Bulletin of Entomological Research (2005) **95**, 179–186 DOI: 10.1079/BER2004349

Winter survival of larvae and pupae of the blowfly, *Lucilia sericata* (Diptera: Calliphoridae)

K.M. Pitts and R. Wall*

School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 IUG, UK

Abstract

The mortality of larvae and pupae of the blowfly *Lucilia sericata* (Meigen) were examined during winter and spring, for two or three years. In soil cores in the field, 70–95% of the larvae died overwinter. Larvae congregated in the top 10 cm of the soil core and did not move extensively throughout the column during the winter. Larvae and pupae at greater depth were less likely to pupariate and emerge successfully than larvae closer to the surface. Under semi-natural conditions, where pupae were placed outside in sawdust filled tubes, in the absence of the usual biotic mortality factors, the mortality of larvae was considerably lower and was also unaffected by low winter temperatures. Hence, low temperatures did not appear to be the primary cause of high overwintering mortality in the field which, it is suggested, is more likely to be the result of the action of biotic mortality factors, such as entomopathogenic nematodes and fungi.

Introduction

Overwintering mortality has been shown to be one of the most important factors controlling abundance in some insect species (Knight & Bale, 1986; Walsh, 1990). Quantification of winter mortality levels is therefore essential in the development of a full understanding of the long-term population ecology of any insect species in a seasonal environment.

The blowfly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) is the primary agent of sheep myiasis in northern temperate habitats and, in these areas, is a pest of major economic and welfare importance (Hall & Wall, 1995). An extensive questionnaire study of 1600 sheep farmers in England and Wales, showed that in 1988–89 blowfly-strike affected over 80% of farms, where an average of 1.5% of sheep were struck each year (French *et al*., 1992). A similar questionnaire study of 164 farms in The Netherlands in 2000, showed that 52.4% of the farms reported at least one case of myiasis with, overall, 2.9% of the sheep infested (Snoep *et al*., 2002).

During late September and early October in northern Europe, under the influence of declining temperatures and photoperiod, acting on both the adult and juvenile stages, larvae of *L. sericata* that have completed feeding on their ovine host do not form puparia, but instead enter a state of diapause and overwinter in the soil as fully mobile third-stage larvae (Davies, 1929; Cragg & Cole, 1952; Ring, 1967; Saunders *et al*., 1986). This state of diapause is thought to promote the survival of the juvenile stage of *L. sericata* over winter, allowing pupation and emergence in the spring of the following year (Wall *et al*., 1992a).

The aim of the current study was to examine the behaviour of larvae of the blowfly *L. sericata* throughout the period of diapause under field conditions and to determine levels of mortality experienced during diapause and by the first spring generation of pupae.

Materials and methods

Diapausing larvae

To rear diapausing larvae for the study, populations of adult *L. sericata* were maintained in an environmental chamber under a 12:12 h light:dark cycle and at 15°C (range ± 0.5°C). Females were provided with 25 g of roughly chopped pig liver daily, to allow protein feeding, egg-development and then, some days later, oviposition. Eggs and newly hatched first-stage larvae were transferred to a cooled incubator and maintained under a 6:18 h light:dark cycle and at 12°C (range ± 0.5°C). However, even under

*Author for correspondence
Fax: 0117 928 9182
E-mail: richard.wall@bristol.ac.uk

these conditions, a small proportion (< 1%) of the larvae still formed puparia; the remaining larvae were considered to be in a state of diapause if they were still fully-mobile third-stage larvae, one week after the first larvae from the same cohort had formed puparia (Cragg & Cole, 1952). This procedure was continued throughout the study to provide diapausing larvae. The larvae from different egg-batches were mixed prior to use to randomize any possible maternally derived or other egg-batch differences in response.

Winter field mortality

In the autumn of 1998, fifty-two plastic drainpipe tubes, 100 cm in length 10 cm in diameter, were obtained. Each had chicken wire (2.5 cm diameter mesh) over one end, held securely in position using plastic cable ties. This allowed vertical water movement but retained a soil core. The tubes were numbered and then buried vertically, so that 70 cm of the tube was below the soil surface. The tubes were filled with soil until the level inside the core was the same as that of the surrounding earth. All the tubes were in a 2×3 m plot in a grazed pasture at a sheep farm in south-west England in the Woodspring district of north Somerset, 20 km west of Bristol. In the first year of the study, 100 diapausing larvae were first placed in each of 31 tubes (numbered 1–31) on the 31 October (cohort 1). A further 21 tubes (numbered 32–52) each received 100 larvae on the 18 December (cohort 2). At two-week intervals, starting on the 20 January 1999, four tubes, two from each cohort, were selected using a random number table, excavated and returned to the laboratory.

In the autumn of 1999, forty identical tubes were fitted with chicken-wire bases then buried vertically in the field, as described previously. The tubes were divided between two 1×1.5 m plots, 300 m apart, in grazed pastureland at the same sheep farm used the previous year. One hundred diapausing larvae were placed in each of the 40 tubes on the 14 October. Two tubes from each plot were collected on the 21 October to provide an indication of the number of larvae remaining in the tubes after only a week. Then at two-week intervals, starting on the 13 January 2000, four tubes, two from each plot, were selected at random, excavated and returned to the laboratory.

