BEHAVIORAL RESPONSE OF HOUSE FLY, *MUSCA DOMESTICA* L. (DIPTERA: MUSCIDAE) TO NATURAL PRODUCTS

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Abstract. The house fly *Musca domestica* L. (Diptera: Muscidae) is a medically important insect worldwide because adults are an annoyance and mechanical carriers of various pathogens, and the larvae cause myiasis in humans and animals. In order to efficiently control fly populations, information on the most suitable baits to attract adult flies, either for food source or reproductive success of oviposition, is essential. We investigated the behavioral response of this fly species, using a dual-choice wind tunnel (T-box), to natural products, both of animal and plant origins. Correlation between wind speed and fly response showed that the wind speed set at 0.4 m/s was the optimal speed for T-box testing with this species. One hundred 5 to 7 day-old adult males or females deprived of food and water for 15 hours were transferred into a clean rearing cage for 5 minutes. Two hundred grams of a natural product (41 in total) were introduced into the cage, and response to the natural product was considered positive when the flies landed and/or stayed on the natural product within 5 minutes of placement in the cage. The natural products that attracted > 50% of flies were used as the candidate odor sources for further study in the T-box experiments. Only 12/41 natural attracted > 50% of the flies in the 5 minutes observation period, with fresh beef viscera being the most attractive for both sexes (≥74%). These 12 natural products were then assessed for their attractiveness in the T-box using a wind speed of 0.4 m/s, and the assay indicated that fresh beef viscera was still the most attractive product to lure flies. Finally, we compared the three most attractive products for house fly (1st - fresh beef viscera, 2nd - ripe banana, 3rd - fresh beef liver) against each other. Fly preferred the fresh beef viscera as the most attractive product in direct comparison with the other two products. Information on luring and trapping adult house fly can be used in the development of a suitable attractant bait to be used in fly population control programs.

Keywords: *Musca domestica* L., bait, behavioral response, control, house fly

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INTRODUCTION

The house fly *Musca domestica* L. (Diptera: Muscidae) is a medically and agronomically important insect worldwide. Adults are the mechanical carriers of numerous kinds of pathogens, such as bacteria (*eg*, *Escherichia coli* 0157:H7, *Pseudomonas aeruginosa*, *Salmonella typhi*), viruses, fungi, and other parasites (Greenberg, 1973; Chaiwong *et al*, 2014; Phoku *et al*, 2014), which can cause diseases in humans. The larvae cause myiasis, which can be oral (Yadav *et al*, 2014), pelvic (Shaunik, 2008), intestinal (Sehgal *et al*, 2002), cutaneous (Burgess and Davies, 1991), scalp (Ferraz *et al*, 2010), furuncular (Magnarelli and Andreadis, 1981), and umbilical (Ambey and Singh, 2012). Myiasis cases have also been reported in animals, *eg*, sheep, goat (Amin *et al*, 1997) and cat (Gardiner *et al*, 1983). In agricultural settings, adult flies are the prime nuisance pest associated with dairy and other confined animal facilities (Hinkle and Hickle, 1999). Prompt and rapid control measures are needed to reduce the fly population or maintain fly numbers below the action threshold, at which pathogen transmission or nuisance to humans and/or animals occurs (Gerry *et al*, 2011).

Various strategies should be incorporated in an integrated pest management (IPM) of house flies, including chemical, mechanical and/or biological controls. For short term strategy, applying chemical insecticides is the mandatory management; however, extensive use of insecticides for house fly control could lead to insecticide resistance (Cao *et al*, 2006; Jandowsky *et al*, 2010; Scott *et al*, 2013). In Thailand, pyrethroid resistance was detected in house fly populations in suburban and rural areas of northern Chiang Mai Province. In 2005, permethrin [50% lethal concentration (LC_{50})] for house fly (wild-strain Chiang Mai) was 1.9-3 μg/l and to deltamethrin 0.0185-0.1251 mg/l (Sukontason *et al*, 2005). A recent study in 2014 with the field colonies suggested that the LC_{50} level has increased to 26.13-322.58 mg/l for permethrin and 86.15-382.56 mg/l for deltamethrin (Sukontason K, unpublished), indicating a large increase in insecticide resistance. Non-chemical strategies for control of house fly population are needed for incorporation in IPM programs.

