Boll. Zool. agr. Bachic.
Ser. II, 27 (1), 1995: 61-70

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Identification by meconia of four species of egg parasitoids of *Thaumetopoea pityocampa* (Den. & Schiff.) (Insecta Lepidoptera Thaumetopoeidae)

Abstract - The meconia and remains of four species of chalcidoid egg parasitoids of the mediterranean pine processionary moth, *Thaumetopoea pityocampa* (Den. & Schiff.) were studied in about 200 egg-batches collected at different places in Spain and Portugal. All parasitoid species could be determined by their meconia inside the egg shell after emergence. In this way, species specific and quantitative recordings can be made throughout year. The meconia of larvae of *Baryscapus servadeii* (Dom.) (Eulophidae) resemble either single caps, or globules, or globular aggregations depending on the time of parasitism. The larvae of *Ooencyrtus pityocampae* (Mercet) (Encyrtidae) form yellow flat discs in freshly laid eggs, and dark balls in older eggs. The larvae of *Anastatus bifasciatus* (Fonsc.) (Eupelmidae) produce a meconium, resembling corn-grain aggregations and the polyembryonic *Trichogramma* sp. adults emerge from eggs with a sooted inner shell surface.

Zusammenfassung - *Identifizierung von vier Eiparasitoiden-Arten von* Thaumetopoea pityocampa *(Den. & Schiff.) (Insecta Lepidoptera Thaumetopoeidae) anhand ihrer Mekonien.*

Die Mekonien und Rückstände von vier chalcidoiden Eiparasitoiden-Arten des mediterranen Kiefernprozessionsspinners *Thaumetopoea pityocampa* (Den. & Schiff.) wurden bei etwa 200 Eigelegen aus verschiedenen Regionen Spaniens und Portugals untersucht. Alle Eiparasitoiden-Arten konnten mit Hilfe ihrer, nach dem Schlupf in der Eihülle zurückgelassenen Mekonien determiniert werden. Auf diese Weise lassen sich artspezifische und quantitative Ermittlungen über die Gelegeparasitierung unabhängig von der Jahreszeit durchführen. Die Mekonien der Larven von *Baryscapus servadeii* (Dom.) (Eulophidae) erscheinen als einzelne Kappe, kugelförmig oder als globuläre Ansammlung. Dies ist abhängig vom Zeitpunkt der Parasitierung. Die Larve von *Ooencyrtus pityocampae* (Mercet) (Encyrtidae) bildet gelbe flache Scheibchen bei früher Parasitierung der Eier und dunkle Kügelchen, wenn die Parasitierung bei älteren Eiern erfolgte. Die Larve von *Anastatus bifasciatus* (Fonsc.) (Eupelmidae) produciert als Meconium eine Gruppe von Roggenkorn ähnlichen Ausscheidungen, und die Adulten der polyembryonalen

Trichgramma sp. schlüpften aus Eiern, deren Schale innen dunkle Flecken aufweist oder tief geschwärzt ist.

Riassunto - *Identificazione di quattro parassitoidi oofagi di* Thaumetopoea pityocampa *(Den. & Schiff.) (Insecta Lepidoptera Thaumetopoeidae) mediante i loro meconii.*

Sono stati presi in esame i meconii ed i residui di quattro specie di Chalcidoidea parassitoidi oofagi in circa 200 ovature della «Processionaria mediterranea del pino» *Thaumetopoea pityocampa* (Den. & Schiff.) provenienti da regioni differenti della Spagna e del Portogallo. Si sono potute così determinare queste specie mediante i loro meconii lasciati nel guscio dopo lo sfarfallamento. In tal modo gli studi quantitativi e relativi alla determinazione delle specie si possono eseguire indipendentemente dalla stagione dell'anno. I meconii delle larve di *Baryscapus servadeii* (Dom.) (Eulophidae) si presentano come un singolo cappuccio a forma di globo, oppure come un assembramento globulare, in funzione del momento della parassitizzazione. Le larve di *Ooencyrtus pityocampae* (Mercet) (Encyrtidae) formano dischi gialli piatti in uova appena deposte, mentre in uova più vecchie formano globi scuri. Le larve di *Anastatus bifasciatus* (Fonsc.) (Eupelmidae) producono un meconio che assomiglia ad un raggruppamento di granelli, mentre gli adulti dei poliembrionali *Trichogramma* sp. sfarfallano lasciando l'interno dell'uovo con macchie scure o del tutto nerastro.

