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An efficient method to sample *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae) using colored pan-traps in cage poultry facilities

Bianca P. Valério, Bruno S. Nunes, Thiago M. Alvarenga, César F. Carvalho†, Leopoldo F. O. Bernardi, Stephan M. Carvalho, Lívia M. S. Ataíde

Universidade Federal de Lavras, Lavras, MG, Brazil. †In memorian.
Corresponding author: liviaataide@gmail.com

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Abstract. When houseflies find optimal conditions to develop, they rapidly increase their population size negatively impacting both humans and animals through nuisance and the transmission of pathogens. *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae) shows a preference for animal faeces and for this reason it is a serious pest in animal breeding facilities. To prevent proliferation of houseflies and to evaluate the efficiency of the control methods, it is important to routinely monitor the activity of this dipteran in such facilities. There are several types of traps for sampling houseflies in breeding sites, here we used colored pan-traps to evaluate the efficiency of the trap and its color in sampling *M. domestica* in a cage poultry facility located in the city of Nepomuceno, Minas Gerais, Brazil. To do so, we set up yellow, white, blue and red pan-traps next to the cages of poultries and collected flies once a week for a period of a year. Although the density of *M. domestica* was high during the entire period of sampling, more flies were collected in white (monthly average of 470.83 flies) than in yellow (327.55), blue (267.14) and red traps (199.63). Hence, we argue that this poultry farm needs to improve its housefly monitoring program and we suggest a continuous use of white pan-traps to monitor the efficiency of the pest control methods implemented in the facility.

Keywords: Housefly, monitoring, management, color trap, control methods.

Adult flies belonging to the Muscidae family (Insecta: Diptera) can become a pest (any population of organisms causing significant economic damage), in places where they find great developmental conditions, especially in regions with high temperature and humidity, reaching a huge population in a short period of time (Prado 2003). Among them, *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae) stands out as one of the most important synanthropic pest species in our society and are even popularly called as "houseflies", due to its common presence inside our houses (Marchiori et al. 2000; Barnes et al. 2008).

The development of immature stages of *M. domestica* occurs in almost all types of decaying organic matter, but they show a preference for faeces from horses, cattle, pigs and chicken (Brito et al. 2008). For this reason, houseflies are often associated with urban sites such as waste handling facilities and with country sites such as farms of animal production (Lole 2005), negatively impacting both humans and animals through nuisance and the transmission of pathogens. Due to its ability to quickly spread in facilities with animal production, housefly's proliferation can promote a decrease in productivity due to the inconvenience caused to workers and animals. Because they can easily spread to neighboring houses and facilities it can also result in costly citations, fines and lawsuits (Brasil 2008).

The negative impact of houseflies in our society can be reduced following implementation of area-wide housefly management programs aiming to decrease the pest population to acceptable levels (Rodrigueiro et al. 2002). Within the management program, the first step is to survey the pest population level in the area, because without pest monitoring, control often starts late, *i.e.*, when the fly population is already high enough to cause negative impacts in the facility or the surroundings. Therefore, caution is needed in the beginning of the infestation and a control method should be introduced before an outbreak, *i.e.*, point at which the housefly population causes economic injury or other negative impact in the area (Gerry 2020). Furthermore, routine monitoring of housefly activity is important to confirm the effectiveness of fly control efforts used in the area avoiding the use of continuous ineffective measures such as insecticides to which houseflies can develop a high degree of resistance (Freeman et al. 2019).

Although there are several methods used to monitor the population level of *M. domestica* in the field, it is important to mention that there is not a unique or a standard protocol for housefly monitoring. The most common methods used to sample adult houseflies are sticky fly ribbons (Anderson & Poorbaugh 1965), sticky fly cards (Gerry 2020), scudder fly grid (Urech et al. 2011), attractant-baited traps (Zahn et al. 2019), spot cards (Lysyk & Axtell 1985) and pan-traps. This last method is the method that we used in our experiments, the so-called pan-traps. Pan-traps are simple traps made of plastic pots filled with a low concentration of saline solution of water and detergent and often rely on color as attractant to the fly. Once the fly is attracted to the color of the pot, it gets trapped inside and dies in the solution. This method is highly efficient once visual cues are one of the most important stimuli used by the flies when they seek for shelter, for food and even to decide whether they should stay in a patch and where to locate more resources within it (Zuker 1996). In fact, this makes pan-traps an efficient and cost-effective method widely used for sampling flower-visiting insects (Vrdoljak & Samways 2012). Because pan-traps have proved to be an efficient and economical sampling method, the present study aimed to evaluate the efficiency of colored pan-traps in monitoring changes in housefly abundance over time in a large caged-layer poultry facility.

