forensic sciences

MDPI

Article

The Influence of Substrates on Blow Fly (Diptera: Calliphoridae) Development

Kenya Moore [1], Kelly Bagsby [2,*] and Krystal R. Hans [2]

[1] Lexachrom Analytical Laboratory, 30 S Ocean Ave, Suite 203, Freeport, NY 11520, USA; kenya.moore272@gmail.com
[2] Department of Entomology, Purdue University, 901 W State St., West Lafayette, IN 47907, USA; hans3@purdue.edu
* Correspondence: kellybagsby18@gmail.com

Abstract: Blow flies (Diptera: Calliphoridae) can be used in forensic entomology to provide information, including an estimate of the time of colonization and minimum postmortem interval, based on insect development. This study examined the development of third instar *Calliphora terraenovae* Macquart in four substrates: pine shavings, soil, sand, and clay. Pupation time, survival to adult, and wing vein length were measured to examine the substrate influence. There was a significant difference in the time to pupation ($F_{3,36} = 11.87$, $p < 0.0001$) and the number of flies that eclosed ($F_{3,36} = 4.716$, $p = 0.007$) among the substrates. Blow flies pupated faster in pine shavings and eclosed as adult flies faster in sand than in other substrates. Adults eclosed in the sand in 21.9 days, followed by pine shavings (22.8), clay (24.2), and soil (26.6). Although overall survivorship was low (10–46%), the greatest number of flies eclosed in sand, and the fewest in clay. Understanding the factors that impact blow fly development can help forensic entomologists improve rearing protocols and apply this information to death investigations, especially in cases with buried remains.

Keywords: forensic entomology; *Calliphora terraenovae*; pupation; postmortem interval; burial

Citation: Moore, K.; Bagsby, K.; Hans, K.R. The Influence of Substrates on Blow Fly (Diptera: Calliphoridae) Development. *Forensic Sci.* **2024**, *4*, 409–416. https://doi.org/10.3390/forensicsci4030025

Academic Editor: Matteo Nioi

Received: 16 July 2024
Revised: 10 August 2024
Accepted: 14 August 2024
Published: 23 August 2024

1. Introduction

In forensic entomology, insect evidence can be analyzed and applied to investigations [1]. In the medicolegal forensic entomology subdiscipline, forensic entomologists are asked to examine insect evidence to provide information relating to time of colonization (TOC), movement of the decedent, and the presence of wounds [1–3]. Time of colonization is one of the most important determinations that a forensic entomologist can provide in death investigations. TOC is the period of time between insects depositing eggs and the discovery of the remains [1,4]. To determine TOC, it is imperative to understand the life cycle of the blow fly. There are four main life stages: egg, larva, pupae, and adult [5–7]. The blow fly life cycle begins with an adult fly laying eggs. Those eggs hatch into larvae, the larvae consume food until they pupate, and during pupation the larvae metamorphosize into adult flies and eclose from the pupal casing [8].

Development data is derived from studying these stages at different temperatures [9]. Understanding the duration of each development stage in a laboratory setting can be useful when applying this information to insects recovered from death investigations where the temperature of the development at the scene is used to calculate the TOC based on the known development rates. Many environmental variables have been studied to understand how they affect development including the food source [10–12], photoperiod [13,14], interspecies competition [15–17], and most importantly temperature [9–11,16,18,19]. Insects are poikilothermic, which means that their development is highly dependent on the ambient temperature of their environment; the warmer the temperature, the faster development can occur [13].

Remains can be found in a variety of conditions and habitats, and understanding how environmental conditions affect the dispersion and pupariation phase is crucial for death investigations. Many factors affect this dispersion phase and subsequent pupation including moisture, temperature, interspecies interactions, and substrate [20,21]. Substrate type can affect dispersion and pupation. A study found that decayed fruits and vegetables and fresh leaves were the most favorable, whereas folded clothes were the least favorable [6]. Studies have shown that pupation tends to occur close to the larvae's food source but they can disperse 0.8–10 m away and bury themselves at a depth of 2.5–10 cm, depending on the species [6,21–24]. Substrate compaction is a significant factor in the ability of the post-feeding larvae to bury themselves to pupate [25]. Vertical dispersion, the process when a larvae burrows into the soil to pupate, can be affected by soil compaction [25,26]. A 2010 study described how the depth of pupation was inversely related to the degree of compaction, with larvae burrowing only 0.5 cm into highly compacted soil treatment [25]. Soil compaction can affect gas exchange and temperature which affects the development of the larvae [25].

