OVER-WINTERING ECOLOGY OF *LUCILIA CUPRINA* IN SOUTH-EASTERN AUSTRALIA

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ABSTRACT

Flystrike is a major problem for the Australian sheep industry, estimated to cost at least $280 m annually. A review of blowfly strike and the ecology of the primary sheep blowfly (*Lucilia cuprina*) in south-eastern Australia revealed that some aspects of the biology of *L.cuprina* were still poorly understood. In addition, no recent studies had been conducted in Western Victoria, a high winter rainfall area with a large population of Merino sheep.

Consequently, a 2-year study on the seasonal timing of larval over-wintering and spring emergence of flies was undertaken on a farm near Ballarat, in Western Victoria, during 2005 and 2006. Replicated cohorts of postfeeding larvae of *L.cuprina* were deposited regularly, and the daily emergence of flies and meteorological observations were recorded.

Larvae deposited during spring, summer and early autumn developed rapidly, with the time to median emergence of flies taking 30 days in spring, decreasing to 10 days as soil temperatures increased in summer. A transitional phase of larval development was observed during mid-autumn of both years (11-26 April), slightly later than in a previous study at Canberra. Some larvae deposited in this period pupated immediately, whereas others entered an arrested development, emerging as flies the following spring. Induction of this arrested development was associated with sustained low soil temperatures ($\leq 10^{o}$C). In both years, over-wintering larvae resumed their development in late winter after soil temperatures exhibited a rising pattern or consistently stayed above 11^{o}C. The mean dates for the first and median emergence of flies in spring were 4 and 21 October in 2005, and 1 and 12 October in 2006, respectively. This emergence of flies from over-wintering larvae was synchronous, regardless of the date larvae were deposited. Mortality of over-wintering larvae was high, although quite variable between deposits, being 95% in 2005 and 68% in 2006.

Sequential sampling of larvae deposited in May 2006 indicated that pupation of over-wintering larvae occurred between 29 August and 14 September. Trapping of free-

ranging flies at the site found that fly numbers followed a bimodal pattern, with a large peak in November and a smaller peak in early March.

Validation of six predictive models of *L.cuprina* development, using data from this study, showed none could predict the last generation of flies in autumn, the time when larvae entered arrested development, or the occurrence of a split emergence. A simple linear model ('Temsum'), using actual soil temperatures and 1 July as start date, was best able to predict the first generation of flies in spring.

Ecological studies such as this will help to refine Integrated Parasite Management (IPM) programs for the control of flystrike. A number of aspects of *L.cuprina* biology were identified that deserve further study, in particular the development of immature stages at low temperatures. This information would also support the development of more complex models simulating the population dynamics of *L.cuprina*.

DECLARATION

This is to certify that:

(i) *this thesis comprises only my original work,*

(ii) *due acknowledgement has been made in the text to all other material used,*

(iii) *no part of this thesis has been submitted for any other degree.*

Sandra De Cat

The Lord in His wisdom made the fly,

And then forgot to tell us why.

Ogden Nash 1902-1971

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Two and a half years ago, my passion for sheep drew me to Australia. Little did I know that I would be looking at maggots and flies instead of the red dirt and woolly sheep I had fantasized about. This research opened my eyes and helped me realize that science is a fascinating and never-ending journey. I would love to take this opportunity to thank a few people who guided me on this trip.

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LITERATURE REVIEW

1.1 Development of Sheep & Wool Production in Australia

The first sheep brought to Australia in 1788 had plain bodies and short, fine wool (Belschner 1976; Massy 1990). The introduction of the Vermont bloodline from the USA in the 1880s increased the wool bearing capacity of Australian Merinos, although the considerable disadvantages of this bloodline soon became obvious. The skin of Vermont sheep was characteristically thrown into folds, which increased the wool density, but these wrinkles made these sheep much more susceptible to blowfly attack. Consequently, after the introduction of the Vermont bloodline, a movement started to breed more 'plain-bodied' sheep (Abbott et al. 2002; Graham 1979; Massy 1990).

The Australian Merinos that we now know can be divided into 4 'strains', bred for specific climatic regions and classified mainly according to the diameter of their wool: superfine (Saxon), medium fine (Spanish/Saxon), medium-medium/ strong (Peppin), and strong/ extra strong (South Australian). Traditionally, they have been bred and run in areas of decreasing rainfall, from superfine/ fine wools in the wettest to strong wools in the driest regions (Massy 1990; Short and Carter 1955). Throughout all sheep producing regions, these Merino sheep, as compared with British breeds and crossbred sheep, are highly susceptible to flystrike. Their dense compact fleeces deflect light rainfall and remain dry, but heavy persistent rain penetrates these fleeces and takes longer to dry out, rendering them more susceptible to body strike. In addition, the higher number of skin folds on the breech and body predisposes these Merino sheep to flystrike (Arundel and Sutherland 1988; Belschner 1976).

Since the introduction of sheep, Australia has become the world's leading producer of fine wool. There were just over 100 million sheep in 2005-2006 (ABARE 2006). More than 85% of these were Merinos, with many of the remainder being Merino-derived. Blowfly strike is a major problem in the Australian sheep flock, estimated to cost at least

$280m each year (Sackett et al. 2006). The prevention and management of flystrike has been a major challenge for Australian wool producers since the first major outbreaks of flystrike around the turn of the 19th century (Wardhaugh 2001).

1.2 Blowflies in Australia

Blowflies can be separated into 3 groups: primary, secondary and tertiary (Belschner 1956; Norris 1959; Tillyard and Seddon 1933). Primary blowflies are able to initiate strikes on susceptible live sheep and they are the first flies to visit fresh carrion. In Australia, the primary blowflies of most importance are *Lucilia cuprina, Calliphora augur*, *Calliphora stygia, Calliphora albifrontalis, Calliphora dubia* (formely *Calliphora nociva*) and *Lucilia sericata*. Secondary blowflies are unable to initiate a strike on live sheep, but can lay eggs in strikes previously established by primary flies. This usually leads to an increase in the severity of the strike. Secondary blowflies oviposit on carrion after the primary flies have laid their eggs, but their larvae are more vigorous and usually destroy the larvae of *Lucilia spp* (Fuller 1934; Mackerras 1930; Waterhouse 1947). In Australia, the important secondary blowflies are *Chrysomya rufifacies* and *Chrysomya saffranea* (formely *Chrysomya micropogon*). Tertiary flies only participate in the end stages of a blowfly attack, on live sheep, or when carrion is old and dried up. They cause little or no damage to live sheep. The most common tertiary carrion fly is *Hydrotoea rostrata* .

The sheep blowfly, *Lucilia cuprina,* is thought to have arrived in Australia in the late 1800s, most likely with sheep imported from South Africa (Norris 1990; Waterhouse and Paramonov 1950). The earliest records of flystrike in Australia date from 1870, with the first major fly waves recorded around the turn of the 19th century. By 1915, flystrike was a widespread problem for sheep farmers of mainland Australia (Graham 1979; Tillyard and Seddon 1933). At the time, the problem was believed to originate from a changed behaviour of the native blowflies and the widespread introduction of the Vermont bloodline. However, Mackerras (1930) showed that an introduced *Lucilia* species was the chief cause of flystrike in Australia. It was not until Fuller (1932b) made the distinction between the two very similar species, *L.cuprina* and *L.sericata,* that it became clear that *L.cuprina* was the principal Australian sheep blowfly (Barton 1982; Mackerras and Fuller 1937; McQuillan et al. 1983; Tillyard and Seddon 1933; Watts et al. 1976).

1.3 Identification of *Lucilia cuprina*

1.3.1 Adult Flies

L.cuprina adults are about 8 to 9 mm in length and easily recognized by their metallic coppery green appearance (Barton 1981) (Figure 1-1). The femora of the forelegs are bright metallic green, in contrast to the similar *L.sericata* and *Ch.rufifacies* which have dull dark blue to black forelegs (Hardy 1940; Waterhouse and Paramonov 1950). *L.cuprina* can further be distinguished from *Ch.rufifacies* by its abdomen, which is more slender and lacks the transverse dark bands of the other species (Barton 1981; Tillyard and Seddon 1933).

1.3.2 Larval & Pupal Stages

The larvae of *L.cuprina* are smooth and cream in colour, although sometimes have a slight pink tinge. The average length of full-grown maggots is 12 mm (Fuller 1932b). The puparia are smooth, fairly slim and red to brown in colour (Figure 1-2) (Tillyard and Seddon 1933).

Figure 1-1 The female (left) and male (right) *Lucilia cuprina* fly

Figure 1-2 *L.cuprina* life cycle stages - from left to right: eggs, 1st instar larvae, 2nd instar larva, 3rd instar feeding larva, 3rd instar postfeeding larva, prepupa and pupa.

1.4 Life Cycle of *L.cuprina*

The life cycle of *L.cuprina* has several stages (eggs, larvae, postfeeding larvae, pupae and adult flies). The development rates of each stage depend primarily on the temperature experienced in their immediate environment, either on live sheep, in soil or as free ranging flies. Under summer conditions, the complete development from egg to adult ranges from 14-21 days (Mackerras 1933).

1.4.1 Egg Stage

Eggs are laid in batches of around 70-260 eggs, depending on the size of the female (Webber 1955). Eggs are very susceptible to desiccation and require a moist environment for hatching. Under laboratory conditions, survival is high within the temperature range of 15-40°C and at 100% humidity. Survival drops rapidly outside this temperature range or with decreasing humidity. Eggs fail to hatch if saturation deficits exceed 10 mm Hg (Vogt and Woodburn 1980). Humidity alters the egg shape, making it easier for larvae to rupture the shell with increasing humidity. Temperature does not seem to affect this process (Davies 1950). Egg survival and development rates are maximal around 35°C (Foster et al. 1975; Vogt and Woodburn 1980). These optimal temperatures correspond to those in the fleece of sheep which functions as an air blanket creating a constant temperature zone of 35 to 38°C. However, fleece tip temperatures can easily rise over 40°C during the hot summer months (Murray 1957). Eggs usually hatch within 12-24 hours, if the oviposition site remains moist (Mackerras 1933).

1.4.2 Larval Stages

1.4.2.1 Larval Feeding Stage

After hatching, *L.cuprina* passes through three larval feeding stages before dropping off the sheep as postfeeding larvae. First instar larvae move down the wool, close to the skin and feed on serous exudate, already present on the surface of the skin or released by the action of larval proteolytic enzymes (Mackerras and Freney 1933; Sandeman et al. 1987). These young maggots are very susceptible to desiccation and require a moist environment to survive (Foster et al. 1975). Unlike first instars, second and third instar larvae possess large mouth hooks which allows them to penetrate deep into the dermis,

damaging healthy tissue and creating exudate and a moist environment for further egg and larval development (Norris 1959; O'Flynn 1982; Sandeman et al. 1987).

1.4.2.2 Postfeeding Larval Stage

Once third instar larvae are fully fed, they wander away from the strike, drop off the sheep and subsequently burrow into the soil. From this point, the larvae do not feed and consequently empty the contents of their crop and gut. The cuticle becomes more cream and opaque in colour (Fraenkel and Bhaskaran 1973; Tillyard and Seddon 1933).

The larvae leave the sheep at night, usually between the third and the seventh night after eggs are laid, with the greatest proportion leaving on the fourth and fifth night (O'Flynn 1982; Smith et al. 1981; Wardhaugh 2001). The larvae enter the soil close to where they leave the host, the median dispersal distance ranges from 0.8 to 1.6 m (Vogt and Woodburn 1982). As a result, the highest concentration of postfeeding larvae and pupae will be found in and around 'sheep camps', areas within a paddock where sheep tend to rest at night (Smith et al. 1981).

The depth to which postfeeding larvae burrow varies from 1.5 to 6 cm. This is strongly influenced by temperature, and most likely, by soil moisture and soil structure. Larvae are usually found deeper in the soil in summer and closer to the surface in winter when temperatures are lower (McLeod 1997; Vogt and Woodburn 1982; Wardhaugh 2001). The larvae remain mobile and can move to drier areas when the soil is too moist (Foster et al. 1975).

In the final phase of the postfeeding larval stage, the larvae contract to form the so called 'white prepupae'. Prepupae therefore denotes the stage between the completion of the white puparium and the change to the brown pupa (Fraenkel and Bhaskaran 1973) (Figure 1-2).

The duration of the postfeeding larval stage is highly variable. During summer, the median time from drop off to pupariation is 2 days and ranges between 1 and 4 days (Dallwitz and Wardhaugh 1984; Mackerras 1933). The time to pupariation increases with decreasing temperatures in autumn. Dallwitz and Wardaugh (1984) found that in the Canberra region during late March and early April, pupation occurred either within 15 days of the cessation of feeding or was delayed until the following spring.

1.4.3 Pupal Stage

Once pupariation has started, the pupae are immobile and become more vulnerable to adverse environmental conditions, such as waterlogging (Rumbo 1979). Pupal development rates increase linearly between 15°C (25 days) and 30°C (6 days) (Foster et al. 1975). Pupae held under fluctuating or constant temperature regimens show similar development rates and survival between 15 and 30°C. At constant temperatures, the lower and the upper thermal limits for survival are 15 and 35°C, respectively, whereas under fluctuating conditions pupae can survive short exposures to -10°C or 46°C. Survival of pupae, exposed daily for 7 hours to 38 and 0°C, was 78 and 98%, respectively (Dallwitz 1984).

1.4.4 Adult Stage

1.4.4.1 Emergence

Immature flies emerge from the pupae between midnight and 9am (Browne 1979; Norris 1959). The first flies to emerge tend to be all males; nevertheless, the overall sex-ratio is found to be approximately 1:1 (Mackerras 1933). The period of emergence of flies from a single cohort of postfeeding larvae varies from 4 days in summer to about seven weeks in spring (Foster et al. 1978; Vogt and Woodburn 1979).

1.4.4.2 Nutritional Requirements

L.cuprina flies need water and carbohydrates to maintain life (Webber 1957; 1958). In addition, females need protein in this diet to support egg maturation (Mackerras 1933; Webber 1958). Protein may be obtained from carrion, struck sheep or animal faeces, particularly sheep faeces (Clift and McDonald 1976; Webber 1958). Provided protein is abundant, the rate of ovarian development increases with temperature and females require a minimum of 57 day degrees above 8°C to produce their first batch of eggs (Vogt et al. 1985c). The number of eggs matured is dependent on the size of the female, which, in turn, is determined by the availability of food in the larval stages (Foster et al. 1975; Mackerras 1933; Vogt et al. 1985a; Webber 1955). Males are ready to mate within a day of emergence. They do not need a protein feed, although it is known to increase their sexual activity (Browne 1979; Mackerras 1933; Vogt and Woodburn 1979).

