**Focus on:**

Macropholus pygmaeus (Rambur) as an efficient predator of the tomato leafminer *Tuta absoluta* (Meyrick) in Europe. A review

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The tomato leafminer, *Tuta absoluta* (Meyrick), originates from South America, and remains one of the main tomato pests in this continent. Since its introduction to Europe in 2006, Mediterranean countries have also been exposed to this pest. Because of the endophytic habits of the larvae and ability of adults to reproduce parthenogenetically, chemicals and sexual pheromone-based control methods generate poor results. Recently, the use of biocontrol agents, such as *Macropholus pygmaeus* Rambur (Heteroptera: Miridae), has been investigated as an alternative means of control, the results of which are presented in this review. *Macropholus pygmaeus* is a mirid bug that is widely used to control different phytophagous insects in integrated pest management strategies through Europe. Several studies have confirmed the high predation potential of *M. pygmaeus* on *T. absoluta* under laboratory and semi-field conditions. This predator spontaneously colonizes tomato greenhouses in the southern Mediterranean countries. The use of banker plants (i.e., plants that provide a habitat to the predator) improves the colonization ability of this natural enemy. Hence, if the local population size is low, an augmentative strategy could be adopted. Predators may be released before or after the onset of pest infestation, with recommendations varying depending on natural population densities of both the pest and predator. The efficiency of *M. pygmaeus* has also been evaluated when used in combination with other biocontrol agents or with chemicals. This work presents an overview of different types of control strategies using *M. pygmaeus* against the tomato leafminer, *T. absoluta*.

**Keywords.** Gelechiidae, *Macropholus*, biological control, integrated pest management, predatory insects, greenhouses.

**Macropholus pygmaeus** (Rambur), prédateur efficace de la mineuse de la tomate *Tuta absoluta* (Meyrick) en Europe (synthèse bibliographique). La mineuse de la tomate, *Tuta absoluta* (Meyrick), est considérée comme l’un des principaux insectes ravageurs des cultures de tomates en Amérique du Sud, d’où elle est originaire. Depuis son introduction sur le continent européen en 2006, les pays méditerranéens doivent également faire face aux infestations de ce nouveau ravageur. À cause du caractère endophyte des larves et de leur capacité à se reproduire par parthénogénèse, les méthodes de lutte chimiques et sémiiochimiques ne sont pas totalement efficaces. L’utilisation d’insectes auxiliaires, tels que *Macropholus pygmaeus* Rambur (Heteroptera : Miridae) représente une alternative explorée récemment et dont les résultats sont rassemblés dans cette synthèse bibliographique. En effet, *M. pygmaeus* est un prédateur de *T. absoluta* couramment utilisé dans les programmes de lutte contre plusieurs insectes phytophages. Les différentes études de laboratoire et de terrain qui lui sont dévolues reconnaissent toutes le grand potentiel de *M. pygmaeus* dans la lutte contre *T. absoluta*. Le prédateur est natif de la région paléarctique et colonise spontanément les serres de tomates de la région méditerranéenne. Des méthodes dites de conservation visent à améliorer la colonisation naturelle des prédateurs à l’aide de plantes refuges (i.e. plantes qui fournissent un habitat au prédateur). Quand la conservation n’est pas suffisamment efficace, des lâchers de prédateurs peuvent être effectués pour augmenter la population locale. Les lâchers se font soit en prévision des infestations ou en applications curatives. Les recommandations varient en fonction des densités de prédateurs et de ravageurs. Dans le but d’améliorer les résultats de la lutte biologique, il est possible de combiner *M. pygmaeus* avec d’autres moyens de lutte, que ce soit d’autres prédateurs ou parasitoides, ou des méthodes chimiques. L’ensemble des approches visant la lutte biologique contre *T. absoluta* à l’aide de *M. pygmaeus* est présenté ici.

