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Effects of Parasitism and Soil Compaction on Pupation Behavior of the Green Bottle Fly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae)

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Entomology

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by
Jonathan Alan Cammack
August 2009

Accepted by:
Dr. Peter H. Adler, Committee Co-Chair
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ABSTRACT

Although the pupation behavior of blow flies has been widely studied, my work is the first to examine the effects of parasitoids and soil compaction on pupation behavior. The objectives of my research were to provide insight into a host-parasitoid system involving *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) and *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) and to develop a predictive model of pupation depth for *L. sericata*, with respect to soil compaction. Two experiments were conducted examining the effects of parasitoids and soil compaction on the pupation behavior of *L. sericata*. In all experiments, larvae of *L. sericata* were introduced to containers with soil of different compaction levels. Development time, depth of pupation, pupal orientation, and spatial distribution of puparia were recorded after emergence of adult flies. Although females of *N. vitripennis* did not significantly affect the burrowing depth of *L. sericata*, they increased the rate of pupal development by 15.0–23.7 hours at 28.4°C ±1.20 and increased the clumping of puparia. Burrowing depth of *L. sericata* is negatively related to soil compaction. Mean depth of pupation was 4.4 cm in low-compaction soil and 0.5 cm in high-compaction soil. In high-compaction soil, rate of pupal development decreased by 10.5–18.8 hours at 25.2°C ±0.30 and puparia were clumped. Based on these results, I suggest that forensic entomologists should add a pocket penetrometer, ruler, and garden trowel to their evidence collection kit, allowing efficient location of blow fly puparia at a body-recovery scene. Future research should address the influence of parasitoids and other properties of soil on the development of additional forensically important insects.

DEDICATION

For the three who believed in me enough to make me leave: Drs. Jimmy K. Olson, Jeffery K. Tomberlin, and James B. Woolley; thank you.

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INTRODUCTION

In a natural setting, 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 sedentary pupal stage occupies approximately 50% of the total duration of blow fly development, and many organisms have evolved to exploit this life stage (Greenberg & Kunich 2002). Knowledge of pupation behavior, therefore, is critical in developing methods to control blow flies or locate them in forensic investigations.

The green bottle fly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) is a cosmopolitan species of medical, veterinary, and forensic importance. It can transmit pathogens (Nelder et al. 2008), clean non-healing wounds (Sherman et al. 2000), cause myiasis in humans (Merritt 1969) and other animals (Tillyard & Seddon 1933), and be used to determine the minimum time since death in forensic investigations (Catts & Haskell 1990).

Lucilia sericata has been studied in many regions of the world because of its interactions with humans and animals. Because it is a pest of livestock, efforts have been made to control *L. sericata* with pesticides and biological control agents such as *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). To date, little research has focused on the pupation behavior 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 *Nasonia vitripennis* (Ullyett 1950). Understanding the pupation behavior of *L. sericata* is

imperative because forensic entomology depends on locating the oldest insects that developed on remains in order to provide the most accurate minimum postmortem interval. If the remains are in a late stage of decomposition, larvae from the first sere of succession might have left the remains and pupated in the surrounding soil. If so, investigators must be able to locate these puparia.

To provide insight into a host-parasitoid system, with respect to soil compaction, and develop a predictive model of pupation depth of *L. sericata*, I addressed the following questions: How does soil compaction affect the ability of parasitoids to parasitize *L. sericata*, and how does soil compaction affect the pupation behavior of *L. sericata*? To answer these questions, I tested the following research hypotheses:

H_{A1}: Larvae of *L. sericata* burrow deeper into soil in the presence of the pupal parasitoid *N. vitripennis*.

H_{A2}: Larvae of *L. sericata* burrow deeper into less compacted soil.

H_{A3}: Soil compaction influences the rate of fly development.

H_{A4}: Soil compaction influences the spatial distribution of *L. sericata* puparia in soil.

LITERATURE REVIEW

Little work has been conducted on the interaction between blow fly pupae and their parasitoids, and none on the effect of soil compaction on blow fly pupation. The two species in my study, *Lucilia sericata* and *Nasonia vitripennis*, have historically affected humans agriculturally as a pest and biological control agent, respectively. These species also are used to determine the minimum time since death in forensic investigations. In the following literature review, I address the pupation behavior of blow flies, the influence of soil compaction on pupation behavior, the parasitoids of blow flies, and the two species under study: *Lucilia sericata* and *Nasonia vitripennis*.

Pupation Behavior of Blow Flies

When larval blow flies near the end of the third instar, they typically leave the food source in search of a site to pupate; this phase is known as the post-feeding stage. In a forensic investigation, failure to locate these pupae, which likely are the oldest insects that fed on the decomposing remains, will result in an inaccurate estimation of the minimum period of insect activity (PIA), as it relates to the time since initial insect colonization (Greenberg 1990, Amendt et al. 2007).

Therefore, knowledge of where blow flies pupate is critical to accurate analysis of insect evidence. In Australia, pupation behavior differs between two subfamilies of the Calliphoridae: the Chrysomyinae (*Chrysomya*) pupate on or near the food source on the surface of the ground, and the Calliphorinae (*Lucilia, Calliphora*) disperse from the food source and burrow into the soil before pupating (Norris 1959). Since this initial observation on behavioral differences, many studies have been conducted on the dispersal

behavior of post-feeding larvae of blow flies. In cardboard channels filled with wood shavings, 98% of the larvae of *Phormia regina* (Meigen) and 90% of *Chrysomya rufifacies* (Macquart) pupate at the food site, while 99% of the larvae of *L. sericata* and 84.5% of the larvae of *Calliphora vicina* Robineau-Desvoidy pupate between 3.0 and 8.1 m from the food source (Greenberg 1990). During the summer in Louisiana, 59% and 52% of *Cochliomyia macellaria* (Fabricius) are found within 0.9 m of the food source in a wooded and pasture habitat, respectively (Tessmer & Meek 1996). In the pasture habitat, larval blowflies dispersed to the southeast in 3 of 4 seasonal tests, and dispersed to the southwest in the other. In the wooded habitat, dispersal of larvae was to the southeast and southwest in the spring and fall, respectively, but no difference in direction was observed in the summer or winter (Tessmer & Meek 1996). In Brazil, post-feeding larvae of *Chrysomya albiceps* (Wiedemann) disperse 22.0 cm and larvae of *Chrysomya megacephala* (Fabricius) disperse 31.9 cm in wood shavings (Gomes & Von Zuben 2005), which contradicts the observations by Norris (1959).

After dispersing, larval blow flies 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 & Von Zuben 2005). The effect of temperature on burrowing behavior of *Chrysomya albiceps* and *Lucilia cuprina* Wiedemann in vermiculite was investigated by Gomes et al. (2009). The burrowing behavior in sand of three blow fly species responsible for sheep strike in South Africa was studied by Ullyett (1950) who found that larvae of *L. sericata* burrow up to 14 cm deep, with most (54.7%) at 1.3-3.8 cm. *Chrysomya chloropyga* Wiedemann

burrows no deeper than 6.4 cm, with most (57%) puparia found on the surface, and 98.7% of *Chrysomya albiceps* puparia are found on the surface, with none pupating deeper than 3.8 cm. The variability and few number of studies on dispersal and burrowing behavior of blow fly species illustrates that continued study of these phenomena are needed.

Effect of Soil Compaction on Pupation

Many holometabolous insects complete their development as pupae in the soil. For those of agricultural, medical, veterinary, or forensic importance, knowledge of where in the soil these insects pupate is critical in developing methods to control or locate them. Much of the work on the effect of soil compaction on insect pupation behavior has been done on insects of agricultural and economic importance. The small hive beetle *Aethina tunida* Murray (Coleoptera: Nitidulidae) is unable to pupate in compacted dry soil and thus unable to complete development, and emergence of adults is significantly lower in compacted wet soil than uncompacted soil for three of the six soil types tested by Ellis et al. (2004). Laboratory experiments on fruit flies (Diptera: Tephritidae) suggest that soil compaction negatively affects the burrowing ability of larvae. Larve 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). However, soil compaction is negatively correlated with emergence of adult Mediterranean fruit flies *Ceratitis capitata* (Wiedemann); mortality increases in soils with high bulk densities (and

thus high compaction) in comparison to soils with lower bulk densities (Eskafi & Fernandez 1990). Depth of pupation by olive fruit flies, *Dacus oleae* (Gmelin), in different substrate types also is affected by compaction. In compact substrates, larvae of *D. oleae* pupate no deeper than 5 cm while larvae pupate up to 8 cm deep in uncompacted substrates (Tsitsipis & Papanicolaou 1979).

Overall, the level of soil compaction affects the pupation behavior of insects; they pupate deeper in less compact soil than in more compact soil. In the laboratory, larvae of *A. suspensa* crawl for approximately 5 hours across compact soil before burrowing or pupating on the surface (Hennessey 1994). This behavior probably occurs in the field and crawling larvae are at a greater risk of mortality from predation, parasitism, or desiccation, which likely is true for larval blow flies dispersing from decomposing remains.

Parasitoids of Blow Flies

Many species of parasitic Hymenoptera are parasitoids of larvae or pupae of blow flies and are commonly reared from field-collected puparia. In his landmark decomposition studies of fetal pigs, J.A. Payne recorded nine species (representing five families) of parasitic Hymenoptera reared from, observed parasitizing, or known to parasitize blow flies (Payne & Mason 1971). In collections from poultry houses, swine barns, refuse dumps, and garbage dumpsters in South Korea, four species of Pteromalidae were reared from puparia of *Chrysomya megacephala* (Fabricius) and two species from puparia of *L. sericata* (Rueda et al. 1997). In Malaysia, four species of Pteromalidae were reared from puparia of *C. megacephala* from 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 control of blow flies responsible for sheep strike (myiasis) in Australia, New Zealand, and the United Kingdom (Tillyard & Seddon 1933, Davies 1934, & Bishop et al. 1996), where multiple species of Hymenoptera representing four families were used to control blow flies. A project in France, in collaboration with the sheep blow fly projects of Australia, found two species of Braconidae and one species of Pteromalidae parasitizing *Calliphora vicina* (as *erythrocephala*), *Calliphora vomitoria* (Linnaeus), *Lucilia sericata*, and *Lucilia illustris* (as *caesar*) (Meigen) (Evans 1933).

Lucilia sericata

The green bottle fly *Lucilia sericata* is of agricultural, medical, veterinary, and forensic importance. It is cosmopolitan in distribution and is the most abundant species of blow fly in North America (Hall 1948), where is completes 4-8 generations per year and overwinters as larvae or pre-pupae (Hall 1948). It is a pest of both livestock and humans in confined animal facilities where the flies breed in large numbers in manure and other refuse (Sulaiman et al. 1990, Rueda et al. 1997). *Lucilia sericata* can cause myiasis in sheep (Tillyard & Seddon 1933), three-toed box turtles (McAllister 1987), and humans (Merritt 1969, Daniel et al. 1994). Specimens of *L. sericata* collected in South Carolina tested positive for the presence of *Coxiella burnetii*, implicating this species as a vector of pathogenic bacteria (Nelder et al. 2008). Greenberg (1971) lists many viruses, bacteria, protozoa, and nematodes associated with *L. sericata*.

Lucilia spp. might be capable of anticipating death (Davis 1928) and are often found on dead animals. *Lucilia sericata* is an early colonizer of decomposing remains (Hall & Doisy 1993, Byrd & Castner 2001, Watson & Carlton 2003), making it useful in predicting the minimum PIA as it relates to the time since initial insect colonization, which may or may not correlate with the post-mortem interval (Amendt et al. 2007). The temperature-related development of *L. sericata* has been studied by Kamal (1958), Anderson (2000), Grassberger & Reiter (2001), and Clark et al. (2006). Larvae of *L. sericata* are able to ingest drugs from the tissues on which they are feeding, which can alter their development (Bourel et al. 1999, Campobasso et al. 2004). Molecular identification techniques also have been developed and tested on *L. sericata* (Wallman & Donnellan 2001, Harvey et al. 2003, Saigusa et al. 2005). *Lucilia sericata* has been at the center of many studies that have influenced forensic entomology.

Nasonia vitripennis

Nasonia vitripennis is one of the most common parasitoids of calliphorids and other cyclorrhaphan Diptera associated with carrion. The cosmopolitan *N. vitripennis* is gregarious and externally parasitic on cyclorrhaphan pupae (Whiting 1967). However, *N. vitripennis* probably represents a species complex (J.B. Woolley, personal communication). Prior to 1990, *N. vitripennis* was the only species in the genus. Sampling of bird's nests in North America from 1986 to 1988 revealed two new species associated with the bird blow flies, *Protocalliphora* spp. (Darling & Werren 1990). Increased sampling in other regions of the world likely would reveal more species. *Nasonia vitripennis* was a known parasitoid of blow flies as early as 1914 (Tillyard &

Seddon 1933) and has been recorded as a parasitoid of at least 29 species of calliphorids (Noyes 2009).

