Impact of pesticides on an insect with double ecosystem services:
the case of *Exorista larvarum* (Linnaeus)

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Pollinator's biodiversity and their importance

Animal pollination is a key ecosystem service that allows the maintenance of wild and cultivated plant species. Pollinators such as bees, birds, bats and mammals have an extremely important role in determining food availability for other animals. In terms of human food supply, it has been estimated that approximately 35% of global crops benefit from animal pollination services (Klein *et al*. 2007). Insect pollination for food production has an evaluated value of around $361 billion USD/year, worldwide (Lautenbach *et al*. 2012). Within the impressively diverse Insecta, there is thought to be approximately 150,000 flower-visiting species throughout the world, including bees, flies, butterflies, moths, wasps, ants and beetles (FAO 2008).

Among all the insect pollinators, bees are undoubtedly the most valuable. The managed western honey bee, *Apis mellifera* L., is the most commonly used insect due to its versatility and ubiquitous distribution (Winfree *et al*. 2007, Arena & Sgolastra 2014). Yet, the great majority of pollinators are wild species. In fact, wild bees can improve seed set and quality of agricultural goods, and even enhance the honey bee effectiveness, thereby increasing the commercial value of several crops (Chagnon *et al*. 1993, Greenleaf & Kremen 2006, Rader *et al*. 2016). In addition, a meta-analysis of 39 field studies, across five continents, showed that non-bee insects were responsible for 39% of the total number of crop flower visits, strongly demonstrating the importance of other insect orders for pollination (Rader *et al*. 2016). Wild bees and non-bee insects can therefore provide these services, and even in some cases more efficiently than honey bees (Garibaldi *et al*. 2013, Orford *et al*. 2015). Moreover, Diptera families, such as Syrphidae and Tachinidae, not only provide pollination services as adults, but are also major biocontrol agents as larvae due to their entomophagous activity towards insect pests. This well demonstrates the importance of biodiversity in increasing and stabilizing ecosystem services.

Pollinators in decline

Worldwide, high losses in honey bee colonies and in the diversity and abundance of wild pollinators have been noticed both in agricultural landscapes and natural habitats (Burkle *et al*. 2013). Recent studies have associated these declines with a variety of interacting factors, such as climate change, pathogen and parasite loads, pesticide use and habitat loss (Goulson *et al*. 2015). These stressors are collectively reducing the resources used by pollinators for foraging, nest opportunities and shelter. High profile examples of the negative impacts of agricultural intensification and land-use changes include many native European butterflies (FAO 2008) and the North American monarch butterfly (*Danaus plexippus* (L.)) (Olaya-Arenas & Kaplan 2019), which are facing serious long-term declines of their populations.

The role of wild bees and non-bee insects in providing pollination has often been overlooked and there are few studies currently available on the potential effects of agricultural practices on these insects (Uhl & Brühl 2019). Agricultural intensification characterized by a high use of pesticides may expose wild pollinator species to a plethora of pesticide mixtures. In addition, until now, the current risk assessment of pesticides regarding pollinators has relied only on honey bees (OEPP/EPPO 2010). The risk assessment schemes for pesticides have assumed that the worst-case scenarios projected for honey bees are sufficiently conservative to protect other pollinator species, or even that the predictions for these other species can be extrapolated directly from honey bees. However, different bee species have different sensitivities to pesticides and routes of exposure, which depend on their specific life history traits (Arena & Sgolastra 2014, Sgolastra *et al*. 2019). The extrapolation from honey bees could be even more hasty if we consider non-bee insects (Uhl & Brühl 2019). These differences highlight the necessity to properly evaluate the effects of pesticide use on these important ecosystem service providers.

Figure 1. Adult male and female of *E. larvarum*. (Photos by C. Martins.)

Case study: *Exorista larvarum* (Linnaeus) (Diptera: Tachinidae) (Fig. 1)

