Study on ascending and descending vertical dispersal behavior of third instar larvae of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae): An evidence that blowflies survive burial

Anika Sharma, Samy Sayed, Madhu Bala, Jaroslav Kmet, Marek Horvath

A. Department of Zoology and Environmental Sciences, Punjabi University, Patiala, India
B. Department of Science and Technology, University College-Ranyah, Taif University, B.O. Box 11099, Taif 21944, Saudi Arabia
C. Department of Integrated Forest and Landscape Protection, Faculty of Forestry, Technical University in Zvolen, T. G. Masaryka 24, 960 53 Zvolen, Slovakia
D. University of Security Management in Košice, Slovakia

**Abstract**

Although the pupation behavior of blowflies has been widely studied, this preliminary study was done on the vertical dispersal behavior (both ascending and descending) and fly emergence rate of third instar larvae of *Chrysomya megacephala* (Fabricius) to evaluate whether immature stages of blowflies survive burial and emerge out as adults. Third instar larvae of *Chrysomya megacephala* were placed at three different depths (5 cm, 25 cm and 45 cm) of soil under laboratory conditions to determine the impact of soil depth on the ascending and descending vertical dispersal behavior and the subsequent emergence of adults. The results of this study, although preliminary, but valuable to the field of forensic entomology because they provide new information about both ascending and descending vertical dispersal behavior of *Chrysomya megacephala* forensically important species of blowfly. In all the cases, maximum number of pupae recovered at the depth of 0 to 5 cm are 35.5 ± 4.5, 34 ± 1, 25 ± 5, when food was located at 5 cm, 25 cm and 45 cm depth respectively. This means that maximum no of larvae reached to the depth of 0 to 5 cm by ascending dispersal irrespective of at which depth they are placed. Paramount pupae were recovered from shallow burial depth of 0–5 cm in ascending dispersal and showed highest eclosion success i.e. 90.1% followed by 25 cm and 45 cm i.e. 71.7% and 55% respectively. While the number of pupae recovered as well as eclosion success was less in descending dispersal with an average of 62.8%, 39.25% and 33.9% at depths of 5, 25 and 45 cm respectively. This manifests if larvae disperse ascendingly, it increases their chance of survival.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Blowflies belonging to the family Calliphoridae are ecologically diverse and occupy various habitats. These flies develop in various substrates, from decomposing organic matter to live animal tissues (Zumpt 1965). Blowflies use deceased body as an oviposition and feeding substrate. Understanding the behavior of carrion-feeding insects is an important component in determining the time since death or minimum postmortem interval (PMImin) of discovered human remains (Amendt et al. 2004). While investigating a murder, every clue helps. Present research sheds light on how – and whether – blowflies survive when buried underground during their development. It’s an advancement that will help forensic investigators to understand how long a body may have been left above ground before being buried – or possibly whether remains were moved from one grave to another. Blow flies are probably the most important insects to forensic entomology. They are usually the first insects to arrive after an animal – or a person – has died. These flies can arrive at a dead body in less than 10 min and lay their eggs within an hour.

Larval dispersal is an important process in the life cycle of blow flies during which the larvae leave their food substrate and search for a suitable place to pupariate (Levot et al., 1979; Godoy et al.,...
Horizontal dispersal is very common phenomenon when third instar larvae leave food substrate and move along the surface. Vertical dispersal is occur when larvae buried themselves for pupation, and emerge out as adult from soil. Hence, a knowledge about where blow flies pupate is critical for an accurate analysis of insect evidence. Research shows that in Australia, pupation behavior differs between two subfamilies of the Calliphoridae: the Chrysomyinae (Chrysomya) pupate on or near the food source on the ground surface, and the Calliphorinae (Lucilia, Calliphora) disperse from the food source and burrow into the soil before pupation (Norris 1959). After this initial observation on behavioral differences, many studies have been conducted on the dispersal behavior of post-feeding larvae of blow flies.

Godoy et al. investigated experimentally and theoretically the dispersal behavior of post feeding larvae of Chrysomya megacephala, Chrysomya putoria and Cochliomyia macellaria in an earlier study and found that most larvae pupariated close to the food source (Godoy et al., 1995). Some studies, specifically on this post-feeding larval dispersal phase have emphasized the patterns and spatial distribution of larval movement (Gomes and Von Zuben 2005a; Tessmer and Meek 1996; Von Zuben et al. 1996). Whilst this is of considerable use in forensic entomology, particularly in identifying the most probable areas to look for puparia in relation to a corpse, only Gomes and Von Zuben investigated post-feeding radial dispersal behavior of Chrysomya albiceps in a circular arena and results have shown a positive correlation between burial depth and distance (Gomes and Von Zuben, 2005b). Singh and Bala studied post-feeding larval dispersal behavior of Chrysomya megacephala and Chrysomya rufifacies in a circular arena and found that the larvae of Chrysomya megacephala are more dispersive than that of Chrysomya rufifacies. Secondly, most larvae of Chrysomya rufifacies remained near the food site as also observed by Greenberg (Singh and Bala 2010; Greenberg 1990).

