STUDIES ON THE OVER-WINTERING ECOLOGY OF THE SHEEP BLOWFLY IN SOUTH-EASTERN AUSTRALIA

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Summary
Ecological studies of the over-wintering and spring emergence of the Australian sheep blowfly (*Lucilia cuprina*) were undertaken over two consecutive years (2005-06). Replicated cohorts of post-feeding larvae of *L. cuprina* were placed in containers at an experimental site on a farm in central western Victoria. The numbers of flies emerging each day was used to describe the pattern of emergence, especially for the spring generation of flies. A data-logger was used to obtain detailed measurements of soil and weather conditions. This enabled environmental factors to be related to the emergence of flies.

Larvae deposited during spring, summer and early autumn developed rapidly, with median development to adult flies taking 30 days in spring, decreasing to 10 days as soil temperatures increased in summer. A transitional phase of development was observed in larvae deposited during mid-autumn of both years (11-26 April). Some larvae pupated immediately, whilst others entered a state of arrested development and emerged as adults the following spring. The date when the first flies emerged, and when 50% of flies had emerged ('median emergence'), was similar for larvae deposited in late autumn and winter, regardless of the date they were deposited. This synchronous emergence, in late Sep and early October, was earlier than reported in other studies in Canberra. A high mortality of over-wintering larvae was observed, with from 0-50% of these deposits emerging as flies the following spring. Serial sampling of larvae deposited in May 2006 indicated that pupation of over-wintering larvae commenced after 29 Aug, with 42% of the surviving stages having pupated by 14 Sep.

The emergence and environmental data was used to validate existing models of blowfly development and a number of discrepancies were identified.

Introduction
Flystrike is a major problem for the Australia sheep industry, estimated to cost at least $280 m annually.[1] The Australian sheep blowfly, *Lucilia cuprina*, breeds preferentially on sheep and initiates the majority of strikes.

The use of insecticides is an important component of an integrated approach to controlling flystrike when combined with management procedures that make sheep less susceptible to strike. The latter include crutching, shearing, mulesing, tail docking, effective worm control, and genetic selection against risk factors, such as fleece rot and breech soiling ('dag'). However, in contrast to control programs for internal parasites, the application of insecticides to control blowflies is based more around farm management practices, such as time of shearing, lambing and cropping, than any specific knowledge of the blowfly life cycle. Consequently, chemical treatments are often applied in response to the occurrence of flystrike rather than to control the population of the primary pest.

An Integrated Parasite Management (IPM) approach has the potential to give more effective control of blowflies, and could also reduce the use of chemicals on some farms. There is considerable knowledge about the biology of blowflies to formulate IPM strategies for flystrike, although there are several gaps in this knowledge.[2] Results from one study in south-eastern Australia showed that a more timely insecticide treatment would be to target reduction of the first generation of flies that emerge from over-wintered larval populations.[3] Despite this, a preventive approach to control has not been widely adopted.

The objective of this study was to obtain more information about the over-winter survival of blowfly larvae in south-eastern Australia, and to describe the pattern of emergence of flies in the spring. This data was then used to validate models of blowfly development and emergence that had been devised from previous studies.

Materials and methods

A field study was conducted over two years on a 1300 ha farm at Rokewood (600 mm annual rainfall), 40 km south of Ballarat. The experimental site was a fenced 900m^2 area within a 15 ha paddock. A weather station and data logger located at the centre of the site recorded air temperature, soil temperature (5 cm) and soil moisture each hour, and measured rainfall over the previous 24 hours.

About 100 wild adult *L. cuprina* were caught in traps (Lucitrap®) at the start of each year and transferred to the laboratory where they were maintained at 27^{o}C under a constant light regime. The flies were provided with liver on which to lay eggs, and the larvae that hatched were reared on reconstituted meat meal.