In the framework of my doctoral thesis, under the guidance of Prof. Fabio Sgolastra and in collaboration with Prof. Maria Luisa Dindo (both at University of Bologna, Italy), I aim to assess the impacts of pesticides on non-*Apis* pollinators and develop new test methods that can be used in the risk assessment scheme. To this end, we selected the tachinid *Exorista larvarum* as a study species. *Exorista larvarum* is a gregarious larval parasitoid recorded as a natural enemy of several lepidopteran pests of forest and agricultural interest (Cerretti & Tschorsnig 2010, Tschorsnig 2017, Dindo *et al*. 2019). It is widespread in Europe, Asia and North Africa, and is established in North America where it was introduced as a biological control agent of the gypsy moth, *Lymantria dispar* (L.) (Sabrosky & Reardon 1976). Although it is well-known as a parasitoid, *E. larvarum* can also provide pollination services, since it visits flowers as an adult to feed. It is well known that most adult parasitoids (including *E. larvarum* and other tachinids) feed on pollen and nectar and these provide them with energy and may also positively influence egg maturation (Thompson & Hagen 1999, Wäckers 2003). European tachinids visit many flowering plants, especially in the family Apiaceae (Mellini & Coulibaly 1991). Interestingly, laboratory studies have shown that honey bee-collected pollen is a highly suitable food for captive *E. larvarum* adults (Dindo *et al*. 2019). Therefore, similarly to other Diptera, *E. larvarum* can perform a double ecosystem service, although its role as pollinator has been unexplored.

As with bees, tachinid flies may be exposed to pesticides or other toxic chemicals either topically, during pesticide application, or via consumption of residue-containing food in treated flowers. To address this potential risk, we performed an acute contact toxicity study following, as much as possible, the standard protocol used for honey bees.

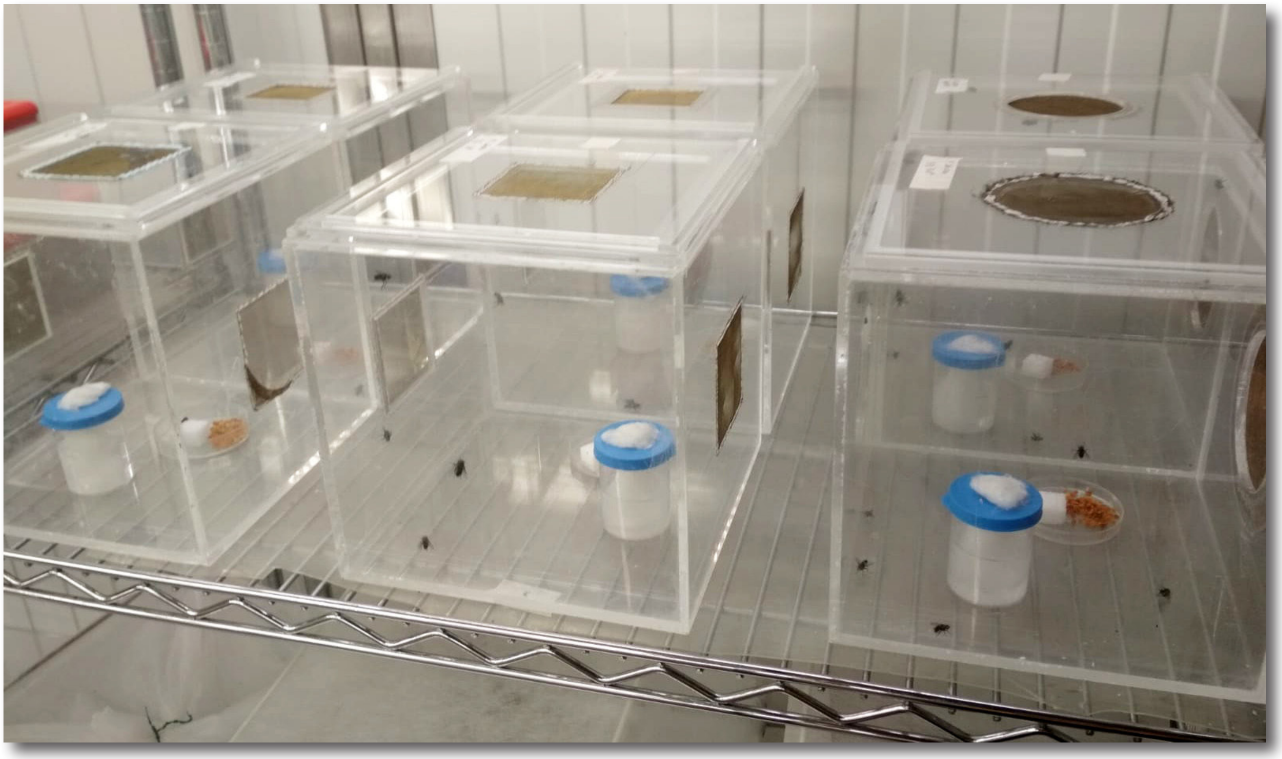

Figure 2. Test cages of treated adult mated females of *E. larvarum*. (Photo by C. Martins.)

