1}$ (16–193 d, SD = 38.6). The sex ratio for 600 adults was 1:1.7 (female: male).

*Litosermyle ocanae* had a total of four instars. Nymphs and adults had small anatomical differences differing in colour in each instar, first being light yellow and gradually becoming greenish brown. The duration of each nymphal instar was as follows: for male nymph I, 33.8 d (25–38 d, SD = 4.4, $n$ =23), nymph II, 30.5 d (25–37 d, SD = 4.3), nymph III, 26.1 d (22–31 d, SD = 2.3) and for nymph IV, 31.1 d (18–36 d, SD = 4). For females, the nymphal stage I was 29.8 d (19–38 d, SD = 6.8, $n$ = 55), nymphal stage II was 28.0 d (17–37 d, SD = 6.8), nymphal stage III was 26.7 d (19–46 d, SD = 4.2) and for nymphal stage IV was 32.1 d (18–38 d, SD = 4.4).

The *L. ocanae* eggs (Figure 3b) were on average 2.5 mm (2.1–2.8 mm, SD = 0.1, $n$ = 100) long and 2.0 mm (1.7–2.5 mm, SD = 0.1, $n$ = 100) wide. Adults and the fourth instar nymphs differed in size and the nymphs also lacked wings. Emerged nymphs of the first instar had a nymph to egg size ratio of 6:1, giving the impression that they had been rolled inside the eggs. The range of body length for the first instar nymphs was 12.0 mm (10.1–13.2 mm, SD = 1.1, $n$ = 20). Nymphal stage II had a body length of 15.3 mm (10.3–19.2 mm, SD = 2.4, $n$ = 21). Third instar nymphs were 27.4 mm (23.6–31.1 mm, SD = 2.3, $n$ = 17) and the fourth instar nymphs were 32.2 mm (29.8–36.9 mm, SD = 1.8, $n$ = 21) long. The average body length for adult males was 33.3 mm (29.8–36.9 mm, SD = 2.3, $n$ = 11),

