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Food quality influences ovarian development in *Scyphophorus acupunctatus* (Coleoptera: Dryophthoridae)

Maritza Vega-Petlcalco¹, René Arzuffi¹, Jorge Valdez², Mario Rodríguez-Monroy¹, Alfredo Jiménez-Pérez¹, and Norma Robledo^{1,*}

Abstract

This paper describes the anatomy of the female reproductive system of the agave weevil, *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Dryophthoridae). This insect is considered a primary pest of agave (*Agave* spp.) (Asparagaceae) and tuberose (*Polianthes tuberosa* L.) (Asparagaceae) in Mexico. Developmental biology and ovarian maturation of laboratory-reared *S. acupunctatus* fed a meridic diet was compared with individuals fed tuberose bulbs. Ovarian maturation was classified according to ovariole and follicle differentiation, as well as the presence of follicular relics. This classification yielded 1 parous and 3 nulliparous stages. We found that ovarian maturation was delayed for those weevils fed the meridic diet compared with those fed tuberose bulbs. Also, the greatest number of ovarian follicles occurred in individuals fed tuberose bulbs; however, this relationship changed with the age of the insect. Proximal length and follicular area also varied according to age and type of diet. We believe that the ovarian developmental stages described here for *S. acupunctatus* could be a useful method to monitor parity status of field populations when assessing the effectiveness of control programs for this weevil pest.

Key Words: female reproductive system; *Polianthes tuberosa*; agave weevil; meridic diet; tuberose bulbs

Resumen

En este trabajo se describe por primera vez la morfología del sistema reproductor femenino del picudo del agave, *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Dryophthoridae). Este insecto es considerado la principal plaga del agave (*Agave* spp.) (Asparagaceae) y nardo (*Polianthes tuberosa* L.) (Asparagaceae) en México. Se comparó el desarrollo biológico y la maduración ovárica de individuos alimentados con dieta meridica y los alimentados con bulbos de nardo. La clasificación de la maduración ovárica se basó en la diferenciación de ovariolas y folículos, la presencia de reliquias foliculares u ovulación. Esta clasificación comprende 1 paro y 3 estadios nuliparos. Encontramos que los picudos alimentados con dieta meridica sufren un retraso en su maduración ovárica en comparación con los alimentados con nardo. Además, el mayor número de folículos se presentó en individuos alimentados con nardo. Sin embargo, esta relación cambió con la edad. La longitud y el área del folículo próximo se modificaron dependiendo de la edad y el tipo de alimento. La información presentada en este estudio permite conocer la madurez sexual de *Scyphophorus acupunctatus* obtenidos del campo, la cual proporciona una valiosa guía para monitorear la efectividad de las acciones de su manejo.

Palabras Clave: sistema reproductor femenino; *Polianthes tuberosa*; picudo del agave; dieta meridica; bulbos de nardo

Knowledge of the relationship between age and physiological maturity of adult insects in a population allows one to determine reproductive maturity, develop life tables, predict population fluctuations, calculate dispersion rates, and develop prediction models for the management of insect pests (Tyndale-Biscoe 1984). Age and physiological maturity is determined by changes in the reproductive system, as well as somatic cells (fat body, cuticle, malpighian tubules, trachea, etc.), or external factors (e.g., “wear and tear”) (Tyndale-Biscoe 1984; Hayes & Wall 1999; Klowden 2007). Understanding this relationship helps us better comprehend population structure and identify periods when control tactics would be most successful.

The agave weevil, *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Dryophthoridae), is considered a primary pest of agave (*Agave* spp.) (Asparagaceae) and tuberose (*Polianthes tuberosa* L.) (Asparagaceae) in Mexico (Solís-Aguilar et al. 2001; Camino et al. 2002; Figueroa-Castro et al. 2013). Currently, we maintain a colony of this weevil in or-

der to develop and evaluate pest management methods for its control. Continuous mass rearing of this species under laboratory conditions is possible using an artificial meridic diet rather than rearing individuals on tuberose bulbs (Valdés-Estrada et al. 2012). However, it is unknown if ovarian development is affected by the artificial diet. Here we describe the sequence and stages of ovarian maturation in virgin *S. acupunctatus* weevils, as well as the influence that meridic and tuberose bulb diets may have on this developmental process.

