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Influence of parasitism and soil compaction on pupation of the green bottle fly, *Lucilia sericata*

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Abstract

The influence of parasitoids and soil compaction on pupation behavior of blow flies was examined in a host–parasitoid system involving *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) and *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). Larvae of *L. sericata* were introduced to containers with soil of different compaction levels, with or without parasitoids. Although females of *N. vitripennis* did not significantly affect the pupation depth of *L. sericata*, they increased the rate of pupal development by 15.0–23.7 h at 28.4 ± 1.2 °C, and increased the clumping of puparia. Pupation depth of *L. sericata* was negatively related to soil compaction; mean depth of pupation was 4.4 cm in uncompacted soil and 0.5 cm in high-compaction soil. In high-compaction soil, pupal development increased by 10.5–18.8 h at 25.2 ± 0.3 °C, and puparia were clumped. These results provide a framework for locating puparia in forensic investigations and releasing appropriate parasitoids for biological control of blow flies.

Introduction

Larval blow flies (Diptera: Calliphoridae) that have developed on decaying organic matter leave the food source near the end of the third instar to pupate on or in the surrounding soil (Norris, 1959; Greenberg, 1990). Most studies of the wandering phase and subsequent pupation behavior have investigated the distance and direction that larvae disperse from the food source but not how deep they pupate in the soil (Gomes et al., 2006).

The sedentary pupal stage occupies approximately half of the total duration of blow fly development, and many organisms (e.g., parasitic Hymenoptera) have evolved to exploit this life stage (Greenberg & Kunich, 2002). In South Carolina (USA), nine species in five families of parasitic Hymenoptera parasitize blow flies (Payne & Mason, 1971). In South Korea, four species of Pteromalidae attack puparia of *Chrysomya megacephala* (Fabricius) and two species parasitize puparia of the green bottle fly, *Lucilia sericata* (Meigen) (both Diptera: Calliphoridae) (Rueda et al., 1997). In Malaysia, four species of Pteromalidae parasitize puparia of *C. megacephala* in poultry houses and refuse dumps (Sulaiman et al., 1990). Throughout the world, multiple species of hymenopteran parasitoids have been imported, mass-reared, and released as biological control agents of blow flies. The most notable of these programs involved multiple species of Hymenoptera to control blow flies responsible for sheep strike (myiasis) in Australia, New Zealand, and the UK (Tillyard & Seddon, 1933; Davies, 1934; Bishop et al., 1996).

Lucilia sericata is a cosmopolitan species of medical (Merritt, 1969; Sherman et al., 2000; Nelder et al., 2008), veterinary (Tillyard & Seddon, 1933), and forensic importance (Catts & Haskell, 1990). Because it is a pest of livestock, *L. sericata* has been controlled with pesticides and biological control agents such as *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). To date, little research has focused on the pupation behavior

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of *L. sericata* (Greenberg, 1990; Tessmer & Meek, 1996), and no study has examined the influence of soil compaction on this behavior. Only one study has mentioned the pupation behavior of *L. sericata* with respect to parasitism by *N. vitripennis* (Ullyett, 1950). Knowledge of pupation behavior and the effects of parasitism on pupation are critical for controlling blow flies and locating them in forensic investigations. To provide an accurate estimate of the period of insect activity, the oldest insects that developed on a body must be located, including those that have left the remains and pupated in the surrounding soil.

Our objectives were to investigate the effects of soil compaction on the pupation behavior of *L. sericata*, with and without parasitoids (*N. vitripennis*), and develop a predictive model for the pupation depth of *L. sericata*. The following research hypotheses were tested: (1) larvae of *L. sericata* pupate deeper into soil in the presence of the pupal parasitoid *N. vitripennis*, (2) larvae of *L. sericata* pupate deeper in less compacted soil, (3) soil compaction influences the rate of fly development, and (4) soil compaction influences the spatial distribution of puparia of *L. sericata* in soil.