Calliphora terraenovae Macquart is a species that has been found to be forensically relevant, but there is limited research regarding its development [27,28]. A 1958 study demonstrated that in *Calliphora terraenovae* development, the time from oviposition to eclosion as an adult took longer than that of other forensically relevant blow fly species [29]. Research has demonstrated that *C. terraenovae* has long pupation periods [29]. Kamal (1958) analyzed thirteen species of flies in the Calliphoridae and Sarcophagidae families: three of those species being of the *Calliphora* genus [29]. The three *Calliphora* species had the longest prepupal period, with *Calliphora vomitoria* Linnaeus taking a maximum of 21 days to complete and *C. terraenovae* taking a maximum of 17 days to complete. Comparatively to other forensically relevant species, such as *Phormia regina* Meigen and *Lucilia sericata* Meigen, the prepupal period took a maximum of 7 and 8 days, respectively. The entire immature lifespan of the three *Calliphora* species was also considerably longer than that of *P. regina* and *L. sericata*. *Calliphora terraenovae*, *Calliphora vicina* Robineau-Desvoidy, and *C. vomitoria* took a maximum of 23, 25, and 27 days, respectively, to complete their development. *P. regina* and *L. sericata* took a maximum of 12 and 15 days, respectively, to develop from egg to adult. Understanding the factors that affect dispersion and pupation can improve collection techniques at scenes [30].

The purpose of this study was to examine the development of *C. terraenovae* in four different substrates. Time to pupation, time to eclosion, survival to adult, and final adult size were observed and measured. We expected that clay and sand substrates would result in soil compaction, making blow fly pupation and eclosion more difficult.

2. Materials and Methods

The blow flies used in this study were first generation and originated from wild caught flies collected in 2017. They were housed in colonies at Delaware State University in Dover, Delaware. Each colony was kept in an aluminum cage (46 cm^3, BioQuip 1450C, BioQuip Products, Inc., Rancho Dominquez, CA, USA.), and provided with water, sugar and pork liver to promote ovarian development. Pork liver was used as a substrate for oviposition and egg masses were removed from the cage, placed onto a fresh piece of pork liver and contained inside of a glass mason jar with pine shavings. The mean temperature of the laboratory was 18.3 $\pm$ 0.05 °C with 33.48 $\pm$ 0.50% relative humidity during this study. Five third-instar post-feeding *Calliphora terraenovae* larvae were randomly selected from the colonies and placed in an experimental unit, which consisted of a glass mason jar (473 mL) filled with 275 mL of each of the experimental substrates, topped with landscape tarp and a metal ring in order to contain the larvae. Only five maggots were selected to eliminate other factors that could influence development, such as the density of individuals. This set up was replicated ten times for each of the four substrates: sand, soil, clay, and pine shavings (control). Each substrate was purchased for this study. The sand used in the study was premium play sand manufactured by Quikrete (Atlanta, GA, USA), the soil was

topsoil that did not contain any fertilizers or other additives, the clay was a clay burrowing substrate used in reptile tanks, and pine shavings were pine pet bedding shavings.

All experimental units were observed every 24 h to document the time of pupation and eclosion, for a total of 31 days. Once flies had eclosed and expired, they were removed, and the left wing of each adult was detached to measure the posterior cross-vein (dm-cu) length. Temperature and humidity were recorded hourly for the duration of the study (HOBO UX100-11A, Onset Computer Corporation, Bourne, MA, USA).

Statistical Analysis

Percentage eclosion data were square root transformed in order to meet the assumptions of homogeneity. Time to pupation, time to eclosion, and wing vein length were normally distributed.

