

SESSION II

BASIC BIOLOGICAL CHARACTERISTICS
OF NATURAL ENEMIES

Morphometry and molecular markers to discriminate between *Macrolophus pygmaeus* and *Macrolophus melanotoma*

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Macrolophus melanotoma (= *M. caliginosus*) and *Macrolophus pygmaeus* have been referred to be important predators of pests in vegetable crops in the Palaearctic region. Due to their high morphological similarity these two mirid species have been confused, with important consequences for their use, both in inoculative and/or conservative biological control programs. In this study we review the taxonomic characters that resulted reliable to separate the two species, as the profiles of cuticular hydrocarbons, a discriminant function of four morphometric ratios of males, and specific primers to distinguish both species through conventional PCR. We also discuss the correct assignation of *Macrolophus* species on historical records. All tested *Macrolophus* samples coming from old IRTA's lab colonies were identified as *M. pygmaeus*, indicating that the articles we have published on '*M. caliginosus*' using laboratory colonies should actually refer to *M. pygmaeus*. When samples from commercial companies were analyzed using the specific primers previously mentioned, in all cases the species identified was *M. pygmaeus*. This result indicates that the species being released commercially is *M. pygmaeus* in most of the cases. We can hypothesize that most of the field work on '*M. caliginosus*' in the Mediterranean Basin and presumably also in the rest of Europe, which is mainly centred in tomato crops, may actually refer to *M. pygmaeus*. Identification of native plants that are sources of the predator that colonises the tomato crop is a key element in the implementation of conservation biological control programmes for this crop. However, most of the host plants of both species still remain largely unknown.

Influence of phytophagous behaviour on prey consumption by *Macrolophus pygmaeus*

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Omnivorous insects in Heteroptera constitute an important component of predatory guilds with high potential for biological control. Understanding the relative effects of plant feeding on the suppression of prey on omnivores could be an important element for improving biological control strategies. In the current paper, the effect of different plant food sources on the predation rate of the omnivorous predator *Macrolophus pygmaeus* (Hemiptera: Miridae) were examined. In all the experiments, nymphs of the second instar of the aphid *Myzus persicae* (Homoptera: Aphididae) were used as prey at different densities. First the consumption rate of the predator on *M. persicae* at various prey densities on leaf of pepper or eggplant was evaluated. Then, using as additional food sources, flower or pollen of eggplant, predator's efficiency was estimated on three densities of prey. The predation rate was not affected by the type of plant leaf used. However, the results showed that the predation rate of *M. pygmaeus* was significantly decreased by the provision of flower or pollen at high prey densities. The importance of these results in understanding the role of phytophagy in the predation rates of omnivorous predators is discussed.

Effect of supplemental food on the fitness of four omnivorous predator species

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The predators *Orius laevigatus*, *O. majusculus*, *Macrolophus pygmaeus* and *Nesidiocoris tenuis* are extensively used in biological controls in vegetable crops. The fitness and local density of these predators, which are omnivores and feed on arthropods and plants, may be enhanced by the provision of supplemental food, especially when prey is scarce, and this may improve biological control results. The aim of our study was to evaluate the effect of four supplemental foods: *Ephestia kuehniella* eggs, dry *Artemia* sp. cysts, the mite *Tyrophagus putrescentiae* and commercial multifloral bee pollen, on the reproduction and longevity of these four predators. Our results show that using dry cysts of *Artemia* sp. as factitious prey had positive effects on the reproduction of the predators *O. majusculus*, *O. laevigatus*, *M. pygmaeus* and *N. tenuis*. Furthermore, dry commercial multifloral bee pollen improved the longevity of the females of *O. laevigatus*, *O. majusculus* and *M. pygmaeus* as much as eggs of *E. kuehniella*. Dry cysts and dry pollen need little or no handling to prepare them for use, are very easy to disperse in the crop and maintain their quality for a reasonable time. Further experiments need to be conducted under semi-field conditions to better evaluate the positive effect of these two supplemental foods on predator establishment in the crop. It will also be important to evaluate the effect of these foods on life parameters of pest species and on intraguild relationships among predators.

