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Life cycle of *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae) in Santiago, Chile, and sex fenotype differentiation of adults

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Periodical samples of eggs, larvae, pupae and adults of *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae) were obtained to determine its life cycle on urban trees affected in municipalities of Santiago, Metropolitan Region, Chile. The duration of the pupae and adult stages were determined also in the laboratory. Adults were collected to determine the sex ratio and to study sex phonological differences, which were corroborated trough analysis of genitalia. Four generations of *X. luteola* a year were determined for Santiago, Chile, from October through April with duration of one to two months each, and also a hibernation period for adults lasting four months. The *X. luteola* individuals were reared successfully in the laboratory, and the duration of the pupa and adult stages were similar to those in the literature. Phenotypical sex differences were established between adult *X. luteola* individuals, which were verified by analysis of genitalia, and a 1:1 sex ratio was determined.

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Key words: Elm leaf beetle, *Ulmus*, genitalia.

INTRODUCTION

Diverse insect pests species have entered in Chile, related to the openness of foreign commercial exchange and the greater travel of people. One of the most severe defoliators in the world for elms (*Ulmus* spp., Ulmaceae), the elm leaf beetle *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae) has been detected in city trees in central Chile. These valuable trees are used mainly as ornamentals resistant to cold, adaptable to diverse soils, and are planted frequently in green areas and avenues of the municipalities in Santiago (HERNÁNDEZ, 2004; MARTÍNEZ, 2005). This mainly monophagous pest affects all kinds of elms of all ages (ROMANYK and CADAHIA, 2002). According to MUÑOZ *et al.* (2003), some infestations have been found also in the genus *Aesculus* (*e.g. A. hippocastanum* L., or horse chestnut).

X. luteola is distributed all over Europe, northern Africa, the Caucasus and the near East (ROMANYK and CADAHIA, 2002). In the US, this beetle was introduced from Europe in 1830; the first report of the pest in California was near 1920 (ROMANYK and CADAHIA, 2002; MAISTRELLO *et al.*, 2005), and has develop since in one of the most serious urban forest insect problem in several states (CLAIR *et al.*, 1987; DREISTADT *et al.*,

2001), as well as in Canada (ROMANYK and CADAHIA, 2002), and Australia (LEOFE, 2002). It occurs also in Germany (WEGENER *et al.*, 2001; MEINERS *et al.*, 2005), Iran (SENDI *et al.*, 2005; SHEKARI *et al.*, 2008), Spain (MARTÍN *et al.*, 2001), Portugal (ESCADA *et al.*, 1979), and Argentina (DEFAGÓ *et al.*, 2006). In Chile, *X. luteola* was first detected in 1994 in the city of Los Andes, Valparaiso Region (SAG, 2005), although Askevold (1991) mentions that the first record of an individual of *X. luteola* goes back to April, 1982, in Ritoque (Valparaiso). From there, it has reached south down to the Araucanía Region (SAG, 2010).

The egg of this insect pest (Figure 1B) is small, oval, with an opaque creamy yellowish color. The larva grows up to 11 mm in length. When emerged, it has a brownish color. From the second stadium on, it has four longitudinal yellowish stripes on the dorsum. The pupa, approximately 6 mm in length, looks orange-yellow, and has strong thin hairs. The adult (Figure 1A), has a short olive-green oval body measuring 5 a 7 mm, with lines in el margin and centre of the elytra, and black spots on the thorax. The head is yellow, with two black spots, and black eyes. The filiform antennae are yellowish on the underside, and light brown in the upperside. The thorax is much wider than the head. The elytra are round in the apex and have a densely micro punctured and wrinkled surface. The legs are robust and yellow. The hibernating individuals present the body covered by short sparse hairs (DE LIÑÁN, 1998; ROMANYK and CADAHIA, 2002).