Materials and methods

Source of insects

A colony of *L. sericata* was initiated with approximately 100 puparia from a colony in its sixth generation, maintained by JD Wells at West Virginia University. Flies were wild-caught in June 2007 in Morgantown, WV, USA (39°38′N, 79°57′W). A colony of *N. vitripennis* was initiated with 12 males and 54 females that emerged on 15–17 December 2007 from puparia of *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) collected in Clemson (SC, USA), on 27 November 2007 (Cammack & Nelder, 2010). Voucher specimens of *L. sericata* and *N. vitripennis* were deposited in the Clemson University Arthropod Collection.

Insect rearing

Each species was reared in separate environmental rooms maintained at 24.1 ± 0.05 °C (range: 17.4–30.1 °C), approximately 60% r.h., and L16:D8 photoperiod (Grassberger & Reiter, 2001; Nabity et al., 2006). Adult flies were provided ad libitum with granulated sugar, powdered milk, and distilled water. Larvae of *L. sericata* were reared on beef liver on which the eggs were deposited (Byrd & Castner, 2000). Wasps were provided with distilled water and a supersaturated solution of distilled water, sugar, and honey. The colony was maintained on puparia from the colony of *L. sericata* (Smith, 1969).

Soil collection and preparation

Soil for experiments was collected in Clemson (34°40′N, 82°50′W) and was Ap horizon Cecil Sandy Loam (Taxonomic class Fine, kaolinitic, thermic, Typic Kanhapludult), with pH 5.5. This soil series (Cecil) is found throughout the piedmont region from Virginia to Alabama, USA, covering more than 4.05 million ha (National Cooperative Soil Survey, 2007). Soil aggregates were broken up and poured through a 3.0-mm screen. Once sieved, the soil was stirred daily and air dried. Six to sixteen hours before each experiment, the soil was rehydrated with distilled water to 16% moisture by weight and stored overnight to allow even dispersal of the water throughout the soil. The soil was sieved again with a 3.0-mm screen to break up clumps that formed when the soil was rehydrated.

Experiment designs

Experiments were performed at 25.2 ± 0.3 °C (range: 20.6–27.0 °C), approximately 60% r.h., and L16:D8 photoperiod.

Effect of *Nasonia vitripennis* on pupation behavior of *Lucilia sericata*

Fifty 1.4-l plastic containers with screened lids were used in five treatments (10 containers per soil treatment). The treatments were uncompacted soil (0.016 ± 0.0005 kg cm^{-2}), uncompacted soil (0.0183 ± 0.001) + *Nasonia*, high-compaction soil (4.26 ± 0.06), high-compaction soil (4.38 ± 0.05) + *Nasonia*, and no soil or *Nasonia*. For the uncompacted treatments, 1.1 l of soil was poured into the containers to a level of 11 cm; larvae of *L. sericata* did not pupate deeper than 11 cm in preliminary experiments. In the high-compaction treatments, 1.5 l of soil was compacted to a level of 11 cm, using wooden tamps and a rubber mallet. Five post-feeding larvae of *L. sericata* were placed on the soil (or container bottom in the no-soil treatment) in the center of each container. Parasitism rate of *Calliphora* species by *N. vitripennis* begins to level off when host density reaches 25 puparia per 484 cm^2 (Jones & Turner, 1987), which is equivalent to five puparia per 105 cm^2 in our arenas. For the two parasitoid treatments, five females of *N. vitripennis* were added to each container after introduction of larvae of *L. sericata*. Five females of *N. vitripennis* were used to increase the likelihood of a parasitoid contacting a host puparium. The containers were randomized on shelves in the rearing room. A 1-dram (3.7 ml) vial filled with a supersaturated sugar solution stoppered with cotton was placed on the lids of the 20 containers with parasitoids and refilled ad libitum. Each container was considered an experimental unit, with 10 experimental units (or replications) per treatment.

The experiment was repeated, and an additional treatment was added: no soil + *Nasonia*. Ten 1.4-l plastic

containers were used for this treatment, bringing the total number of containers (replications) in this repetition to 60.