In both years, after removal of the mesh base in the laboratory, the soil cores were gently pushed out of the plastic tubes. The cores were then measured and sliced into 10-cm sections. These sections were searched individually by hand and the number, depth and status of larvae or puparia was recorded. The searching of each 10-cm section lasted for 10 min. If after this time larvae or puparia were still being found then searching would continue for a further 3 min. If a larva or puparium was discovered during this time then searching would continue for a further 3 min commencing from the time of discovery of the last specimen. The process continued until no more larvae or puparia were discovered. The larvae and puparia recovered from the cores were counted and transferred to labelled individual 250 ml plastic containers, half-filled with sawdust and maintained in an insectary, at 25°C (range $\pm$ 1°C), 55% r.h. and a 18:6 h light:dark cycle, to determine their viability. The resulting adult flies were then identified to ensure that the cores had not been colonized by another species of fly.

Adult emergence

In both years of the field study, within one week of the first puparium being recovered from a soil core, a fine mesh cover was fitted to the top of the remaining tubes in the field. From the centre of this mesh a strip of plastic (20×10 cm) coated in non-setting polybutene adhesive (Oecotak A5®, Oecos Ltd, Kimpton, UK), was suspended. The fine mesh prevented emerging flies from escaping from the tube and as a result the majority became stuck to the sticky surface and could be collected and counted. The surface of the soil core was also searched and any dead adult flies found were recovered. To assess the time of first emergence of wild adult flies, in late April each year, liver-baited, sticky traps (Wall *et al*., 1992b) were placed in the surrounding fields and inspected for *L. sericata* at 3- to 4-day intervals.

Weather and soil water content

During the 1999/2000 winter season the water content of the soil at each of the 10-cm depths was determined. For this, immediately following recovery from the field and the removal of larvae and puparia, 200 g of soil from each 10-cm section was weighed using a top-pan balance (Mettler-Toledo PG80001-S) and then transferred to an oven at 60°C for a minimum of 48 h, after which it was re-weighed. The difference in the two weights expressed as the proportion of the wet weight of the soil gave a measure of the water content of the soil section. In addition, soil was also collected from three areas surrounding the plots used in the investigation. This soil was also dried and weighed, as described, to obtain an estimate of the comparative water content outside the tubes. During both years a weather station (DataHog, Skye Instruments Ltd, Llandrindod Wells, UK) was used to record average air temperature and relative humidity.

Winter mortality under semi-natural conditions

Ten diapausing larvae were placed in each of between 24 and 35 glass tubes (150×17 mm) approximately 10 mm from the base of the tube. Sawdust filled the tube above and below the larvae and the tubes were then placed upright in a sawdust-filled bucket (30 cm in diameter, 25 cm in depth). The bucket was placed on the roof of the School of Biological Sciences, University of Bristol, on the 19 December, each year of study. The bucket was protected from direct rain by a cover 1 m above it, but was otherwise exposed to ambient weather conditions. At the same time a weather station (DataHog, Skye Instruments Ltd, Llandrindod, Powys) was placed within 1 m of the bucket. After an initial period of three weeks, the tubes were emptied once a week *in situ* and the larvae examined visually. Dead larvae were removed and recorded and the number that had pupariated was recorded. All live larvae and puparia were returned to their respective tubes and the tubes returned to the bucket in randomized positions. In March, fine mesh was placed over the tubes to collect emerged flies which were sexed, killed and recorded until no more flies emerged for a month, following which any remaining larvae and pupae were considered dead. Flies that died during emergence were not considered to have been able to emerge successfully and were included in subsequent analyses as having died as a pupa. This investigation was continued over three successive winter field seasons, 1997/98, 1998/99, and 1999/2000.

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In 1999/2000 ten glass tubes, each containing ten larvae, were also filled with a loam-based compost (John Innes, Norwich, UK).

Statistical methods

Data were analysed by stepwise multiple linear regression using S-Plus 2000 (MathSoft Inc., Seattle, USA), exponential regression and generalized linear modelling (GLM) were performed using SPSS (SPSS Inc. Chicago, USA). Ryan-Joiner normality tests were performed using Minitab (Minitab Inc., Pennsylvania, USA). All means are accompanied by standard errors in parentheses. Percentage mortality data were arcsine transformed and normality verified before inclusion in statistical tests. Where time is included in generalized linear models it is as a covariate, other variables, unless stated otherwise, were included as fixed factors.

Results

There were no significant differences in the winter average temperatures or relative humidities in the two years of the field trials. In 1998/99 the mean winter temperature experienced was 8.3°C (±0.08) and the mean relative humidity was 81.6% (±0.2). In 1999/2000 the mean winter temperature was 8.5°C (±0.08) and the mean relative humidity was 79.5% (±0.2). All the insects recovered from the soil cores were *L. sericata*.

Winter field mortality

During 1998/99 and 1999/2000, 18.1% (±2.8%) and 27.6% (±3.8%) of the diapausing larvae were recovered from the soil cores respectively. Of the larvae recovered, only 46.1% (±4.4%) and 38.1% (±3.9%) successfully pupated and emerged, during 1998/99 and 1999/2000 seasons, respectively. Hence, from all the larvae introduced to tubes at the start of the two investigations only 6.4% (±0.6%) during 1998/1999 and 11.3% (±1.2%) during 1999/2000, emerged successfully.

In 1998/1999, overall, the proportion of larvae that were recovered alive declined significantly over time in a non-linear manner ($F_{1,39} = 37.2$, $P < 0.001$), indicating that the rate of mortality increased over winter. This significant negative trend was strongly evident in the data from the second cohort ($F_{2,16} = 34.0$, $P < 0.001$, fig. 1a) where, by the time larvae initiated pupariation, only about 20% of the cohort remained alive. However, the relationship was non-significant for the larvae of the first cohort. Notably, a significantly greater proportion of the larvae from the second cohort (30.1% ±3.8) were recovered compared to the first cohort (6.1% ±1.1%) ($F_{1,39} = 64.2$, $P < 0.001$).