Sugar as nutritional supplement for the zoophytophagous predator *Nesidiocoris tenuis*: practical implications

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Nesidiocoris tenuis (Hemiptera: Miridae) is a zoophytophagous predator widely used in integrated pest management programs in both greenhouse and open-field tomato crops in the Mediterranean basin. Its use, both by inoculation or conservation, may cause some drawbacks. First, to establish *N. tenuis*, inoculative releases are usually combined with the addition of *Ephestia kuehniella* (Lepidoptera: Pyralidae) eggs. The use of this factitious prey increases the cost of the release. Second, *N. tenuis* can cause some plant damage due to its phytophagous diet when prey is scarce. The aims of this work were: (i) to evaluate the effect of sugar addition on the biology and prey consumption of *N. tenuis* under laboratory conditions; (ii) to test whether the quantity of *E. kuehniella* eggs added as factitious prey could be reduced when sugar sources were also added under greenhouse and open-field conditions; (iii) to test whether the addition of sugar reduce the damage inflicted by *N. tenuis*. Our results showed that the addition of sugar in an *E. kuehniella*-based diet increased *N. tenuis* progeny and reduced the predation rate of *E. kuehniella* eggs. In the field, the addition of sugars reduced the number of *E. kuehniella* eggs necessary to establish *N. tenuis* and reduced the damage produced by *N. tenuis*. These findings may have practical implications, both in *N. tenuis* mass-rearing systems and in biological control programs where *N. tenuis* is released.

Analysing predation of *Orius majusculus* (Hemiptera: Anthocoridae) in lettuce crops by PCR

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Orius majusculus (Hemiptera: Anthocoridae) is an important generalist predator known to feed on two of the main pests in lettuce crops, *Frankliniella occidentalis* (Thysanoptera: Thripidae) and *Nasonovia ribisnigri* (Hemiptera: Aphididae). Diagnostic molecular gut-content analysis has been conducted to study predation of *O. majusculus* on these pests, as well as on the springtails of the genus *Entomobrya* sp. (Collembola: Entomobryidae), the most abundant non-pest prey in lettuce crops of the studied area. Two pairs of specific primers were designed for *N. ribisnigri* and *F. occidentalis*, amplifying 331 bp and 277 bp fragments of the mitochondrial Cytochrome *c* Oxidase I (COI) region, respectively. Collembola detection was conducted with a pair of a group-specific primers previously designed that amplify an amplicon of 177 bp of the 18S rDNA region. Prey was detected in 100% of predators immediately after prey consumption (t=0h). PCR analysis of *O. majusculus* collected in the field revealed a strong relationship between prey density in the crop and consumption. Sixty per cent of these field-collected *O. majusculus* were positive for *N. ribisnigri* in spring (when this pest species was present) and up to 40% were positive for *F. occidentalis* in summer (when the density of this pest was the highest). Collembola detection ranged from 9.4% to 28.2% depending on the season and predator stage. A higher proportion of immature *O. majusculus* ingested more than one prey species simultaneously compared to adults of the same predator species. These findings suggest that *O. majusculus* is an important predator for the control of both lettuce pests, being springtails an important alternative prey, which could maintain *O. majusculus* populations when both pest species are not present in the crop or are not abundant enough.

Do oviposition substrates and different materials as hiding-places influence reproductive parameters of *Orius insidiosus*?

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Orius insidiosus is a commercially produced natural enemy used as a biological control agent of thrips in greenhouses. Mass production is strongly influenced by the type of oviposition substrate, as well as by hiding materials that reduce the contact between individuals and avoid cannibalism in the rearing unit. The objective of this study was to evaluate two oviposition substrates, bean sprouts and bean pods of *Phaseolus vulgaris*, and different types of materials (rice hulls, popcorn, shredded paper towel and Styrofoam white flakes) to provide hiding places and prevent cannibalism in the rearing system of *O. insidiosus*. Bean sprouts were accepted and more suitable for *O. insidiosus* females as an oviposition substrate in the laboratory. The total number of eggs/rearing unit/30days, the number of eggs/female/day/rearing unit and the number of emerging adults were significantly higher with bean sprouts as oviposition substrate compared to bean pods. No significant differences were found in the number of eggs and number of emerging adults/rearing unit with different types of materials to provide hiding-places and avoid cannibalism.

