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Damien Charabidze, Valery Hedouin, Didier Gosset. An experimental investigation into the colonization of concealed cadavers by necrophagous blowflies. *Journal of Insect Science*, 2015, *Journal of Insect Science*, 15 (1), 10.1093/jisesa/iev129 . hal-04262851

**HAL Id: hal-04262851**

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Submitted on 27 Oct 2023

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## RESEARCH

# An Experimental Investigation Into the Colonization of Concealed Cadavers by Necrophagous Blowflies

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Subject Editor: Marc De Meyer

J. Insect Sci. (2015) 15(1): 149; DOI: 10.1093/jisesa/iev129

**ABSTRACT.** We used seven baited boxes with different combinations of access holes and odor diffusion surfaces to study the arrival of necrophagous flies. During laboratory experiments, 30 gravid *Lucilia sericata* females were kept in a chamber with one of the boxes. The box with the largest odor diffusion surface (99 cm<sup>2</sup>) combined with the lowest accessibility (one 1 cm<sup>2</sup> entrance hole) was entered least (5 ± 3.7 flies per run). In contrast, the most frequently entered box (one 9 cm<sup>2</sup> entrance hole with no additional odor diffusion surface) caught a mean of 24.6 ± 3.4 flies per run. These results indicate that 1) *L. sericata* entered nearly inaccessible places and 2) both odor diffusion and accessibility impacted the number of flies caught. During field experiments, the seven boxes were placed together outdoors. The box with the most entrances (ten 9-cm<sup>2</sup> holes) caught the most flies (55.6–99.4% of the total). Only a few flies entered the other boxes. Access to the less accessible boxes (poor odor diffusion and small entrances) was also delayed. The major conclusions of the field experiments are that 1) boxes with low accessibility took longer to be accessed; 2) larger odor diffusion surfaces were more attractive to flies; and 3) flies accessed boxes more readily through larger holes than through an equivalent surface area made up of smaller holes. With these conclusions in mind, attempts to quantify the preappearance interval or to interpret the number of flies observed in indoor forensic entomology cases should be approached with caution.

**Key Words:** indoor forensic entomology, postmortem interval, delayed colonization, body concealment, *Lucilia sericata* (Meigen 1826)

Forensic entomology is used to estimate the age of insects sampled from cadavers and subsequently approximate the time of the death. However, flies do not always immediately colonize corpses after death (Greenberg 1991, Campobasso et al. 2001). If a corpse is difficult to reach, especially in the case of wrapping, concealment, or burying, the arrival of necrophagous insects may be delayed (Ahmad et al. 2011, Gunn and Bird 2011, Martín-Vega et al. 2011, Voss et al. 2011, Bhadra et al. 2014). Such a delay is also observed when bodies are discovered inside dwellings or vehicles (Campobasso et al. 2001, Reibe et al. 2008, Bonacci et al. 2009, Frost et al. 2010, Anderson 2013, Voss et al. 2008, 2011). A 2009 study by Reibe and Madea (2009) called attention to potential bias affecting postmortem interval estimation in indoor forensic entomology. In this experiment, 1–2 kg piglets that had been placed in a room with a slightly opened (0.18 m<sup>2</sup>) window were colonized later and less often than piglets placed 50 m away in an outdoor location. Another study by Anderson (2013) of decomposition rates in indoor and outdoor environments obtained similar results; corpses located indoors were colonized 5 days later than those located outdoors, and fewer insects were observed. Finally, a review of nine indoor forensic entomology cases published by Pohjoismäki et al. (2010) highlighted the same issues, and the authors also noticed that buildings affect both the diffusion of odors and access to corpses.

The main consequences of the concealment of a corpse are 1) an increased preappearance interval (i.e., the time before the arrival of the first insect) and 2) a decreased number of flies accessing the corpse. Oviposition behavior can also be affected. Bhadra et al. (2014) experimentally demonstrated that blowflies are able to colonize resources placed inside closed suitcases, and if the bait was not accessible to their ovipositor, the flies laid their eggs on the surface of the zipper. This egg-laying behavior was also affected by several parameters, including not only the size and shape of the zipper but also the contact between the zipper and the bait.