Females usually mate once in their lifetime (Barton Browne 1958), and can produce a batch of 70-260 eggs every 4 to 8 days (Foster et al. 1975). They live from 8 to 28 days (Gurney and Woodhill 1926), with an average life expectancy of less than 3 weeks. Therefore, females rarely produce more than two batches of eggs (Vogt and Woodburn 1979).

1.5 Dormancy among Blowflies

It is common amongst insect species to go through a state of dormancy during unfavourable environmental conditions, such as cold temperatures in late autumn and winter. Dormancy can be classified as either quiescence or diapause (Gullan and Cranston 2005; Mansingh 1971).

Quiescence is a state of suppressed development as a direct response to unfavourable environmental conditions. Low winter temperatures can arrest growth, but development resumes as soon as temperatures start rising again (Gullan and Cranston 2005; Mansingh 1971).

On the other hand, diapause is programmed well before the adverse conditions occur. The arrested growth is usually longer than quiescence and the return of favourable environmental conditions does not terminate the diapause immediately. Diapause is a complex phenomenon, involving a number of morphological, physiological, behavioural and biochemical changes which makes the insects more resistant to adverse environmental conditions (Gullan and Cranston 2005; Mansingh 1971; Tauber and Tauber 1976).

The blowfly species, *Lucilia sericata*, *Lucilia caesar* and *Calliphora vicina*, all show a true diapause in the postfeeding larval stage. From mid- to late autumn, the majority of postfeeding larvae will cease their development and enter a diapause, even when conditions are still favourable for pupation (Cragg and Cole 1952). Cragg and Cole (1952) were the first to note that a maternal factor influenced the onset of diapause in *L.sericata*. A similar maternal influence is seen in *L.caesar* and *C.vicina* (Fraser and Smith 1963; Ring 1967a; Vinogradova and Zinovjeva 1972). Towards the end of the blowfly season free ranging females generated more diapausing offspring than laboratory bred females, even when the larvae were raised under standard laboratory conditions.

Photoperiod and/or temperature seem to be the major factors controlling this maternal influence (Ring 1967b; Tachibana and Numata 2004b; Vinogradova and Zinovjeva 1972). Additionally, diapause is also influenced by environmental cues acting directly on the larvae, such as temperature, photoperiod and overcrowding (Fraser and Smith 1963; Mellanby 1938; Ring 1967b; Tachibana and Numata 2004b; Vinogradova 1974). It remains unclear if parental age influences the onset of diapause. Cragg and Cole (1952) and Tachibana and Numata (2004a) did not find a significant effect of parental age on the proportion of offspring of *L.sericata* that entered diapause, whereas Ring (1967a) did in *L.caesar*.

Mortality, exceeding 70%, has been recorded for *L.sericata* during the over-wintering period. It appears that low temperatures are not the primary cause of this high mortality, but depletion of conserved energy stores and actions of pathogens, such as nematodes and fungi are more likely to be responsible for the low survival (Pitts and Wall 2005; 2006).

1.6 Dormancy in *L.cuprina*

In south-eastern Australia, *L.cuprina* passes the winter in the postfeeding larval stage (Dallwitz and Wardhaugh 1984; Foster and Helman 1979; Foster et al. 1975; Mackerras 1933; McKenzie 1990; 1994; Norris 1959). A true diapause, such as the one that occurs in *L.sericata*, has not been described for *L.cuprina* (Dallwitz and Wardhaugh 1984; Norris 1959). Postfeeding larvae, kept at low temperatures, resume their development as soon as they are transferred to higher temperatures. Dallwitz and Wardhaugh (1984) described the dormancy as a distinct state of developmental arrest, initiated by soil temperatures experienced by the larvae themselves, but possibly also by the maternal photoperiod. The presence and onset of dormancy seems to vary regionally. In the Canberra region, there is a transitional phase from late March to early April; pupation either takes place within 15 days or is delayed until spring (Dallwitz and Wardhaugh 1984). In contrast, in Queensland (O'Sullivan et al. 1983) and western New South Wales (McLeod 1997), postfeeding larvae continue their development in winter when temperatures remain mild.

The mechanisms for the termination of the arrested devlopment have not been fully identified. It is believed that temperature is the main trigger for resumption of

development, most likely mediated by absolute temperature and/or a specific pattern of temperature changes (McLeod 2001).

Emergence of flies from larvae entering the ground between late March and September is synchronous, although the spread is greater than at other times of the year (Dallwitz and Wardhaugh 1984; McKenzie 1990).

Mortality of larvae and pupae during the over-wintering period is higher than during other times of the year, exceeding 85% in studies done by McKenzie in Heidelberg, a suburb in north-eastern Melbourne, Victoria (1990; 1994). The proportion of larvae reaching adulthood decreased with time spent in the ground and mortality was greater in genotypes that were resistant to either dieldrin or diazinon (McKenzie 1990; 1994).

1.7 The Distribution & Abundance of *L.cuprina* Flies

1.7.1 Oviposition

Female *L.cuprina* seek suitable oviposition sites on live sheep. Several factors predispose sheep to blowfly attack such as fleece rot, dermatophilosis, urine staining, scouring, foot rot and pre-existing strikes (Seddon and Albiston 1967; Tillyard and Seddon 1933). All these predisposing conditions feature moisture, which is an essential factor for oviposition (Tillyard and Seddon 1933). Female flies are attracted by odours associated with faecal material and bacterial activity (Browne 1979; Emmens and Murray 1982; 1983; Morris et al. 1998), as well as oviposition pheromones produced by gravid females (Barton Browne et al. 1969). Visual cues also play a part in the search for an oviposition site, but the relative importance of visual and olfactory cues remains unclear (Browne 1979; Tillyard and Seddon 1933).

Although *L.cuprina* is known to be one of the first flies to visit carrion, very few flies result from this infestation due to high competition for food and space from other blowfly species (Anderson et al. 1988; Barton 1982; Farquharson 1999; Fuller 1934; McLeod 1997; Waterhouse 1947). The burial of carcasses seems to favour *L.cuprina* and it has been therefore recommended to burn or poison carcasses before burial (Belschner 1957; Fuller 1932a).

In summary, it is believed that sheep are the principal breeding ground for *L.cuprina* and its distribution in south-eastern Australia appears to be limited to the presence of susceptible sheep (Anderson et al. 1988; Waterhouse 1947). In contrast, *L.sericata* has been frequently recorded in urban areas, in particular near garbage, and so its distribution has no close relationship with the presence of sheep (Monzu 1979; Waterhouse and Paramonov 1950).

1.7.2 Dispersal of Flies

Local variation in fly numbers seems mainly affected by weather and pasture conditions. In south-eastern Australia, flies prefer open pastures to bushland (Vogt and Woodburn 1979). In contrast, Norris (1959) found flies, in the Canberra region, equally common in timbered areas as in open pastures. Flies tend to be more abundant in places where sheep congregate, such as sheep camps, dams, and water troughs (Tillyard and Seddon 1933), and relative densities at these sites remain similar throughout the fly season (Denwood et al. 1999; Vogt and Woodburn 1979). A Tasmanian study found that significantly more flies were trapped when commercially available traps (Lucitraps®) were attached to a post, close to water or sheltered from the wind (Denwood et al. 1999).

L.cuprina flies tend not to travel far. Mark-recapture studies done by Gilmour et al. (1946) in Canberra, found flies up to 7.5 km from the release site within 30 h, but the mean dispersal of flies ranged from 0.7 to 3.5 km within 60 h of fly release. Foster et al. (1975) reported similar results 30 years later for the same area, with average dispersal distances ranging from 0.6 to 4.2 km over the same time interval of 60 h. Gurney and Woodhill (1926) found flies 6.4 km away from the release point, after 16 days, in north-west New South Wales. Similar results were cited by Norris (1959), with some flies trapped 6.4 km away from the release centre within 48 h. The mean dispersal distances varied from 1.2 to 1.6 km after 2 and 9 days, respectively. In summary, these studies concluded that *L.cuprina* flies do not disperse far, with most lingering within 1 to 2 km of their emergence site. However, later studies suggest that fly dispersal can occur more rapidly and over a larger distance when protein sources and oviposition sites are limited, such as in areas of low sheep density (Wardhaugh 2001).

Intrinsic to fly dispersal is fly activity, which is primarily affected by temperature, humidity and wind (Tillyard and Seddon 1933). The threshold for activity lies around 15°C, and fly activity decreases when temperatures exceed 30°C (Kitching 1977;

Nicholson 1934). It has been suggested that flies increase their activity when looking for a protein meal, a mate or a suitable oviposition site (Browne 1979). Liver-baited traps attract primarily young flies looking for a protein meal and mature flies looking for a suitable oviposition site (Kitching 1981). Caution is necessary with the interpretation of these results, given that the over representation of flies seeking protein might reflect a shortage of these resources (Kitching 1977).

1.7.3 Seasonal Abundance

In south-eastern Australia, flies are not present all year round, but over-winter as postfeeding larvae in the soil and emerge as flies during the following spring (Dallwitz and Wardhaugh 1984; Foster and Helman 1979; Foster et al. 1975; Mackerras 1933; McKenzie 1990; 1994; Norris 1959). The first appearance of flies in spring is directly linked to soil temperatures, and therefore differs from location to location and from year to year (Fuller 1934). The results of trapping during one year may not give a typical picture for a particular area because the number of flies trapped is influenced by several factors, including prevailing seasonal conditions which can profoundly affect fly activity (Fuller 1934; Gilmour et al. 1946). However, by averaging the results over a number of years the effects of abrupt changes in weather on fly activity is minimised and a better representation of fly abundance is achieved (Gilmour et al. 1946). In addition, Vogt and Woodburn (1983) were able to standardise trap catches to a 'standard' set of weather conditions, thereby providing relative measures of fly abundance, irrespective of the weather conditions.

Field results show two different patterns in seasonal abundance of *L.cuprina* for most of south-eastern Australia. The most common is a bimodal pattern, with two peaks each year; a primary, larger one in spring, and a secondary, smaller one in autumn. In Canberra, flies appear in October, increase numerically and reach a maximum in late November or early December. Abundance drops rapidly in January and February, and a secondary rise occurs in late March or early April. Flies usually disappear by mid April (Dallwitz and Wardhaugh 1984; Fuller 1934; Gilmour et al. 1946; Tillyard and Seddon 1933). In warmer areas, the spring maximum occurs earlier and the autumn maximum later than in Canberra (Gurney and Woodhill 1926; Tillyard and Seddon 1933). Flies were absent in winter in all areas studied, with the exception of north-western New South

Wales (Gurney and Woodhill 1926) and Western Australia (Monzu 1979) where flies were trapped all year round.

In contrast, Foster et al. (1975) and Barton (1982) did not find a bimodal pattern in the seasonal abundance of *L.cuprina*. Instead, flies were present from October to May, reaching one clear peak in late January or February. The fly densities recorded by Foster et al. (1975) in Canberra were significantly higher than those by Gilmour et al. (1946) in Murrumbateman, only 40 km north of Canberra. The difference in seasonal abundance, as well as in fly densities, between the studies done by Foster et al. (1975) and Barton (1982), and the studies done more than 30 years earlier by Fuller (1934) and Gilmour et al. (1946), could be accounted for by an increase in sheep numbers due to pasture improvement (Barton 1982), or changes in seasonal weather patterns.

One explanation for the different patterns of seasonal abundance of *L.cuprina* was given by Whitten et al. (1976). Fly numbers over summer are influenced by weather conditions, with low numbers during hot, dry summers and high numbers during wet summers. Summer rainfall decreases the soil temperatures, resulting in a higher survival of larvae and pupae. In addition, wet summers are characterized by higher numbers of susceptible sheep due to a higher prevalence of fleece rot (Belschner 1956) and scouring from new pasture growth and worm infections (Morley et al. 1976).

1.8 Blowfly Strike in Merino Sheep

1.8.1 Breech Strike

Breech strike refers to all strikes on the breech, crutch and tail. It is the commonest form of strike in Merino sheep. Initially, susceptibility to breech strike was associated with the wetting of the breech of ewes with urine (Belschner 1956; Tillyard and Seddon 1933). Subsequently, breech strike became more strongly associated with diarrhoea and breech soiling. This can arise from a number of causes, including worm infestations, change of feed or the widespread use of improved pastures (Larsen et al. 1994; Morley et al. 1976; Watts and Marchant 1977; Watts et al. 1979). Continuous wetting of the breech with urine or faeces causes skin irritation, accompanied by exudation of serum and bacterial decomposition, rendering this area attractive for oviposition by blowflies and providing

an ideal environment for the development of their eggs and larvae (Seddon and Albiston 1967; Tillyard and Seddon 1933).

Susceptibility to breech strike depends on the presence of moisture. Further, the retention of moisture varies with the degree and position of wrinkles of the skin. Plain, Mulesed and crutched breeches are generally less predisposed to breech strike (Graham 1979; Tillyard and Seddon 1933). In addition, any management practice that decreases diarrhoea and breech soiling in sheep will reduce their susceptibility to breech strike.

1.8.2 Body Strike

Body strike affects any part of the body apart from the breech, pizzle and head, and most commonly occurs on the withers, back, loin or sides of the sheep (Belschner 1937; Tillyard and Seddon 1933). Young sheep, regardless of sex, are more prone to body strike than older sheep (Raadsma 1987; Watts et al. 1979). Body strike occurs predominantly during periods of prolonged wet, humid weather, and is most frequently associated with fleece rot and dermatophilosis (Belschner 1956; Gherardi et al. 1981; Raadsma 1987; Watts et al. 1979).

1.8.3 Other Strikes

Pizzle strike occurs in wethers and young rams. It is associated with urine soiling of the wool around the prepuce, which can initiate inflammation and bacterial infection (infectious balanoposthitis) (Anonymous 1989; Belschner 1956; Tillyard and Seddon 1933). Pizzle strike often goes undetected, and can be a major source for increasing fly populations early in the season (Simpson 1990; Wardhaugh and Dallwitz 1984).

Poll strike occurs around the horns of rams. It is usually associated with the accumulation of skin secretions and debris at the base of the horns, or with skin damage and wounds from fighting (Belschner 1956; Tillyard and Seddon 1933; Watts et al. 1979).

