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Notes on the biology of *Anagrus breviphragma* (Hymenoptera, Mymaridae), naural enemy of the corn leafhopper *Dalbulus maidis* (Hemiptera, Cicadellidae) and others plant diseases vectors in South America

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Anagrus breviphragma Soyka 1955 is a wide distributed and polyphagous species that, in northern Argentina is a common egg parasitoid of the corn leafhopper *Dalbulus Maidis* De Long & Wolcott. Considering the lack of information about this wasp and the economic significance of its hosts, the purpose of this contribution was to provide data of its biology. *A. breviphragma* culture was established in a climatic cell (25 ± 2°C, 70-75% RH and 14L:10D h of artificial photoperiod). Data obtained from distinct maturation stages of the exposed host eggs were analyzed. The eggs-laying behavior was described. This wasp is solitary and proovigenic, without a preovipositional period. *A. beviphragma* parasitized eggs that were only 1-3 day-old, and was not able to mature in eggs containing advances embryos. In laboratory, the measured average of parasitims was 66.5%. The developmental time of *A. breviphragma* is around 16.5 days, and females reached the adult stage significantly faster than males. This species also developed faster in one-day-old eggs than in two-old, and in those of two-days-old faster than in three-days-old eggs. Adult longevity averages 5 days while few individuals surviving more than 12 days; females lived longer than males. The obtained sex ratio showed a predominance of melaes over males.

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INTRODUCTION

The Hemiptera Auchenorrhyncha has an agronomic importance because of its specific ability to transmit viruses and other pathogens and the damages that cause by feeding or oviposition (necrosis and hydric stress) (Nielson, 1968; Harris, 1979; Nault and Ammar, 1989; Remes Lenicov and Virla, 1999). These characteristics are increased by their high reproductive potential, short life cycle and elevated dispersion and adaptability rates.

Maize (*Zea mays* L.) is one of the most economical important cereals in South America. The diseases transmitted by different species of Cicadellidae (leafhoppers) and Delphacidae (planthoppers) show considerable incidence in maize agroecosystems. The "Corn Stunt Spiroplasm" (CSS), "Maize Bushy Stunt Mycoplasm" (MBSM) or the "Maize Rayado Fino Virus" (MRFV) are

common and important in Central and South America and they are frequently registered in northern Argentina (Giménez Pecci *et al.* 1998).

Parasitoids have been the most investigated leaf and planthoppers natural enemies, because of their potential as biological control agents. At a global scale, among the natural enemies that limit different Auchenorrhyncha vector populations, it was suggested that only certain taxa, like egg predator and egg parasitoid Hymenoptera, play an important role (Döbel and Denno, 1993).

The mymarid wasps or "fairy flies" are the best-known egg parasites of leafhoppers. Representatives of the family have been successfully utilized in several instances for the control of crop pests (see Meyerdirk and Moratorio, 1987a, for a review).

The species belonging to *Anagrus* genera are proovigenic and solitary parasitoids, very small (< 1mm) and delicate in structure, and live relatively short (Corbet and Rosenhein, 1996). They have high reproductive rates, short life cycles and are somewhat specific in host selection (Witsack 1973, cited by Meyerdirk and Moratorio, 1987b). Two species of *Anagrus* Haliday occur in Argentina: *A. (Anagrus) breviphragma* Soyka 1955 and *A. (Anagrus) flaveolus* Waterhouse 1913 (Triapitsyn, 1997).

Anagrus breviphragma, formerly known as *Anagrus silwoodensis* Walker, is a wide distributed and polyphagous species. The earlier distributional data indicates that this species was neartic (with records for Italy, France, Germany, Great Britain and Austria), but a recent review of the genus *Anagrus* in America south of the United States demonstrated that this species is present in the Neotropical Region too, with registers in Guadeloupe, Guiana, Brazil and Argentina (Triapitsyn, 1997). The known host are the leafhoppers (Cicadellidae) *Cicadella viridis* (L.) (Europe), and *Dalbulus maidis* De Long and Wolcott (South America), and the planthoppers (Delphacidae) *Peregrinus maidis* (Ashmead) and *Delphacodes kuscheli* Fennah (South America) (Triapitsyn, op. cit.). Moratorio (1987) studied the effect of host on this species (as *A. silwoodensis*) however, its biology is still unknown.