**Mots-clés.** Gelechiidae, *Macropholus*, contrôle biologique, gestion intégrée des ravageurs, insecte prédateur, serre.
1. INTRODUCTION

The tomato leafminer moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is currently considered as a serious agricultural threat to European tomato crops, causing losses of 80% to 100% in areas without control measures (Desneux et al., 2010; Desneux et al., 2011a). The pest pressure varies among the European countries, being higher in the Eastern Mediterranean countries while it has declined to medium levels in Western Mediterranean countries. In Northern European countries, infestations are limited to glasshouses (EPPO, 2011). This moth is 6–7 mm in length, and is native to South America, but was introduced to Spain in 2006. It has a reproduction rate of 10 to 12 generations a year under greenhouse conditions, facilitating the fast invasion of new territories (Desneux et al., 2010).

The larvae of this moth preferentially develop on tomato plants (*Solanum lycopersicum* L.) (Desneux et al., 2010); however, larvae and adults may also feed and reproduce on a wide range of solanaceous plants (Carpóros Megído et al., 2013a) and other plants such as cape gooseberry (*Physalis peruviana* L.), bean (*Phaseolus vulgaris* L.) and Malva sp. (Tropéa Garzia et al., 2012). Immediately after egg hatching, the larvae consume the leaves and fruits creating galleries through the mesophyll layer, reducing the ability of plants to photosynthesize, which hinders plant development. These galleries also allow the entry of a diverse array of pathogens (Urbaneja et al., 2009; Desneux et al., 2010). The most common control method of this pest is the use of insecticides, such as abamectin, spinosad, tebufenozide, and chlorfenapyr (Lietti et al., 2005), and sex pheromones (Carpóros Megído et al., 2013b). However, both control strategies generate poor or non lasting results (Michereff Filho et al., 2000; Hassan et al., 2009; Vacas et al., 2011). For instance, the efficacy of the sex pheromone strategies is negatively affected by the ability of *T. absoluta* females to reproduce parthenogenetically (Carpóros Megído et al., 2012), while the endophytic (*i.e.*, ability to live within the plant) nature of the larvae reduces the effectiveness of insecticides (Lietti et al., 2005). Moreover, the larvae quickly develop resistance to insecticides (Guedes et al., 2012). Consequently, other strategies are being investigated such as the use of entomopathogenic nematodes (Batalla-Carrera et al., 2010), *Bacillus thuringiensis* Berliner (González-Cabrera et al., 2010) or sterile male techniques (Cagnotti et al., 2012). Among those strategies under evaluation, a promising alternative form of pest management is the use of biocontrol agents (Desneux et al., 2011b; Cabello et al., 2012; Zapalpà et al., 2013).

*Macrolophus pygmaeus* Rambur (Heteroptera: Miridae) is a mirid bug that occurs in the Palaeartic region (Sanchez et al., 2012), and is considered as a principal natural enemy of tomato pests in this region (Perdikis et al., 2011). This bug is a highly polyphagous predator, and is already used as a biocontrol agent against various phytophagous insect pests under greenhouse conditions (Nannini et al., 2012). Both nymphs and adults have piercing-sucking mouthparts, and feed on whitefly eggs, aphids, mites, thrips, and moth eggs (Albajes et al., 2002; Perdikis et al., 2008).

The taxonomy of some *Macrolophus* spp. has been discordant and very dynamic. *Macrolophus pygmaeus* and *Macrolophus melanotoma* (Costa) are sympatric species and cryptic owing to their very similar morphology. These two species differ mainly for few and variable features, such as the lateral band behind the eye and differ in the color pattern of the first antennal segment, being white and black in *M. melanotoma* and entirely black in *M. pygmaeus* (Castañé et al., 2013). Moreover, according to the synonymization by Carapezza (1995), *M. melanotoma* was often quoted its synonym *M. caliginosus* (Wagner). To clarify the situation, researchers have developed molecular techniques and resolved this misidentification using an integrative approach such as crossing experiments, multivariate morphometry and mitochondrial DNA analysis (Perdikis et al., 2003; Martinez-Cascales et al., 2006; Machetelincx et al., 2009; Castañé et al., 2013; Evangelou et al., 2013). It is now an admitted fact that the predators commercially available for biological control are *M. pygmaeus* (Castañé et al., 2013). In the following studies, data have been obtained for *Macrolophus caliginosus*, but will be treated in this review as *M. pygmaeus*: Arnò et al. (2003), Castañé et al. (2004), Castañé et al. (2007), Malausa et al. (1987), Sampson et al. (1999), Tedeschi et al. (2001) and Van Schelt et al. (1996).