Prevention of diapause induction in a Japanese strain of *Aphidoletes aphidimyza*

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The aphidophagous gall midge *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae), a dominant natural enemy of aphids, is used in many countries to control aphids in greenhouses. Since developmental arrest during diapause reduces the effectiveness of natural enemies, we studied the effect of photoperiod and temperature on the incidence of diapause in a Japanese strain of *A. aphidimyza* by examining diapause induction under different day-length and temperature conditions. The critical day length for diapause induction was 12.7 h at 20°C. Diapause incidence was completely prevented at 30°C, even under a photoperiod of 11L : 13D. Diapause induction in *A. aphidimyza* could be prevented by providing light during the scotophase in the laboratory. However, diapause induction was not prevented by providing light for 1 h at night in the field experiments from September to November 2011.

A comparison of three *Encarsia* species as controlling agents for *Bemisia tabaci* in greenhouses

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The study constitutes part of an effort to locate potentially efficient agents for biological control against the whitefly *Bemisia tabaci*. Three parasitoid species, *Encarsia inaron*, *E. lutea* and *E. sophia*, which attack *B. tabaci* under natural conditions, were studied comparatively in order to evaluate the most suitable species for use as a biological control agent against *B. tabaci* in greenhouses. The tested parameters were: developmental durations, longevity and oviposition characteristics. All three species lived ca. 20 days each, and the two former ones developed to adulthood within ca. 14 days and *E. lutea* within 15. The differences between oviposition rates were not significant (66.00 ± 36.27 , 32.84 ± 26.19 and 42.73 ± 21.74 average and standard deviation for *E. inaron*, *E. lutea* and *E. sophia*, respectively). *Encarsia inaron* had the highest percentage of females that lived longest whereas *E. lutea* had a higher semelparity. Our findings failed to reveal the superiority of one species over the others as candidates for the intended biological control. Additional parameters, including their competitive behaviour and compatibility with other natural enemies in the greenhouse, should thus be considered.

The effect of pollen on the predation of *Polyphagotarsonemus latus* by the predaceous mites *Amblyseius swirskii* and *Euseius scutalis*

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The broad mite *Polyphagotarsonemus latus* (Acarina: Tarsonemidae) is a polyphagous pest that infests almost 60 plant families including sweet peppers. Several chemical treatments are used to control its population. However, there is an agriculture movement towards ecological sustainability in agriculture that has led to an increased interest in using biological control. The predatory mites *Amblyseius swirskii* (A.s.) and *Euseius scutalis* (E.s.) have been shown to control the populations of several arthropods and thus can be used as biological pest control. The object of this research is to determine the effect of adding pollen on the efficiency of the predators. The presence of pollen is expected to increase the efficiency of the predator by allowing predators to establish before the presence of the pests. In order to test this hypothesis we first tested if pollen increased the efficacy of the predators when prey was available. Potted sweet pepper plants (*Capsicum annuum*) were infested with broad mites and then randomly assigned to one of the five treatments: control (no predatory mites); E.s. plus pollen; E.s. without pollen; A.s. plus pollen; A.s. without pollen. Plants were sampled once a week to check for the presence of broad mites and predatory mites. Five plants from each treatment were randomly chosen and were cut at ground level and placed in a plastic container that contained 80% EtOH. In the laboratory, plants were rinsed and arthropods were counted using a dissecting microscope. Preliminary results showed that although the same number of predatory mites was released initially, there were fewer *E. scutalis* than *A. swirskii*. The pollen had a positive effect on both of the predator populations.