Materials and Methods

INSECTS

Initially, *S. acupunctatus* larvae were obtained from tuberose crops in Mazatepec, (18.7334778°N, 99.3172222°W) and Yauhtepec

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(18.8289444°N, 99.09291667°W), Morelos, Mexico, from Dec 2015 to Dec 2016 and reared on tuberose bulbs in the laboratory. Upon emergence, adults were maintained in an environmental chamber at 25 ± 2 °C, 60% RH, 12:12 h (L:D) photoperiod. Individuals were separated by sex based on the criteria of Ramírez-Choza (1993) and females were kept individually in 30 mL plastic containers.

OVARIAN MATURATION

To determine the effect of diet on ovarian maturation, virgin *S. acupunctatus* were fed a meridic diet composed of agar, vitamins, sucrose, brewer's yeast, wheat bran, cholesterol, Wesson's salts, sorbic acid, ascorbic acid, methyl p-hydroxybenzoate, and distilled water (Valdés-Estrada et al. 2012). Another cohort was fed 5 mm wide tuberose bulb slices, previously hydrated in water for 20 min.

All females were dissected at 0, 5, 10, 15, 20, 30, 45, and 60 d of age ($n = 12$ for each age) under a stereoscopic microscope (Zeiss Discovery-V20, Thornwood, New York, USA). Ovaries were removed by pulling the ovipositor in a posterior direction until they were freed from the other tissues. Samples were stored at 4 °C in 5 mL Eppendorf tubes with water and thymol (May & Baker Ltd., Ongar, United Kingdom) solution of 0.5 mg per mL. Photomicrographs of dissected material were prepared using a Canon EOS 5D Mark II camera (Canon, Inc., Tokyo, Japan).

The proximal follicle area, length, and presence of mature follicles or relics were obtained from micrographs of dissected material. Only data from an ovariole of each weevil with the largest number of follicles were recorded. Length and area of the proximal follicle in each ovariole was measured using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). Follicle area was calculated using the formula of an ellipse (πab , where $\pi = 3.1416$, $a = \text{length}/2$, and $b = \text{width}/2$) (Perez-Mendoza et al 2004).

Ovarian development was classified based on the degree of follicular differentiation, maturity of the proximal follicle, and presence of follicular relics (or mature follicles) in the oviducts as reported by Grodowitz & Brewer (1987). Development was divided into 1 parous and 3 nulliparous stages. Nulliparous stages were classified as: nulliparous 1 (where the germarium and vitellarium are not differentiated and are without follicles); nulliparous 2 (well-defined ovarioles with multiple follicles, the proximal follicle transparent indicating that it had not completed maturation); and nulliparous 3 (proximal follicle more opaque [mature] and close to ovulation). In the parous stage the follicles pass through the lateral oviduct (ovulation) or have follicular relics at the base of the ovarioles.

STATISTICAL ANALYSIS

The mean number of follicles, as affected by diet and age (as well as mean length and area of the proximal follicle in each ovariole), was subjected to a 2-way ANOVA and differences determined by Tukey's test ($P < 0.05$). Females (on d of emergence) did not contain follicles, so this age was not included in analysis. Therefore, only length and area of the proximal follicle of individuals ≥ 10 d of age were included in the analysis (75% females possessed follicles). All analyses were performed using SigmaPlot 12.5 (Systat Software, Inc., San Jose, California, USA).

Results

OVARIAN MATURATION

We found that the female reproductive system of virgin *S. acupunctatus* was composed of a pair of ovaries with 2 tubular ovarioles each (Fig. 1). Only 0.01% of the dissected females possessed

an ovary with 3 ovarioles; when this occurred the ovary was not fully developed and possessed no mature follicles. Follicle oocytes were surrounded by a layer of follicular cells that constituted the follicular epithelium (Fig. 2A). Follicles were observed to develop gradually into the vitellarium, where the least mature follicles were located distally whereas the mature ones occurred proximally, close to the common oviduct (Fig. 2B). Mature follicles were filled with yolk that contained a germinal vesicle or defined nucleus (Fig. 2C).

