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CONTRIBUTION TO THE KNOWLEDGE OF THE BIOLOGY OF *EUPLECTRUS PLATYHYPENAE* (HYMENOPTERA: EULOPHIDAE), A PARASITOID OF *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE) IN ARGENTINA

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ABSTRACT. This report is the first record of *Euplectrus platyhypenae* Howard, (Hymenoptera: Eulophidae) as a parasitoid of the fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), in Argentina. Because of the importance of this pest, the need of establishing a rational control method to eliminate the excessive use of pesticides, and the lack of information about the behavior and bionomics of this parasitoid in Argentina, we carried out laboratory studies in order to establish some relevant parameters of its life cycle. *S. frugiperda* larvae were obtained from laboratory cultures maintained on artificial diet; rearing and testing of the insects were done at 25 ± 2 °C, 70-75% RH and 14L:10D photoperiod in a climatic chamber. Duration of the different stages, pre-ovipositional period, longevity, fertility, fecundity, localization of the egg masses, and sex ratio, were assessed. This species parasitized all instars, except the first. Larvae of the fifth or sixth instar of FAW are capable of removing a great proportion of the eggs and/or newly emerged parasite larvae. The fecundity of the parasitoid is low and the sex ratio always favored females and only males were produced from unmated females.
KEY WORDS: *Euplectrus platyhypenae*, *Spodoptera frugiperda*, life cycle, behavior, Southern hemisphere population, corn pests, Argentina.

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RESUMEN. En este estudio se cita por primera vez a *Euplectrus platyhypenae* Howard (Hymenoptera: Eulophidae) como un parasitoide del cogollero del maíz, *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae), en Argentina. Debido a la falta de información sobre el comportamiento y bionomía de este parasitoide en Argentina, se realizaron estudios de laboratorio con la finalidad de establecer alguno de los parámetros relevantes del ciclo de vida de este parasitoide. Larvas de *S. frugiperda* fueron obtenidas de crías de laboratorio y mantenidas sobre dieta artificial. Los estudios sobre los insectos fueron realizados a 25 ± 2ºC, 70-75% HR y 14L:10º de fotoperíodo en cámaras climatizadas. Se registraron los diferentes estados de desarrollo, período de pre-oviposición, longevidad, fertilidad, fecundidad, localización de las posturas y proporción de sexos. Esta especie, parasita todos los estadíos larvales, excepto el I. Larvas del cogollero, de V a VI estadio son capaces de remover de su cuerpo los huevos y/o las larvas recién emergidas del parasitoide. La fecundidad de *E. platyhypenae* es baja y la proporción de sexos siempre favoreció a las hembras y a los machos cuando estos provenían de hembras vírgenes.
PALABRAS CLAVE: *Euplectrus platyhypenae*, *Spodoptera frugiperda*, ciclo de vida, comportamiento, población del hemisferio sur, plaga del maíz, Argentina.

The fall armyworm (FAW), *Spodoptera frugiperda* (Smith, 1797) (Lep.: Noctuidae), is an important pest of many crops and is widely distributed in the New World (Sparks, 1979), affecting corn crops in both tropical and subtropical regions. This species is currently controlled by pesticide applications (García Roa *et al.*, 1999; Yépez *et al.*, 1993; Clavijo, 1993; Gerónimo Gómez *et al.*, 1999). In northern Argentina, FAW infestations result in yield losses that fluctuate between 17 % to 72 % of the total production (Perdiguero *et al.*, 1967).

FAW is a native neotropical pest with a diverse complex of parasitoids like natural enemies. Ashley (1979), Ashley *et al.* (1982) and Molina Ochoa *et al.* (2003) registered 35 species of parasitoids for South America. In Argentina, 12 species of Hymenoptera and eight Diptera are known parasitoids on this species (Vera *et al.*, 1995; Virla *et al.*, 1999).