All these consequences of concealment can impact the decomposition timeline and, in a forensic context, the estimation of postmortem

interval. It may seem rather obvious to say that if a corpse is less accessible, flies will arrive later, and there will be fewer of them. However, to our knowledge, no experimental evidence has been obtained to quantify this assertion. Furthermore, delayed or altered insect access to concealed corpses could actually result from two different reasons. First, low odor diffusion (meaning the escape of gases to the outside) may affect the capacity of necrophagous insects to locate a corpse. Second, the inherent difficulty of accessing a concealed corpse (low accessibility, i.e., the difficulty of finding a way to a bait/cadaver) may prevent, or at least delay, insect arrival. In other words, two overlapping processes may influence the colonization of concealed corpses: detection and accessibility. We hypothesized that both factors could strongly influence the colonization process and must be considered together.

We performed experiments under controlled (laboratory) and field conditions to address this issue. We used boxes baited with beef liver to test how different combinations of accessibility and odor diffusion affect the ability of necrophagous calliphorid flies to access bait (Bilaniuk and Beresford 2010). Although such an experimental design is not directly connected to “real” forensic cases, it provides general information on the behavior of necrophagous blowflies.

## Materials and Methods

**Boxes.** Under field conditions, flies use both olfaction and visual cues to locate their food/egg-laying substrates (Cragg 1956, Barton Browne 1960, Broce et al. 1991, Spivak et al. 1991, Urech et al. 1994, Hall et al. 1995, Wall and Fisher 2001). However, the visualization of a cadaver is generally limited when a body is concealed. Our experiments were designed to prevent visual or tactile detection of the bait and instead focused on the olfactory mechanism. Green opaque polypropylene boxes were used as concealment chambers. Each box measured 42 cm in length, 30 cm in width, and 23 cm in height and was closed by a translucent polypropylene cover. Different parameters were considered including 1) the entrance surface, which is defined as the total open surface (holes) allowing insects to enter the box; 2) the entrance

length, which is defined as the total length of the edges of the entrance holes (i.e., cumulative perimeters); 3) the odor diffusion surface, which is defined as the meshed surface allowing odor diffusion but preventing any access by the insect, and 4) the total odor diffusion surface, which is defined as the sum of the entrance and odor diffusion surfaces (Table 1).

Openings were cut in the boxes and either covered by mesh (to create odor diffusion surfaces) or left uncovered as open access holes (Table 1). Round access holes of 1 cm<sup>2</sup> or 9 cm<sup>2</sup> were drilled on the bottom of one of the large sides of the boxes 1 cm above ground in one or two rows. Holes were lengthened on the inside by a 5-cm long, gray opaque plastic pipe that was oriented 45° upward to reduce the possibility of flies exiting. Meshed odor diffusion surfaces were created by cutting out squares on the left side of the entrance side of the box, which were covered with a thin gray plastic insect screen (1 by 1 mm mesh). The inside of each box was coated with transparent insect glue (Eco Glue, Greece Oli), and according to specifications, this glue is odorless for both cockroaches and rodents. The glue inside the box was renewed between each trial, and the surfaces were cleaned with 90% ethanol between the removal and reapplication of the glue.

In forensic entomology studies, pig cadavers are generally considered to be the most relevant model of human corpse decomposition (Schoenly et al. 2007), but for obvious reasons, it was not possible to use such large cadavers for this study. Because of ethical considerations, baits consisting of 100 g of fresh minced beef liver combined with 20 ml of water were used for each trial (Hayes et al. 1999, Hall et al. 2003, Wooldridge et al. 2007, Gunn and Bird 2011). Such small bait traps have been proven to be accurate predictors of dipteran early colonizers in forensic entomology studies (Farinha et al. 2014). This experimental design allowed for comparisons between boxes and replicates (same baits = same bias), but bias must be kept in mind before attempting to extrapolate the results of this study to large cadavers, especially human corpses.

**Laboratory Experiments.** The laboratory experiments were performed on adult *Lucilia sericata* flies bred at Lille, France. Inbreeding was reduced by adding 200 wild-strain individuals each month. The

breeding substrate for the larvae was minced beef liver, and the rearing temperature was 25°C. The day of emergence was counted as day 0, and adult flies were placed in gauze cages at 21 ± 1°C with a photoperiod of 12:12 (L:D) and fed sugar and water ad libitum. Minced beef liver was added from days 0 to 7 and from days 12 to 15 to provide the proteins needed for vitellogenesis. Only gravid females aged between 7 and 12 d and from 15 to 20 d were used for the experiments, and each female was used only once (Wall 1993).