Data for time to pupation, percentage of flies eclosed, time to eclosion, and wing vein length were analyzed using a one-way ANOVA (aov function) to examine the effect of substrate. All significant results were followed by pairwise comparisons to determine which substrates had an effect. All analyses were performed in GraphPad Prism version 10.2.3 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com) and R 4.2.1 (R Project for Statistical Computer, http://www.R-project.org (accessed on 17 June 2024) [31,32].

3. Results

Development to adult was completed within 30 days for all treatments. Pupation occurred within 13 days, with pupation occurring more quickly in the pine shavings and slowest in the clay (Figure 1; $F_{3,36} = 11.87$, $p < 0.0001$). After pupation, the blow flies began to eclose within 30 days. Eclosion was significantly different among substrates and occurred fastest in pine shavings (Figure 2; H(3) = 14.65, $p = 0.002$). Pairwise comparisons using Dunn's test indicated that sand substrates resulted in significantly faster development when compared to soil ($p = 0.001$). The percentage of eclosion was also high in the sand substrate (Figure 3; $F_{3,36} = 4.72$, $p = 0.007$). Out of the four substrates, the blow flies in the sand substrate were larger than those in any other substrate (Figure 4; $F_{3,40} = 4.03$, $p = 0.01$).

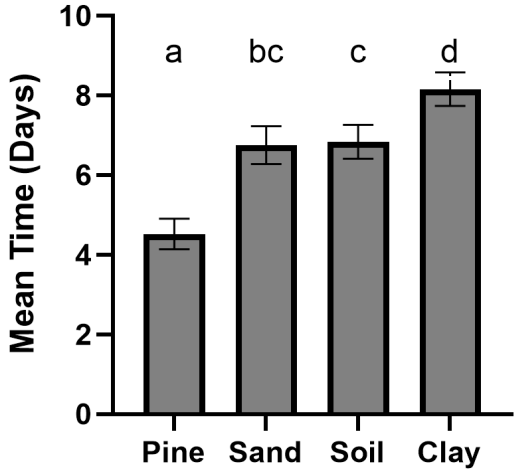

Figure 1. Mean time in days (±SE) to pupation in each substrate. There was a significant effect of substrate on pupation time ($F_{3,36} = 11.87$, $p < 0.0001$). Pupation in pine shavings occurred quickly, while pupation in clay occurred the slowest. Means with the same letter are not significantly different. **a**: Pine shavings are significantly different from sand ($p = 0.009$), soil ($p = 0.006$), and clay ($p < 0.001$); **bc** = Sand is significantly different from pine shavings ($p = 0.009$) and clay ($p = 0.0292$); **c**: Soil is significantly different than pine shavings ($p = 0.006$) and clay ($p = 0.0420$); **d** = Clay is significantly different than pine shavings ($p = <0.001$), sand ($p = 0.292$), and soil ($p = 0.0420$).

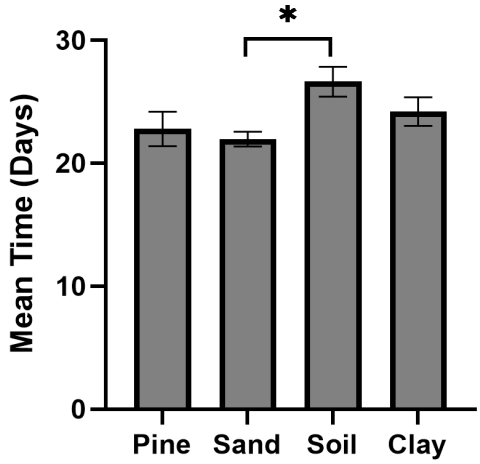

Figure 2. Mean time in days (±SE) to eclosion in each substrate. There was a significant effect of substrate on time to eclosion (H(3) = 14.65, $p = 0.002$). Pairwise comparisons of substrates containing an asterisk (*) above indicate a significant difference with $p < 0.01$.