Host location by females of *N. vitripennis* has been widely studied but is not entirely understood. Edwards (1954) studied host-locating behavior of females of *N. vitripennis* in the laboratory, using an olfactometer. He determined that females of *N. vitripennis* were more attracted to decomposing rabbit liver that was fed on by larvae of *Calliphora* sp. than to rabbit liver decomposed by bacteria, or a milk pad on which larvae of *Musca* had fed. However, the actual attractant is unknown, whether it is a product of decomposition from feeding activity of larvae or an excretory product of the larvae. This attraction provides support for a possible cue used by females of *N. vitripennis* in locating hosts: a volatile compound associated with the host and its feeding activity. Because larvae are not the host of *N. vitripennis* and females of *N. vitripennis* presumably cannot detect a host until they are within 2-3 mm of it, Edwards (1954) investigated the location of host puparia by *N. vitripennis*. Using three types of puparia (washed *Musca* sp., washed *Calliphora* sp., and unwashed *Calliphora* sp.), he found that a single, unwashed puparium of *Calliphora* sp. produced results similar to those of the larvae feeding on rabbit liver, and the washed puparia were unattractive to *N. vitripennis*. He also found that in the presence of the odor from rabbit liver fed on by larvae of *Calliphora* sp., females of *N. vitripennis* are attracted to artificial puparia and attempt to oviposit on objects that resemble puparia in size and shape. From these studies, Edwards (1954) concluded that puparia have no attractive odor of their own and instead must be “dirty” with odors from the food source of the larvae, and oviposition is stimulated by visual

cues such as shape and size. These results suggest that females of *N. vitripennis* should be able to locate a host puparium in the soil by following the trail of odors left by the burrowing larvae. Ullyett (1950) stated that flies pupating beneath the soil surface were inaccessible to *N. vitripennis* and thus protected from parasitism. However, he used no *N. vitripennis* in his experiments and cited no literature to support the claim.

The association with carrion-feeding insects makes *N. vitripennis* an insect of forensic importance. Because females of *N. vitripennis* lay their eggs in the space between the pupa and puparium, they are unable to successfully parasitize puparia less than 24 hours old (Edwards 1954). If adults of *N. vitripennis* emerge from puparia collected at a body-recovery scene, these puparia are at least 24 hours old when collected, and if estimation of the PIA is based on collection time of the puparia, the PIA should be increased by 24 hours. Like all insects, the development of *N. vitripennis* is influenced by temperature, which makes these insects useful in determining an extended PIA. When reared on puparia of *Protophormia terraenovae* (Robineau-Desvoidy) at temperatures between 15 and 30ºC, the mean minimum time from oviposition to emergence of adults is 217-226 accumulated degree days (43.5-11.3 days, respectively) (Grassberer & Frank 2003). However, this development is not as straightforward as previously thought. Mello & Aguiar-Coelho (2009) used *Chrysomya megacephala* as a host and found that increasing the density of host puparia per female parasitoid and increasing the density of female parasitoids per host puparium leads to an increase in duration of development of the progeny. Density of host puparia under natural conditions might be high due to the pupation behavior of blow flies. A decomposition study by Cammack & Nelder (in

review) might support this claim. They collected puparia of *Chrysomya rufifacies* around the dry remains of a deer carcass and isolated them in the laboratory. After adults of *N. vitripennis* emerged, Cammack & Nelder used the development data set of Grassberger & Frank (2003) to determine the minimum PIA on the deer carcass. Based on this data set, the calculated time of parasitism was after the puparia had been isolated in the laboratory, which possibly could be explained by the results of Mello & Aguiar-Coelho (2009).

MATERIALS & METHODS

Source of Insects

A colony of *Lucilia sericata* was initiated from approximately 100 puparia obtained from a colony maintained by J.D. Wells at West Virginia University. Flies were wild-caught in June 2007 in Morgantown, West Virginia, and the colony was in its 6th generation when I received puparia to start my colony. A colony of *Nasonia vitripennis* was initiated with 66 individuals (12 ♂, 54 ♀) that emerged on 15-17 December 2007 from field-collected puparia of *Chrysomya rufifiacies*. These puparia were recovered from around the dry remains of a deer in Clemson, South Carolina, on 27 November 2007 (Cammack & Nelder, in review). Pinned voucher specimens of both species were deposited in the Clemson University Arthropod Collection (CUAC); additional specimens were fixed in 95% ethanol and placed in the CUAC.

Insect Rearing

Each species of insect was reared in a separate environmental room to prevent *N. vitripennis* contamination of the colony of *L. sericata*. Environmental conditions in each room were 24.1°C ± 0.05 (17.4-30.1°C) and approximately 60% relative humidity, with a light:dark cycle of 16h:8h. The colony of *L. sericata* was maintained in a 0.227-m^3 aluminum rearing cage (Part# 1450D, BioQuip Products®, Rancho Dominguez, CA). Adult flies were provided *ad libitum* with granulated sugar, powdered milk, and distilled water. A piece of beef liver (ca. 50 g) was placed in the bottom of a 473-ml clear plastic cup and covered with a moist paper towel. This container was placed in the colony and removed when egg masses were noted. As the larvae of *L. sericata* developed, more liver

was added until the larvae had completed development. Before the larvae reached the post-feeding stage, the cup in which they were feeding was placed in a 1.4-l container of soil (identical to that used in experiments) in which to pupate.

The colony of *N. vitripennis* was maintained in a BugDorm-2 Insect Tent® (Part# BD2120, MegaView Science Co. Ltd., Taiwan) and provided with distilled water and a supersaturated solution of distilled water, sugar, and honey *ad libitum*. The colony was maintained on puparia from the colony of *L. sericata* by placing a petri dish of puparia on the floor of the cage or by isolating each female of *N. vitripennis* in a 26-ml plastic vial with 8-10 puparia. The latter method was most effective because it prevented an over-abundance of male progeny (Werren 1980, 1983).

Soil Collection and Preparation

Soil was collected from the Calhoun Field Laboratory (GPS: 34°40’88.6”N 82°50’38.97”W) on the Clemson University campus, Clemson, South Carolina, USA. The soil used in experiments was Ap horizon Cecil sandy loam (Taxonomic class Fine, kaolinitic, thermic, Typic Kanhapludult) with a pH of 5.5. This soil was chosen because clay soils used in preliminary experiments caused the developing flies to enter diapause, presumably due to the lack of moisture (Greenberg & Kunich 2002).

Soil aggregates were broken up with a rubber mallet, concrete block, or garden trowel. This mixture was poured through a 3.0-mm screen to obtain a particle size of less than 3.0 mm. Once sieved, the soil was stirred daily and allowed to air dry. Six to sixteen hours before each experiment, the soil was rehydrated with distilled water to approximately 16% moisture by weight (the approximate field capacity, JA Cammack,

personal observation) and stored overnight to allow even dispersal of the water throughout the soil. The following day, the rehydrated soil was again sieved with the 3.0-mm screen to break up clumps that formed when the soil was rehydrated.

Experiment Design

Experiments were performed at 25.2ºC ± 0.3 (20.6-27.0 ºC) and approximately 60% relative humidity, with a light:dark cycle of 16h:8h. Before each experiment, all containers and lids were washed in a dilute solution of hot tap water and Alconox® Powdered Precision Cleaner (VWR International) and air dried. Temperature in each room was recorded hourly with a HOBO® U12 4-External Channel Data Logger (Part# U12-006, Onset Computer Corporation, Bourne, MA).

Effect of Nasonia vitripennis on pupation behavior of Lucilia sericata

Fifty-two 1.4-l plastic containers were used in five treatments (10 containers per treatment) and two controls. The treatments were low compaction (0.016 ± 0.0005 kg/cm^2), low compaction (0.0183 ± 0.001 kg/cm^2) + *Nasonia*, high compaction (4.26 ± 0.06 kg/cm^2), high compaction (4.38 ± 0.05 kg/cm^2) + *Nasonia*, and no soil or parasitoids. The low-compaction treatment represents the lowest level of compaction attainable (uncompacted) and the high-compaction treatment is the maximum compaction measurable with the pocket penetrometer. For the low-compaction treatments, 1.09 l of soil was poured into the containers, to a level of 11 cm. This depth was chosen because larvae of *L. sericata* did not burrow deeper than 11 cm in preliminary experiments. For the high-compaction treatments, soil was compacted into the containers using tamps (Fig. 1.1) of different diameters (10.9, 11.2, and 11.6 cm) constructed of 0.64-cm plywood for

the tamp surface and 2.54 x 5.08 cm white pine board for the handle. Three diameters of tamps were used because the containers used in the experiment tapered slightly from top to bottom. In the high-compaction treatments, 1.5 l of soil was compacted to two-thirds of the starting volume, to a level of 11 cm.

Figure 1.1. Wooden tamps used to compact soil into containers to determine the effects of parasitism and soil compaction on pupation behavior of *Lucilia sericata* from West Virginia; 2008-2009.

Five post-feeding larvae of *L. sericata* were placed on the soil surface in the center of each container, except the two controls in which no larvae were introduced. Parasitism rate of *Calliphora* sp. by *N. vitripennis* begins to level off when density of hosts is 25 puparia/484 cm^2 (Jones & Turner 1987), which is equivalent to 5 puparia/105 cm^2 in my arenas. For the two parasitoid treatments, five females of *N. vitripennis* were added to the container, following introduction of larvae of *L. sericata*. Five females of *N. vitripennis* were used to increase the likelihood of a parasitoid contacting a host puparium. All containers had lids with an 8.5-cm diameter screened hole. The containers were placed randomly on shelves in the rearing room. A 1-dram vial filled with a supersaturated sugar and distilled water solution stoppered with cotton was placed on top of the lid screens of the 20 containers with parasitoids and refilled *ad libitum*.

The experiment was replicated, 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 in this replicate to 60.

Effect of soil compaction on pupation behavior of Lucilia sericata

To model the response of burrowing depth to soil compaction, five levels of soil compaction were used, with five replicates per treatment. A statistical power analysis (SAS, PROC POWER) was performed on preliminary data (mean burrowing depth and standard deviation from the "*Effect of N. vitripennis on pupation behavior of L. sericata*" experiment). These power analyses indicated that 5 replicates of each compaction level would give power of 0.7 or greater for detecting a regression relation between burrowing depth and soil compaction.

Containers of soil were prepared in a manner similar to those in the previous experiment. Thirty-five 1.4-l plastic containers were used for six treatments (five per treatment) plus five controls. The compaction treatments were low (0.019 ± 0.0006 kg/cm^2), medium-low (0.46 ± 0.05 kg/cm^2), medium (0.95 ± 0.14 kg/cm^2), medium-high (2.49 ± 0.12 kg/cm^2), and high (4.47 ± 0.006 kg/cm^2). For the low-compaction treatment, 1.09 l of soil was poured into the containers to a level of 11 cm. For the remaining compaction treatments, tamps from the previous experiment were used to compact soil to a level of 11 cm. In the medium-low treatment, 1.23 l of soil was compacted to seven-eighths of the starting volume. In the medium-compaction treatment, 1.26 l of soil was compacted to four-fifths of the starting volume. In the medium-high treatments, 1.34 l of soil was compacted to three-fourths of the starting volume. In the high-compaction treatments, 1.5 l of soil was compacted to two-thirds of the starting volume. Five additional containers were prepared as controls (one per compaction) to measure the initial compaction of each treatment. Five containers were used as a no-soil treatment.

Five post-feeding larvae of *L. sericata* were placed on the soil surface, in the center of each container, except the five controls in which no larvae were introduced. All containers were covered with lids with an 8.5-cm diameter screened hole. The containers were placed randomly on shelves in the rearing room.

Replicate two was set up in the same manner as the first, with one exception. Instead of using the control containers to measure initial compaction, a HOBO® Water/Soil Temperature sensor (Part# TMC6-HD, Onset Computer Corporation, Bourne, MA) was placed in the soil in each control container at the mean depth of pupation for

that compaction treatment (determined from experiment two). Soil temperatures at the mean depths of pupation were recorded hourly with a HOBO® U12 4-External Channel Data Logger (Part# U12-006, Onset Computer Corporation, Bourne, MA) to determine the effect of compaction on soil temperature and the resulting effect on development of *L. sericata*.

A blank experiment (no larvae of *L. sericata*) was run for 12 days to examine the change in soil moisture at the mean depth of pupation over time. Five readings were taken for the low- and high-compaction treatments, and four readings were taken for the medium-low-, medium-, and medium-high-compaction treatments.

Field validation of the model for burrowing depth of Lucilia sericata in soil

Laboratory experiments on the *effect of soil compaction on pupation behavior* were validated in the field in four different locations and soil compactions around the Cherry Farm Insectary, Clemson University, Clemson, SC: a forested area, a sandy beach on the shore of Lake Hartwell, a fallow field, and a highly compacted with frequent foot and automobile traffic. Arenas were constructed from lids of the 1.4-l containers used in the laboratory experiments, except these lids had an 8.5-cm diameter hole that was not screened. Five arenas were placed in each habitat for a total of 20 per replicate. The arenas were secured with wire hooks pressed into the ground outside the arena. Five post-feeding larvae of *L. sericata* were introduced to the center of each arena. Replicate two was set up in the same manner as the first, with one exception: no sandy beach treatment was used.