Wound strike refers to strikes associated with infected wounds of any sort, such as tail docking, Mulesing, cuts from shearing or crutching, scabby mouth lesions, foot rot and foot abscesses, grass seed abscesses and conjunctivitis (Anonymous 1989; Belschner 1956; Watts et al. 1979).

1.8.4 Covert & Overt Strikes

By definition, covert strikes are only detected upon close physical examination of individual sheep. The strikes tend to be small and most commonly occur around the pizzle, crutch and horns. However, they can be 5 to 14 times more prevalent than overt strikes. In a two year study, the yield of larvae from most covert strikes was only 20% of that from overt strikes but their persistence for up to 3 or 4 months probably made a substantial contribution to subsequent fly populations (Anderson et al. 1988; Wardhaugh and Dallwitz 1984).

Overt strikes are easily detected during a routine inspection of the mob. Struck sheep have patches of discoloured wool, usually associated with an unpleasant odour; they appear restless, hold their head close to the ground and frequently try to bite the infected area (Tillyard and Seddon 1933). Physiological changes in affected sheep include an increase in temperature and respiratory rate, and loss of appetite and body weight (Broadmeadow et al. 1984; Walkden-Brown et al. 2000). Bacteria usually proliferate in the wound. If sheep are not treated, death can occur, as a result of a bacterial toxaemia and the systemic effects of large amounts of ammonia, released by 3rd instar larvae (Broadmeadow et al. 1984; Guerrini et al. 1988; Seddon and Albiston 1967).

1.9 Control of Flystrike

The acute nature of flystrike, and the losses that can occur from it, means that farmers must inspect susceptible sheep regularly during the flystrike season to detect and treat struck sheep. Consequently, measures to prevent and control flystrike are routinely undertaken on all Merino wool producing farms. These include management practices to reduce the susceptibility of sheep, such as crutching, shearing, Mulesing, selection of sheep resistant to blowfly strike and the application of effective insecticides, as well as techniques to reduce the abundance of flies, such as flytraps.

1.9.1 Crutching and Shearing

Crutching is the removal of wool stained with urine or caked with faeces from around the breech area. Traditionally, crutching is undertaken before lambing and/or 4 to 8 weeks before shearing (Anonymous 1989; Watts et al. 1979). The short breech wool, remaining after crutching, is not favourable for oviposition by *L.cuprina* as short wool

dries more quickly and is less likely to be soiled by urine and faeces. This dramatically decreases the sheep's susceptibility to breech strike. Shearing and crutching provides up to 8 weeks protection against breech strike in Mulesed sheep, except in scouring sheep, which can still be struck soon after these procedures (Morley et al. 1976; Morley and Johnstone 1983; Raadsma 1988; Watts et al. 1979). However, the timing of crutching does not necessarily coincide with the period of highest risk of flystrike (Reeve and Thompson 2005).

Practices usually carried out in conjunction with crutching include ringing (the removal of urine stained wool from around the pizzle and belly of wethers), and wigging (the removal of wool from the head) (Anonymous 1989; Armstrong et al. 2001; Seddon and Albiston 1967; Watts et al. 1979).

Recently shorn sheep have a considerably reduced risk of strike, especially during wet periods which favour the development of fleece rot and dermatophilosis, which are the major predisposing factors for body strike (Morley 1994; Raadsma 1987). However, time of shearing is often determined by factors such as time of joining and lambing, availability of shearers, wool quality factors, and convenience, which again may not coincide with periods of high blowfly activity (Campbell 2006; Irving 1991).

1.9.2 Mulesing

Mulesing is a surgical operation, which removes, by clean sharp shears, wool-bearing skin from the breech and tail of sheep. This increases the size of the natural bare area around the vulva and anus. The recommended procedure is to perform one cut on each side of the vulva, combined with the removal of all but a 'V' shaped piece of wool-bearing skin extending one-third of the length of the docked tail (Beveridge 1984; Morley and Johnstone 1983; Morley and Johnstone 1984). This operation is usually carried out on lambs at marking time and provides a substantial lifetime reduction in risk of breech strike (Douglass 1965a; 1965b; Dun and Donnelly 1965; Lightfoot and McGarry 1964; Luff 1976; Morley and Johnstone 1983; Rothwell et al. 2007; Watts and Luff 1978). Correct application of the Mules operation has been shown to reduce the prevalence of breech strike by up to 90% (Barton 1982; Morley and Johnstone 1983; Watts and Luff 1978; Watts et al. 1979).

Despite the long term benefits of the Mules operation, the practice was slow to be adopted (Morley and Johnstone 1983) and has recently been strongly criticized by animal rights activist groups (Lee and Fisher 2007). This led to the declaration in November 2004 by the Australian Sheep and Wool Industry Taskforce to phase out Mulesing by 2010 (Dorrian 2006b). Work funded by Australian Wool Innovation P/L (AWI) has since developed two alternative procedures to create a similar effect to Mulesing: clips and intra-dermal injection. Specially designed plastic clips are applied to folds of skin on the breech and tail. The loss of blood supply to these skin folds causes death of the skin and leaves a low-profile scar (Dorrian 2006a; 2006b). The second procedure being evaluated is the intra-dermal injection of the antiseptic, cetrimide, using a needleless gun. This causes necrosis and contraction of the skin, adopting a pattern similar to Mulesing (Dorrian 2006a; Rothwell et al. 2007).

1.9.3 Tail Docking

The docking of tails, to reduce the accumulation of faeces around the tail and breech, is a common practice in Australia. It is performed on lambs at marking time, usually when lambs are 3 to 6 weeks of age. The recommended length is at the third palpable joint, level with the tip of the vulva in ewes (Morley and Johnstone 1983). Sheep with shorter tails are unable to lift their tail sufficiently, leaving the skin in this area moist when experiencing diarrhoea and therefore making this region highly attractive to blowflies (Watts and Luff 1978; Watts and Marchant 1977). Sheep with tails docked at the correct length are consequently less susceptible to breech strike than sheep with any other tail length (Graham et al. 1947; Morley and Johnstone 1983; Riches 1941).

1.9.4 Insecticides

Farmers have relied on insecticides for both prevention and treatment of flystrike, since the 1950s. Many characteristics of insecticides, such as their mode of action, method of application, length of protection provided, withholding periods for wool and meat, and the amount of residues in wool, affect their use on farms. Insecticides, can be broadly grouped into those that directly kill the feeding stages of *L.cuprina,* and those with indirect actions on their morphogenesis.

1.9.4.1 Insecticides that Directly Kill Larvae

Organophosphates, of which diazinon is the most common, were introduced in the 1950's for use against blowfly strike as a replacement for the cyclodiene compounds to which significant resistance had occurred (Shanahan, 1958). They are contact poisons and therefore quite useful as wound dressings when treating struck sheep (Joshua 1999b; Levot 1990; Levot 1993; 1995). When first introduced, jetting or dipping provided a protection period of 12 to 14 weeks (Levot 1993; 1995). However, resistance was detected in 1965 (Shanahan and Hart 1966) and is now widespread in field populations of *L.cuprina*, reducing the protection period to about 4 to 6 weeks (Levot 1993; 1995). On average, wool residues exceed the standards set for the European market 8 to 10 times, and their side-effects are particularly harmful to aquatic insects (Brightling 1999; Joshua 1999b). For this reason, together with the hazards associated with the application methods, registration of diazinon for dipping and jetting was suspended in May 2007 (Anonymous 2006). Nevertheless, diazinon is still the main ingredient in fly dressings and its use for treatment of strikes will be continued for the foreseeable future.

Spinosad, the first member of the spinosyn class of insecticides, has a unique effect on the insect nervous system, causing muscle contractions, paralysis and death in target pests. Spinosad provides 4 to 6 weeks protection against flystrike and is also available as a fly dressing to protect or treat wounds for blowfly strike. A reduced period of protection may result when used on sheep with less than 6 weeks wool. It is an extremely valuable agent for use against strikes close to shearing because it has a nil withholding period for both wool and meat (eMIMS 2006).

Synthetic pyrethroids act on the nervous systems of target pests, causing paralysis and death, and also suppress oviposition. They are principally used to control lice on sheep. However, alpha-cypermethrin is registered for use against flystrike, giving up to 10 weeks protection against body strike. It is administered as a backline pour-on and has a withholding period of 2 months for wool (eMIMS 2006; Joshua 1999c; Levot 1990; Tellam and Bowles 1997).

Macrocyclic lactones are rarely used for blowfly control in sheep. However, field trials have demonstrated that jetting sheep with ivermectin can give up to 12 weeks protection against blowfly strike (Eagleson et al. 1993).

1.9.4.2 Insect Growth Regulator Pesticides

Cyromazine prevents moulting of larvae. It provides up to 14 weeks protection as a dipping or jetting fluid and is also available as a low volume spray-on. The protection period against blowfly strike may be reduced when cyromazine is administered to sheep with less than 6 weeks wool. Since the introduction of cyromazine in 1979, no cases of resistance have been detected in field populations of *L.cuprina*. Cyromazine cannot be used less than 2 months before shearing (eMIMS 2006; Hart et al. 1979; Joshua 1999a; Levot 1990; Levot and Sales 2004).

Diflubenzuron interferes with the chitin production of *L.cuprina* larvae. It is mainly used as an insecticide against lice (Joshua 1999a; Levot and Sales 2004). However, it provides up to 12 weeks protection against blowfly strike when used as a dipping or jetting fluid. In addition, this compound is available as a pour-on formulation for use on sheep with long wool (eMIMS 2006). There have been several reports of resistance to diflubenzuron in field populations of *L.cuprina*, mainly in Queensland (Levot and Sales 2002). The withholding period for wool is 6 months (eMIMS 2006).

Dicyclanil, the most recently released IGR pesticide, prevents moulting of *L.cuprina* maggots (Joshua 1999a). The low volume spray-on formulation provides 18 to 24 weeks protection against blowfly strike and can be used 'off-shears' (eMIMS 2006). So far, no field resistance to dicyclanil has been reported. However, laboratory strains of *L.cuprina*, selected for resistance to diflubenzuron, an unrelated compound, have also been shown to exhibit a low-level of resistance to dicyclanil (Levot and Sales 2004).

1.9.4.3 New Insecticides

The search for new insecticides is an continual challenge. However, the precise mode of action and pathways of resistance of insecticides are often poorly understood. To address this, AWI is providing considerable funding to the blowfly genome project, a collaborative project between the University of Melbourne and Massey University in New Zealand. The immediate goal is to map the genome of the sheep blowfly, *Lucilia cuprina*, thereby creating the potential to identify new targets for insecticides and vaccines (Dorrian 2006a; Lee et al. 2007).

1.9.4.4 Use of Insecticides

The use of insecticides by farmers varies greatly, presumably in response to their experience with flystrike. Some choose to treat all sheep routinely at about the same time each year, before the start of the expected 'fly season', whereas others delay treatment until a certain % of sheep in a mob have been struck (Lottkowitz et al. 1984). The threshold for treatment is typically 1-2% of new strikes per week (Webb Ware, pers comm.). Another common strategy is to just treat struck sheep as they are detected in a mob (Lottkowitz et al. 1984).

A survey of sheep farmers in Victoria, conducted as part of the AWI IPMs project, in September 2005, showed that 50% routinely treated their weaners for the prevention of breech and body strike, most commonly in December. About a third of the respondents routinely jetted their ewes and wethers, most commonly in November to prevent breech strike (De Cat et al., unpublished). The same survey identified that 97% of farmers used the Mules operation on Merino lambs. These figures were similar to the results found in the national survey conducted in 2004 (Reeve and Thompson 2005).

Timing of treatment and the use of chemical compounds is also determined by characteristics such as the length of wool when a chemical can be applied, the time of shearing in relation to permissible chemical residues in wool, and management factors that restrict the handling of sheep, such as lambing.

Rather than using chemicals during the peak of seasonal fly abundance, alternative treatment strategies, such as early treatment in spring to kill larvae from the first generation of flies that emerge from over-wintering larvae, have the potential to alter the population dynamics of *L.cuprina*, reducing fly numbers throughout the 'fly season', as well as the prevalence of flystrike (McKenzie and Anderson 1990).

1.9.5 Selection of Sheep Less Susceptible to Flystrike

The use of genetic selection to reduce the susceptibility of sheep to both body and breech strike is attractive, because this produces a permanent and cumulative change and hopefully reduce reliance on insecticides to control flystrike.

The effectiveness of selection of Merino sheep with less wrinkle on the breech has been known for many years (Belschner 1976). However, it is doubtful that breeding alone will

provide the degree of protection similar to that achieved by Mulesing and tail docking (James 2006; Morley 1949). The traits most likely to be successful in breeding programs include a larger natural bare area around the breech, less breech wrinkle, and a greater bare area on the ventral surface and sides of the tail (James 2006; Karlsson et al. 2001; Scobie et al. 2002). In addition, reduced scouring and dag formation (Larsen et al. 1999) and an increased immunological response to internal parasites and blowfly larvae will also be useful (James 2006).

The two main predisposing factors for body strike are fleece rot (Belschner 1937; McGuirk 1983; Raadsma 1987; Watts et al. 1979) and dermatophilosis (Gherardi et al. 1981; Raadsma 1990), of which resistance to fleece rot is the more heritable trait (heritability of up to 0.40, compared to up to 0.12) (Atkins and McGuirk 1979; Lewer et al. 1987; Raadsma and Rogan 1987; Rogan 1983). Direct selection against fleece rot and body strike demonstrated that, after 10 years of selection, the prevalence of body strike was reduced to 1% in the resistant flock compared to 19% in the susceptible flock (Raadsma 1987). Alternatively, selection can be indirect, using traits which are genetically linked to resistance to fleece rot, such as measured greasy wool colour, fibre diameter and clean fleece weight (Hayman 1953; Raadsma 1987; 1990). However, these characteristics do not always show a consistent relationship with resistance to fleece rot (McGuirk et al. 1978; Raadsma 1987).

Obviously, resistance to flystrike cannot be the only objective in a breeding program and it has to be weighed against production objectives, such as increased clean fleece weight, reduced fibre diameter and reproductive traits, all of which have direct financial benefits to the producer (Atkins and McGuirk 1979).

