



Research article

Integrating predatory mites and pupal parasitoids for biological control of house fly (*Musca domestica*)

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Abstract

Importance of the work: Combining beneficial organisms provides strong biocontrol of house flies, highlighting the potential of integrated pest management in livestock farming.

Objectives: To evaluate and propose the biocontrol of filth flies when they are resistant to all available insecticides.

Materials and Methods: The efficiency was checked of the parasitoids alone, predatory mites alone and both at the same time, to control the emergence of house flies from 1,000 eggs compared to a control, in a laboratory-controlled experiment.

Results: The use of predatory mites (*Macrocheles muscaedomesticae*) in addition to parasitoids (*Spalangia cameroni* and *Muscudifurax raptorellus*) was more efficient than using only parasitoids.

Main finding: The benefit was demonstrated of combining predatory mites targeting the egg and larval stages of house flies, with pupal parasitoid wasps. These findings support the potential of this biological control strategy and indicate that it merits further evaluation under field conditions in livestock farming systems for the management of house flies and stable flies.

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Introduction

The house fly *Musca domestica* L. (Diptera: Muscidae) is one of the most widespread pests of humans and livestock worldwide, as it breeds in decomposing manure and other organic substrates and thrives in animal production systems (Geden et al., 2021). House flies are notorious nuisance pests, reducing animal welfare and productivity through stress and irritation, as well as being recognized mechanical vectors of a wide range of pathogens affecting both animals and humans (Thomas and Skoda, 1993; Pospischil et al., 1994; Chavassee et al., 1999; Iwasa et al., 1999). Their close association with livestock farming systems makes them particularly problematic in poultry, swine, dairy and beef facilities (Geden et al., 2021). In the USA alone, the annual economic costs attributed to house flies in livestock operations have been estimated to exceed USD 1 billion, considering veterinary treatments, reduced production efficiency, increased use of insecticides and complaints from neighboring communities (Greene et al., 1998; Geden et al., 2021). In addition, other synanthropic flies, such as the stable fly *Stomoxys calcitrans* (L.) (Diptera: Muscidae), contribute to economic losses in livestock (Skovgård, 2004); however, in most production systems, the house fly is by far the dominant pest, with its rapid reproductive cycle (approximately 15 d), capacity to disperse and its role in pathogen transmission making it one of the most challenging insect pests to manage sustainably (Geden et al., 2021).

Chemical insecticides remain the most common approach to house fly control; however, resistance to nearly all major insecticide classes is now widespread (Meyer et al., 1987; Keiding, 1999). Beyond resistance, repeated insecticide use can disrupt ecological balance by reducing non-target beneficial arthropods, such as dung beetles, and poses risks to farm workers exposed during application (Agrican, 2017). Furthermore, large-scale evaluations by the French national health institute INSERM have documented associations between chronic pesticide exposure and adverse human health outcomes, including neurotoxicity, endocrine disruption and increased cancer risk (INSERM, 2013 and 2021). These drawbacks reinforce the urgent need for sustainable alternatives.

Biological control has long been considered a promising solution for pest management; already in the late 1970s and early 1980s, the use of natural enemies was expanding rapidly in crop protection, with well-known successes such as *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) against spider mites (Bolckmans and van Houten, 2006;

van Lenteren, 2012; International Biocontrol Manufacturers Association, 2015). Not only does plant protection benefit from biological control, with the use of beneficial organisms being extended to livestock farming, where several species of hymenopteran parasitoids are used to control filth flies such as the house fly. In most cases, these parasitoids target the pupal stage of the fly, thereby interrupting its development. In animal production systems, the USDA Workshop on the Status of Biological Control of Filth Flies (Patterson, 1981) highlighted that substantial reductions in house fly populations could be achieved if the right parasitoid species were used in combination with good manure management. Since then, parasitoid wasps in the families Spalangidae and Pteromalidae have become the most widely studied and applied natural enemies of house flies. Species such as *Spalangia cameroni* Perkins (Hymenoptera: Spalangidae) and *Muscidifurax raptorellus* Kogan & Legner (Hymenoptera: Pteromalidae) attack the pupal stage of the fly and have demonstrated efficacy in poultry and cattle systems (Skovgård, 2004; Taylor, 2021). However, their performance is sometimes inconsistent, reflecting challenges of species selection, release timing and matching parasitoid biology to fly development.