Enough literature and data are available about the development of blow flies when bodies are exposed above the ground, but not many studies have been done for larval development when the body is buried. Thus, efforts have been made to know how their development would be affected if a body is buried after the blow flies had laid their eggs. Objective of this research is to scrutinize the impact of soil depth on the dispersal behavior as well as on further development of feeding third instar larvae of Chrysomya megacephala and subsequent eclosion success.

2. Materials and method

To begin the culture, wild type specimen of Chrysomya megacephala were collected from Botanic garden, Punjabi University, Patiala Punjab, India by using 5 kg Pork as bait. These specimens were reared in rearing cage under controlled temperature 25 ± 1 °C, 60% Relative humidity and 12L-12D photoperiod to establish pure colony. The flies were fed sugar and water and fresh piece of liver was kept as oviposition medium. The eggs of these flies were collected and placed in new rearing jar filled with moistened sawdust to prevent desiccation. Fresh meat was provided as food for the emerging larvae.

Clay soil, common in southeastern Punjab, was gathered from nearby area of Punjabi University, Patiala, India. Sand and pebbles were mixed in soil and frozen to kill any existing microbes, ants and other insects. Three White Polyvinylchloride (PVC) tubes, 60 cm in length and 150 mm in diameter, were used to survey both ascending and descending vertical dispersal behavior of larvae (Fig. 1 a). A stable platform was provided to the PVC tubes by attaching a plywood base. To achieve a proper depth, soil was added in each tube, bringing levels up to 5, 25 and 45 cm. 60 feedings of liver was kept as oviposition medium. The eggs of these larvae were collected and placed into new rearing jar filled with food source (25gms of grounded goat meat) were buried under soil and covered with 45, 25 and 5 cm of soil respectively (Fig. 1 b & c). The tubes were left undisturbed for 7 days so that the process of pupation got completed. Larval dispersal was inspected by analyzing the soil to determine at which depth larvae pupated. The soil was pushed out of the PVC tube in 5 cm increments and examined for pupae. Depth of pupation site was measured with measuring tape. After removing from soil, pupae were placed in rearing cage for adult emergence. After eclosion, pupae were scrutinized to discern eclosion success. The experiment was terminated when no fly emerged from pupae for 3 days. Experiment was replicated under similar conditions.

3. Results

Third instar Chrysomya megacephala larvae move through loose soil and show ascending as well as descending vertical dispersal behavior. Maximum number of larvae have shown ascending vertical dispersal and eclosion success was also high in case of ascendingly dispersed larvae (Table 1). Depth has an inverse relationship with eclosion success. Eclosion success decreases with increase in placement depth of larvae in soil (Fig. 5, 6). The data was analyzed by using Chi-square test to determine:

1. Effect of depth on the dispersal of third instar larvae of Chrysomya megacephala.
2. Effect of depth on eclosion success of Chrysomya megacephala.

3.1. Ascending and descending vertical dispersal

Ascending dispersal was investigated by examining soil with 5 cm incremental removal to determine where larvae pupated. It was observed that maximum number of larvae pupated between 0 and 5 cm soil depth (Fig. 2, 4). In all the cases, maximum number of pupae recovered at the depth of 0 to 5 cm are 35.5 ± 4.5, 34 ± 1, 25 ± 5, when food was located at 5 cm, 25 cm and 45 cm depth respectively as shown in Table 1. This means that maximum no of larvae i.e. up to 50% reached to the depth of 0 to 5 cm by ascending dispersal irrespective of at which depth they are placed. An average number of pupae recovered in case of descending vertical dispersal was fewer than ascending vertical dispersal (Table 2, Fig. 3, 4). X^2 (chi-square) fit with P < 0.01 in experiment 1 shows that there is significant difference between the tendency of larvae to disperse ascendingly and descendingly. A similar conclusion is reached in the replicate as well.