Dalbulus maidis is the most common corn leafhopper and causes great losses to maize in Latin America, because it is widely distributed and capable of transmitting three pathogens: the "maize rayado fino virus", the "corn stunt spiroplasm" (*Spiroplasma kunkelii*) and the "maize bushy stunt mycoplasm" (Nault and Madden, 1985; Nault, 1990, 1993). The economic importance of *P. maidis* and *D. kuscheli* in corn fields in America was discussed in the works of Remes Lenicov *et al.* (1997) and Remes Lenicov and Virla (1999).

Considering the lack of information on the biology of this wasp and the economic significance of its hosts, the purpose of this contribution was to provide data on its biology (as eggs-laying behavior, egg viability, duration of developmental stages, sex ratio, longevity) using *Dalbulus maidis* as the leafhopper host.

MATERIALS AND METHODS

Young corn plants bearing *Dalbulus maidis* eggs were exposed in corn fields near Cabeza de Buey (Salta province, Argentina) to become attacked by egg parasites. Parasitized eggs were brought into the laboratory. A colony of field-collected *A. breviphragma* was established in a climatic cell under the following conditions: 25 2°C, 70-75% RH and 14L:10D h of artificial photoperiod. *D. maidis* colony were reared and maintained as described by Remes Lenicov and Virla (1993)

The parasitoid breading was made in PET (Polyethylen-Terephtalathe) cylindrical cages (35-cm high x 18-cm diam). For aeration, the top was closed by nylon mesh cloth, and one hole was made on a lateral side. The hole was fitted with cotton plugs, which were moistened with water and honey as needed.

For studying the behavior and life cycle, fertilized females were placed in glass tubes of 12 x 2,5 cm with eggs of *D. maidis* laid at the edge of the distal portions of corn leaves. After exposure, the tubes containing the host eggs within the leaves where held in the climatic chamber under the conditions mentioned above. These tubes were daily checked to ensure the quality of the vegetal leaves, until the emergence of the adult wasps. Adult longevity was monitored twice daily, in the vials and without host material.

Host eggs were readily visible under the dissecting stereoscopic microscope when the leaf was illuminated from underneath. Those that changed to orange or reddish, after five to seven days, were considered as "parasitized eggs", meanwhile those developing eyespots were considered as "unparasitized".

Leafhopper nymphs hatched from the unparasitized eggs and the number of hatchling was counted daily. When the hatching was nearly over, each leaf was dissected and the host eggs remaining in the plant tissues were counted. By this time, *A. breviphragma* attained the pupal stage, so it was easy to distinguish parasitized eggs from unparasitized ones. Consequently, the percentage of parasitism may be calculated. Developmental data were analyzed by the analysis of variance and *t* test for mean separation at 0.05 level of significance

Voucher specimens are deposited in the entomological collections of M. Lillo Institute (Tucumán, Argentina) and University of California at Riverside (California, USA) (Triapitsyn, 1997).

RESULTS AND DISCUSSION

During studies in Northern Argentina on the natural enemies of *Dalbulus maidis* was recovered from exposed eggs a mymarid wasp. Some specimens were sent to Dr. S. Triapitsyn (University of California) for taxonomic examination, resulting to be *Anagrus breviphragma* Soyka 1955. Moreover, this wasp was obtained from *Delphacodes kuscheli* egg masses in oats crops at Rio Cuarto (Córdoba province). Triapitsyn (op. cit.) mentioning the specimens obtained in this work said (*sic*) "This is the first record of *A. breviphragma* from outside of Europe. The identification is based solely on morphological features; however, additional studies, including the use of molecular methods, are needed to demonstrate its correctness".

As was recorded for others species of *Anagrus* there was not observed a premating or preovipositional period; the oviposition of *A. breviphragma* begins immediately after adult emergence, so it is proovigenic. The egg is laid into that of the host and the entire larval and pupal life is spent there. These species is solitary, producing only one adult per host egg.

The Mymaridae are true egg parasites in the sense that they normally attack the host eggs before an appreciable development of the embryo has taken place. As was reported for another species, *A. breviphagma* limits theirs attacks to eggs that are only one to three-days-old; it is not capable to mature in eggs containing advanced embryos.