Data Collection and Analysis

Adult emergence of *L. sericata* and *N. vitripennis* was recorded for each experiment at 15-minute to 8-hour intervals for the entire 16-hour duration of the daylight period in the rearing room. An experiment ended when no adults had emerged over a two-day period.

When emergence of adults of *L. sericata* and *N. vitripennis* ceased, the puparia of *L. sericata* were located in the soil column. First, compaction of the soil in each container was measured using a pocket penetrometer (Part # 49015, Lab Safety Supply). The tip of the device (or 2.54-cm diameter adapter foot) was pushed into the soil to a depth of 0.635 cm, and the compaction of the soil was read where the sliding collar of the device stopped. Because the device only had markings every 0.25 kg/cm^2, compactions were subjectively read to the nearest tenth when necessary. The adapter foot was used in soils of low compaction and the measured compaction was multiplied by 0.0625 to account for the difference between the surface area of the adapter foot and the tip of the device. A modified plastic spoon was then 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 was recorded, and for all but the first experiment, the horizontal location in the container was recorded, using a 205 x 205 cm piece of plexiglass with a 14 x 14 cm grid divided into 1 x 1 cm squares. Orientation of each puparium (horizontal or vertical) also was recorded. This process was repeated until the fifth puparium of *L. sericata* was located or the bottom of the container was reached.

Puparia in the field-validation study were located in a manner similar to the laboratory studies. After larvae in the colony that dispersed at the same time as experiment larvae had pupated, puparia were located in the soil in each arena. Before the soil was disturbed, compaction was measured using a pocket penetrometer. A modified plastic spoon and garden trowel were used to remove soil from the arenas in a radial fashion at a depth of approximately 0.25 cm per rotation. This process was repeated until the fifth puparium of *L. sericata* was located, or a depth of 11 cm was reached. The depth at which each puparium was located was recorded. Samples from the sandy beach and forest floor of the first replicate were removed from the ground with a post-hole digger, placed in 1.4-l plastic containers, and processed in the laboratory.

ANOVA followed by Fisher's Least Significant Difference test (PROC GLM, GLIMMIX) of the Statistical Analysis Software (SAS) package (SAS Institute®, Cary, NC) was used to determine the following:

- Effect of females of *N. vitripennis* on burrowing depth of larvae, spatial distribution of puparia, and rate of pupal development of *L. sericata*
- Effect of soil compaction on burrowing depth of larvae of *L. sericata*
- Effect of soil compaction on rate of pupal development of *L. sericata*
- Effect of soil compaction on orientation (horizontal or vertical) of puparia of *L. sericata*
- Effect of soil compaction on the spatial distribution of puparia of *L. sericata* in the soil.

All tests were based on $\alpha=0.05$ to reduce the probability of a type I error. The no-parasitoid treatments (low compaction, 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 check of randomization of containers indicated that they were sufficiently distributed in the experiment chamber so that there was no position effect. A regression analysis (PROC GLM, SAS) was used to determine the relationship between soil compaction and burrowing depth of larvae.

To determine the effect of females of *N. vitripennis* and soil compaction on spatial distribution of puparia in the soil, the average pairwise distance between all puparia in a container was calculated using the actual depth of pupation (cm) and x-y coordinates measured on scaled data-recording sheets.

RESULTS

Effect of Nasonia vitripennis on pupation behavior of Lucilia sericata

Larvae of *L. sericata* burrowed to approximately 5.4 cm in soil of low compaction and to approximately 0.8 cm in soil of high compaction (Table 1.1); raw data in Appendix A. When females of *N. vitripennis* were present, no significant effect on burrowing depth of larvae was observed in soil of low compaction ($F_{1,192}$=0.12, p=0.7255) or soil of high compaction ($F_{1,188}$=0.7, p=0.4055). Rate of parasitism was 0% for all treatments except the high compaction treatment in replicate two (10%). Parasitism was 98% in the no soil + *Nasonia* control in replicate two. Puparia parasitized by *N. vitripennis* were either partially exposed (i.e., with part of the puparium above the soil surface) or completely exposed.

Under some conditions, the presence of females of *N. vitripennis* might influence the spatial distribution of puparia of *L. sericata* in the soil. In low-compaction treatments, no significant difference ($F_{1,18}$=1.10, p=0.3091) in the mean inter-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 females of *N. vitripennis* were present than when absent ($F_{1,18}$=4.74, p=0.043) (Table 1.2).

In replicate one, the presence of females of *N. vitripennis* had no significant effect on the rate of pupal development in either the low-compaction ($F_{1,88}$=1.45, p=0.2319) or high-compaction treatments ($F_{1,88}$=2.52, p=0.1158) (Table 1.3). However, in replicate two, the presence of females of *N. vitripennis* had a significant effect on the rate of pupal

development in both the low ($F_{1,70}$=49.74, p<0.0001) and high ($F_{1,71}$=4.29, p=0.042) soil compaction treatments (Table 1.3); raw data in Appendix B. Rate of pupal development was significantly faster when females of *N. vitripennis* were present in both soil compaction treatments. Mean rate of emergence of adults was 90.8% ± 0.4 for all treatments in both replicates, except the no soil control in replicate two (emergence = 0%) (Table 1.4).

Table 1.1. Mean burrowing depth (cm) ± SE of larvae of *Lucilia sericata* from West Virginia, with and without *Nasonia vitripennis* from South Carolina, at two different soil compactions; 2008.

	Soil-Compaction Treatment			
Replicate	Low	Low + *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

Low=0.016 ± 0.0005 kg/cm^2, Low + *Nasonia*=0.0183 ± 0.001 kg/cm^2, High=4.26 ± 0.06 kg/cm^2, High + *Nasonia*=4.38 ± 0.05 kg/cm^2; 10 containers/treatment with 5 larvae of *L. sericata*/container.

Table 1.2. Mean inter-pairwise distances[1] between puparia of *Lucilia sericata* from West Virginia, with and without *Nasonia vitripennis* from South Carolina, at two different soil compactions; 2008.

	Soil-Compaction Treatment	
	Low	High
Nasonia absent	9.78[a]	3.79[a]
Nasonia present	10.5[a]	2.04[b]

Low=0.016 ± 0.0005 kg/cm^2, Low + *Nasonia*=0.0183 ± 0.001 kg/cm^2, High=4.26 ± 0.06 kg/cm^2, High + *Nasonia*=4.38 ± 0.05 kg/cm^2. Different letters within a column (low or high) indicate significant differences (α=0.05, Fisher's Least Significant Difference test); 20 containers/compaction for each *Nasonia* treatment (present or absent), with 5 larvae of *L. sericata*/container and 5 females of *N. vitripennis*/container.

[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 measured from a data-recording sheet.

Table 1.3. Mean time of development (hours) from egg to adult of *Lucilia sericata* from West Virginia, with and without females of *Nasonia vitripennis* from South Carolina, at two different soil compactions; 2008.

		Soil-Compaction Treatment			
Replicate	Mean Temperature (°C) ± SE	Low	Low + *Nasonia*	High	High + *Nasonia*
1	24.4 ± 0.02	328.2[a]	330.6[a]	336.6[a]	343.7[a]
2	28.4 ± 1.20	355.7[a]	332.0[b]	372.3[a]	357.3[b]

Low=0.016 ± 0.0005 kg/cm^2, Low + *Nasonia*=0.0183 ± 0.001 kg/cm^2, High=4.26 ± 0.06 kg/cm^2, High + *Nasonia*=4.38 ± 0.05 kg/cm^2. Different letters within a row (replicates 1 & 2) and within a compaction and parasitoid treatment indicate significant differences (α=0.05, Fisher's Least Significant Difference test).

Table 1.4. Percent emergence and male:female ratios[1] of adults of *Lucilia sericata* from West Virginia at two different soil compactions; 2008.

			Soil Compaction Treatment			
Replicate	Low	Low + *Nasonia*	High	High + *Nasonia*	No Soil	No Soil + *Nasonia*
1	90%, 26:19	98%, 31:15	94%, 27:19	94%, 24:22	98%, 17:32	-
2	84%, 17:17	86%, 27:13	94%, 25:15	74%, 17:14	96%, 14:14	0%

Low=0.016 ± 0.0005 kg/cm^2, Low + *Nasonia*=0.0183 ± 0.001 kg/cm^2, High=4.26 ± 0.06 kg/cm^2, High + *Nasonia*=4.38 ± 0.05 kg/cm^2; 10 containers/ treatment with 5 adults of *L. sericata*/container

[1]Ratios might not equal 50 because some flies escaped or were damaged before sexing.

Effect of soil compaction on pupation behavior of Lucilia sericata

As soil compaction increased, burrowing depth decreased for larvae of *L. sericata* (Fig. 2.1). Larvae burrowed to a mean of approximately 4.4 cm in soil with low compaction, 2.0 cm with medium-low compaction, 1.9 cm with medium compaction, 0.9 cm with medium-high compaction, and 0.5 cm with high compaction (Table 2.1); raw data in Appendix A. The relation of burrowing depth to soil compaction was expressed with a linear regression model, 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:

$$y = \beta_o + \beta_1 x + \varepsilon$$

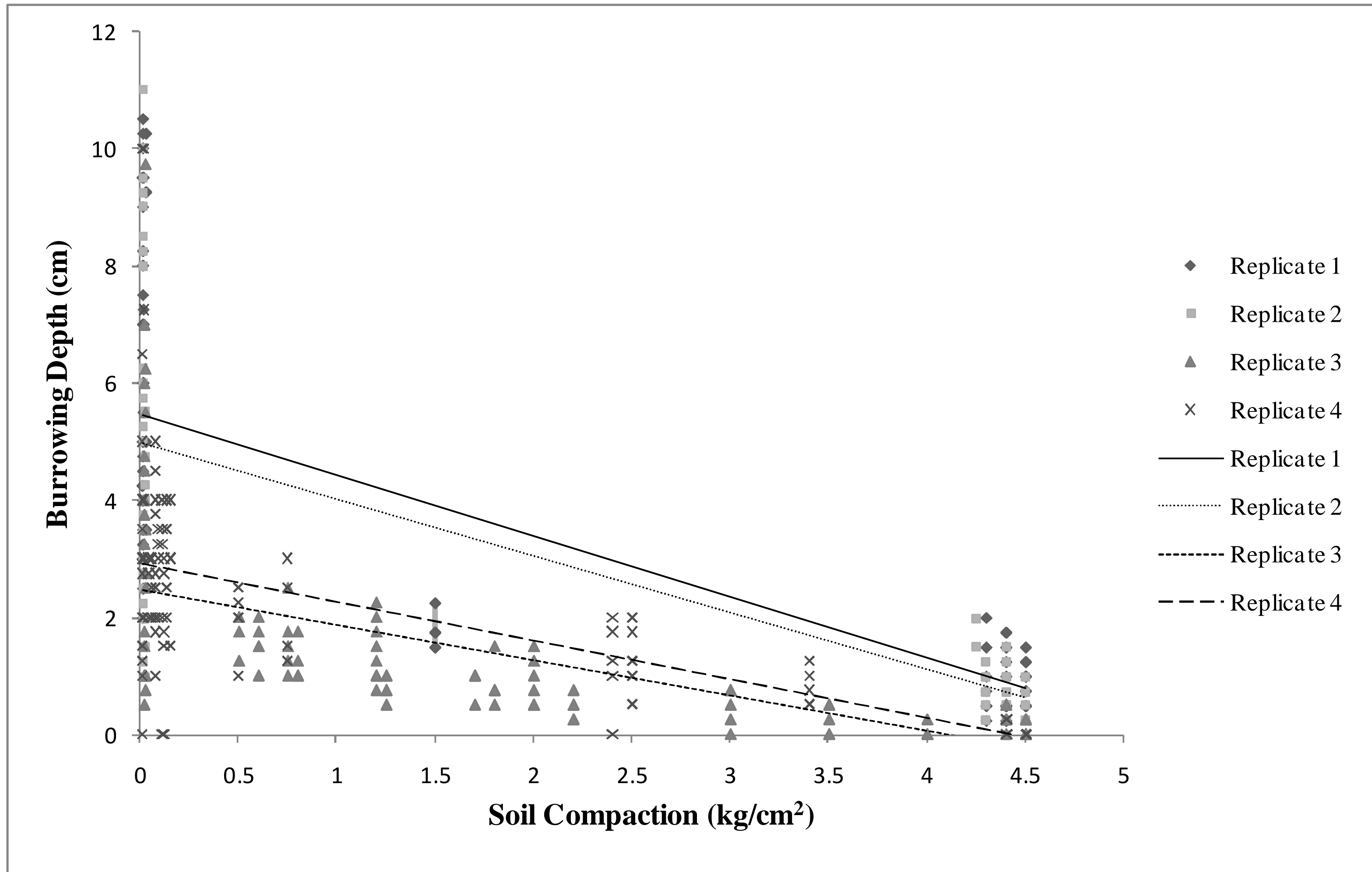
The intercepts of the regression differed significantly ($F_{3,429}$=38.66, p<0.0001) for each replicate but the slopes did not ($F_{3,429}$=0.72, p=0.54) (Table 2.2). From these regression equations, a predictive model of burrowing depth for *L. sericata* was developed (SAS, PROC GLM), where d is depth (cm), e is inverse natural log, and C is soil compaction (kg/cm^2):

$$d = e^{[(1.462 \pm 0.03) - (0.255 \pm 0.01)C]}$$

The spatial distribution of puparia in the soil was affected by soil compaction. When larvae of *L. sericata* encountered soil that was compacted to more than 0.025 kg/cm^2 (all treatments except low compaction), the mean inter-pairwise distance between puparia (4.42) was less than that of larvae that burrowed into soil compacted to less than or equal to 0.025 kg/cm^2 (9.51) ($F_{1,2}$=40.5, p=0.0238) (Table 2.3). Soil compaction had no significant effect on the orientation of puparia in the soil (either vertical or horizontal) ($F_{5,41}$=1.66, p=0.1658).