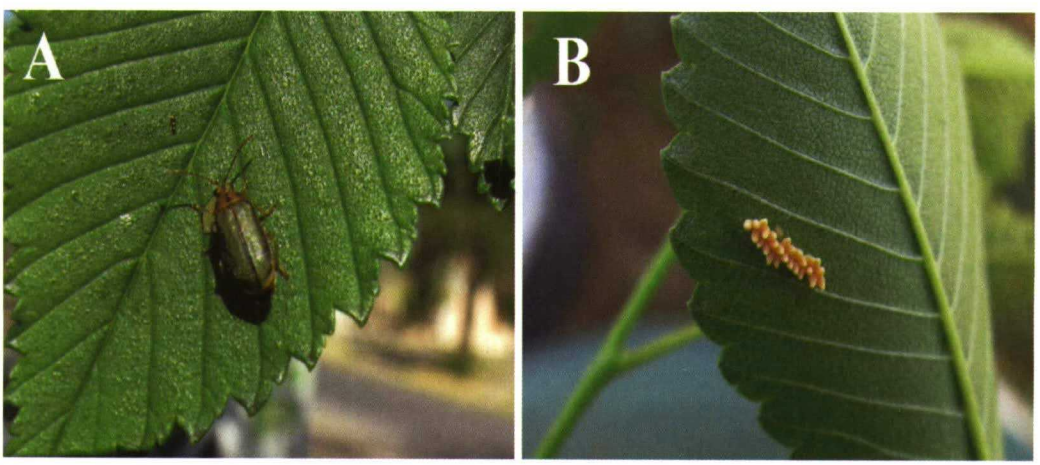

Figure 1. A) Adult of *X. luteola* affecting elm leaves. B) Eggs from *X. luteola* set on the underside o fan elm leaf

Both the adults and larvae of *X. luteola* feed on the parenchyma of the leaves, without consuming the veins; en occasionally all the leaves may be affected, and the trees look brown (MARTÍN *et al.*, 2001). If damage is severe and occur in several years, the trees develop a deformed canopy, vigour loss, physiological disorders, and reduce their photosynthesis, which leaves them susceptible to other pests, disease agents and stress factors. They become particularly susceptible to escolytid beetles carrying the spores of the fungus *Ceratocystis novo ulmi* Brasier, the cause of the Dutch elm disease, a disease threatening with the disappearance of elms (MARTÍN *et al.*, 2001; ROMANYK and CADAHIA, 2002; MUÑOZ *et al.*, 2003). Defoliation causes also the elimination of

shade in the summer, and reduces the aesthetical value of the trees (DREISTADT *et al.*, 2001).

Commonly the adults of *X. luteola* hibernate in diverse sites, in cracks in the bark, under plant residues in the base of the trunk, walls, homes or roofs. In spring they fly to the foliage, where they feed and lay 1-30 eggs, generally on the underside of the leaves. In approximately 8 days the larvae emerge and feed on the foliage, leaving only the veins; when reaching maturity, in two to four weeks, they descend the trunk to pupate. In about ten more days, the adults emerge near the base of the tree, and fly to the leaves to feed and lay their eggs, which initiates a new generation (DREISTADT *et al.*, 2001; ROMANYK and CADAHÍA, 2002; MUÑOZ *et al.*, 2003). In regions where the weather conditions are favourable, these beetles may reach up to three generations a year, as in Spain and Portugal (ESCADA *et al.*, 1979; MARTÍN *et al.*, 2001), and some areas in California (DREISTADT *et al.*, 2001).

Given the absence of information of the life cycle of *X. luteola* in Chile and the need to differentiate the sexes of the insect to pursue the integrated management of this pest, this study had the objective of determining its life cycle in Santiago, and the duration of the pupae and adults under laboratory conditions, and to establish the determination of sexes of the adult stage and their ratio.

MATERIALS AND METHODS

The study was conducted on individuals of *X. luteola* larvae and adults obtained from infested adult elm trees (n=35) in central and western municipalities of Santiago (33°32'S; 70°42'W), Metropolitan Region, central Chile, from July 2008 through June 2009.