Dispersal of TSWV by *Frankliniella occidentalis* (Thysanoptera: Thripidae) in presence of natural enemies

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Biological control reduces the population of arthropod vectors on crops. However, natural enemies might induce antipredator behaviour in vector prey, thus enhancing vector dispersal and therefore virus spread. In a previous study we showed that the spread of a non-persistent virus, *Broad bean wilt virus 1* (BBWV-1), by its aphid vector *Myzus persicae* was not affected by the presence of natural enemies as compared to the control without predators. However, predators inducing stronger antipredator behaviour, such as the coccinellid *Adalia bipunctata*, promoted higher transmission efficiency of BBWV-1 than larvae of the syrphid fly *Sphaerophoria rueppellii*, which induced softer antipredator behaviour. The effect of antipredator behaviour on transmission efficiency is affected by the mode of transmission of the virus, since different acquisition and inoculation periods are required to transmit them. Antipredator behaviour might involve the emission of alarm pheromone by prey. Both aphids and thrips are able to emit alarm pheromone in presence of natural enemies. Predators eliciting the emission of alarm pheromone in vectors could promote vector dispersal and virus spread more than those natural enemies not inducing alarm pheromone emission. We studied the antipredator behaviour of the thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae) in presence of two predators commonly used in greenhouse crops as biological control of thrips: the predatory bug *Orius laevigatus* and the predatory mite *Amblyseius swirskii*. To compare antipredator behaviour of thrips with a predator not feeding normally on thrips, we tested behaviour of thrips in presence of larvae of the aphidophagous syrphid fly *S. rueppellii*. Behaviour of predators and prey was observed under the stereoscope on arenas consisting of a piece of bean pod on a Petri dish, where adult thrips were allowed to settle before one predator was released. Continuous observations were recorded during 20 minutes with the behaviour program Etholog. In another experiment, dispersal of thrips towards receptor plants and transmission efficiency of *Tomato spotted wilt virus* (TSWV) by thrips in presence and absence of predators was compared in a semi-field study. The predators tested were the same as in the behaviour observations. Dispersal and transmission experiments were performed by placing a TSWV-infected source pepper plant, where viruliferous thrips were released, surrounded by healthy receptor pepper plants in a cage. Two predators were placed on each source plant, after the thrips were let to settle during 30 minutes. The number of thrips on the source and on the receptor plants was recorded 24 h and 48 h after the experiment started. An insecticide was sprayed then to prevent further movement of thrips between plants. Transmission of TSWV by thrips was measured 15-25 days post inoculation as the number of infected receptor plants divided by the number of total receptor plants. Transmission experiments were performed both with thrips larvae and adult thrips, even though thrips larvae are less efficient transmitters than adults. Preliminary results indicate that thrips displayed antipredator behaviour less frequently in front of syrphid larvae than in presence of predatory mites or *O. laevigatus*. The implications of our results on the epidemiology of TSWV are discussed within the context of a multi-predator system and a plant virus transmitted in a persistent propagative manner. To our knowledge, this is the first description of predation of *F. occidentalis* by the aphidophagous syrphid fly *S. rueppellii*.

Life history parameters for *Nesidiocoris tenuis* and *Macrolophus pygmaeus* (Hemiptera: Miridae) preying upon *Tuta absoluta* eggs (Lepidoptera: Gelechiidae)