Effect of soil compaction on pupation behavior of *Lucilia sericata*

To model the response of pupation depth of *L. sericata* to soil compaction, five levels of compaction were used, with five containers (replications) per level. Containers of soil with screened lids were prepared in a manner similar to those in the previous experiment. Thirty 1.4-l plastic containers were used for six treatments (five per soil treatment and five per no-soil treatment). The soil compaction treatments were uncompacted (0.019 ± 0.00 kg cm^{-2}), medium-low (0.46 ± 0.05), medium (0.95 ± 0.14), medium-high (2.49 ± 0.12), and high (4.47 ± 0.01). For the uncompacted treatment, 1.1 l of soil was poured into the containers to a depth of 11 cm. For the remaining compaction treatments, tamps were used to compact soil to a depth of 11 cm. Five empty containers were used as a no-soil treatment. Five post-feeding larvae of *L. sericata* were placed on the soil (or container bottom in the no-soil treatment) in the center of each container. The containers were randomized on shelves in the rearing room.

This experiment was repeated in the same manner, with one exception. Five additional containers were used to determine the effect of soil compaction on soil temperature and the resulting effect on development of *L. sericata*. HOBO® Water/Soil Temperature sensors and U12 4-External Channel Data Loggers (Onset Computer Corporation, Bourne, MA, USA) were used to record the soil temperature at the mean depth of pupation for each compaction treatment (determined from repetition one of this experiment).

An experiment with five compaction levels but no larvae was run for 12 days, under similar environmental conditions as before, to examine the change in soil moisture at the mean depth of pupation. Readings of soil moisture were taken every 3 days (four or five total readings) for each compaction treatment. For each reading at each level of compaction, approximately 15 g of soil was collected at the mean depth of pupation and oven dried at 105 °C to determine the percentage moisture.

Data collection and analysis

Adult emergence of *L. sericata* and *N. vitripennis* was recorded for each experiment at intervals of 8 h or less during daylight in the rearing room. An experiment ended when no adults had emerged after 2 days. Compaction of the soil in each container was measured using a pocket penetrometer (Lab Safety Supply, Janesville, WI, USA). A plastic spoon was used to remove soil in a radial fashion around the container at a depth of approximately 0.25 cm per rotation. When a puparium was located, its depth and orientation (horizontal or vertical) were recorded, and for all but the first experiment, the horizontal location in the container was determined using a piece of plexiglass divided into 1×1 cm squares, and recorded on scaled data-recording sheets.

All statistical analyses were performed using the Statistical Analysis Software (SAS) package (SAS Institute, Cary, NC, USA). ANOVA followed by Fisher's Least Significant Difference test (Proc GLM, GLIMMIX) was used in all analyses. All tests were based on $\alpha = 0.05$ to reduce the probability of a type I error. The models used for the ANOVA and regression analysis had error terms associated with the repetition to repetition variation as well as the replicate to replicate variation. Attention was paid to ensuring that the correct error term was used for testing the treatment and/or time effects. The no-parasitoid treatments (uncompacted, high compaction, and no-soil control) from the first experiment were included in the analyses of the effect of soil compaction on pupation behavior of *L. sericata*. A regression analysis (Proc GLM) was used to determine the relationship between soil compaction and pupation depth of larvae. To ensure that the data met necessary assumptions for valid estimation and significance testing, the regression analysis was performed on the log (pupation depth + 1). To determine the effect of females of *N. vitripennis* and soil compaction on spatial distribution of puparia in the soil, the mean pairwise distance between all puparia in a container was calculated using the actual depth of pupation (cm) and the x–y coordinates measured on scaled data-recording sheets (x-coordinate = distance from left to right on scaled data-recording sheet, and y-coordinate = distance from top to bottom on scaled data-recording sheet).

Results

Effect of *Nasonia vitripennis* on pupation behavior of *Lucilia sericata*

No significant effect on pupation depth was observed in uncompacted soil ($F_{1,19} = 0.5$, $P = 0.70$) or soil of high compaction ($F_{1,18} = 0.36$, $P = 0.56$) when females of *N. vitripennis* were present (Table 1). Parasitism was 98% in the no soil + *Nasonia* control, and 0% for all treatments except the high compaction in repetition two (10%). All puparia parasitized by *N. vitripennis* were at least partially exposed above the soil surface. Mean mortality in control treatments was 3 ± 0.003%.