In 1999/2000 again, overall, the number of larvae that were recovered alive declined significantly over time in a non-linear manner ($F_{1,39} = 31.4$, $P < 0.001$) showing that the rate of mortality increased over winter. However, this significant negative trend was strongly evident in the data from the second field site ($F_{2,19} = 21.5$, $P < 0.001$, fig. 1b) but not the first. A significantly greater number of *L. sericata* were recovered from site 2 (36.8% ±5.8) than site 1 (17.8% ±3.8), ($F_{1,39} = 14.2$, $P < 0.001$).

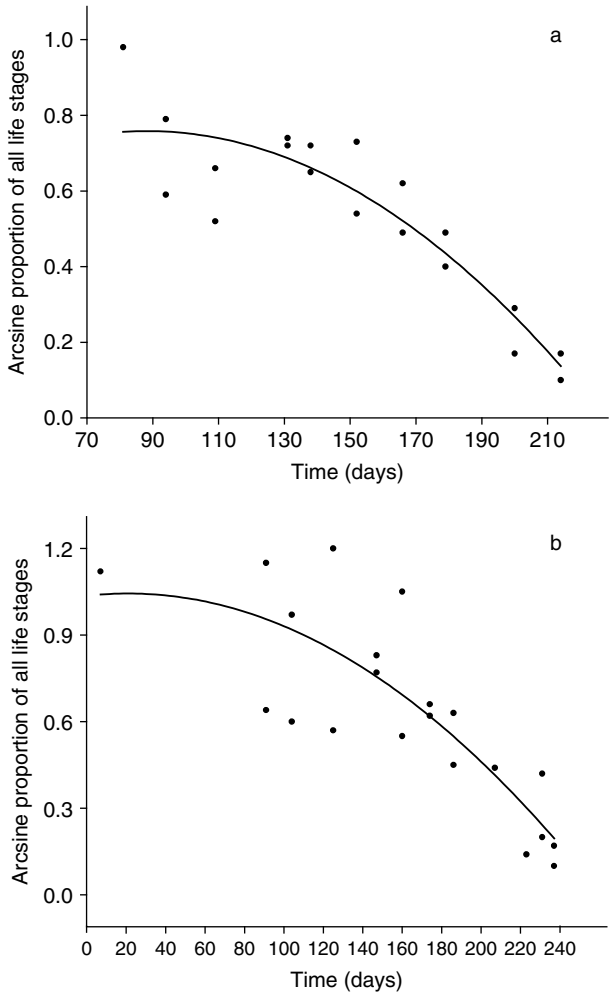

Fig. 1. The arcsine proportion (radians) of *Lucilia sericata* of all life-cycle stages recovered over time during the winter of (a) 1998/1999 field season (second cohort) ($Y = 0.4513 + 0.00695X - 0.000039X^2$, $F_{2,16} = 34.0$, $P < 0.0001$, $r^2 = 80.9\%$, day 81 = 20/01/99) and (b) winter of 1999/2000 field season (site 2) ($Y = 1.036 + 0.00077X - 0.000018X^2$, $F_{2,19} = 21.5$, $P < 0.0001$, $r^2 = 69.4\%$, day 1 = 14/10/99). The arcsine transformed value in radians of 0 is 0 and of 1 is 1.57.

Effects of soil depth

Examination of the number of larvae recovered in the field at each depth, expressed as a proportion of all larvae recovered from each individual tube, shows that the proportion of larvae recovered was significantly related to depth, for both 1998/1999 ($F_{1,237} = 101$, $P < 0.001$, fig. 2a) and 1999/2000 ($F_{1,237} = 204.5$, $P < 0.001$, fig. 2b). There were no effects of cohort, time or site on these relationships. The largest proportion of larvae were recovered from the top 10 cm of the core and the maximum depth from which larvae were removed was 60 cm in both years. Depth had a significant effect on the successful pupation of larvae; larvae recovered from deeper in the soil were less likely to form puparia than larvae nearer the surface ($F_{1,159} = 18.9$, $P < 0.001$, fig. 3a). The depth from which larvae were recovered also had a significant effect on adult emergence, the deeper

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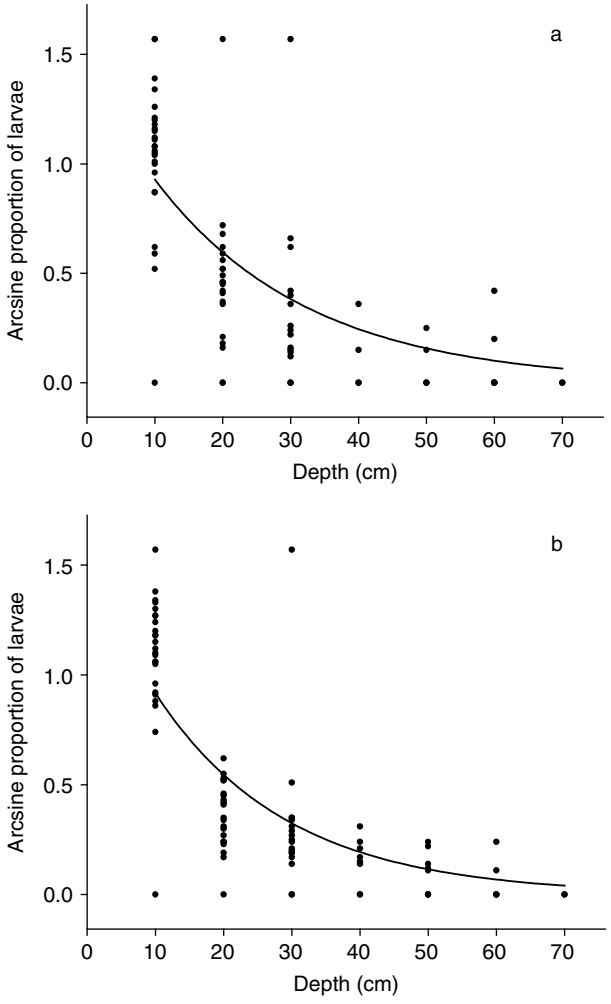