1.9.6 Flytraps

Trapping of flies to reduce blowfly populations is an alternative approach to decrease flystrike. Bait bins filled with liver and sodium sulphide have been used to attract flies (Anderson et al. 1990) and more recently, commercial traps and lures (Lucitraps® and Lucilures®) have been designed to specifically attract *L.cuprina* (Urech et al. 2001). Experiments by Mackerras (1936) demonstrated that intensive trapping can significantly lower the prevalence of strike. Later studies in the arid zone of New South Wales and southern Queensland supported these results (Anderson et al. 1990; Ward 2001). However, the question then and now remains: is intensive trapping an economical and

effective means of controlling flystrike and does a reduction in the fly population, lead to a decrease in the strike rate of susceptible sheep (Belschner 1957)? More recent evidence from Tasmanian studies suggest that reducing fly numbers by intense trapping will not always reduce the prevalence of flystrike (Horton et al. 2001).

In addition, the use of flytraps may not be the most effective way to reduce fly numbers. However, they are useful to detect the presence of flies, monitor fly numbers, and identify high-risk areas on a farm. Consequently, their use can be integrated with other management procedures to reduce the risk of strike, thus forming part of an Integrated Parasite Management (IPM) program to control blowfly strike (Anderson et al. 1990; Armstrong et al. 2001; Horton et al. 2001; Ward 2001).

1.9.7 Other Potential Methods to Control Flystrike

A number of alternative methods to control flystrike by disrupting the life cycle of *L.cuprina* have been investigated. These include vaccination against *L.cuprina* larvae, biological control of larvae and genetic modifications to the fly populations.

Natural strikes do not result in a strong immune response to larval infestations (East and Eisemann 1993; Sandeman et al. 1992). However, Sandeman (1992) showed that individual sheep could develop some resistance against blowfly strike after frequent larval exposures, although this immunity was only transient. A range of larval antigens have been identified for possible use in vaccines (Bowles et al. 1987; East and Eisemann 1993; Tellam et al. 1994; Tellam et al. 2001) and Bowles et al. (1996) showed that blowfly control through vaccination was feasible. However, the challenge remains to convert these promising experimental results into a cost-effective vaccine against blowfly strike for use on farms.

The biopesticide, *Bacillus thuringiensis* has been used worldwide for over 30 years to control various pests (Hill and Pinnock 1998; Schnepf et al. 1998). This organism is a common gram-positive bacterium characterized by its ability to produce a range of insecticidal toxins (Cooper 1994). Several isolates of *B.thuringiensis* are known to produce crystals toxic to *L.cuprina* larvae (Gough et al. 2002; Gough et al. 2005; Heath et al. 2004). However, trials demonstrated that the protection against natural strikes obtained with *B.thuringiensis* strains is considerably lower than that from the use of insecticides (Heath et al. 2004). A great advantage of *B.thuringiesis* preparations is the absence of chemical

residues (Levot 1993; Schnepf et al. 1998). Nevertheless, the commercial application of this technology is probably some time away.

Parasitoids and predators of *L.cuprina* have the potential to suppress the natural blowfly population. They have been considered since the early days of blowfly control, but no single species was identified that would successfully reduce the blowfly population (Gurney and Woodhill 1926; Tillyard and Seddon 1933). One explanation for this is that *L.cuprina* breeds principally on sheep, whereas the natural enemies of *L.cuprina* are more likely to be associated with carcasses and so encounter only a small proportion of the *L.cuprina* population (Foster et al. 1975). Smallridge (1995) demonstrated that the microsporidium *Octosporea muscaedomesticae* reduced the survival and reproductive capacity of adult *L.cuprina* in the laboratory. However, no experiments have yet been published evaluating the effect of *O.muscaedomesticae* on field populations of *L.cuprina* flies.

The genetic control method involves the release of genetically altered individuals to suppress and ultimately eradicate the local blowfly population (Foster et al. 1975; Whitten and Maddern 1983). A successful example of this approach is the sterile male release program for the control and eradication of the New World Screwworm fly, *Cochliomyia hominovorax,* from southern USA and central America (Baumhover 1966; Gullan and Cranston 2005).

A number of trials towards the genetic control of *L.cuprina* were undertaken in the Canberra region in 1976-1979 (Foster and Smith 1991; Foster et al. 1985; Vogt et al. 1985b), and on 'little' Flinders Island, off the Eyre Peninsula of South Australia, in 1985-1986 (Foster and Smith 1991; Mahon 2001). Subsequently, a larger trial was conducted in 1989-1991 on 'big' Flinders Island, which is part of the Furneaux Islands group in Bass Strait (Foster 1990; Foster et al. 1993). Mass reared males, carrying partial sterility and eye colour mutation, successfully mated with wild type females, resulting in the introduction of these harmful genes in their offspring. These field trials demonstrated the successful suppression of field populations in these geographically isolated areas. However, practical difficulties associated with the mass rearing of flies was a major problem during the large scale trial on Flinders Island. In addition, there were problems with the immigration of wild flies into the suppressed areas and lower survival of the offspring of released males in the field (Foster and Smith 1991; Foster et al. 1993; Tellam and Bowles 1997). This lead to the abandonment of the program in the early 1990s (Mahon 2001).

1.9.8 Integrated Pest Management

Integrated Pest Management (IPM) is the co-ordinated application of different methods for strategic pest control programmes that are effective, practical, economically viable and protective of both public health and the environment (Dent 1995). IPM requires a detailed knowledge of the ecology of the pest species and the application of ecological principles.

1.10 Conclusion

There is a large amount of information available on the biology and ecology of *L.cuprina*. However, several gaps remain in this knowledge and can be divided into two kinds. Firstly, some aspects of the biology are still unclear and need further research. These include the development of immature stages of the life cycle, in particular of postfeeding larvae, key factors for induction and termination of the arrested development of over-wintering larvae, causes of mortality during over-wintering and the importance of breeding sites other than sheep (McLeod 1997; Wardhaugh 2001).

Secondly, most of the information on the biology and ecology of *L.cuprina* has been collected in either Canberra or Western New South Wales, since 1920. However, this information might not be all relevant in the dry summer - high winter rainfall areas of south-eastern Australia. Detailed studies conducted in this area are limited to the early work of Mackerras and Fuller (1937), the later studies of Barton (1982), a survey of flystrike by Murray (1980), a survey of farmer attitudes and practices (Lottkowitz et al. 1984), and 2 major studies on insecticide resistant *L.cuprina* flies during winter in Heidelberg, Victoria (McKenzie 1990; 1994).

The specific objectives of this study were:

1) to obtain quantitative information about the over-winter survival of *L.cuprina* larvae in south-eastern Australia,

2) to describe the pattern of emergence of flies in spring in south-eastern Australia, and

3) to validate available models that predict the emergence of *L.cuprina* flies in spring from over-wintering larvae.

CHAPTER 2

MATERIALS & METHODS

The experimental design was based on previous studies done by Dallwitz and Wardhaugh (1984) and McKenzie (1990; 1994), but incorporated more frequent deposits and more detailed observations.

2.1 Experimental Site

The study was conducted over two years on a 1300 ha farm at Rokewood, 40 km south of Ballarat in the Western District of Victoria. The sole enterprise on the farm was wool production from a self-replacing flock consisting of 5800 fine-wool Merino ewes.

The experimental site was a fenced 900m^2 area within a 15 ha paddock (Figure 2-1). The site had pastures consisting predominantly of phalaris (*Phalaris aquatica*), tall fescue (*Festuca arundinacaea* cv. Demeter), and subterranean clover (*Trifolium subterraneum*). The soil at the site was a grey clay loam and classified as a yellow duplex, Dy3.43 (Northcote 1979). A solar powered weather station and data logger (Monitor Sensors Aust Pty Ltd.) were located at the centre of the site (Figure 2-6).

Rokewood has a typical climate for south-eastern Australia, characterized by dry summers and winter rainfall. The monthly averages of temperature and rainfall from the nearest Bureau of Meteorology station at Lismore (number: 089018) are listed in Table 2-1. The average annual rainfall at Lismore is 625 mm, slightly higher than the 60-year average of records kept on the farm (600 mm).

2.2 Field & Laboratory Pots

The design of the field pots followed that of McKenzie (1990). PVC plumbing pipe, 10 cm in diameter, was cut into lengths of 20 cm and sealed at one end with domestic flywire. The top of the pot was fitted with a removable lid with an 8 cm hole covered by

domestic flywire to prevent the escape of emerged flies (Figure 2-2). Similar but smaller 250 ml pots (height 7 cm, diameter 5 cm) were prepared for the laboratory studies. The laboratory pots had a removable flywire lid but an enclosed base (Figure 2-2).

In 2005, the field pots were filled to 3 cm from the lid with soil taken from the experimental site. The soil was sifted and autoclaved to kill any immature stages of *L.cuprina* that may have been present. In 2006, the pots were filled with coarse river sand following recommendations made by colleagues to facilitate the recovery of larvae and pupae (Wardhaugh, Mahon and Woodburn, pers comm). The sand was purchased from a nursery supplier and was not autoclaved. Before the change, a pilot study was conducted on five replicates of the same batch of postfeeding larvae transferred immediately into either soil or sand and incubated at 23°C. This found no difference between the proportion of flies emerging from larvae placed in either sand or soil (94% vs. 92%, t-test P = 0.37).

The pots for the field studies were placed in the ground so that the surface of the soil or sand in the pots was level with the soil on the experimental site. The pots were placed in line and 10 cm apart from each other (Figure 2-1).

2.3 Breeding of *L.cuprina* Larvae

The postfeeding larvae used for these experiments were bred from *Lucilia cuprina* flies caught in flytraps (Lucitrap®). In 2005, a single collection of over 100 adult flies took place on 31 January at a farm near Ballarat, 30 km north of the experimental site. In 2006, several collections were made at the farm in Rokewood and the farm near Ballarat during February and March to make a total pool of 100 adult flies. Each pool of flies was considered large enough to ensure sufficient variation of genotypes was present. The flies were reared and maintained at a constant temperature of 27°C and under a 24-hour light schedule in the fly laboratory of the Department of Genetics at the University of Melbourne.

The *L.cuprina* flies were housed in plastic flywire framed cages and had unlimited access to water and a protein biscuit (Figure 2-3). The flies were encouraged to lay eggs on a small piece of liver, covered with moist cotton wool to prevent the liver from drying out.

The eggs and first instar larvae were transferred to a container with a removable flywire lid and raised on reconstituted meat meal (Figure 2-4). Each container contained about 500-1000 larvae. The larvae took about 4 to 5 days in the medium to complete their development and leave the food source, entering the postfeeding wandering stage. At the time of collection, it was assumed that all larvae were fully-fed and `crop-full` and to be at the same stage of the life cycle. These postfeeding larvae were either counted and transferred to the experimental pots, or kept in the container until they emerged as flies, when they were transferred to the plastic cages. This ensured a sufficient population of flies was available for breeding successive batches of larvae.

The postfeeding larvae were collected around 9am, counted into groups of 20 and consecutively transferred to the randomly arranged field and laboratory pots (20 to pots 1, 2, 3 etc, then another 20 to pot 1, 2, 3 etc). Each field pot contained 5 lots of 20 larvae and each laboratory pot contained 1 lot of 20 larvae and 1 lot of 30 larvae. In 2006, five groups of 20 larvae were weighed to check if larval size influenced larval survival.

2.4 Experimental Design

Replicated samples of 100 postfeeding larvae were collected and transferred to field pots that were placed in the soil at the experimental site at intervals of 1 to 6 weeks. Deposits took place from 16 March to 30 November 2005 ('Year 1') and from 10 January to 24 October 2006 ('Year 2').

The dates of deposits and number of replicates made over the two years are summarized in Table 2-2. Three to five experimental pots were placed in the ground at times when temperatures were such that rapid development of postfeeding larvae to pupae was expected (spring, summer and early autumn). The number of replicates was increased to 10 from April to June, the times when transition to over-wintering occurred and increased mortality was expected (McKenzie 1990; 1994).

At the time of each field deposit, 3 to 5 laboratory pots, each containing 50 postfeeding larvae from the same batch, were placed in an incubator at 23°C in complete darkness ('laboratory controls'). The controls indicated the viability of the larvae at the start of each deposition. At certain times, there were fewer postfeeding larvae available and so the number of field and control pots had to be reduced (Table 2-2).

In 2006, additional replicates were added on four occasions in late autumn (12 replicates on 19 April, 1 and 9 May, and 13 replicates on 30 May). These pots were randomly selected and removed over time to determine the survival and the developmental stage of the larvae. For the first three dates, four pots were removed at 2, 4 and 8 weeks after the deposition of larvae, whereas four replicates of the 30 May deposit were removed after 2 weeks and three replicates each after 12, 13 and 15 weeks, respectively. After removal, the content of each pot was carefully sifted and the numbers of postfeeding larvae, dead larvae and pupae recorded. Larvae were classified as dead if they appeared dried out, yellow and immobile.

In summer 2006, the field pots deposited on 26 April 2005, 18 May 2005 and 8 June 2005, were removed from the experimental site and the content of each pot was carefully sifted. The number of pupae was recorded and pupae were classified as either open or closed. Pupae were defined as closed when the puparium was intact and open when the puparium was fractured into two or more pieces. Open pupae either contained remnants of flies or were empty. This was done to check that parasitoids were not responsible for larval mortality.

2.5 Emergence of Flies

The expected date of emergence of flies from each deposit of larvae was estimated from the data of Dallwitz (1984). Starting at least 4 days before these estimated times, the field pots were inspected daily between 11am and 2pm for the presence of flies. Any flies present were anaesthetized using a portable CO_2 dispenser and collected into numbered pots for subsequent counting. Separate counts were made for each replicate pot at each inspection. The laboratory pots were inspected daily for flies after 8 days, and any flies present were anaesthetized and counted.