The genus *Euplectrus* Westwood (Hym.: Eulophidae) is cosmopolitan. Thirty five species have been recorded from the Neotropical region but only three are present in Argentina: *E. platyhypenae* Howard, 1885, *E. furnius* Walker, and *E. puttleri* Gordh (De Santis and Esquivel, 1966; De Santis, 1967, 1979, 1980, 1981, 1989; De Santis and Fidalgo, 1994; Virla *et al.*, 1999, Shauff and Janzen, 2001; Noyes, 2003).

The biology of the genus *Euplectrus* is based on studies of *E. platyhypenae* Howard, *E. bicolor* Swederus, *E. agaristae* Crawford, *E. laphygmae* (Ferrière), *E. comstockii* Howard, *E. plutteri* Gordh, and *E.* nr. *laphygmae* (Ferrière) (Zimin, 1930; Noble, 1938; Melin, 1969; Wall and Berberet, 1974; Gerling and Limon, 1976; González, 1985; Noyes, 2003). All of these species are gregarious ectoparasites of Lepidoptera (Arctiidae, Gelechiidae, Geometridae, Hesperiidae, Limacodidae, Lymantriidae, Noctuidae, Pyralidae, Sphingidae, Tortricidae and Zygaenidae) and are potentially valuable biological control agents (Puttler *et al.*, 1980; Jones and Sands, 1999; Noyes, 2003). *Euplectrus platyhypenae* has been reported to attain 20% parasitization of lepidopteran pests on alfalfa in Kansas (Smith, 1927); this species was also introduced from continental U.S.A. and established in Hawaii, originally against *Spodoptera mauritia* Boisd. and once there, it was mass-reared and released against *Cirphis latuiscula* H. S. and *C. unipuncta* Haw (Lep.: Noctuidae) (Gerling and Limon, 1976).

Females deposit eggs in clusters on the dorsum of the host, attaching them by a pedicel inserted under the cuticle but above the epidermis (Chattergee, 1945). Larval development is completed at the oviposition site. *Euplectrus puttleri* and *E. kuwanae* (Crawford) are host specific (monophagous) attacking *Anticarsia gemmatalis* (Hübner) and *Argyrogramma albostriata* (Bremer and Grey) (Lep.: Noctuidae) respectively, but others have several hosts (Puttler *et al.*, 1980; Uetmatsu, 1980; Coudron and Puttler 1988; Shauff and Janzen, 2001; Noyes, 2003).

Species of *Euplectrus* inhibit molting and arrest the development of the host (Puttler *et al.*, 1980; Coudron and Puttler, 1988; Coudron *et al.*, 1990). Molting inhibition usually occurs without host paralysis.

In northern Argentina, the only species of Eulophidae known from *S. frugiperda* is *E. furnius*, but its biology and the effects on FAW populations are unknown (Virla *et al.*, 1999). *Euplectrus platyhypenae* was cited utilizing *Pseudaletia adultera* (Schaus) (Lep., Noctuidae) in Salta province (De Santis, 1967).

Despite the fact that *E. platyhyenae* is a well-known species, there is partial information on its life cycle, fertility and others biological parameters. Furthermore this knowledge was obtained from holartic populations (Smith, 1927; Luginbill, 1928; Vickery, 1929; Wilson, 1932; Wall and Berberet, 1974; Gerling and Limon, 1976; Couldron *et al.*, 1990). In parasitoids, geographically isolated populations can show differences in their life history characteristic (see Acosta and

O´Neil, 1999, as example).

Considering the economic importance of FAW, this study sets out collect data about *E. platyhypenae* behavior and other biological parameters based on a Southern hemisphere population, and summarizing known biological data about this and others *Euplectrus* species.