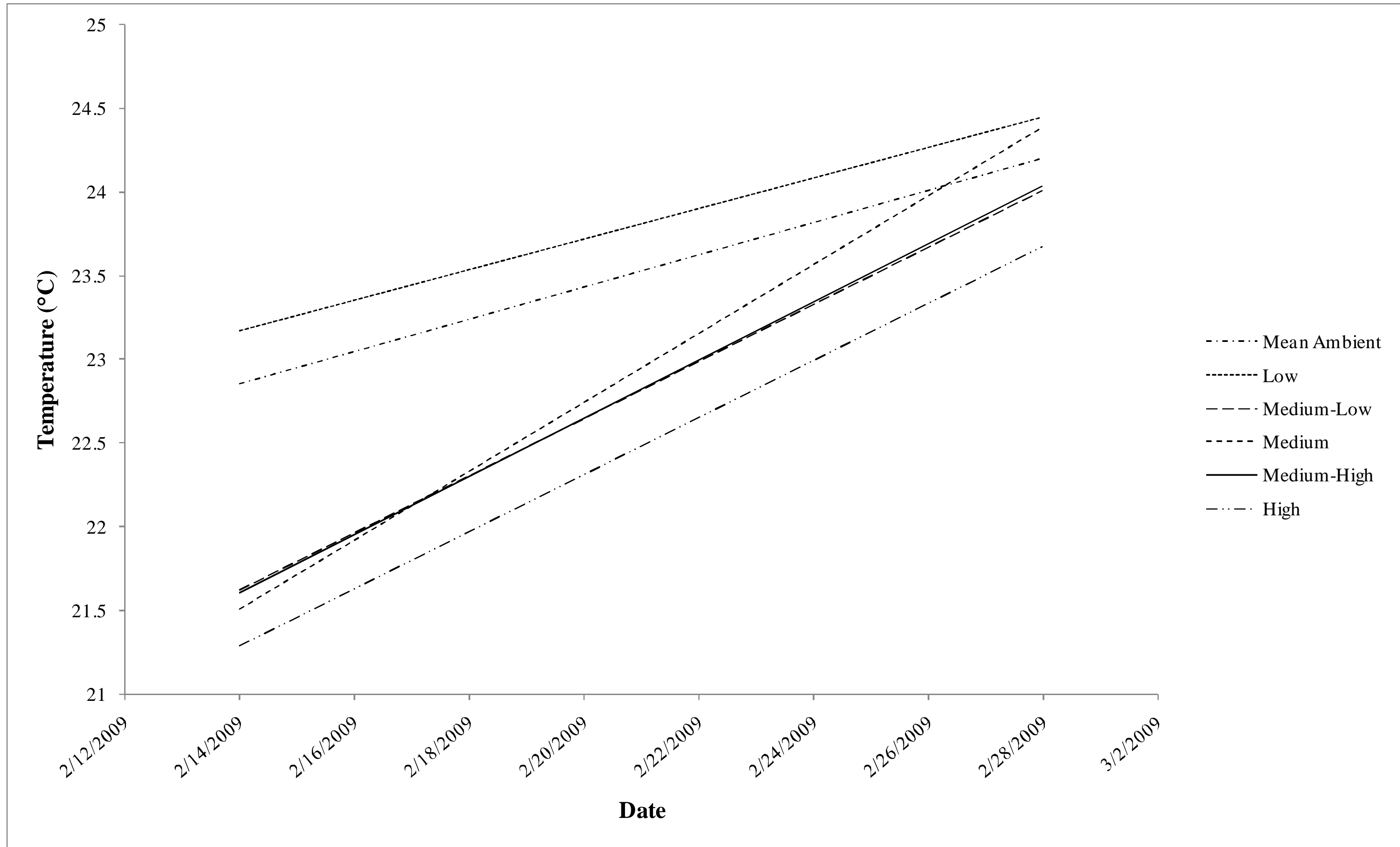
Soil compaction also affected the rate of pupal development of *L. sericata*, with rate of development decreasing with increasing soil compaction (Table 2.4). In general, flies in soil of low compaction took less time to develop than did flies in soil of high compaction. However, when all replicates were analyzed together, only the high-compaction treatment differed significantly from the others ($F_{5,21}$=6.67, p=0.0007). This difference in development rate was supported by the soil temperature recorded at the mean depth of pupation (Fig. 2.2). The mean development time of females (357.5 hours) was significantly longer than that of males (350.4 hours) ($F_{1,21}$=7.08, p=0.0146), and the effect of soil compaction on development time was the same for both females and males ($F_{5,21}$=0.09, p=0.9932); raw data in Appendix B. Mean rate of emergence of adults for all treatments and replicates was 94% ± 0.2 (Table 2.5).

Figure 2.1 Burrowing depths (cm) of larvae of *Lucilia sericata* from West Virginia in response to soil compaction (kg/cm^2), with linear regression for each replicate; 2008-2009.



Bullets represent depths at which puparia were found, and can represent multiple puparia recovered at that same depth.

Figure 2.2. Mean ambient and soil temperature (ºC) at the mean depth of pupation of *Lucilia sericata* from West Virginia, at different levels of soil compaction; 2009.



Low=0.017 ± 0.0004 kg/cm^2, Medium-Low=0.46 ± 0.05 kg/cm^2, Medium=0.95 ± 0.14 kg/cm^2, Medium-High=2.49 ± 0.12 kg/cm^2, High=4.33 ± 0.04 kg/cm^2.

Table 2.1. Mean burrowing depth (cm) ± SE of larvae of *Lucilia sericata* from West Virginia at different levels of soil compaction; 2008-2009.

Soil-Compaction Treatment					
Replicate	Low	Medium-Low	Medium	Medium-High	High
1	5.6 ± 0.4	-	-	-	1.0 ± 0.07
2	4.9 ± 0.4	-	-	-	0.7 ± 0.07
3	3.5 ± 0.5	1.5 ± 0.09	0.8 ± 0.08	0.5 ± 0.09	0.2 ± 0.03
4	3.6 ± 0.5	2.4 ± 0.1	2.9 ± 0.2	1.3 ± 0.13	0.02 ± 0.01

Low=0.017 ± 0.0004 kg/cm^2, Medium-Low=0.46 ± 0.05 kg/cm^2, Medium=0.95 ± 0.14 kg/cm^2, Medium-High=2.49 ± 0.12 kg/cm^2, High=4.33 ± 0.04 kg/cm^2. Replicates 1 and 2 had 10 containers/treatment, and replicates 3 and 4 had 5 containers/treatment with 5 larvae of *L. sericata*/container.

Table 2.2. Intercepts and slopes for each replicate of linear regression equations relating log (burrowing depth + 1) of larvae of *Lucilia sericata* from West Virginia to soil compaction; 2008-2009.

Replicate	Intercept	Slope
1	1.79 ± 0.045^{a}	-0.26^{a}
2	1.68 ± 0.056^{b}	-0.26^{a}
3	1.17 ± 0.045^{c}	-0.25^{a}
4	1.36 ± 0.034^{d}	-0.28^{a}

Different letters within a column (intercept or slope) indicate significant differences (α=0.05, Fisher’s Least Significant Difference test).

Table 2.3: Mean inter-pairwise distances[1] between puparia of *Lucilia sericata* from West Virginia in uncompacted ($\leq$ 0.025 kg/cm^2) and compacted (> 0.025 kg/cm^2) soil; 2008-2009.

	Soil Compaction (kg/cm^2)	
Replicate	$\leq$ 0.025	> 0.025
2	9.78[a]	3.79[b]
3	9.76[a]	3.82[b]
4	9.00[a]	5.64[b]

Different letters within a row (Replicates 2-4) indicate significant differences (α=0.05, Fisher's Least Significant Difference test). Each replicate had 10 containers/compaction category and replicates 3 and 4 had 5 containers/uncompacted and 20 containers/compacted category, with 5 larvae of *L. sericata*/container.

[1]No unit of measure is used, as this distance was calculated using the actual depth at which a puparium was found and x-y coordinates measured from a data-recording sheet.

Table 2.4. Mean time of development (hours) from egg to adult of *Lucilia sericata* from West Virginia at different levels of soil compaction; 2008-2009.

		Soil-Compaction Treatment					
Replicate	Mean Temperature (°C) ± SE	Low	Medium-Low	Medium	Medium-High	High	No Soil
1	24.4 ± 0.02	328.2[a]	-	-	-	336.6[b]	332.8[c]
2	28.4 ± 1.20	355.7[a]	-	-	-	372.3[b]	361.3[a]
3	24.2 ± 0.03	340.0[a]	345.7[abc]	352.1[cd]	350.7[bcd]	356.6[d]	342.5[ab]
4	23.6 ± 0.04	368.8[a]	368.0[a]	366.7[a]	377.1[b]	404.2[c]	364.3[a]

Low=0.017 ± 0.0004 kg/cm^2, Medium-Low=0.46 ± 0.05 kg/cm^2, Medium=0.95 ± 0.14 kg/cm^2, Medium-High=2.49 ± 0.12 kg/cm^2, High=4.33 ± 0.04 kg/cm^2. Different letters within a row (Replicates 1-4) indicate significant differences (α=0.05, Fisher's Least Significant Difference test).

Table 2.5. Percent emergence and male:female ratios[1] of adults of *Lucilia sericata* from West Virginia at different levels of soil compaction; 2008-2009.

	Soil-Compaction Treatment					
Replicate	Low	Medium-Low	Medium	Medium-High	High	No Soil
1	90%, 26:19	-	-	-	94%, 27:19	98%, 17:32
2	84%, 17:17	-	-	-	94%, 25:15	96%, 14:14
3	92%, 9:13	96%, 8:11	100%, 6:19	92%, 8:12	88%, 9:13	84%, 9:3
4	96%, 14:8	92%, 12:10	96%, 11:13	100%, 17:6	100%, 10:10	96%, 11:12

Low=0.017 ± 0.0004 kg/cm^2, Medium-Low=0.46 ± 0.05 kg/cm^2, Medium=0.95 ± 0.14 kg/cm^2, Medium-High=2.49 ± 0.12 kg/cm^2, High=4.33 ± 0.04 kg/cm^2. Replicates 1 and 2 had 10 containers/treatment, and replicates 3 and 4 had 5 containers/treatment, with 5 larvae of *L. sericata*/container.

[1]Ratios might not equal 50 because some flies escaped or were damaged before sexing.

Field validation of the model for burrowing depth of Lucilia sericata in soil

In replicate one, 4 of 100 puparia were located. The high-compaction treatment (> 4.5 kg/cm^2) prevented the larvae of *L. sericata* from burrowing. The larvae did not burrow within 20 hours after being placed in the arenas, and were preyed on by adults of *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (A.S. Tebeau, personal observation). Therefore, no puparia were recovered from the high-compaction treatment. One larva was recovered from the sandy beach (0.40 kg/cm^2) and fallow-field treatments (1.5 kg/cm^2), and two larvae were recovered from the forest-floor site (0.75 and 1.25 kg/cm^2). In replicate two, 1 of 75 puparia was located. Again the high-compaction treatment (> 4.5 kg/cm^2) prevented the larvae from burrowing. The larvae had not burrowed within one hour of being placed in the arenas, when it began to rain heavily. Thirteen hours later, no larvae were visible in the arenas; suggesting that they either escaped from the arenas, were drowned by the rainfall or preyed upon. Within 10 minutes after being placed in the arenas all larvae in the forest-floor treatment had burrowed, and all but 2 larvae in the fallow-field treatment had burrowed. The remaining two larvae were preyed on by *S. invicta*. The one puparium recovered was from the forest-floor site (0.80 kg/cm^2). The model of larval burrowing depth, where d is depth (cm), e is inverse natural log, and C is soil compaction (kg/cm^2):

$$d = e^{[(1.462 \pm 0.03) - (0.255 \pm 0.01)C]}$$

overestimated the depth of burrowing by larvae of *L. sericata* in the field (Table 3.1).

Table 3.1. Observed and predicted (95% confidence interval) burrowing depths (cm) of larvae of *Lucilia sericata* from West Virginia, with respect to soil compaction (kg/cm^2) in field trials in Upstate South Carolina; 2009.

