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DETERMINATION OF THE LIFE CYCLE OF *SCYPHOPHORUS ACUPUNCTATUS* (COLEOPTERA: CURCULIONIDAE) UNDER LABORATORY CONDITIONS

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ABSTRACT

In this study of the development cycle of *Scyphophorus acupunctatus* Gyllenhal, during the month of Feb the incubation period of eggs averaged of 5.9 d. There were 6 instars, and larval development was completed in an average of 34.9 d. Adults lived an average of 413.8 d. During Sep, eggs hatched in 5.5 d, and there were 8 instars, lasting 54.2 d; adult longevity averaged of 433.7 d. There were 7 instars. The size of the head capsule was 0.7 mm for L1 and up to 2.8 mm for L7. Measurements of head capsule width used to determine instar in the field fell into 9 numerical groups, indicating there are 9 well-defined larval stages for *S. acupunctatus*. There is high mortality in the egg stage and of larvae in the first stages; while in the final larval stages and in the emergence of the adults there is a long period of stability in which the mortality is reduced to the minimum, and increases noticeably at the end of the adult stage.

RESUMEN

En este estudio de ciclo de desarrollo se observó que para el período de febrero la incubación de los huevos fue de 5.9 días en promedio, se detectaron seis estadios larvales, con una duración de 34.9 días, mientras que la longevidad del adulto fue de 413.8 días en promedio. En el periodo de septiembre la incubación de los huevos fue de 5.5 días, con ocho estadios larvales, que tuvieron una duración de 54.2 días; la longevidad del adulto fue de 433.7 en promedio. Se determinaron siete estadios larvales para *S. acupunctatus*. El tamaño de la cápsula cefálica que fue de 0.7 para L1 hasta 2.8 mm, para L7. Con el método de medición de la anchura de cápsula cefálica de larvas obtenidas en campo para determinar los estadios larvales, se determinaron nueve grupos numéricos, lo que indica que los valores representan a nueve estadios larvales bien diferenciados para *S. acupunctatus*. En las curvas de sobrevivencia se observó que hay una alta mortalidad en la etapa de huevo y de larvas en los primeros estadios; mientras que en los últimos estadios larvales y en la emergencia de los adultos se presenta una etapa larga de estabilidad en la que la mortalidad se reduce al mínimo y después aumenta marcadamente al final de la etapa adulta.

Translation provided by the authors.

In the state of Morelos, Mexico, the cultivation of ornamental plants is an important component of the economy. The warm climate of the southern and central zones of this state (18°26' to 18°52' and 900-1330 msnm) favors commercial production of ornamentals such as the tuberose, *Polianthes tuberosa* L. (Liliales: Agavaceae). The black weevil *Scyphophorus acupunctatus* Gyllenhal, is a major pest of this plant, infesting 51% of the bulbs (Camino et al. 2002). This insect also has been reported as an important pest of other commercial crops (*Agave tequilana* Weber, *Agave americana* var. *expansa*) (Valenzuela 1994; Waring & Smith 1986), as well as wild yuccas and agaves (Pott 1975; Morton & Dawling 1992).

The weevil is considered a multivoltine species with generations overlapping throughout the

year (Solís et al. 2001; Waring & Smith 1986). The larvae as well as the adults feed on the bulbs of tuberose, where they form a series of chambers before they pupate. Pupation occurs within a cocoon formed from fiber and mud. After emergence, the adults remain among the bulbs where they copulate, and where the females lay eggs (Hernández et al. 2001). In addition to the damage caused by the boring of larvae in the plant tissue, some phytopathogens associated with the weevil may cause wilting and later death of the plant (Waring & Smith 1986).

Siller (1985) studied development of the weevil in the laboratory on *Agave atrovirens* and found that on this food plant the incubation period of the eggs was 8 d, the average duration of the larval stage was 58 d and the average pupal period

was 13 d, and the total period from oviposition to adult emergence was 81 d. Lock (1969) indicated that the larval stage of this species may have a duration of 21 to 58 d with 5 instars on sisal depending on the relative humidity of the environment. In henequen (*Agave fourcroydes*), Ramírez (1993) reported 11 larval stages with a life cycle averaging 108 d. This differs, however, from the life cycle duration observed in tuberose.