Figure 2-1 The experimental site showing the location of the field pots and the weather station

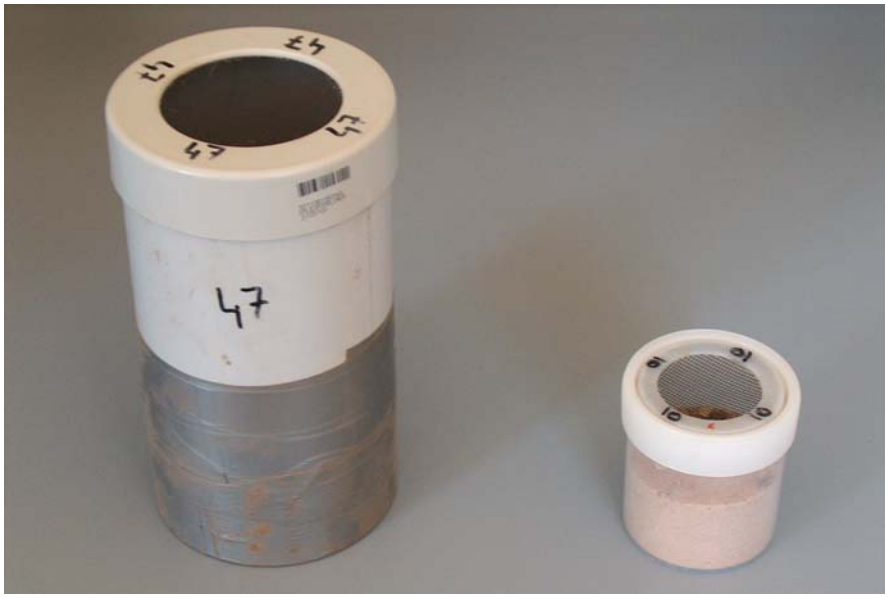


Figure 2-2 Field pot (left) and laboratory pot (right)

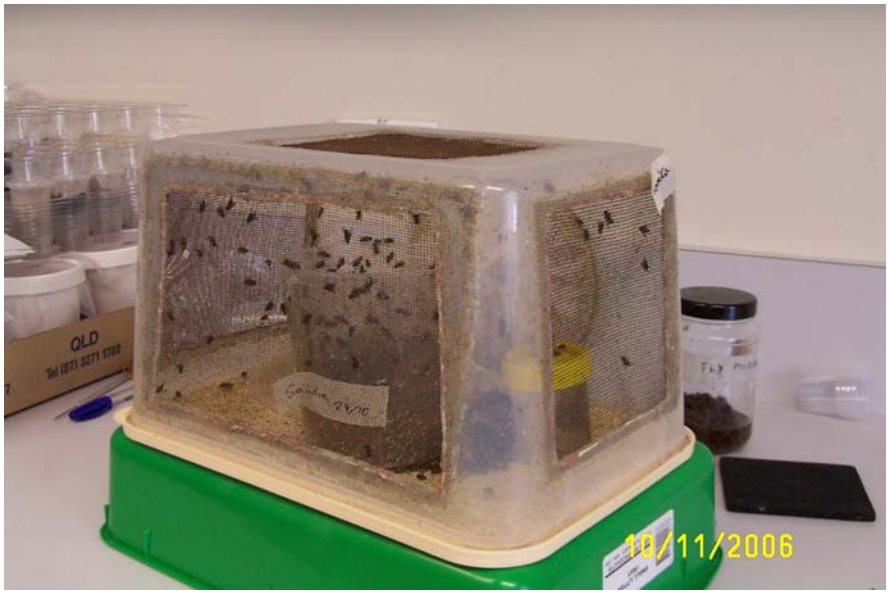


Figure 2-3 *L.cuprina* flies were maintained in a plastic flywire framed cage at 27°C and under a 24-hour light schedule

Figure 2-4 *L.cuprina* larvae were raised on reconstituted meat meal in a plastic container with a flywire lid, kept at 27°C

Table 2-1 Average measures for temperature, humidity and rainfall at Lismore, 30 km west of the experimental site (Bureau of Meteorology Station no. 089018, 1919-2004; Latitude: 37.9558 S, Longitude: 143.3422 E, Elevation: 160m)

Element	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Mean daily maximum temperature - deg C	26.7	26.1	23.8	19.3	15.8	13.1	12.4	13.6	15.7	18.3	20.7	23.8	19
Mean no. of days when Max Temp ≥ 40.0 deg C	0.5	0.3	0	0	0	0	0	0	0	0	0	0.1	0.8
Mean no. of days when Max Temp ≥ 35.0 deg C	3.7	2.8	0.7	0	0	0	0	0	0	0	0.4	1.6	9.3
Mean no. of days when Max Temp ≥ 30.0 deg C	9	8.5	4.8	0.6	0	0	0	0	0	0.4	2.2	5.1	30.6
Highest daily Max Temp - deg C	43.3	42.2	39.4	34.3	26.4	22.2	21.6	25	29.4	33	38.8	41.5	43.3
Mean daily minimum temperature - deg C	11.5	12.1	11	8.8	7.1	5.2	4.6	5	6	7.2	8.4	10.1	8
Mean no. of days when Min Temp ≤ 2.0 deg C	0	0	0	0.7	1.4	4.3	5.4	3.6	2.1	1.4	0.3	0.1	19.3
Mean no. of days when Min Temp ≤ 0.0 deg C	0	0	0	0	0.2	1.1	1.5	0.9	0.4	0.2	0	0	4.3
Lowest daily Min Temp - deg C	2.8	1.7	1.3	-1.1	-0.6	-3.3	-3.9	-3.7	-1.1	-1.9	0.6	2	-3.9
Mean 9am air temp - deg C	18.5	18.1	16.6	13.4	10.4	8	7.3	8.5	10.8	13.1	14.7	16.9	12.9
Mean 9am wet bulb temp - deg C	14.2	14.4	13.5	11.2	9.1	7	6.3	7.2	8.9	10.5	11.7	13.1	10.5
Mean 9am dew point – deg C	10.8	11.3	10.9	9.3	7.6	6	5.2	5.7	6.8	8	8.8	9.8	8.3
Mean 9am relative humidity - %	61	65	69	76	83	87	86	82	77	72	68	63	74
Mean 9am wind speed - km/h	14.5	14.2	13.6	12.7	12.7	13.1	13.9	14.6	15.9	14.7	14.5	14.1	14
Mean 3pm air temp - deg C	25.1	24.7	22.4	18.4	14.5	12.3	11.3	12.5	14.5	16.6	19.2	22	17.7
Mean 3pm wet bulb temp - deg C	16.7	16.8	15.7	13.3	11.3	9.8	8.9	9.4	10.9	12.2	13.9	15.2	12.7
Mean 3pm dew point – deg C	10.2	10.8	10	8.7	8	7	6.1	6	6.9	8.2	8.9	9.2	8.3
Mean 3pm relative humidity - %	39	42	47	55	66	70	71	65	61	58	52	45	56
Mean 3pm wind speed - km/h	16.4	15.7	15.5	16.1	16.2	16.5	17.8	17.9	18	16.2	16.4	16.5	16.6
Mean monthly rainfall – mm	37.4	36.7	35.7	51.1	54.4	54	58	68	63	64.1	55.3	47.1	624.9
Median (5th decile) monthly rainfall – mm	32.2	23.5	32	42.5	52.5	51.8	56.5	71.1	60.4	59	52	41.4	628.2
9th decile of monthly rainfall – mm	73.7	88.2	73.6	100	92.2	84.2	85.7	96.3	96.6	101.3	97.5	89.5	766.1
1st decile of monthly rainfall – mm	9.2	4.3	8	11.9	20.6	24.2	29.3	34	35.1	23.8	23.9	13.8	473.1
Mean no. of raindays	7.2	6.8	8.5	11.9	15.1	15.9	17.4	18.3	16	14.6	12.3	9.9	153.9
Highest monthly rainfall - mm	164.6	140.3	121.5	128.2	137	131.8	131.9	117.3	113.8	160.6	158.8	175.2	
Lowest monthly rainfall - mm	2.3	1.1	2.5	0	11.2	8.2	17.2	12.9	25.4	11.3	9.9	3.3	
Highest recorded daily rainfall - mm	102	122.6	45.7	80	38.9	42.7	31.8	42.9	57.4	49.6	59	105.2	122.6
Mean no. of clear days	7.9	7.3	5.9	4.8	3.3	3.6	3.1	2.5	3.1	3	3.3	5.2	52.9
Mean no. of cloudy days	9.9	9.5	13.1	13.9	17.1	15.1	16	16.5	14.4	17.3	16.8	14.4	174

Figure 2-5 Flytraps (Lucitraps®) were used to monitor the presence of *L.cuprina* flies at the experimental site

Figure 2-6 The solar powered weather station and data logger located at the centre of the experimental site, immediately adjacent to the field pots

2.6 Presence of Free-ranging *L.cuprina* Flies

The presence of free-ranging *Lucilia cuprina* flies was monitored by using flytraps (Lucitrap®) fixed to posts on the perimeter of the experimental site (Figure 2-5). Two traps were used between 13 October 2005 and 10 October 2006, and four traps between 10 October and 28 November 2006.

The three lures in each trap were replaced every 6 months as recommended by the manufacturer. These consisted of three separate bottles: Lure A (120 g/L sodium sulphide); Lure B (1055 g/L 2-mercaptoethanol & 47 g/L indole); and Lure C (960 g/L butanoic acid).

The traps were inspected daily when fly activity was considered more likely (spring, summer and autumn), and twice weekly when fly activity was less likely (late autumn and winter). The traps were emptied weekly when flies were active. A portable CO_2 dispenser was used to anaesthetize live flies before emptying the traps. The flies were sorted by species and the number of *L.cuprina* was recorded.

2.7 Weather Data

The centre of the site contained a solar powered weather station and data logger (Monitor Sensors Aust Pty Ltd.) (Figure 2-6). The station made hourly recordings of air temperature, soil temperature, soil moisture and solar radiation, and measured rainfall over the previous 24 hours. For hourly observations, the logger interrogated the sensor once every hour and logged the value read at that time.

Soil and air temperatures were measured in the range from -20 to 60°C (± 0.1°C). Air temperature was measured at a height of 2 m and soil temperature was measured at a depth of 5 cm under pasture. Soil moisture was recorded at a depth of 10 cm under pasture and measured as the percentage saturation by volume.

A tipping bucket rain gauge measured rainfall in 0.2 mm increments over the previous 24 hours (from 9 am the previous day to 9 am the current day). The system had a maximum capacity of 720 mm/hr.

The hourly weather data were also summarized as the daily mean, minimum and maximum. The mean or average value of a set of 24 observations within one day was the sum of all the observations divided by the number of observations, in this case 24. The daily minimum was the lowest number in the set of 24 readings within one day. The daily maximum was the largest number recorded in that day. This differs from the standard method in which the daily minimum and maximum is the value read at 9 am and 3 pm, respectively.

The 7-day rolling average temperature was the average temperature of the current daily average temperature and the daily average temperatures of the 6 previous days. The 7-day rolling minimum temperature was the average temperature of the current daily minimum temperature and the daily minimum temperatures of the 6 previous days. Similarly, the 7-day rolling maximum temperature was the average temperature of the current daily maximum temperature and the daily maximum temperatures of the 6 previous days.

A standard rainday was a day with a daily rainfall of at least 0.2 mm.

2.8 Statistical Methods

The Levene`s test for equal variances was used to describe the variance of the mean number of flies emerging from pots set up at different times. The Levene's test was the preferred test because it is less sensitive to data that depart from a normal distribution.

The mortality of the over-wintering larvae was calculated from the deposits made from May to mid-July. The deposits exhibiting a split emergence were excluded from these calculations because the number of larvae that entered arrested development in these deposits was not known. Deposits made later in winter were also not used as flies are normally not present in south-eastern Australia at that time of the year.

Table 2-2 Deposit dates and number of laboratory and field pots for each deposit in 2005 and 2006

Year	Deposit date	No of laboratory pots [a]	No of field pots	Year	Deposit date	No of laboratory pots [b]	No of field pots
2005	16-Mar	5	5	2006	10-Jan	3	3
	6-Apr	2	3		24-Jan	2	4
	26-Apr	5	10		7-Feb	3	3
	18-May	4	10		1-Mar	3	5
	8-Jun	5	10			3[c]	3[c]
	12-Jul	6	10		22-Mar	3	3
	10-Aug	3	5			3[c]	5[c]
	19-Sep	4	5		11-Apr	5	4
	5-Oct	3	5		19-Apr	4	10
	26-Oct	3	5		1-May	4	10
	30-Nov	3	4		9-May	6	10
					30-May	3	9
					18-Jul	6	9
					22-Aug	3	6
					19-Sep	2	4
					12-Oct	3	5

[a] 50 postfeeding larvae/pot; [b] 100 postfeeding larvae/pot; [c] larvae derived from recently caught wild flies

CHAPTER 3

RESULTS – FIELD STUDY

3.1 Emergence of Flies

The percenrages of flies that emerged from the larvae in the laboratory and field pots are shown in Table 3-2.

3.1.1 Laboratory Pots

The number of laboratory pots, each containing 50 larvae, ranged from 2 to 6 pots for each deposit time. The average percentage of larvae emerging as flies in the laboratory pots was 93%, ranging from 49 to 100%. Emergence of flies from larvae in the laboratory pots consistently occurred over a 3 to 4 day interval.

The data contained two outliers (values more than 1.5 times away from the interquartile range), 6 April 2005 and 19 September 2006 (Figure 3-1). After excluding the two outliers, the average emergence of flies in the laboratory pots was 95% (95% CI: 92.4, 96.8). When the two outliers were excluded, the variance of the mean number of flies emerging from pots set up at different times was not significantly different (Levene's test, $p = 0.975$).

Five batches of twenty postfeeding larvae, from each of 8 collections in 2006, were weighed and their average weight is shown in Table 3-1. The average weight of 20 larvae, over all the collections, was 0.755g. Larvae collected on 19 September and 12 October were the lightest, averaging 0.603g and 0.524g, respectively.