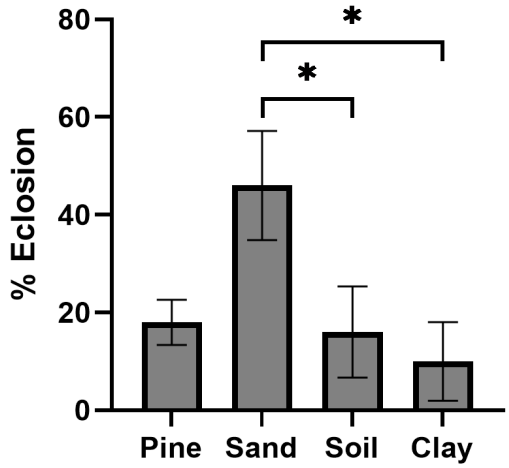

Figure 3. Percentage of flies that eclosed in each substrate. There was a significant effect of substrate on the percentage of flies eclosed ($F_{3,36} = 4.72$, $p = 0.007$). Pairwise comparisons of substrates containing an asterisk (*) above indicate a significant difference with $p < 0.05$.

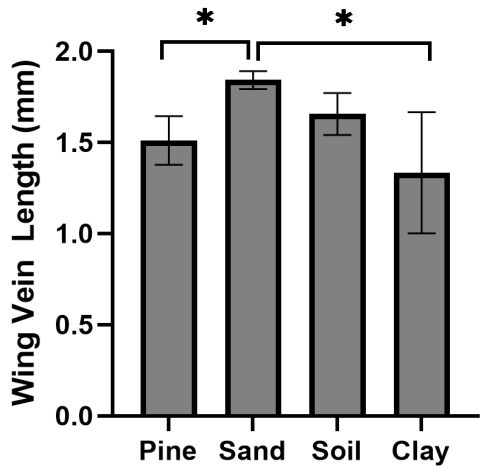

Figure 4. Mean wing vein length in mm (±SE) of eclosed flies in each substrate ($F_{3,36} = 4.03$, $p = 0.01$). The flies that eclosed from the sand were the largest, whereas flies from the clay were the smallest. Pairwise comparisons of substrates containing an asterisk (*) above indicate a significant difference with $p < 0.05$.

4. Discussion

Insects play a crucial role in determining TOC estimates. Collection of the oldest insects at a scene is pertinent in order to calculate an estimate of how long the remains have been available for colonization. In certain circumstances, the oldest insects may be overlooked because they have dispersed from the remains and have begun to burrow into the surrounding substrate to pupate. If the oldest insects are not collected from a scene, there is a risk of underestimating the time of colonization.

Previous research has analyzed the horizontal and vertical dispersal behavior of various blow flies. Depending on the species, they dispersed different lengths away from the food source. *Phormia regina* has been documented to have the shortest horizontal dispersal and pupate near the food source, but *L. sericata* and *C. vicina* can disperse further to pupate from 3 m to 8.1 m away [26,30]. *Phormia regina* and *L. sericata* also burrow into the soil to pupate at different distances as well. *Phormia regina* often disperses to pupate on the surface and at shallower depths such as up to 2 cm, whereas *L. sericata* can burrow down to 11 cm [30,33]. A study analyzing larval survival after burial of *Cochliomyia macellaria* Fabricius and *Protophormia terraenovae* Robineau-Desvoidy determined that second instar larvae, third instar larvae, and pupae were able to eclose as adults after being buried in clay soil up to 50 cm deep [26]. Burial did affect survival with fewer adults reaching the surface when buried at a depth of 50 cm. Other studies have analyzed the effect of soil type and moisture content [7]. These two abiotic factors affect development time, dispersal, and the survival of larvae and pupae. Clay soil with a high moisture content resulted in longer development periods and low survival. These differences among species show the importance of analyzing the behavior of each species in a variety of environmental conditions.