In order to implement IPM, regular monitoring of the house fly population should be conducted. Bait-trapping is a useful method, based on its easy application in monitoring fly population and its target specificity. For an efficient trapping, a suitable bait is needed to lure the target fly species. In Thailand, fly surveys indicated that the blow fly, *Chrysomya megacephala* (F.), and house fly are of the predominant species associated with urbanized and/or suburban areas (Sucharit *et al*, 1976; Tumrasvin *et al*, 1978; Ngoen-klan *et al*, 2011). A recent experiment conducted with a dual-choice wind tunnel (T-box) indicated the most suitable bait for *C. megacephala* is a one-day tainted pork viscera (Bunchu *et al*, 2008). For house flies, various kinds of bait have been used to attract the adults, based on their availability at different locations, *eg*, raw fish (Kano *et al*, 1964), putrid fish (Sucharit *et al*, 1976), mixture of putrefied fish intestine and Korean rice liquor (Park 1977), decomposed meat (Tumrasvin *et al*, 1978), salted fish preserved in oil (Nurita *et al*, 2008), and fresh sheep meat (Akbarzadeh *et al*, 2012).

In nature, house flies have been found on various fresh, dead or tainted animal and plant materials. It is difficult to determine the most attractive material for house flies as a result of these regional variability.

The present study determined the most appropriate natural product to be used as an attractant to adult house fly using a T-box and products of three natural origin, namely, fresh animal materials, fresh plant materials and 1- and 3-day tainted animal or plant materials. Such information is important for the development of an effective odor/bait house fly trap.

MATERIALS AND METHODS

Flies

M. domestica adults were obtained from a colony maintained at the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand, established from individuals collected in Chiang Mai (located at 17-21°N, 98-99°E: 325 m above sea level). Flies were reared according to the method previously described (Sukontason *et al*, 2004): at 24-28°C, natural light/dark photoperiod and relative humidity of ~70-80%. Adults were reared on two kinds of food: 1) a mixture of 10% (w/v) sugar solution and multivitamin syrup solution and 2) fresh pork liver (used as both a food source and oviposition site).

Evaluation of house fly behavioral response to natural products in rearing cage

Natural products ($n = 41$) with strong odor were purchased from local retailers and either used immediately or kept in a plastic bag at ambient condition for 1 or 3 days to allow natural tainting. The assay followed the method by Chaudhury *et al* (2002) with some modifications and were conducted in rearing cages (30x30x30 cm) between 12:00 and 03:00 PM at room temperature (~25-30 °C) and relative humidity (~70-80%) of the laboratory.

We conducted the experiment with 3 categories of strong odor natural products: 1) fresh animal materials, 2) fresh plant materials, and 3) 1- and 3-day tainted animal or plant materials. Fresh animal materials were pork liver, beef liver, minced pork, pork viscera (comprising stomach, pancreas, and small intestine), beef viscera (same as pork), chicken viscera (comprising liver, kidney, small intestine, and large intestine), swine blood (liquid and clotted), walking-catfish meat, marinated crab (*Sesarma* sp) and *Pla ra* (fermented several kinds of fish, such as Java barb or freshwater cyprinid fish). Plant materials included ripe banana (*Musa sapientum*), coconut meat, Khao Hom Mali rice (*Oryza sativa*), cooked Khao Hom Mali rice, glutinous rice (*Oryza sativa* var. *glutinosa*), cooked glutinous rice, molasses (dark brown, moist granular sugar), sugar, pickled cabbage, and tapioca flour. To prepare tainted materials, animal or plant materials were placed in the plastic bag and kept at 37°C for 1 day and 3 days.

For each assay, 100 5-7-day old adult male or female flies deprived of food and water for 15 hours (ideal for these experiments as determined in preliminary study), were transferred to a clean rearing cage. After 5 minutes of acclimatization, 200 g of a natural product was placed on a clean glass plate (15 cm in diameter) and introduced into the cage. Flies were considered as responding to the natural product when they had landed and/or stayed still on the natural product within 5 minutes of placement in the cage. Only the responding flies were counted and recorded, and each natural product was tested in 3 replicates using different groups of flies. At the end of each trial, the cages were cleaned by rinsing with hot water and fabric sleeves were replaced. The natural products that attracted > 50% of flies were used as candidate odor sources for further study in the T-box (Bunchu *et al*, 2008).