This review summarizes available data about *M. pygmaeus* in the particular event of *T. absoluta* biocontrol. The review discusses laboratory and field assay results as well as current information on the relation between the predator and its pest.

An extensive review of the literature has been performed using SCOPUS database, using all keywords associated with the title and abstract, including the biological materials and methods presented in this review.

2. NATURAL OCCURRENCE OF M. PYGMAEUS IN OPEN FIELDS AND GREENHOUSE COLONIZATION

*Macrolophus pygmaeus* is native to the Palaeartic ecozone. It is distributed from Finland to Algeria, the Azores Islands, and Tajikistan (Sanchez et al., 2012). In Spain, *M. pygmaeus* is among the most abundant natural enemies of *T. absoluta* (Arnò et al.,
3. LABORATORY AND FIELD EVIDENCE OF M. PYGMAEUS PREYING ON T. ABSOLUTA

Several laboratory experiments have been conducted to evaluate the ability of *M. pygmaeus* to prey on *T. absoluta*. Under laboratory conditions, starved *M. pygmaeus* preyed on over 30 *T. absoluta* eggs daily (Urbaneja et al., 2009). The predation rate recorded in this experiment was higher compared to that reported on other lepidopteran prey, such as *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), 20 eggs, probably because of the smaller size of *T. absoluta* eggs (Urbaneja et al., 2009). Arnó et al. (2009) reported a predation exceeding 100 *T. absoluta* eggs per *M. pygmaeus* adult and per day. Chailleux et al. (2013a) also showed the active predation of *M. pygmaeus* on *T. absoluta*. *Macrolophus pygmaeus* shows a type II functional response; namely, the predation rate increases with the offered prey density leveling off to a plateau at higher densities. More *M. pygmaeus* females than males preyed on *T. absoluta*, with this difference between the sexes becoming stronger with increasing prey density (Urbaneja et al., 2009).

Urbaneja et al. (2009) also investigated under laboratory conditions the predation rate of *M. pygmaeus* on different *T. absoluta* instars. Although it is able to feed on all developmental stages, *M. pygmaeus* preferentially feeds on first instars (Mollá et al., 2009; Urbaneja et al., 2012), with a mean number of two first instar larvae being eaten per day (Urbaneja et al., 2009). Arnó et al. (2003) obtained the same predation rate when *M. pygmaeus* preyed on third and second instars of *Liriomyza trifolii* Burgess (Diptera: Agromyzidae). These larvae are of similar size to the first instar larvae of *T. absoluta* (Urbaneja et al., 2009). Jaworski et al. (2013) investigated *M. pygmaeus* preference between *T. absoluta* and *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) using entire tomato plants. The results showed a similar preference between whitefly nymphs and leafminer eggs, but a lower predation rate on leafminer larvae. Adult predators showed a higher predation rate than juveniles. The presence of the leafminers increased adult predation on both pests and decreased juvenile predation. However, these results are obtained by laboratory investigations and may vary under field conditions. Bompard et al. (2013) showed a beneficial effect of *M. pygmaeus* on the tomato leafminer in the greenhouse. The study assessed predator-mediated interactions between two pests, *T. absoluta* and *B. tabaci* in greenhouse, by monitoring the pest and predator populations when released on crops either separately or combined. They showed that the predator significantly reduced both pest populations separately, 30-fold for adult whiteflies and 3.7-fold for the leafminer (Bompard et al., 2013).

