



Journal of  
**Entomology**

ISSN 1812-5670



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**Biological Characterization of Two Sibling Species in *Sycophila mellea* (Hymenoptera: Chalcidoidea: Eurytomidae) in Britain, Parasitoids of *Tetramesa* spp. (Hymenoptera: Chalcidoidea: Eurytomidae) in Poaceae**

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**Abstract:** The taxonomic status of *Sycophila mellea* (Curtis) species complex (Hymenoptera: Eurytomidae), reared from *Tetramesa linearis* (Walker) and *T. brevicornis* (Walker) feeding in the grasses: *Elymus repens* (Viv) and *Festuca rubra* (L.), respectively, were investigated using host preference study. The results of host preference experiments demonstrated that the *S. mellea* (ex: *T. linearis* and ex: *T. brevicornis*) were attracted predominantly to their native host plants, *E. repens* and *F. rubra*, respectively. In conclusion, the results from host preference study strongly suggest that members of *S. mellea* (ex: *T. linearis* and *T. brevicornis*) represent different taxa.

**Key words:** Biological species, sibling species, host preference, Hymenoptera, Eurytomidae, *Tetramesa*, *Sycophila*, Poaceae

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## Introduction

Parasitic organisms generally and herbivorous insects and insect parasitoids in particular, are rich in sibling species (Claridge, 1988; Dawah *et al.*, 2002) and they demonstrate that within a genus, diversification occurs with little morphological differentiation; this makes taxonomic separation of the species very difficult. Chalcidoid wasps are generally very difficult to identify because of little apparent morphological differentiation.

*S. mellea* is a parasitoid of *T. linearis* and *T. brevicornis*, feeding on two host plants, *E. repens* and *F. rubra*, respectively (Dawah *et al.*, 1995). In the literature it is regarded as a single biological species (Fitton *et al.*, 1978), yet the fact that it attacks hosts specific to plants growing in different habitats suggests that it might be a complex of species (Anga, 1991).

Parasitoid specificity to their host habitat has been used to support the taxonomic separation of closely-related species of Hymenoptera (Dawah *et al.*, 2002). For example, Dawah (1988) in his host choice experiments, demonstrated that species of the *Pediobius eubius* (Walker) (Hymenoptera: Eulophidae) complex were attracted to their particular host plants through olfactory responses to odours emanating from them. He found that females prefer the odour of the host food plant from which they were reared, i.e., known host to non-host food plant. He found that the *Pediobius* ovipositing on the grass *Elymus repens* was a different species to that ovipositing on *Dactylis glomerata* L. He stated that this result clearly supported the taxonomic separation of species of the *Pediobius* complex.

Vet *et al.* (1984) showed that *Asobara tabida* (Nees) (Hymenoptera: Braconidae) appeared to be either attracted to fruit or to decaying leaves. Further investigation revealed that this species was in

fact composed of two closely-related sibling species, each specialized in its own micro habitat (Kenis and Mills, 1998).

Campan *et al.* (2002), Mattiacci *et al.* (2000), Havill and Raffa (2000), Rutledge and Wiedenmann (1999) and Ding *et al.* (1989) reported the role of plants and other cues characteristic of habitats containing hosts and these have been shown to be important in host habitat location.

More evidence for the importance of host plant odours has been obtained from behavioural studies in the laboratory, using Y-tube olfactometers and wind tunnel experiments (Zanen and Carde, 1991; Potting *et al.*, 1995; Nealis, 1986).

Dawah *et al.* (2002) examined the behavioural responses of female *Pediobius obscurus* Dawah and Al-Haddad and *P. planiventris* (Thomson) to four grass species; *E. repens*, *B. sylvaticum*, *B. pinnatum* (L.) Beauv and *D. glomerata*, infested with larvae of their *Tetramesa* host. They found that closely related species of *P. planiventris* reared from *T. fulvicollis* in *B. sylvaticum* and *P. obscurus* reared from *T. angustipennis* in *A. pratensis* (L.) are associated with their host food plant in a way supporting their genetic and taxonomic separation. Since no parasitoid larvae or larval remains were found in *D. glomerata*, they assumed that no oviposition by either of the *Pediobius* species occurred. They also found that female *Pediobius* oviposited only on the host species from which they were normally reared as natural host food plants.