The use of predatory mites as complementary biocontrol agents against flies has also been developed. Predatory mites of the family Macrochelidae provide a complementary form of control by targeting earlier life stages of flies. For example, *Macrocheles muscaedomesticae* (Scopoli) is a cosmopolitan predator of fly eggs and larvae, commonly found in livestock manure (Geden and Axtell, 1988; Azevedo et al., 2018). By attacking flies in their immature stages, predatory mites can reduce the number of hosts available to parasitoids and exert early pressure on fly population growth. One advantage of these predatory mites is their shorter life cycle compared to pupal parasitoids, allowing a faster population response.

A field study further supported this integrated approach. González et al. (2024) demonstrated that combining parasitoids with the predatory mite *Macrocheles robustulus* substantially reduced stable fly populations, highlighting the potential of predator–parasitoid combinations in filth-fly management.

Recently, international institutions have highlighted the strategic importance of biocontrol. In November 2024, the International Biocontrol Manufacturers Association and the Food and Agriculture Organization signed a Letter of Intent to accelerate the uptake of biological control tools worldwide and transition toward more sustainable agrifood systems, underscoring the growing recognition of biocontrol

as a cornerstone of future pest management (International Biocontrol Manufacturers Association and Food and Agriculture Organization, 2024).

The idea of combining natural enemies to achieve stronger fly suppression has been suggested for decades. For example, Ho (1985), in his work on the mass production of *M. muscaedomesticae*, emphasized that predators and parasitoids could be complementary because they act on different developmental stages of the fly. Later reviews also pointed to this potential (Chen et al., 2018; Azevedo et al., 2022; International Biocontrol Manufacturers Association and Food and Agriculture Organization, 2024); however, to date no published study has experimentally tested the combined release of predatory mites and parasitoids against the house fly.

Therefore, the objective of the current study was to evaluate, under controlled-laboratory conditions highly favorable to fly development, the combined impact of predatory mites and parasitoids on house fly emergence. It was hypothesized that dual releases of *M. muscaedomesticae* with *S. cameroni* and *M. raptorellus* would provide greater suppression of house fly populations than either natural enemy alone. This represented the first experimental test of the long-standing idea that predation and parasitism could be integrated to improve the biological control of house flies.

Materials and Methods

All experiments were conducted at the Entomology Laboratory of BESTICO France (Nantes, France) between January and September 2025.

Origin and rearing of arthropods

A laboratory colony of *M. domestica* was maintained at $27.1 \pm 1.0^\circ\text{C}$ and $65 \pm 3.3\%$ relative humidity (RH) under a 12 hr:12 hr (light:darkness) photoperiod. Adults were kept in rearing cages (BugDorm-2400 Insect Tent; 96 cm $\times$ 26 cm) and provided with water and a dry diet consisting of powdered milk and granulated sugar. Oviposition was stimulated using egg traps consisting of a black cloth impregnated with fish sauce (Nuoc Mam) and covered with a perforated black pot. Traps were left in the cages for 24 hr and the collected eggs were transferred to complete the cycle onto a house fly larval diet composed of 1 part ground soybean, 1 part sow feed, 1.8 parts wheat bran and 2.7 parts water. This routine was observed to maintain the colony at the lab.

Individuals of the predatory mite (*M. muscaedomesticae*) originated from a goat farm near the insectarium in Montbert, Nantes, France. Laboratory colonies were maintained with a method adapted from Ho (1985), consisting of using plastic containers (Sunware; 35 cm diameter $\times$ 12 cm height) to culture the mite. The plastic container was filled with wet vermiculite and the mites were fed daily with a mixture of house fly eggs and larvae. The stock colony was reset every 2 wk to maintain good quality and sanitation. Colonies were maintained in darkness at $26\text{--}28^\circ\text{C}$ and 70–90% RH.

Adult parasitoids (*Spalangia cameroni* and *Muscidifurax raptorellus*) were obtained directly from the commercial producer BLOWASP at Bestico, France, consisting of house fly (*M. domestica*) pupae parasitized by these species. For experimental needs, the parasitized pupae were held in the laboratory until adult emergence and newly emerged parasitoids were provided with house fly pupae as a food source prior to their use in experiments. Parasitoids were used in the experiment within age 2–3 d. Random collection of adult wasps prior to experimentation was managed using a mouth vacuum.