Life cycle: The elms in the centre and western communes were visited weekly to sample their foliage and trunks to collect diverse stages of *X. luteola*, depending on the date. The information gathered in the field was used to elaborate a scheme of the duration of the stages in the cycle of the insect.

Duration of pupa and adult stages in the laboratory: Last stage larvae of *X. luteola* were collected from different elm trees during the summer of 2008, the season when they may be defoliated completely by the elm leaf beetle (De Liñán, 1998). These larvae were taken in cloth bags together with elm leaves for food, to the Forestry Entomology Laboratory, Department of Silviculture and Nature Conservation, College of Forestry and Nature Conservation Sciences, University of Chile, for rearing. The larvae were placed on Petri dishes with the bottom lined with filter paper slightly wet with distilled water, and fresh elm leaves as food until reaching the pupa stage. The pupae were provided only with humidity and a cover of leaves to set low light conditions. When emerging, the adults were fed fresh leaves. Both larvae and adults were maintained under natural light, at 22±3 °C and 61±4% RH. The duration of pupae and adults were recorded from 7 cohorts of 5 individuals each.

Determination of fenotypes of sexes and sex ratio of adults: Adults (n = 250) were observed exhaustively under stereoscopic magnification. Once individuals were separated empirically by sex, a sample of them (n= 25) were dissected and their processed on warm KOH at 10% v/v (CARRERA and OSUNA, 1996), to corroborate that the observations under magnification corresponded with those of the genitalia, thus obtaining a visual and non destructive method to identify males and females. When this process was completed, a random sample of other 250 adult individuals were separated by sex to determine the sex ratio.

RESULTS AND DISCUSSION

Life cycle of *X. luteola* in Santiago, Chile

In the study area, adults of *X. luteola* emerged alter hibernation at the beginning

of October, to feed and mate. The eggs deposited by the hibernated females appeared from the second week of October onwards. The duration of this first cycle of egg laying lasted about one month (from mid October through mid November). The first larvae emerged a week after the first eggs appeared. The life cycle is presented in Figure 2.

The larvae have three stadia, and undergo two moltings during their growth; they are initially yellowish and acquire gradually more definite yellow tones, with two lateral stripes of dark spots; the larval period lasted 3-4 weeks. Starting the second half of November and through mid December, prepupae and pupae were found, mainly at the base of elm trees, covered by litter. The first adults appeared at the beginning of December, initiating thus a second generation. The eggs and ensuing larvae developing in December and January gave rise to another generation of adults at the end of January (Figure 3).

The observations of the stages of development in the field allowed to elaborate the scheme presented in Figure 2, where a third and even a fourth generation appear on trees in the city of Santiago, with some adults of *X. luteola* through April, which hibernate until the weather conditions become again favorable.

In the study area, *X. luteola* had four generations a year, which coincides with observations by ESCADA *et al.* (1979) in Portugal, where this insect has generally three generations, but up to four cycles in exceptional cases, These authors stated that in the northern hemisphere the first generation develops from May through June, the second from