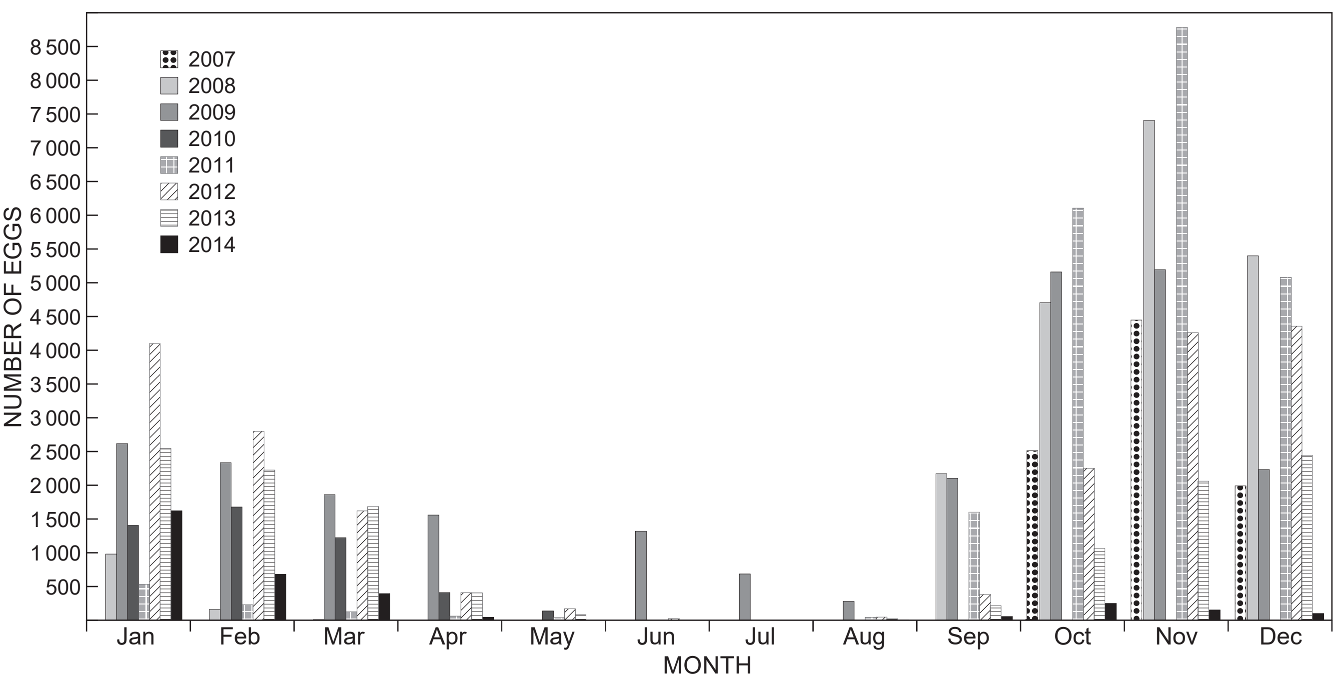


**Figure 2:** Postures of *Litosermyle ocanae* per year. Each bar represents the number of egg postures per month per year