Ovarian development of virgin *S. acupunctatus* consisted of 4 stages: 3 nulliparous (without ovulation) and 1 parous (with ovulation) (Fig. 3). Ovulation occurred when the mature oocyte passed through the lateral oviduct (Fig. 3 PA). Follicle reabsorption and ovulation can be estimated by the presence of follicular relics at the base of the ovariole (Fig. 3 PB). Follicular relics were primarily light yellow, spotted, or ring shaped whereas very few were dark brown.

Females fed the meridic diet experienced a delay in ovariole maturation compared with those fed on tuberose bulbs (Fig. 4). We noted that all females fed the meridic diet at 0 and 5 d of age possessed undifferentiated ovaries without follicles (nulliparous 1), whereas 17% of those that fed on tuberose bulbs at 5 d of age presented well-defined ovarioles with follicles (nulliparous 2). At 10 d, 75% of females fed the meridic diet were in stage nulliparous 2 as well as 83% of females fed on tuberose; however, the rest of the females (17%) had mature follicles close to ovulation (nulliparous 3). At 15 d, 92% of the females fed on tuberose had already ovulated or had follicular relics (parous), but only 17% of the females fed on a meridic diet reached that stage (Fig. 4). At 20 d of age, all females fed on tuberose reached the parous stage, whereas 17% of those fed the meridic diet were in nulliparous 3 stage then later reached the parous stage at 30 d.

We also found that the number of follicles found in ovaries depended on the type of diet ($F = 31.22$; $df = 1, 154$; $P < 0.001$), the age of the individual ($F = 76.71$; $df = 6, 154$; $P < 0.01$), and the interaction between these 2 variables ($F = 2.42$; $df = 6, 154$; $P < 0.05$). Females fed tuberose bulbs gradually increased their number of follicles up through 20 d of age followed by a decrease; individuals fed the meridic diet showed similar development but with a delay of 10 d (Fig. 5). The production of follicles in females fed on tuberose was significantly greater than that of females fed on a meridic diet at 10, 20, and 45 d of age.

Length of the proximal follicle of individual weevils containing the most mature oocyte (Fig. 6A) was not affected by diet ($F = 2.73$; $df = 1, 129$; $P > 0.05$), but was affected by age ($F = 22.75$; $df = 5, 129$; $P < 0.001$), as well as the interaction between these 2 factors ($F = 3.16$; $df = 5, 129$; $P = 0.010$). The length of the proximal follicle increased progressively with respect to age until 30 and 20 d of age in females fed the meridic diet and tuberose bulb, respectively. At 30 d of age, the proximal follicle length of females fed the meridic diet was significantly greater compared with individuals that fed on tuberose (Fig. 6A); however, at 45 d of age this relationship was reversed.

The proximal follicular area was not affected by diet ($F = 0.05$; $df = 1, 119$; $P > 0.05$) but was affected by age ($F = 19.21$; $df = 5, 129$; $P < 0.001$), and interaction of diet and age ($F = 4.24$; $df = 5, 129$; $P < 0.001$). The proximal follicle area increased gradually with time until 30 and 20 d of age in individuals fed on the meridic diet and tuberose bulbs, respectively (Fig. 6B). Maximum increase was observed at 30 and 60 d of age in females fed the meridic diet, whereas those fed on tuberose was 45 d. Moreover, females that fed on tuberose bulbs had a significantly smaller proximal follicle area than those fed the meridic diet at 30 d, reversing this relationship at 45 d of age.