Fig. 2. The arcsine proportion (radians) of larvae of the blowfly *Lucilia sericata* recovered from soil-filled tubes and the depth at which they were recovered during winter in (a) 1998/99 ($Y = 1.447e^{(-0.044X)}$, $F_{1,73} = 78.46$, $P < 0.0001$, $r^2 = 51.87\%$) and (b) 1998/1999 ($Y = 1.155 - 0.01625X$, $F_{1,73} = 8.3$, $P < 0.01$, $r^2 = 10.2\%$). The arcsine transformed value in radians of 0 is 0 and of 1 is 1.57.

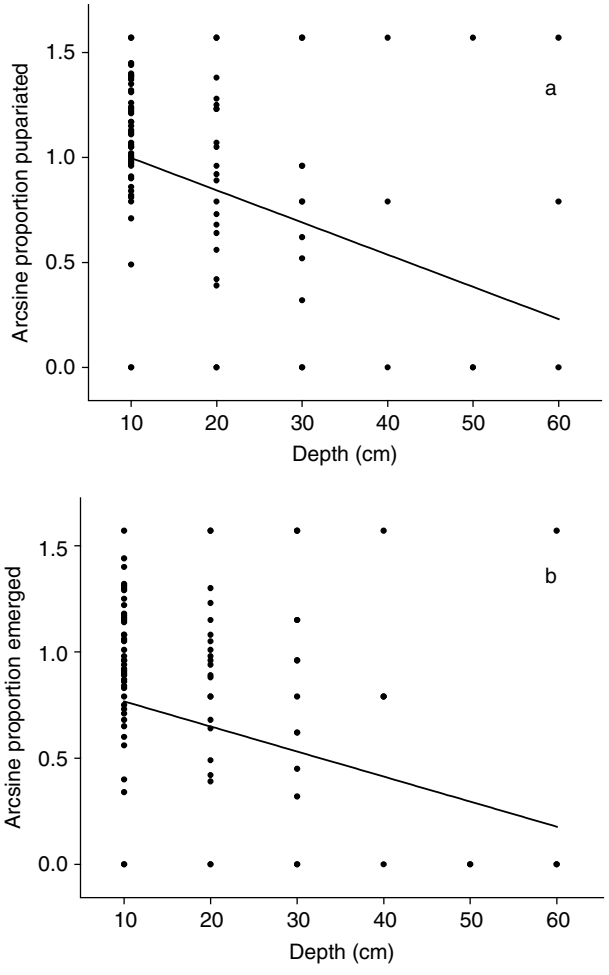

Fig. 3. The arcsine proportion (radians) of larvae of the blowfly *Lucilia sericata* and the depth from which they were recovered during both winter field seasons which (a) pupated successfully ($Y = 1.151 - 0.0153X$, $F_{1,159} = 18.9$, $P < 0.0001$, $r^2 = 10.6\%$) and (b) emerged ($Y = 0.885 - 0.0118X$, $F_{1,84} = 13.3$, $P < 0.0005$, $r^2 = 7.7\%$). The arcsine transformed value in radians of 0 is 0 and of 1 is 1.57.

a larva, the less likely it was to emerge successfully ($F_{1,159} = 13.3$, $P < 0.001$, fig. 3b).

For pupae, in 1998/99, 72.7% were recovered from the top 10 cm of the core, 24.3% were recovered from a depth of 10–20 cm and the remainder (3%) were recovered from 20–30 cm. During the 1999/2000 winter field season 97.5% of all pupae were recovered from the top 10 cm of the soil core, the remainder (2.5%) were recovered from a depth of 10–20 cm. The proportion of larvae and pupae recovered was not related to either temperature in each year ($F_{2,78} = 0.12$, $P = 0.89$) or relative humidity ($F_{2,78} = 0.04$, $P = 0.96$).

The time when larvae were removed from the field had no effect on whether or not they pupariated successfully in either year ($F_{1,160} = 0.103$, $P = 0.75$ and $F_{1,160} = 0.02$, $P = 0.89$ respectively) nor on whether or not they emerged successfully ($F_{1,160} = 0.103$, $P = 0.75$ and $F_{1,160} = 0.02$, $P = 0.89$ respectively).

Soil water content

During 1999/2000, soil water content was different at the two field sites ($F_{1,290} = 52.6$, $P < 0.001$). This difference did not change significantly over time ($F_{1,290} = 7.75$, $P < 0.05$) and there was no significant effect of depth on water content ($F_{5,290} = 1.33$, $P = 0.25$). The mean soil water content at site 1 was 26.3% (± 0.4%, range: 17–35%) and at site 2 it was 21.2% (± 0.2%; range 17–25%). There was no relationship between water content of the soil and the probability of a larvae successfully forming a puparium or emerging at either site 1 or site 2. There was no significant difference overall, in the water content of soil collected from the top 10 cm of the ground around the plots used in the investigation compared with soil at an equivalent depth in the tubes.