3.2. Eclosion success

Burial depth has a significant impact on mean number of adults emerging successfully. It is seen that the burial depth is inversely related to the eclosion success. In fact, the dispersal path has also impacted emergence rate. Ascending vertical dispersal have high success rate in case of a depth of 5 cm followed by 25 cm and 45 cm i.e. 90.1%, 71.7% and 55% respectively (Table 1, Fig. 5). Even though the same pattern of depth was followed in case of descending vertical dispersal, eclosion success was less in this case with an average of 62.8%, 39.25% and 33.5% respectively (Table 2, Fig. 6). Chi-square was applied to compare the level of significance for eclosion success in both cases of dispersion. The p value for both experiment and replicate was (P = 0.001, 0.03) respectively which signifies that the level of eclosion success shows significant value in ascending dispersal as compare to descending.
4. Discussion

Blow fly larvae (Diptera: Calliphoridae) that have fed and developed on a decomposing body or other decaying organic matter will leave the food source to pupate on or in the surrounding soil. The pupal stage is sedentary and occupies approximately 50% of the total duration of blow fly development, and many organisms have evolved to exploit this life stage (Greenberg and Kunich, 2002). Knowledge of all life stages is foremost for utilizing insects in forensic investigation and developing new innovative methods to estimate minimum Post-mortem Interval. Postfeeding larvae starts dispersing from food resource. After dispersion, Post feeding larvae often burrow into the ground to pupate, but little information exists on this behavior. In Brazil, Chrysomya albiceps and Chrysomya megacephala burrow into wood shavings to an average depth of 4.0 cm (Gomes and Von Zuben, 2005b).

In this study we have analyzed both ascending and descending vertical dispersal behavior of feeding third instar larvae as well as eclosion success of Chrysomya megacephala. More than 75% larvae dispersed ascendingly and pupated not more than 25 cm in all the
cases of provided depth. As the soil depth escalate, oxygen availability diminishes, therefore it might be the reason that larvae prefer to move upward towards the soil surface for more oxygen availability. Eclosion success rate has increased when the larvae move ascendingly. Number of larvae emerged successfully as adults was significantly greater in ascending dispersal, which reveals larvae preferred to move upward in clay soil (Compact soil). Laboratory experiments on fruit flies (Diptera: Tephritidae) suggest that soil compaction negatively affects the burrowing ability of larvae. Larvae of the Caribbean fruit fly *Anastrepha suspensa* (Loew) burrow deeper in less compacted soil. The deepest mean depth of pupation for *A. suspensa* is 3.3 cm in low compaction soil and the shallowest is 0.7 cm in high compaction soil, with no significant difference in percentage of adult emergence based on soil compaction (Hennessey 1994). Pupation depth by olive fruit flies, *Dacus oleae* (Gmelin), in different substrate types is affected by compaction. In compact substrates, larvae of *D. oleae* pupate within 5 cm while larvae pupate up to 8 cm deep in uncompacted substrates (Tsitsipis and Papanicolaou 1979). Therefore, soil compaction affects the pupation behavior of insects; they pupate deeper in less compact soil than in more compact soil. Pore space of soil is negatively correlated with soil compaction; pore space decreases as compaction increases (Babercheck 1992). This results in reduction of gas exchange in the soil and thus less oxygen is available for the developing fly (Brady and Weil 2008).

Several studies have investigated the vertical dispersal of post feeding larvae burrowing into the soil to pupate and to emerge as adult flies several days later (Greenberg 1990; Cammack et al. 2010). Such behavior further characterizes blow flies as r-strategists. The depth at which puparia are located varies with species: *P. regina* about 2 cm, *C. macellaria* at 4–5 cm and *L. sericata* at depths of 11 cm (Greenberg 1990; Cammack et al. 2010; Ulljett 1950). Balme et al. investigated ascending vertical dispersal behavior of *P. terraenovae* and *C. macellaria* and found no significant differences in the number of emerging adults at depths of 5 and 25 cm. However, significantly fewer adults were collected from immatures buried at the 50-cm depth (Balme et al. 2012).