Post-feeding ('wandering') larvae were collected around 9am and replicate counts of 100 transferred into ventilated containers made from PVC pipe and flywire. The containers, filled with soil (Year 1) or sand (Year 2), were then placed in the ground at the experimental site so that the removable top was 3cm above the soil surface.

Deposits were made at intervals of 1 to 6 weeks, from 16 Mar 2005 to 30 Nov 2005 ('Year 1') and 10 Jan to 24 Oct 2006 ('Year 2') (Table 1). From 3-5 replicates were deposited when rapid development of wandering larvae was expected (spring, summer and early autumn), and 10 from Apr to Jun, when over-wintering and higher mortality occurs.[4,5] At the time of each field deposit, 3-5 replicates of 50 larvae from the same batch were incubated at 23^{o}C to assess their viability ('controls').

Additional replicates were deposited on four occasions in late autumn of Year 2 (19-Apr, 1-, 9-and 30-May). Four containers were recovered from the first 3 deposit dates at 2, 4 and 8 weeks after deposition, and 3 containers were recovered from the 30-May deposits 2, 12, 13 and 15 weeks after deposition. The soil in each pot was carefully sieved and the numbers of wandering larvae, dead larvae and pupae recorded. The presence of *L. cuprina* flies at the experimental site was recorded using 2 traps (Lucitrap®).

Results

Emergence of flies

A summary of control and field deposits is given in Table 1 and the patterns of emergence of flies from field deposits are shown in Figures 1 and 2. The average emergence of laboratory controls was 93%, with two deposits having a much lower emergence (49 and 75%) due to a heating failure in the rearing facility.

Emergence of adult flies was more rapid and less dispersed in summer and early autumn. One deposit in Year 1 (26-Apr) and 2 deposits in Year 2 (11- and 19-Apr) exhibited a pattern of split emergence between autumn and spring. Larvae in these deposits had a high mortality, with an average emergence of 50%, 8% and 17%, respectively.

Table 1. Date of deposits of wandering larvae, number of containers deposited (n), mean proportion of larvae that emerged (% emerging), and time taken for larvae to develop to flies during Years 1 (2005) and 2 (2006).

Year	Date of deposit	Controls		Field deposits		Days to emergence of:		
		n[a]	% emerging	n[b]	% emerging (SD)	First fly	Median (50% of flies)	Last fly
1 (2005)	16-Mar	5	95	5	96 (1.3)	19	19	22
	6-Apr[e]	2	49	3	53 (13.1)	16	19	27
	26-Apr[c]	5	93	10	45 (15.4)	30	41	72
					5 (5.7)	159	180	184
	18-May	4	93	10	0 (0)	-	-	-
	8-Jun	5	94	10	5 (6.9)	126	141	152
	12-Jul	6	97	10	9 (7.1)	82	93	112
	10-Aug	3	95	5	17 (4.1)	52	70	102
	19-Sep	4	90	5	68 (7.5)	24	32	62
	5-Oct	3	91	5	43 (19.3)	24	30	47
	26-Oct	3	98	5	63 (16.1)	14	18	24
	30-Nov	3	93	4	47 (8.1)	12	14	17
2 (2006)	10-Jan	3	93	3	5 (4.5)	10	11	14
	24-Jan	2	91	4	52 (7.3)	10	10	12
	7-Feb	3	98	3	88 (6.1)	11	12	14
	1-Mar	3	97	5	81 (7.2)	10	11	16
		3[d]	98	3[d]	90 (1.2)	10	11	17
	22-Mar	3	94	3	66 (3.6)	13	18	24
		3[d]	99	5[d]	85 (3.2)	17	19	26
	11-Apr[c]	5	96	4	7 (2.6)	42	47	68
					1 (1.0)	177	179	179
	19-Apr[c]	4	99	10	4 (1.8)	44	55	69
					13 (9.0)	166	177	205
	1-May	4	97	10	11 (7.7)	155	165	192
	9-May	6	95	10	21 (12.9)	140	153	179
	30-May	2	100	9	45 (15.7)	121	133	155
	18-Jul	6	98	9	50 (12.3)	73	84	99
	22-Aug	3	95	6	72 (7.2)	43	63	87
	19-Sep[e]	2	75	4	54 (10.1)	20	24	30
	12-Oct[e]	3	93	5	24 (19.0)	20	21	25

