ECOLOGY, BEHAVIOR AND BIONOMICS

Life-History of The Guava Weevil, *Conotrachelus psidii* Marshall (Coleoptera: Curculionidae), Under Laboratory Conditions

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Ciclo Biológico do Gorgulho-da-Goiaba, *Conotrachelus psidii* Marshall (Coleoptera: Curculionidae), em Condições de Laboratório

RESUMO - O gorgulho-da-goiaba, *Conotrachelus psidii* Marshall, é uma das principais pragas da goiabeira no Brasil. As fêmeas ovipositam em frutos pequenos e as larvas desenvolvem-se acompanhando o amadurecimento dos frutos. Quando as larvas atingem o desenvolvimento máximo, abandonam o fruto severamente danificado e pupam no solo. Este trabalho foi realizado para determinar o ciclo biológico do gorgulho-da-goiaba em laboratório. Goiabas atacadas por gorgulhos foram coletadas em pomares e colocadas em caixas plásticas com areia umedecida. Depois da emergência, os adultos foram colocados individualmente em caixas de acrílico e lhes foi fornecido água e alimento. Os adultos imediatamente após a emergência foram colocados juntos para possibilitar os acasalamentos, depois dos quais, as fêmeas foram isoladas. Os ovos produzidos foram colocados em placas de Petri contendo lâminas de goiaba madura sobre papel de filtro umedecido. A eclosão das larvas foi monitorada diariamente e o número de ínstares foi determinado através da medição da largura da cápsula cefálica. A duração da fase ovo foi de 3,9 ± 0,58 dias. A duração da fase larval foi de 16,0 ± 3,80 dias, sendo identificados quatro ínstares. Larvas no máximo desenvolvimento (pré-pupas) enterraram-se no solo. A fase de pré-pupa foi de 142,0 ± 32,00 dias e o período pupal foi de 16,0 ± 0,90 dias. Os adultos permaneceram enterrados por 34,0 ± 18,00 dias e, após saírem do solo, viveram 148,0 ± 89,00 dias. A fecundidade variou entre 539 e 793 ovos/fêmea e a porcentagem de ovos férteis foi de 96.5%.

PALAVRAS-CHAVE: *Psidium guajava*, ciclo biológico, ínstar

ABSTRACT - The guava weevil, *Conotrachelus psidii* Marshall, is a severe pest of guava fruits in Brazil. The mated females lay eggs in small unripe fruits. As the fruits develop, so do the larvae. Mature larvae abandon the ripe fruits and pupate underground. Larval feeding causes extensive damage to the fruit. We conducted this study to understand the weevil biology under laboratory conditions. Weevil-infested ripe guava were collected in orchards and placed inside cages with moist sand. After emergence, the adults were individually placed in acrylic boxes with food and water. Recently emerged adults were also placed inside plastic boxes for mating, after which the females were isolated. The eggs were placed on moist filter paper in petri dishes containing slices of ripe guava fruit. Egg-hatching was monitored daily and the number of larval instars established by measuring the width of their head capsules. The egg incubation period lasted 3.9 ± 0.58 days. The larval period was 16.0 ± 3.80 days and four instars were identified. After burrowing in the soil, the mature larvae (pre-pupa) remained underground for 142.0 ± 32.00 days and then pupated. The pupal period lasted 16.0 ± 0.90 days, but the adults remained underground for a further 34.0 ± 18.00 days. After emerging from the soil, adults lived 148.0 ± 89.00 days. Fecundity varied from 539 to 793 eggs/female, and the percent egg hatch was 96.5%.

KEY WORDS: *Psidium guajava*, bionomics, instar

Guava, *Psidium guajava* L., is a fruit of the Myrtaceae family native to America and widely cultivated in many tropical and subtropical countries because of its great economic importance (Menzel 1985). In Brazil, important limitations for producing guava include diseases incited by fungi (Medina 1991), and insect pests, notably fruit flies, *Ceratitis capitata* (Macquart) and *Anastrepha* spp. (Cavalcante 1980) and the guava weevil, *Conotrachelus psidii* Marshall (Orlando *et al.* 1974, Sampaio 1975, Medina 1991).

As a serious pest of guava, the guava weevil has only been registered in Venezuela (Rubio 1974, Boscán de Martinez & Casares 1980) and Brazil (Bondar 1923, Sampaio 1975) where up to 80% of the fruits may be attacked in orchards untreated with insecticides (Orlando *et al.* 1974, Boscán de Martinez & Casares 1980). When recently attacked by the weevil, a hardened, black depression is formed externally on the unripe fruits at the egg laying site (Sampaio 1975) and the pulp dries and blackens internally.