Pesticide trials

Under laboratory conditions, we topically exposed adult mated females to a neonicotinoid pesticide. The tests were carried out with a population of *E. larvarum* reared and maintained at the DISTAL (University of Bologna) laboratory, using *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) as factitious host (Figs. 2, 3). The experiment was conducted to obtain the median lethal dose (LD50) and to detect potential sub-lethal effects at the different tested doses. As sub-lethal endpoint, we assessed the oviposition rate on the host larva, which is a relevant ecological parameter. The results showed a lower sensitivity of *E. larvarum* to the tested pesticide compared to honey bees. However, sub-lethal effects on the fecundity of the females were observed at doses 13 times lower than the LD50 value. More complete details about the findings of this study will be published upon completion of my thesis.

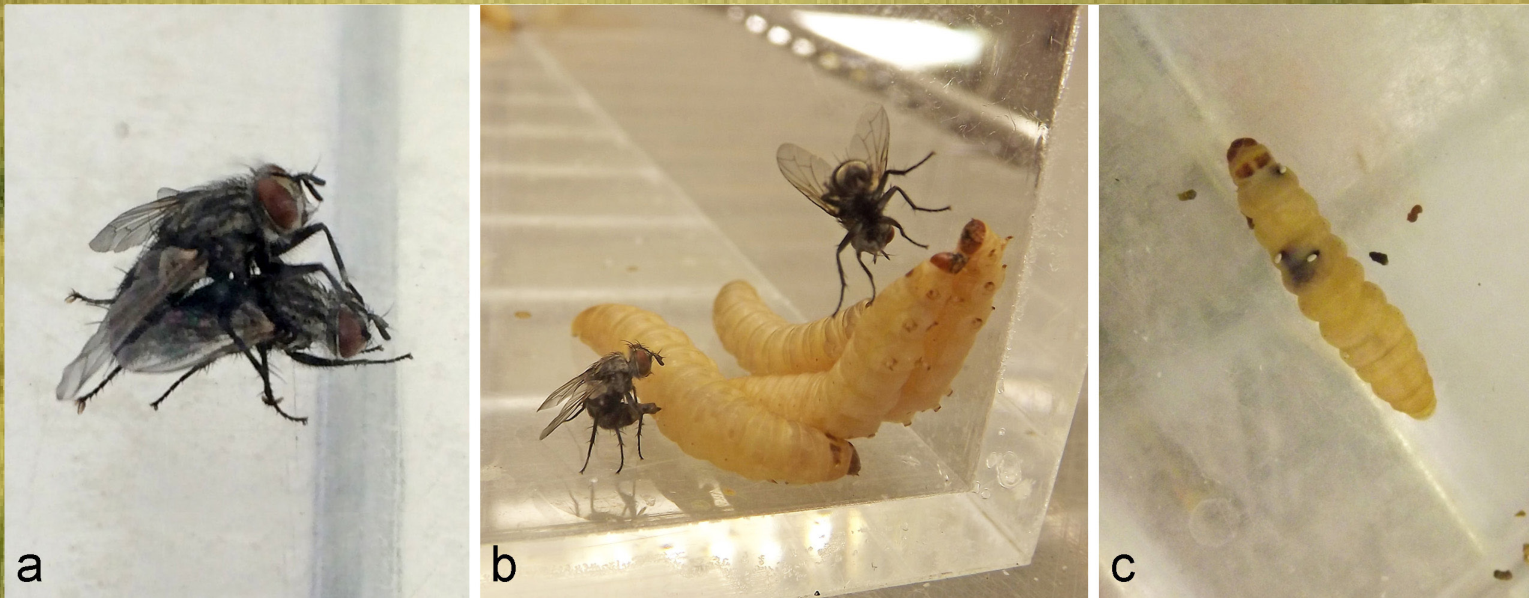

Figure 3. a. Mating activity of *E. larvarum*, before treatment with pesticide. **b**. Oviposition (exposure to *G. mellonella* after treatment with pesticide of the *E. larvarum* females). **c**. Eggs laid on *G. mellonella* caterpillars. (Photos by S. Francati.)

Final thoughts

To our knowledge, this was the first attempt to perform toxicological tests on a tachinid species under laboratory conditions. We believe this could be the first step to better understanding the potential risks that tachinid and other dipteran species face in intensive agro-ecosystems, as well as an opportunity to highlight their conservation value.

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