The present study investigate whether members of *S. mellea* (ex: *T. linearis*) and *S. mellea* (ex: *T. brevicornis*), important primary parasitoids of two phytophagous *Tetramesa*, *T. linearis* on *E. repens* and *T. brevicornis* on *Festuca rubra*, respond to the host food plant in a way that could support their taxonomic separation.

## Materials and Methods

### Collection Sites

Grasses were collected from 1999 to 2000 from the following numbered localities in South Wales and England (Ghajarieh, 2003).

### Rearing Individual Larvae from Stems

Rearing techniques of *Tetramesa* and *Sycophila* species larvae employed here was described by Dawah (1987). Grasses were collected in November 2000, when the larvae within had completed feeding (Graham and Claridge, 1965). The stems were dissected by scalpel without damaging the larvae when the larvae were fully fed and in diapause and placed in individual gelatine capsules and labeled. They were placed in a shed where they were constantly checked until *Sycophila* larvae were reared to adulthood. All males and females of *Sycophila* and *Tetramesa* used in all experiments were fed with 10% (w/v) honey solution. Grasses were identified using Hubbard (1954). Larvae were identified to genus using Dawah and Rothfritz (1996) and host plant association.

### Host Preference Tests

The behavioural responses of the host food plant preferences of *S. mellea* female were tested for three grass species: *Elymus repens*, *Festuca rubra* and *Phalaris arundinacea* (L.), infested with larvae of the following *Tetramesa* hosts: *T. linearis*, *T. brevicornis* and *T. longicornis*, respectively. The *Phalaris arundinacea*/*T. longicornis* association was used as a control. There is no report that *S. mellea* species complex attack *P. arundinacea*.

The technique used here is described in Dawah *et al.* (2002). Rearing larvae of *Tetramesa* and *Sycophila*. The plants used were at the same developmental stage, with a compact shoot on each. The grasses were planted in pots (15 cm in diameter) in a greenhouse. The host plants *E. repens*, *F. rubra* and *P. arundinacea* were infested with their natural hosts, *Tetramesa linearis*, *T. brevicornis* and *T. longicornis*, respectively at the mid of June 2000. This was done by releasing mated females of the three species into a cage measuring 25 × 25 × 50 cm<sup>3</sup>. Whilst in the vials, mating was assumed to occur naturally and insects were left at least for 72 h. being introduced for oviposition. At the end of this period, the three species were released separately into cages containing their natural host plants for oviposition. The plants were then left for ten days (Dawah *et al.*, 2002), to allow the *Tetramesa* larvae to emerge from the eggs and whilst one infested stem of each the three grass species was then positioned at random inside the experimental cage. The cages used for this part of experiment were wooden-framed, measured 60×40×40 cm, with the top and the sides covered with nylon mesh. Sleeves were fitted to permit access without allowing the insects to escape.

The general experimental procedure was as follows: Both sexes of *S. mellea* that were used in these experiments were virgin and have no experience with odour of the grasses. Rearing the larvae separately ensured the virginity of wasps. A mating period of 2-3 days was allowed (Dawah, 1988), a sufficient time to allow successful mating in a C.T. room (26±°C). One mated female of each *Sycophila* was placed inside the cage at a point equidistant from each of the stems and the insect allowed to select the host food plant. There were eight experimental replicates for each *S. mellea* population (ex: *T. linearis* and *T. brevicornis*).

## Results and Discussion

The dissections of stems of the three grasses, *E. repens*, *T. linearis* and *P. arundinacea* confirmed that all stems used in the experiment were infested with *Tetramesa*. Larvae of *Sycophila* were recorded however only in the natural host food plants. No evidence of the presence of larvae was found in the stems of other plant species. No *Sycophila* larvae were found from *P. arundinacea* (Table 1), so it could be assumed that no oviposition by either of the two population of *Sycophila* took place in that grass.

The preference of insect parasitoids towards the food plant of the host, rather than to the host itself, has been reported by many authors. Parasitoids, in the absence of their host, may be attracted to the micro habitat of their preferred host by chemical cues. van Alphen and Jervis (1996) suggested that two phases in host finding behaviour can be recognised for parasitoids: 1) habitat location and 2), host location. They stated that two sorts of stimuli operate at each level - attractant stimuli and arrestant stimuli. Attractant stimuli elicit orientation to the general area of hosts and for reduction in the distance, are involved with arrestant stimuli by parasitoids moving around host areas (Waage, 1978).