Compaction (kg/cm^2)	Observed Depth	Predicted Depth
1.50	1.0	2.9-3.0
1.25	2.0	3.1-3.2
0.80	2.5	3.4-3.6
0.75	2.5	3.5-3.6
0.40	3.0	3.8-4.0

DISCUSSION

Effect of Nasonia vitripennis on pupation behavior of Lucilia sericata

The lack of a parasitoid effect on burrowing depth of *L. sericata* is opposite expectation. The sedentary pupal stage occupies approximately 50% of blow fly development, which puts the developing fly at risk of predation and parasitism. Many organisms have evolved to exploit this life stage (Greenberg & Kunich 2002), and the presence of parasitoids should influence the behavior of blow fly larvae, which would be under pressure to escape parasitism. These changes in host behavior in response to parasitoid presence are known as non-consumptive effects (Peckarsky et al. 2008). Ullyett (1950) stated that *L. sericata* is the preferred host of *N. vitripennis* and burrows into soil to escape parasitism. However, no experimental evidence or citation supported that claim. Ullyett (1950) studied only the burrowing depth of larvae of *L. sericata* and inferred from where they pupated in the soil that they would be safe from parasitism. This conclusion was based on the assumption that females of *Nasonia* do not burrow. Possibly these species have coexisted long enough that *L. sericata* has evolved a behavioral mechanism, burrowing into the soil, to avoid parasitism by this common parasitoid. Although *L. sericata* is the preferred host of *N. vitripennis* (Ullyett 1950), it is not the only host (Noyes 2009). Differences in pupation behavior exist between *L. sericata* and these other host species (Norris 1959). As seen in my experiments, some larvae of *L. sericata* will pupate on the surface of soil in which conspecific larvae have burrowed. In the New World, introduced *Chrysomya* spp. that pupate on the soil surface are hosts of *N. vitripennis* (Cammack & Nelder in review). These other hosts and surface-pupating

calliphorines and chrysomyines are probably abundant enough to sustain populations of *N. vitripennis*.

The lack of parasitism of puparia below the soil surface also is opposite expectation. Based on the experiments by Edwards (1954), I predicted that parasitism should occur both above and below the soil. When larvae of *L. sericata* were placed in arenas, their crawling and burrowing activity presumably would have left chemical trails of the decomposing liver on which they were feeding. These trails could have led females of *N. vitripennis* to the host puparia, but possibly were diluted by the soil, or the decomposition fluids from the liver were wiped from the larvae by their movements. However, these diluted or incomplete scent trails might be an artifact of my laboratory experiment. In a natural setting, larval blow flies disperse from their food source en masse. Large numbers of larvae transport material from the food source and appear to create a trail of decomposition fluids leading to the spot where they burrow and pupate (JA Cammack, personal observation). Because of the possible large amount of this material, these decomposition fluids likely would seep into the ground, especially through larval burrowing. Thus, a scent trail would exist for females of *N. vitripennis* to follow.

The lack of an effect of parasitoids on the spatial distribution of puparia in the low-compaction treatment is expected. However, the effect in the high-compaction treatment (clumped pupation when females of *N. vitripennis* were present) is opposite expectation. I would predict that larvae of *L. sericata* should show either no difference or an increase in spacing when *N. vitripennis* is present. Females of *N. vitripennis* locate

host puparia through chemical and visual cues (Edwards 1954). If puparia are clumped, the chemical cues emanating from them would be stronger than if the larvae had pupated singly. Clumped pupation behavior would increase the likelihood that a female of *N. vitripennis* would locate a host puparium. Once the female wasp finds a puparium, it then could find others in close proximity through visual cues. The strategy of pupating closer together, therefore, has a potential negative effect on the developing flies. Although the developing flies are at a higher risk of parasitism, the parasitoids might also 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. 2008). If host puparia are clumped, multiple female parasitoids likely will find the puparia, which can result in superparasitism or parasitism by multiple females. If superparasitism occurs, the sex ratio of the resulting offspring will be male-biased (Werren 1980, Barbosa et al. 2008b), and the developing parasitoids likely will compete for resources within the host. Both a reduction in the number of progeny per host and the increase in proportion of males would have negative fitness effects for *N. vitripennis*.

The difference in development rate of *L. sericata* in the presence and absence of *N. vitripennis* provides insight into the ability of a parasitoid to manipulate host biology and behavior (non-consumptive effects). In both soil-compaction treatments in replicate two when *N. vitripennis* was present, the duration of fly development was significantly shorter than when *N. vitripennis* was absent. Larvae in the presence of females of *N. vitripennis* might have expended more energy while burrowing to escape the parasitoids than would larvae in the absence of *N. vitripennis*. An increase in energy expenditure

could have caused pupation to occur sooner because emptying of the crop is thought to trigger pupation in blow flies (Grenberg & Kunich 2002). However, the difference in rate of development across replicates suggests that more work on this system is warranted before conclusions can be drawn on the effects of parasitoids on development of *L. sericata*.

In conclusion, the presence of *N. vitripennis* affects some aspects of the pupation behavior of larvae of *L. sericata*. Burrowing depth by larvae is not affected. Other hosts of *N. vitripennis*, such as *Chrysomya rufifacies*, with different pupation habits are possibly becoming preferred hosts because they are more accessible for parasitism. This hypothesis could be tested on *C. rufifacies*, using similar experiments. The dispersal behavior of blow fly larvae in the field might facilitate host location by females of *N. vitripennis.* In higher levels of soil compaction, larvae of *L. sericata* pupate closer together when females of *N. vitripennis* are present than when absent, which might have negative effects on both the pupal stage of *L. sericata* and the parasitoids. My research also suggests that development rate of *L. sericata* is increased by parasitoid presence, possibly as a means to avoid parasitism.

Effect of soil compaction on pupation behavior of Lucilia sericata

Burrowing 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 (Cammack, unpublished data). Ullyett (1950) found puparia of *L. sericata* up to 14 cm deep, but 80% burrowed no deeper than 8.9 cm, which is similar to my results (86% of larvae pupated at less than 9 cm deep). As soil compaction increases, the larva requires more energy to penetrate the soil surface and continue burrowing. Due to this increase in energy expenditure, larvae might stop burrowing when they have depleted the contents of their crop, which is believed to trigger pupation (Greenberg & Kunich 2002). As the larvae disperse from the food source to locate a pupation site, the contents of the crop are used and also become stored as fat to supply the developing pupa with energy. Once the crop is empty, pupation begins (Greenberg & Kunich 2002). Perhaps if more food were available in the crop, the larvae might have burrowed deeper in more compact soil.

The available pore space in the soil also might contribute to the negative correlation between soil compaction and burrowing depth. Pore space is negatively correlated with soil compaction; as compaction increases, pore space decreases (Babercheck 1992). This decreased pore space reduces gas exchange in the soil and thus less oxygen is available (Brady & Weil 2008) for the developing fly. Larvae of *L. sericata* and other cyclorrhaphan Diptera might have evolved a behavioral response to this decreased availability of oxygen in compact soil by pupating closer to the soil surface

where more oxygen is available. However, pupating closer to or on the soil surface can have a negative effect on the developing fly because the pupa is more susceptible to predation and parasitism (Guillen et al. 2002).

The spatial distribution of puparia of *L. sericata* in soil is affected by soil compaction. Larvae that burrow in uncompacted soil (≤ 0.025 kg/cm^2) are less clumped in distribution than larvae that burrow in soil compacted more than 0.025 kg/cm^2. The clumped distribution of puparia in compact soil might be the result of one or more larvae following the burrow of another larva. When a larva penetrates the soil surface and burrows, a tunnel is created that has less resistance than the surrounding soil. When other larvae encounter an area of less resistance, they likely would use it to enter the soil. The clumped distribution also might be the result of communal burrowing. In compacted soil, larvae might work in the same spot to try to penetrate the surface. This cooperation might be a form of kin selection because it likely would increase the rate at which the larvae enter the soil and reduce the probability of predation and parasitism. However, larvae possibly burrow in one area because it has less resistance than the rest of the soil (either because a larva has already broken through the surface or from the physical characteristics of the soil). I observed communal burrowing behavior in soil of high compaction (Figure 2.3).

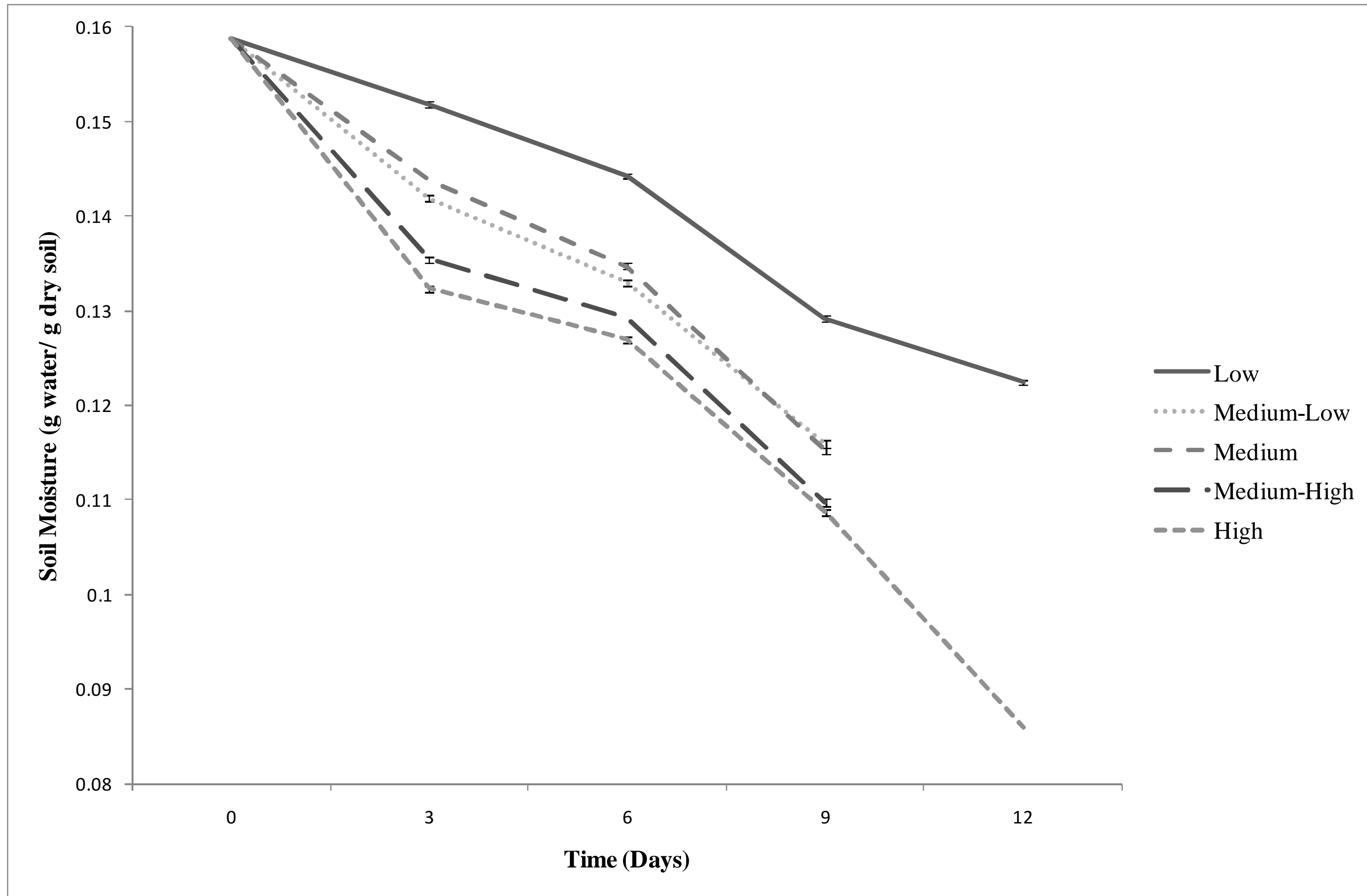
The rate of pupal development of *L. sericata* also is influenced by physical properties of the soil. Development took longer in high-compaction soil. Soil of low compaction has larger pores than soil of higher compaction, which means more air is present in soil of low compaction (Babercheck 1992). Because 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. Soil of high compaction will have a temperature lower than ambient and less compact soils. I predicted that more compact soils retain more water than soils that are less compact due to capillary forces and would have a lower temperature due to the difference in the specific heat between water, soil, and air (water > soil > air). However, the soil moisture-content at the mean depth of pupation was opposite expected; soil of lower compaction retained more water than did compact soil (Fig. 2.4). Because of this difference in moisture levels, I suspect the difference in soil temperature at the mean depth of pupation (Fig. 2.2) is due to the amount of air in the soil.

In conclusion, soil compaction affects the pupation behavior of *L. sericata*. Burrowing by larvae to locate a pupation site is negatively affected by soil compaction; as soil compaction increases, burrowing depth decreases. Soil compaction greater than 0.025 kg/cm^2 results in larvae of *L. sericata* pupating closer together, but the mechanism driving this behavior is unknown. Pupal development also is affected by soil compaction, presumably due to differences in pore space between uncompacted and compacted soils; flies that develop in highly compacted soil (4.33 ± 0.04 kg/cm^2) take significantly longer to complete the pupal stage than do flies that develop in soils with lower compaction.