Key words: *Thaumetopoea pityocampa*, egg batches, egg parasitoids, meconia, *Baryscapus servadeii, Ooencyrtus pityocampae, Anastatus bifasciatus, Trichogramma* sp.

INTRODUCTION

The pine processionary caterpillar, *Thaumetopoea pityocampa* (Den. & Schiff.) is widely spread in pine forests of the western mediterranean countries and the most important defoliator of the pine trees. Its most frequent chalcidoid egg parasitoids are Baryscapus *(= Eutetrastichus) servadeii* (Dom.) (Eulophidae) and *Ooencyrtus pityocampae* (Mercet) (Encyrtidae). Additionally, *Anastatus bifasciatus* (Fonsc.) (Eupelmidae) and *Trichogramma* sp. (Trichogrammatidae) are also common (Bellin et al., 1990; Masutti & Battisti, 1990; Schmidt, 1990; Tiberi, 1990; Tsankow, 1990).

Host phenology is to a large extent influenced by climatic factors. Temperature primarily determines moth ecclosion, which lasts from June to November, depending on the latitude and longitude of the site (Milani, 1990). After emergence copulation and oviposition follow in the same night (Maksymov, 1978). Each female lays only one egg-batch, mainly on a pair of needles or small twigs

(Schmidt, 1988). The number of eggs in one egg-batch differs widely: 70-300 in France (Huchon & Demolin, 1970), about 223 in Bulgaria (Tsankow, 1972), 93-349 in Greece (Schmidt & Douma-Petridou, 1989).

Parasitism of the eggs may occur immediately after oviposition, or up to shortly before hatching of the caterpillars. However, during the last third of host embryogenesis, parasitism and parasitoid development were reduced (Kitt & Schmidt, 1993).

Some of the egg parasitoids may have two generations during the univoltine life cycle of the host (Kitt & Schmidt, 1993). The first generation emerges within 2-3 weeks after oviposition (Halperin, 1970; Halperin, 1990), but many parasitoids enter diapause, which may last for several months, or until the next flight period of the host (Schmidt & Kitt, 1993, for *O. pityocampae*).

Quantitative observations based on the collection of all parasitoids and determination of annual rates of parasitism are extremely time consuming. On the other hand, quantitative records are easily made when the parasitoid meconia are analysed inside the egg-shells. Kitt and Schmidt (1993) and Schmidt and Kitt (1994) were able to show that the meconia and remains are characteristic for *B. servadeii* and *O. pityocampae* in eggs of T. wilkinsoni Tams. In this study, the differences in the meconia of each parasitoid species found in eggs of *T. pityocampa* were identified.

MATERIAL AND METHODS

About 200 egg-batches of *T. pityocampa* (Den. & Schiff.) were collected throughout the Iberian peninsula (from the Pyrenees to the Sierra Nevada), shortly before, or after hatching of the caterpillars, in 1991. Additionally, 40 egg-batches were obtained in January 1992 which had been laid in August 1991. All the egg-batches were singled in 6.5 ml plastic tubes, closed by a foam stopper and stored at 20-25 ºC in the laboratory. A daily hatching control was necessary to remove the emergent parasitoids, and separate them according to species and sex. An egg from which a parasitoid has emerged can be recognized by the presence of a small hole (Schmidt, 1988). After removing the scale cover, the parasitoid could be attached to the emerging hole and the meconium inside the egg-shell was studied.

The meconia and remains of the parasitized eggs were prepared, determined and attached to the emerged parasitoids. The egg contents were photographed using a binocular. Additionally, the meconia were observed under a stereo microscope (40x magnification), then some were put on an alu-slide (4828B Specimen Grid Box, 4 KB- Produktor AB, Stockholm-Bromma 1, Sweden) with conductor tabs for electron microscopy scanning. After drying in a freezer for 72 h, the ob-

ject was spattered with gold for 2 min. at 20 mA. Micrography was carried out under an ETEC-antiscan SEM with 25 kV.