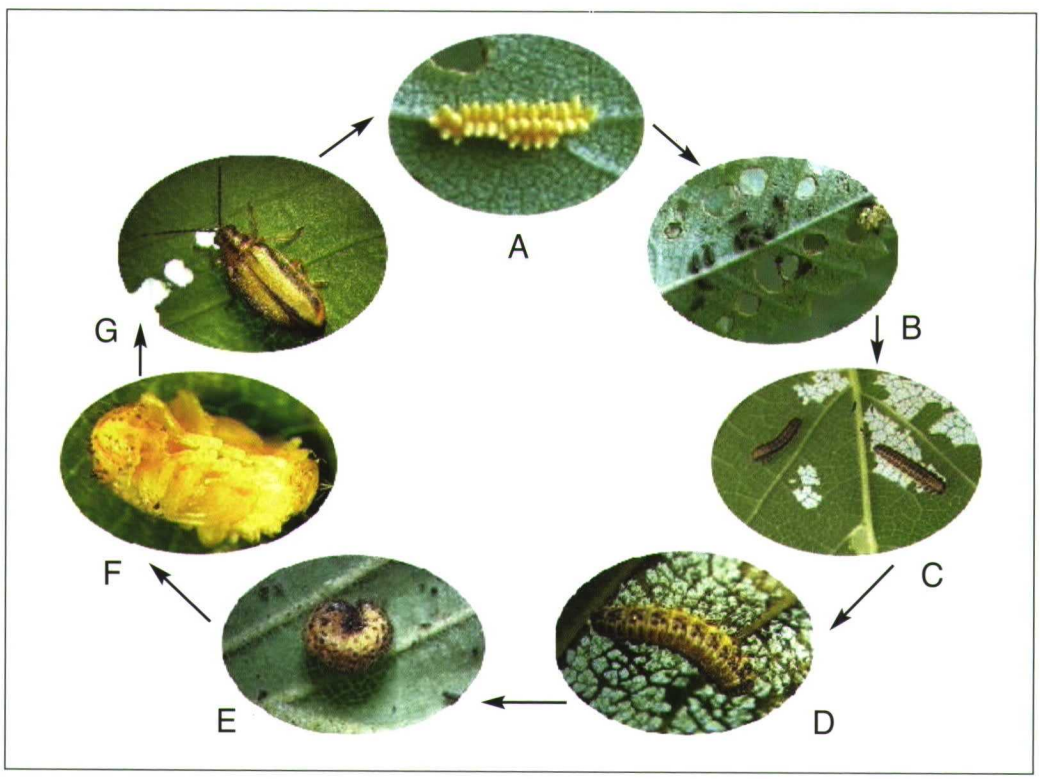

Figure 2. Stages and life cycle of *X. luteola* in Santiago, Chile. A) Eggs; B) First stadium larvae; C) Second stadium larvae; D) Third stadium larvae; E) Pre-pupae; F) Pupae; G) Adults

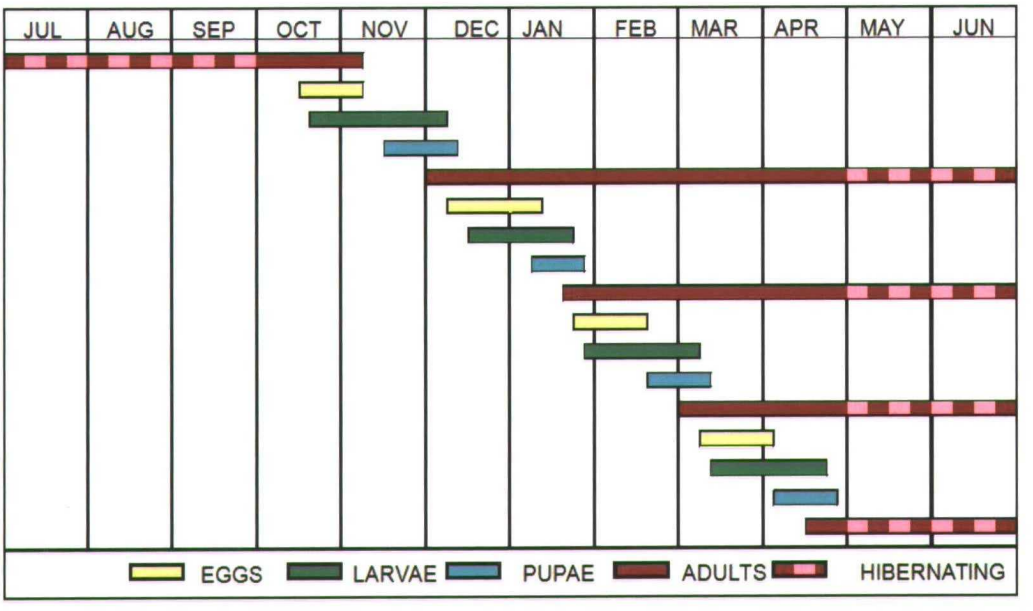

Figure 3. Life cycle of *X. luteola* in Santiago, Chile, 2008-2009

June through July, and the third from August through September, with a duration for each generation in that hemisphere very similar to the one to two months observed in Santiago, Chile.