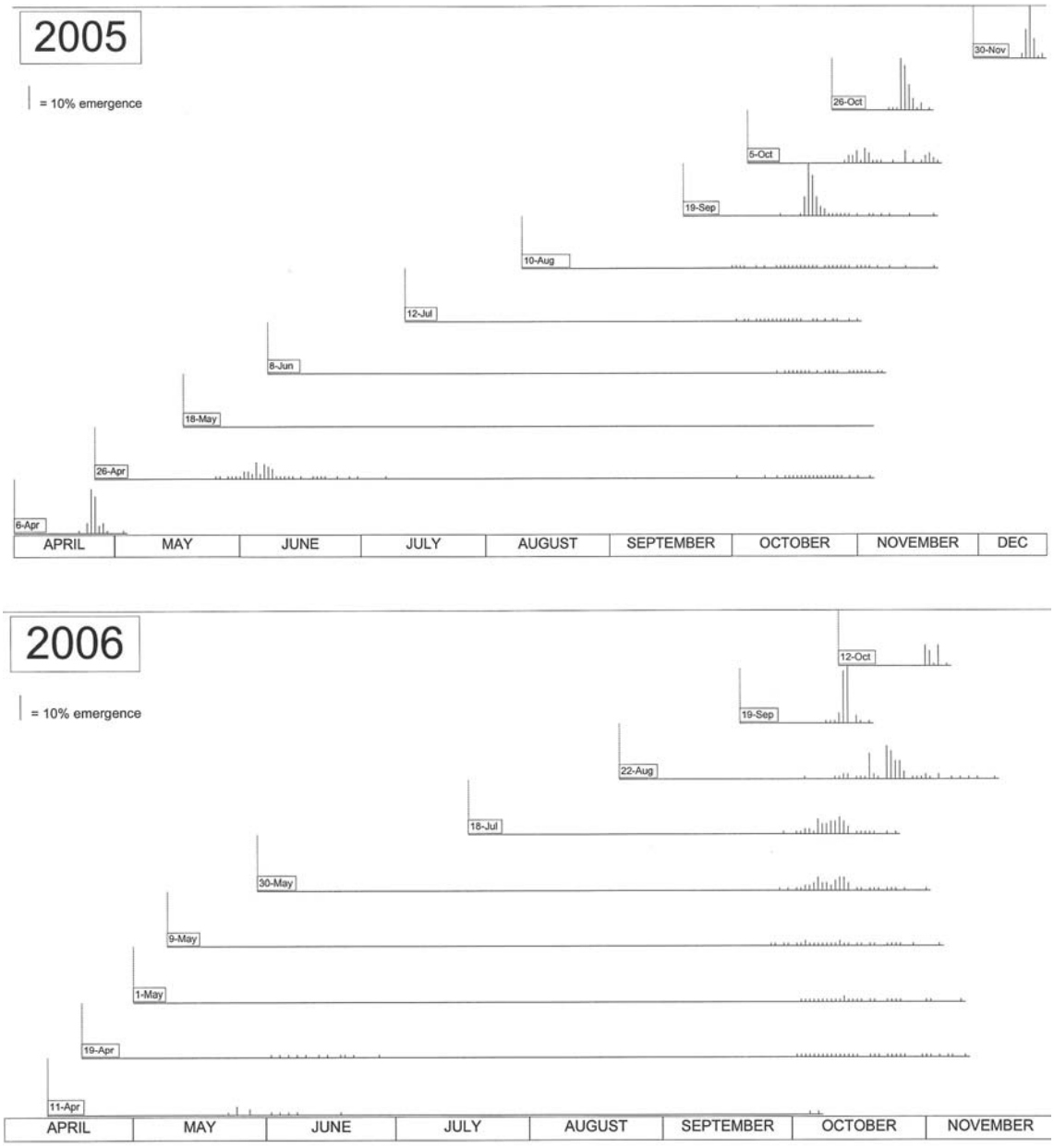
[a] 50 larvae/ container; [b] 100 larvae/ container; [c] split emergence of flies in autumn and spring; [d] larvae derived from recently caught wild flies; [e] small post-feeding larvae

