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Biological data on *Megaphragma amalphitanum* Viggiani and *Megaphragma mymaripenne* Timberlake (Hymenoptera: Trichogrammatidae), egg-parasitoids of *Heliothrips haemorrhoidalis* (Bouché) (Thysanoptera: Thripidae) in southern Italy

Abstract – The biparental species *Megaphragma amalphitanum*, and the usually thelytokous species *M. mymaripenne*, are egg-parasitoids of the greenhouse thrips, *Heliothrips haemorrhoidalis* in Campania (southern Italy). The longevity of fed and unfed adults were compared in laboratory studies at five different temperatures (3°C, 10°C, 15°C, 20°C, 25°C). Pre-imaginal stage development time, potential fecundity and progeny were evaluated at 25°C. Data on the duration of the egg-adult cycle were collected at 15°C and at 25°C. Sex-ratio of *M. amalphitanum* was evaluated at 25°C. *M. amalphitanum* showed higher potential fecundity (44.3 eggs in comparison with 24.3), longevity (13.6 days in comparison with 6.1 at 3°C) and a shorter egg-adult cycle (21.6 days in comparison with 24.95 at 25°C) than *M. mymaripenne*. The pre-imaginal stages of *M. mymaripenne* were more tolerant to low temperature.

Key words: development, longevity, parasitoid, potential fecundity, progeny, sex ratio.

INTRODUCTION

The greenhouse thrips, *Heliothrips haemorrhoidalis* (Bouché) (Thysanoptera: Thripidae) is a pest of viburnum [*Viburnum tinus* L. (Angiospermae: Caprifoliaceae)] and of other ornamental plants, both in natural and cultivated habitats, as well as of fruit crops, such avocado [*Persea americana* Miller (Lauraceae)], several species in the genus *Citrus* L. (Rutaceae) and is also a pest of tea [*Camellia sinensis* (L.) Kuntze (Theaceae)]
High density can result in complete defoliation and eventual plant death and this may be extremely serious in public gardens, where viburnum and other plants as myrtle [Myrtus communis L. (Myrtaceae)] constitute thick hedges. Chemical control of this pest is very difficult in public areas due to their usage by the general public; consequently, pesticide use is limited (Del Bene et al., 1998). Moreover, on fruit crops, such as avocado, chemical control of thrips interferes with the activity of natural enemies. For these reasons, studies on the natural enemies of H. haemorrhoidalis and other thrips pests have increased in recent years and attempts are being made to bring H. haemorrhoidalis under biological control by the use of larval- or egg-parasitoids (McMurtry et al., 1991; Viggiani & Bernardo, 1996; Wysoki et al., 1996; Froud & Stevens, 1997).

In southern Italy, and for the first time in Europe, the presence of two species of Megaphragma Timberlake (M. mymaripenne Timberlake and M. amalphitanum Viggiani), egg-parasitoids of H. haemorrhoidalis was recorded in 1992 (Viggiani, 1994; Viggiani & Bernardo, 1997). Later Pintureau et al. (1999) collected M. amalphitanum from eggs of the same host in southern France (Antibes) and Portugal (Faro).

In the present paper, some biological parameters of these two parasitoids have been studied in the laboratory and results are presented here.

MATERIALS AND METHODS

Rearing of the parasitoids

M. mymaripenne and M. amalphitanum were reared on their natural host, eggs of H. haemorrhoidalis. For this purpose, adult H. haemorrhoidalis were collected from viburnum leaves in the field and allowed to oviposit on leaves of the same host plant kept in petri dishes. Viburnum leaves containing thrips eggs were maintained with the petiole wrapped in cotton wool and inserted in Eppendorf tubes filled with water. Viburnum leaves with H. haemorrhoidalis eggs, established in this manner, were used for the experiments. Stock cultures of M. mymaripenne and M. amalphitanum were obtained by using adults that emerged from parasitised eggs of H. haemorrhoidalis collected in Vietri sul Mare (Salerno, Italy). Rearing of both hosts and parasitoids were maintained in a climatic chamber at 25°C and 15°C, 70% ± 10 r.h. and 12L:12D.

Longevity

Newly emerged adults were isolated in gelatin capsules. Data on daily longevity were recorded for females and males of M. amalphitanum and females of M. mymaripenne (n = 15) either fed with honey or unfed, at 3, 10, 15, 20 and 25°C (±1). Food was provided in the form of drops of honey placed on small pieces of viburnum leaves every 3 days.
Duration of the egg-adult cycle

Fully-grown viburnum leaves taken from the second node from the top (from the same host plant) were removed in April and May in order to collect data on developmental time (egg to adult) of parasitoids. Thirty females of *H. haemorrhoidalis* were exposed to each leaf and placed in plastic boxes (120 mm in diameter and 120 mm height) for 24hrs. After oviposition leaves were transferred to plastic cages of similar dimensions. Test tubes with leaves and water were placed in a sheet of foam polystyrene, to avoid problems with excessive humidity. Four female parasitoids were confined on each leaf inside the experimental boxes for 24hrs. Then leaves were transferred to petri dishes (100 mm in diameter), lined with a layer of cotton wool and a sheet of blotting paper that exceeded the petri dish diameter and closed with elastic bands. This allowed moistening of petri dish inferiors daily without opening them. On the fifteenth day, thrips larvae emerging from unparasitised eggs of *H. haemorrhoidalis* were eliminated with a brush in order to prevent the leaf from excessive injury due to larval feeding.