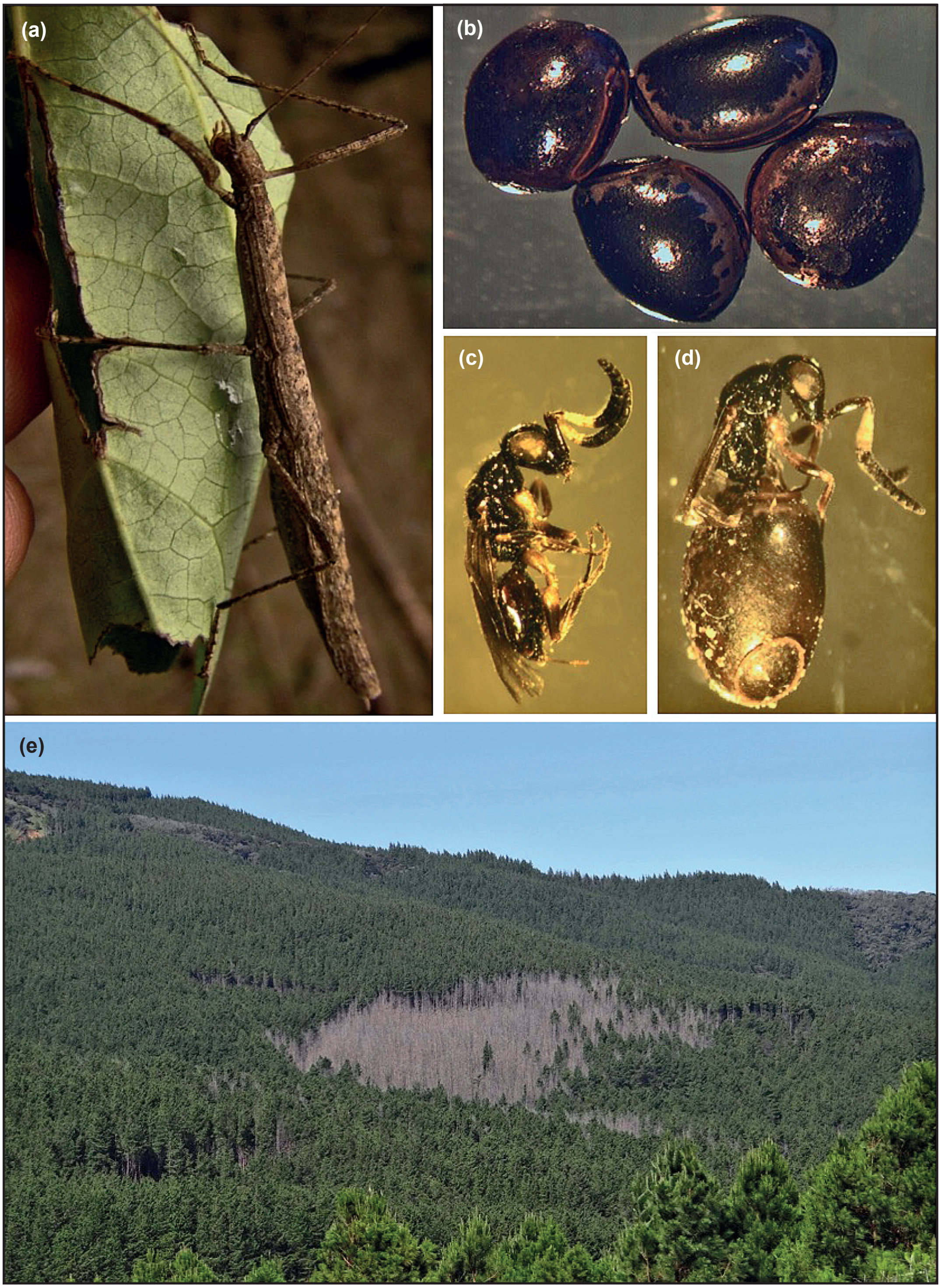


**Figure 3:** *Litosermyle ocanae*: (a) adult female; (b) eggs; (c) *Adelphe* sp. female; (d) emerging female of *Adelphe* sp. from *L. ocanae* egg; (e) area affected by *L. ocanae* in Cauca Department

whereas the female body length was an average of 31.9 mm (Figure 3a; 30.7–32.7 mm, SD = 1.1, $n = 8$).

Evaluation of the impact of defoliation on the three categories (dominant, co-dominant and suppressed) of trees in three evaluated areas with 300 trees in each area showed variable results for trees under different levels of stress (Table 1). The dominant trees were the most common in all three areas, but showed the lowest percentage mortality from the insect in 2008 with 0–2.4%. The suppressed trees were the least common at all of the sites but showed the highest mortality for all three years, with complete defoliation at two sites in 2010. Mortality

**Table 1:** Defoliation on the three categories (dominant, co-dominant and suppressed) of trees in three evaluated areas with 300 trees from 2008 until 2010

| Year | Study area | Dominant | | Co-dominant | | Supressed | |
|---|---|---|---|---|---|---|---|
| | | Number of trees evaluated | Mortality (%) | Number of trees evaluated | Mortality (%) | Number of trees evaluated | Mortality (%) |
| 2008 | 1 | 204 | 0.0 | 29 | 0.0 | 28 | 53.6 |
| | 2 | 166 | 2.4 | 68 | 36.8 | 34 | 88.2 |
| | 3 | 178 | 0.0 | 61 | 0.0 | 14 | 42.9 |
| 2009 | 1 | 204 | 63.2 | 29 | 41.4 | 28 | 100.0 |
| | 2 | 166 | 4.2 | 68 | 39.7 | 34 | 91.2 |
| | 3 | 177 | 39.5 | 62 | 38.7 | 14 | 85.7 |
| 2010 | 1 | 202 | 65.3 | 29 | 55.2 | 30 | 100.0 |
| | 2 | 166 | 4.2 | 68 | 44.1 | 34 | 94.1 |
| | 3 | 178 | 42.1 | 61 | 68.9 | 14 | 100.0 |
| Total | | 1 641 | | 475 | | 230 | |



increased with time for all tree categories, especially between 2008 to 2009, suggesting the movement and increase of the insect population. From the 2 700 observations made, a total of 118 trees were not included in the analyses as the cause of their death could not be attributed to *L. ocanae.*

