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Two Low-cost Devices to Evaluate the Reproductive Capacity in *Musca domestica* (Diptera: Muscidae)

ABSTRACT

Musca domestica L. (Diptera: Muscidae) is a relevant domestic, medical and veterinary pest against which new active ingredients must be continuously developed and tested. A key feature of these ingredients is the ability to interfere with the reproductive capacity, causing sterility in both sexes. The authors have developed two simple low-cost devices to evaluate the reproductive capacity in both sexes of *M. domestica*. The use of these devices for experimental tests could be extended to other fly species.

Keywords: fecundity box, fertility box, *Musca domestica*, low-cost devices, reproductive capacity test

INTRODUCTION

The house fly, *Musca domestica* L. (Diptera: Muscidae) is probably the most widely distributed insect pest, strongly connected to human populations and to their domesticated animals[1]. It is a polyphagous and endophilic species[2], very common in cattle and poultry farms[3] where it represents the worst hygienic problem[4]. The high mobility and reproductive rate of adults not only cause heavy infestations in livestock farms or in landfills, but also in nearby residential areas, leading to complaints and lawsuits[4,5]. The life cycle of *M. domestica* consists of six stages: egg, three larval instars, pupa and adult. For its extreme ubiquity and strong connection to humans, *M. domestica* plays a key pathogenic role in both larval and adult stages[6]. The larvae may cause facultative myiasis[7] and the adults, mechanical vectors of a wide range of pathogens (virus, bacteria, protozoans and parasite nematodes), are a serious threat to human and animal health[6]. A relevant issue in the fight against house flies is the continuous search for active ingredients able to sterilize flies of both sexes. Here we present two low-cost devices to evaluate in individual flies and in each gonotrophic cycle the fecundity in *M. domestica* females and the fertility in both sexes. Fecundity is defined as the number of eggs laid by each female and fertility as the number of eggs hatched among them[8].

MATERIALS AND METHODS

Fecundity test device

This device (hereafter indicated as "fecundity box") allows to evaluate the fecundity of individual females for each gonotrophic cycle through a deposition/alimentation (d/a) site, which in turn allows to evaluate the number of hatched eggs and therefore the fertility of both sexes. The fecundity box is composed by two 200ml cylindrical jars of polystyrene, with screw caps (Anicrin, Verona, Italy), of 6cm diameter and about 8 cm height. The first jar is the base of the fecundity box. For easy handling of the d/a site, two rectangular openings (3.5 cm base and 5-6 cm height) are cut on the cylinder sides (Fig.1A), taking care not to damage the screw rim. A round opening about 3cm in diameter is then carved on the cap of the first jar to accommodate the d/a site. A round opening of the same size is carved on the base of the second jar: the cap of the first jar and the base of the second jar, with aligned openings, are glued together with hot melt adhesive (UHU Bison, Milan, Italy) (Fig.1B). A10ml plastic syringe without plunger and handle is then glued on the side of the second jar (Fig.1A). The d/a site is composed of a 50ml polypropylene test tube with screw cap, cut at 25ml height and with a hole of 5mm in diameter at 5ml height.A 5cm flexible silicon tube(JBL,GMBH & Co., Mannheim, Germany), 5.5mm in diameter, is inserted in the hole. A concentric opening about 2.5cm in diameter is then carved on the test tube cap (about 3.4 cm in diameter) (Fig.1C).The inner side of the opening is carved in the way to obtain a row of small notches which will become protected spaces for egg deposition (Fig.1D). The modified 50-ml test tube is filled by a cotton plug soaked with a mixture of 70% raw milk and 30% water, and closed by a piece of filter paper (67 g/m^2, Cordenons, Milan, Italy) on which the cap should be fitted (Fig. 1D, E). After removing the excess filter paper, to prevent leakage of the mixture the cap is sealed all around with laboratory film (Parafilm® Bemis Co., Oshkosh, Wisconsin, USA) (Fig. 1E). On the cap of the second jar (about 6cm in diameter) a round concentric opening (about 4.5cm in diameter) is carved. A round piece of 1x2 mm mesh plastic mosquito net (about 6 cm in diameter) is

glued with hot melt adhesive over the jar opening. Once the glue is solidified, a small 1-cm hole is made on the net side, about 1-cm distance from the rim: this hole allows the safe introduction of flies in the device and prevents their escape.

The small hole can be closed by a 1.5ml Eppendorf test tube (Fig.1A). All parts of the device can now be assembled: the second jar glued to the cap of the first one is screwed on the first jar, then the complete d/a site is fitted in the opening (Fig.1A). The other end of the d/a silicon tube is connected to the lower part of the syringe. The syringe is filled with about 1.5-2 ml of the milk-water mixture, which progressively flows from the syringe to the d/a site, providing not only food for adults but also a suitable egg laying site for fertility tests (Fig.1F).
Once verified the presence of eggs in the d/a system (Fig.1G-I) the adult flies can be anaesthetized with CO_2 and placed in another fecundity box to verify egg-laying in the next gonotrophic cycle. The total cost of one fecundity box is about Euro 0.40 (USD 0.50).