In this study, substrate type was shown to affect pupation, eclosion, survival to adult, and adult size. Pupation was impacted by substrate, except between sand and soil. Pupation in pine shavings, the standard substrate used in laboratories, took the shortest amount of time (4.5 $\pm$ 0.4 d). Pine shavings are a dry substrate with less compaction. This substrate provides ample room between each pine shaving particle for adequate oxygen flow. Clay, on the other hand, took the longest amount of time for pupation to complete (8.1 $\pm$ 0.4 d). This finding is consistent with previous studies that have analyzed clay soils. Clay soils tend to retain more moisture and have small particles measuring smaller than 0.002 mm [34,35]. The higher moisture content and compaction between individual particles can hinder the larvae's ability to disperse and survive [25]. All replicates in this study were able to disperse as third instars and pupate, but not all survived to become adults.

Eclosion was also impacted by substrate. In sand, there was a significant difference regarding the time it took for eclosion to occur than eclosion in soil. The flies that survived eclosed after 21.9 $\pm$ 0.6 days in sand, 22.8 $\pm$ 1.4 days in pine shavings, 24.2 $\pm$ 1.2 days in clay, and 26.6 $\pm$ 1.2 days in soil. These findings are consistent with other development studies [29]. The development data from Kamal (1958) on three different *Calliphora* species, including *C. terranovae*, demonstrated that the time to disperse as third instars and pupate took as little as 12 days and at most 39 days. Because there is limited data regarding *C. terranovae*, it is important to understand how other *Calliphora* species develop as well. *Calliphora vomitoria* took between 21 and 39 days to complete the prepupal and pupae stages. *Calliphora vicina* took between 12 and 27 days. *Calliphora terranovae* took the longest of the three species at 24 and 35 days. Eclosion is a process where the teneral adult fly begins to emerge from the pupal casing [36]. The adult inflates a structure between the eyes called the ptilinum which pushes on the pupal casing causing it to open [36]. As eclosion of the adult flies occurred fastest in sand but slowest in soil, it could have been impacted by the particle size. Sand has larger particles measuring between 0.05 mm and 2 mm, which would allow more soil aeriation and less soil compaction [7,34]. The soil used in this study was topsoil, which contains a combination of sand, clay, and other organic materials. Due to the mixture of different soil types, this would reduce soil aeration and have a higher degree of compaction. As there was a drier environment due to the soil aeration in the sand and less compaction, the eclosion process occurred more readily in sand than in soil. With

more data collected regarding the life histories of *C. terranovae*, more accurate development data can be used for this species.

There was a significant difference among the substrates regarding survival. The blow flies in the sand had the highest survivability, with 46 ± 11.2% of the flies eclosing as adults. Sand does not retain as much moisture as clay or soil due to the larger particle sizes and increased soil aeriation [34]. The other substrates—clay, soil, and pine shavings—had lower rates of survivability, with only 10–18% eclosing. Pupae require environmental conditions that allow them to breathe as they complete their metamorphosis [37,38]. Lower rates of adult eclosion in the clay substrate were not a surprising result, because clay has a higher moisture content, higher degree of compaction, and small particle sizes. These conditions result in lower oxygen concentrations and reduce the ability of the pupae to exchange gas. Lower rates of adult eclosion in pine shavings was a surprising result because of the low moisture content, lesser degree of compaction, and larger particle size.

The type of substrate impacted the overall size of the adult blow flies, as assessed using the wing vein length. Of the adults that survived, clay resulted in the smallest adults and sand resulted in the largest adults. Previous research on *C. vicina* showed that the wing veins of larger adults measured 1.85 mm and smaller adult blow flies measured 1.67 mm [39]. There is little data available regarding the wing lengths of *C. terraenovae* and differences between species should be expected, but the findings in this study are in line with other species of the same genus. Abiotic factors could affect the size of the adult blow fly. Other abiotic factors tested in this study, including moisture content, particle size, or soil aeration of the substrates could have affected the size of the adults. Further research should assess these abiotic factors in different substrates to determine if they have a significant effect on body size.