Figure 2.3. Communal burrowing by larvae of *Lucilia sericata* from West Virginia in soil of high compaction (4.33 ± 0.04 kg/cm^2); 2009.

Figure 2.4. Soil-moisture content (g water/g dry soil) (± SE) at the mean depth of pupation of *Lucilia sericata* from West Virginia, at different levels of soil compaction over 12 days; 2009.



Low=0.017 ± 0.0004 kg/cm^2, Medium-Low=0.46 ± 0.05 kg/cm^2, Medium=0.95 ± 0.14 kg/cm^2, Medium-High=2.49 ± 0.12 kg/cm^2, High=4.33 ± 0.04 kg/cm^2. Each time point per soil-compaction treatment represents one container.

Field validation of the model for burrowing depth of Lucilia sericata in soil

The low proportion (~3%) of puparia recovered from field trials suggests that the larvae either burrowed deeper than 11 cm or burrowed laterally (i.e., outside the confines of the arena). A larva burrowing deeper than 11 cm is unlikely because only one of 650 larvae pupated at 11 cm (the bottom of an experiment container) in my laboratory experiments. Lateral burrowing by the larvae is more likely. In 38 of 130 containers (29%) in my laboratory experiments, tunnels were visible on the sides of the containers. These tunnels suggest that the larvae burrowed laterally and then downward after encountering a side. In the field trials, no container was present to prevent the larvae from continuing to burrow laterally.

In the field, I would expect shallower burrowing by larvae of *L. sericata* than in the laboratory due to the nonhomogenous nature of soil. Soil in the field contains objects such as rocks and roots that would impede larval burrowing. When a burrowing larva encounters these objects, much like when one encountered the container side in my laboratory experiments, it would have to change the direction of burrowing. When burrowing laterally, a larva would use energy that otherwise would be applied to downward burrowing. Because emptying the crop is thought to trigger pupation (Greenberg & Kunich 2002), a larva that burrows laterally should pupate at a shallower depth than one that burrows downward.

Although the model does not accurately predict larval burrowing depth, the overestimation, nonetheless, would result in location of the puparia, because it would not be possible to sample at only the predicted depth without first removing the overlying

soil. The inaccuracy of the model might be a result of the difference in homogeneity between soil in my laboratory and field experiments. A larger sample size, however, might show the model to be more accurate in predicting burrowing depth of *L. sericata*, with respect to soil compaction.

SUMMARY AND SUGGESTIONS FOR FUTURE RESEARCH

Females of *Nasonia vitripennis* and soil compaction both affect the pupation behavior of *Lucilia sericata*. Although parasitoids do not significantly affect burrowing depth, larvae pupate closer together and pupae develop faster in the presence of *N. vitripennis*. Burrowing depth of larvae is inversely related to soil compaction; larvae burrow deeper in less compact soil. Larvae pupate closer together and pupae develop slower in soil of high compaction. An applied goal of this research was to develop a model of burrowing depth for *L. sericata* in soil. Although the model overestimates burrowing depth, it still will allow investigators to locate puparia in the soil at a body-recovery scene.

As a result of my research, forensic entomologists and investigators can now more accurately locate blow fly puparia in the soil at a body-recovery scene. A pocket penetrometer, ruler, and garden trowel should be added to the forensic investigator's evidence-collection kit. From studies by Greenberg (1990) and Tessmer & Meek (1996), forensic investigators can determine the direction and distance post-feeding blow fly larvae travel from a corpse. Once forensic investigators have found the location of pupation, the pocket penetrometer can be used to measure soil compaction. The compaction can be applied to the model $\text{Depth} = e^{[(1.462 \pm 0.03) - (0.255 \pm 0.01)\text{Compaction}]}$ to determine how deep to dig for puparia of *L. sericata*. Once these puparia are located, they should be isolated in vials in the laboratory until emergence of adult blow flies or parasitoids. If parasitoids are reared from the puparia, estimation of the PIA might be inaccurate because of the influence of parasitoid presence on blow fly development. I

recommend that forensic entomologists decrease their PIA estimate of the fly species from which *N. vitripennis* was reared. For example, the PIA estimate of *L. sericata* should be decreased by 276–436 accumulated degree hours. (Development of *L. sericata* was faster by 15.0-23.7 hours when in the presence of *N. vitripennis*; multiplication of these values by the rearing temperature (28.4°C) after subtracting the lower development threshold (10°C) results in this range). If adults of *L. sericata* are collected emerging from soil at a body-recover scene, the compaction of the soil from which they are emerging should be measured. If this compaction is ≥ 4.33 kg/cm^2, the PIA estimate should be increased by 160-286 ADH. (Development of *L. sericata* was slower by 10.5-18.8 hours in soil of high compaction; multiplication of these values by the rearing temperature (25.2°C) after subtracting the lower development threshold (10°C) results in this range).

The interaction between blow flies and their parasitoids, as well as the interaction between these insects and soil, should continue to be studied. Blow flies commonly burrow in soil to pupate, and associated parasitoids of are commonly collected from carrion (Norris 1959). As demonstrated by my research, both of these phenomena influence the development of blow flies. Additional information on the influence of other characteristics of soil, such as moisture and type (i.e., sand, silt, and clay content), on the behavior and bionomics of forensically important insects is needed to advance the practice of forensic entomology. The accuracy of the model of blow fly pupation depth also might be increased by including these characteristics of soil (Hennessey 1994), as well ambient temperature (Gomes et al. 2009), photoperiod (Warman & Lewis 1997) and other environmental characteristics in a multiple regression model. Because of the

increasing use of forensic entomology (Benecke 1998, Greenberg & Kunich 2002) and the likelihood that these insects will be associated with a corpse (Anderson & Cervenka 2002), further study of the effects of soil and parasitoids on the development of blow flies is necessary to estimate the post-colonization interval more accurately.

APPENDICES

Appendix A

Burrowing Depth Data

Burrowing depth (cm) of larvae of *Lucilia sericata* from West Virginia, with or without *Nasonia vitripennis* from South Carolina, at soil of different compactions; 2008-2009. Compaction treatments: L=low (0.017 ± 0.0004 kg/cm^2), LP=low (0.0183 ± 0.001 kg/cm^2) + *Nasonia*, ML=medium-low (0.46 ± 0.05 kg/cm^2), M=medium (0.95 ± 0.14 kg/cm^2), MH=medium-high (2.49 ± 0.12 kg/cm^2), H=high (4.33 ± 0.04 kg/cm^2), HP=high (4.38 ± 0.05 kg/cm^2) + *Nasonia*.

Experiment	Compaction Treatment	Compaction (kg/cm^2)	Depth (cm)
1	L	0.015625	3
1	L	0.015625	2.5
1	L	0.015625	3.5
1	L	0.015625	7
1	L	0.015625	10
1	L	0.01875	3
1	L	0.01875	3.5
1	L	0.01875	5
1	L	0.01875	5.5
1	L	0.01875	7.25
1	L	0.03125	3
1	L	0.03125	3.5
1	L	0.03125	5
1	L	0.03125	9.25
1	L	0.03125	10.25
1	L	0.015625	2.5
1	L	0.015625	3
1	L	0.015625	4.5
1	L	0.015625	6
1	L	0.015625	7
1	L	0.0125	3
1	L	0.0125	4.25
1	L	0.0125	4.25
1	L	0.0125	5
1	L	0.0125	9.5
1	L	0.015625	3.25
1	L	0.015625	6

1	L	0.015625	6
1	L	0.015625	10
1	L	0.015625	10.25
1	L	0.01875	2.5
1	L	0.01875	3.5
1	L	0.01875	6
1	L	0.01875	7
1	L	0.01875	9.5
1	L	0.015625	3
1	L	0.015625	4
1	L	0.015625	4
1	L	0.015625	8.25
1	L	0.015625	9
1	L	0.015625	3
1	L	0.015625	4
1	L	0.015625	7.5
1	L	0.015625	8
1	L	0.015625	10.5
1	L	0.015625	2
1	L	0.015625	4.75
1	L	0.015625	6
1	L	0.015625	7
1	LP	0.015625	1.5
1	LP	0.015625	2
1	LP	0.015625	6
1	LP	0.015625	6.75
1	LP	0.015625	10
1	LP	0.01875	1.25
1	LP	0.01875	2
1	LP	0.01875	2.5
1	LP	0.01875	9
1	LP	0.01875	9
1	LP	0.015625	3.75
1	LP	0.015625	5
1	LP	0.015625	6.25
1	LP	0.015625	7
1	LP	0.01875	4.5
1	LP	0.01875	5.5

1	LP	0.01875	6.25
1	LP	0.01875	7.5
1	LP	0.01875	8
1	LP	0.21875	4.75
1	LP	0.21875	5
1	LP	0.21875	6.5
1	LP	0.21875	9.5
1	LP	0.21875	10
1	LP	0.015625	3
1	LP	0.015625	3.75
1	LP	0.015625	4.75
1	LP	0.015625	5
1	LP	0.015625	6
1	LP	0.015625	3.5
1	LP	0.015625	4
1	LP	0.015625	5.5
1	LP	0.015625	7
1	LP	0.015625	10
1	LP	0.015625	1.75
1	LP	0.015625	3.25
1	LP	0.015625	8
1	LP	0.015625	8
1	LP	0.015625	8.5
1	LP	0.0125	4
1	LP	0.0125	6
1	LP	0.0125	7.5
1	LP	0.0125	8
1	LP	0.01875	2.5
1	LP	0.01875	4.5
1	LP	0.01875	5
1	LP	0.01875	8.5
1	LP	0.01875	9.5
1	H	4.5	0.75
1	H	4.5	1
1	H	4.5	1
1	H	4.5	1
1	H	4.5	1.25
1	H	4.5	1

1	H	4.5	1
1	H	4.5	1
1	H	4.5	1
1	H	4.5	1.25
1	H	4.5	0.5
1	H	4.5	0.5
1	H	4.5	0.75
1	H	4.5	1
1	H	4.4	1
1	H	4.4	1.25
1	H	4.4	1.5
1	H	4.4	1.5
1	H	4.4	1.75
1	H	4.3	0.5
1	H	4.3	1
1	H	4.3	1.5
1	H	4.3	1.5
1	H	4.3	2
1	H	4.5	0.75
1	H	4.5	0.5
1	H	4.5	0.75
1	H	4.5	1.25
1	H	4.5	1
1	H	4.4	0.25
1	H	4.4	0.5
1	H	4.4	0.5
1	H	4.4	0.5
1	H	4.4	0.5
1	H	4.3	0.25
1	H	4.3	0.5
1	H	4.3	1
1	H	4.3	0.75
1	H	4.3	1.5
1	H	4.5	0.5
1	H	4.5	1
1	H	4.5	1
1	H	4.5	1.25
1	H	4.5	1.5

1	H	1.5	1.5
1	H	1.5	1.5
1	H	1.5	1.75
1	H	1.5	1.5
1	H	1.5	2.25
1	HP	4.5	0.75
1	HP	4.5	1
1	HP	4.5	1
1	HP	4.5	1.25
1	HP	4.5	1.5
1	HP	4.4	0.5
1	HP	4.4	1.75
1	HP	4.4	1.5
1	HP	4.4	0.25
1	HP	4.4	0.5
1	HP	4.4	0.75
1	HP	4.4	1
1	HP	4.4	2
1	HP	4.4	2
1	HP	4.3	0.25
1	HP	4.3	1
1	HP	4.3	1
1	HP	4.3	1.25
1	HP	4.25	0.75
1	HP	4.25	1
1	HP	4.25	1.5
1	HP	4.25	2
1	HP	4.25	1.5
1	HP	3.5	0.25
1	HP	3.5	0.25
1	HP	3.5	0.75
1	HP	3.5	1
1	HP	3.5	1.25
1	HP	4.4	0.5
1	HP	4.4	0.5
1	HP	4.4	0.75
1	HP	4.4	1
1	HP	4.4	1.25

1	HP	4.4	0.5
1	HP	4.4	1
1	HP	4.4	1.25
1	HP	4.4	1.25
1	HP	4.4	1.5
1	HP	4.5	0.25
1	HP	4.5	0.5
1	HP	4.5	0.75
1	HP	4.5	1.25
1	HP	4.5	1.5
1	HP	4.5	0.75
1	HP	4.5	1
1	HP	4.5	1
1	HP	4.5	1
1	HP	4.5	1.5
2	L	0.0125	3
2	L	0.0125	3.5
2	L	0.0125	10
2	L	0.0125	11
2	L	0.0125	3.5
2	L	0.0125	4
2	L	0.0125	8
2	L	0.0125	9
2	L	0.0125	10
2	L	0.0125	2
2	L	0.0125	3.5
2	L	0.0125	8
2	L	0.0125	8.25
2	L	0.0125	10
2	L	0.0125	2.5
2	L	0.0125	3.75
2	L	0.0125	4
2	L	0.0125	5.25
2	L	0.0125	6
2	L	0.015625	2
2	L	0.015625	2.75
2	L	0.015625	3.5
2	L	0.015625	6.25