Calibration of wind speed and determination of behavioral response of flies to olfactory stimuli in T-box

The wind speed used in the wind tunnel experiment was determined as described previously (Bunchu *et al*, 2008), using the relationship between wind speed and the number of responding flies generated by the regression wizard (SigmaPlot version 6.01). This equation was used to calculate the motor speed levels, which provided the wind speeds of 0.1, 0.2, 0.3, 0.4 and 0.5 m/s.

To perform the assay, 200 (100 males and 100 females) 5- to 7-day old adult house flies were used. Only distilled water was offered for 24 hours prior to the experiment instead of the mixture of 10% (w/v) sugar solution and multivitamin syrup normally used in rearing to prevent them from dehydrating. Both ventilating fans in the wind tunnel were turned on at the same speed 30 minutes before transferring the fasting flies into the released partition. All flies were acclimatized within this partition for 30 minutes before the standard odor source, the 1-day tainted beef liver placed on a 20 cm diameter plastic plate, was introduced to one of the stimulus partitions (left or right).In order to reduce bias, the odor source was placed in different stimulus partitions in subsequent experiments. Flies were left in the tunnel for 3 hours (from 12:00 to 03:00 PM) at the room temperature (~25-30°C) and relative humidity (~70-80%) of the laboratory. We tested wind speeds at 0, 0.1, 0.2, 0.3, 0.4, and 0.5 m/s, with three repetitions for each speed experiment. The wind speed with the highest number of fly collected was chosen for the next experiment.

After each assay, the removable nylon fly nets were removed from the tunnel and kept 4ºC for 1 day to kill all flies. The number of flies collected in each partition was counted. The whole tunnel was sanitized with a cleansing solution (Hy-2001, Bangkok, Thailand) and the remaining odor inside the tunnel was eliminated by placing 1 kg of the activated charcoal in each partition of the tunnel.

Determination of house fly behavioral response of natural products in T-box

Fly behavioral response in the T-box was assessed as described above. In order to control for any possible side bias, the position of the test natural product and standard bait (1-day tainted beef liver) were changed in every trial. In addition to the number of responding flies in each partition, the percentage of trapped flies of each sex was calculated. The three most attractive natural products for house fly then were tested in direct comparison against each other in the T-box to ensure the most attractive product was determined.

Data analysis

Correlation between wind speed and motor speed was determined using SPSS 13.0 for Windows (SPSS, Chicago, IL). SigmaPlot version 6.01 was used to test whether number of flies correlated with the wind speed. Number of the attracted flies (responders *versus* non-responders) to different baits from three independent replicates was analyzed using Pearson χ^2 test (SPSS 13.0 for Windows) using a p-value < 0.05 as significance level.

RESULTS

Behavioral response of house fly to natural products in rearing cage

Out of 13 fresh animal materials, 6 attracted > 50% of flies (Fig 1). Beef viscera attracted the most flies, followed by pork liver. *Pla ra* (fermented fishes) attracted the lowest number of flies. Out of 10 fresh