RESULTS

The meconia were studied throughout one year (one or two generations). In addition, some specimens were reared individually in the laboratory. All observations resulted in the same species-specific remains and the meconia preserved species specific characteristics for over one year.

1. Remains in eggs parasitized by Ooencyrtus pityocampae

In 2043 eggs parasitized by *O. pityocampae* a brown skin was observed which coated the inside shell surface of the egg. It was detached from the wall by cutting the egg. On the inner side of the egg cap cut, remnants of the respiratory system of the parasitoid larva were observed. It is a respiratory mask, similar to a cap sticked to a thin respiratory pipe, responsible for gas exchange with the air around the egg (Fig. 1.1-3). The respiratory pipe marks the place of parasitoid oviposition; two respiratory masks were found very seldom. Fig. 1.1 shows the positioning of the parasitoid's egg with the respiratory mask, inside the host. During the egg laying period lasting for about 5 min., the channel was prepared and the egg was surrounded by a small brown skin, from which later on, the respiratory mask originated. In this case, no host embryo developed, and only some dried up yolk was observed.

In many cases dead stages of *O. pityocampae*, diapausing specimens, and remains of the pupal envelope were observed after emergence of the adult parasitoids. The structure and colour of the meconium of *O. pityocampae* varied, depending on the parasitized host stage. Single flat and yellow to orange coloured discs were found in parasitized eggs, in which the larva of the parasitoid could feed on the yolk (Fig. 1.4-7; 2a). In these eggs no remains of the host larva were present. In eggs parasitized in a later embryonic stage the small discs of meconium were round and looked like small darker balls, coloured up to red-brown (Fig. 1.5; 2b). In such eggs, often head capsules and mandibles of the host larvae were observed.

2. Remains in eggs parasitized by Baryscapus servadeii

In 1977, eggs which were parasitized by *B. servadeii* showed no skin inside the egg, and the egg shell was light coloured. The place of egg laying by the parasitoid was observed as a dark point on the egg cap. In many cases dead stages of parasitoids or remnants of the pupal envelope and meconium, were found in the eggs