Experimental setup

The experiments were conducted in circular plastic boxes (Sunware; 35 cm diameter $\times$ 12 cm height) with six ventilation holes drilled on the cap (5 cm diameter) covered with a fine net (0.5 mm). This set up was designed to allow air circulation in the box, to avoid fungal development and to prevent too rapid desiccation of the house fly diet during experimentation.

All boxes receiving the four different experimental treatments were filled with 600 g of the house fly larval diet described above, as well as eventually 1,000 house fly eggs according to the method described below, with predatory mites, parasitoids or both. Each treatment was replicated 35 times. Then, the boxes were maintained in the insectary at $25 \pm 1.0^\circ\text{C}$ and $65 \pm 3.3\%$ RH under a 12 hr:12 hr (light:darkness) photoperiod throughout the experimental period of 15 d. Experiments began with the transfer of house fly eggs into the diet and ended 15 d later. Preliminary experiments indicated that with such abiotic conditions, the house flies developed and hatched as adults in less than 15 d. At the end of the experiment, the boxes were transferred to a freezer prior to counting the numbers of adult house flies. Then, the number of adult house flies between treatments was compared.

For each replication, 1,000 *M. domestica* eggs (approximately age 4 hr) were collected from the oviposition traps exposed in the fly cages. The eggs were rinsed and sieved through a fine mesh (0.125 mm), suspended in water and transferred into the wells of a cell culture plate to facilitate counting under a stereomicroscope. During this step, the eggs were inspected visually and only eggs showing normal morphology (intact, oval-shaped, fully formed, no visible signs of damage or collapse) were selected for transfer. Exactly 1,000 eggs were counted and transferred by pipette onto the larval substrate in each experimental box (Fig. 1).

Experimental treatments

Four treatments were evaluated and replicated 35 times: 1) the control (C) containing 1,000 fly eggs without natural enemies; 2) parasitoids (P) containing 1,000 fly eggs + 21 adult parasitoids (*Spalangia cameroni* and *Muscidifurax raptorellus*) that were introduced 4 d after house fly egg transfer. The number of parasitoids was selected to reflect release densities commonly used in applied biological control programs in livestock environments. Parasitoids aged 2 – 4 d were randomly collected (sex not distinguished), kept at 18°C

for 24 hr in small containers (Fig. 2) with water and honey and then released into the experimental bowls; 3) predatory mites (PM) and 1,000 fly eggs + 24 adult *M. muscaedomesticae* that were introduced at day 0. The mites were transferred 24 hr prior to the experiment into 60 mL plastic vials (24 individuals per vial) and maintained at 18°C without food until use (Fig. 2). The number of predatory mites was chosen based on release densities reported in the literature for macrochelid mites used in fly management and other biological control applications, while maintaining standardized conditions across treatments; 4) predatory mites + parasitoids (PMP) with 1,000 fly eggs + 24 adult *M. muscaedomesticae* introduced at day 0 and 21 parasitoids introduced at day 4.

At 15 d after egg deposition, the bowls were placed at –20°C for 5 hr to kill all insects. Then, number of adult *M. domestica* flies emerging from each bowl was counted under a binocular microscope (Fig. 2D).

The number of emerged flies in each treatment was compared using a Kruskal-Wallis test, followed by Dunn's *post hoc* test with Holm correction for multiple comparisons. No data transformations were applied. Analyses were performed using version 4.2.2 of the R software package (R Core Team, 2022), with significance set at $\alpha = 0.05$.