Samplings were performed at the caged-layer poultry facility called as Aviário Santo Antônio located at Nepomuceno city, Minas Gerais, Brazil (latitude: 21°12'40.6"S, longitude: 45°14'06.3"W). Four sets of pan-traps were randomly placed next to the cages of the poultry farm. Each set of pan-traps consisted of four subsets of cylinder-shaped plastic pots painted in white, yellow, red and blue colors which were suspended by an iron umbrella-shaped structure (Fig. 1A). Pan-traps were set at 110 cm above ground and remained in the same place during the entire sampling period. Inside each pot (1 L) we added 500 mL of water with approximately 10 mL of detergent and 5g of table salt, used to remove the surface tension of the water and to preserve the collected flies in the pots. The 16 pots were left in the open field from September 2017 to July 2018. The sampling of the flies falling in the pots was performed once a week. After collecting the adult flies, the pots were cleaned and placed back in the same position. All flies collected in each of the 16 subsamples were stored separated in sealed glass jars containing absolute alcohol (99%). Flies were quantified and identified in the laboratory by using an optical microscope. The identification was based on the identification keys proposed by Carvalho & Mello-Patiu (2008).

All the statistical analyses were performed using the software R version 3.3.1 (R Development Core Team 2020). Differences in the relative abundance of houseflies between color traps were analysed using a generalized linear mixed model (GLMM) with a Poisson distribution and the *lme4* package. The abundance of flies was included as response variable (y) and the traps color was included as explanatory variable (x) in the model. To avoid the temporal pseudoreplication, the variables day, month and year were included as a random factor in the model. First, residual analysis was performed to check error distribution in the model. Then, a model was built aiming to investigate differences in the abundance of *M. domestica* among the treatments and since we found overall differences, we made contrast analyses comparing two models for significance between colors (Tab. 1).

Table 1. Values for correlation tests performed (GLMM) between different fly abundance in response to color traps.

Color trap	Estimated std error	*z* value	*p*
White trap vs Blue trap	0.566711	0.005909	< 0.001
White trap vs Red trap	-0.858047	0.006516	< 0.001
White trap vs Yellow trap	0.362865	0.005551	< 0.001
Red trap vs- Yellow trap	-0.495181	0.006927	< 0.001
Red trap vs Blue trap	0.291335	0.007218	< 0.001
Yellow trap vs Blue trap	-0.203846	0.006360	< 0.001

The number of *M. domestica* collected in the caged-layer poultry facility differed between color (Chi = 20764; p < 0.001) and we collected more flies in white traps than in other color traps (Fig. 1B). On average, 470.83 flies were captured in white traps, 327.55 in the yellow traps, 267.14 in blue traps and 199.63 in the red ones.

Similar to other studies developed worldwide, here we show that white colored pan-traps are attractive and an efficient method to collect houseflies in the field. For instance, Annoh et al. (2017) evaluated the diversity and abundance of insects on mango farms in southern Ghana and sampled more houseflies in white pan-traps, followed by yellow and blue pan-traps. In India, Painkra (2019) collected houseflies by using white, yellow and blue pan-traps in different blooming seasons (ouset of bloom, full bloom, end of bloom) in a bitter gourd ecosystem and observed that the housefly color preference changed among the plant blooming seasons evaluated. During the onset of boom, highest sampling of houseflies was recorded in blue pan-traps followed by yellow pan-traps and the lowest in white pan-traps. However, during full bloom period, highest values were observed in yellow pan-traps followed by white pan-traps and lowest were recorded in the blue pan-traps. At the end of bloom, higher abundances were recorded in yellow pan-traps followed by blue and then, white pan-traps. In this same study, they also showed that other insects, mostly pollinators, were also affected by plant blooming patterns.

Hanley et al. (2009) also emphasized that the efficacy of the color in capturing houseflies may vary according to the study site, presence of light, odors and other environmental factors. In fact, they found that the color of the trap had no significant effect on the number of houseflies captured in the poultry unit they tested, meaning that is important to consider the whole environment when using color pan-traps to sample houseflies in an open field. In our study, our pan-traps were placed close to the poultry cages, but far enough to be exposed to the biotic and abiotic factors such as sunlight, rain and wind with presence of plants surrounding them. Therefore, although the white pan-traps captured the highest number of houseflies throughout the year in our study, we acknowledge that different a result can be obtained if those same pan-traps are tested in another facility. Since we did not measure the biotic and abiotic factors directly affecting our sampling, we cannot correlate any of these factors with our sampling efficiency.

Nevertheless, pan-traps have been considered an effective method for sampling several groups of insects, not only houseflies, and its efficacy between the taxon depends on the pan-trap color (Vrdoljak & Samways 2012). In general, yellow is considered as the most attractive color for most groups of insects, but this is not a rule of tomb. Saunders & Luck (2013) showed that although yellow is the color that usually attracts the largest number of insects, attraction might change depending on the habitat. They sampled insects in native vegetation and in almond orchards in northwestern Australia and observed that native hymenopterans preferred the yellow color in orchards, but in native vegetation they preferred blue-colored pan-traps. By using white, yellow, red and blue pan-traps, Campbell & Hanula (2007) sampled insects in three forests located in the south-eastern of United States and showed that most flies and bees were captured by blue traps. Interestingly, Campbell & Hanula (2007) also observed that the combination of colors can even increase efficiency of sampling