In uncompacted treatments, no significant difference ($F_{1,18} = 1.10$, $P = 0.31$) in the mean pairwise distance between puparia was observed when females of *N. vitripennis* were present or absent. In high-compaction treatments, however, larvae of *L. sericata* pupated closer together when

Table 1 Mean (± SE) pupation depth (cm) of larvae of *Lucilia sericata*, with and without *Nasonia vitripennis*, at two soil compactions

	Soil-compaction treatment			
Repetition	Uncompacted	Uncompacted + *Nasonia*	High	High + *Nasonia*
1	5.62 ± 0.37	5.77 ± 0.36	0.96 ± 0.06	1.01 ± 0.07
2	4.95 ± 0.40	5.08 ± 0.45	0.76 ± 0.07	0.60 ± 0.06

females of *N. vitripennis* were present than when absent ($F_{1,18} = 4.74$, $P = 0.043$) (Table 2).

The presence of females of *N. vitripennis* significantly increased the rate of pupal development in the uncompacted ($F_{1,70} = 49.74$, $P<0.0001$) and high-compaction ($F_{1,71} = 4.29$, $P = 0.042$) treatments in repetition two, but not in repetition one ($F_{1,88} = 1.45$, $P = 0.23$; $F_{1,88} = 2.52$, $P = 0.12$, respectively) (Table 3). Parasitism accounted for 3.0 ± 0.002% of the total mortality, mean mortality of *L. sericata* for all treatments in both repetitions was 8.2 ± 0.003%, and rate of parasitism in the no soil control in replicate two was 100%.

Effect of soil compaction on pupation behavior of *Lucilia sericata*

Pupation depth of *L. sericata* decreased as soil compaction increased (Figure 1). Larvae pupated at a mean of 4.4 cm in uncompacted soil, 2.0 cm in medium-low compaction, 1.9 cm in medium compaction, 0.9 cm in medium-high compaction, and 0.5 cm in high compaction (Table 4). The relation of pupation depth to soil compaction was expressed with a linear regression model:

$$y = \beta_0 + \beta_1 x + \varepsilon, \quad (1)$$

where y is log(depth + 1), β_o is the intercept, β_1 is the slope, x is soil compaction, and ε is random error. The intercepts of the regression differed significantly ($F_{1,45} = 315.21$, $P<0.0001$) for each repetition but the slopes did not ($F_{3,45} = 0.84$, $P = 0.48$). From these regression equations, a predictive model of pupation depth for *L. sericata* was developed:

$$d = e^{[(1.462\pm0.03)-(0.255\pm0.01)C]}, \quad (2)$$

where d is depth (cm), e is inverse ln, and C is soil compaction (kg cm^{-2}). The mean pairwise distance between puparia (4.42) in soil compacted to more than 0.025 kg cm^{-2} (all treatments except uncompacted) was less than that of puparia in soil compacted to ≤0.025 kg cm^{-2} (9.51) ($F_{1,2} = 40.5$, $P = 0.024$) (Table 5). Soil compaction had no significant effect on the orientation (vertical or horizontal) of puparia in the soil ($F_{5,41} = 1.66$, $P = 0.17$).

Flies in uncompacted soil generally developed faster than did flies in soil of high compaction (Table 6). However, when all repetitions were analyzed together, only the high-compaction treatment differed significantly from the others ($F_{5,21} = 6.67$, $P = 0.0007$). The high-compaction soil was cooler than the other soil-compaction treatments, and *L. sericata* developed at a slower rate than at other compaction levels (Figure 2). Soil of lower compaction retained more water than did compact soil (Figure 3). The mean development time of females (357.5 h) was significantly longer than that of males (350.4 h) ($F_{1,21} = 7.08$, $P = 0.015$), but the effect of soil compaction on development time was the same for both females and males ($F_{5,21} = 0.09$, $P = 0.99$). Mean rate of emergence of adults for all treatments and repetitions was 94 ± 0.2%.

Table 2 Mean pairwise distances[1] between puparia of *Lucilia sericata*, with and without *Nasonia vitripennis*, at two soil compactions

	Uncompacted	High compaction
Nasonia absent	9.78a	3.79a
Nasonia present	10.5a	2.04b

Means within a column followed by a different letter are significantly different (Fisher's Least Significant Difference test: $P<0.05$).