When the eggs were exposed, females searched rapidly over the leaves tapping the surface constantly with the tips of the antennae. Whether host location was by random search or by directional cues was not determined. Some females were observed to walk very close to the position of an egg and not to stop as long as the antennae did not touch the convex surface of the leaf over the egg. When an egg is encountered, the female stops and taps the surface repeatedly with the antennae. The process of oviposition started after the host was examined; the female positioned the tip of the abdomen on the host egg, the ovipositor was then extruded and inserted through the leaf cuticle determining it suitability. If the developmental stage of the egg is adequate, it becomes to oviposit.

Females found the eggs quickly and became in oviposition state in the first five

Table 1.—**Total average of parasitism (percent) by *Anagrus breviphragma* attacking *Dalbulus maidis* eggs considering the developmental stage of the eggs, under laboratory conditions. (x: average)**

General	732 exposed = 487 parasitized	
	Range	26.66 - 95.83
	x	**66.53**
1-day-old	278 exposed = 216 parasitized	
	range	33.33 - 95.83
	x	**70.70**
2-days-old	287 exposed = 195 parasitized	
	range	29.17 - 80.00
	x	**67.94**
3-days-old	167 exposed = 76 parasitized	
	range	26.67 - 78.57
	x	**45.51**

minutes. In laboratory, the average of parasitism was 66.5% and differences were found when eggs of different maturation stage were exposed. Table 1.

It was possible to observe that three days after oviposition, the egg content was in almost constant agitation as a result of the movements of the parasite larva. This movements remains for three or four days and during this time the eggs become first light orange and then turn to reddish. So, those eggs containing advance larvae and pupae become distinct by the orange or red coloration. Pupation takes place inside the host egg. The body of the pupa is visible through the transparent eggshell of the host. Similar observations were made by Otake (1968) studying *Anagrus nr. flaveolus* in Japan.

Not all the parasitized eggs reach the adult stage. In laboratory the viability of *A. breviphragma* is high, an average of 80.49% of adult emergence was obtained. The records show that more imagoes were obtained from two-days-old eggs (83.08%) than from those of one-day-old (79.63%) or three days old eggs (76.32%). Since the exposed eggs of *D. maidis* were laid on the edge of the distal portions of corn leaves, most of them are attacked at this time. Table 2.

Table 2.—**Viability of the parasitized eggs of *Dalbulus maidis* by *Anagrus breviphragma* considering the developmental stage of the eggs, under laboratory conditions. The number of emerged imagoes is considered. (x: average)**

General	487 parasitized = 392 adults	
	range	50.00 - 100%
	x	**80.49%**
1-day-old	216 parasitized = 172 adults	
	range	72.00 - 100%
	x	**79.63%**
2-days-old	195 parasitized = 162 adults	
	range	66.65 - 94.12%
	x	**83.08%**
3-days-old	76 parasitized = 58 adults	
	range	50.00 - 90.00%
	x	**76.32%**

Table 3.—**Viability of *Dalbulus maidis* attacked by *Anagrus breviphragma*, under laboratory conditions and considering the developmental stage of the eggs. (x: average)**

General	732 exposed eggs = 141 hatched nymphs	
	range	0 - 45.83%
	x	**19.26%**
1-day-old	278 exposed eggs = 33 hatched nymphs	
	range	0 - 44.40%
	x	**11.87%**
2-days-old	287 exposed eggs = 57 hatched nymphs	
	range	0 - 45.83%
	x	**19.86%**
3-days-old	167 exposed eggs = 51 hatched nymphs	
	range	20 - 44.44%
	x	**30.54%**

Studies on the biology of *Dalbulus maidis* (Remes Lenicov and Virla, 1993) mentioned that 69.36% of the eggs are hatching. The viability of the exposed host eggs in laboratory was low and showed an average of 19.26%; differences from distinct maturation stage of the exposed eggs were obtained and analyzed, for instance from three-days-old eggs hatched about 30.54% of nymphs, meanwhile from one-day-old eggs hatched only 11.87%. Surely in the field it is possible that the position of the eggs into the host tissue may protect them from attack. Table 3.

It is known that changes in host species produce variations in the morphology, physiology and behavior of their parasitoids. New (1969) demonstrated that the size and developmental time of the mymarid *Alaptus pallidicornis* Foerster were influenced when bred from three different host species. Moratorio (1987) studied the effect of host species on *Anagrus breviphragma* (as *A. silwoodensis* Walker) and *Anagrus mutans* Walker showing that the host influences such parasitoid life-history characteristics as fecundity and fertility.