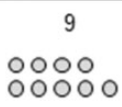


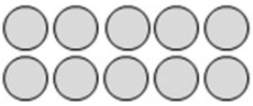

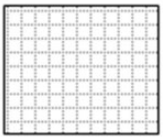
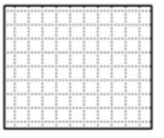
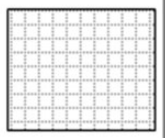

The experimental arena consisted of a 1-m<sup>3</sup> cupboard (200 by 100 by 50 cm) designed to quantify the number of flies accessing the boxes without disturbing them. Twenty 1-cm<sup>2</sup> mesh air entrance holes were made on the bottom front of the cupboard, and an air extractor (140 m<sup>3</sup>/h) was placed on top to create a permanent flow of air inside the enclosure (bottom to top). A white 18-W daylight tube provided light from 0800 to 2200 hours (16D/8N), and the temperature inside the cupboard was constant and equal to the room temperature (21 ± 1°C). Water and sugar were placed inside the setup ad libitum.

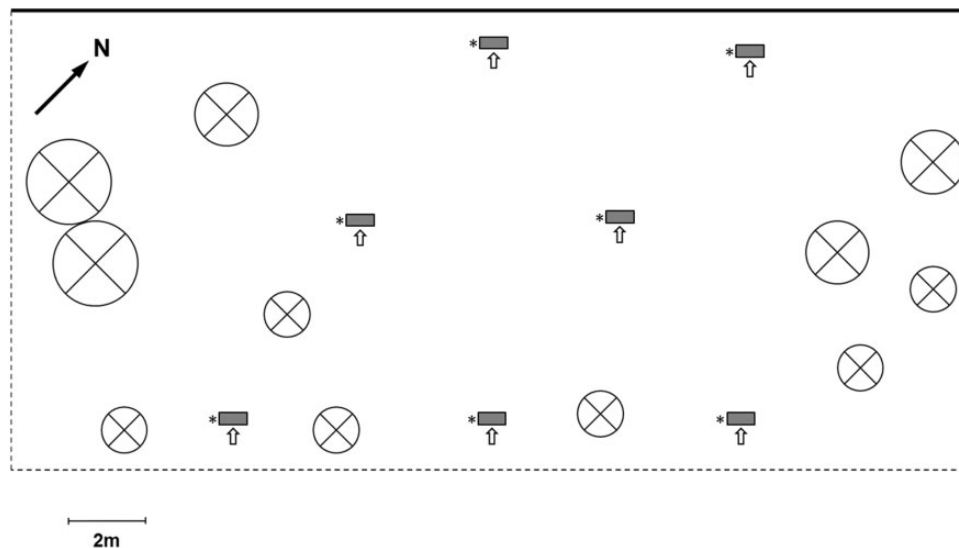
Thirty gravid females were confined inside the cupboard with one of the baited boxes at a time (A, B, C, D, E, F, or G) (Table 1). To prevent bait decomposition/desiccation, the duration of each experiment was limited to 53 h (i.e., 2 whole days and a half day for a total of 33 h of light and 20 h of dark), and five replications were performed for each box. After the end of each run, the cupboard was opened, the number of flies glued inside the box was counted, and the cupboard was fully cleaned with 90% ethanol. Control experiments were also performed with un-baited boxes using the same setup (five replications for each box).

**Field Experiments.** Field experiments were performed in a 25 by 12-m green space located near the forensic institute of Lille, France. All the tested boxes were simultaneously placed on the ground at a distance of 6 m from each other with the same orientation and sun exposure (Fig. 1). The positions of the boxes were randomized between each trial.

All experiments were performed between April and September over a 2-yr period. Each trial started at 10:00 hours (day 1) and lasted until 18:00 hours on day 3 (tot = 56 h), and boxes were checked every day at 10:00 hours and 18:00 hours. Insects that became glued inside the

**Table 1.** Design of the boxes. Boxes were identified by a letter (from A to G) corresponding to a unique combination of access holes (round) and odor diffusion surfaces (square meshed openings). Surfaces are shown to scale.

	A	B	C	D	E	F	G
Surface area of the access hole(s) (cm <sup>2</sup> )	1	1	1	1	9	9	9
Number of holes							
Total access surface area (cm <sup>2</sup> )	1	1	1	9	9	9	90
Total entrance perimeter length (cm)	3,14	3,14	3,14	28,3	10,6	10,6	95,5
Surface area of meshed odor diffusion areas (cm <sup>2</sup> )	0				0		
Total ventilated surface area (cm <sup>2</sup> )	1	9	100	100	9	100	100



**Fig. 1.** Location of the field experiment boxes (symbolized by rectangles). Experiments were performed in a 25 by 12-m green space located near the forensic institute of Lille, France. For each trial, the traps were simultaneously placed on the ground for 3 d, and box positions were randomized between each trial. Bold lines indicate a building wall; dashed lines depict open access; and crossed circles indicate bushes or small trees. The access holes in the boxes are indicated by an arrow and the meshed surfaces with an asterisk.