Timing of emergence

The threshold temperature for the development of post-diapause larvae of *L. sericata* has been found previously to

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Table 1. The predicted and observed date (day/month/year) of adult emergence for *Lucilia sericata* in the field.

Year	Adult emergence		First adult flies caught
	Predicted	Observed	
1998/99 Cohort 1	03 May 99 (152)	08 May 99 (169.8)	10 May 99
1998/99 Cohort 2	09 May 99 (152)	21 April 99 (94.6)	10 May 99
1999/00 Both sites	27 April 00 (152)	28 May 00 (262.9)	30 May 00

The figures in parentheses represent the predicted or observed number of day-degrees accumulated by larvae/pupae since the start of the investigation. Also, the dates on which wild flies were first caught on sticky targets in each of the two years.

be 9.2°C and it has been shown that post-diapause larvae require 29.7 (±0.3) day-degrees above this threshold to initiate pupation (Wall *et al*., 1992a). In addition to the day-degrees needed for post-diapause larvae development, a further 122.3 (±1.9) day-degrees are required to complete pupation and reach adult emergence (Wall *et al*., 1992a).

From the temperatures recorded in the field, the expected time of emergence was predicted (table 1). Since the tubes were examined at two-week intervals it is not possible to obtain a precise value for the day-degrees accumulated at the start of adult emergence. Therefore a figure is presented giving the number of day-degrees accumulated at the mid point between the last inspection when no adults were present and the inspection immediately following, when adults were present (table 1).

In 1998/99, the onset of emergence was between 17.8 day-degrees later and 57.4 day-degrees earlier than predicted, for the first and second cohorts respectively (table 1). Nevertheless, the observed dates of adult emergence were within 5 and 19 days of those predicted, for cohorts 1 and 2, respectively.

In 1999/2000 there were no differences in the observed date of first emergence at the two sites, which are therefore treated together. The observed onset emergence of adult flies was 111 day-degrees later than predicted at both sites (table 1). Adult flies were first collected four weeks after the expected first emergence date.

Comparison between the dates of emergence for flies within the tubes and the first date on which wild flies were recorded on sticky targets in the field, shows that the rate of development in the tubes followed that of wild flies in the field closely (table 1).

Winter mortality under semi-natural conditions

The number of larvae that died during each two-week period between samples, was expressed as the number of dead larvae at the end of any one sample period divided by the number of live larvae plus pupae present at the end of the previous sample period. The data from all tubes were pooled. Survivorship analysis indicated that, in contrast to those seen in the field soil cores, the overwinter larval mortality rates were relatively low and were approximately linear in all three years, although the relationships between percentage survival and time were relatively variable (table 2). The larval mortality observed in 1998/99 was significantly lower than in both 1997/98 and 1999/2000, which were not significantly different from each other ($F_{2,1792} = 126.8$, $P < 0.001$; table 3). Stepwise multiple linear regression did not show any significant relationship between proportionate larval mortality and any weather variable, for any of the three winter seasons.

The mortality rate of developing pupae within puparia could not be determined over time without opening the puparium and therefore killing the developing pupa. Consequently mortality levels experienced as pupae could only be recorded after all the flies had emerged (table 3). There was a significant difference in the pupal mortality between years ($F_{2,77} = 48.7$, $P < 0.01$), being significantly higher in 1997/98 than that observed in 1998/99 which was, in turn, significantly lower than that recorded in 1999/2000. The mean temperatures experienced by pupae were not directly correlated with the percentage pupal mortality observed.

There was no difference in the mortality rates recorded in 1999/2000 between the larvae in sawdust or loam-based compost, suggesting that the medium in which they were placed had no effect on survival.

Discussion

The levels of winter mortality observed for *L. sericata* in the field soil cores in the present study, of between 70 and 95%, are comparable with those obtained for the over-wintering stages of other insect species under natural conditions. Mortalities of up to 96% have been reported for eggs of the pine sawfly, *Neodiprion sertifier* (Geoffroy) (Hymenoptera: Diprionidae) (Austarä, 1971). In the bird cherry–oat aphid, *Rhopalosiphum padi* (Linneaus) (Hemiptera: Aphididae) 100% mortality was observed in viviparous adults and nymphs and 80% mortality of eggs on bird cherry trees (Leather, 1980).

In the present study, under semi-natural conditions, larval winter mortality was lower than that of pupae in

Table 2. The mean percentage survival (±SE) of larvae of the blowfly, *Lucilia sericata*, in tubes exposed to ambient conditions under semi-natural conditions during winter plus the test statistics and coefficients of regression of the arcsine percentage survival plotted against time.

Year	Larval mortality rate (% per day)	Regression coefficients	r^2
1997/98	0.2 (±0.012)	$F_{1,502} = 298.4$, $P < 0.001$, $Y = 0.96 - 0.002X$	37.3%
1998/99	0.05 (±0.006)	$F_{1,746} = 73.9$, $P < 0.0001$, $Y = 0.91 - 0.00051X$	9%
1999/00	0.13 (±0.011)	$F_{1,538} = 146.7$, $P < 0.001$, $Y = 89.9 - 0.129X$	21.4%

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Table 3. The percentage of the initial number of *Lucilia sericata* that died overwinter as larvae or pupae, or that successfully emerged as adults during each of three years under semi-natural conditions.

Year	Larval mortality (%)	Pupal mortality (%)	Successful emergence (%)
1997/98	26.4	57.7	15.9
1998/99	6.0	17.7	76.3
1999/00	27.2	35.9	36.9

spring and a linear rate of larval mortality over time was recorded, indicating that the probability of death remained constant over time. In contrast, during both years of the field study, larval mortality in the soil cores was considerably higher and followed a curvilinear pattern, with the rate of mortality increasing over time. One possible explanation for this difference is the action of pathogens such as entomopathogenic nematodes or fungi in the soil.