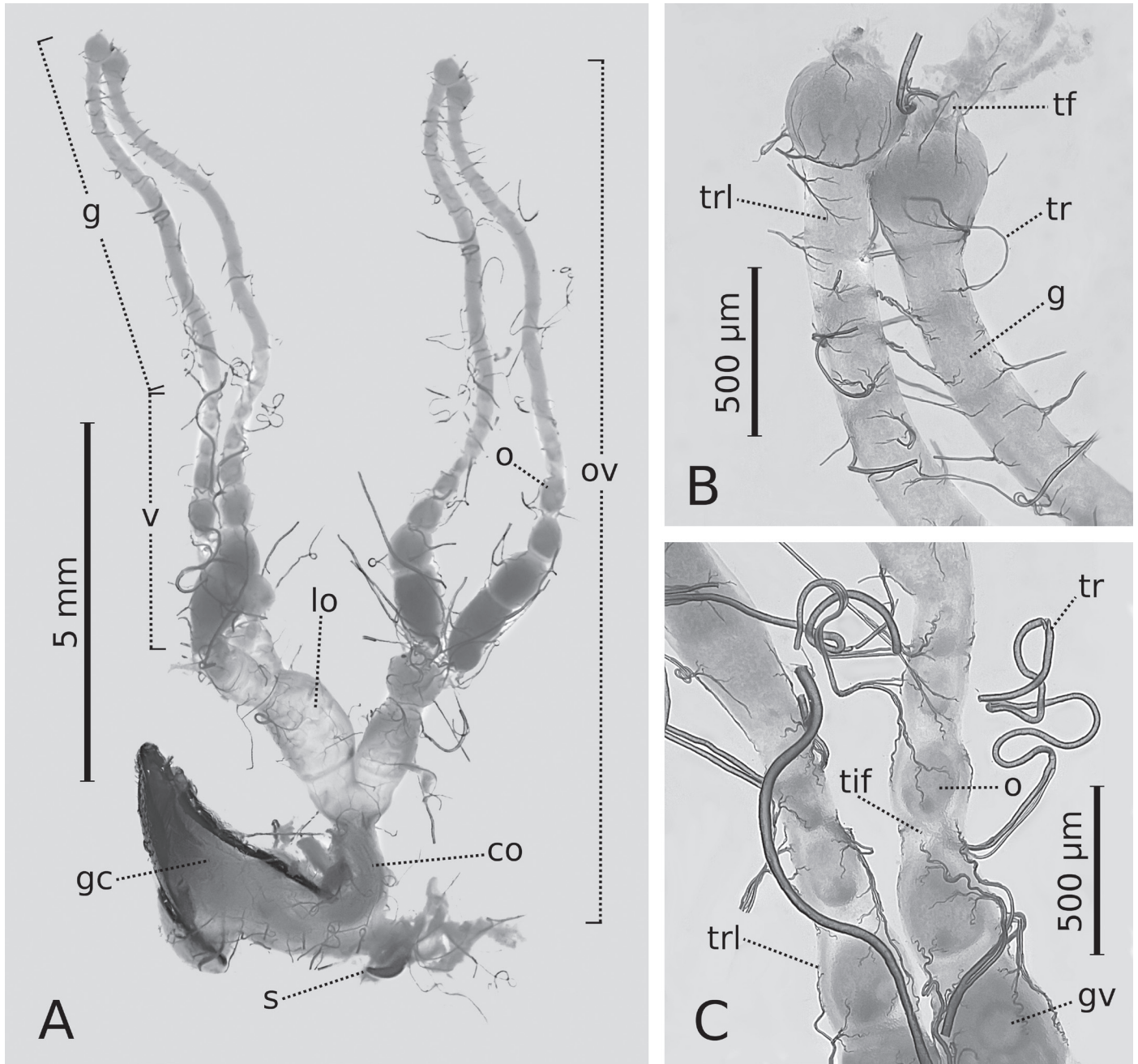


Fig. 1. (A) Female reproductive system of *Scyphophorus acupunctatus* formed by 2 ovaries (ov), germarium (g), vitellarium (v), oocyte (o), lateral oviduct (lo), common oviduct (co), spermatheca (s), and genital chamber (gc); (B) Distal part of the germarium (g) with bulky appearance possibly with trophocytes, prefollicular cells, and oogonia, terminal filament (tf), trachea (tr), tracheoles (trl); and (C) Approach to the onset of vitellarium with oocytes in development, germinal vesicle (gv), interfollicular tissue (tif), trachea (tr), and tracheoles (trl).