boxes were removed, labeled, and frozen, and calliphorid flies were determined to species according to Smith (1986) and Szpila (2012). As males are not directly involved in cadaver colonization, those specimens were separated and pooled together as “males” for the analysis. Sarcophagids were determined to family, and other species of Diptera were pooled together and labeled as “others” (most of the time, it was not possible to determine the species due to stuck or incomplete specimens.) As the experiments focused on the preappearance interval and colonization intensity of calliphorid flies, the presence of unidentified specimens in the datasets did not affect the results.

Twelve replications with all seven boxes together (from A to G) were performed, and 12 separate replications were performed without box G (i.e., with only boxes A, B, C, D, E, and F). The most accessible box (box G for the first experiments and box F for the experiments without G) was used as the reference to calculate the delay in access. For example, a first catch in box G after 8 h followed by a first catch in box A after 48 h was considered a 40-h delay in access to box A.

Weather data were obtained from the local weather station (Meteo France, Lille Lesquin), and three variables were measured for each 3-d trial: thermal sum, defined as the 3-d sum of the average daily temperature; total rainfall duration, defined as the sum of rainfall time in min; and total sunshine duration, the period, in min, during which direct solar irradiance exceeded a threshold value of 120 W/m<sup>2</sup>.

**Statistical Analysis.** The comparison of the number of flies in each box during the laboratory experiments was performed using a Kruskal–Wallis test with a Dunn procedure for multiple comparisons. The comparison of the number of insects in each box during field experiments was performed using a Friedman test with a Nemenyi procedure for multiple comparisons (Hollander and Wolfe 1999), and the weather data were compared using a Mann–Whitney test. All the calculations were made at the  $\alpha = 0.05$  threshold using XLSTAT 2011.3.02 software (Addinsoft).

## Results

**Experimental Setup and Potential Bias.** These experiments were designed to infer the effect of accessibility and odor diffusion by comparing the number of flies entering different baited boxes. Beef liver was used as bait for all experiments, but such meat baits desiccate more quickly than cadavers and lack the guts and associated bacteria known to attract some necrophagous species (Emmens and Murray 1983, Ashworth and Wall 1994, Morris et al. 1997). From a chemical/physical

point of view, the odor diffusion of such baits (i.e., the escape of gases through large openings) depends on several parameters including the size of the odor molecules, the nature of the molecules that compose the air, temperature, and atmospheric pressure (Gorban et al. 2010). Thus, it was not possible to quantify the diffusion of gas blends in complex designs such as those used for these experiments. Therefore, our analysis addresses this question from a comparative rather than a quantitative point of view.

**Laboratory Experiments.** During the laboratory tests, flies entered all the boxes (Fig. 2), but only a few flies entered the unbaited boxes (i.e., controls). Actually, the number of flies caught under control conditions never exceeded 3 per run and did not differ among replications. Under test conditions (baited boxes), the most colonized box was E with a mean of  $24.6 \pm 3.4$  flies per replication, and box C was the least colonized with a mean of  $5 \pm 3.7$  flies per replication. Furthermore, this box had the lowest single value among all the replications with only three flies in one individual run. No significant differences were found between the number of flies in boxes A, B, D, E, F, and G; only C differed significantly from the others ( $K = 19.9$ ,  $P$  value = 0.003).

**Field Experiments: The Effect of Removing Box G.** Considering the three relevant climatic parameters (thermal sum, total sunshine duration, and total rainfall), the weather did not differ statistically between the two field experiments (with and without box G) (Mann–Whitney test;  $P$  values:  $T^\circ = 0.68$ , rainfall = 0.8, sunshine duration = 0.47) (Tables 2 and 3). This similarity allows for a comparison of the two sets of experiments (i.e., box G present or absent). The total number of insects caught with box G was 741, with a mean of  $61.8 \pm 53$  per run (Table 2), but fewer flies were caught without box G, with a total of 153 flies and a mean of  $12.8 \pm 15.2$  flies per run (Table 3). However, the number of insects caught in boxes A, B, C, D, E, and F was very similar between the two sets of experiments (Mann–Whitney test,  $U = 71.5$ ,  $P$  value = 0.99) (except for box F, no statistical tests were performed due to the low number of captured flies). We conclude that the relative attractiveness of the different boxes did not change after box G was removed.