In other regions of the northern hemisphere, this insect has one to three generations a year, depending on the weather conditions (DE LIÑÁN, 1998; MUÑOZ *et al.*, 2003). In northen California, *X. luteola* has at least one generation a year, and two to three generations in central and southern California, respectively (DREISTADT *et al.*, 2001). In Spain only two generations have been observed (MARTÍN *et al.*, 2001).

The 7 day duration of the pupae is similar to the 7.29 day period determined by FOLCIA *et al.* (2005), but shorter than the 10 days registered by ROMANYK and CADAHIA (2002), and DE LIÑÁN (1998). In their study of development of *X. luteola* juveniles at different temperatures, KING *et al.* (1985) found duration of 6.4 days for the pupae, at a 22-29 °C range. These differences with our results may be due to the slightly lower temperature range herein (18-25 °C), which may have lengthened their development.

Duration of pupae and adults in the laboratory

From the 7 *X. luteola* cohorts, the average duration in the laboratory of the pupae was 7 days and 21 days for the adults (Table 1).

The life span of the adult lasted considerably less (21 days) than the 26.29 days registered by Folcia *et al.* (2005). However, Hall (1986) found it to be 16.7 days, even lesser than our result, which leads us to conclude the life span of the adult of *X. luteola* varies a lot with the temperature.

Phenotypical determination of sex of adults and sex ratio of *X. luteola*

The detailed observation of adult individuals of *X. luteola* verified only small differences in morphology in males and females, in the 5th ventral sclerite (Figure 4).

Table 1. **Mean duration (days ± standard deviation) of pupae and adults of *X. luteola* in the laboratory**

	Duration (days ± SD)	
Cohorts	**Pupae**	**Adults**
C1	7 ± 2	23 ± 3
C2	6 ± 1	22 ± 4
C3	7 ± 1	23 ± 2
C4	8 ± 1	22 ± 1
C5	7 ± 2	17 ± 2
C6	7 ± 1	16 ± 3
C7	8 ± 2	21 ± 3
Means	7 ± 2	21 ± 3

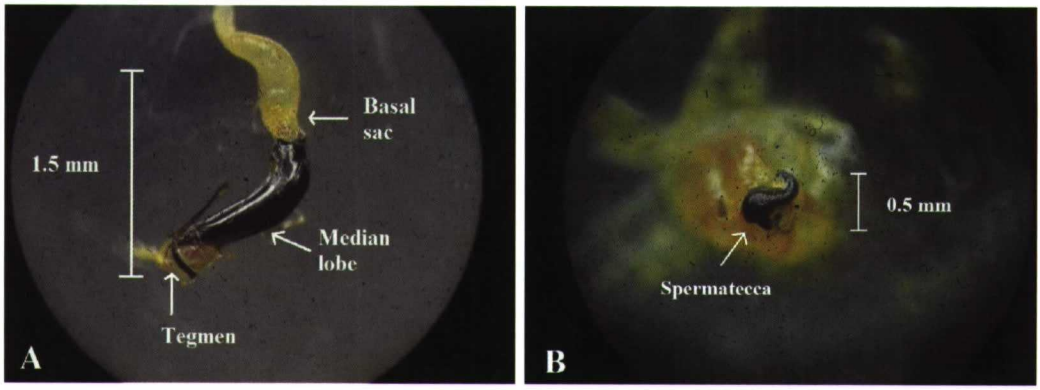

Figure 4. Ventral view of the abdomen of *X. luteola* adults. A.) Male; B) Female

The males (Figure 4A) presented a characteristic form in the borders of the 5th sternite (with a semi circular termination), together with the presence of a yellow internal sclerite (a light colour in the figure). In females (Figure 4B), the borders of that sternite were more abrupt, or straight, evidencing a noticeable groove complete don top by a generally dark sclerite, which allowed to differentiate them from the males.