Discussion

We observed that the female reproductive system of virgin *S. acupunctatus* was formed by a pair of ovaries with 2 ovarioles. These characteristics were similar to that reported for other dryophthorids such as *Cosmopolites sordidus* Germar (Coleoptera: Dryophthoridae) (Gold et al. 2001; Uzakah 2017), *Sitophilus oryzae* L. (Coleoptera: Dryophthoridae) (Perez-Mendoza et al. 2004), *Rhynchophorus ferrugineus* Olivier (Coleoptera: Dryophthoridae) (Naggar et al. 2010), and the curculionids *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) (Burke 1959; Grodowitz & Brewer 1987), *Neochetina eichhorniae* Warner (Coleoptera: Brachyceridae) (Grodowitz et al. 1997),

Sitona cylindricollis Förster (Coleoptera: Curculionidae) (Garthe 1970), and *Sitophilus granarius* L. (Coleoptera: Dryophthoridae) (Dinuță et al. 2009). Ovarioles of *S. acupunctatus* contained a bulky germarium and are probably of the meroistic, telotrophic type as reported in other weevils (Grodowitz et al. 1987, 1997; Naggar et al. 2010; Perez-Mendoza et al. 2004).

Ovaries of virgin *S. acupunctatus* females possessed follicles in different stages of maturation and appearance of relics. We noticed that there was no synchronization of proximal follicle maturation whereas the number of ovulations in each ovariole varied. These results are in agreement with that reported by Adams (2000), Perez-Mendoza et al. (2004), and Lenz et al. (2007) for other insect species. However, in our

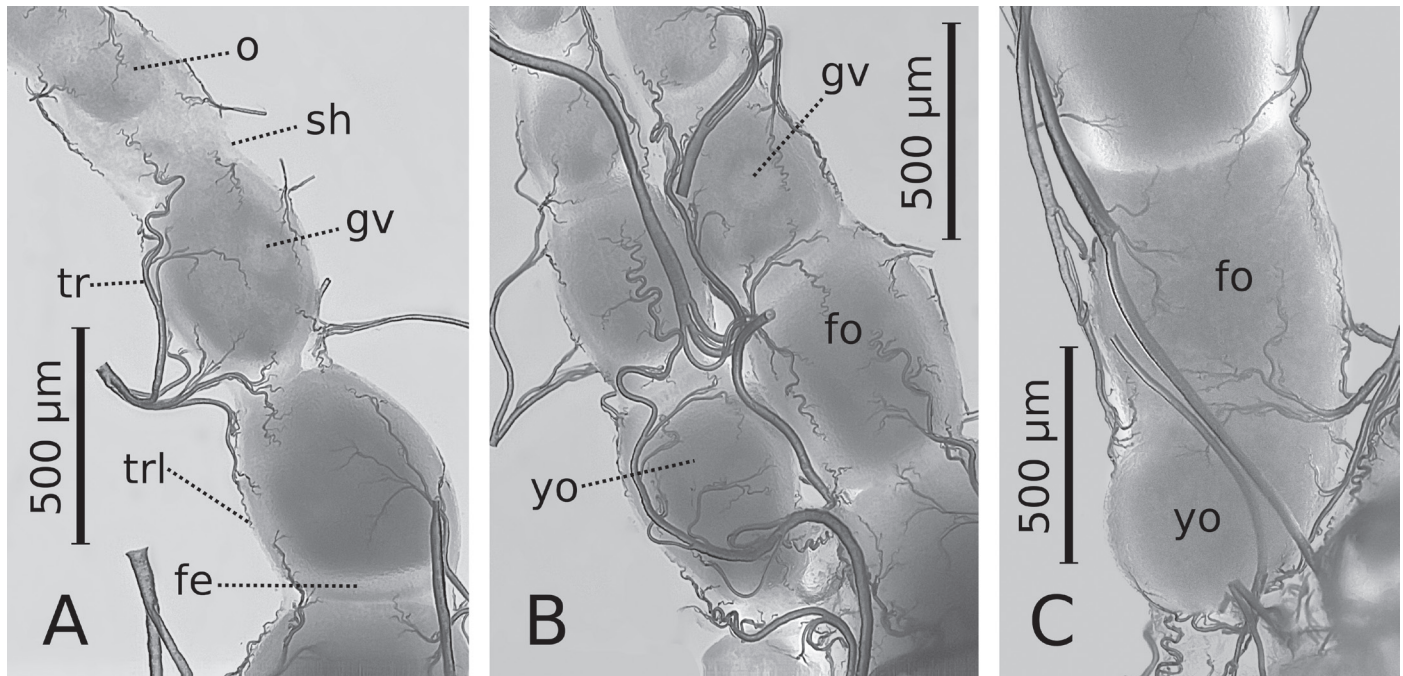


Fig. 2. Follicles of *Scyphophorus acupunctatus* in different stages of development. (A) Oocyte (o) located in the distal part of the vitellarium, further developed oocyte surrounded by follicular epithelium (fe) showing germinal vesicle (gv), peritoneal sheath (sh), trachea (tr), tracheoles (trl); (B) Gradual maturation of follicles (fo) with yolk (yo) and germinal vesicle (gv); (C) Mature follicle located in the proximal part of the vitellarium with yolk (yo).