[1]No unit of measure is used, because this distance was calculated using the actual depth at which a puparium was found and x–y coordinates taken from a unit-free grid.

Discussion

The presence of parasitoids should influence the behavior of blow fly larvae, which would be under pressure to escape parasitism. Ullyett (1950) inferred from where larvae of *L. sericata* pupated in the soil that they would be safe from parasitism, based on the assumption that females of *N. vitripennis* do not burrow. The results of our study confirm that larvae of *L. sericata* escape parasitism by *N. vitripennis* by burrowing in soil. However, burrowing in soil might not protect *L. sericata* from parasitism by other species of Hymenoptera that enter soil to parasitize hosts,

Table 3 Mean time of development (h) from egg to adult of *Lucilia sericata*, with and without females of *Nasonia vitripennis*, at two soil compactions

		Uncompacted		High compaction	
Repetition	Mean ambient temperature (°C)	*Nasonia* absent	*Nasonia* present	*Nasonia* absent	*Nasonia* present
1	24.36 ± 0.018	328.2a	330.6a	336.6a	343.7a
2	28.45 ± 1.20	355.7a	332.0b	372.3a	357.3b

Means within a compaction treatment × repetition followed by a different letter are significantly different (Fisher's Least Significant Difference test: $P<0.05$).

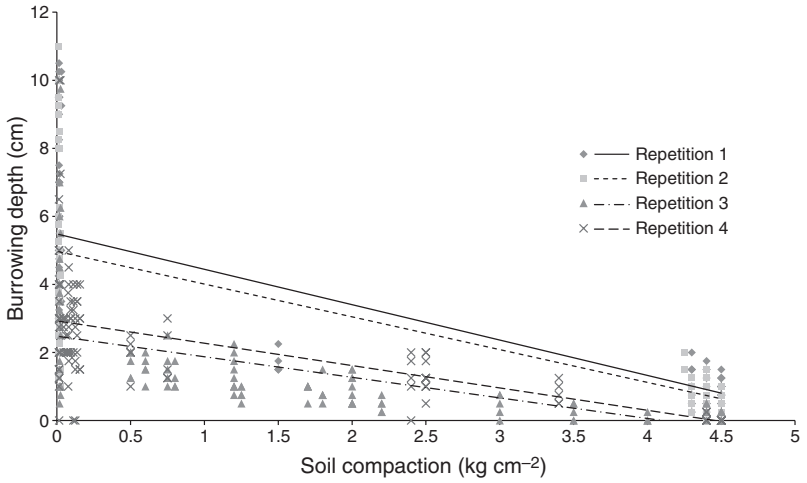

Figure 1 Pupation depths (cm) for *Lucilia sericata* in response to soil compaction (kg cm^{-2}), with linear regression for each repetition; experiments from 2008 and 2009 combined. Symbols represent depths at which puparia were found, and can represent multiple puparia recovered at that same depth.

Table 4 Mean (± SE) pupation depth (cm) for *Lucilia sericata* at different levels of soil compaction

Repetition	Uncompacted	Medium-low	Medium	Medium-high	High
1	5.62 ± 0.37	–	–	–	1.04 ± 0.07
2	4.95 ± 0.40	–	–	–	0.76 ± 0.07
3	3.48 ± 0.52	1.51 ± 0.09	0.84 ± 0.08	0.54 ± 0.09	0.19 ± 0.03
4	3.65 ± 0.52	2.44 ± 0.14	2.97 ± 0.21	1.35 ± 0.13	0.02 ± 0.01

such as *Muscidifurax raptor* Girault & Sanders, *Spalangia cameroni* Perkins, *Spalangia endius* Walker, and *Spalangia gemina* Boucek (all Pteromalidae) (Geden, 2002).