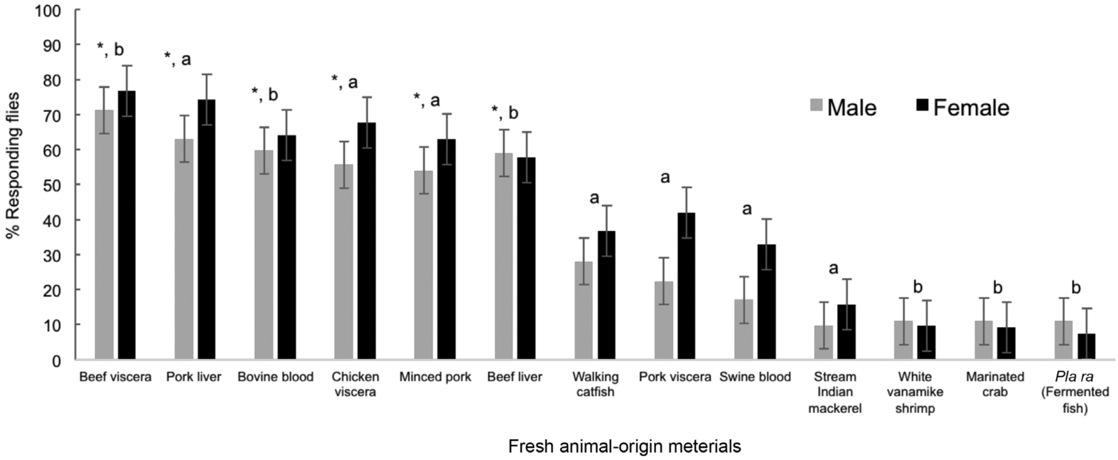

Fig 1–Percent responding *M. domestica* to the fresh animal materials in screening trial. One hundred adults deprived of food and water for 15 hours were transferred into a rearing cage. Flies landed and/or stayed on 200 g natural product within 5 minutes was considered positive. *Natural product that attract > 50% of flies in a 5-minute period. [a]Significantly attracted more females than males ($p < 0.05$). [b]Nonsignificant difference in attraction between males and females.

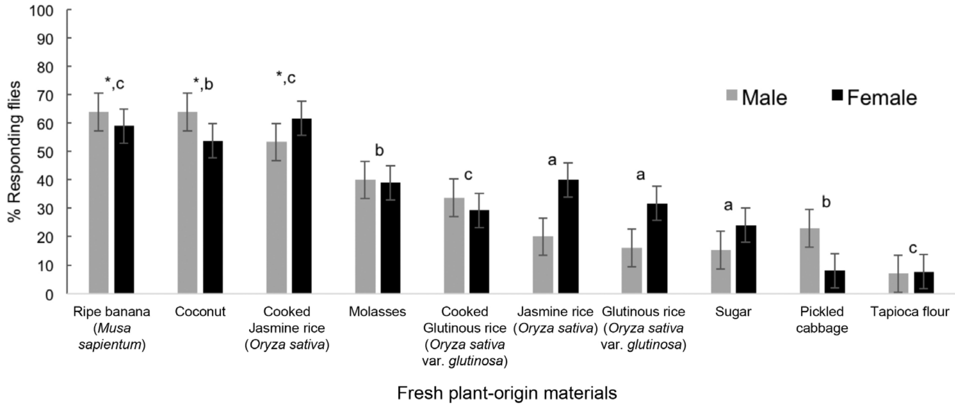

Fig 2–Percent responding *M. domestica* to the fresh plant-origin materials in screening trial. The experimental protocol is described in legend to Fig 1. *Natural product that attract > 50% of flies in a 5-minute period. [a]Significantly attracted more females than males ($p < 0.05$). [b]Significantly attracted more males than females ($p < 0.05$). [c]Nonsignificant difference in attraction between males and females.

plant materials, only 3 (ripe banana, coconut and cooked Khao Hom Mali rice) attracted > 50% of flies, of which ripe banana had the best attraction for both fly sexes (Fig 2). Tapioca flour exhibited the least attraction. Among the tainted materials, only 2 attracted > 50% in both sexes (1-day tainted pork and 1-day tainted pork liver), with better attraction of females than male ($p < 0.05$) (Fig 3).

Calibration of wind speed and behavioral response of house flies to olfactory stimuli

The number of house flies entering the odor-loaded partition gradually increased with increasing wind speeds. There was little difference between number of flies attracted to products with 0.3 and 0.4 m/s wind speed but the highest number of flies entering the odor-loaded partition was ≈68% when wind speed