MATERIALS AND METHODS

A colony of *E. platyhyphenae* was founded with individuals obtained from parasitized FAW collected in corn crops near Piquete Cabado (24° 49' S, 64° 11' W) (Salta Province, Argentina). For this study, FAW larvae were obtained from laboratory cultures maintained an artificial diet of bean flour and wheat germ (Ozores et al., 1982) at the PROIMI's insectary. Rearing and testing of insects were done in a climatic chamber under controlled environmental conditions at 25 ± 2 °C, 70-75% RH and 14L:10D photoperiod.

During the study, different instars FAW was placed in a glass tube (12 cm high x 1.5 cm diameter) with fresh corn leaves as food and them were exposed singly to parasitoids for 24h. After this period, the hosts were replaced with a new healthy (unparasitized) larva. To obtain biological data 69 female parasitoids were used.

The parasitized larvae were observed twice a day, until parasitoids completed development. The number of larval instars was determined by dissecting and counting the attached exuviae. A drop of pure honey was provided in the tubes as food resource for the adult parasitoids. Parthenogenic reproduction was assessed by exposing 10 unmated females to FAW larvae and determining the sex of the resulting progeny. Duration of the different stages, pre-ovipositional period, longevity, fertility, fecundity, localization of the egg masses, and progeny sex ratio were measured.

Voucher specimens (5 ♂♂ and 5 ♀♀) are deposited in the Miguel Lillo Institute entomological collection (IMLA) (Tucumán, Argentina).

RESULTS AND DISCUSSION

Stinging behaviour. According to Coudron *et al.* (1990), when *E. plathypenae* parasitized *Trichoplusia ni* (Lep.: Noctuidae) larvae within 36 h of ecdysis to the IV instar, it arrested molting and prevented new cuticle formation. The ecdysis mechanism is explained in Coudron and Puttler (1988), and Kelly and Coudron (1990).

For FAW larvae exposed to *E. platyhypenae* but not parasitized, we recorded different responses. Most larvae showed arrested molting and died after some days. However, we observed that some larvae were able to molt, increasing the duration of each larval instar, but were not able to reach the pupal stage. Some, however, reached the pupal stage but the adult moth did not emerge or, in those where emergence took place, adults longevity was 13.6 ± 5.54 days with no difference between males and females (P>0.05). Although shorter, when compared with normal longevity of *S. frugiperda* in the laboratory colony (17.54 ± 5.39 days) there was no significant difference (P>0.05).

FAW larvae exposed to *E. platyhypenae*, stung but not oviposited, became sluggish, showing a reduction in their feeding rate and mobility and after six days, the parasitized ones completely stopped movement. Similar observations were reported for this parasitoid by Wall and Berberet (1974), and *E. plathypenae* affecting *T. ni* (Coudron *et. al*, 1990). Gerling and Limon (1976) mentioned that the host stops feeding sometime after parasitoid oviposition and dies thereafter, and the exact timing of death varies within that species and depends upon the number of parasitoid larvae feeding upon the host.

Parasitism behaviour. Like *Euplectrus puttleri* (Puttler *et al.*, 1980; Vera and Fidalgo, 1990), *E. platyhypenae* parasitized all host instars, except the first.

Our observations on the female behavior were similar to the described by Gerling and Limon (1976). The female parasitoid examines the host

with her antennae and then jumps on its back when ready to oviposit; generally, large host (IV to VI instars) tries unsuccessfully to dislodge her while she inserts her ovipositor into the host's body. The host becomes motionless within a short time, and the parasitoid lays her eggs. One minute later, the host resumes its activity.

When larvae of FAW near to molting were exposed, females of *E. platyhypenae* walked over the host body several times, and stung it repeatedly, but no eggs were laid. Larvae of FAW in this condition are not a suitable host for the parasitoid. When the host was in an appropriate stage, during the first or second day after a molt, oviposition occurred immediately after the first stings.

FAW larvae responses. Some adult parasitoids were decapitated by IV, V, and VI instar FAW larvae, suggesting that they are sometimes able to defend themselves against attack by the parasitoids; similar observations were made by Jones and Sands (1999) for *E. melanocephalus* Girault, in the laboratory. We also noted that when *E. platyhypenae* parasitized V or VI instar larvae of FAW on last abdominal segments, they were able to use their mandibles to remove a great proportion of the eggs and/or newly emerged larvae.