Table 1: Results of the host specificity experiment. Individual mated females of *S. mellea* (ex: *T. linearis* and *T. brevicornis*) were given the choice between *E. repens*, *F. rubra* and *P. arundinacea* infested with *T. linearis*, *T. brevicornis* and *T. longicornis*, respectively

<i>S. mellea</i> female introduced	No. replicates (one female per cage)	No. larvae found associated with <i>T. linearis</i> in <i>E. repens</i>	No. larvae found associated with <i>T. brevicornis</i> in <i>F. rubra</i>	No. larvae found associated with <i>T. longicornis</i> in <i>P. arundinacea</i>	$\chi^2$	p-value
<i>S. mellea</i> ( <i>E. repens</i> )	8	14	0	0	69.80	p<0.001
<i>S. mellea</i> ( <i>F. rubra</i> )	8	0	5	0	10.42	p<0.025

Much relevant research has been about orientation to microhabitats, in other words potential sources of host food such as plants (van Alphen and Vet, 1936). Godfray (1993) suggested that stimuli from the host micro habitat or food plant are one of the three broad categories of information that are used in host location. Chemical cues from the host's micro habitat can attract parasitoids in the absence of the host itself (McAuslane *et al.*, 1990; Read *et al.*, 1970; Camors and Payne, 1972).

Volatiles released by other organisms may be used in host location, Greany *et al.* (1977), Spradbery (1970), Lewis and Jones (1971) Ramachandran and Norris (1991). Greany *et al.* (1977) reported that the braconid *Biosteres longicaudatus* (Ashmead), which attacks tephritid fruit flies, is attracted to some chemical cues released by a fungus that grows on peaches (*Prunus persica*). Some ichneumonids in the genera *Rhyssa* and *Megarhyssa* are attracted to volatile chemicals produced by a fungus (Spradbery, 1970). Some other important sources of short-range attractant chemicals include frass and honeydew. Thus for example, Lewis and Norris (1971) demonstrated that the braconid *Microplitis croceipes* (Cresson), a parasitoid of the corn earworm moth, *Helicoverpa zea* (Boddie), responds by antennation to 13-methyl haptaoctane, a chemical in the host frass. Ramachandran *et al.* (1991) identified several active volatiles from frass of the soybean looper moth, *Pseudophusia includens* (Walker) (Lepidoptera: Noctuidae), larvae that attract the braconid, *Microplitis demolitor* Wilkinson.

Most Homopterans produce large quantities of honey dew which both reveal their presence and provide food for parasitoids. The encyrtid, *Microterys nietmeri* (Mutschulsky), responds to fructose and sucrose and some other unidentified compounds in the honeydew secreted by its host, *Coccus hesperidum* (L.) (Homoptera: Coccidae), the brown soft scale (Vinson *et al.*, 1978). Tactile and visual micro habitat cues also are important in host location. The braconid *Opius (alloeum) melleus* (Gahan) is attracted to hawthorn berries, *Crataegus monogyna* Jacq, where its host feeds (Glas and Vet, 1983). Wackers and Lewis (1993) have reported that after attraction to the host plant by long range stimuli such as volatile chemicals, visual cues help parasitoids in the final stages of approach and landing.

The present work demonstrated a very strong preference between parasitoids and their host food plant (here on which its insect-host feeds) as opposed to a non-host food plant. The main conclusions were drawn from this study that females of *S. mellea* (ex: *T. linearis*) and *S. mellea* (ex: *T. brevicornis*) showed a strong response to the host plants that their *Tetramesa* hosts which parasitize *E. repens* and *F. rubra*, respectively. Dissections confirmed that all stems of *E. repens*, *F. rubra* and *P. arundinacea* were infested by their specific parasites, *T. linearis*, *T. brevicornis* and *T. longicornis* in the experiments. No evidence was found for the presence of *S. mellea* larvae in the stems of *P. arundinacea*. Larvae and larval remains of *Sycophila* were recorded in their natural host plants.

These results confirm that *S. mellea* (ex: *T. linearis*) oviposit only in *E. repens* and *S. mellea* (ex: *T. brevicornis*) oviposited in *F. rubra*. No parasitoid larvae or larval remains were observed from *P. arundinaceae*. So that it is assumed that no oviposition by *Sycophila* took place in that grass. The results also clearly show an attraction to the host plant, but not to non-host food plants. As a further step towards understanding the taxonomic status of these potential sibling species, the responses of *S. mellea* (ex: *T. linearis*) and *S. mellea* (ex: *T. brevicornis*) to their hosts supports their taxonomic separation.

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