5. Conclusions

Substrate type affects pupation, eclosion, survival to adult, and adult size. Pine shavings resulted in larvae pupating quickly. Sand resulted in the highest number of adult flies that eclosed from their pupal casings. The adult flies that eclosed from the sand were also measured to be the largest flies out of all four substrates. Larvae that were in the soil substrate also took longer to pupate and eclose but were measured to be second largest. Clay resulted in longer pupation and time to eclosion in addition to a smaller body size. Future studies should analyze the characteristics of the substrate that may affect the rate of pupation and eclosion in addition to survival. Dispersal behavior is important to recognize because collecting the oldest specimens at a scene is imperative for accurate time-of-colonization estimates. Understanding how substrates affect pupation, eclosion, and development aids in time-of-colonization estimates for the forensic entomologist.

Author Contributions: Conceptualization, K.R.H. and K.M.; methodology, K.R.H. and K.M.; formal analysis, K.B. and K.R.H.; investigation, K.M.; resources, K.R.H.; data curation, K.R.H.; writing—original draft preparation, K.M.; writing—review and editing, K.B. and K.R.H.; visualization, K.M. and K.B.; supervision, K.R.H.; project administration, K.R.H.; funding acquisition, K.R.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Department of Biological Sciences, Delaware State University.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data can be accessed at the following link: 10.6084/m9.figshare.26114134.

Acknowledgments: The authors would like to thank the Hans Lab members for their assistance with this research.