A single parasitoid identified as a species of *Adelphe* (Hymenoptera: Chrysididae) (C Sarmiento, Universidad Nacional de Colombia, pers. comm., 2010) (Figure 3c and d) emerged from the collected eggs. From a total of 7 200 evaluated eggs, 2 087 or 68.7% (range: 61.2–93.3%, $n = 8$) had been parasitised by *Adelphe* sp. The sex ratio of males to females for the parasitoid was 1:1.1 (range: 1:0.5–1:5.7, $n = 8$). There were 459 unemerged wasps and 952 emerged nymphs. The remaining unhatched eggs (4 161) were considered not to be viable. In addition to *Adelphe* sp., other natural enemies of *L. ocanae* observed in the field included the entomopathogenic fungus *Beauveria bassiana*, the adult predator *Podisus* sp. (Hemiptera: Pentatomidae), the nymph parasitoid *Anisia* sp. (Diptera: Tachinidae), and the nymph and adult predators *Arilus* sp. and *Zelus* sp. (Hemiptera: Reduviidae).

This study represents the first to consider the life cycle of *L. ocanae* in Colombia or elsewhere in the world. Results showed that *L. ocanae* has a life cycle different to other members of the Diapheromeridae such as *Libethroidea inusitata* (Ramírez 2009; Conle et al. 2011). In the latter insect, adult males lived an average of 217.4 d, adult females lived an average of 198.9 d and it has five nymphal instars (Madrigal and Abril 1994). *Litosermyle ocanae* had similar male and female lifespans (225.8 and 236.6 d, respectively) but with only four nymphal instars. Future studies should consider the life histories of the many other Diapheromeridae in Colombia as these might also show interesting and different patterns of development. For example, in the Phasmatodea, the average number of eggs per female during its life cycle is between 60 and 700 eggs female$^{-1}$ (Key 1970). In Colombia, *L. inusitata* displayed a biological potential of 220 eggs female$^{-1}$ (Madrigal and Abril 1994), whereas in this study *L. ocanae* had an average of 111.7 eggs female$^{-1}$.

Stick insects exhibit localised infestations in plantations (Figure 3e), mostly due to their reduced mobility. However, observations from this study showed that severe defoliation tends not to extend for long periods of time. This is apparently due to competition with other insects and the impact of natural enemies. This is a common characteristic of many native insect pests that damage plantation forestry in Colombia (Gallego 1959; Vélez 1972; Bustillo 1976; Rodas 1994). In this study and over a period of 26 years, *L. ocanae* had between two and three generations before the outbreaks disappeared. In the absence of control measures, this trend will likely continue in the future.

Areas affected by *L. ocanae* have increased in Colombia due to the large range of food sources for this insect. Nymphs and adults are responsible for cutting and feeding on needles of *P. patula* in plantations over eight years old. These are typically stressed trees and silvicultural practices to reduce within-plantation stress through optimal pruning and nutrient management will be needed to reduce levels of damage by *L. ocanae*. In addition, new strategies involving biological control need to be developed to prevent the spread of *L. ocanae* in Colombia.

The discovery of a relatively abundant parasitoid, *Adelphe* sp., holds some promise for the management of *L. ocanae* in Colombia. *Adelphe* spp. are commonly referred to as 'cuckoo' wasps and are well-known stick insect egg parasitoids (Krombein 1956; Anon. 1985; Kimsey 1986). These parasitoids are adapted to feed on the fluid contents of the eggs after oviposition (Krombein 1986). In addition, *Beauveria bassiana*, found infesting *L. ocanae* in this study, is used for the management of a number of forestry pests in Colombia. However, the efficacy of *B. bassiana* is largely dependent on environmental conditions (James et al. 1998), and is ineffective as a biological control agent in areas with low humidity, such as at the site used in the present study.

This study provided information on the life cycle and importance of *L. ocanae* as a pest of *Pinus* species in Colombia. Furthermore, we identified an egg parasitoid of *L. ocanae* that shows promise to be used as a native biological control agent. Knowledge of the behaviour and population dynamics of each of the life stages of *L. ocanae* emerging from this study provides initial data on which to base management strategies such as biological or chemical applications. Continued monitoring is required to assess the spread and impact of this insect. In addition, further work on the rearing and efficacy of the *Adelphe* sp. as a natural enemy is needed.

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