Table 3-1 The average weight (g) of 20 postfeeding larvae at different times of collection in 2006

Collection date	Weight[a] (g)
22-Mar	0.822
22-Mar[b]	0.994
11-Apr	0.705
1-May	0.718
9-May	0.773
30-May	0.823
22-Aug	0.829
19-Sep	0.603
12-Oct	0.524

[a] average weight of 5 groups of 20 postfeeding larvae
[b] larvae derived from newly trapped wild flies

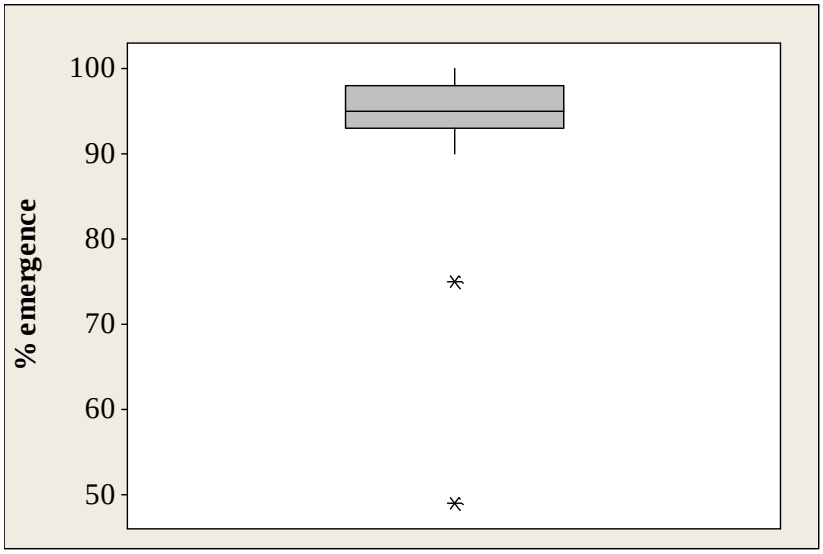


Figure 3-1 Boxplot of the average percentage of larvae emerging as flies from laboratory pots for each deposit time. The rectangular box represents the middle 50% (interquartile range) of the data and the lines extending to either end indicate the general extent of the data (* denotes outliers, points greater than 1.5 times the interquartile range)

Table 3-2 The number of laboratory and field pots (n) prepared for each deposit in 2005 and 2006, and the mean percentage of flies emerged (± standard deviation)

Year	Date of deposit	Laboratory pots		Field pots	
		n[a]	% emerging	n[b]	% emerging (SD)
2005	16-Mar	5	95	5	96 (1.3)
	6-Apr[e]	2	49	3	53 (13.1)
	26-Apr[c]	5	93	10	45 (15.4)[1]
					5 (5.7)[2]
	18-May	4	93	10	0 (0)
	8-Jun	5	94	10	5 (6.9)
	12-Jul	6	97	10	9 (7.1)
	10-Aug	3	95	5	17 (4.1)
	19-Sep	4	90	5	68 (7.5)
	5-Oct	3	91	5	43 (19.3)
	26-Oct	3	98	5	63 (16.1)
	30-Nov	3	93	4	47 (8.1)
2006	10-Jan	3	93	3	5 (4.5)
	24-Jan	2	91	4	52 (7.3)
	7-Feb	3	98	3	88 (6.1)
	1-Mar	3	97	5	81 (7.2)
	1-Mar[d]	3	98	3	90 (1.2)
	22-Mar	3	94	3	66 (3.6)
	22-Mar[d]	3	99	5	85 (3.2)
	11-Apr[c]	5	96	4	7 (2.6)[1]
					1 (1.0)[2]
	19-Apr[c]	4	99	10	4 (1.8)[1]
					13 (9.0)[2]
	1-May	4	97	10	11 (7.7)
	9-May	6	95	10	21 (12.9)
	30-May	3	100	9	45 (15.7)
	18-Jul	6	98	9	50 (12.3)
	22-Aug	3	95	6	72 (7.2)
	19-Sep[e]	2	75	4	54 (10.1)
	12-Oct[e]	3	93	5	24 (19.0)

[a] 50 postfeeding larvae/pot; [b] 100 postfeeding larvae/pot; [c] deposits exhibiting a split emergence of flies between autumn[1] and spring[2]; [d] larvae derived from recently caught wild flies; [e] small postfeeding larvae for this deposit

3.1.2 Field Pots

The number of field pots for each deposit date ranged from 3 to 10. The average percentage of larvae emerging as flies in the field pots ranged from 0 to 95.8%, depending on time of deposit (Table 3-2). The patterns of the emergence of flies from field deposits are shown in Figure 3-2 and Figure 3-3.

3.1.2.1 Summer & Early Autumn Deposits (Dec-Mar)

The average percentage of flies emerging from larvae deposited during summer and early autumn was 64.5% (95% CI: 38.5, 90.5).

Development of larvae was rapid during this period and the interval over which flies emerged was relatively short, ranging from 3 to 12 days (Table 3-3 and Figure 3-4). The first flies emerged 10 to 19 days after the deposition of larvae, the median number of flies emerged after 10 to 19 days, and the last flies appeared 12 to 26 days after larvae were deposited.

3.1.2.2 Mid-autumn & Winter Deposits (Apr-Aug)

One deposit in 2005 (26 April) and two deposits in 2006 (11 and 19 April) exhibited a split emergence of flies. Some larvae pupated and emerged as flies in autumn, whereas others remained dormant during winter and emerged as flies in spring (Figure 3-2 and Figure 3-4).

The percentage of larvae emerging as flies, as well as the pattern of emergence, varied considerably for these three deposits. The total percentage of larvae emerging as flies from the 26 April deposit was 49.8%, with 89% of flies emerging in autumn and 11% in spring. The emergence of flies was much lower the following year, 7.8% and 16.7% for the 11 April and 19 April deposits, respectively. For the 11 April deposit, 90% of the total flies appeared in autumn, whereas for the 19 April deposit only 23% of the total flies emerging appeared in autumn.

The interval, during late autumn, over which flies emerged from these deposits ranged from 26 to 43 days (Table 3-3 and Figure 3-4). The first flies appeared in autumn, 30 to 44 days after the deposition of larvae. The median number of flies emerged in autumn after 41 to 55 days and the last flies appeared 68 to 72 days after the deposit date.

There was a much broader interval for flies emerging in spring, with the time between the first and last emergence ranging from 3 to 40 days (Table 3-3 and Figure 3-4). The first flies emerging after winter appeared 159 to 177 days after the deposition of larvae. The median number of flies emerged after 177 to 180 days, and the last flies appeared 179 to 205 days after the larvae were deposited.

In 2005, no flies emerged from the 18 May deposit and the percentage of flies emerging was also low for the three subsequent deposits; 4.6, 9.3 and 16.8% from the 8 June, 12 July and 10 August deposits, respectively. In 2006, the percentage of larvae emerging as flies from deposits made in late autumn through to winter was higher, with the average emergence of flies from the 1 May, 9 May, 30 May, 18 July and 22 August deposits being 11.1, 21.1, 45.2, 49.7 and 71.1%, respectively.

Emergence of flies from deposits made between mid-autumn and the end of winter was delayed until spring in both years. The first flies emerged from these deposits on 1 October and 26 September in 2005 and 2006, respectively. This emergence of flies was synchronous for all deposits made from mid-autumn to winter (Figure 3-2 and Table 3-3). The interval between the first and last emergence of flies from over-wintering larvae in spring was large, ranging from 27 to 51 days (Table 3-3 and Figure 3-4). In 2005, the first flies from the 26 April, 8 June, 12 July and 10 August deposits emerged on 2 October, 12 October, 2 October and 1 October, respectively. The dates when the median number of flies emerged from the latter deposits were 23 October, 27 October, 13 October and 19 October, respectively. The last flies emerged on 4, 7, 1 and 20 November, respectively. In 2006, the first flies from the 11 April, 19 April, 1 May, 9 May, 30 May, 18 July and 22 August deposits emerged on 5 October, 2 October, 3 October, 26 September, 28 September, 29 September and 4 October, respectively. The median number of flies emerged from these deposits on 7, 13, 13, 9, 10, 10 and 24 October, respectively. The last flies emerged on 7 October, 10 November, 9 November, 4 November, 1 November, 25 October and 17 November, respectively.

3.1.2.3 Spring Deposits (Sep-Nov)

The percentage of larvae emerging as flies from deposits made in spring was higher than from autumn and winter deposits, averaging 50% (95% CI: 28.3, 72.6). The interval between the first and last emergence of flies ranged from 6 to 39 days (Table 3-3 and Figure 3-4). Emergence of flies was more rapid and less dispersed as soil temperatures

increased later in spring. The first flies emerged 14 to 24 days after deposition of larvae, the median number of flies emerged after 18 to 32 days and the last flies appeared 24 to 62 days after deposition of larvae.

3.1.2.4 Variability between Replicates at Each Deposition Time

Overall, the variance around the mean number of flies at each deposit time was not the same ($p = 0.02$) (Figure 3-5). However, means from deposits made in summer, early autumn and spring had equal variances ($p > 0.05$), whereas those made in late autumn and winter had unequal variances ($p < 0.01$).

3.1.2.5 Assessment of Pupae Contained in Deposits made in Autumn 2005

Results from the recovery of the life cycle stages, in summer, from deposits of larvae made in autumn 2005 are given in Table 3-4. Six months after the 18 May deposit, more than 80% of the deposited larvae were recovered as pupae of which 16% appeared opened and 84% closed.

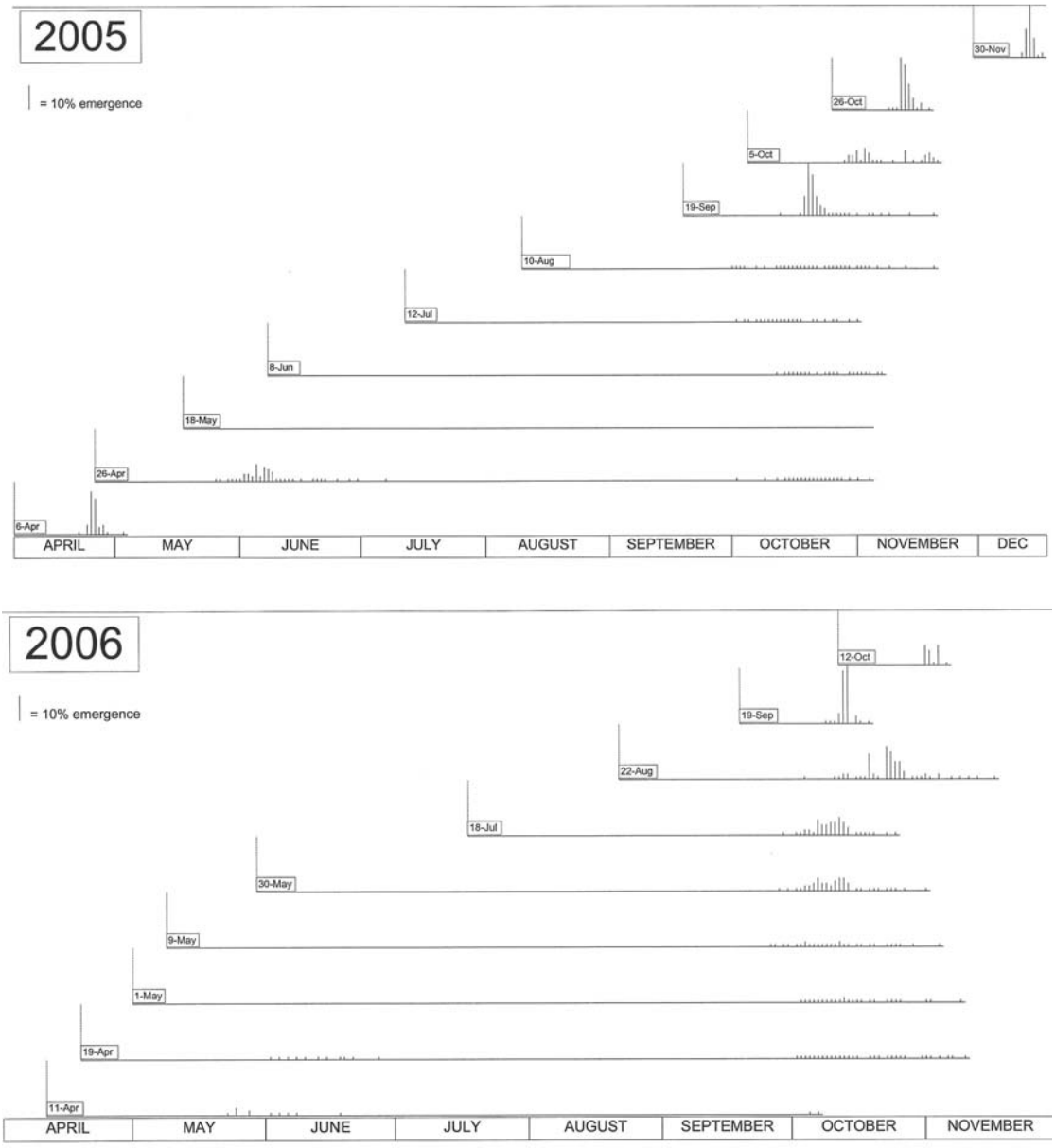


Figure 3-2 The pattern of emergence of flies from replicated deposits made in autumn, winter and spring during 2005 and 2006. Vertical lines on the left indicate the date of deposit. Vertical lines on the right of each main vertical line represent the daily proportion of flies that emerged

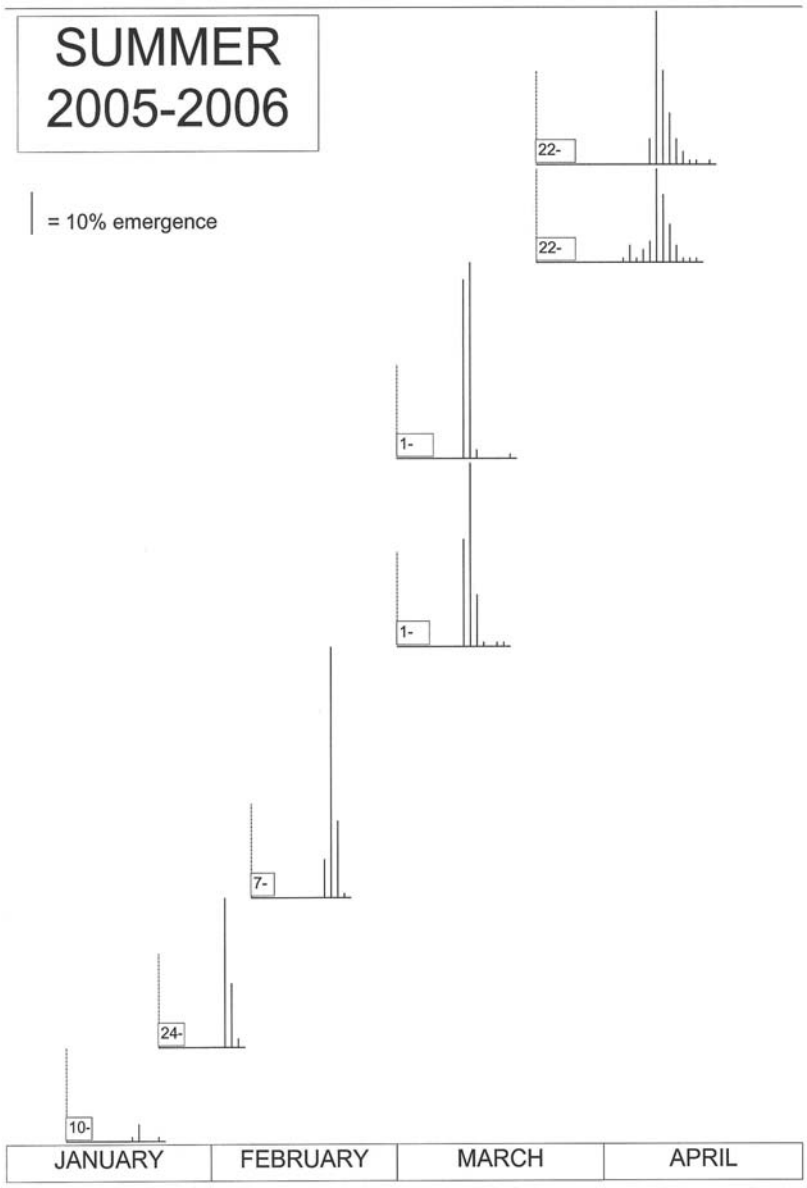


Figure 3-3 The pattern of emergence of flies from deposits made in Jan-Mar of 2006. Vertical lines on the left indicate the date of deposit. Vertical lines on the right of each main vertical line represent the daily proportion of flies that emerged. Upper graphs for March and April show the emergence of flies from larvae derived from newly trapped wild flies and the lower graphs represent the emergence of flies from larvae derived from the 1-year old laboratory stock