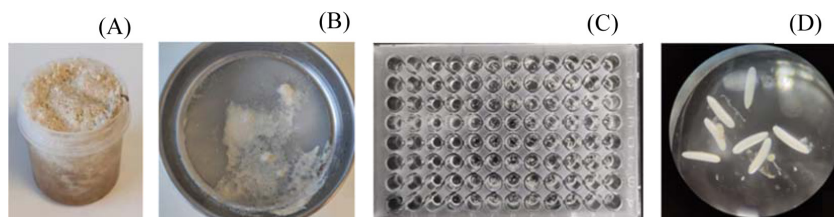


Fig. 1 Egg counting procedure: (A) oviposition trap; (B) collection of eggs; (C, D) transfer of eggs into wells of the cell culture plate for counting prior to pipetting onto experimental substrate

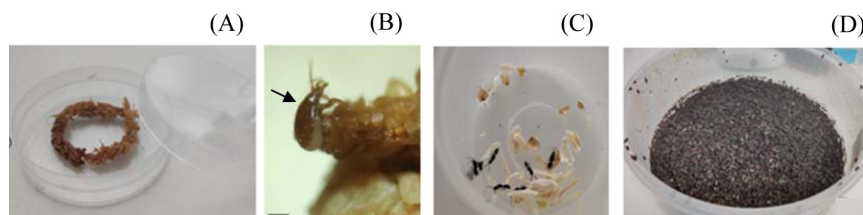


Fig. 2 (A) Small cup with sphagnum used for the transfer of predatory mites; (B) adult of *Macrocheles muscaedomesticae*, where scale bar = 1 mm; (C) small cup with parasitoids prior to transfer in experimental boxes; (D) frozen bowl for counting number of developed adult house flies.

Ethics statements

The authors declare that ethical approval was not required for this type of research in France.

Results

The control treatment showed very good house fly development, with a mean $\pm$ SE of 729 ± 8.0 adults emerging from 1,000 eggs (approximately 73% survival). The Kruskal-Wallis test revealed a highly significant treatment effect on the number of emerged flies (chi-squared = 128.8, degrees of freedom = 3, $n = 140$, $p < 0.0001$). The *post hoc* pairwise comparisons using Dunn's test with Holm correction showed that all treatments differed significantly from one another ($p < 0.001$ in every comparison).

The mean number ($\pm$ SE) of emerged adult flies per treatment was 729.3 ± 8.0 for the control, 516.2 ± 14.9 for the parasitoid treatment, 380.2 ± 4.8 for the predatory mite treatment and 277.5 ± 11.4 for the combined treatment ($n = 35$), as shown in Fig. 3.

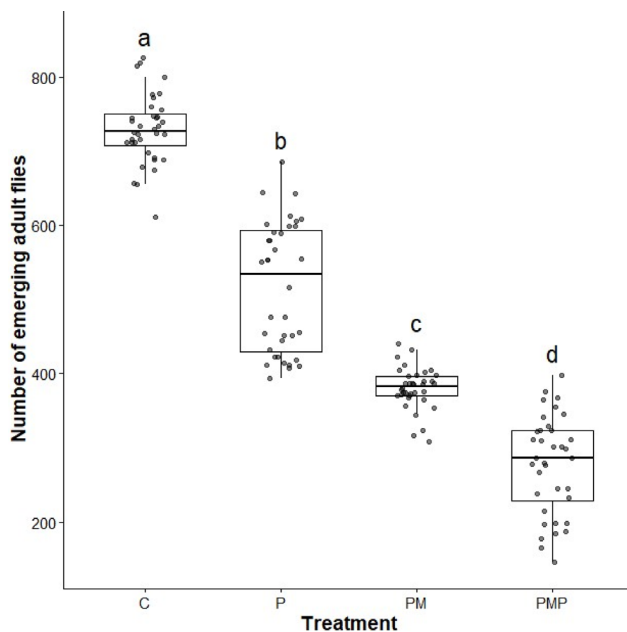


Fig. 3 Number of adult house flies (*Musca domestica*) emerging from 1,000 eggs after 15 d under four treatments: control (C), parasitoids (P), predatory mites (PM) and combined PM + P (PMP), where boxes represent interquartile ranges, horizontal lines are medians, whiskers indicate $1.5 \times$ interquartile range and dots represent outliers. Different lowercase letters above boxes indicate significant differences between treatments (Dunn's test, Holm correction, $p < 0.05$).

Discussion

For practical reasons, a standardized artificial substrate was used to rear the flies rather than animal manure. It was considered that this choice had limited impact on the relative parasitism and predation rates of the natural enemies tested and therefore the results could be extrapolated reasonably to livestock facilities. Furthermore, because the experimental conditions favored fly development, the relative efficacy of combined releases may be even greater under farm conditions, where environmental constraints would naturally limit fly population growth.