An extensive survey of entomopathogenic nematodes occurring in British soils showed that there was no evidence of seasonality in the number of late instar larvae of *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) larvae infected when exposed in the laboratory, to soil samples collected from field sites. It was suggested that Britain's temperate and moist climate provides conditions suitable for a year-round presence of *Steinernema bibionis* (Bovien) (Rhabditida: Steinernematidae), a rhabditid nematode that is an obligate and lethal parasite of insects. The nematode invades the haemocoele by entering the insect through one of its body cavities or actively penetrating the cuticle. Bacteria are then released from the nematode intestine causing septicaemia which rapidly kills the insect (Hominick & Briscoe, 1990), often within 48 h (Strong *et al*., 1996). The bacteria establish suitable conditions for nematode growth and reproduction and inhibit colonization of other microorganisms (Poinar, 1979). *Steinernema bibionis* was shown to be the most prevalent soil-dwelling entomopathogenic nematode in Britain (Hominick & Briscoe, 1990) while *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) was not recorded from this survey, although it had previously been shown to persist in British soil throughout the year (Georgis & Hague, 1981). *Steinernema feltiae* (cited *as Neoaplectana carpocapsae*) has been recovered from both soil samples and parasitized pre-pupa of the larch sawfly, *Cephalcia lariciphila* (Wachtl) (Hymenoptera: Pamphiliidae) (Georgis & Hague, 1981). Furthermore, *S. bibionis* has been shown to be capable of infecting third-stage non-feeding larvae of *Lucilia cuprina* (Weidemann) (Diptera: Calliphoridae) at a temperature of 3°C (Molyneux, 1986). At this temperature, however, reproduction of the nematode was not possible although the nematodes may remain in the host's haemocoele as infective juvenile stages until temperatures rise allowing bacterial growth and nematode reproduction (Molyneux, 1986). Soon after pupariation, *L. cuprina* become almost completely invulnerable to parasitism by nematodes (Molyneux, 1984). Nematodes have been shown to aggregate in areas where patches of overwintering insect larvae occur (Gouge *et al*., 2000; Mrácek & Becvár, 2000).

A survey of entomopathogenic fungi freely occurring within UK soils showed that the most common species were *Beauveria bassiana* (Balsamo) (Ascomycetes: Sordariomycetidae), and *Paecilomyces farinosus* (Holmsk) (Ascomycetes: Eurotiomycetidae), but with greater prevalence in soil samples from woodland and hedgerow habitats than from arable habitats (Chandler *et al*., 1997). However, after an initial inoculation, *B. bessiana* was found to persist poorly in soils (Vänninen *et al*., 2000). In comparison, *Metarhizium anisopliae* (Metschnikoff) (Ascomycetes: Sordariomycetidae) persisted extremely well and after three years remained in sufficient numbers of infectious propagules to infect over 80% of the *Tenebrio molitor* (Linnaeus) (Coleoptera: Tenebrionidae) larvae used as baits in the soil. The highest concentration of *M. anisopliae* was found in the top 5 cm of the soil column (Vänninen *et al*., 2000). However, although it is clear that entomopathogenic fungi overwinter and remain viable (even when the soil temperature fell to 0°C and air temperature fell to −25°C, Vänninen *et al*., 2000) it is not known whether they are capable of infecting soil dwelling insects at this stage.

Under the semi-natural conditions used in the present study, larval mortality was lower in 1998/99 than 1997/98 or 1999/2000. Although the results showed that there was no significant effect of temperature on mortality over time within a season, it is possible that temperature had an effect on overall levels of mortality when compared between winters. During 1997/98 and 1999/2000 the temperatures experienced by the larvae prior to the onset of pupation were generally higher than in 1998/99, regularly exceeding the developmental threshold of 12°C. In comparison, temperatures rarely exceeded 12°C during 1998/99. It has been shown that mild winter temperatures can reduce survival in some overwintering insects; overwintering larvae of the goldenrod gall fly, *Eurosta solidaginis* (Fitch) (Diptera: Tephritidae) suffered 70% mortality when maintained at 12°C for three months in comparison to only 11% and 30% mortality observed in larvae maintained at 0°C and −22°C respectively (Irwin & Lee Jr., 2000). In the current study, it is possible that the warmer temperatures experienced by diapausing larvae during the 1997/98 and 1999/2000 winter seasons caused an increase in metabolic rate and a resultant decrease in conserved energy stores. With no means of replenishing their nutrient reserves these larvae may not have been able to pupate (Wigglesworth, 1972). Those larvae that did pupariate may not have had the reserves to complete metamorphosis and may have died as pupae. As a result, the effects of temperature acting on the larvae may not have manifested themselves until the larvae had pupariated. Further investigation would be required to support this hypothesis.

In all three winter seasons, under the semi-natural conditions, the highest level of mortality was observed at the pupal stage. A previous laboratory study showed that houseflies, *Musca domestica* (Linnaeus) (Diptera: Muscidae), which had just pupariated or pupae which were approaching emergence, were the least cold tolerant (Leopold *et al*., 1998).

In the field, during the 1998/99 winter season, there was a significant difference in the rate of loss of larvae between the two cohorts, with higher mortality rates for larvae from the first cohort. Again, the early winter temperatures experienced by these larvae were regularly above that of the developmental threshold for diapausing larvae and this initial relatively warm period may have resulted in the greater depletion of metabolic reserves. It is also possible that the warmer temperatures were more suitable for pathogenic nematode or fungal activity.