Females of *N. vitripennis* locate host puparia through chemical and visual cues (Edwards, 1954). Clumped pupation in the presence of *N. vitripennis* in the high-compaction treatment is opposite expectation. Clumping should increase the chemical signal available to females of *N. vitripennis*. The strategy of pupating closer together, therefore, might increase the risk of parasitism. The parasitoids also might suffer negative consequences. When only one female of *N. vitripennis* is parasitizing hosts, increased host density results in a lower mean number of progeny per host (Barbosa et al., 2008a). If host puparia are clumped, multiple parasitoids likely will find the puparia, resulting in superparasitism. If superparasitism occurs, the sex ratio of the resulting offspring will be male-biased (Werren, 1980, 1983; Barbosa et al., 2008b) and the developing parasitoids likely will compete for resources within the host.

The difference in development rate of *L. sericata* in the presence of *N. vitripennis* provides insight into the ability of a parasitoid to manipulate host biology and behavior. The duration of fly development was significantly shorter in one repetition when *N. vitripennis*

Table 5 Mean pairwise distances[1] between puparia of *Lucilia sericata* in uncompacted (≤0.025 kg cm^{-2}) and compacted (>0.025 kg cm^{-2}) soil

	Soil compaction	
Repetition	Uncompacted	Compacted
2	9.78a	3.79b
3	9.76a	3.82b
4	9.00a	5.64b

Means within a row followed by a different letter are significantly different (Fisher's Least Significant Difference test: $P<0.05$).
[1]No unit of measure is used, because this distance was calculated using the actual depth at which a puparium was found and x–y coordinates taken from a unit-free grid.

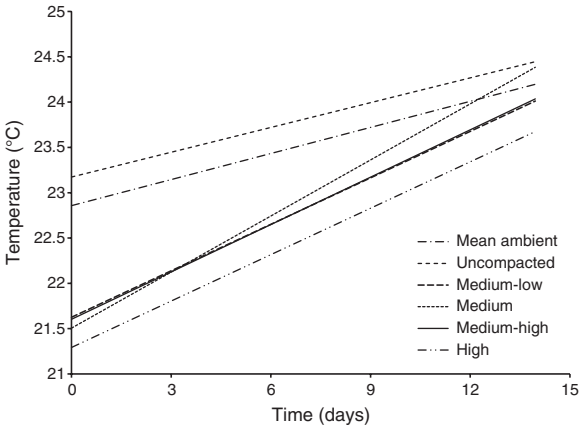

Figure 2 Mean ambient and soil temperature (°C) at the mean depth of pupation for *Lucilia sericata*, at different levels of soil compaction.

was present. Larvae in the presence of females of *N. vitripennis* might expend more energy while burrowing to escape the parasitoids than would larvae in the absence of *N. vitripennis*. An increase in energy expenditure could cause pupation to occur sooner because emptying of the crop is thought to trigger pupation in blow flies (Greenberg & Kunich, 2002). However, the difference in rate of development across repetitions suggests that more work on this system is warranted before conclusions can be drawn on the effects of parasitoids on development of *L. sericata*.

Pupation depth of larvae of *L. sericata* is inversely related to soil compaction. The only previous study investigating the burrowing activity of *L. sericata* used sifted river sand as a pupation medium (Ullyett, 1950). The compaction of that sand is unknown, but dry sand along a lake shore has a compaction of approximately 0.04 kg cm^{-2} (JA Cammack, unpubl.). Ullyett (1950) found puparia of *L. sericata* up to 14 cm deep, but 80% pupated no deeper than 8.9 cm, which is similar to our results: 86% of larvae pupated <9 cm deep. As soil compaction increases, larvae require more time and energy to penetrate the soil. Larvae, therefore, might stop burrowing when they have depleted the contents of their crop.

The available pore space in the soil also might contribute to the negative relation between soil compaction and pupation depth. As compaction increases, pore space decreases (Babercheck, 1992), reducing gas exchange in the soil; thus, less oxygen is available (Brady & Weil, 2008). Larvae, therefore, might pupate closer to the soil surface where more oxygen is available. However, pupating closer to the surface increases susceptibility to predation and parasitism (Guillen et al., 2002).