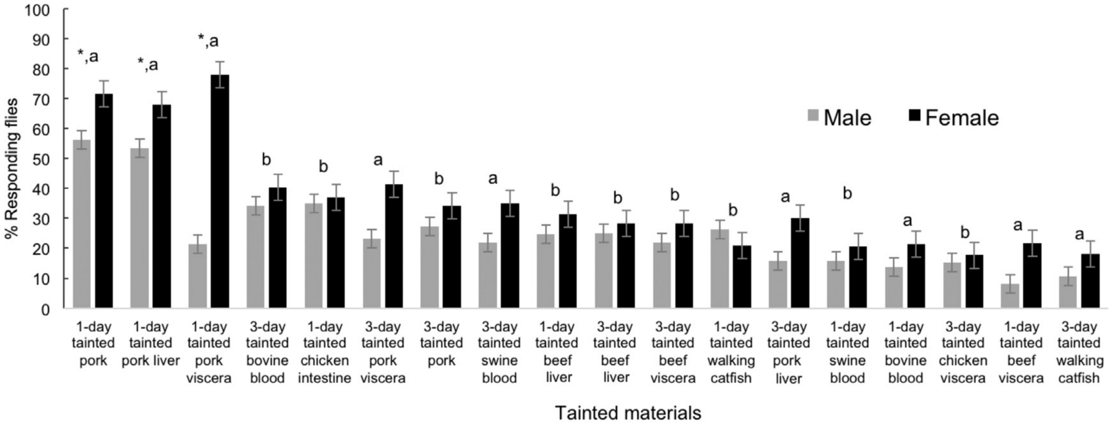

Fig 3–Percent responding *M. domestica* to tainted materials in screening trial. The experimental protocol is described in legend to Fig 1. *Natural product that attract flies > 50% in 5-minute period. [a]Significantly attracted more females than males ($p < 0.05$). [b]Nonsignificant difference in attraction between males and females.

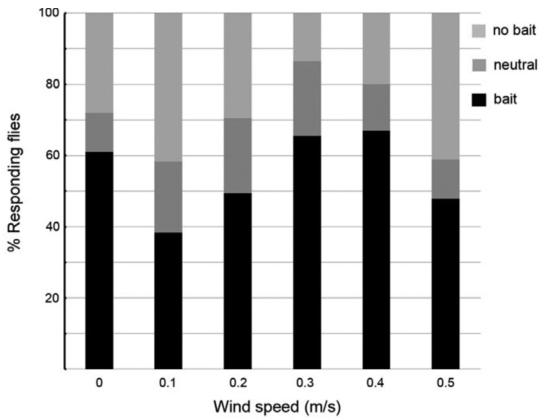

Fig 4–Behavior response of *M. domestica* to 200 g of 1-day tainted beef viscera at different wind speed levels, showing that wind speed set at 0.4 m/s was the optimal speed for T-box testing. Experiments were performed for 3 hours (12:00 to 03:00 PM) at the room temperature (~25-30°C) and laboratory relative humidity (~70-80%).

was 0.4 m/s (Fig 4). The wind speed was set at 0.4 m/s within the wind tunnel for subsequent experiments.

Behavioral response of house fly to natural products in T-box

Fresh beef viscera attracted the highest number of flies, followed by ripe banana and fresh beef liver, respectively (Fig 5). This was confirmed in direct comparison between the products (Fig 6).

DISCUSSION

Knowledge of the most suitable bait to attract adult house flies is essential for the study of several complex population characteristics (*eg*, population dynamics, longevity, development or reproductive potential) and remains a fundamental element in designing an efficient control strategy. Trials of the most attractive natural products demonstrated that more house flies flew upwind toward fresh animal material odor source than toward fresh plant materials, or 1- and 3-day tainted animal or plant materials. These findings agree with the study by Daenghan *et al* (1998) who showed that house flies prefer junk meat (protein-source) than ripe banana, stream rice and sugar (carbohydrate-source). Consistent results were found in Colombia, where adult house flies showed preference for chicken viscera (58.9%), followed by fish (39.5%), human feces (1.3%) and decomposing