Many aspects of the life-history of the following species of *Conotrachelus* have already been studied: *C. humeropictus* (Fiedler) on cupuassu, (D'Avila Lopes 2000), *C. nenuphar* (Herbst) on plum (Jacklin & Yonce 1970, Racette *et al.* 1991), *C. schoofi* (Papp) on *Carya* spp. (Tedders & Payne 1986) and *C. neomexicanus* (Fall) on *Pinus* spp. (Bodenham *et al.* 1976). With regard to *C. psidii*, besides the study undertaken by Boscán de Martinez & Casares (1981) on its seasonal occurrence in guava orchards, no other study dealing with its biology has been published.

Understanding the life-history of insect pests is essential for the development of better techniques for their monitoring and control. Thus, aiming to facilitate future studies for the monitoring and control of the guava weevil, by means of semiochemicals, this study was undertaken to develop a preliminary method of rearing and to elucidate some aspects of its life-history under laboratory conditions.

Material and Methods

Insects. Six hundred ripe guavas were collected displaying the characteristic signals of attack by the guava weevil in February 1999, in an orchard in "São José de Ubá" county, in the state of Rio de Janeiro. The fruits were placed in trays (60x40x10 cm) with 7 cm of moistened, sterilised sand in the bottom. The trays were taken to the Laboratório de Proteção de Plantas of the Universidade Estadual Norte Fluminense. Room conditions were maintained at 25 ± 3°C and 12:12 (L:D) photoperiod, with scotophase starting at 18:00h. Rotten or dried up fruits were eliminated daily. After one week, when all the fruits were eliminated, the sand was sifted and the guava weevil larvae recovered and counted., The larvae were placed immediately in smaller trays (30x20x10 cm) with moist sand in the bottom and maintained in the same room conditions until all the adult weevils emerged.

As they emerged from the sand, the adults were placed individually in boxes (Gerbox®, 10x10x5 cm). They were fed with slices of ripe guava fruit and water. The boxes, food and water were changed at two or three day intervals.

Weevils Mating and Egg Incubation. As soon as the adults emerged in the laboratory, they were placed daily inside the Gerbox® boxes (4/box) for 4h, in order to facilitate the observation of mating. When coupled, males and females were marked with water based ink. Then the females were confined in Gerbox® boxes with unripe fruits, slices of ripe fruit and water. The eggs were removed daily from the unripe fruits and placed individually on moist filter paper in petri dishes. The petri dishes were then covered and placed in an incubator (25 ± 1°C, 65-75% UR). The eggs were inspected daily and the incubation period consisted of the number of days elapsed from the removal of the eggs until the newly hatched 1st instar was seen.

Larval Period. The newly hatched 1st-instar larvae were individually placed in petri dishes with a guava slice (2x5x10 mm) as food. The slice was renewed and increased in size according to the development of the larva. The number of days comprising the 1st instar hatching and the abandonment of the guava slice by the fully grown larva (the larva stopped feeding and moved around searching for a place to pupate) was considered as the larval period.

During the larval period, the width of head capsules of the larvae (n = 200) were measured daily with the aid of an micrometer reticle coupled to a stereoscopic microscope and, at the same time, the presence of exuviae inside the petri dishes was also investigated. After measurement, the larvae were returned individually to the petri dishes with a new guava slice and maintained in an incubator (25 ± 1°C and 65-75% RH). Daily measurements and observations were made until the larvae became pre-pupae and then they were transferred individually to plastic cups (50 ml) with moist sand and placed in the same incubator. Pre-pupae measurements continued monthly until pupation. The number of instars was established by means of the graphic method (Taylor 1931) and confirmed by the confidence intervals of the measurements.

Pre-Pupal and Pupal Period (Larva Underground). The fully grown larva (pre-pupa) were arranged individually between two guava slices and placed in a plastic cup (50 ml) over a layer (4 cm) of moist sand. To confirm the pupation, the larvae were inspected daily and returned to their respective containers. The number of days comprising the day that the larvae abandoned the fruit and burrowed in the sand until pupation was considered as the pre-pupal period. The number of days elapsed between pupation and the emergence of the adult was considered as the pupal period.

Adult Underground (Inactive Adults). The adult underground period was considered as the number of days that the adults stayed buried in the sand.

Fecundity, Fertility and Longevity. After emerging from the sand, the adults were maintained under the same conditions as those originated from larvae collected in the field but fed with the better food indicated in the feeding test. They were mated at regular intervals (25 - 30 days) during their lifetime. To determine fecundity, fertility and longevity, after mating each female (n = 10) was confined in a plastic container (Gerbox®, 10x10x10 cm) with a slice (0.5x1x2 cm) of ripe guava and a fresh unripe fruit, which were substituted periodically.