### Table 1

| Vertical dispersal | Depth of food source below soil (cm) | Pupation depth (cm) | No. of pupae recovered | Mean no. of pupae recovered±SD | Eclosion success | Average Eclosion success |
|-------------------|--------------------------------------|---------------------|------------------------|--------------------------------|-----------------|--------------------------|
|                   | 1st Sampling                         | 2nd Sampling        |                        |                                |                 |                          |
| Ascending vertical dispersal | 5 | 0–5 | 31 | 40 | 35.5 ± 4.5 | 90.3% | 90% | 90.1% |
|                   | 0–5 | 35 | 33 | 34 ± 1 | 69.5% | 73.9% | 71.7% |
|                   | 5–10 | 3 | 5 | 4 ± 1 | 45.1% | 45.1% | 45.1% |
|                   | 10–15 | 3 | 4 | 3.5 ± 0.5 | 60% | 60% | 60% |
|                   | 15–20 | 3 | 3 | 3 ± 0 | 30% | 30% | 30% |
|                   | 20–25 | 2 | 1 | 1.5 ± 0.5 | 15% | 15% | 15% |
|                   | 0–5 | 20 | 30 | 25 ± 5 | 54.7% | 55.3% | 55% |
|                   | 5–10 | 8 | 11 | 9.5 ± 1.5 | 45.1% | 45.1% | 45.1% |
|                   | 10–15 | 10 | 7 | 8.5 ± 1.5 | 45.1% | 45.1% | 45.1% |
|                   | 15–20 | 5 | 4 | 4.5 ± 0.5 | 45.1% | 45.1% | 45.1% |
|                   | 20–25 | 3 | 0 | 1.5 ± 1.5 | 15% | 15% | 15% |
|                   | 25–30 | 3 | 2 | 2.5 ± 0.5 | 15% | 15% | 15% |
|                   | 30–35 | 2 | 1 | 1.5 ± 0.5 | 15% | 15% | 15% |
|                   | 35–40 | 0 | 0 | 0 ± 0 | 0% | 0% | 0% |
|                   | 40–45 | 2 | 1 | 1.5 ± 0.5 | 15% | 15% | 15% |
|                   | 45 | 0–5 | 20 | 30 | 25 ± 5 | 54.7% | 55.3% | 55% |
|                   | 5–10 | 8 | 11 | 9.5 ± 1.5 | 45.1% | 45.1% | 45.1% |
|                   | 10–15 | 10 | 7 | 8.5 ± 1.5 | 45.1% | 45.1% | 45.1% |
|                   | 15–20 | 5 | 4 | 4.5 ± 0.5 | 45.1% | 45.1% | 45.1% |
|                   | 20–25 | 3 | 0 | 1.5 ± 1.5 | 15% | 15% | 15% |
|                   | 25–30 | 3 | 2 | 2.5 ± 0.5 | 15% | 15% | 15% |
|                   | 30–35 | 2 | 1 | 1.5 ± 0.5 | 15% | 15% | 15% |
|                   | 35–40 | 0 | 0 | 0 ± 0 | 0% | 0% | 0% |
|                   | 40–45 | 2 | 1 | 1.5 ± 0.5 | 15% | 15% | 15% |
|                   | 45 | 0–5 | 20 | 30 | 25 ± 5 | 54.7% | 55.3% | 55% |

**Fig. 3.** Pupae recovered in descending dispersal with food depth at 5, 25 and 45 cm.
The evaluation of PMImin is one of the most important aspects of legal medicine and it could be underestimated if the older dispersing larvae or those that disperse longer and faster or deeper, are not taken into account (Smith 1986). Because of this, it is necessary to investigate the pattern of larval dispersal on the pupation site, as demonstrated in this study with larvae of Chrysomya megacephala.

4.1. Conclusion and suggestion

Future research could focus on identifying the furthest distance pupae can travel under a variety of environmental conditions, soil types, and seasonal temperature. Little work has been conducted on the effect of soil on larval dispersal and blowfly pupation. Cammack (2009) demonstrated that when a larva penetrates the soil surface and burrows, a tunnel is created that has less resistance than the surrounding soil. When another larva encounters an area of less resistance, they would most likely use it to enter the soil. The clumped distribution also might be the result of communal burrowing. This might be the reason of preference of ascending vertical dispersal also. Thus, further study of the effects of soil and depth on the dispersal and development of blow flies is necessary to estimate the post-colonization interval more accurately.

**Fig. 4.** Ascending as well as Descending vertical dispersal of third instar larvae of Chrysomya megacephala with food depth at 5, 25, and 45 cm. D.D. - Descending dispersal; A.D. - Ascending dispersal; marker is showing ascending dispersal; marker is showing descending dispersal; marker is indicating food depth. Color of food source is matched with corresponding ascending and descending dispersal behavior.

**Fig. 5.** Eclosion success in case of ascending vertically dispersed larvae.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Authors are thankful to the department of Zoology and environmental Sciences for providing laboratory to conduct the experiment. This study was financed by Taif University Researchers Supporting Project number (TURSP -2020/92), Taif University, Taif, Saudi Arabia.