The objective of the present study was to determine the life cycle of the black weevil *S. acupunctatus* feeding on tuberose bulbs under laboratory conditions, and to develop life tables, which are useful for understanding the population dynamics (Cividanes 2002) of a species and to study some aspects of the biology of the insect (Kazak et al. 2002). This information will support development of better strategies for management of this pest.

MATERIALS AND METHODS

Study Site

The work was done in the Entomology Laboratory of the Plant-Insect Interactions Department, Biotic Products Development Center, Instituto Politécnico Nacional in Yautepec, Morelos, Mexico. A Precision model 818 incubator (USA) was used to maintain the following conditions: temperature of $27 \pm 2^\circ\text{C}$, relative humidity 60% and a photoperiod of 12-12 h.

Insects

Laboratory cultures of *S. acupunctatus* were established with weevils in various stages of development from collections in a commercial tuberose plantation in the Municipality of Emiliano Zapata, Morelos. In the laboratory, the larvae and adults were fed separately on tuberose bulbs, until F2 adults were obtained. Eggs were then collected from 45 pairs of adults.

Development Cycle

The study was done during 2 periods, one beginning in Feb 2000 and the other beginning in Sep 2001. Life tables were used to estimate the duration of the different stages of the life cycle. A cohort of 64 eggs was used for the period beginning in Feb 2000 and 59 eggs for the period beginning in Sep 2001. In both studies, the eggs were separated and placed on moistened filter paper in plastic Petri dishes (60 mm in diameter). These dishes were held in the incubator at constant temperature and relative humidity. Observations were made daily during the incubation period.

As they emerged, each larva was placed in a hole 3 mm deep made with a dissecting needle in a 5-g tuberose bulb. Larvae were transferred to

these holes with a No. 0 camel's hair brush, and the bulbs containing larvae were placed in 70-ml plastic containers with small perforations in the cover to allow for air circulation to prevent accumulation of excess moisture. The containers with the bulbs and larvae were placed in the incubator at the specified environmental conditions. The bulbs were replaced every 3 d so that the larvae would always have fresh food. As the larvae grew (stages 5 to 8) heavier bulbs were used (15 to 17 g). Observations were made daily and the following events were recorded: successive larval molts, pupation, adult eclosion, and mortality. Pupae were retained in the plastic containers in which they had developed, but the bulbs were removed. The pupae were inspected daily and changes recorded until adult eclosion. The adults obtained were fed in groups with tuberose bulb and their longevity was recorded. The life table and survivorship curves were constructed from these observations. The Mann-Whitney test was applied ($P \leq 0.05$) to compare the development cycle in the 2 periods of the year.

The number of larval stages was determined by 2 methods: (a) direct observation and quantification of larval molts in the laboratory and (b) quantification of the width of the head capsule of 400 larvae obtained in field collections. Direct observations in the laboratory were made daily and molts were recorded. Molts were indicated by the presence of exuviae, in which the head capsule was the most evident structure. The width of each head capsule was measured at its widest point. For the second method, 400 larvae collected in the field were fixed in alcohol at 70%, and then were classified by the different sizes detected and the width of the cephalic capsules. The measurements in both methods were done with the aid of a vernier and a Nikon stereoscopic microscope. The data were used to construct a frequency distribution for graphic representation. For this purpose, the number of intervals was determined by the equation proposed by Sturges: $K = 1 + 3.322 \log n$, where K is the number of class intervals and n is the number of values in the data set. In addition, the amplitude of the interval was calculated by the relationship, $W = R/k$, where R is the amplitude and W is the dimension of the class intervals (Pagano & Gauvreau 2001).