Results

Insects. Out of the 600 collected fruits, 120 fully grown larvae were found buried in the sand. These pre-pupae suffered high mortality, since only 40 adult weevils were obtained. The reason for this high mortality was not clear, but might be due to the involuntarily excessive drying of the sand.

Weevil Mating and Egg Incubation. Except for four eggs, all eggs were found in the egg chamber built by the females. At the moment of removing the eggs from the unripe guava fruits, the eggs were ovoid shaped, turgid, pearly-white and measured 0.7 ± 0.06 mm by 1.2 ± 0.06 mm on average. Within 36h, the mandibles of the larvae could be visualized. At the end of the third and beginning of the fourth day the larval head capsules and mandible movements were observed inside the eggs. The incubation period lasted 3.9 ± 0.60 days (Table 1).

Larval Period. The newly hatched 1st-instar larvae, (1.5 ± 0.15 mm of length) began to fed immediately after hatching and remained feeding inside the fruit until the end of the 4th instar when they abandoned the fruits and burrowed into the sand after 24h to 48h. The duration of the larval period was 16 ± 3.80 days (Table 1).

The graphic method allowed the establishment of four well defined frequency peaks at 0.44; 0.61; 0.83 and 1.19 mm of width of head capsule, indicating four larval instars (Fig. 1). Table 2 shows the measurements of the larvae, confirming the result. The average measurements of each instar was similar to the value indicated by the frequency peaks shown in Fig. 1. The growth ratio (Dyar's Rule) was practically 1.4 for all instars (Table 2). The duration of each of the four instars is shown on Table 3. The 4th instar was of considerable length, because after abandoning the fruit the larvae buried themselves in the sand where they stayed for more than four months without moulting into pupae.

Pre-pupal and Pupal Period (Larva Underground). The duration of the pre-pupal period was 142 ± 32 days. The pupal period of 16 ± 0.9 days was shorter and less variable than the pre-pupal period (Table 1).

Adults Underground. After the pupal period the newly emerged adult weevils continued buried in the sand for a period of 34 ± 18 days, however, this period was quite variable, from 6 to 71 days (Table 1).

Fecundity, Fertility and Longevity. The first and the last adult weevil emerged from the sand, approximately, five and ten months, respectively, after the larvae became buried in the sand. After their emergence from the sand, the adult weevils remained sedentary, rarely feeding, and did not mate until the end of the second week, when the first courtship behaviour was observed. From the third week onward, the weevils intensified their mobility, feeding, mating, and oviposition started two days later. During the reproductive period the females mated several times (at least five times). The maximum oviposition was 15 eggs/female/day and the number of eggs laid (fecundity) varied from 539 to 793 eggs/female during a reproductive period of four to ten months, respectively. The percent egg hatch (fertility) was 96.5%. The average adult longevity was 150 days, but was quite variable, ranging from 3 to 418 days. The oldest individual was still alive after 13 months (Table 1).

Table 1. Length in days of different development stages of *C. psidii* reared at 25 ± 1°C and a photoperiod of 12:12 (L:D) h.

	Development stages (days)					
	Egg	Larva	Pre-pupa	Pupa	Adult	
					Inactive[1]	Active
Mean ± SD	3.9 ± 0.60	16.0 ± 3.80	142.0 ± 32.00	16.0 ± 0.90	34.0 ± 18.00	153.0 ± 92.00
Range	2 - 6	8 – 27	84 - 229	14 – 18	6 – 71	3 - 418
n	400	306	65	60	55	52

[1]Adult underground

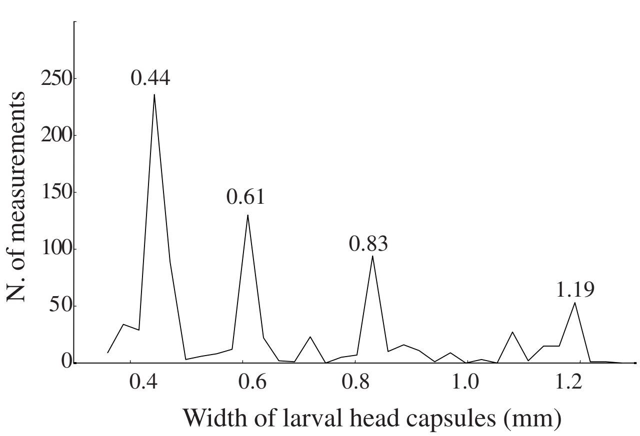

Figure 1. Frequency distribution of head capsule width in different larval stages of *C. psidii* reared at 25 ± 1°C and 65-75% RH.