Fertility test device

This device (hereafter indicated as "fertility box") is a hatchery allowing to evaluate the number of hatched eggs among those laid by a single female. According to the experimental procedure, the fertility box allows to evaluate the fertility of either sex. This box is composed of a 6-cm plastic Petri dish (Fig. 2A), whose bottom is filled to half by a pressed cotton layer (Fig.2B). A round concentric opening (3-4cm in diameter) is carved in the Petri dish lid (Fig. 2A) and covered with a piece of cotton tissue glued with all-purpose adhesive (New Pritt, Henkel, Bologna, Italy) to prevent the escape of hatched larvae (Fig.2B). The Petri dish bottom with the cotton layer is covered by round filter paper (Fig.2C) kept in place by the lid. For easy egg handling and counting, four square pieces cut from the previously mentioned 1x2mm mosquito net (5-7 mm in side, 25 small squares for each piece) are placed over a piece of filter paper under the lid opening and kept in place by four steel insect pins (Fig.2C). The filter paper and the cotton layer are then soaked with 2-2.5 ml of the milk-water mixture. The eggs laid by the same female in the fecundity box are collected by a fine brush, counted under a stereomicroscope and distributed over the mosquito nets in the fully assembled fertility boxes, one egg for each net square (for a maximum of 25 eggs for each square and 100 eggs for each Petri dish) (Fig.2D, E). The lid and the bottom of the Petri dish are laterally sealed with plastic tape (Fig.2F). The total cost of one fertility box is about Euro 0.15 (USD 0.19).

Device testing

To verify the efficiency of the two devices, fecundity and fertility tests were carried out using a *M. domestica* population from a susceptible strain (SRS/WHO - Standard Reference Strain/World Health Organization)[9], maintained since 2009 at the Department of Life Sciences and Biotechnology, University of Ferrara (Ferrara, Italy). The tests required insects of the same age which had no previous contact with each other. An adequate number of flies (around 50) at the pupal stage were individually placed in test tubes closed by a net. Adults newly emerged from cocoons were sexed according to the morphology of external genitalia and a female and a male of the same age were simultaneously placed in each one of 5 fecundity boxes. Every 5 hours the box was checked to verify the presence of eggs in the d/a site for three gonotrophic cycles (about 8-10 days). Eggs were collected, counted under a stereomicroscope and placed in the fertility box: hatching was verified at 8-10 hours from collection. To detect significant differences in the number of eggs laid among the three gonotrophic cycles, the one-way ANOVA test was performed by the STATISTICA 7.1 program (StatSoft, Tulsa, Okhlahoma, USA) with significance level $p < 0.05$.

RESULTS AND DISCUSSION

The results of the test showed that the flies were not stressed because no deaths were detected and all females completed all three gonotrophic cycles with an average number of eggs not significantly different from each other ($p < 0.05$). Concerning the fertility (expressed as percent average of hatched eggs in comparison to those laid), this value was always equal or above 97% and no significant differences were detected among average values of the three gonotrophic cycles (Table 1). According to the above results, the simple and low-cost devices appear suitable to evaluate in a reliable way the reproductive capacity of *M. domestica*.

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The two devices are interesting because their components are easily available in most laboratories and the total cost of each device does not exceed Euro 0.40 (USD 0.50). Moreover, no specials tools are required to shape and assemble the devices and most of their re-usable components can be easily cleaned in a common dishwasher machine.
Because of the low cost and the availability of materials, it is possible to use a high number of these devices to conduct large-scale experiments on many individuals. Concerning the fecundity box, the presence of small notches in the d/a site allows the female to laid eggs in a protected place and the central position of the d/a site at the base of the second jar allows the operator to easily verify the presence of eggs. In the case of abnormal drying of the d/a site, it is possible to add the milk-water mixture by the syringe without opening the cage, therefore preventing fly escape. In the fertility box the mosquito net fragments ease the process of egg counting, which can be performed very rapidly and correctly, given the fixed number of 25 small squares per fragment. The presence of a tissue piece closing the circular opening of the Petri dish lid prevents the escape of larvae, which may be used for further tests.
In conclusion, the two devices appear useful for the evaluation of reproductive capacity in the house fly, but they could be employed as well for other Diptera (such as Muscidae and Calliphoridae) with a diet similar to that of *M. domestica*.

Table 1. Synthesis of reproductive capacity of *Musca domestica* L. (Diptera: Muscidae). Fecundity ± standard deviation (SD) = average number of eggs laid in each gonotrophic cycle. Fertility ± SD = (total number of eggs hatched per cycle x 100)/ total number of eggs laid per cycle.