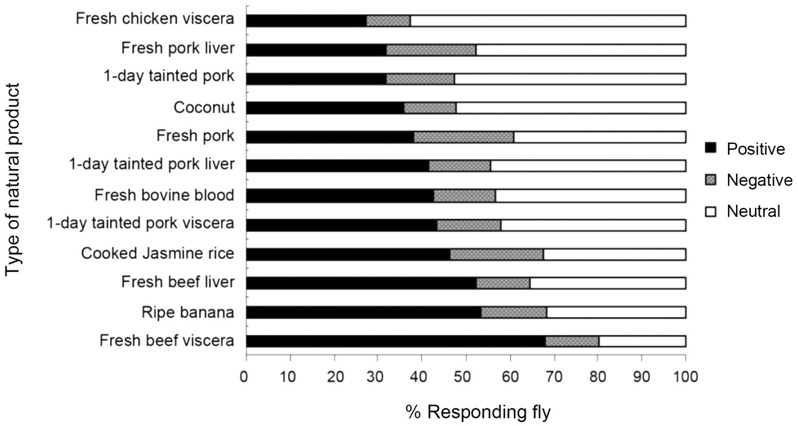

Fig 5–Behavior response of *M. domestica* to 12 materials in T-box using a wind speed of 0.4 m/s, indicating that fresh beef viscera was the most attractive product to lure flies. Experiments were performed for 3 hours (12:00 to 03:00 PM) at room temperature (~25-30°C) and laboratory relative humidity (~70-80%).

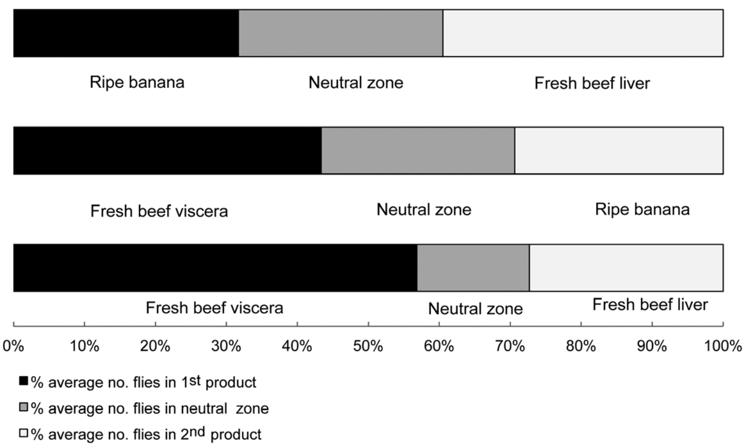

Fig 6–Behavior response of *M. domestica* to three most attractive natural products in T-box operated at 0.4 m/s, indicating that fresh beef viscera was the most attractive product in direct comparison with the other two products in each opposite partition of the T-box. Experiments were performed for 3 hours (12:00 to 03:00 PM) at room temperature (~25-30 °C) and laboratory relative humidity (~70-80%).

onion (*Allium cepa*, 0.3%) (Urib *et al*, 2010). Adult house fly attraction to other natural products (*eg*, vinegar) also has been documented (Qian *et al*, 2013). Many factors may affect attractiveness difference between animal and plant materials. Fresh animal materials likely contain blood, which greatly attracts adult flies (Daengharn *et al*, 1998).

Hung *et al* (2015) determined the attraction to volatiles from fungal culture and showed that house flies are attracted to odors from the fungus *Aureobasidium pullulans* growing on honeydew melons. We did not attempt to qualify the volatile substances emitted from the fresh beef viscera, nor did we evaluate the species of microorganisms associated with any of the tested materials. Future experiments will address the identity of volatile compounds emanating from the materials we tested for potential use as effective attractants, and possible synergistic action among such compounds as an effective house fly bait.

The optimal wind speed used to investigate the behavioral responses in wind tunnels vary with different insects. Our results demonstrated that more house flies flew upwind to volatile emitted from natural products at the wind speed set at 0.4 m/s. This wind speed was similar to that experiment of the fruit fly *Drosophila melanogaster* (Diptera: Drosophilinae) (Budick and Dickinson, 2006). However, the optimal wind speed 0.4 m/s obtained from our study was lower than that previously reported (0.5 m/s) from blow fly *C. megacephala*, (Bunchu *et al*, 2008), or blow fly *Lucilia sericata* (Wall and Fisher, 2001).

In conclusion, our results confirm fresh beef viscera as an optimum natural product for attracting adult house fly. This information would be useful in implementing an effective bait-trap device for integration into fly management strategies.

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