Table 3-3 Dates of first, median, and last emergence of flies for each deposit in 2005 and 2006. The number of days between the deposit date and date of emergence of flies is given in brackets

Year	Date of deposit	First emergence of flies	Median emergence of flies	Last emergence of flies	Range [a]
2005	16-Mar	4-Apr (19)	4-Apr (19)	7-Apr (22)	4
	6-Apr	22-Apr (16)	25-Apr (19)	3-May (27)	12
	26-Apr	26-May (30)	6-Jun (41)	7-Jul (72)	43
		2-Oct (159)	23-Oct (180)	4-Nov (192)	34
	18-May				0
	8-Jun	12-Oct (126)	27-Oct (141)	7-Nov (152)	27
	12-Jul	2-Oct (82)	13-Oct (93)	1-Nov (112)	31
	10-Aug	1-Oct (52)	19-Oct (70)	20-Nov (102)	51
	19-Sep	13-Oct (24)	21-Oct (32)	20-Nov (62)	39
	5-Oct	29-Oct (24)	4-Nov (30)	21-Nov (47)	24
	26-Oct	9-Nov (14)	13-Nov (18)	19-Nov (24)	11
	30-Nov	12-Dec (12)	14-Dec (14)	17-Dec (17)	6
2006	10-Jan	20-Jan (10)	21-Jan (11)	24-Jan (14)	5
	24-Jan	3-Feb (10)	3-Feb (10)	5-Feb (12)	3
	7-Feb	18-Feb (11)	19-Feb (12)	21-Feb (14)	4
	1-Mar	11-Mar (10)	12-Mar (11)	18-Mar (17)	8
	22-Mar	8-Apr (17)	10-Apr (19)	17-Apr (26)	10
	11-Apr	23-May (42)	28-May (47)	18-Jun (68)	27
		5-Oct (177)	7-Oct (179)	7-Oct (179)	3
	19-Apr	2-Jun (44)	13-Jun (55)	27-Jun (69)	26
		2-Oct (166)	13-Oct (177)	10-Nov (205)	40
	1-May	3-Oct (155)	13-Oct (165)	9-Nov (192)	38
	9-May	26-Sep (140)	9-Oct (153)	4-Nov (179)	40
	30-May	28-Sep (121)	10-Oct (133)	1-Nov (155)	35
	18-Jul	29-Sep (73)	10-Oct (84)	25-Oct (99)	27
	22-Aug	4-Oct (43)	24-Oct (63)	17-Nov (87)	45
	19-Sep	9-Oct (20)	13-Oct (24)	19-Oct (30)	11
	12-Oct	1-Nov (20)	2-Nov (21)	6-Nov (25)	6

[a] between first and last fly emergence

Table 3-4 Percentage of opened and closed pupae recovered from pots removed in summer after deposition of larvae in the previous autumn

Deposit date	Percentage recovered*			
	Closed pupae	Opened pupae	Opened pupae with content of flies present	Total
26 Apr 05	2	43	5	50
18 May 05	68	13	0	81
8 Jun 05	57	17	0	74

* 100 postfeeding larvae per pot

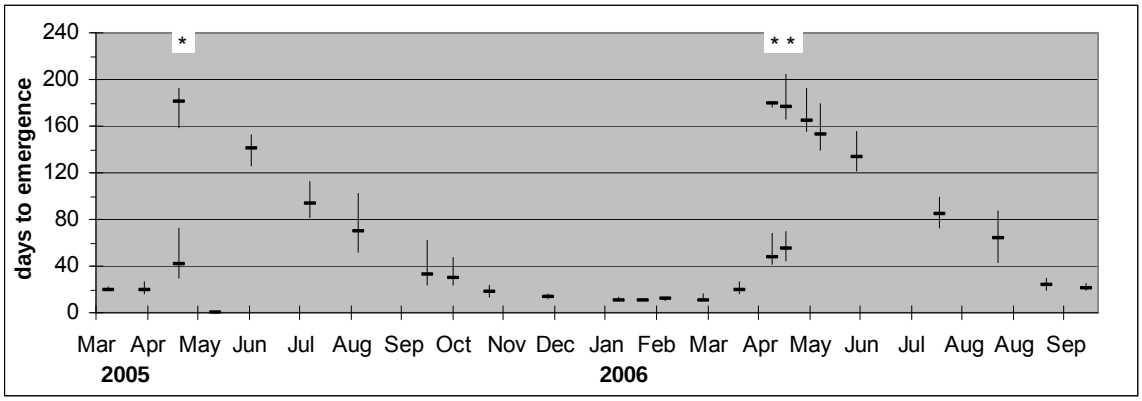


Figure 3-4 The number of days between deposition of larvae and emergence of flies. The vertical lines indicate the date of deposit, the bottom end of each line is the day when the first fly appeared, the cross bar is the day when the median number of flies emerged and the top end of each line is the day of emergence for the last fly (* indicates a split emergence between autumn and spring)

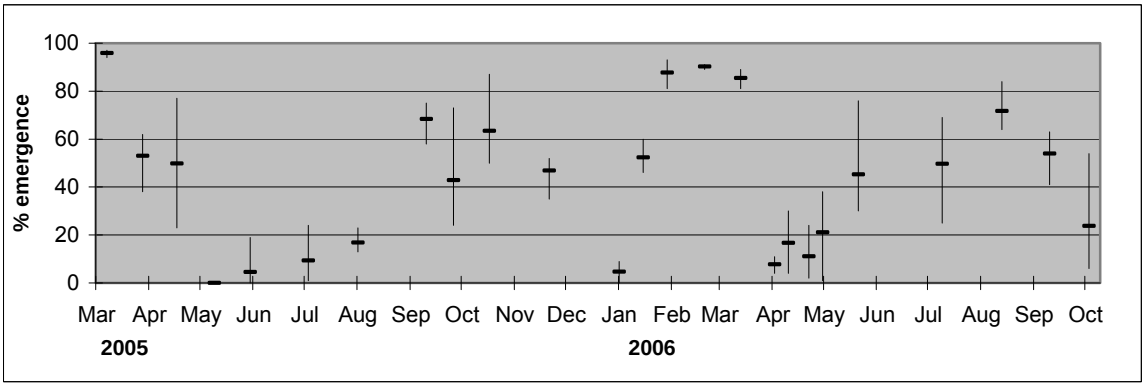


Figure 3-5 The percentage of flies that emerged from larvae in each field deposit. The vertical line indicates the date of deposit, the bottom end of each line is the replicate with the lowest percentage, the crossbar is the average percentage of all replicates and the top end of each line is the replicate with the highest percentage of flies that emerged from the deposit

3.2 Sequential Development of Larvae in Pots Removed in Winter & Early Spring

Results from the recovery of larvae and pupae from the additional deposits of larvae made in late autumn 2006 are set out in Table 3-5. The percentage of all life cycle stages that were recovered varied from 76 to 98%.

The proportion of pupae, recovered two weeks after the deposition of larvae on 19 April, 1 May, 9 May and 30 May, declined progressively from 23.6 to 15.2 to 7.6 and to 0% of the total life cycle stages recovered. For deposits made on 1 May, the proportion of pupae recovered gradually increased as pots were removed 2, 4 and 8 weeks after deposition (15.2, 21.9 then 22.7%, respectively). In contrast, the proportion of pupae from larvae deposited on 19 April and 9 May remained constant at 25 and 7%, respectively, irrespective of the time after deposition when the pots were recovered.

Dead larvae were common in the 1 May deposit, comprising 13, 12 and 26% of the life cycle stages recovered 2, 4 and 8 weeks after deposition of larvae, respectively. In contrast, the proportion of dead larvae did not exceed 5% in pots deposited on 19 April and 9 May.

The only time when flies were recovered was 8 weeks after the 1 May deposit, when they comprised 5% of the total life cycle stages recovered.

Nine pots, set up on 30 May, were examined in late winter before fly emergence could be expected. Pupae comprised less than 3% of all life cycle stages recovered at 13 weeks after deposition of larvae (31 August). This increased to 42% at 15 weeks after deposition of larvae (14 September). There was evidence of increased mortality on resumption of development of larvae, with 68% of the live larvae recovered on 14 September emerging as flies when incubated at 23ºC, compared to 83% of those recovered on 31 August (Table 3-6).

Table 3-5 The number of live and dead larvae and pupae, and percentage of total numbers recovered from pots removed in winter and early spring after deposition of larvae in late autumn 2006

Deposit date	No. of pots[a]	Time after deposition	No. of larvae and pupae (% of total recovered)			Total (% of larvae deposited)
			Live larvae	Dead larvae[b]	Pupae	
19-Apr	4	2 weeks	230 (74.4)	6 (1.9)	73 (23.6)	309 (77%)
1-May	4		248 (72.3)	43 (12.5)	52 (15.2)	343 (86%)
9-May	4		331 (90.2)	8 (2.2)	28 (7.6)	367 (92%)
30-May	4		375 (95.9)	16 (4.1)	0	391 (98%)
19-Apr	4	4 weeks	248 (69.3)	19 (5.3)	91 (25.4)	358 (90%)
1-May	4		223 (65.0)	45 (13.1)	75 (21.9)	343 (86%)
9-May	4		317 (91.1)	10(2.9)	21 (6.0)	348 (87%)
19-Apr	4	8 weeks	207 (67.9)	9 (3.0)	68 (22.3)	305 (76%) [c]
1-May	4		158 (51.3)	80 (26.0)	68 (22.7)	308 (77%)
9-May	4		309 (89.0)	11 (3.2)	27 (7.8)	347 (87%)
30-May	3	12 weeks	205 (75.9)	53 (19.6)	12 (4.4)	270 (90%)
30-May	3	13 weeks	218 (87.2)	25 (10.0)	7 (2.8)	250 (83%)
30-May	3	15 weeks	112 (46.3)	29 (12.0)	101(41.7)	242 (81%)

[a] 100 postfeeding larvae/pot - [b] larvae visually classified as dead when removed from soil - [c] includes 19 flies which emerged in the field and two dead newly emerged flies

Table 3-6 The number of flies that developed from larvae and pupae incubated at 23°C after their recovery from pots removed 13 and 15 weeks after the deposition of larvae on 30 May 2006

Time after deposition	No. of flies emerging from total recovered		
	Live larvae	Dead larvae[a]	Pupae
13 weeks	181/ 218 (83%)	1/ 7 (12%)	2/ 7 (29%)
15 weeks	76/ 112 (68%)	1/ 29 (3.4%)	64/ 101 (64%)

[a] Larvae visually classified as dead when removed from soil

3.3 Trapping of Flies

In 2005, the first flies after winter were trapped on 17 October. Flies were continuously present in the traps from then until the start of April 2006. The last fly before winter was trapped on 28 April. No flies were trapped during the winter of 2006 and the first flies after that winter were trapped on 7 October, 10 days earlier than in the previous year.

In each year, fly numbers were relatively low until mid-November. Numbers then increased rapidly and peaked in late November (Figure 3-6, A & B). Subsequently, fly numbers declined rapidly in January 2006, with only a few flies present in February. There was a slight increase in fly numbers in early March, followed by a steady decrease until 28 April, when the last flies were trapped (Figure 3-6 A).