Discussion

The forty guava weevils adults obtained from the 120 field collected larvae represented a survival rate of 33.3 percent. If the mortality was a consequence of the low moisture content of the sand substrate as it appears to be, it could be substantially reduced by the proper moisture maintenance. This factor, as well as the temperature, is considered by Jacklin & Yonce (1970) to be decisive for the survival of *C. nenuphar* in the laboratory.

The incubation period, of almost four days was similar to the incubation period of other species, such as: four to five

Table 2. Width of the head capsule in different larval stages of *C. psidii* reared at 25 ± 1°C and 65-75% RH.

	Width of head capsule (mm)			
	1st instar	2nd instar	3rd instar	4th instar
Mean ± SD	0.44 ± 0.03	0.59 ± 0.04	0.84 ± 0.07	1.15 ± 0.09
Confidence Interval	0.43 – 0.45	0.58 – 0.61	0.81 – 0.87	1.11 – 1.21
n	200	74	50	44
Growth ratio	1.34	1.42	1.36	Mean: 1.37

Table 3. Length in days of different instars of *C. psidii* instars reared at 25 ± 1°C and 65-75% RH.

	Duration of ínstars (days)			
	1st instar	2nd instar	3rd instar	4th instar
Mean ± SD	2.7 ± 0.60	3.8 ± 1.20	3.5 ± 1.10	171.0 ± 26.00
Range	1-5	1-5	1-8	127-235
n	200	74	50	35

days for *C. neomexicanus* (Bodenham *et al.* 1976); 4.5 days for *C. schoffi* (Tedders & Payne 1986).

Unripe guava was the best diet for the larvae, especially for preventing high mortality of the 1st instars. Furthermore, we observed a preference of the 1st instars for the green part of the fruit (close to the pericarp). Ripe fruits were not suitable for 1st instars, because their mortality increased as a consequence of an attack by entomopathogenic fungi, facilitated by the higher moisture in the fruits. Last instars should be fed with ripe guava, because it was noticed that the larvae fed with ripe fruits developed faster (8 days to become pre-pupa). For this reason, in the course of the study, we decided to feed the 1st and 2nd instars with unripe fruits and the other instars with ripe fruits. Based on field observations by Boscán de Martinez & Casares (1980), we believe that the great variability of the pre-pupal period observed can be attributed to the feeding of the larvae, because the degree of ripening of the fruits offered as food was only controlled with the progress of this study.

The duration of the larval period of 16 days does not coincide with the duration observed in the field during our regular collections of guava fruits infested with the weevils. Under natural conditions, the larvae usually start their development in small fruits (< 4 cm diameter) that take more than two months to ripen, the moment at which the larvae abandon the fruit to pupate underground. However, the average of 142 days for the pre-pupal period is included in the value of 120 to 210 days that we deducted from the guava weevil seasonal occurrence data obtained by Boscán de Martinez & Casares (1981).

The period that the weevils remained underground as adults may be due to the need for completing the sclerotization process and the absence of a stimulus for their emergence from the soil. The stimulus could be provided by precipitation (Boscán de Martinez & Casares 1981), which would also facilitate the weevils exit through the soil.

The reason for such a large variation in the adult longevity is not clear. However, male and female data were grouped together, and food quality and excessive manipulation might also account for variability. Therefore, it is necessary to confirm the longevity under natural conditions, but it may well be superior to 150 days, allowing the same reproductive females to infest two consecutive crops. Although the floral buds and small fruits are considered as sources of the adults' food in the field (Boscán de Martinez & Casares 1980), ripe guava was their sole source of nourishment in the laboratory.

There was little variation in the results of head capsule measurements of each instar, and no superposition of their confidence intervals. Similarly *C. nenuphar* (Amis & Snow 1985), *C. neomexicanus* (Bodenham *et al.* 1976) and *C. humeropictus* (D'Avila Lopes 2000) *C. psidii* developed through four larval instars. The 1.37 average growth ratio is close to the growth constant of Dyar's rule (Dyar & Rhinebeck 1890), that states that after each moult the growth increases geometrically with a constant ratio for each species, which on average is by a factor of 1.4.

The efficiency of our rearing technique could be improved with few alterations. The correct feeding of the larval instars and the control of the moisture content of the sand when the weevil is underground (pre-pupa, pupa and adult) were the crucial factors. Considering these factors, perhaps 60% of the weevil eggs could render adult progeny. This value is close to the obtained by Yonce *et al.* (1971) in their study of artificial and fresh fruit based diets for *C. nenuphar*.

Until further studies are carried out to achieve greater success in the rearing of *C. psidii* in the laboratory, our technique and the data obtained will contribute to the study of behaviour and control which are urgently needed for the establishment of a management program to the control this pest.

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