In the only previous studies of the behaviour of diapausing *L. sericata* larvae (Davies, 1929, 1934), earthen pots were filled with soil and sunk into the ground. Larvae placed on the soil surface burrowed into the soil and one week later were found at the bottom of the pots, a depth of 12.7 cm. Periodic examination of these pots showed that the larvae remained quiescent throughout the winter and that they remained viable even though the soil around them became completely frozen. Towards the end of March the larvae migrated to within 0.6 cm of the surface; the first puparia were observed at the start of April and the first adult fly emerged at the end of April. The study suggested that no movement of larvae occurred until the mean daily soil temperature at 20 cm reached 7.2°C (Davies, 1934). In the current investigation, larvae generally remained in the top 10 cm of the soil throughout winter and there was no detectable movement of larvae within the soil cores throughout the winter field season. It has been shown that in some Diptera species diapausing as a winter-active stages, for example *Bibio rufiventris* (Duda) (Diptera: Bibionidae), larvae are able to move within the soil column and therefore escape to deeper layers should lower winter temperatures occur (Hoshikawa *et al*., 1988). In the case of *L. sericata* larvae overwintering in soil in southern England, it is likely that the temperatures they experience remain relatively constant throughout the winter and that these temperatures are higher than aerial temperatures (Bale, 1987). Furthermore, most overwintering larvae can supercool to between −20 or −30°C (Sømme, 1982) and diapausing larvae of *L. sericata* have a supercooling point of −14°C (Ring, 1972). This level of cold-hardiness in combination with the protection afforded by overwintering sites may be enough to ensure survival. It therefore seems likely that diapausing larvae of *L. sericata* do not experience temperatures that would be sufficiently low to result directly in their deaths.

Examination of the larvae recovered throughout the winter season showed a significant relationship between depth and the probability of successfully forming a puparium and emerging as an adult. The precise cause of this apparent effect of depth is not known. However, in winter larvae and pupae at greater depth will have been maintained at relatively higher temperatures than those near the soil surface (Wu & Nofziger, 1999). As a result, they will have had a higher metabolic rate and depleted their nutrient reserves more quickly, thereby compromising their chances of successful completion of development. It is possible also that at greater depths where temperature fluctuations are smaller in amplitude, entomopathogenic nematodes and fungal spores remain active and able to infect larvae. The precise cues causing some larvae to burrow to greater depths than other larvae from the same cohort, such as the presence or absence of light for example, are not known.

During the second field season the two field sites, 300 m apart, displayed very different mortality patterns; site 1 had lower levels of winter mortality compared to site 2. The only apparent difference between the sites was that the soil water content was significantly higher at site 1 than at site 2. Precisely how this might have affected the mortality levels is not known, though it may be that the lower water content was associated with increased activity of entomopathogenic nematodes (Gouge *et al*., 2000; Koppenhöfer *et al*., 1995).

It is notable that the first wild flies were caught in the field at approximately the same time as the first flies emerged from the tubes in this investigation. This suggests there was little difference in the rate at which day-degrees were accumulated by larvae/pupae in experimental tubes and wild individuals in the surrounding pasture. Using the known day-degree requirements for post-diapause larvae and pupae, the dates of adult emergence could be predicted. In 1998/99 the predictions for both cohorts were very close to the observed patterns of pupation and emergence, the first adult *L. sericata* were caught on sticky traps within a week of the predicted first emergence. In contrast, however, in 1999/2000 the number of day-degrees accumulated was over 100 day-degrees higher than the predicted value and observed adult emergence was four weeks later than predicted. This result was common to both field sites. It is possible that the ground temperatures remained cooler that year causing a slower rate of day-degree accumulation that that recorded at the surface; in future studies more detailed recordings of both surface and soil temperatures should be undertaken to resolve this question.

Overall, this study emphasizes the high rates of winter mortality that affect this species of blowfly, but suggests that these rates are more likely to be associated with biotic pathogens than low temperatures. Indeed, it may be that warmer weather in autumn and winter increases mortality by increasing the activity of pathogens or causing a more rapid depletion of the larval metabolic reserves required for pupation; further work will be required to consider these possibilities in detail.

Acknowledgements

The authors are grateful to the University of Bristol for a University studentship awarded to KMP.

References

Austarä, Ø. (1971) Cold-hardiness in eggs of *Neodiprion sertifer* (Geoffroy) (Hym. Diprionidae) under natural conditions. *Norsk Entomologist Tidsskrift* **18**, 45–48.

Bale, J.S. (1987) Insect cold hardiness: freezing and supercooling – an ecophysiological perspective. *Journal of Insect Physiology* **33**, 899–908.

Chandler, D., Hay, D. & Reid, A.P. (1997) Sampling and occurrence of entomopathogenic fungi and nematodes in UK soils. *Applied Soil Ecology* **5**, 133–141.

Cragg, J.B. & Cole, P. (1952) Diapause in *Lucilia sericata* (Mg.) Diptera. *Journal of Experimental Biology* **29**, 600–604.

Davies, W.M. (1929) Hibernation of *Lucilia sericata*, Mg. *Nature* **123**, 759–760.

Davies, W.M. (1934) The sheep blowfly problem in North Wales. *Annals of Applied Biology* **21**, 267–282.

French, N.P., Wall, R., Cripps, P.J. & Morgan, K.L. (1992) Prevalence, regional distribution and control of blowfly strike in England and Wales. *Veterinary Record* **131**, 337–342.

Georgis, R. & Hague, N.G.M. (1981) A neoaplectanid nematode in the larch sawfly *Cephalcia lariciphila* (Hymenoptera: Pamphiliidae). *Annals of Applied Biology* **99**, 171–177.