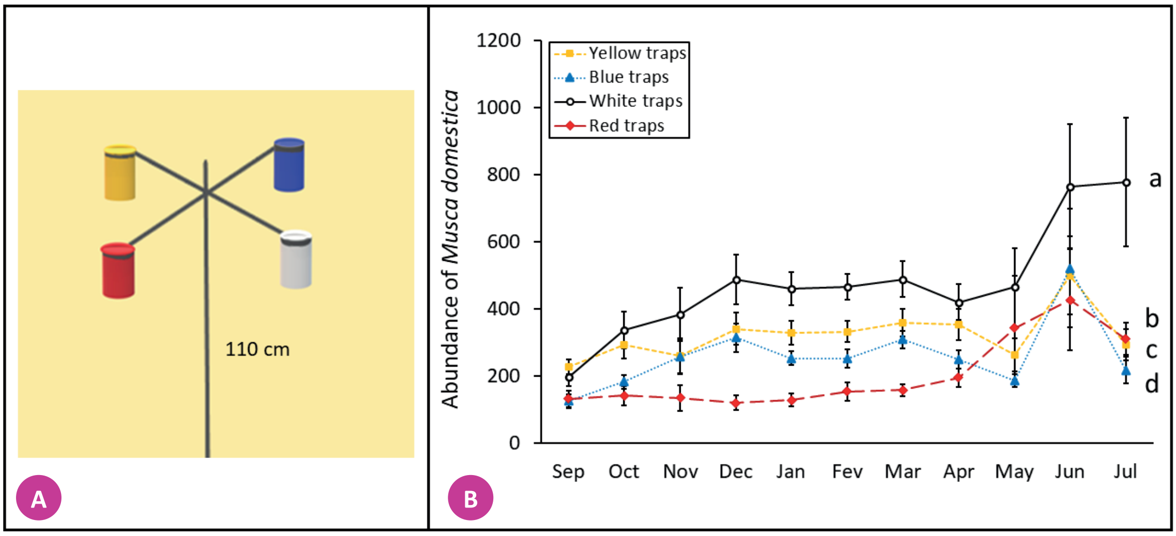

Figure 1. Abundance of houseflies sampled by pan-traps at Aviário Santo Antônio, a caged-layer poultry facility. The schematic drawing (A) represents the pan-trap built for sampling the houseflies in the field. Each set of the pan-trap consisted of four subsets of plastic pots painted in white, yellow, red or blue. The graphic (B) shows the monthly average number (±SE) of Musca domestica Linnaeus, 1758 collected during the sampling period of one year. Different letters denote significant (P≤ 0.05) differences among the treatments.

and insects mostly attracted to a combination of colors are the ones that usually visit flowers, *i.e.*, the pollinators. Taken together, these studies demonstrate that color pan-traps are an efficient method to collect insects in open fields and the degree of attraction for a specific color depends on biotic and abiotic factors and is detrimental for the number of insects that will be captured by the pan-traps (Dafni et al. 2005). Although it is important to prevent that the housefly population reaches an outbreak, to date there are no protocols available for guidance of the number of houseflies that should be considered above the acceptable level of nuisance complaints (Gerry 2020). Since the number of flies may vary to define an outbreak depending on the facility, comparisons are not appropriate in all cases. For instance, for caged-layer poultry farms, Axtell (1970a) first suggested that 200 flies/ribbon/week would be an acceptable number of houseflies to sampled in a place, but in a subsequent survey, Axtell (1970b) estimated that 100 flies/ribbon/week would be enough to cause an outbreak. Other outbreak limits proposed were 300 flies/ribbon/week (Lysyk & Axtell 1985), 50 spots/card/week (Lysyk & Moon 1999), and either 350 flies/trap/week (Axtell 1986) or 300 flies/trap/week (Lysyk & Moon 1999) for baited jug traps. These results demonstrate that parameters to measure housefly outbreaks are not standardized but instead, are specific to the facilities surveyed, the monitoring methods used, and even to the specific site where monitoring devices should be placed at each facility.

Here we demonstrate that the density of *M. domestica* can be monitored by using white pan-traps in places and organizations seeking to initiate or improve pest management programs in their avian breeding facilities. Pan-traps have proved to be suitable for the assessment of housefly abundance with minimum sampling biases and efforts. They are environmentally friendly, cheap, easy to use and deliver reliable results. Because we found a high density of *M. domestica* during the entire period of sampling, we argue that this poultry facility needs to improve housefly monitoring and control. We also suggest them to routinely use the white pan-traps to monitor the efficiency of the pest control methods implemented in the facility. Other facilities can use this protocol to monitor their level of houseflies as well, but we advise them to make pilot experiments testing different colors first. Moreover, due to its ability to quickly develop and reproduce, preferentially, monitoring of houseflies should be performed during the entire year.

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Authors' Contributions

All authors contributed to the study conception and design. Material preparation, data collection was performed by Bruno S. Nunes and Thiago M. Alvarenga. Analysis of the data was performed by Livia M. S. Ataíde and Leopoldo F. O. Bernardi. The first draft of the manuscript was written by Bianca P. Valério and Livia M. S. Ataíde. All authors commented on previous versions of the manuscript, read and approved the final manuscript.

Conflict of Interest Statement

All authors have declared that there is no conflict of interest.

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