**Field Experiments: The Number of Flies Entering the Boxes.** The total number of flies caught per box varied from 6 to 157 with box G present (Table 2) and from 0 to 46 after removing box G (Table 3). With all seven boxes together, the results showed a clear asymmetry; box G caught significantly more flies than the others (Fig. 3). The number of flies in box G varied from 4 to 156 between trials, i.e., from 55.6 to

99.4% of the total number of flies caught during a single run. On the contrary, boxes A, B, C, D, E, and F proportionally caught only a few flies, never exceeding 23 flies in a single box, i.e., a maximum of 29% of the total number of flies caught during a single run (Tables 2 and 3). No significant differences were found between boxes A, B, C, D, and E; only boxes F and G differed significantly from the others ( $Q = 64.1$ ;  $P$  value  $< 0.0001$ ).

After removing box G, box F caught the most flies (Fig. 1). Box F caught from 0 to 100% of the total and differed statistically from the other boxes (A, B, C, D, and E) ( $Q = 20.2$ ;  $P$  value = 0.001).

More females were trapped than males; there were only 104 males out of a total of 894 flies, i.e., less than 15%. The species richness was greater in boxes D, E, F, and G (Fig. 4), but *L. sericata* was the most common species with a total of 412 flies. *Lucilia* sp. (unidentified specimens other than *L. sericata*) was the second most important taxa, but it was almost only collected in box G (140 out of a total of 151). Other species constituted the majority in boxes A, B, and C.

**Field Experiments: Preappearance Interval.** The catch rate was constant throughout the experiments and thus not affected by bait

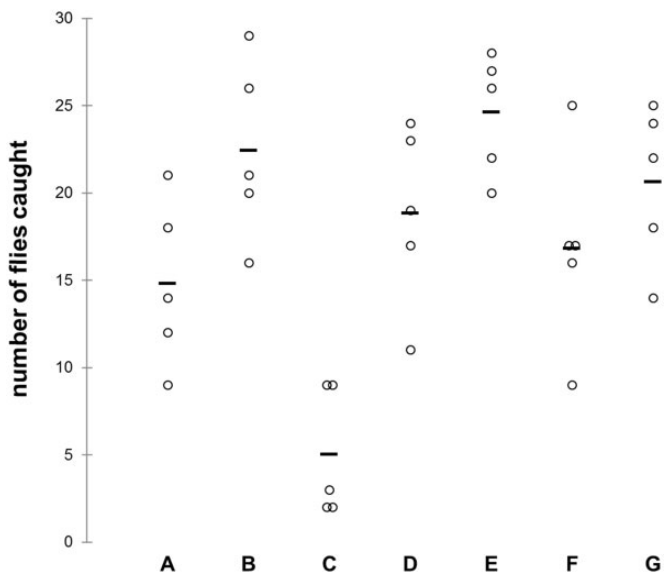
decomposition, and no differences were observed between the five sampling times (mean  $\pm$  SD; 8 h:  $15.9 \pm 24.2$ , 24 h:  $17.7 \pm 20.4$ , 32 h:  $21.3 \pm 21.4$ , 48 h:  $11.5 \pm 17.5$ , and 56 h:  $29.6 \pm 29.7$  with and without G combined).

Differences in the time before the observation of the first insect, i.e., the preappearance interval, were observed between boxes (Fig. 5). Boxes A, B, C, D, and E were not accessed at all during most of the runs, i.e., the preappearance interval in these boxes exceeded 56 h, which was the duration of a run. When flies entered these boxes, the first catch was delayed from 8 to 48 h compared with the reference box, but during one run, the first flies were observed in boxes A and B 16 h prior to those in the reference box (F). Entrance into boxes D and F before box G was only observed twice.

## Discussion

**Laboratory Experiments.** The results demonstrated that flies were able to enter all the boxes, including the minimally accessible ones. This was even true under control conditions as some flies entered unbaited boxes, but the number was always very low and did not differ among boxes. On the contrary, the same boxes baited with beef liver attracted a significant number of flies. Even with only a single 1-cm<sup>2</sup> round hole and without any other odor diffusion surface, 50% of the flies were able to find the only access hole and enter box A. This result highlights the ability of necrophagous flies to detect and access concealed resources; previous studies have shown that, under certain conditions, Calliphoridae flies can even enter through holes less than 5–7 mm in diameter (Hall et al. 2003, Wooldridge et al. 2007). Boxes with openings of 1 or 9 cm<sup>2</sup> and a total access surface area ranging from 1 to 90 cm<sup>2</sup> were similarly colonized; only box C differed significantly with only a few flies trapped (Fig. 2). It is likely that the combination of a large meshed odor diffusion surface with low accessibility (only a single small entrance hole) in this box resulted in flies being attracted to rest on the insect screen, effectively diverting them from the small opening. This conclusion is especially interesting as this condition can be regarded as a model for corpses discovered inside a dwelling with insect screens on the windows.