The first flies from over-wintering larvae emerged on 1-Oct in Year 1 and 26-Sep in Year 2. The emergence of flies was synchronous from all deposits made between May and Aug in both years. The date when 50% of the total number of flies had emerged ('median emergence') occurred between 13- and 27-Oct for 4 autumn-winter deposits in Year 1, and between 7- and 24-Oct for 7 deposits in Year 2.

Recovery of larvae and pupae

Nine pots deposited on 30-May were examined in late winter and early spring before fly emergence. Pupae comprised <3% of the stages recovered on 29-Aug, but this increased to 42% on 14-Sep. During this time, minimum, average and maximum daily soil temperatures ranged from 7.5-11.2, 9.1-12.5 and 11.0-14.4^{o}C, respectively. There was evidence of increased mortality after the resumption of development, with only 66% of the stages recovered on 14-Sep emerging as flies when incubated at 23^{o}C, compared with 81% from pots removed on 29-Aug.

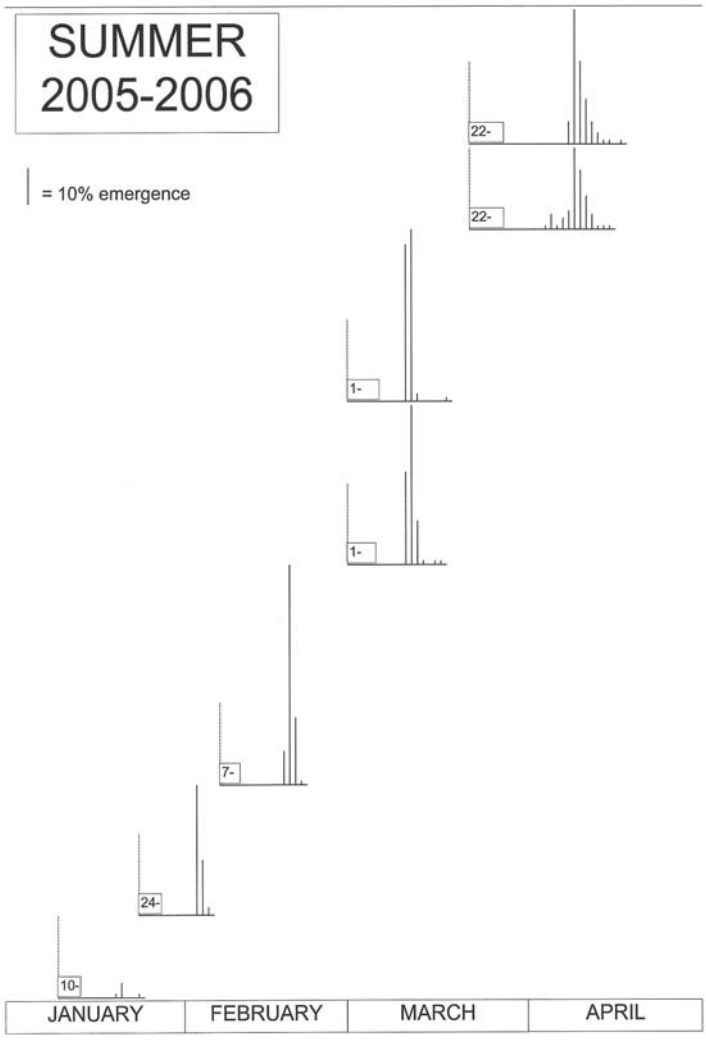
Figure 1. The pattern of emergence of flies from replicated deposits made in autumn, winter and spring of Years 1 (2005) and 2 (2006).