Gouge, D.H., Smith, K.A., Lee, L.L. & Henneberry, T.J. (2000) Effect of soil depth and moisture on the vertical distribution of *Steinernema riobrave* (Nematoda: Steinernematidae). *Journal of Nematology* **32**, 223–228.

Hall, M.J.R. & Wall, R. (1995) Myiasis in humans and domestic animals. *Advances in Parasitology* **35**, 258–334.

Hominick, W.M. & Briscoe, B.R. (1990) Survey of 15 sites over 28 months for entomopathogenic nematodes (Rhabditida: Steinernematidae). *Parasitology* **100**, 289–294.

Hoshikawa, K., Tsutsui, H., Honma, K. & Sakagami, S.F. (1988) Cold resistance in 4 species of beetles overwintering in the soil with notes on overwintering strategies of some soil insects. *Applied Entomology and Zoology* **23**, 273–281.

Irwin, J.T. & Lee Jr, R.E. (2000) Mild winter temperatures reduce survival and potential fecundity of the goldenrod gall fly *Eurosta solidaginis* (Diptera: Tephritidae). *Journal of Insect Physiology* **46**, 655–661.

Knight, J.D. & Bale, J.S. (1986) Cold hardiness and overwintering of the grain aphid *Sitobion avenae*. *Ecological Entomology* **11**, 189–197.

Koppenhöfer, A.M., Kaya, H.K. & Taormino, S.P. (1995) Infectivity of entomopathogenic nematodes (Rhabditida: Steinernematidae) at different soil depths and moistures. *Journal of Invertebrate Pathology* **65**, 193–199.

Leather, S.R. (1980) Egg survival in the bird cherry–oat aphid, *Rhopalosiphum padi* L. *Entomologia Experimentalis et Applicata* **27**, 96–97.

Leopold, R.A., Rojas, R.R. & Atkinson, P.W. (1998) Post-pupariation storage of three species of flies: increasing chilling tolerance by acclimation and recurrent recovery periods. *Cryobiology* **36**, 213–224.

Molyneux, A.S. (1984) The influence of temperature on the infectivity of heterorhabditid and steinernematid nematodes for larvae of the sheep blowfly, *Lucilia cuprina*. pp. 344–351 *in* Bailey, P.T. & Swincer, D.W. (*Eds*) *Proceedings of the Fourth Australian Applied Entomological Research Conference*. Adelaide, Govt. Printer.

Molyneux, A.S. (1986) *Heterorhabditis* spp. and *Steinernema* (=*Neoaplectana*) spp.: temperature, and aspects of behaviour and infectivity. *Experimental Parasitology* **62**, 169–180.

Mrácek, Z. & Becvár, S. (2000) Insect aggregations and entomopathogenic nematode occurrence. *Nematology* **2**, 297–301.

Poinar, G.O. (1979) *Nematodes for biological control of insects*. Florida, CRC Press.

Ring, R.A. (1967) Maternal induction of diapause in the larva of *Lucilia caesar* L. (Diptera: Calliphoridae). *Journal of Experimental Biology* **46**, 123–136.

Ring, R.A. (1972) Relationship between diapause and supercooling in the blowfly, *Lucilia sericata* (Mg.) (Diptera: Calliphoridae). *Canadian Journal of Zoology* **50**, 1601–1605.

Saunders, D.S., Macpherson, J.N. & Cairncross, K.D. (1986) Maternal and larval effects of photoperiod on the induction of larval diapause in two species of fly, *Calliphora vicina* and *Lucilia sericata*. *Experimental Biology* **46**, 51–58.

Snoep, J.J., Sol, J., Sampimon, O.C., Roeters, N., Elbers, A.R.W., Scholten, H.W. & Borgsteede, F.H.M. (2002) Myiasis in sheep in the Netherlands. *Veterinary Parasitology* **106**, 357–363.

Sømme, L. (1961) On the overwintering of houseflies (*Musca domestica* L.) and stable flies (*Stomoxys calcitrans* L.) in Norway. *Norsk Entomologisk Tidsskrift* **11**, 191–223.

Strong, D.R., Kaya, H.K., Whipple, A.V., Child, A.L., Kraig, S., Bondonno, M., Dyer, K. & Maron, J.L. (1996) Entomopathogenic nematodes: natural enemies of root-feeding caterpillars on bush lupine. *Oecologia* **108**, 167–173.

Vänninen, I., Tyni-Juslin, J. & Hokkanen, H. (2000) Persistence of augmented *Metarhizium anisopliae* and *Beauveria bassiana* in Finnish agricultural soils. *BioControl* **45**, 201–222.

Wall, R., French, N. & Morgan, K.L. (1992a) Effects of temperature on the development and abundance of the sheep blowfly *Lucilia sericata* (Diptera: Calliphoridae). *Bulletin of Entomological Research* **82**, 125–131.

Wall, R., Green, C.H., French, N. & Morgan, K.L. (1992b) Development of an attractive target for the sheep blowfly *Lucilia sericata*. *Medical and Veterinary Entomology* **6**, 67–74.

Walsh, P.J. (1990) *The influence of site factors on the community structures of pine beauty moth predators*. PhD thesis, University of Ulster.

Wigglesworth, V.B. (1972) *The principles of insect physiology*. New York, Wiley and Sons Inc.

Wu, J. & Nofziger, D.L. (1999) Incorporating temperature effects on pesticide degradation into a management model. *Journal of Environmental Quality* **28**, 92–100.

(Accepted 25 November 2004)