2	L	0.015625	8.5
2	L	0.0125	2
2	L	0.0125	5.75
2	L	0.0125	6
2	L	0.0125	8
2	L	0.0125	9.5
2	L	0.01875	2
2	L	0.01875	2
2	L	0.01875	2.5
2	L	0.01875	4
2	L	0.01875	4.75
2	L	0.0125	1.25
2	L	0.0125	3
2	L	0.0125	3.5
2	L	0.0125	6
2	L	0.0125	9.25
2	L	0.025	1
2	L	0.025	1.5
2	L	0.025	3
2	L	0.025	4.25
2	L	0.025	5.5
2	L	0.015625	2
2	L	0.015625	2.25
2	L	0.015625	2.5
2	L	0.015625	5
2	L	0.015625	8
2	LP	0.015625	1.25
2	LP	0.015625	4.5
2	LP	0.015625	4.5
2	LP	0.015625	6
2	LP	0.015625	6.5
2	LP	0.01875	0.75
2	LP	0.01875	2.25
2	LP	0.01875	2.25
2	LP	0.01875	4
2	LP	0.01875	9.5
2	LP	0.0125	2.25
2	LP	0.0125	8.25

2	LP	0.0125	9.5
2	LP	0.0125	9.5
2	LP	0.0125	10
2	LP	0.015625	1.75
2	LP	0.015625	2.25
2	LP	0.015625	2
2	LP	0.015625	7
2	LP	0.015625	9.5
2	LP	0.025	2
2	LP	0.025	6.25
2	LP	0.025	8
2	LP	0.025	9
2	LP	0.03125	2.75
2	LP	0.03125	3
2	LP	0.03125	3
2	LP	0.03125	8
2	LP	0.03125	9
2	LP	0.0125	1.5
2	LP	0.0125	2.75
2	LP	0.0125	3
2	LP	0.0125	4.5
2	LP	0.0125	9
2	LP	0.01875	1
2	LP	0.01875	1.75
2	LP	0.01875	2
2	LP	0.01875	3
2	LP	0.01875	7.5
2	LP	0.03125	0.5
2	LP	0.03125	3
2	LP	0.03125	4.5
2	LP	0.03125	8.5
2	LP	0.03125	8.5
2	LP	0.01875	2.75
2	LP	0.01875	4
2	LP	0.01875	6.75
2	LP	0.01875	9.5
2	LP	0.01875	10.5
2	H	4.4	1

2	H	4.4	1
2	H	4.4	1
2	H	4.4	1.25
2	H	4.4	1.5
2	H	4.5	0.25
2	H	4.5	0.5
2	H	4.5	0.5
2	H	4.5	0.5
2	H	4.5	1
2	H	4.4	0.5
2	H	4.4	0.75
2	H	4.4	1
2	H	4.4	1
2	H	4.4	0.5
2	H	4.4	0
2	H	4.4	0.25
2	H	4.4	0.5
2	H	4.4	0.5
2	H	4.4	1
2	H	4.5	0.25
2	H	4.5	0.25
2	H	4.5	0.25
2	H	4.5	0.75
2	H	4.5	0.75
2	H	4.3	0.25
2	H	4.3	0.75
2	H	4.3	0.75
2	H	4.3	0.75
2	H	4.3	1
2	H	4.25	1.5
2	H	4.25	2
2	H	4.25	2
2	H	4.25	1.5
2	H	4.25	2
2	H	4.4	0.25
2	H	4.4	0.5
2	H	4.4	0.5
2	H	4.4	0.5

2	H	4.4	0.75
2	H	4.4	0
2	H	4.4	0
2	H	4.4	0.5
2	H	4.4	0.75
2	H	4.4	1
2	H	4.3	0.25
2	H	4.3	0.5
2	H	4.3	0.75
2	H	4.3	1
2	H	4.3	1.25
2	HP	4.5	0.5
2	HP	4.5	0.75
2	HP	4.5	1
2	HP	4.5	1.25
2	HP	4.4	0.5
2	HP	4.4	0.5
2	HP	4.4	0.75
2	HP	4.4	0.75
2	HP	4.4	1
2	HP	4.5	1
2	HP	4.5	0.75
2	HP	4.5	1
2	HP	4.5	0.75
2	HP	4.5	1
2	HP	4.4	0.25
2	HP	4.4	0.25
2	HP	4.4	0.25
2	HP	4.4	0.5
2	HP	4.4	0.75
2	HP	4.5	0
2	HP	4.5	0
2	HP	4.5	0
2	HP	4.5	0
2	HP	4.5	0.25
2	HP	4.3	0.5
2	HP	4.3	0.75
2	HP	4.3	1

2	HP	4.3	1
2	HP	4.3	1.5
2	HP	4.4	0.5
2	HP	4.4	0.75
2	HP	4.4	1
2	HP	4.4	1
2	HP	4.4	2
2	HP	4.5	0
2	HP	4.5	0
2	HP	4.5	0.25
2	HP	4.5	0.5
2	HP	4.5	0.5
2	HP	4.5	0.25
2	HP	4.5	0.25
2	HP	4.5	0.5
2	HP	4.5	0.5
2	HP	4.5	0.5
2	HP	4.5	0
2	HP	4.5	0.25
2	HP	4.5	0.5
2	HP	4.5	0.75
2	HP	4.5	1
3	L	0.01875	1.5
3	L	0.01875	1.75
3	L	0.01875	4
3	L	0.01875	4.5
3	L	0.01875	6
3	L	0.025	1
3	L	0.025	1
3	L	0.025	2.75
3	L	0.025	3.5
3	L	0.025	9.75
3	L	0.025	0.75
3	L	0.025	2.5
3	L	0.025	5.5
3	L	0.025	6.25
3	L	0.01875	0.5
3	L	0.01875	1

3	L	0.01875	3.75
3	L	0.01875	4.75
3	L	0.01875	7
3	L	0.01875	1
3	L	0.01875	3.25
3	L	0.01875	4.5
3	ML	0.8	1
3	ML	0.8	1.25
3	ML	0.8	1
3	ML	0.8	1.75
3	ML	1.2	1
3	ML	1.2	1
3	ML	1.2	1
3	ML	1.2	2
3	ML	1.2	2.25
3	ML	0.5	1.25
3	ML	0.5	1.25
3	ML	0.5	1.75
3	ML	0.5	2
3	ML	0.5	2
3	ML	0.75	1
3	ML	0.75	1.25
3	ML	0.75	1.5
3	ML	0.75	1.75
3	ML	0.75	2.5
3	ML	0.6	1
3	ML	0.6	1.5
3	ML	0.6	1.5
3	ML	0.6	1.75
3	ML	0.6	2
3	M	1.2	0.75
3	M	1.2	1.25
3	M	1.2	1.5
3	M	1.2	1.75
3	M	1.25	0.5
3	M	1.25	1
3	M	1.25	0.75
3	M	1.25	0.75

3	M	1.25	1
3	M	1.8	0.75
3	M	1.8	0.5
3	M	1.8	0.75
3	M	1.8	0.75
3	M	1.8	1.5
3	M	1.7	0.5
3	M	1.7	1
3	M	1.7	1
3	M	1.7	1
3	M	1.7	1
3	M	3	0
3	M	3	0.25
3	M	3	0.5
3	M	3	0.75
3	M	3	0.75
3	MH	2	0.5
3	MH	2	0.5
3	MH	2	0.75
3	MH	2	0.5
3	MH	2	0.5
3	MH	2.2	0.25
3	MH	2.2	0.25
3	MH	2.2	0.5
3	MH	2.2	0.5
3	MH	2.2	0.75
3	MH	3.5	0
3	MH	3.5	0
3	MH	3.5	0.25
3	MH	3.5	0.5
3	MH	3.5	0.5
3	MH	4	0
3	MH	4	0
3	MH	4	0
3	MH	4	0.25
3	MH	2	1
3	MH	2	1.25
3	MH	2	1.25

3	MH	2	1.5
3	MH	2	1.5
3	H	4.5	0.25
3	H	4.5	0.25
3	H	4.5	0.25
3	H	4.5	0.25
3	H	4.5	0.25
3	H	4.4	0.25
3	H	4.4	0.25
3	H	4.4	0.25
3	H	4.4	0.5
3	H	4.4	0.5
3	H	4.4	0
3	H	4.4	0.25
3	H	4.4	0.25
3	H	4.4	0.25
3	H	4.4	0.25
3	H	4.5	0
3	H	4.5	0
3	H	4.5	0
3	H	4.5	0.25
3	H	4.5	0.25
3	H	4.5	0
3	H	4.5	0
3	H	4.5	0
3	H	4.5	0
3	H	4.5	0.25
4	L	0.015	1
4	L	0.015	1.5
4	L	0.015	1.5
4	L	0.015	5
4	L	0.015	6.5
4	L	0.01625	1.25
4	L	0.01625	1.25
4	L	0.01625	2
4	L	0.01625	4
4	L	0.01625	5
4	L	0.025	2

4	L	0.025	3
4	L	0.025	4
4	L	0.025	7.25
4	L	0.025	10
4	L	0.0156	2
4	L	0.0156	2
4	L	0.0156	2.75
4	L	0.0156	3.5
4	L	0.0156	10
4	L	0.0156	2
4	L	0.0156	3
4	L	0.0156	3
4	L	0.0156	4
4	L	0.0156	.
4	ML	0.0775	1
4	ML	0.0775	2
4	ML	0.0775	2
4	ML	0.0775	2.5
4	ML	0.0775	3.75
4	ML	0.0625	2
4	ML	0.0625	2
4	ML	0.0625	2.5
4	ML	0.0625	2
4	ML	0.0625	3
4	ML	0.5	1
4	ML	0.5	2.25
4	ML	0.5	2
4	ML	0.5	2
4	ML	0.5	2.5
4	ML	0.09375	2
4	ML	0.09375	3
4	ML	0.09375	3
4	ML	0.09375	3.5
4	ML	0.09375	3.25
4	ML	0.0468	2
4	ML	0.0468	2.75
4	ML	0.0468	3
4	ML	0.0468	3

4	ML	0.0468	3
4	M	0.08	1.75
4	M	0.08	2.75
4	M	0.08	4
4	M	0.08	4.5
4	M	0.08	5
4	M	0.15625	1.5
4	M	0.15625	1.5
4	M	0.15625	3
4	M	0.15625	3
4	M	0.15625	4
4	M	0.125	1.5
4	M	0.125	1.75
4	M	0.125	2.75
4	M	0.125	3
4	M	0.125	
4	M	0.1375	2.5
4	M	0.1375	2
4	M	0.1375	3.5
4	M	0.1375	3.5
4	M	0.1375	4
4	M	0.1125	2
4	M	0.1125	3.5
4	M	0.1125	3.25
4	M	0.1125	4
4	M	0.1125	
4	MH	3.4	0.5
4	MH	3.4	0.75
4	MH	3.4	1
4	MH	3.4	1.25
4	MH	3.4	0.5
4	MH	2.5	1.25
4	MH	2.5	1.25
4	MH	2.5	1.75
4	MH	2.5	2
4	MH	2.5	2
4	MH	0.75	1.25
4	MH	0.75	1.25

4	MH	0.75	1.5
4	MH	0.75	3
4	MH	0.75	2.5
4	MH	2.4	1.25
4	MH	2.4	1
4	MH	2.4	1.75
4	MH	2.4	2
4	MH	2.4	
4	MH	2.5	1
4	MH	2.5	1
4	MH	2.5	0.5
4	MH	2.5	1
4	MH	2.5	1.25
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0
4	H	4.4	0
4	H	4.4	0
4	H	4.4	0
4	H	4.4	0.25
4	H	4.4	0.25
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0
4	H	4.5	0

Appendix B

Development Data

Development time (hours) from egg to adult of *Lucilia sericata* from West Virginia, with or without *Nasonia vitripennis* from South Carolina, at different levels of soil compaction; 2008-2009. Compaction treatments: L=low (0.017 ± 0.0004 kg/cm^2), LP=low (0.0183 ± 0.001 kg/cm^2) + *Nasonia*, ML=medium-low (0.46 ± 0.05 kg/cm^2), M=medium (0.95 ± 0.14 kg/cm^2), MH=medium-high (2.49 ± 0.12 kg/cm^2), H=high (4.33 ± 0.04 kg/cm^2), HP=high (4.38 ± 0.05 kg/cm^2) + *Nasonia*, Ma=no soil, no *Nasonia*.