References

Amendt, J., Krettek, R., Zehner, R., 2004. Forensic entomology. Naturwissenschaften 91, 51–65.
Babercheck, M.E., 1992. Effect of soil physical factors on biological control agents of soil insect pests. Fla Entomol. 75, 539–548.
Balme, G.R., Denning, S.S., Cammack, J.A., Watson, D.W., 2012. Blow flies (Diptera: Calliphoridae) survive burial: Evidence of ascending vertical dispersal. Forensic Sci. Int. 216, e1–e4.
Brady NC, Weil RR (2008) The nature and properties of soils. 14th edition. Pearson Education, Inc. Upper Saddle River, New Jersey. 965 pp.
Cammack, J.A., Adler, P.H., Tomberlin, J.K., Arai, Y., Bridges Jr, W.C., 2012. Blow flies (Diptera: Calliphoridae) survive burial: Evidence of ascending vertical dispersal. Forensic Sci. Int. 216, e1–e4.

Table 2
Descending vertical dispersal and eclosion success of third instar larvae of Chrysomya megacephala.

| Vertical dispersal below soil (cm) | Depth of food source (cm) | Pupation depth (cm) | No. of pupae recovered | Mean no. of pupae recovered± SD | Eclosion Success 1st Sampling | Eclosion Success 2nd Sampling | Average Eclosion Success |
|----------------------------------|--------------------------|---------------------|-----------------------|-------------------------------|-----------------------------|-----------------------------|--------------------------|
| 5                                | 5–10                     | 13                  | 10                    | 11.5 ± 1.5                    | 65.6%                       | 60%                         | 62.8%                    |
| 10–15                            | 1                        | 1                   | 1                     | 1 ± 0                         | 65.6%                       | 60%                         | 62.8%                    |
| 15–20                            | 2                        | 2                   | 1                     | 1 ± 1                         | 65.6%                       | 60%                         | 62.8%                    |
| 20–25                            | 2                        | 2                   | 1                     | 1.5 ± 0.5                     | 65.6%                       | 60%                         | 62.8%                    |
| 25–30                            | 2                        | 2                   | 1                     | 1.5 ± 0.5                     | 65.6%                       | 60%                         | 62.8%                    |
| 30–35                            | 4                        | 4                   | 3                     | 3.5 ± 0.5                     | 65.6%                       | 60%                         | 62.8%                    |
| 35–40                            | 2                        | 2                   | 1                     | 1.5 ± 0.5                     | 65.6%                       | 60%                         | 62.8%                    |
| 40–45                            | 1                        | 0                   | 0                     | 0.5 ± 0.5                     | 65.6%                       | 60%                         | 62.8%                    |
| 45–50                            | 1                        | 0                   | 0                     | 0.5 ± 0.5                     | 65.6%                       | 60%                         | 62.8%                    |
| 25                                | 25–30                    | 2                   | 2                     | 2 ± 0                         | 35.7%                       | 42.8%                       | 39.25%                   |
|                                  | 30–35                    | 2                   | 2                     | 2 ± 0                         | 35.7%                       | 42.8%                       | 39.25%                   |
|                                  | 35–40                    | 2                   | 2                     | 2 ± 0                         | 35.7%                       | 42.8%                       | 39.25%                   |
|                                  | 40–45                    | 5                   | 5                     | 5 ± 0                         | 42.8%                       | 25%                         | 33.9%                    |
|                                  | 45–50                    | 5                   | 5                     | 5 ± 0                         | 42.8%                       | 25%                         | 33.9%                    |

Fig. 6. Eclosion success in case of descending vertically dispersed larvae.

Fig. 6. Eclosion success percentage in case of descending vertically dispersed larvae.
Smith, K.G.V., 1986. A Manual of Forensic Entomology. Cornell University Press, Ithaca, p. 475.
Tsitsipis, J.A., Papanicolaou, E.P., 1979. Pupation depth in artificially reared olive fruit flies Dacus oleae (Diptera, Tephritidae), as affected by several physical characteristics of the substrates. Ann. Zool. Ecol. Anim. 11, 31–40.
Tessmer, J.W., Meek, C.L., 1996. Dispersal and distribution of Calliphoridae (Diptera) immatures from animal carcasses in Southern Louisiana. J. Med. Entomol. 33, 665–669.
Ullyett, G.C., 1950. Pupation habits of sheep blow flies in relation to parasitism by Mormoniella vitripennis (Hymenoptera: Pteromalidae). Bull. Entomol. Res. 40, 533–537.
von Zuben, C.J., Bassanezi, R.C., Dos Reis, S.F., Godoy, W.A.C., Von Zuben, F.J., 1996. Theoretical approaches to forensic entomology. 1 Mathematical model of postfeeding larval dispersal. J. Appl. Entomol. 120, 379–382.
Zumpt, F., 1965. Myiasis in man and animals in the Old World. Butterworths, London, p. 267.