RESULTS AND DISCUSSION

Description of the Developmental Stages

Recently laid eggs are white, turning yellow as the embryo develops. They are elongate measuring 1.2 to 1.5 mm in length and 0.5 to 0.6 mm diameter (Fig. 1a); incubation requires 4-5 d. The larvae are legless, yellowish white in color (Fig. 1b). Recently hatched larvae are the same size as the egg, with spiracles on the abdominal

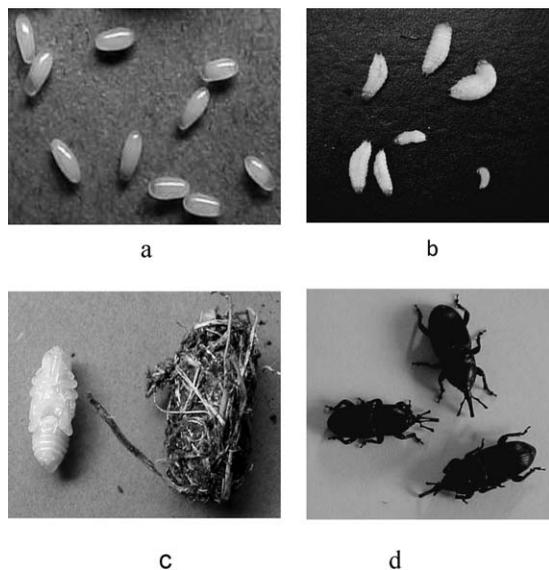


Fig. 1. Developmental stages of *S. acupunctatus* (a) eggs, (b) larvae of different stages, (c) pupa and cocoon (d) adult.

segments. Seta of the meso- and metathoracic epipleurites are subequal in length; the back edge of abdominal segment 9 with a pair of projections (longer than wide), each one with 3 elongated setae (Woodruff & Pierce 1973). Larvae develop through 7-9 instars with an average development time of 34.9 to 54.2 d. In this stage great losses occur, given that it passes its entire larval stage feeding on the interior of the tuberose bulb. The larva in the final stage forms a cocoon, with fibers of the tuberose bulb or with mud. In the prepupal stage, it stops feeding (it still has the larval form), is almost immobile, and makes only circular movements of its terminal segments, passing from 1 to 2 d in this stage.

The pupa is exarate, measuring an average of 16 mm in length, cream colored, turning dark as it matures (Fig. 1c). The pupal stage lasts 9-10 d. Developing wings are apparent on the dorsal side of the pupa, and the head, snout, eyes, and antennae on the ventral side. In the thorax the legs can be seen in process of formation. Adults are robust and shiny black, finely punctate, measuring 12-15 mm in length, (Fig. 1d), without scales or dorsal setae, elytra striated without pubescence; the face (snout) is thick and curved, nearly as long as the pronotum, antenna geniculate, inserted at the base of the snout, and the antennal club corneous is compact with the apical part of a spongy appearance and concave. The adults are relatively long lived, surviving from 414 to 433 d under laboratory conditions. The observed sex ratio (male:female) was 2:1. The eggs are laid singly in small perforations made by the female with the

snout in the tuberose bulbs and covered with a sticky substance that dries after a few minutes, turns black, and hardens. This cover protects the larva when it hatched and begins to boring into a bulb. This description agrees with that of Siller (1985) for the same species.

Development Cycle

For the period of development in Feb 2000, the eggs hatched on average at 5.9 d. Six 6 instars were detected, with larval development requiring 34.9 d, and longevity of adults was an average of 413.8 d. In Sep 2001 eggs hatched in 5.5 d, and there were 8 instars. Larval development occurred in 54.2 d and adults lived an average of 433.7 d (Table 1). The results indicated a significant difference between the 2 periods of the year in the duration of the egg, larval, and adult stages. These differences are probably due to the fact that although the insects were maintained under controlled conditions of temperature and humidity, the environmental conditions were different at the start of the observations, given that in Sep (the rainy season) the humidity and temperature are higher than in Feb. The prepupal and pupal stages are probably less vulnerable or sensitive to the influence of environmental factors, because they are within the cocoon and their metabolism is diminished.