Duration of the egg-adult cycle in *M. mymaripenne* and *M. amalphitanum* was evaluated at 25°C and 15°C, 70 % ± 10 r.h. and 12L:12D.

Development time of the preimaginal stages

Data on duration of the parasitoid larval development in *M. mymaripenne* only (n = 30) at 25°C, 70 % ± 10 r.h. and 12L:12D were obtained by direct observation under a stereomicroscope with transmission light through both egg chorion and leaf epidermis. Rearing was performed using the same method described for duration of the egg-adult cycle.

Potential fecundity and progeny

Newly emerged females (< 24hrs from rearing at 25°C) (n = 15) were dissected under a stereomicroscope in order to collect data on potential fecundity.

Evaluation the progeny of the two species of *Megaphraagma* performed at 25°C, 70%±10 r.h. and 12L:12D with both mated females (n = 10) (< 24 hrs) throughout her life time and virgin females of *M. amalphitanum* (n = 10), and with virgin females of *M. mymaripenne* (n = 10). Each female was allowed to oviposit on a *Viburnum* leaf with more than 50 host eggs.

Statistical analysis

All data were tested against the ANOVA assumption: non-homogeneity of variance and non-normality. Data which met the above assumptions were analysed using ANOVA with log-transformed data, if necessary, otherwise a non-parametric test (Kruskal – Wallis) was used. A multiple range test (Tukey HSD) was used to discriminate among means at the 0.05 level of significance (STATGRAPHICS PLUS 4.0).
Potential fecundity data were analysed by using one-way analysis of variance (ANOVA) without log-transformation.
All data are presented non-transformed.

RESULTS

Longevity

Collected data are shown in Fig. I – II.

Honey-fed females of both species lived longer than those starved. On the contrary, starved males of *M. amalphitanum* showed a greater longevity at 10°C and 20°C but not at all other temperatures. The highest longevity was found at 3°C for *M. amalphitanum* with 13.6±0.52 (S.E.) days when the females were fed; this longevity was greater than *M. mymaripenne* with 6.1±1.01 days, at the same temperature. However, honey-fed females of *M. mymaripenne* lived longer at 15°C (8.8±1.32 days). In general, for both species and sexes, longevity decreased with increasing temperatures.

Females of *M. mymaripenne* that had starved were more short-lived than those of *M. amalphitanum* at all temperatures tested.

Development of the preimaginal stages and duration of the egg-adult cycle

At 25°C parasitoid egg hatching requires about 24hrs.

![Graph showing longevity of starved adults at different temperatures.](image)

**Fig. I -** Mean longevity (±S.E.) of starved adults without hosts at different temperatures. Means within each group followed by the same letter are not significantly different (*P* > 0.05) (*f.* = females; *m.* = males).
Parasitised eggs of *H. haemorrhoidalis* are indistinguishable from those non-parasitised until the eleventh day. On the twelfth day a white spot, corresponding to the parasitoid larva midgut, is observed. The full-grown larva, which is sacciform, without mandibles, tracheal system and apparent segmentation, was observed 12-20 days after oviposition. Pupae with red eyes appeared from day 14 until day 23 and those with black eyes from the seventeenth day, reaching a maximum on day 21 after oviposition (63.33%). Emergence of adults started on day 22, with the maximum on day 25 and day 26 (50%) (Fig. III).

At 15°C the developmental time from egg to adult was 45.2±2.07 days for females of *M. amalphitanum* (n=16) and 75.8±0.41 days for females of *M. mymaripenne* (n=138). Very few larvae of the first species, however, developed into adults, and no males were produced, with high mortality at the black-eye pupal stage. At 25°C, the developmental time from egg to adult for both male and female of *M. amalphitanum* and female of *M. mymaripenne*, was respectively of 21.6±0.16 days (n=265) and 22.5±0.09 days (n=283) and 24.95±0.07 (n=414) days (Fig. IV).

**Potential fecundity and progeny**

In the ovarioles of newly emerged females of *M. amalphitanum* and *M. mymaripenne* an average of 44.3±1.08 and 24.0±0.88 mature oocytes were present respectively; \( P < 0.05 \); \( F = 201.24 \).
Fig. III - Percentage of different stages in development from egg deposition till emergence of adults of *M. mymaripenne* (n = 30).