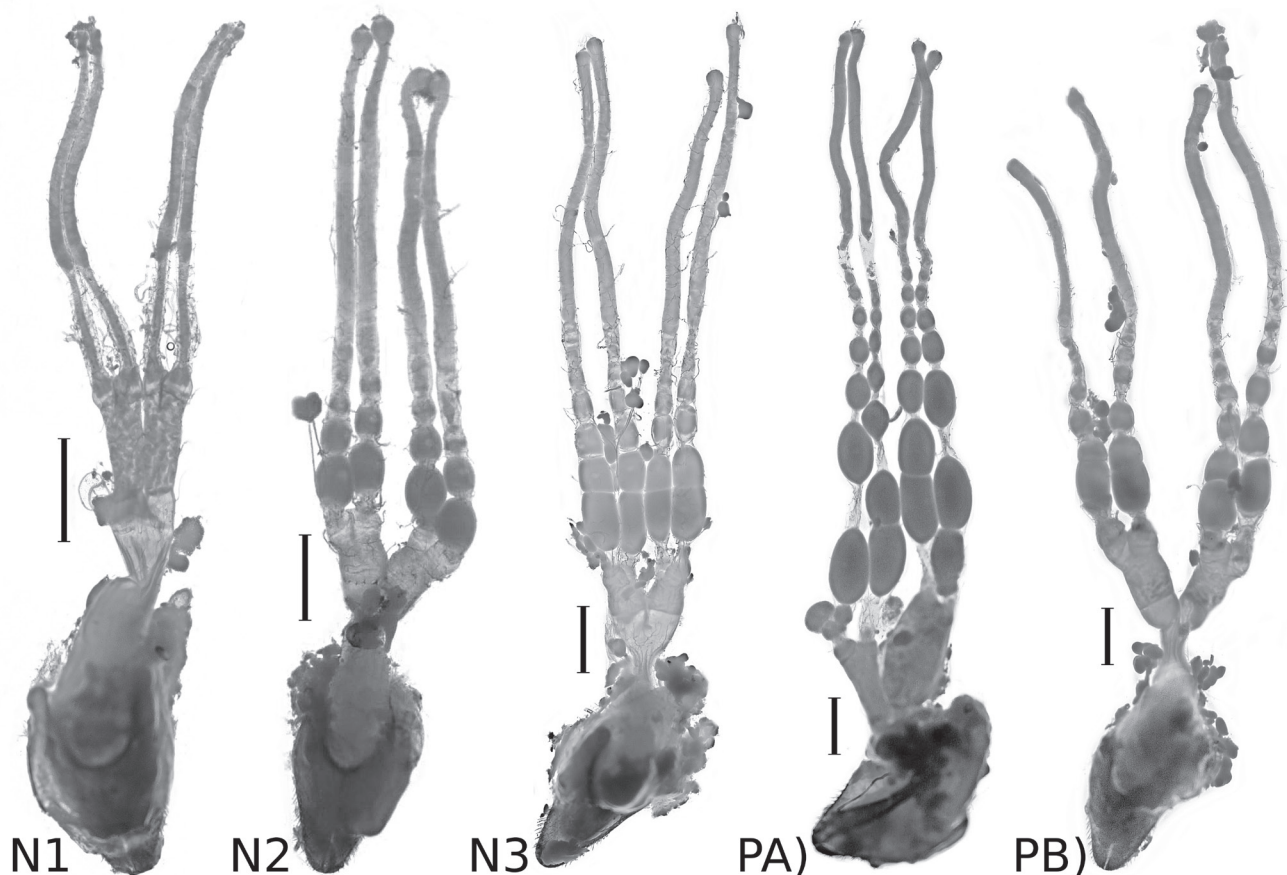


Fig. 3. Sequence of developmental stages of ovarian maturation of *Scyphophorus acupunctatus* females. N1: without follicles, no clear differentiation between germarium and vitellarium; N2: ovarioles begin to differentiate (germarium and vitellarium), follicles begin to form but not yet mature; N3: ovarioles completely differentiated, proximal follicle mature and ready to be ovulated, no follicular relics present; PA: follicles in oviducts; PB: presence of follicular relics. N = nulliparous.