4. BIOLOGICAL CONTROL USING M. PYGMAEUS

4.1. Conservation: colonization improvement

Conservation biological control consists in habitat manipulation to enhance the action of endemic or exotic natural enemies (Gurr et al., 1999). Usually, predator occurrence is related to the occurrence of target prey. Being zoophytophagous, *M. pygmaeus* offers the advantage of remaining on the crop, even when prey insects are scarce. The plants have a major influence on the survival of *M. pygmaeus* (Ingegno et al., 2011). To effectively establish the population of this predator in the crop, one or several alternative host plants could be planted near the crop requiring protection. These banker plants could also enable the bug population to remain in the vicinity between two crop cycles (Parolin et al., 2012).
**Solanum nigrum** L. is a major host for *M. pygmaeus* (Perdikis et al., 2006; Lykouressis et al., 2008; Ingegno et al., 2011). *Macrolophus pygmaeus* completes its developmental cycle by feeding exclusively on this plant species, although its fecundity is lower than when also feeding on insect prey (Perdikis et al., 2006; Lykouressis et al., 2008). In Crete, *M. pygmaeus* is also abundant on many non-cultivated plants, including *Dimorphotheca aurantiaca* DC., *Cistus creticus* L., *Amaranthus blitum* L., and *Datura stramonium* L. (Roditakis et al., 2003). In Italy, large numbers of *M. pygmaeus* are found on *Artemisia vulgaris* L. (Ingegno et al., 2009).

Ingegno et al. (2011) investigated the fitness of *M. pygmaeus* inhabiting different plants, and plant preferences based on olfactometric assays. The plants selected for the study had been previously reported as host plants for *Dicyphini*, in Piedmont, Italy (Ingegno et al., 2009), and are common in Italy where the experiment was conducted. *Capsicum annuum* L., *Calendula officinalis* L. and *S. nigrum* allowed the predator to survive longer without prey than *Salvia officinalis* L. and *P. officinalis*. The predator reached the fourth instar on the first three plants while it died before the second instar on the last two plants. *Parieteria officinalis* was the least attractive plant to *M. pygmaeus*, but still served as a suitable refuge in the absence of tomato crops (Ingegno et al., 2011). Alomar et al. (2006) reported a high number of predators on *C. officinalis* in fields confirming the potential of this plant. Hilly landscapes might also favor the entry of this predator into greenhouses (Gabarra et al., 2004).

### 4.2. Augmentative strategy

Augmentative strategies are based on the release of predators into greenhouses to increase their numbers. This method is widely used for *M. pygmaeus* in the Mediterranean basin. This species has been commercially available since 1994 for use in integrated pest management (IPM) programs, and is being increasingly used in European tomato greenhouses (Castañé et al., 2011). Although *M. pygmaeus* is a zoophytophagous mirid, it only damages crops at very high population densities and when prey is scarce (Van Schelt et al., 1996; Castañé et al., 2011). Sampson et al. (1999) reported the only case of damages caused by the predator, and highlighted the fact that the number of individuals is a factor influencing the importance of damage. Here, two alternative augmentative strategies are described. The inundative strategy aims to suppress the pest by releasing a large amount of biocontrol agents. The inoculation strategy aims to establish the predator population on the crop before pest infestation. The initial number of predators that are released should be evaluated to reach a sufficient population size before pest establishment. If the pests begin to damage the plants, more natural enemies must be released within the framework of the second strategy.

Alternative food sources, such as *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs and *Artemia* cysts may be provided to *M. pygmaeus* to accelerate population growth (Perdikis et al., 2008; Put et al., 2012). Indeed, Mollá et al. (2014) proved that the progeny production of the predator feeding on a diet based exclusively on *T. absoluta* and tomato plants in laboratory conditions, was too low to lead to a significant and stable population. Similar observations have been made in the field (Bompard et al., 2013).

A semi-field experiment was conducted in tomato greenhouses, and showed that *M. pygmaeus* inoculation reduced leaflet and fruit infestation of *T. absoluta* by up to 76% and 56%, respectively (Mollá et al., 2009). In Sardinian greenhouses, the weekly application of two *M. pygmaeus* per m² allowed the infesting *T. absoluta* population to be controlled (Nannini et al., 2012). In north-east Spain, where *T. absoluta* causes major damage, one to two *M. pygmaeus* adults per m² are released in greenhouses, when natural predator populations are low (Urbanø et al., 2012).

*M. pygmaeus* may also be released at the early stages of crop growth, even before transplanting the plant crop to the greenhouse (Trottin-Caudal et al., 2012). Adult predators are released on the seedlings, and initially fed with *Ephesia* eggs. The eggs laid by *M. pygmaeus* hatch very soon after transplantation, allowing population growth to start earlier (Trottin-Caudal et al., 2012).

### 4.3. Integration in IPM schemes

The use of a natural enemy in combination with other biological control agents, predators and parasitoids, or chemical insecticide treatments is also common, and may help attain higher levels of effectiveness.