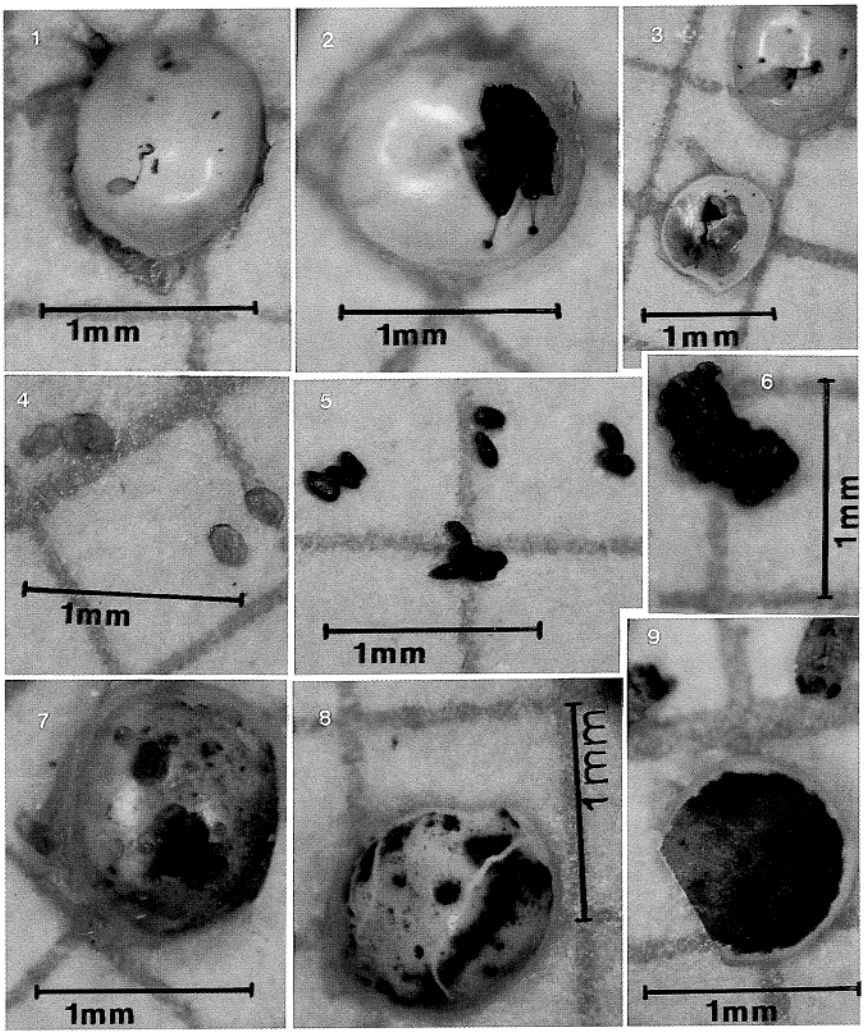

Fig. 1 - Caps of *Thaumetopoea pityocampa* (Den. & Schiff.) eggs parasitized by *Ooencyrtus pityocampae* (Mercet), inside view: respiratory channel with a small sac, in which the egg of the parasitoid died (1); egg cap with two respiratory masks and respiratory channels, left by two developed and emerged parasitoids (2); egg caps each showing a respiratory mask, and a brown skin in the lower one (3). Isolated meconium of *O. pityocampae*, at early (4) and at late (5) stage of parasitism. Meconium of *Baryscapus servadei* (Dom.), at late stage of parasitism (6) and of *O. pityocampae* (Mercet) on the brown skin inside of the egg-cap at early stage of parasitism (7). Caps of *T. pityocampa* (Den. & Schiff.) eggs parasitized by *Trichogramma* sp.; the colouration of the inside of the egg-shell depends on the number of grown-up individuals; the black meconium looks like dust (8-9).

after parasitoid emergence. At an early developmental stage of the host egg the parasitoid produced a liquid meconium, which dried up and attached to the egg-shell, appearing like a bowl (Fig. 3a). Parasitism of a later stage resulted in solid meconia looking like small balls, sticked together inside the egg-shell (Fig. 1.6; 3b+c). When parasitism took place at an advanced stage of embryonic development of the host, the sclerotized cuticle of the head capsule was additionally present. Depending on the developmental stage of the host embryo, the colour of the meconium changed from light and dark grey, to almost black. The meconium appeared always dull because of the rough surface (Fig. 1.6).

3. Remains in eggs parasitized by Anastatus bifasciatus

In 93 eggs parasitized by *A. bifasciatus*, there was no brown skin inside the egg. Dead stages of the parasitoid could be observed, as well as remnants of the pupal envelope and the meconium of the developed parasitoid. The meconium was similar to that of *B. servadeii*, when the host embryo was parasitized at a late stage. Differences were detected only through careful examination. The structure of the meconium of *A. bifasciatus* was similar to corn grains, and longer than the ball shaped one of *B. servadeii*. It has a smooth surface and a brighter glue (Fig. 2c+d), is black coloured, and darker than that of *B. servadeii*.

4. Remains in eggs parasitized by Trichogramma sp.

The observation of 653 eggs parasitized by *Trichogramma* sp., showed that the egg-shell has a sooted inner surface, which is sometimes only black spotted, but often dusty and coloured deep black (Fig. 1.8-9). The dead adults found were coloured yellow to brown, and of variable size.

DISCUSSION

Our investigations established that each egg parasitoid leaves a different meconium and remains, thus allowing for the determination of quantitative records on rates of egg parasitism, and for an assessment of parasitoid impact throughout the developmental period of the host. To this purpose, every egg must be opened, and for each population, a minimum of 50 egg-batches of *T. pityocampa* are needed, to calculate biologically reliable mean values.