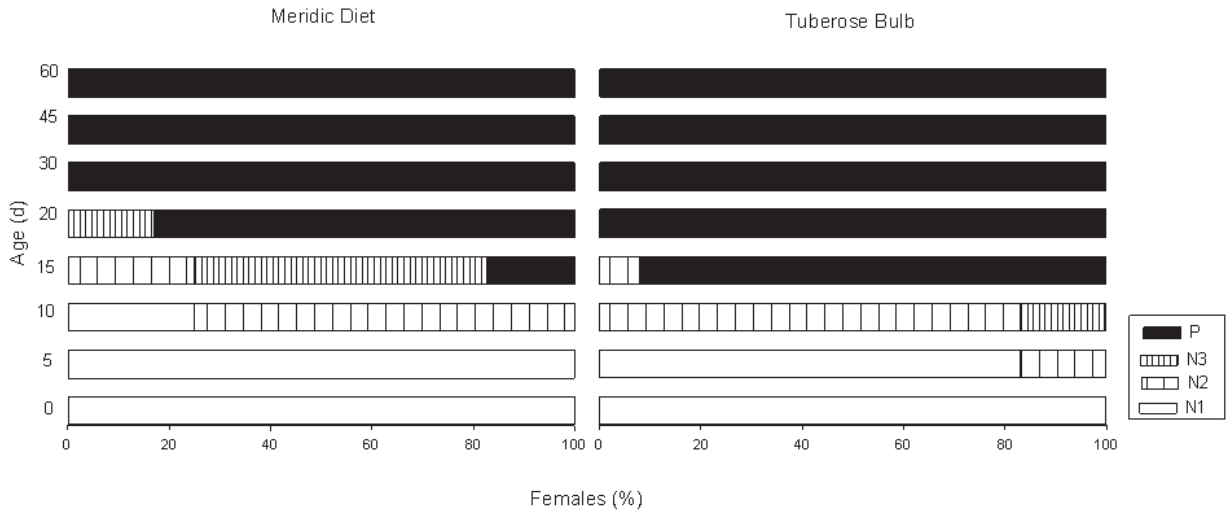


Fig. 4. Ovarian maturation of *Scyphophorus acupunctatus* females (%) at different ages fed on meridic diet (left) and tuberose bulb (right), n = 12 of each age and type of diet.

study it was not possible to determine if *S. acupunctatus* follicular relics were the result of reabsorption or ovulation as reported for *N. eichhorniae* and *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae) (Grodowitz et al. 1997; Scholz et al. 1998).

We found that *S. acupunctatus* females fed on a tuberose or meridic diet contained close to mature follicles in ovarioles (Nulliparous 3) when 10 and 15 d old, respectively. These data are in agreement with Hernández et al. (2012) where 14-d-old females were ready to mate. Our study also showed that females fed on tuberose bulbs were parous between 10 and 15 d old whereas those that fed on the meridic diet reached this stage between 15 and 20 d old. This developmental