Soil compaction affects the spatial distribution of puparia of *L. sericata*. Larvae that pupate in uncompacted soil (≤0.025 kg cm^{-2}) are less clumped than are larvae that pupate in more compacted soil. The clumped distribution in compacted soil might be the result of larvae following the burrow of another larva, which has less resistance than the surrounding soil. The clumped distribution also might be the result of cooperation, increasing the rate at which larvae enter the soil and reducing the probability of predation and parasitism. Fire ants, *Solenopsis invicta* Buren, for example, attack larvae within 10 min of being placed on the soil in the field (Cammack, 2009). Communal burrowing occurred only in soil of high compaction.

Table 6 Mean time of development (h) from egg to adult of *Lucilia sericata* at different levels of soil compaction

Repetition	Mean (± SE) ambient temperature (°C)	Uncompacted	Medium-low	Medium	Medium-high	High	No soil
1	24.36 ± 0.018	328.2a	–	–	–	336.6b	332.8c
2	28.45 ± 1.20	355.7a	–	–	–	372.3b	361.3a
3	24.17 ± 0.031	340.0a	345.7abc	352.1cd	350.7bcd	356.6d	342.5ab
4	23.65 ± 0.037	368.8a	368.0a	366.7a	377.1b	404.2c	364.3a

Means within a row followed by different letters are significantly different (Fisher's Least Significant Difference test: $P<0.05$).

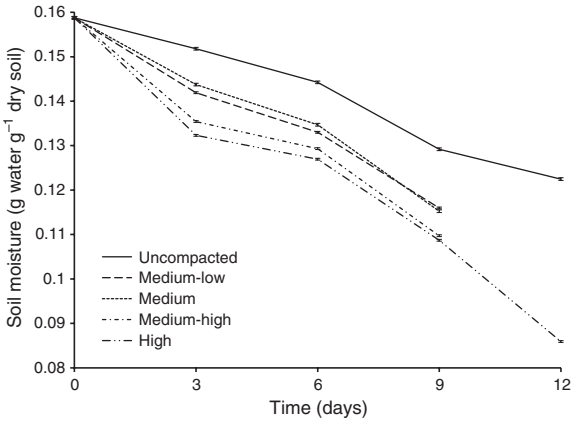

Figure 3 Mean (± SE) soil-moisture content (g water g^{-1} dry soil) at the mean depth of pupation for *Lucilia sericata*, at different levels of soil compaction over 12 days.

Physical properties of the soil influence the rate of pupal development of *L. sericata*. Development takes longer in high-compaction soil. Uncompacted soil has larger pores than soil of higher compaction and, therefore, has more air (Babercheck, 1992). Because the specific heat of soil is higher than that of air (Brady & Weil, 2008), soil with more pore space (i.e., lower compaction) will have a temperature closer to ambient than will soil with less pore space (i.e., higher compaction).

Because soil in the field is less homogeneous than in the laboratory, larvae might not pupate as deeply. When a burrowing larva encounters objects, it changes direction. Larvae in the field, therefore, might burrow laterally more often than larvae in the laboratory. Field validation of the predictive model of pupation depth showed that the laboratory-developed model over-predicted pupation depth in the field by a mean of 74%; however, only 3% of puparia (n = 175) were recovered in the field (Cammack, 2009).

Information from this study can be applied in urban and agricultural settings (e.g., refuse dumps and confined animal facilities) where these flies might require management. The lack of a parasitoid effect on host pupation depth suggests that the release of *N. vitripennis* in the field (e.g., a refuse dump or poultry house) will not reduce the efficacy of the control program, and could increase the efficacy because larvae pupate closer together in the presence of parasitoids. The predicted depth of pupation can be used to determine which species of parasitoid would be most efficient at locating puparia in the substrate, based on the depth the wasp can burrow and how efficiently they search for host puparia (Geden, 2002).

Information from our study can aid forensic investigators in locating the oldest insects to establish the period of insect activity. Direction and distance that post-feeding larvae move from a corpse can be determined based on studies by Greenberg (1990) and Tessmer & Meek (1996). Soil compaction then can be measured with a pocket penetrometer and used in model (2) to determine how deep to dig for puparia. Accuracy of the model of pupation depth might be increased by incorporating additional characteristics of soil such as pH and moisture content (Hennessey, 1994; Hodge & Caslaw, 1998), ambient temperature (Gomes et al., 2009), and photoperiod (Warman & Lewis, 1997).

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