Conflicts of Interest: Author Kenya Moore is employed by the Lexachrom Analytical Laboratory. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Lutz, L.; Zehner, R.; Verhoff, M.A.; Bratzke, H.; Amendt, J. It Is All about the Insects: A Retrospective on 20 Years of Forensic Entomology Highlights the Importance of Insects in Legal Investigations. *Int. J. Leg. Med.* **2021**, *135*, 2637–2651. [CrossRef] [PubMed]
2. Weidner, L.M.; Byrd, J.; Sutton, L.; Hans, K.R. Forensic Entomology: An Introduction to the Literature of the United States. *WIREs Forensic Sci.* **2021**, *3*, e1435. [CrossRef]
3. Kotzé, Z.; Aimar, S.; Amendt, J.; Anderson, G.S.; Bourguignon, L.; Hall, M.J.R.; Tomberlin, J.K. The Forensic Entomology Case Report—A Global Perspective. *Insects* **2021**, *12*, 283. [CrossRef] [PubMed]
4. Weidner, L.M.; Nigoghosian, G.; Yoho, K.C.; Parrott, J.J.; Hans, K.R. An Analysis of Forensically Important Insects Associated with Human Remains in Indiana, U.S.A. *J. Forensic Sci.* **2020**, *65*, 2036–2041. [CrossRef]
5. Brundage, A. General Entomology and Basic Arthropod Biology. In *Forensic Entomology: The Utility of Arthropods in Legal Investigations*; Byrd, J.H., Tomberlin, J.K., Eds.; CRC Press: Boca Raton, FL, USA, 2020; pp. 1–14. [CrossRef]
6. Ghafoor, A.; Akbar, M.; Ansar, M.; Amjad, I.; Khan, M.S.; Ashraf, M. Morning Responses of Calliphorid and Sarcophagid Prepupae towards Different Pupation Materials. *J. Anim. Plant Sci.* **2010**, *20*, 281–285.
7. Kökdener, M.; Şahin Yurtgan, M. The Effect of Soil Type and Moisture Level on the Development of *Lucilia sericata* (Diptera: Calliphoridae). *J. Med. Entomol.* **2022**, *59*, 508–513. [CrossRef]
8. Tarone, A.; Benoit, J. Insect Development as It Relates to Forensic Entomology. In *Forensic Entomology: The Utility of Arthropods in Legal Investigations*; Byrd, J.H., Tomberlin, J.K., Eds.; CRC Press: Boca Raton, FL, USA, 2020; pp. 226–245. [CrossRef]
9. Nabity, P.D.; Higley, L.G.; Heng-Moss, T.M. Effects of Temperature on Development of *Phormia regina* (Diptera: Calliphoridae) and Use of Developmental Data in Determining Time Intervals in Forensic Entomology. *J. Med. Entomol.* **2006**, *43*, 1276–1286. [CrossRef] [PubMed]
10. Boatright, S.A.; Tomberlin, J.K. Effects of Temperature and Tissue Type on the Development of *Cochliomyia macellaria* (Diptera: Calliphoridae). *J. Med. Entomol.* **2010**, *47*, 917–923. [CrossRef]
11. Bambaradeniya, Y.T.B.; Karunaratne, W.A.I.P.; Tomberlin, J.K.; Goonerathne, I.; Kotakadeniya, R.B.; Magni, P.A. Effect of Temperature and Tissue Type on the Development of the Forensic Fly *Chrysomya megacephala* (Diptera: Calliphoridae). *J. Med. Entomol.* **2019**, *56*, 1571–1581. [CrossRef]
12. Clark, K.; Evans, L.; Wall, R. Growth Rates of the Blowfly, *Lucilia sericata*, on Different Body Tissues. *Forensic Sci. Int.* **2006**, *156*, 145–149. [CrossRef]
13. Bauer, A.M.; Bauer, A.; Tomberlin, J.K. Effects of Photoperiod on the Development of Forensically Important Blow Fly *Chrysomya rufifacies* (Diptera: Calliphoridae). *J. Med. Entomol.* **2020**, *57*, 1382–1389. [CrossRef] [PubMed]
14. Nabity, P.D.; Higley, L.G.; Heng-Moss, T.M. Light-Induced Variability in Development of Forensically Important Blow Fly *Phormia regina* (Diptera: Calliphoridae). *J. Med. Entomol.* **2007**, *44*, 351–358. [CrossRef] [PubMed]
15. Hanski, I. Carrion Fly Community Dynamics: Patchiness, Seasonality and Coexistence. *Ecol. Entomol.* **1987**, *12*, 257–266. [CrossRef]
16. Okpara, P.; VanLaerhoven, S. Density, Temperature, and Comingled Species Affect Fitness within Carrion Communities: Coexistence in *Phormia regina* and *Lucilia sericata* (Diptera: Calliphoridae). *Insects* **2023**, *14*, 139. [CrossRef]
17. MacInnis, A.E.; Higley, L.G. Competition among Three Forensically Important Blow Fly Species (Diptera: Calliphoridae): *Phormia regina*, *Lucilia sericata*, and *Chrysomya rufifacies*. *Environ. Entomol.* **2020**, *49*, 1473–1479. [CrossRef]
18. Byrd, J.H.; Butler, J.F. Effects of Temperature on *Cochliomyia macellaria* (Diptera: Calliphoridae) Development. *J. Med. Entomol.* **1996**, *33*, 901–905. [CrossRef]
19. Weatherbee, C.R.; Pechal, J.L.; Stamper, T.; Benbow, M.E. Post-Colonization Interval Estimates Using Multi-Species Calliphoridae Larval Masses and Spatially Distinct Temperature Data Sets: A Case Study. *Insects* **2017**, *8*, 40. [CrossRef]
20. Turpin, C.; Kyle, C.; Beresford, D.V. Postfeeding Larval Dispersal Behavior of Late Season Blow Flies (Calliphoridae) in Southern Ontario, Canada. *J. Forensic Sci.* **2014**, *59*, 1295–1302. [CrossRef]
21. Reigada, C.; Godoy, W.A.C. Dispersal and Predation Behavior in Larvae of *Chrysomya albiceps* and *Chrysomya megacephala* (Diptera: Calliphoridae). *J. Insect Behav.* **2005**, *18*, 543–555. [CrossRef]
22. Gomes, L.; Godoy, W.A.C.; Von Zuben, C.J. A Review of Postfeeding Larval Dispersal in Blowflies: Implications for Forensic Entomology. *Naturwissenschaften* **2006**, *93*, 207–215. [CrossRef]
23. Lima, E.A.B.F.; Ferreira, C.P.; Bernardes, A.M.; Godoy, W.A.C. Neighborhood Interactions and Larval Dispersal Behavior in Blowflies. *J. Insect Behav.* **2009**, *22*, 245–255. [CrossRef]
24. Tessmer, J.W.; Meek, C.L. Dispersal and Distribution of Calliphoridae (Diptera) Immatures from Animal Carcasses in Southern Louisiana. *J. Med. Entomol.* **1996**, *33*, 665–669. [CrossRef] [PubMed]
25. Cammack, J.A.; Adler, P.H.; Tomberlin, J.K.; Arai, Y.; Bridges, W.C. Influence of Parasitism and Soil Compaction on Pupation of the Green Bottle Fly, *Lucilia sericata*. *Entomol. Exp. Appl.* **2010**, *136*, 134–141. [CrossRef]
26. Balme, G.R.; Denning, S.S.; Cammack, J.A.; Watson, D.W. Blow Flies (Diptera: Calliphoridae) Survive Burial: Evidence of Ascending Vertical Dispersal. *Forensic Sci. Int.* **2012**, *216*, e1–e4. [CrossRef]
27. Dicke, R.; Eastwood, J. The Seasonal Incidence of Blowflies at Madison, Wisconsin (Diptera-Calliphoridae). Transactions of the Wisconsin Academy of Sciences, Arts and Letters. Available online: https://digital.library.wisc.edu/1711.dl/5JSPLWNXCEESJ8W (accessed on 5 May 2024).