The developmental time (from oviposition to adult emergence) of *A. breviphragma* is around 16,5 days (n: 370, x: 16.65 +/-3.09). This parameter was analyzed taking in account the maturation of the host eggs and the sex of the offspring. Females reached the adult stage significantly faster than males ($P < 0.05$) (females: n: 234, x: 16.16 +/- 2.72; males: n: 136, x: 17.50 +/- 3.49). Also *A. breviphragma* developed significantly faster in one-day-old eggs than in two-days-old ($P < 0.05$), and in those of two-days-old faster than in three-days-old eggs ($P < 0.05$). Table 4.

This result are in contrast with those obtained by Meyerdirk and Moratorio (1987a) for *A. giraulti* Crawford where not significant differences were observed between males and females developmental rates, held at the same temperatures.

Once emerged, the males rushed for the females, and mating occurred as soon as they managed to reach the females and position themselves appropriately. It was common to observe as many as 2-4 males massing around one female. Each mating lasted 4-10 seconds. After she finished mating, the female departed and became unattractive to males for some days.

Adult longevity averages around 5 days (n: 370, x: 5.16 +/- 3.77); it was variable, with few individuals surviving more than 12 days (Fig. 1). Females lived longer than males and those imagoes emerged from one-day-old egg seem to live longer. Table 5.

Table 4.—**Developmental time of *Anagrus breviphragma* under laboratory conditions depending on the maturation stage of the exposed eggs and the sex of the progeny. (n: number of observations; x: average; ds: standard deviation)**

		Developmental stage of the host eggs		
		1 day	2 days	3 days
General	n	172	140	58
	range	12-20	14-24	14-27
	x	**14.71**	**17.41**	**20.60**
	sd	1.60	2.25	3.63
Females	n	122	86	26
	range	12-20	14-24	14-27
	x	**14.78**	**17.13**	**19.46**
	sd	1.59	2.28	3.91
Males	n	50	54	32
	range	12-18	15-24	14-26
	x	**14.54**	**17.85**	**21.53**
	sd	1.63	2.14	3.14

The sex ratio obtained for other *Anagrus* species showed a predominance of females over males (Meyerdirk and Moratorio, 1987a). In the laboratory culture, the offspring of *A. breviprhagma* had a sex ratio of 1.72: 1 (females/male). This relation changed when the parasitoid attacked 3-days-old eggs, where the progeny contains more males than females. Table 6.

CONCLUSIONS

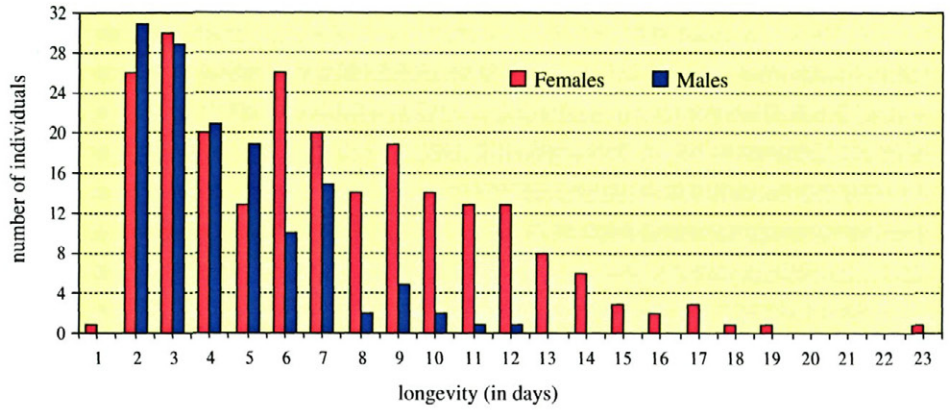
A. breviphagma prefers hosts with an embryo which is still under-differentiated and never attacks host eggs which have already developed the eye spots stage, limiting their attacks to eggs that are only one to three days old.