**Field Experiments.** Far more flies entered the most accessible box (G) than any other during the field experiments. In comparison, box F, which had the same total odor diffusion surface area as G but just a single 9-cm<sup>2</sup> access hole (instead of 10 holes), trapped six times fewer flies on average. The others boxes (A, B, C, D, and E) caught relatively few flies. The low ability of some calliphorids to detect/enter some trap designs has been reported by Hall et al. (2003), and the comparison between the D and F boxes in this study provides more details about the importance of the shape of the access points. During our experiments, box F was extensively colonized, unlike box D, and these two boxes had the same accessibility and total odor diffusion surface. However, box D had nine small 1-cm<sup>2</sup> access holes and a longer total entrance



**Fig. 2.** The number of flies caught in each box during the laboratory experiments. Thirty gravid females were confined for 53 h (33 h of day and 20 h of night) inside a cupboard containing one of the baited boxes. Each point corresponds to the total number of flies caught in a given box during a single trial, and bold horizontal lines represent means. Only box C differed significantly from the others (Kruskal–Wallis:  $K = 19.9$ ,  $P$  value = 0.003).

**Table 2. Outdoor experiments with box G**

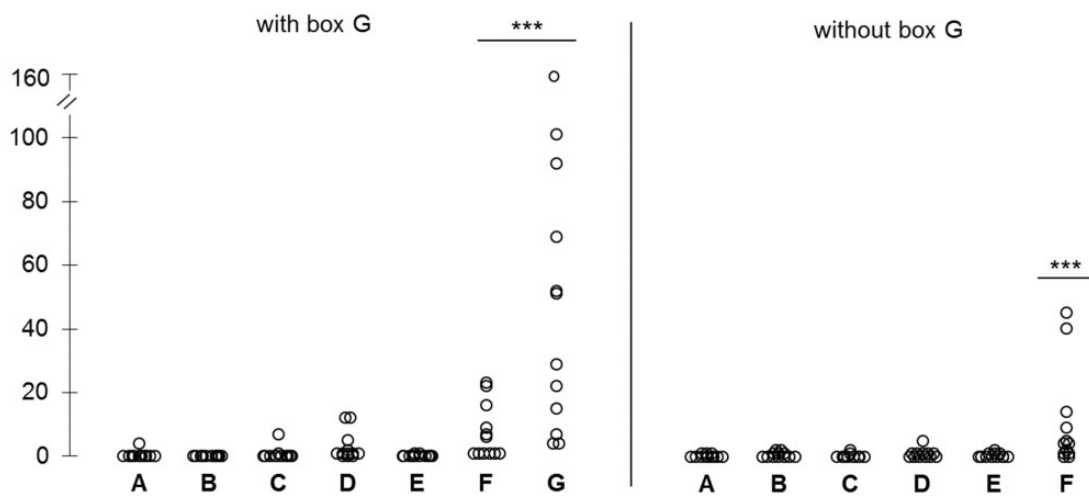
Starting date	Thermal sum (°C)	Total rainfall (min)	Total sunshine duration (min)	Number of flies caught
21 July 2010	48.1	277	738	136
26 July 2010	50.7	464	605	8
4 August 2010	56.6	184	1,000	31
11 August 2010	51.4	174	1,327	77
24 August 2010	53.5	789	782	55
31 August 2010	33.9	0	1,534	157
2 May 2011	45.2	0	2,415	6
9 May 2011	42.0	215	1,722	6
16 May 2011	40.4	40	809	27
23 May 2011	43.1	0	2,568	91
30 May 2011	55.5	197	1,637	116
8 June 2011	45.1	297	1,541	31
Mean $\pm$ SD	47.1 $\pm$ 6.8	219.8 $\pm$ 228.4	1,389.8 $\pm$ 643.7	61.8 $\pm$ 53

Weather data from the nearest weather station (Lille Lesquin). The total numbers of flies caught are reported for each trial.