Gonotrophic cycle	Fecundity ± SD	Fertility ± SD (%)
1°	107.4 ± 8.5	97.0 ± 0.7
2°	102.2 ± 4.9	98.8 ± 4.5
3°	98.2 ± 3.0	97.6 ± 1.1

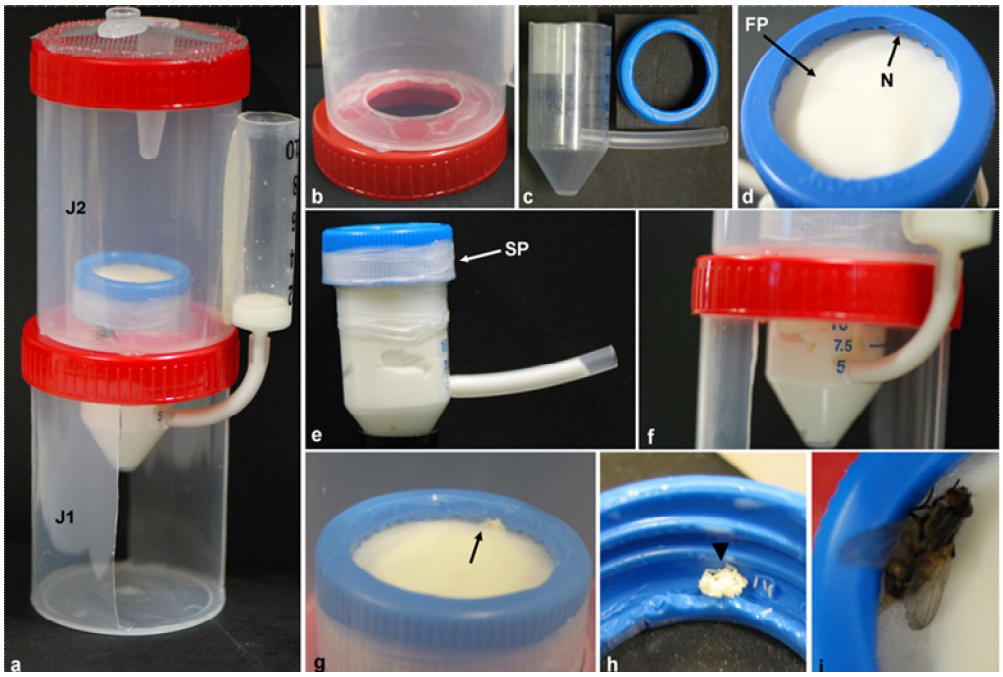

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Figure 1. Fertility box. (A) Overview of fertility box. J1: jar 1; J2: jar 2. (B) Connection between the cap of the first jar and the base of the second one. (C) A 50-ml polypropylene test tube, cut at 25-ml height, with a flexible silicon tube inserted and the cap carved with a concentric opening about 2.5cm in diameter. (D) Detail of the top of the deposition/alimentation (d/a) site. N: notches; FP: filter paper. (E) Side view of the d/a site ready for use. SP: seal of laboratory film. (F) Detail of the fertility box showing the connection between the d/a site and the syringe reservoir providing hydration. (G) A d/a site with freshly laid eggs (arrow). (H) Device open to show the eggs laid near the small notches (arrowhead). (I) Adult female of *Musca domestica* L. (Diptera: Muscidae) laying eggs in the d/a site.

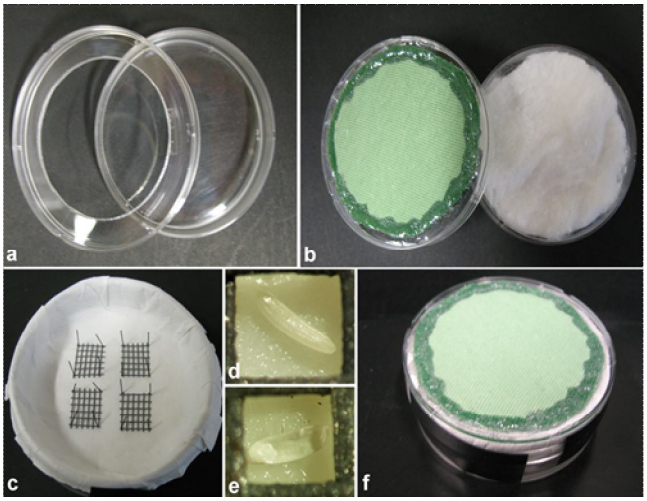

Figure 2. Fecundity box. (A) Plastic Petri dish and lid with a concentric opening. (B) Plastic Petri dish filled by pressed cotton and lid with a concentric opening covered by a piece of glued cotton tissue. (C) Top of the fecundity box showing the filter paper covering the cotton layer and the four mosquito net fragments. (D) Detail of a mosquito net square showing an unhatched egg. (E) Detail of a mosquito net square showing a hatched egg. (F) Overview of the assembled fecundity box.

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CONFLICT OF INTEREST

The authors declare no conflict of interest related this work.

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