Experiment	Compaction Treatment	Development Time (hours)	Sex
1	L	332.5	F
1	L	332.75	F
1	L	328.5	M
1	L	330.25	M
1	L	330.25	F
1	L	331.5	F
1	L	330.25	F
1	L	331.5	F
1	L	332.5	F
1	L	328.5	F
1	L	328.5	M
1	L	328.5	M
1	L	328.5	F
1	L	328.5	M
1	L	328.5	M
1	L	328.5	F
1	L	328.5	F
1	L	328.5	M
1	L	328.5	M
1	L	328.5	F
1	L	328.5	M
1	L	328.5	M
1	L	328.5	M
1	L	328.5	F
1	L	328.5	M
1	L	328.5	M
1	L	328.5	M

1	L	328.5	F
1	L	328.5	M
1	L	328.5	M
1	L	328.5	F
1	L	328.5	M
1	L	304.25	M
1	L	310	M
1	L	310	M
1	L	310	M
1	L	310	M
1	L	310	M
1	L	336	M
1	L	336	M
1	L	336	F
1	L	336	F
1	L	382	F
1	L	310	M
1	L	314.75	F
1	LP	310	M
1	LP	310	M
1	LP	310	M
1	LP	310	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	F
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	F

1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	328.5	M
1	LP	336	M
1	LP	336	F
1	LP	336	M
1	LP	336	F
1	LP	336	F
1	LP	352.5	F
1	LP	352.5	F
1	LP	329.75	F
1	LP	329.75	F
1	LP	329.75	M
1	LP	331.5	F
1	LP	331.5	M
1	LP	331.5	F
1	LP	331.75	M
1	LP	331.5	F
1	LP	330.5	F
1	LP	328.75	F
1	LP	332.5	F
1	H	328.5	F
1	H	331.75	M
1	H	328.5	M
1	H	331.5	M
1	H	330.25	F
1	H	332.5	F
1	H	332	M
1	H	331.5	F
1	H	329.75	F
1	H	329.75	M
1	H	330.25	F

1	H	330.25	F
1	H	331.5	M
1	H	331.5	M
1	H	330.25	M
1	H	332	M
1	H	329	F
1	H	328.5	M
1	H	328.5	M
1	H	328.5	M
1	H	328.5	M
1	H	328.5	M
1	H	328.5	M
1	H	328.5	M
1	H	328.5	M
1	H	328.5	M
1	H	328.5	M
1	H	328.5	M
1	H	328.5	M
1	H	352.5	F
1	H	352.5	F
1	H	352.5	F
1	H	336	M
1	H	336	M
1	H	336	M
1	H	336	M
1	H	336	F
1	H	336	F
1	H	359	F
1	H	359	F
1	H	359	F
1	H	352.5	F
1	H	352.5	F
1	H	352.5	F
1	H	354.75	M
1	H	312	M
1	HP	328.5	M
1	HP	328.5	M
1	HP	328.5	M

1	HP	328.5	M
1	HP	328.5	M
1	HP	328.5	M
1	HP	328.5	M
1	HP	328.5	M
1	HP	328.5	M
1	HP	328.5	M
1	HP	328.5	M
1	HP	328.5	M
1	HP	328.5	M
1	HP	328.5	M
1	HP	328.5	M
1	HP	328.5	M
1	HP	328.5	M
1	HP	328.5	F
1	HP	336	F
1	HP	331.5	F
1	HP	331.75	F
1	HP	328.75	M
1	HP	328.75	M
1	HP	331.5	F
1	HP	336	F
1	HP	359	F
1	HP	352.5	F
1	HP	352.5	F
1	HP	328.75	M
1	HP	328.75	F
1	HP	352.5	M
1	HP	328.75	F
1	HP	328.75	F
1	HP	352.5	F
1	HP	330.25	F
1	HP	330.5	F
1	HP	329.75	F
1	HP	377.5	F
1	HP	424.5	F
1	HP	424.5	F
1	HP	448.5	F

1	HP	448.5	F
1	HP	381.75	F
1	HP	332.75	M
1	HP	330.5	F
1	HP	332	M
1	Ma	331.5	F
1	Ma	331.5	F
1	Ma	331.5	M
1	Ma	331.75	F
1	Ma	331.75	F
1	Ma	328.5	F
1	Ma	329.75	M
1	Ma	329.75	F
1	Ma	329.75	F
1	Ma	332	F
1	Ma	330.25	M
1	Ma	330.25	M
1	Ma	331.5	M
1	Ma	332.5	F
1	Ma	332.5	F
1	Ma	332.75	F
1	Ma	332.75	F
1	Ma	331.5	M
1	Ma	332.75	M
1	Ma	331.75	F
1	Ma	331.5	F
1	Ma	331.5	F
1	Ma	331.5	F
1	Ma	310	M
1	Ma	310	M
1	Ma	336	M
1	Ma	336	F
1	Ma	336	F
1	Ma	336	M
1	Ma	336	F
1	Ma	336	F
1	Ma	336	M
1	Ma	336	F

1	Ma	336	F
1	Ma	336	M
1	Ma	336	F
1	Ma	336	M
1	Ma	336	M
1	Ma	336	F
1	Ma	336	M
1	Ma	336	M
1	Ma	336	F
1	Ma	336	F
1	Ma	336	M
1	Ma	336	F
1	Ma	336	F
1	Ma	336	F
1	Ma	336	F
1	Ma	359	F
2	L	330.49	M
2	L	330.49	M
2	L	350	.
2	L	350	F
2	L	350	.
2	L	350	F
2	L	350	F
2	L	350	F
2	L	350	M
2	L	350	F
2	L	350	F
2	L	350	F
2	L	350	F
2	L	350	F
2	L	350	M
2	L	350	M
2	L	350	M
2	L	350	M
2	L	350	M
2	L	350	M
2	L	350	M
2	L	350	M

2	L	358	.
2	L	358	M
2	L	358	M
2	L	358	F
2	L	358	M
2	L	358	M
2	L	358	.
2	L	358	.
2	L	358	.
2	L	358	.
2	L	358	F
2	L	350	F
2	L	361	M
2	L	374	F
2	L	379.5	F
2	L	379.75	F
2	L	380	M
2	L	380	F
2	L	380	F
2	L	409	.
2	LP	326	M
2	LP	326	M
2	LP	326	M
2	LP	326	M
2	LP	326	M
2	LP	326	F
2	LP	326	M
2	LP	326	M
2	LP	334	M
2	LP	334	M
2	LP	334	F
2	LP	334	F
2	LP	326	M
2	LP	326	M
2	LP	326	M
2	LP	334	M
2	LP	334	M
2	LP	326	F

2	LP	334	F
2	LP	334	F
2	LP	334	F
2	LP	326	M
2	LP	334	F
2	LP	334	M
2	LP	306.5	M
2	LP	306.5	F
2	LP	306.5	M
2	LP	306.5	M
2	LP	306.5	M
2	LP	306.5	M
2	LP	306.5	M
2	LP	306.5	M
2	LP	356	M
2	LP	356	M
2	LP	356	F
2	LP	356	F
2	LP	355.5	M
2	LP	350	M
2	LP	356	F
2	LP	355.75	F
2	H	350	M
2	H	350	M
2	H	350	M
2	H	350	M
2	H	350	F
2	H	350	F
2	H	350	M
2	H	350	M
2	H	358	.
2	H	358	M
2	H	358	M
2	H	358	M
2	H	358	.
2	H	374	M
2	H	374	M
2	H	374	M

2	H	374	M
2	H	374	F
2	H	374	M
2	H	374	F
2	H	374	M
2	H	374	M
2	H	374	F
2	H	374	M
2	H	374	M
2	H	374	F
2	H	379.16	F
2	H	379.16	M
2	H	379.16	F
2	H	379.16	F
2	H	379.16	F
2	H	379.66	F
2	H	379.66	F
2	H	379.66	M
2	H	379.66	F
2	H	380	M
2	H	380	F
2	H	380	M
2	H	380	M
2	H	380	M
2	H	380	M
2	H	385	F
2	H	409	.
2	H	409	.
2	H	409	.
2	H	409	.
2	H	409	F
2	HP	326	M
2	HP	326	M
2	HP	326	M
2	HP	326	M
2	HP	326	M
2	HP	326	M
2	HP	350	F

2	HP	350	F
2	HP	350	F
2	HP	350	F
2	HP	350	M
2	HP	350	F
2	HP	334	M
2	HP	334	F
2	HP	334	M
2	HP	355.25	F
2	HP	355.25	F
2	HP	355.5	M
2	HP	355.5	M
2	HP	355.5	F
2	HP	355.5	F
2	HP	355.75	M
2	HP	355.75	M
2	HP	355.75	M
2	HP	356	M
2	HP	356	F
2	HP	356	F
2	HP	356	F
2	HP	355.25	M
2	HP	355.25	F
2	HP	355.25	F
2	HP	574.75	F
2	HP	361	M
2	HP	448.5	M
2	Ma	358	M
2	Ma	358	M
2	Ma	358	F
2	Ma	358	F
2	Ma	358	F
2	Ma	358	F
2	Ma	358	M
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2	Ma	358	.
2	Ma	358	.
2	Ma	358	.

2	Ma	358	.
2	Ma	358	.
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2	Ma	358	F
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2	Ma	358	M
2	Ma	358	F
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2	Ma	358	.
2	Ma	358	.
2	Ma	358	M
2	Ma	358	F
2	Ma	358	M
2	Ma	358	F
2	Ma	358	F
2	Ma	358	.
2	Ma	350	M
2	Ma	374	F
2	Ma	379.75	M
2	Ma	379.75	.
2	Ma	379.75	.
2	Ma	379.75	.
2	Ma	379.5	M
2	Ma	379.5	F
2	Ma	379.75	.
2	Ma	379.75	.
2	Ma	379.75	M
2	Ma	409	.
3	L	338	M

3	L	338	M
3	L	338	F
3	L	338	.
3	L	338	M
3	L	338	M
3	L	338	F
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3	L	338	M
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3	L	338	M
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3	L	338	F
3	L	338	F
3	L	365	F
3	L	365	F
3	ML	338	M
3	ML	338	M
3	ML	338	.
3	ML	338	F
3	ML	338	F
3	ML	338	M
3	ML	338	F
3	ML	338	F
3	ML	338	M
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3	ML	338	M
3	ML	338	F
3	ML	338	F
3	ML	338	F
3	ML	338	F
3	ML	338	F

3	ML	357	M
3	ML	365	M
3	ML	365	F
3	ML	365	F
3	ML	365	F
3	ML	357	F
3	ML	357	F
3	ML	365	F
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3	M	338	F
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3	M	357	F
3	M	365	F
3	M	365	F
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3	M	365	M
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3	MH	338	M

3	MH	338	F
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3	MH	338	F
3	MH	338	F
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3	MH	365	M
3	MH	367.5	F
3	MH	365	M
3	MH	365	F
3	MH	365	F
3	MH	365	F
3	MH	365	F
3	MH	365	M
3	MH	365	F
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3	H	338	M
3	H	338	F
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3	H	365	F
3	H	365	F
3	H	365	F
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3	H	365	F
3	H	365	M
3	H	365	F
3	H	365	M

3	H	365	F
3	H	365	F
3	Ma	338	.
3	Ma	338	.
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3	Ma	338	M
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3	Ma	338	F
3	Ma	338	.
3	Ma	338	.
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3	Ma	338	.
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4	L	362.5	M
4	L	362.5	M
4	L	362.5	F
4	L	362.5	M
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4	L	362.5	M
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4	L	362.5	M
4	L	362.5	F
4	L	362.5	F

4	L	364	M
4	L	364	M
4	L	377	.
4	L	377	F
4	L	377	F
4	L	377	.
4	L	377	M
4	L	377	M
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4	L	382	F
4	L	382	F
4	ML	353	M
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4	ML	362.5	F
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4	ML	377	F
4	ML	377	F
4	ML	377	M
4	ML	377	F
4	ML	377	.
4	ML	382	F
4	ML	382	F
4	ML	382	F
4	ML	382	F
4	M	353	M
4	M	353	M
4	M	362.5	F
4	M	362.5	F

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4	M	362.5	F
4	M	362.5	F
4	M	362.5	M
4	M	362.5	M
4	M	362.5	F
4	M	362.5	M
4	M	362.5	F
4	M	362.5	F
4	M	362.5	M
4	M	362.5	M
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4	M	377	M
4	M	401	F
4	MH	353	M
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4	MH	362.5	M
4	MH	362.5	.
4	MH	362.5	.
4	MH	362.5	M
4	MH	362.5	M
4	MH	362.5	M
4	MH	362.5	F
4	MH	362.5	M
4	MH	377	M
4	MH	377	M
4	MH	377	M
4	MH	377	M
4	MH	382	M
4	MH	382	M
4	MH	382	M
4	MH	382	F

4	MH	382	F
4	MH	382	F
4	MH	382	F
4	MH	382	M
4	MH	382	M
4	MH	395	F
4	MH	401	M
4	H	362.5	M
4	H	382	F
4	H	382	.
4	H	382	M
4	H	382	M
4	H	382	.
4	H	382	.
4	H	382	F
4	H	382	.
4	H	382	F
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4	H	412	M
4	H	412	F
4	H	412	M
4	H	412	M
4	H	412	F
4	H	412	F
4	H	401	.
4	H	412	M
4	H	412	F
4	H	412	F
4	H	412	M
4	H	412	F
4	H	412	M
4	H	456	F
4	Ma	338	M
4	Ma	338	M
4	Ma	362.5	M
4	Ma	362.5	F
4	Ma	362.5	F
4	Ma	362.5	M

4	Ma	362.5	M
4	Ma	362.5	F
4	Ma	362.5	M
4	Ma	362.5	F
4	Ma	362.5	M
4	Ma	362.5	M
4	Ma	362.5	F
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4	Ma	362.5	M
4	Ma	362.5	M
4	Ma	362.5	F
4	Ma	382	F
4	Ma	382	F
4	Ma	382	F
4	Ma	382	F
4	Ma	382	F
4	Ma	382	.

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