LIFE CYCLE

Pre-oviposition. Females of *E. platyhypenae* have a variable pre-ovipositional period that ranges between 1 and 34 days (12.59 ± 9.13 days); during this time, females ignore host larvae. The observed period exceeds those recorded for *E. melanocephalus* (Jones and Sands, 1999) where pre-oviposition period last approximately 2.5 days.

Egg stage. After the pre-ovipositional period, female parasitoids lay eggs sporadically within an interval that had a mean of 3.95 ± 2.98 days. Others species, as *E. melanocephalus* and *E. laphygmae*, laid eggs regularly (Table 1).

Mated females deposited eggs from 1 to 6 times during their lifetime. Mean daily egg production ranged from 5 to 73 eggs/female/day. The mean of the laid eggs/female/day is the lowest recorded for the genus *Euplectrus* (Table 1).

Smith (1927) accurately described the eggs. This stage lasts 3.7 ± 0.8 days (Table 1). During the first days, changes in its shape and/or size are not evident but a remarkable alteration occurs in coloration. Newly laid eggs are creamy white and turn dark brown within 24 h; approximately 48 h later most are black.

The total number of eggs and cluster laid were 34.22 ± 17.54 and 1.91 ± 1.8 per female respectively, with an average of 18.31 ± 7.48 eggs per host.

Unmated females deposited eggs only once in their lifetime and the number of eggs laid averaged 22.5 ± 9.33. The bibliography indicated that the number of eggs laid per host is variable (Table 1) depending on host size, the species of the parasitoid and the host, and the oviposition pressure in the female parasitoid (Gerling and Limon, 1976).

It is important to remark that the females of *E. platyhyphenae* survive 7.3 ± 6.4 days after the last oviposition. Some females (nine mated and two unmated) did not oviposit during their lifetime.

Distribution of egg masses. The average number of egg/clusters was 17.95 31 ± 7.26. We recorded that 96.95 % of the eggs were deposited on the dorsal and/or dorso-lateral surface of the host's body, and only occasionally (3.05 %) on the ventral surface. Most were laid between the second and fourth abdominal segments or between the metathoracic and the first abdominal segment (Fig. 1). Other authors reported similar observations for this species and for *E. melanocephalus* (Table 1).

Larval stage. This stage lasts 6.74 ± 1.5 days, longer than those previously reported for this species and others (Table 1). During egg hatch, an antero-posterior rupture line forms in the cho-

FIGURE 1. Distribution of the egg masses of *Euplectrus platyhypenae* on FAW larvae in percent), breeding in laboratory at 25 ± 2°C, 70-75% RH, and 14L: 10D photoperiod (HD: head, C: collar, TH1: prothorax, TH2: mesothorax, TH3: metathorax, A: abdominal segments).

rion. It is possible to see the body of the first stage larva through this line, after 2 h. The neonate larvae are creamy green and are enclosed within the chorion that remains attached to the host's in tegument. After 24 h, the body size increased and turned to light green although body segmentation was not evident.

As were reported for *E. laphygmae* (Gerling and Limon, 1976), *E. platyhypenae* have four larval instars.

Two-day-old larvae were bigger and dark green. In the last or mature larval instar (IV), segmentation was clearly evident and larval coloration became yellowish or whitish.

Three or four days after emergence, when the larvae reach the III instar, some of them had such a considerable size that their bodies covered the less developed individuals, which subsequently die. From 1168 larvae II, 557 reached the IV instar and 491 attained the pupal stage. Wall and Berberet (1974) noted in *E. platyhypenae*, a possible competition for food among parasitoid larvae in large clusters. We noted that the surviving larvae show a characteristic arrangement similar to "rosette" (similar to Fig. 3 (Pl. IX) in Smith, 1927; or Fig. 41 in Luginbill, 1928).