In Spain, an IPM program based on the combination of *M. pygmaeus* and *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae) has been used for many years (Urbanø et al., 2012). *Nesidiocoris tenuis* is also a predator of *T. absoluta* which allows to provide higher leaflet and fruit damage reduction than *M. pygmaeus* (Mollá et al., 2009), although this predator naturally occurs only in southern countries (Urbanø et al., 2012). Arnó et al. (2009) evaluated the effectiveness of the IPM program based on the augmentation and conservation of both mirid predators, compared to a conventional plant protection strategy based on insecticide use, in tomato greenhouses and open field. The number of *T. absoluta*, and the percentage of damaged plants and young fruits were significantly lower in greenhouses and open fields treated with the IPM program. Damage was reduced by 80%
after planting. The parasitoids were introduced as parasitized E. kuehniella eggs at a density of 25 individuals per m² eight times during three months after planting. Plant damage was reduced to a similar extent by each of M. pygmaeus and T. acheae alone. However, the best protection level was achieved when both agents were released simultaneously (Trottin-Caudal et al., 2012). Similar results were achieved in microcosms assays (Chailleux et al., 2013a) and in greenhouse cages (Chailleux et al., 2013b).

To reach better control levels with two combined biocontrol agents, intraguild predation (IGP) must be avoided. Macrolophus pygmaeus does not attack T. acheae directly but may decrease the control level if IGP occurs on parasitized eggs. Chailleux et al. (2013a) tested the preference of M. pygmaeus between parasitized and unparasitized eggs in no-choice and double-choice assays. Two parasitized egg stages were included in the test, the newly parasitized eggs, which remain yellow, coloured and 4–5 days parasitized eggs which turn to black. In a no choice situation, the predator fed at the same rate on unparasitized and newly parasitized eggs and at a lower rate on old parasitized eggs. In a choice situation, no preference between yellow and unparasitized eggs was observed, but fewer eggs were consumed when black eggs were offered (Chailleux et al., 2013a).

Macrolophus pygmaeus is a valuable agent to control T. absoluta in an IPM program if no broad spectrum insecticides are used (Arnó et al., 2009). Predator releases in nursery plants are the first step to ensure a good establishment of the population (Trottin-Caudal et al., 2012). On the crop, inoculations of 1–2 adults per m² can be applied (Trottin-Caudal et al., 2012; Urbaneja et al., 2012) and the environment can be manipulated to attract predators from outside or make the population build up easier (Perdikis et al., 2011). Also, E. kuehniella eggs can help building up the population faster (Perdikis et al., 2008; Put et al., 2012; Mollá et al., 2014). In southern countries, N. tenuis and T. acheae can be used in combination with M. pygmaeus to increase the impact of each agent when used alone (Arnó et al., 2009; Trottin-Caudal et al., 2012; Chailleux et al., 2013a). In northern countries, where temperatures are too low for the development of T. acheae and N. tenuis (Urbaneja et al., 2012), B. thuringiensis based formulations can be reasonably used (González-Cabrera et al., 2010; Urbaneja et al., 2012).

5. PERSPECTIVES

When searching for insect hosts or prey, predators and parasitoids have been shown to rely on host plant and prey related odorant cues. The use of these
semiochemicals in pest management is a rapidly growing field of research (Arab et al., 2007; Moayeri et al., 2007). *Macrolophus pygmaeus* has been shown to be attracted to herbivore induced volatiles (HIPVs) emitted by sweet pepper (*Capsicum annuum*) that had been infested by two pests *Tetranychus urticae* Koch (Acarina: Tetranychidae) and *Myzus persicae* Sulzer (Homoptera: Aphididae) (Moayeri et al., 2007). *Macrolophus pygmaeus* is also attracted to tomato plants infested by whiteflies, whereas whiteflies alone do not attract the predator (Ingegno et al., 2011). At present, the attraction of this predator by HIPVs induced by *T. absoluta* has not been investigated. If the compounds responsible for the attraction of *M. pygmaeus* could be identified, they could be incorporated into integrated biocontrol strategies focusing at attracting the predators from natural populations occurring outside the greenhouses, guiding them into the greenhouses to infestation hotspots.

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