These results agree, except for the number of instars, with those reported by Siller (1985), who pointed out that in pulque maguery *A. atrovirens* the incubation period of the eggs varies from 3 to 8 d, with 3instars that require 58 d to complete their development in the fall period. Lock (1969) indicated that the larval stage in sisal *Agave sisalana* depends on the moisture content in the environment and can require a duration of 21 to 58 d with five instars, whereas in the henequen crop *Agave fourcroydes*, Ramírez (1993) reported 11 instars with a life cycle duration of 108 d on average. In addition, he pointed out that the life cycle duration can be affected by nutrition, and the pupal stage required approximately 3 d.

TABLE 1. DURATION IN DAYS (AVERAGE \pm STANDARD ERROR) IN THE DEVELOPMENT STAGES OF *S. ACUPUNCTATUS* FOR 2 SEASONS OF THE YEAR.

Stages/periods	Feb	Sep
Egg	5.9 \pm 0.10*	5.5 \pm 0.07
Larva	34.9 \pm 0.24*	54.2 \pm 0.26
Prepupa	2.2 \pm 0.06	2.1 \pm 0.07
Pupa	9.7 \pm 0.08	9.7 \pm 0.07
Adult longevity	413.8 \pm 4.68*	433.7 \pm 5.74

*Significant differences are indicated for each stage when comparing the 2 periods of the year (Mann-Whitney $n_1 = 64$, $n_2 = 59$. $P \leq 0.05$).

Determination of the Number of Instars

Based on direct quantification of molts and width of cephalic capsule of laboratory larvae, 7 instars were determined for *C. acupunctatus*. The size of the cephalic capsule was 0.7 mm for L1, and up to 2.8 mm for L7 (Table 2). The average larval life was 47 d, while the pupal stage had an average duration of 10.5 d. Based on cephalic capsule widths on larvae obtained from the field, we observed nine numerical groups, indicating 9 well differentiated instars for *S. acupunctatus* (Fig. 2). These data do not coincide with what was reported by Siller (1985) in pulque maguey, in which he found 3 instars after measuring the cephalic capsules.

One disadvantage of the daily inspection of insects is that larvae can be damaged by manipulation, exposure to light, and loss of moisture. Because they are so small, the exuviae of the larvae of the 2 instars can be lost among the debris or the decayed parts of the bulb.

The difference with respect to the number of instars and the width of the cephalic capsule (Table 2 and Fig. 2) from our laboratory study compared with the measurement from the field population is that the field population was not manipulated on a regular basis, and cephalic capsules are larger in the insects collected in the field and smaller in the insects maintained in the laboratory. Our study indicates that the number of instars in this species varied from 6 to 9 instars. According to Salas & Frank (2001), this is due to the fact that Curculionidae, in contrast to other families of insects, do not present a fixed number of larval stages. Thus Hill (1983) reported 5 instars lasting 21 to 53 d for *S. acupunctatus* feeding on sisal *A. sisalana*, whereas Ramírez (1993) found that in henequen *A. fourcroydes*, the cycle is completed in an average of 108 d with 11 instars, and mentioned that this can be affected by nutrition during the larval stage. In contrast, Siller (1985) and Ramos-Elorduy et al. (1986) indicated 3 instars for this weevil in pulque maguey *A. atrovi-*

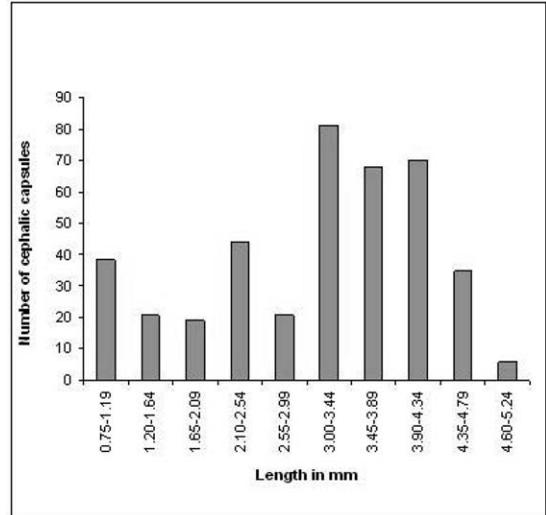


Fig. 2. Frequency distribution of head capsule width in larvae of *S. acupunctatus* $n = 400$.