These emergence values were substantially higher than those typically observed under field or semi-field conditions. For example, Azevedo et al. (2022) reported an average of only approximately 250 adults per 1,000 eggs when larvae developed in hydrated manure placed on soil, whereas natural field populations are usually much lower due to environmental constraints. Similarly, Geden et al. (2021) noted that egg-to-adult survival under optimized laboratory rearing conditions often approached 70–90%, considerably higher than in natural habitats. Such differences confirmed that artificial diets and controlled conditions were more favorable to fly development than natural substrates.

Predatory mites are key natural regulators of house fly populations in animal facilities, with three families of mites—the Macrochelidae, Uropodidae and Parasitidae—being known to feed on fly eggs and larvae (Axtell, 1961 and 1963). Among them, *M. muscaedomesticae* is the most widespread and specialized predator, capable of consuming up to 20 fly eggs/d (Geden and Axtell, 1988). Reductions in fly numbers in cattle and poultry manure have been documented under semi-field conditions with this species (Axtell, 1986) and literature reviews have emphasized its importance for natural suppression of *M. domestica* (Axtell, 1969; Geden, 1990). Despite this, *M. muscaedomesticae* has not been mass-produced or marketed as a biocontrol agent. Instead, the closely related but more generalist species, *M. robustulus* is the only macrochelid mite currently available commercially, primarily for use in horticultural systems. Nevertheless, its biology, phoretic dispersal and close association with fly habitats make *M. muscaedomesticae* a particularly promising candidate for future development (Ho, 1985).

Based on the current results, the combination of predatory mites and parasitoid wasps produced stronger suppression of house flies than either group alone. This outcome likely

reflected both stage specificity and differences in developmental cycles, as the mites target eggs and larvae with a generation time of approximately 4 d, while pupal parasitoids, such as *S. cameroni* and *M. raptorellus*, attack pupae and require 14–28 d for development.

When released together, these complementary agents can provide immediate, as well as sustained control, provided releases are repeated regularly.

In the current study, the efficacy of predatory mites and parasitoid wasps was assessed using adult house fly emergence as the primary endpoint. Although these biological control agents target different developmental stages (eggs and pupae, respectively), adult emergence provides an integrative measure of fly suppression that reflects the combined effects of predation, parasitism and natural mortality throughout development. Direct quantification of parasitized pupae was not included in the current experimental design. Future studies, specifically designed to facilitate pupal recovery, could incorporate stage-specific measurements to refine further understanding of parasitoid activity and predator-parasitoid interactions.

In the current experiment, fly suppression ranked as: predatory mite + parasitoids < predatory mites alone < parasitoids alone < control, confirming that the combined release produced the lowest emergence of adult flies. These patterns indicated that the combined release of predatory mites and parasitoids resulted in the strongest reduction of house fly emergence, outperforming either beneficial species when they were used alone. Even under conditions highly favorable to house fly development, the dual-enemy treatment achieved the consistently lowest emergence rates.

The choice of predator species is critical. While González et al. (2024) demonstrated that the combination of parasitoids with *M. robustulus* reduced *S. calcitrans* populations under field conditions, this mite is considered a generalist, with Bamière et al. (2025) recently confirming its broader feeding preferences, including substantial consumption of nematodes. In contrast, *M. muscaedomesticae* showed a marked association with filth flies, with both stronger phoretic behavior and greater preference for fly eggs, making it a more specialized and potentially more effective control agent for *M. domestica*.

Conclusion

Resistance to insecticides of filth flies has been demonstrated in many countries. It has also been shown that trapping alone could not control those fly populations and had a strong impact on non-target fauna. Biocontrol with beneficial arthropods could be a control solution in such cases. The current study tested a sustainable alternative based on parasitoid wasps and predatory mites, with the findings showing that combining these two beneficial organisms provided stronger control of house flies in a laboratory-controlled experiment. Future studies should extend these findings to other species of synanthropic flies, such as the stable fly (*Stomoxys calcitrans*) and should test the combined release of natural enemies in commercial livestock facilities. Furthermore, field trials would provide essential validation of the laboratory results and help to establish practical protocols for augmentative release.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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