Fig. IV - Mean development time (±S.E.) of *M. amalphitanum* (males and females), *M. mymaripenne* (females) at 15 and 25°C. Means within each group followed by the same letter are not significantly different (P > 0.05). n = number of adult parasitoids (f. = females; m. = males).
Females of *M. amalphitanum* produced a progeny of 18.6±5.88 adults (males and females) when mated and of 12.3±2.25 males when virgin. For *M. mymaripenne* an average of 12.5±1.08 females was obtained.

The sex-ratio (male/male+female) for *M. amalphitanum*, reared under laboratory conditions (25°C), was 0.3. This was rather different from that observed from field material (0.4 in 1994; 0.47 in 1996). No males were obtained rearing *M. mymaripenne* but males were known to be rare in the field; only one was collected. (VIGGIANI & BERNARDO, 1998).

All attempts to mate males of *M. amalphitanum* with females of *M. mymaripenne* were unsuccessful so the two species are isolated.

**DISCUSSION**

The genus *Megaphragma* includes 13 species world-wide, but several are poorly characterized and others are still undescribed (VIGGIANI, 2002). A revision of the genus is underway (POLASZEK, VIGGIANI, pers. comm.).

The species of *Megaphragma* are very small, even less than 0.2 mm in length (DELVAIRE, 1993), and are obtained from thrips whose eggs are embedded in the leaf tissue, i.e. species belonging to the family Thripidae (LOOMANS & VAN LENTEREN, 1995). Both species of *Megaphragma* in this study are about 0.2 mm in length, so they are only visible without a microscope when moving on white paper. Until now, probably due to the microscopic size, very little was known about their biology, such as their development time and behaviour (HESSEIN & McMURTRY, 1988; McMURTRY ET AL., 1991). Simultaneously it is difficult to collect data in the field. The fact that both the insects of this study may co-exist on the same host (two host eggs, on the same leaf about 2 mm apart, are often parasitised by different species) renders it hard to discriminate the two species in the field. Laboratory studies were indispensable in order to improve our knowledge.

This is the first report about the biological comparison of two different species of *Megaphragma*. In laboratory tests *M. amalphitanum* showed a higher potential fecundity and longevity, and a shorter egg-adult cycle than *M. mymaripenne* both at 25°C and at 15°C. Despite these differences *M. mymaripenne* has a wider distribution in Italy than *M. amalphitanum* (VIGGIANI & BERNARDO, 1998). In Europe (France and Portugal), yet, the latter species was the only species recorded (PINTUREAU ET AL., 1999). At 25°C, the life cycle of both species of *Megaphragma* was about one-third shorter than that of their host *H. haemorrhoidalis* (33 days). At 15°C, the life cycle of *M. amalphitanum* was shorter than that of *H. haemorrhoidalis*, 45.2 days in comparison with 70.3 days. HESSEIN & McMURTRY (1988), working with avocado leaves, reported that the egg-adult cycle of *M. mymaripenne* reared at 22-23°C lasted 41.4 days and adult parasitoids lived approximately 48hrs after emergence. These data differ from that
emerged in the present study and it is probably linked to the influence of the host plant. Different results were also recorded for the host *H. haemorrhoidalis* using viburnum instead of citrus (Rivnay, 1935; Del Bene et al., 1998; Reboredo & Jordana, 2001).

The higher longevity that was obtained at 3°C explains the fact that several specimens of both species of *Megaphragma* were collected in winter.

The faster development at 25°C of both *Megaphragma* and at 15°C of *M. amalphitanum* in comparison with those of *H. haemorrhoidalis* did not result in an efficient pest control. In fact, in spite of the high rate of parasitism, in some Italian places reaching 60-70% (Vigliani & Bernardo, 1998) *H. haemorrhoidalis* remains still a pest as in other countries (Hessein & McMurtry, 1988). Also Shibao et al. (2000) reported high percentage of parasitism (53.2%) of *Megaphragma* spp. on *Scirtothrips dorsalis* Hood with the same results, but clearly, parasitism by *Megaphragma* differs according to the locality, host and host plant. In fact, different data were recorded on cowpea [*Vigna unguiculata* (L.)Walp (Leguminosae)], where the level of parasitism by *Megaphragma* spp. was generally very low with a maximum of nearly 30% in west Africa (Tamò et al., 1993) and in citrus plantations in Japan with the highest level of parasitism of 10% (Takagi, 1983).

Biological control of *H. haemorrhoidalis* may be improved by the introduction of the larval parasitoid *Thripobius semiluteus* Boucek (Hymenoptera: Eulophidae) (Froud et al., 1996; Wysoki, 1999; Vigiani et al., 2000; Bernardo et al., *in prep.*).

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