rens. These results indicate that *S. acupunctatus* is able to vary the number of larval stages, larval length, and width of head capsule according to the type of host and environmental conditions such as nutrition.

Life Table

In the life table of *S. acupunctatus* for the 2 seasons, the highest values of life expectancy for both cases are found in the final instars, in the pupal stage, and at the beginning of the adult stage. In this case we believe that the key mortality factor was the manipulation of the individuals in the experiments. The survival curves show that there was high mortality in the egg stage and early instars. On the other hand, in the final instar and in the emergence of the adults there is a long period of stability in which mortality was reduced to a minimum, but later increased considerably at the end of the adult stage, which lasted for up to 445 d in the Feb study and 475 d in the Sep study (Fig. 3). The above indicates that the resulting curves could be placed between type III and type IV, respectively, according to Krebs (1985), because according to these curves, the mortality is concentrated in the early stages of development, while in the adult phase the mortality tends to be lower. According to the results obtained, the tuberose black weevil could be catalogued as a “K” strategist, along with the black banana weevil *Cosmopolites sordidus*, which has a prolonged life span, given that the adults can live more than 1 year and is considered to have “K” strategy, according to Gold & Messiaen (2000).

TABLE 2. MEASUREMENTS OF THE CEPHALIC CAPSULE OF THE LARVAL STAGES OF *S. ACUPUNCTATUS*.

Stage	Cephalic capsule mm
L1	0.7
L2	1.4
L3	1.5
L4	1.7
L5	2
L6	2.4
L7	2.8

Beginning of the study Feb of 2000, $n = 64$.

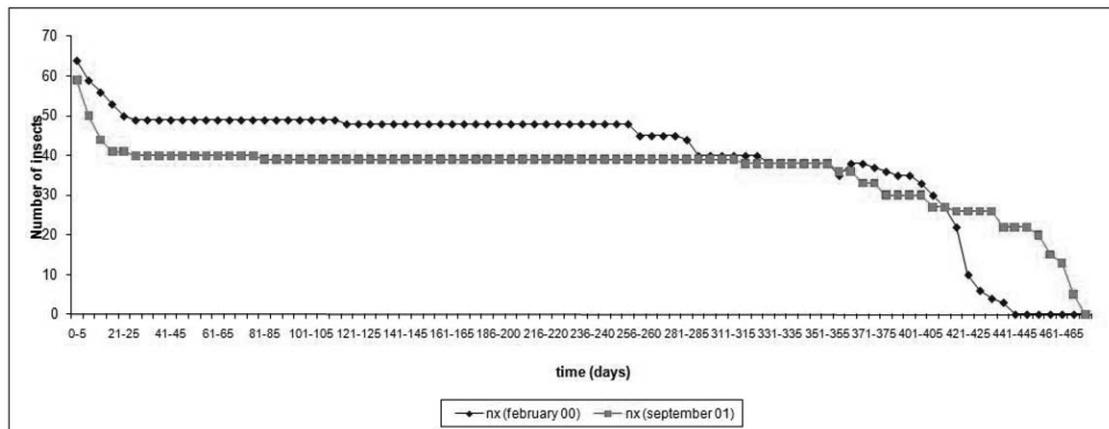


Fig. 3. Survival curve of *S. acupunctatus* under laboratory conditions (temperature of $27 \pm 2^\circ\text{C}$ relative humidity 60%).

The results obtained in this work are very important for designing a management program in which several strategies are used within a program of integrated pest management, according to the biology of the species in different hosts, and regions with different conditions temperature and humidity.

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