Trapping of wild flies

In Year 1, flies emerging from over-wintering larvae were first trapped on 17-Oct-05 and then were continuously present until 28-Apr-06. No flies were trapped during winter of 2006 and the first flies after winter were trapped on 7-Oct-06. In each year, fly numbers were relatively low until mid-November, then increased rapidly to peak in late November.

Figure 2. The pattern of emergence of flies from deposits made in Jan-Mar of Year 2 (2006); upper graphs of Mar and Apr deposits are wild trapped *L.cuprina*



Discussion

Data from this study are consistent with previous work on the development of *L.cuprina* in south-eastern Australia,[4,5] but indicate that there are some important regional differences.

First, there was rapid development and a concentrated fly emergence when wandering larvae (pre-pupae) were deposited during spring, summer and early autumn. Second, there was a transitional phase of larval development in deposits made during mid-autumn of both years (11-26 Apr). Some larvae pupated and emerged as flies, 41-55 days after deposition, whereas other larvae entered a state of arrested development and did not pupate or emerge until spring, 177-180 days after deposition. Entry into this transitional phase was later than described for the Canberra region, where it occurred from late March to early April.[4] Comparison of soil temperatures between the two studies showed that the minima for Canberra were consistently 1-5^{o}C cooler than at Rokewood from late March onwards.

Thirdly, larvae entering arrested development emerged synchronously the following spring. The first flies emerged in late September or early October and the dates of median emergence were consistent between all the autumn-winter deposits; from 13-27 Oct in Year 1 and 7-24 Oct in Year 2. A similar, but later, synchronous emergence of over-wintering stages has been described for Canberra.[4]

There was a high and quite variable mortality of over-wintering larvae, with <20% emerging as flies from 8 of 13 deposits made between April and August of both years. Again, this is consistent with previous studies in suburban Melbourne which found <10% of larvae deposited in May and June emerged as flies.[5,6]

Finally, serial sampling of replicates deposited on 30 May in Year 2 revealed that most over-wintering larvae pupated in early to mid-September.

Subtle differences between this and previous studies are most probably related to differing soil temperatures, as mentioned above, but may also have been influenced by differences between the strains of flies used. In the current study, wild flies were trapped in late-summer each year, so that the parents of the wandering larvae were maintained in the laboratory for no more than 10-12 generations. In contrast, flies used in previous studies were laboratory stock that had been maintained for 47-80 generations[4] or for 5-7 years.[5]

Validation of existing models

Several attempts have been made to develop models of sheep blowfly development and have highlighted the lack of knowledge about key aspects of the biology of *L. cuprina*.[2,7,8] For example, there is little information about the development of wandering larvae of *L. cuprina*, that is the time from cessation of feeding until pupation, especially under low temperatures. Thus, models must use observations from other species, notably *Lucilia sericata*, and so are unlikely to be able to accurately predict the over-wintering of *L. cuprina*.

It is also unclear what influences the termination of over-wintering and resumption of development, although environmental factors, such as soil temperature and changes in soil temperature, are probably the main triggers.

The data collected in the current study was used to validate several models of development and spring emergence. These included:

a) 'TEMPSUM' - a thermal summation model derived from pupal development under constant temperatures.[9]
b) 'FLYALERT', a program developed by CSIRO Division of Entomology, Canberra, in 1989-91.
c) An accumulated development model (the 'Vogt model') which uses hourly soil temperature data.[2]
d) A modified Vogt model.

TEMPSUM calculates soil temperatures from daily minimum and maximum air temperatures and requires accumulation of 100 degrees above a development threshold of 10^{o}C. It predicted fly emergence on 1- and 12 August in 2005 and 2006, respectively, far earlier than was observed.