Our results agree with those obtained by Kitt and Schmidt (1993) and Schmidt and Kitt (1994) for Israel populations of *T. wilkinsoni*, if the time of parasitism is considered.

In *B. servadeii*, the meconia of the larvae showed globular aggregations of feces, when development took place in late embryonic stages of the host. These

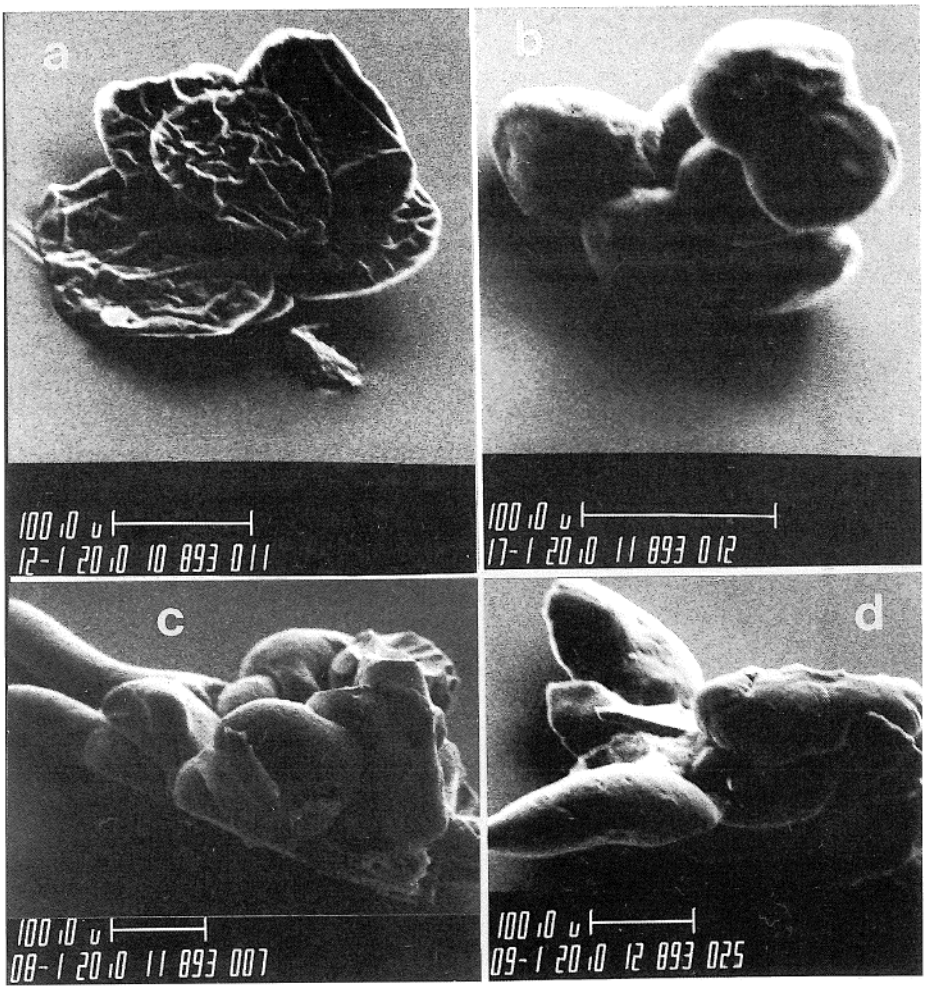

Fig. 2 - Meconia of *Ooencytus pityocampae* (Mercet), a: at early stage of parasitism, b: at late stage of parasitism, and meconia of *Anastatus bifasciatus* (Fonsc.) (c,d).

aggregated feces are very similar to those of a diapausing matura larva found 11 months later. On the other hand, in many eggs parasitized by *B. servadeii* there were sclerotized head capsules and remains of mandibles of the host, indicating that parasitism took place in an advanced embryonic stage of the host, as Tsankov (1990) observed. Taking into account these observations, it seems possible that the meconia can also be used for the determination of the factors involved in the process of diapause. If the meconium is shaped like a ball or cap, para-