28. Williams, R.W. Studies on the Filth Flies at the University of Michigan Biological Station, Douglas Lake, Michigan-Summer 1954. *Am. Midl. Nat.* **1956**, *55*, 126. [CrossRef]
29. Kamal, A.S. Comparative Study of Thirteen Species of Sarcosaprophagous Calliphoridae and Sarcophagidae (Diptera)1 I. Bionomics. *Ann. Entomol. Soc. Am.* **1958**, *51*, 261–271. [CrossRef]
30. Greenberg, B. Behavior of Postfeeding Larvae of Some Calliphoridae and a Muscid (Diptera). *Ann. Entomol. Soc. Am.* **1990**, *83*, 1210–1214. [CrossRef]
31. Software Mackiev. *GraphPad Prism*; Graphpad Software LLC: San Diego, CA, USA, 12 January 2024. Available online: https://www.graphpad.com/features (accessed on 11 January 2024).
32. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 12 January 2024. Available online: http://www.R-project.org/ (accessed on 11 January 2024).
33. Ullyett, G.C. Pupation Habits of Sheep Blowflies in Relation to Parasitism by *Mormoniella Vitripennis*, Wlk. (Hym., Pteromalid.). *Bull. Entomol. Res.* **1950**, *40*, 533–537. [CrossRef]
34. Kovačević, M.; Bajat, B.; Gajić, B. Soil Type Classification and Estimation of Soil Properties Using Support Vector Machines. *Geoderma* **2010**, *154*, 340–347. [CrossRef]
35. S.U., S.L.; Singh, D.N.; Shojaei Baghini, M. A Critical Review of Soil Moisture Measurement. *Measurement* **2014**, *54*, 92–105. [CrossRef]
36. Martín-Vega, D.; Simonsen, T.J.; Hall, M.J.R. Looking into the Puparium: Micro-CT Visualization of the Internal Morphological Changes during Metamorphosis of the Blow Fly, *Calliphora vicina*, with the First Quantitative Analysis of Organ Development in Cyclorrhaphous Dipterans. *J. Morphol.* **2017**, *278*, 629–651. [CrossRef] [PubMed]
37. Sert, O.; Ergil, C. A Study on the Pupal Development of *Calliphora vomitoria* (L., 1758) (Calliphoridae: Diptera) at Different Temperatures. *Turk. J. Zool.* **2021**, *45*, 277–295. [CrossRef]
38. Houlihan, D.F. Some Effects of Low Oxygen Partial Pressures on the Development of *Calliphora vomitoria*. *J. Insect Physiol.* **1974**, *20*, 1367–1387. [CrossRef]
39. Hwang, C.C.; Turner, B.D. Small-scaled Geographical Variation in Life-history Traits of the Blowfly *Calliphora vicina* between Rural and Urban Populations. *Entomol. Exp. Appl.* **2009**, *132*, 218–224. [CrossRef]