FLYALERT requires the 5 day rolling average soil temperature to stay above 15^{O}C before pupation can start, if not development is reset to zero. This means that minor variations in soil temperature can reset development, causing large differences in the predicted emergence dates. We found that the formula used to convert air temperatures to soil temperatures in this model gave significantly different values to those actually recorded. Using the observed daily maximum and minimum air temperatures from each year, and a 1 July start date, FLYALERT predicted emergence dates of 7-Sep and 19-Sep in 2005 and 2006, respectively, about a month earlier than observed. Conversely, using the observed soil temperatures, either hourly or the daily minima and maxima, predicted a later emergence on 6-Nov and 2-Nov, respectively.

The Vogt model uses a non-linear (exponential) function to accumulate development for all stages and hourly soil temperatures. A total of 100 arbitrary development units ('adu') is needed from oviposition to emergence of female flies. A key assumption in this model is that minimum soil temperature must be >16^{o}C

until pre-pupae can accumulate development to pupate. It is not clear where this assumption is derived from, but it causes considerable inaccuracies in the model as development is constantly reset if soil temperatures fall below 16°C. No emergence occurred if this rule was applied to the soil temperature data from this study.

Modification of the Vogt model was investigated by varying the threshold temperatures at which development of pre-pupae and pupae occurred. Either the minimum, 5-day rolling average or maximum temperature for accumulation of development of pre-pupae was changed in 1°C increments between 5-16°C, and the predicted emergence dates compared with the observed median emergence for each year (13-27 October in 2005,7-24 October in 2006). A reasonable fit was found using thresholds for development of 7-8°C for minimum, 9-10°C for 5-day rolling average and 10°C for maximum, but only when autumn-winter deposits were assumed to have been made on 1 July.

Subsequently, the actual deposit dates and soil temperatures were used in a modified Vogt model that used 11°C as the threshold below which no development accrued and any previous development was reset to zero. No split emergence was predicted for the April deposits and there were considerable discrepancies between the predicted and observed emergence times for non-over-wintering larvae. For example, for deposits made in April of each year the model predicted emergence from 14-39 days after it actually occurred.

Strategic application of insecticides to prevent flystrike

Better prediction of fly emergence for different parts of south-eastern Australia could support more precise timing of a strategic treatment strategy for sheep blowflies. At the moment there are two opportunities for this treatment; early spring or mid- to late-autumn.

Strategic treatment in autumn could dramatically reduce the numbers of larvae entering the over-wintering phase. During this time there are high mortalities, confirmed in the current study, with as few as 6% of larvae emerging as adult flies in spring (McKenzie 1990; 1994). Thus, any further decrease in the populations entering the over-wintering phase has the potential to significantly reduce the first generation of flies that emerge the following spring.

Early spring treatment will prevent development of eggs and larvae derived from the first generation of flies emerging from over-wintered larval populations. One study showed that this could significantly decrease the number of adult *L. cuprina* and the prevalence of fly-strike later in the season.[3] However, no further attempts at evaluating this strategy have been made and it is not commonly used on farms in south-eastern Australia.

Members of the Insect Growth Regulator (IGR) family are the most commonly used class of insecticides on farms, being used for over 80% of routine treatments in a recent survey of 500 woolgrowers in Victoria (De Cat, Larsen and Anderson, unpublished). The IGR insecticides are ideal candidates for strategic use, as they provide sustained protection from flystrike (10-12 weeks for cyromazine, 20-22 weeks for dicyclanil).

In conclusion, this study has emphasised that considerably more information about the biology of the sheep blowfly is needed to support the development of models that can accurately describe over-wintering survival and spring emergence in south-eastern Australia. Notable deficiencies are what induces larvae to enter a state of arrested development and over-winter, then what triggers the resumption of development after over-wintering.

There is also a need to more systematically investigate options for strategic treatment, particularly in view of the industry's decision to phase out mulesing by 2010.

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