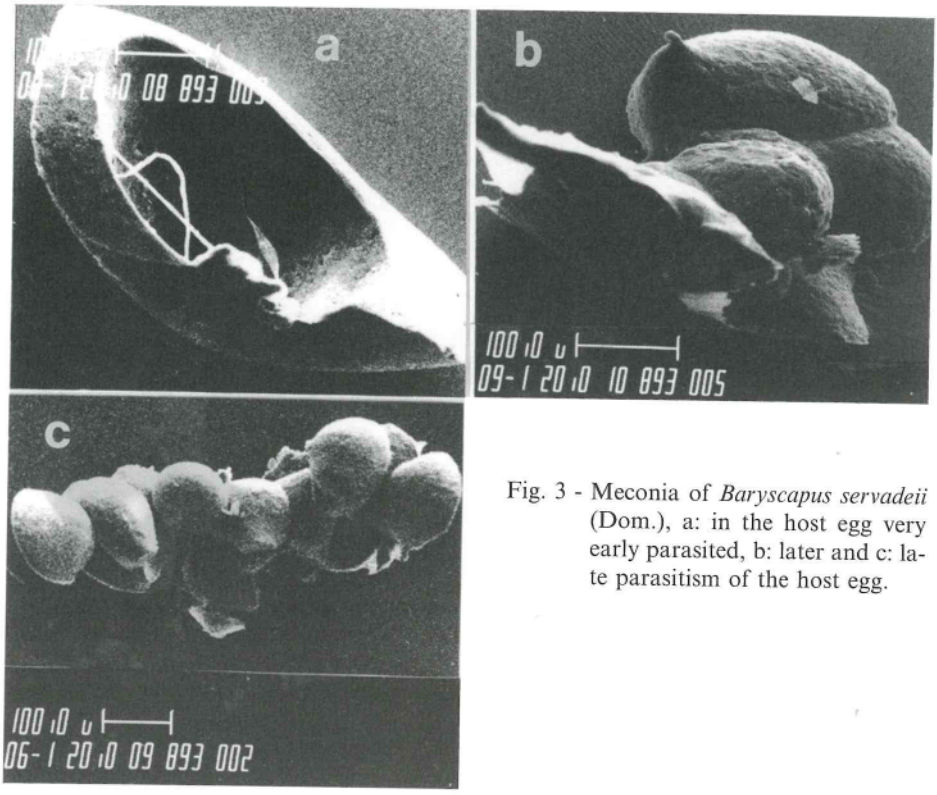

Fig. 3 - Meconia of *Baryscapus servadeii* (Dom.), a: in the host egg very early parasited, b: later and c: late parasitism of the host egg.

sitism took place in an early embryonic stage of the host and no diapause will occur.

Also in *O. pityocampae* larvae, the meconia differred depending on the time of parasitism. If parasitization takes place in the early stages of host development, small yellow discs are produced as meconium in *Thaumetopoea* eggs, and also in artificial eggs, as Masutti et al. (1992) reported. In the latter the meconium appeared after about 11 days of development, produced by the prepupal stage of the parasitoid as small yellow discs when rearing occurred at 22-25 °C. In later embryonic stages of the host, the meconia became darker and globular and the adult parasitoids emerged in the following year. Concerning our egg material, there were seldom remains of the host embryo parasitized by *O. pityocampae*. This indicates that most of the eggs were parasitized shortly after oviposition (Kitt & Schmidt 1993).

In some eggs superparasitism could be observed as reported by Halperin (1970, 1990) and Battisti et al. (1988). In these cases two respiratory masks (Fig. 1.2) and two meconia were found. Hyperparasitism was not observed.

The meconia of *A. bifasciatus* and *Trichogramma* sp. were studied for the first time. More detailed investigations will have to be carried out, to decide whether changes also occur in the meconia, depending on the time of parasitism and on factors related to diapause.

ACKNOWLEDGEMENTS

We would like to thank Dr. R. Stelzer, Institute of Botany, Veterinary School of Hannover (Germany), for his assistance with the SEM technique.

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Ricevuto il 15 marzo 1995; pubblicato il 30 giugno 1995.