The obtained rate of parasitism in laboratory is about 66.5% and disparity of data

Table 5.—**Longevity of *Anagrus breviphragma* under laboratory conditions depending on the developmental stage of the exposed eggs and the sex of the progeny. (n: number of observations; x: average; ds: standard deviation)**

		Developmental stage		
		1 day	2 days	3 days
General	n	172	140	58
	range	1-22	0.5-17	1-10
	x	**5.77**	**4.98**	**3.77**
	ds	4.07	3.67	2.58
Females	n	122	86	26
	range	1-22	0.5-17	1-10
	x	**6.75**	**5.91**	**4.5**
	ds	4.31	3.95	2.96
Males	n	50	54	32
	range	1-9	1-11	1-8
	x	**3.4**	**3.5**	**3.19**
	ds	1.92	2.58	2.09

Fig. 1. ***Anagrus breviphagma:*** **Frecuency of the observed longevity under laboratory conditions (25 ± 2°C, 70-75% RH and 14L:10D of artificial photoperiod)**



was found when eggs of different developmental stage were exposed. New eggs were more parasitized reaching an average of 70.7%. Taking in account all the parasitized eggs, a mean of 80.5% produced adult individuals, overtaking frequently 100% of adult offspring.

The developmental time (from oviposition to adult emergence) of *A. breviphragma* is around 16.5 days, and females reached the adult stage significantly faster than males. Also this species developed significantly faster in one-day-old eggs than in two-days-old, and in those of two-days-old faster than in three-days-old eggs.

The sex ratio obtained showed a predominance of females over males; this relation changed when the parasitoid attacked 3-days-old eggs, where the progeny contains a ratio (males/female) of 1.23:1.

The average number of developmental days from egg to adult and data on longevity and sex ratio are not very different from those recorded for other species of *Anagrus*.

Developmental time (from oviposition to adult emergence) of *A. breviphragma* is shorter (16.5 days) than the developmental time for *D. maidis* (27.8-38.6 days) (data obtained from Remes Lenicov and Virla, 1993) growing at a rate of nearly two gen-

Table 6.—**Sex ratio of *Anagrus breviphragma* progeny obtained under laboratory conditions depending on the developmental stage of the exposed eggs.**

	Numbers of individuals		Proportion
	Females	Males	
General	234	136	1.72 : 1
1 day-old eggs	122	50	2.44 : 1
2 days-old eggs	86	54	1.59 : 1
3 days-old eggs	26	32	1 : 1.23

erations for every one generation of the host.

In the field, *Anagrus breviphragma* help in keeping *Dalbulus maidis* populations below economic thresholds. The laboratory observations suggest that the potential of this mymarid as a biological control agent is large and further studies will allow this species to be used for control of economic leafhopper species.

RESUMEN

Anagrus breviphragma Soyka 1955 es un parasitoide de huevos polífago de amplia distribución, que en el norte de Argentina ataca frecuentemente huevos de la Chicharrita del maíz *Dalbulus maidis* De Long & Wolcott. Teniendo en consideración la falta de información acerca de este antagonista y la importancia económica de sus hospedadores, el propósito de esta contribución fue ampliar el conocimiento sobre su biología. Se estableció una cría experimental de *A. breviphragma* bajo condiciones ambientales controladas (25 +/- 2°C, 70-75% HR y 14:10 hs (luz/oscuridad) de fotoperíodo artificial). Se analizaron datos biológicos tomados a partir del desarrollo del parasitoide cuando ataca huevos de distintos estados de maduración, y se describe el comportamiento de oviposición. Se trata de avispas solitarias y proovigénicas que carecen de un período pre-oviposicional. *A. breviphragma* atacó solamente huevos que tenían entre uno y tres días de desarrollo, y no fue capaz de desarrollarse en huevos que contenían embriones. En laboratorio se obtuvo un promedio de parasitoidimo del 66,5%. El tiempo de desarrollo de esta especie ronda los 16,5 días, y las hembras alcanzan el estado adulto significativamente más rápido que los machos. También, esta especie se desarrolló mas rápidamente en huevos de un día de edad que en los de dos días, y en estos últimos más que en los de tres días de desarrollo. La longevidad de los adultos es de aproximadamente 5 días y solo unos pocos individuos sobreviven más allá de los 12 días de edad; las hembras viven más que los machos. En laboratorio, la relación de sexos obtenida favorece a las hembras.

Key Words: *Anagrus breviphragma*, *Dalbulus maidis*, life cycle, egg parasitoid, corn, biological control.

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