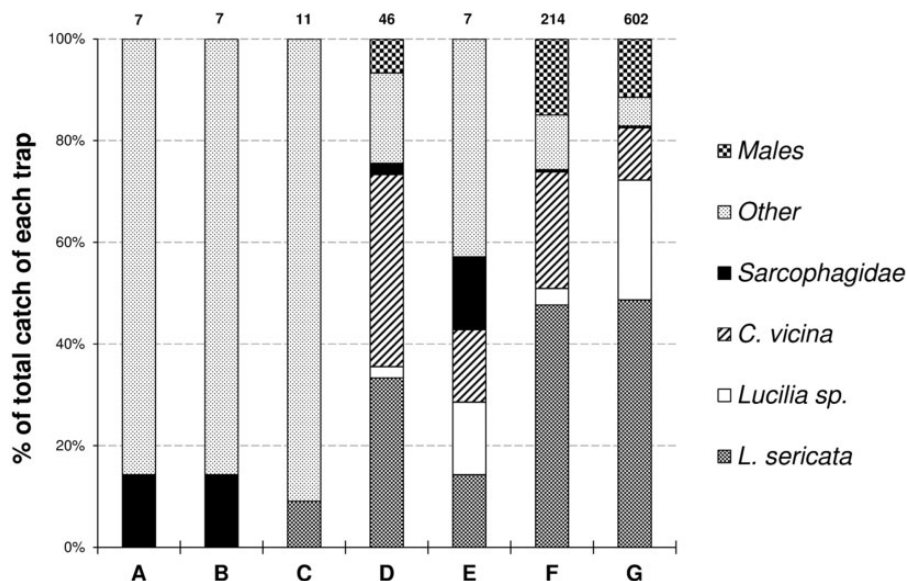
**Table 3. Outdoor experiments without box G**

Date	Thermal sum (°C)	Tot. rainfall (min)	Tot. sunshine duration (min)	Number of flies caught
14 September 2010	32.6	345	568	5
20 September 2010	45.5	0	1,483	4
22 March 2011	46.5	0	1,949	2
4 April 2011	56.3	178	1,015	3
11 April 2011	36.5	76	1,445	0
14 June 2011	31.2	229	959	15
21 June 2011	52.8	137	745	12
27 June 2011	48.8	210	1,845	7
4 July 2011	69.0	152	1,885	42
25 July 2011	47.0	570	34	9
3 August 2011	57.6	497	611	8
10 August 2011	53.5	367	1,290	46
Mean ± SD	48.1 ± 10.9	230.1 ± 182.5	1,152.4 ± 601	12.8 ± 15.2

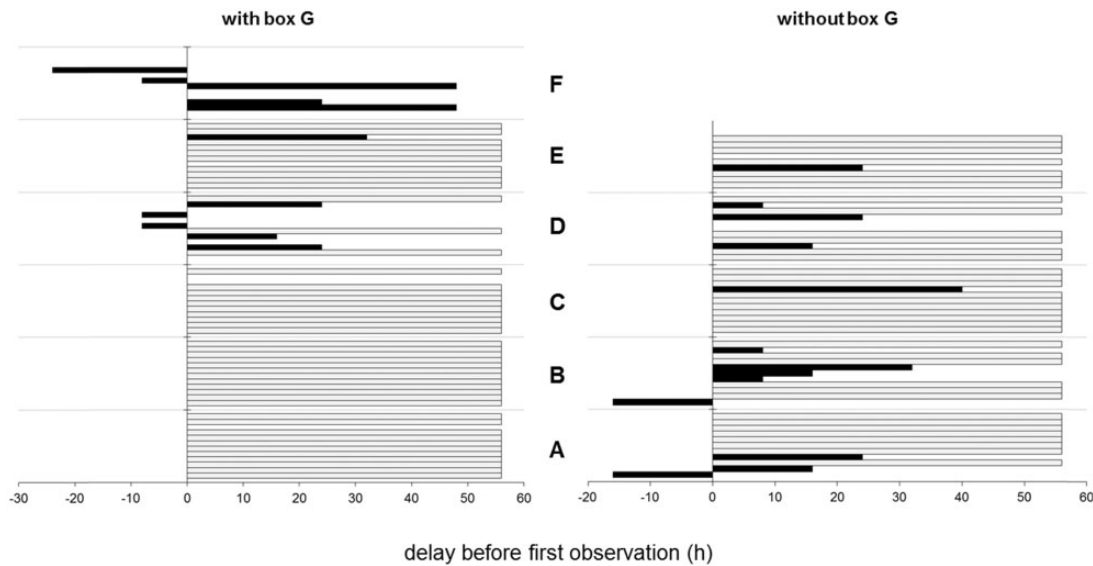
Weather data from the nearest weather station (Lille Lesquin) and the total number of flies caught are reported for each trial.