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The polyphagous predators *Nesidiocoris tenuis* and *Macrolophus pygmaeus* (Hemiptera: Miridae) are endemic natural enemies that commonly inhabit tomato crops in the Mediterranean basin. Both predators are mass-reared and released mainly to control whiteflies, although both have recently been observed contributing to the control of the invasive tomato pest *Tuta absoluta* (Lepidoptera: Gelechiidae). Life-history traits of these two predators were studied and compared when they were fed exclusively with eggs of *T. absoluta* on tomato plants. The experiments were performed under laboratory conditions (25 °C, 60% RH and 16:8 h L:D). The immature survival for both mirids was similar (about 90%), although the nymphal development time was significantly faster for *N. tenuis* than for *M. pygmaeus* (13.0 ± 0.1 d and 16.0 ± 0.2 d, respectively). The progeny was also higher for *N. tenuis* than for *M. pygmaeus* (38.9 ± 6.9 and 3.0 ± 1.7 nymphs per female, respectively). These differences resulted in higher selected life history parameters for *N. tenuis* when compared to *M. pygmaeus* (e.g. the intrinsic rate of increase was 0.135 for *N. tenuis* and 0.006 for *M. pygmaeus*). Our results may explain the higher efficacy of *N. tenuis* controlling *T. absoluta* and the increasing prevalence of *N. tenuis* in those tomato crops infested by *T. absoluta*.

Biology and behaviour of the indigenous parasitoid *Bracon nigricans* on the invasive South American tomato pinworm *Tuta absoluta*

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Bracon (Habrobracon) nigricans (Hymenoptera: Braconidae) was collected in Italy on *Tuta absoluta* (Lepidoptera: Gelechiidae), a Neotropical key tomato pest. The parasitoid was successfully reared under laboratory and semi-field conditions for more than fifty generations, with no apparent adverse effects on the parasitoid performances, such as fertility, progeny sex-ratio and parasitization rates. We studied the biology and behaviour of the wasp under laboratory conditions and we documented its life history. *Bracon nigricans* proved to be a gregarious, idiobiont, arrhenotokous ectoparasitoid of *T. absoluta* mature larvae. Laboratory studies showed that adults fed on a sugary-proteinic nutrient lived longer and produced more progeny than those provided only with *T. absoluta* host larvae. The parasitoid progeny was correlated to host densities, and the parasitism rate was higher at lower host densities. Since females should mate multiple times to be able to produce females throughout their life span, progeny sex-ratio mostly depended on male availability. Both females and males were reproductively active soon after emergence. Parasitoid females showed intense stinging activity before effectively starting to lay eggs and/or to feed on the host haemolymph. During this pre-oviposition activity, female inject venom for permanently paralyzing hosts and very likely for assessing host suitability (based on host size and/or host haemolymphatic kairomone recognition) for the larval development. The proportion of hosts that were killed was constant in all the trials, while the progeny amount was correlated with the host density. Although semi-field and field studies would be needed, the data obtained so far in laboratory conditions suggest that this parasitoid could be considered a potential biological control agent of *T. absoluta* on tomato in Europe.

Do earthworms increase oviposition of the tiger-fly, *Coenosia attenuata* (Diptera: Muscidae)?

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Tiger-fly, *Coenosia attenuata*, is a promising predator of adults of important greenhouse pests such as whiteflies, leafminers and fungus gnats. The larvae live in the soil preying soil-dwelling organisms such as sciarid larvae. The adult attacks insects in flight and they kill more individuals than they actually feed on. Open rearing units could be used as a strategy for increasing the populations of this predator and enhancing conservation biological control. In this study, the influence of earthworm mucus on tiger-fly oviposition was evaluated with the aim of using earthworms as prey in greenhouse tiger-fly rearing units. In laboratory conditions, mucus significantly induced the females to extend more times their ovipositors and to lay more eggs.

Morphometry and biology of a new anthocorid *Montandoniola indica*, a potential predator of *Gynaikothrips uzeli*

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A new anthocorid predator, *Montandoniola indica* was recorded for the first time as a predator of *Gynaikothrips uzeli* infesting *Ficus retusa* in Karnataka (India). The morphometry and biology of this predator were studied. *M. indica* nymphs and adults could be reared on UV irradiated eggs of the rice moth *Corcyra cephalonica*. The feeding potential of *M. indica* nymph was 27 *C. cephalonica* eggs and in the case of adult male and female, 38 and 56 eggs, respectively. The adults of *M. indica* were provided with thrips and pollen in addition to *C. cephalonica* eggs, for better survival. The adult male and female longevity was 23 and 31 days, respectively and fecundity 37 eggs per female.