When larvae were ready to pupate, they moved to the host's venter and arranged themselves transversally to the long axis of the host's body remaining mostly between the host pro-legs.

Pupal stage. When full-grown larvae moved ventrally to pupate, less than the 10% of the immature larvae remained on the host's dorsum. These smaller individuals were unable to complete their development due to the death of the host.

After 4-5h, the mature larvae started to spin a delicate cocoon. Each consisted of threads of silk that fastened the dead host to substrate. Cocoons were amber brown in color. When the cocoon was finished, and the parasitoid larva was completely immobile, the meconium was released. As was stated by Jones and Sands (1999), we considered that with this action it begins the "prepupal" stage. In the first hours, the meconium was reddish brown, but when it dried it became darker and it not have a defined shape or structure. Smith (1927) made an accurate description on cocoon spinning.

Pupation lasted between 8.77 ± 2.84 days; this period was longer than the reported for other authors for *E. platyhypenae* and *E. laphygmae* (Table 1).

Table 1

Summary of the average duration (days) of the egg, larval, pupa stage, adult longevity, sex ratio (F:M), N°/egg/female/day, N° egg/host, distribution of egg masses in the host and instars of hosts parasitized, according to different *Euplectrus* species (n: total of observations; r: range)

STUDY	*EUPLECTRUS* SPECIES	EGG	N°/EGG/ FEMALE/ DAY	N° EGG/HOST	DISTRIBUTION OF EGG MASSES IN THE HOST	HOST
Present paper	***platyhypenae***	**3.72 ± 0.81 n: 1528 r: 3-8**	**1.62 ± 1.26 n: 69 r: 5-73**	**18.31 ± 7.48 n: 108 r: 4-35**	**Dorsal and dorsolateral surface and occasionally on the ventral surface**	***Spodoptera frugiperda***
Smith (1927)	*platyhypenae*	-	-	26	Dorsal surface	*S. frugiperda* (as *Laphygma frugiperda)*
Luginbill (1928)	*platyhypenae*	2-3	-	-	Thorax	*S. frugiperda* (as *Laphygma frugiperda)*
Vickery (1929)	*platyhypenae*	3-6	-	-	-	*Spodoptera frugiperda*
Wilson (1932)	*platyhypenae*	2.28	-	3-30	Thoracic or first three abdominal segments	*S. exigua* (as *Laphygma exigua*)
Wall & Berberet (1974)	*platyhypenae*	-	-	-	-	*Spodoptera frugiperda*
Coudron *et al.* (1990)	*platyhypenae*	-	-	-	-	*Trichoplusia ni*
Gerling & Limon (1976)	*platyhypenae*	-	-	37	laid anywhere on the dorsum of young hosts; but on older hosts, oviposition is limited to the dorsal aspect of the thorax and anterior-abdomen.	-
	agaristae	-	-	60		-
	bicolor	-	-	37 and varying with host size		-
	laphygmae	2	4.6 ± 0.3	8 or 10 (on *S. exempta*)		*Spodoptera littoralis*
	nr. *laphygmae*	-	-	16 (medium larvae) and 25 (large larvae)		-
Puttler *et al.* (1980)	*puttleri*	-	-	-	-	*Anticarsia gemmatalis*
Jones & Sands (1999)	*melanocephalus*	-	11.1	-	Dorsal and dorsolateral surface	Larvae of fruit-piercing moths

Table 1. Continue. . .