**Fig. 3.** The number of flies caught in boxes (y axis) during the field experiments with (left) and without (right) box G. Each point corresponds to the total number of flies caught in a box during a single trial, and asterisks indicate statistical significance (Friedman test: \*\*\**P* value < 0.0001).



**Fig. 4.** The species composition observed during the field experiments. Boxes are reported on the x axis, and the contribution (%) of each taxon to the total number of flies caught in each box is reported on the y axis. The number of males includes all the Calliphoridae species. The numbers on top of the columns indicate the total number of flies caught in the box.



**Fig. 5.** Comparison of the precolonization time (delay before the first insect was observed) between the boxes. The most accessible box (box G for the first set of experiments and box F for the experiments without G) was used as the reference; for each replication, the delay before insect arrival was calculated as the time of the first catch in a box minus the time of the first catch in the reference box (G or F). Bars correspond to trials. Black bars indicate the delay in colonization, and the gray bars represent any colonization, i.e., a delay in fly arrival extending over 56 h (the duration of one run). No bar indicates no difference, i.e., colonization at the same time as the reference box.

perimeter (28.3 vs. 10.6 cm) than F (single 9-cm<sup>2</sup> hole). We conclude that resources accessible through many small openings tend to be accessed by fewer flies than those accessible by fewer but larger openings. Finally, our results demonstrated that greater odor diffusion increased the number of flies entering the box. With exactly the same access opening but with an additional 91-cm<sup>2</sup> odor diffusion meshed surfaces, box F caught significantly more flies than box E. It can be seen from this result that necrophagous flies likely use odor diffusion to detect potential resources as well as to orientate and access them. Such behavior involves a complex set of stimuli and responses that allow flies to follow odor gradients to their source (Cragg 1956, Barton Browne 1960, Spivak et al. 1991, Urech et al. 1994, Wall and Fisher 2001). Accordingly, low odor diffusion or high odor diffusion with low accessibility may prevent flies from accessing concealed corpses.

**Effect of Concealment on Colonization Time.** During field experiments, we observed delayed access to the less open (less odor diffusion and accessibility) boxes (A-E). For most of the replications, no flies were observed in these boxes at all, but when flies entered, it was later than in the more open boxes. In other words, the box with the largest entrance holes and the greatest accessibility was most often the first colonized. The increased preappearance interval on concealed resources may be due to a reduced ability of the flies to detect and orientate toward weak odors in field conditions. As the laboratory experiments demonstrated the ability of the flies to enter all the boxes, the lack of flies in some during the field experiments can be considered as an increased colonization time of at least 3 d (the entire duration of the experiments). Such a delay in access time should be considered in indoor forensic-entomology cases.

**Field Versus Laboratory Experiments.** Comparing the laboratory and the field experiments, one may wonder why experiments performed with exactly the same boxes and baits produced such different results. However, the two experiments have some important differences. During the laboratory experiments, gravid females were locked in the cupboard for 53 h with only a baited box and food ad libitum. Under such conditions, we postulate that these gravid females spent as much time as possible trying to reach the bait as there were few competing stimuli. In contrast, the field experiments took place under different weather conditions in the presence of predators, wild flies, and many competing stimuli, so it is likely that the flies spent less time trying to

enter a given box. The easiest box to detect and access was extensively colonized and the others hardly at all. Similar observations were made by Hall et al. (2003) during field experiments; flies were attracted to the vicinity of boxes by odor lures but failed to enter through the small openings. They spent less than 30 s exploring a trap and explored a maximum of seven entry holes (of a total of 52). Such results are consistent with our hypothesis and confirm the importance of the ground exploration behavior of flies during the colonization process.

In conclusion, this study demonstrates the interaction between accessibility and odor diffusion on the access by necrophagous flies to concealed baits under laboratory and field conditions. Our results proved that *L. sericata* can enter into barely accessible places, but flies more readily accessed boxes through larger holes than through an equivalent surface area made up of smaller holes. We also demonstrated that larger odor diffusion surfaces attracted more flies, so we conclude that both odor diffusion and accessibility affect the number of flies accessing a given resource. Finally, this study demonstrated that low accessibility increased the preappearance interval, but the complexity and variability in the process highlight the difficulty of quantifying it with precision in an experimental situation, let alone under practical conditions. With these conclusions in mind, attempts to quantify the preappearance interval or to interpret the number of flies observed in indoor forensic entomology cases should be approached with caution.

### Acknowledgments

We thank the Nord Pas-de-Calais Region for funding this study and E. Boulleaux for his assistance in the laboratory.

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Received 5 March 2015; accepted 29 September 2015.