Jackson y Jackson (2008) indicates that females of *X. luteola* present a subconical termination of their last tergite, giving them a larger form than in males. However, herein some females were difficult to identify using this character, on the contrary, its form was rather rounded, which make difficult to determine the sex using that description. Once the beetles were separated by sex using the phenotypical characteristics indicated above, a sample (n=25) of the adults were processed in 10% hot KOH to determine their genitalia to validate the recognition factor suggested (Figure 5).

The reproductive structure of males (*Aedeagus*) was observed under stereoscopic magnification, but not that of females because the soft tissues were destroyed during the process. However, the spermateca was still identified. These results corroborate the effectiveness of using the phenotypical keys to determine the sex of *X. luteola* adults.

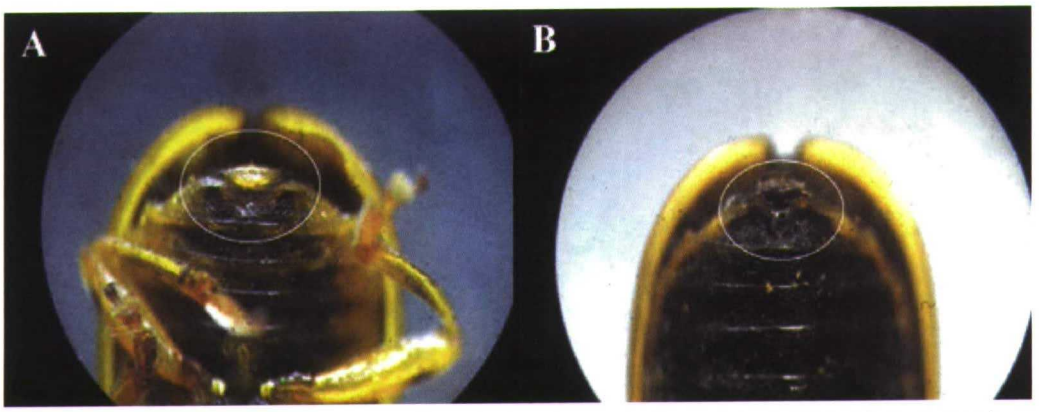

Figure 5. **Reproductive structures of *X. luteola* adults. A) Male (detail of the *Aedeagus*); B) Female (spermateca)**

Of the 250 adults of *X. luteola* examined, 52% were females (130 individuals), that is, approximately half of them, which gives a 1:1 sex proportion, different from that of 5:1 (98 males and 21 females) found by Jackson and Jackson (2008) in a sample of 119 beetles collected.

RESUMEN

HUERTA, I. CHIFFELLE, A., K. PUGA, F. AZÚA, R. JIMÉNEZ, J. E. ARAYA. 2011. Ciclo de vida de *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae) en Santiago de Chile y diferenciación fenotípica sexual de adultos. *Bol. San. Veg. Plagas*, **37**: 57-64.

Se hicieron muestreos periódicos de huevos, larvas, pupas y adultos de *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae) para determinar su ciclo de vida en olmos urbanos afectados en comunas de Santiago, Región Metropolitana, Chile. También se determinó la duración del estado de pupa y adulto en laboratorio. Se colectaron individuos adultos para determinar la razón sexual y estudiar las diferencias fenotípicas sexuales, que se corroboraron mediante análisis de genitalia. Para Santiago de Chile se determinaron cuatro generaciones de *X. luteola* al año, desde octubre hasta abril con una duración de uno o dos meses cada una, más un período de hibernación de adultos cercano a cuatro meses. Los individuos de *X. luteola* se criaron con éxito en laboratorio y la duración de sus estados de pupa y adulto fue similar a la encontrada en la literatura. Se establecieron diferencias sexuales fenotípicas entre adultos de *X. luteola*, que se ratificaron mediante análisis de genitalia, y se determinó una razón sexual de adultos de 1:1.

Palabras clave: vaquita del olmo, *Ulmus*, genitalia.

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