STUDY	*EUPLECTRUS* SPECIES	LARVA	PUPA	FEMALE	MALE	SEX RATIO (F:M)	INSTAR OF HOSTS PARASITIZED	HOST
Present paper	***platyhypenae***	**6.74 ± 1.50 n: 1101 r: 4-11**	**8.77 ± 2.84 n: 491 r: 2-21**	**19.22 ± 8.78 n: 382 r: 2-52**	**10.15 ± 6.25 n: 105 r: 2-32**	**3.62:1 n: 482**	**II to VI**	***Spodoptera frugiperda***
Smith (1927)	*platyhypenae*	-	4-7	14	-	-	-	*S. frugiperda* (as *Laphygma frugiperda)*
Luginbill (1928)	*platyhypenae*	3-5	6-7	-	-	-	-	*S. frugiperda* (as *Laphygma frugiperda)*
Vickery (1929)	*platyhypenae*	3.5-5	5-8	-	15	-	The later instar	*Spodoptera frugiperda*
Wilson (1932)	*platyhypenae*	3.53	4.39	11.53	9.65	3.20:1	II or IV	*S. exigua* (as *Laphygma exigua*)
Wall & Berberet (1974)	*platyhypenae*	-	-	-	-	-	-	*Spodoptera frugiperda*
Coudron *et al.* (1990)	*platyhypenae*	-	-	-	-	-	IV	*Trichoplusia ni*
Gerling & Limon (1976)	*platyhypenae*	-	-	-	-	in the most of the species the sex ratio varies greatly, but is always in favor of the females	III & IV	-
	agaristae	-	-	-	-		III & IV	-
	bicolor	-	-	-	-		-	-
	laphygmae	3.4 ± 0.02	6.4 ± 0.04	45	29.1		II to IV	*Spodoptera littoralis*
	nr. *laphygmae*	-	-	-	-		II to V	-
Puttler *et al.* (1980)	*puttleri*	-	-	-	-	-	all host instar, except the I instar	*Anticarsia gemmatalis*
Jones & Sands (1999)	*melanocephalus*	-	-	-	-	4:1	-	Larvae of fruit-piercing moths

During the first 24 h, the pupae were whitish or creamy white but after this time, the bodies became more pigmented. By the seventh day after pupation, the pupae had a definitive black coloration.

After the mature larva moved down the host body, the host died and slowly its body became flaccid and began internal decomposition as noted from the black coloration. We confirm the data provided by Smith (1927) who pointed out that the host larvae always dies within a day of the pupation of the parasitoids.

Sex ratio and longevity. For *E. platyhyphenae*, we recorded a sex ratio of 3.6:1 females/male. Similar observations were made for others specie (Table 1). Gerling and Limon (1976) mentioned that in most of the species of *Euplectrus* the sex ratio varies greatly, but is always in favor of the females.

Adult emergence occurs in the morning, not exceeding three hours after dawn, and most of the first emerged adults were males.

Longevity of adults averaged 16.07 ±9.08 days, this was longer than reported for other authors (Table 1). Significant difference between both sexes were recorded ($P < 0.001$); the oldest female lived until day 52 after emergence meanwhile the oldest male died at day 32.

Under laboratory conditions, the life cycle of *E. platyhypenae* lasted a mean of 35.6 days (from the egg to death of adult). Gerling and Limon, 1976, reported a life cycle of 26-35 days for *E. laphygmae* affecting larvae of *S. littoralis* (Boisd.).

CONCLUSIONS

This is the first record of *Euplectrus platyhypenae* utilizing *Spodoptera frugiperda* as host in Argentina.

Using FAW as host, *E. platyhypenae* parasitized all instars, except the first, but the larvae were suitable only during the first or second day after molting. Larvae close to molting were rejected as hosts. The V or VI instars of FAW were capable of removing a great proportion of the eggs and/or newly emerged parasitoid larvae with their mandibles if they were deposited on the apical abdominal segments.

Euplectrus platyhypenae females had variable pre-ovipositional period that, in general, exceed those recorded for other species among the genus *Euplectrus*. The sex ratio always favored females. The parthenogenic reproduction is arrhenotokous.

The differences found on several life history parameters among this Southern hemisphere population and the literature data on holartic population encourage further studies to determine the possible existence of biotypes with different potential as biocontrol agents.

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