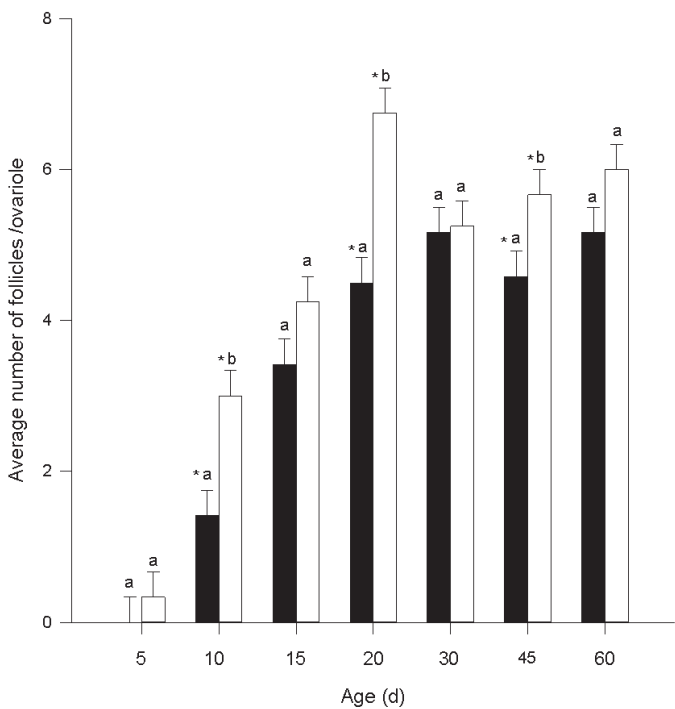


Fig. 5. Average number (\pm SEM) of follicles per ovariole in *Scyphophorus acupunctatus* at 5, 10, 15, 20, 30, 45, and 60 d of age fed on meridic diet (solid bars) and tuberose bulbs (empty bars). Bars within the same age with different letters indicate significant differences (Tukey multiple comparison test, $*P < 0.05$).

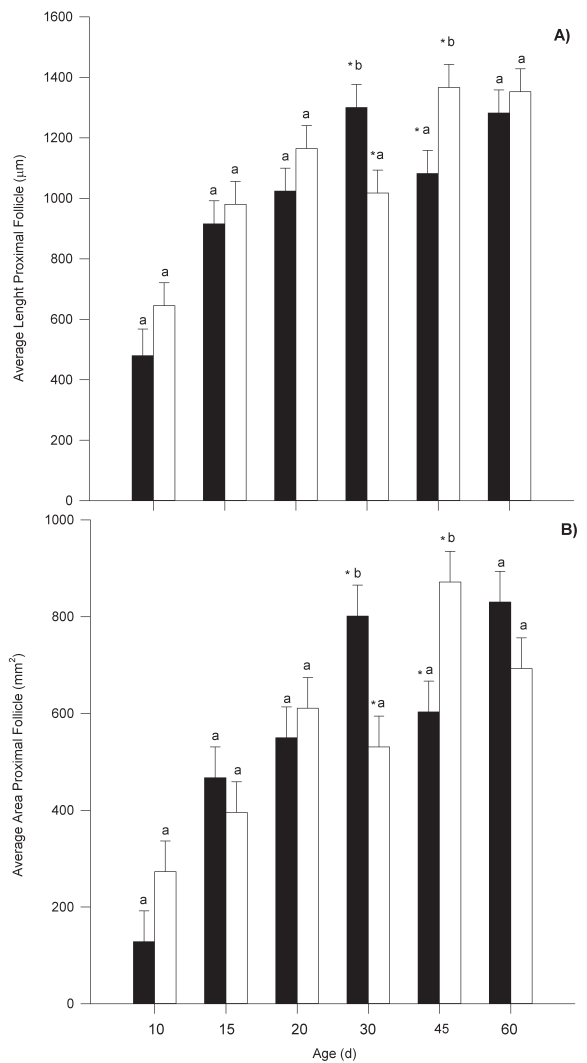


Fig. 6. (A) Average length (\pm SEM) and (B) area (\pm SEM) of proximal follicles in female of *Scyphophorus acupunctatus* at 10, 15, 20, 30, 45, and 60 d of age fed on meridic diet (solid bars) and tuberose bulbs (empty bars). Bars within the same age with different letters indicate significant differences (Tukey multiple comparison test, $*P < 0.05$).

difference is consistent with that reported for other weevils, such as *Ceutorhynchus assimilis* Paykull (Coleoptera: Curculionidae), wherein females complete their ovarian development by feeding on their host plant, but not when they are fed a diet of water and sugar (Ni et al. 1990).

In summary, the ovarian classification described in this paper will help to assess sexual maturity and provide quantitative information about the physiological condition of field populations of *S. acupunctatus* females. This knowledge could provide valuable guidance in monitoring the effectiveness of pest management suppression programs for this weevil pest.

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