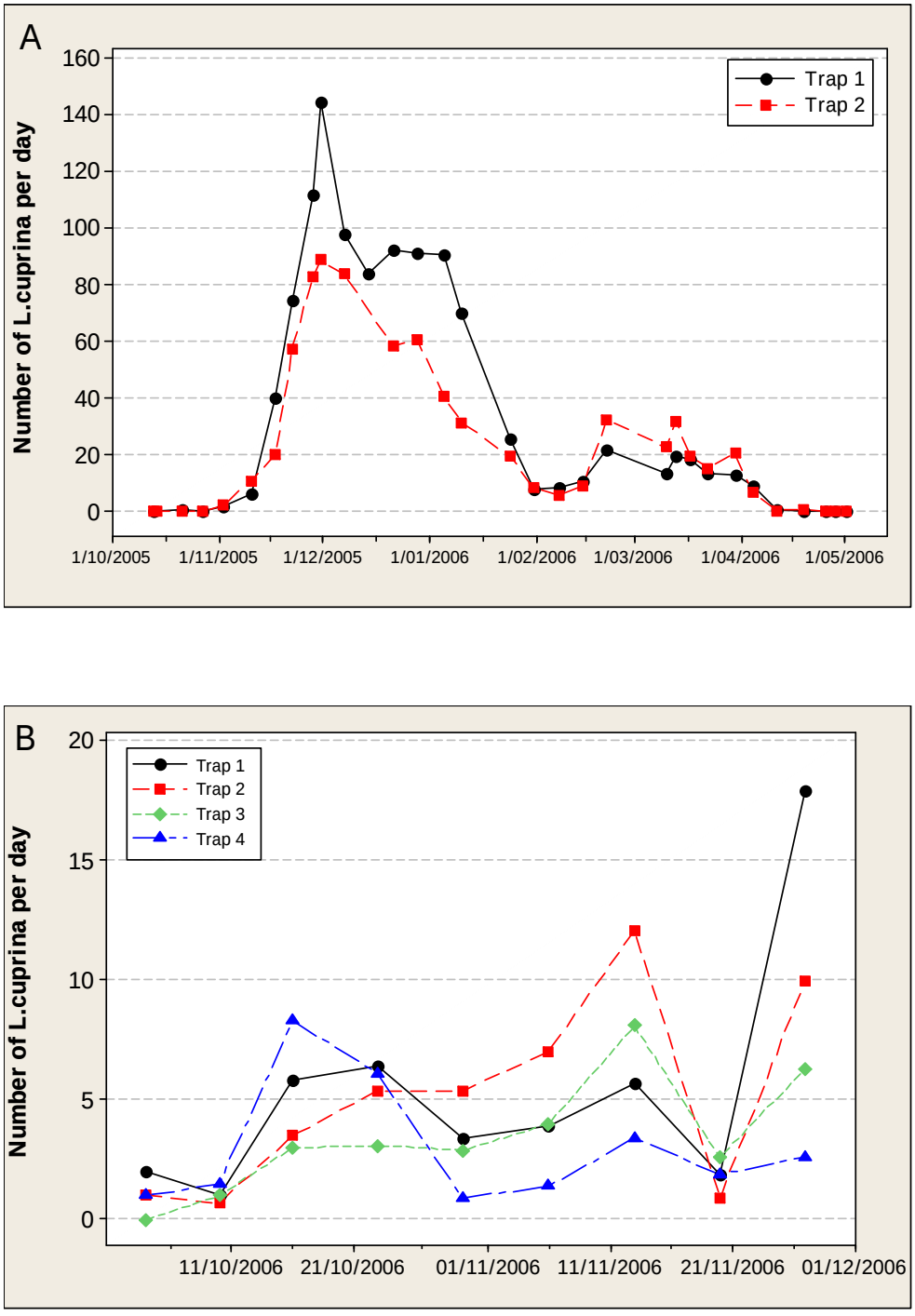


Figure 3-6 The number of *L.cuprina* flies trapped at the experimental site from spring 2005 to autumn 2006 (A) and during spring 2006 (B). Traps were emptied at 6-8 day intervals and the number of flies per day derived from the number of flies trapped divided by the number of days in the collection period

3.4 Weather Data

3.4.1 Air Temperature

Air temperatures from mid-autumn to mid-winter were consistently higher in 2005 compared to 2006. In 2005, the monthly means of the average daily air temperatures were 16.4, 12.1, 10.5 and 9.7°C in April, May, June and July, respectively. These means were 4.2, 2.0, 2.1 and 0.9°C warmer than the corresponding means in 2006 (Table 3-7). In particular, the average maximum temperature in April 2005 was 23.0°C, more than 5°C higher than in 2006.

The average air temperatures during late winter and spring were similar in both years, although the range between the minimum and maximum air temperatures was greater in 2006 than in 2005. The monthly means of the average daily air temperatures for August, September, October and November varied less than 1°C between both years and were 9.9, 11.0, 13.1 and 15.8°C in 2005, respectively (Table 3-7).

3.4.2 Soil Temperature

Consistent with the warmer air temperatures, soil temperatures during autumn and winter were higher in 2005 than in 2006. The monthly means of the average daily soil temperatures were 16.9, 12.8, 10.1 and 9.4°C in April, May, June and July 2005, respectively. These monthly means were 3.2, 1.5, 1.1 and 0.3°C warmer than in 2006 (Table 3-7).

The soil temperatures during late winter and spring were similar in both years (Table 3-7). The monthly means of the average daily soil temperatures for August, September, October and November varied less than 1°C between both years and were 9.7, 11.7, 14.5 and 17.7°C in 2005, respectively.

Soil temperatures varied less throughout the day than air temperatures, with the monthly means of the daily minimum soil temperatures being closer to the monthly means of the daily maximum soil temperatures (Table 3-7).

The maximum daily soil temperatures did not exceed 15°C during July and August in either year. The maximum daily soil temperature exceeded 15°C for the first time on 8

September in 2005 and on 2 September in 2006. The average daily soil temperature was above 15°C for the first time on 17 October in 2005. However, in 2006, this occurred almost a month earlier on 19 September.

The average daily minimum, mean and maximum soil temperatures for the week following each deposit date are summarized in Table 3-9. The highest temperatures were recorded after deposits were made on 10 and 24 January 2006. The lowest temperatures occurred following the deposits made on 12 July 2005, 10 August 2005 and 18 July 2006.

To investigate the relationship between soil temperature and the induction of arrested development of larvae, the 7-day average, minimum and maximum soil temperatures were computed for each day of the week following the three deposits exhibiting a split emergence (26 April 2005, 11 April 2006 and 19 April 2006) and those immediately following these deposits (18 May 2005, 1 May 2006) (see Figure 3-7).

The 7-day rolling average soil temperatures for the week following the deposits exhibiting a split emergence ranged from 12.3 to 16.4°C. The 7-day rolling average and minimum soil temperatures were similar for the deposits immediately after the deposits exhibiting a split emergence (18 May 2005 and 1 May 2006), but the 7-day rolling maximum soil temperatures were about 1.5°C higher in the week following the 18 May 2005 deposit compared to 1 May 2006 deposit. In 2005, the 7-day rolling average, minimum and maximum soil temperatures for the deposit exhibiting a split emergence (26 April) were considerably higher compared to the next deposit (18 May). However, in 2006, the 7-day rolling average soil temperatures for the last deposit exhibiting a split emergence (19 April) and the next deposit (1 May) were similar. The 7-day rolling minimum soil temperatures were even slightly lower for the week following the 19 April deposit than those of the 1 May deposit, whereas the 7-day rolling maximum temperatures for the week following the 19 April deposit were slightly higher than those following the 1 May deposit.

To examine the relationship between soil temperature and the resumption of development of arrested larvae, the 7-day rolling average soil temperatures were calculated for each day from mid-August until the end of September. The results are shown in Figure 3-8, in relation to the period in 2006 when the percentage of pupae developed from arrested larvae increased from 3 to 42% (Table 3-5).

In 2005, starting at 6 September, the 7-day rolling average soil temperatures rose 1.9°C over a 4 day period, then dropped 1.9°C over the next 7 days and rose for a second time with 2.0°C over the next 8 days. The 7-day rolling average soil temperature exceeded 11°C for nine consecutive days, starting on 8 September. In 2006, a similar soil temperature pattern was seen, starting a week earlier than in 2005. From 30 August onwards, the 7-day rolling average soil temperatures first increased 1.5°C over a 4-day period, than dropped 1.5°C over the next 7-day period and then subsequently increased again 2.3°C over the next 8 days. The 7-day rolling average soil temperatures surpassed 11°C for 7 consecutive days, starting on 2 September.

Table 3-7 Monthly means of daily average (minimum, maximum) air and soil temperatures (°C) measured at the experimental site in 2005 and 2006

Month	Air temp		Soil temp	
	2005	2006	2005	2006
Jan	18.1 (11.7, 25.9)	19.7 (12.6, 28.2)	22.1 (18.2, 28.0)	23.2 (19.5, 28.7)
Feb	16.2 (10.7, 22.7)	17.6 (11.7, 24.9)	18.9 (16.5, 22.6)	21.3 (18.0, 26.3)
Mar	15.1 (9.6, 21.4)	17.1 (11.1, 24.7)	17.8 (14.8, 22.1)	19.6 (16.8, 23.6)
Apr	16.4 (10.5, 23.0)	12.2 (8.1, 17.2)	16.9 (14.0, 20.7)	13.7 (11.9, 16.2)
May	12.1 (8.3, 16.6)	10.1 (6.5, 14.0)	12.8 (10.9, 15.5)	11.3 (10.1, 12.5)
Jun	10.5 (7.2, 14.2)	8.4 (5.0, 12.2)	10.1 (8.6, 12.2)	9.0 (8.0, 10.2)
Jul	9.7 (6.8, 13.1)	8.8 (5.8, 12.1)	9.4 (8.0, 11.3)	9.1 (8.2, 10.1)
Aug	9.9 (6.5, 14.0)	10.1 (6.5, 14.5)	9.7 (8.3, 11.9)	10.0 (8.8, 11.4)
Sep	11.0 (7.3, 15.4)	11.6 (6.4, 17.3)	11.7 (9.9, 14.3)	11.8 (10.1, 13.7)
Oct	13.1 (8.3, 18.2)	13.3 (6.2, 21.2)	14.5 (12.4, 17.3)	15.2 (12.5, 18.2)
Nov	15.8 (9.5, 22.5)	15.1 (8.2, 23.4)	17.7 (15.0, 21.3)	17.9 (15.0, 21.5)
Dec	18.5 (10.5, 27.2)	18.0 (9.4, 27.2)	20.2 (16.4, 25.0)	21.6 (17.9, 25.9)

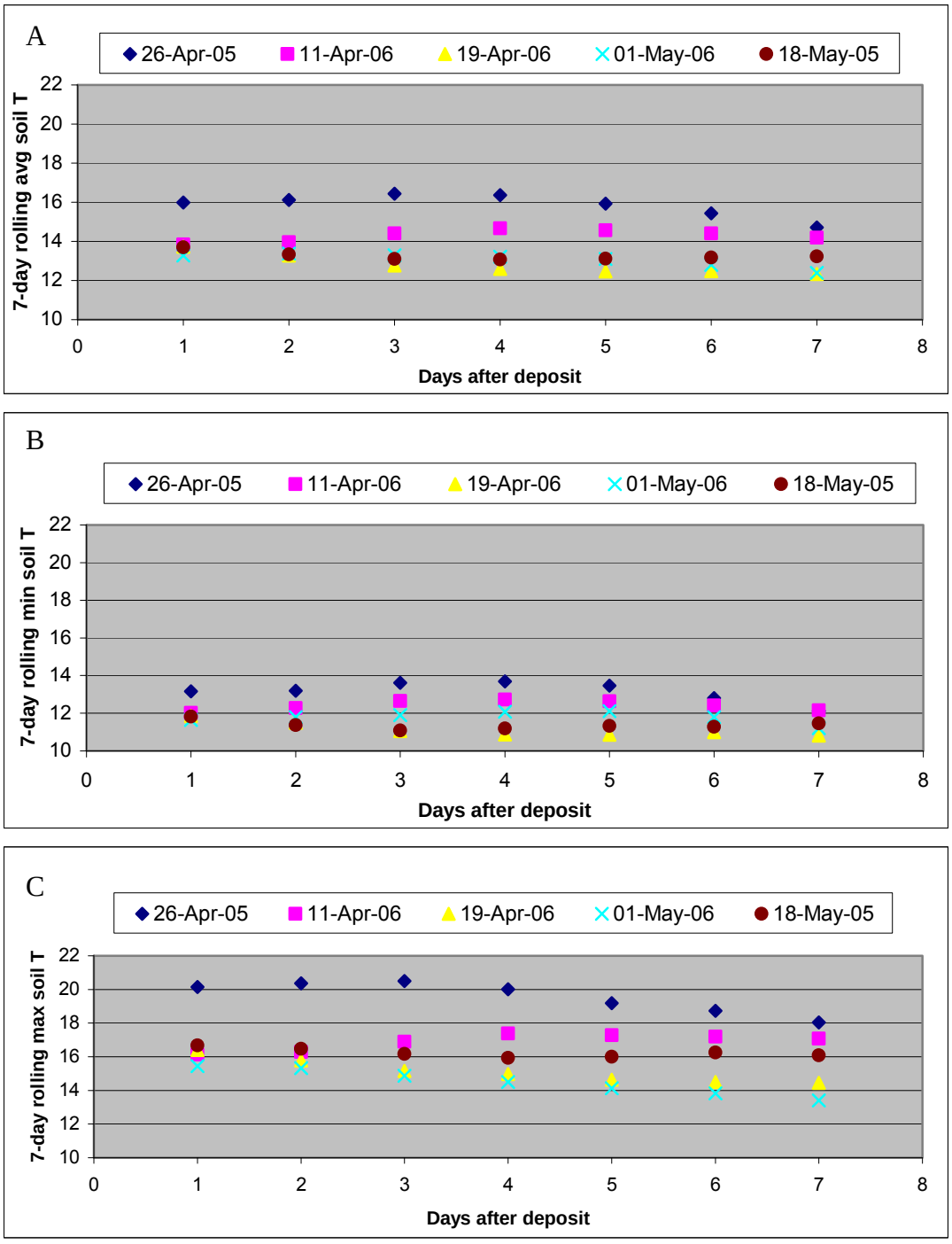
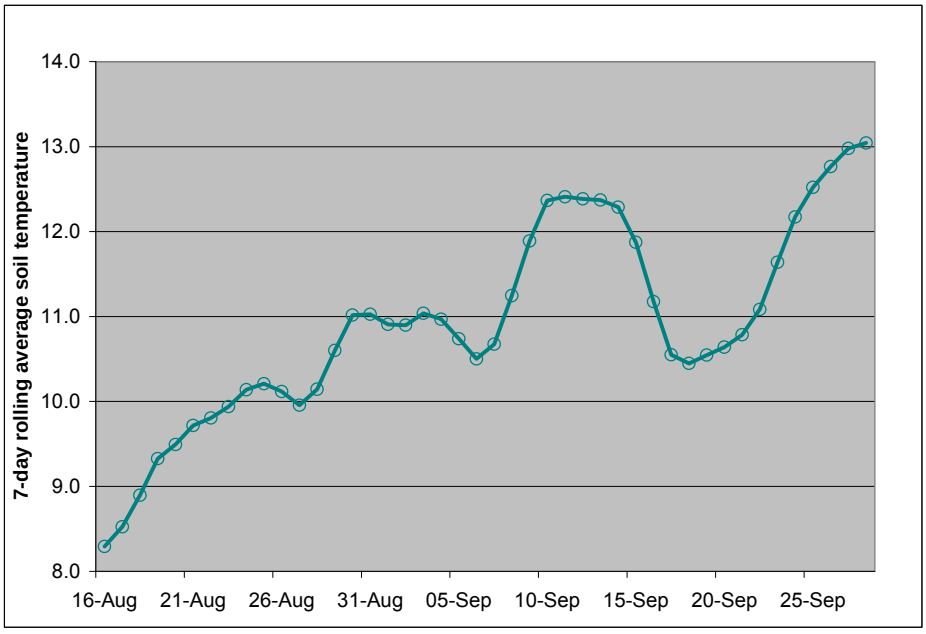


Figure 3-7 The 7-day rolling average (A), minimum (B) and maximum (C) soil temperatures (°C) for each day of the week following the deposits on 26 April 2005, 18 May 2005, 11 April 2006, 19 April 2006, and 1 May 2006

A)



B)

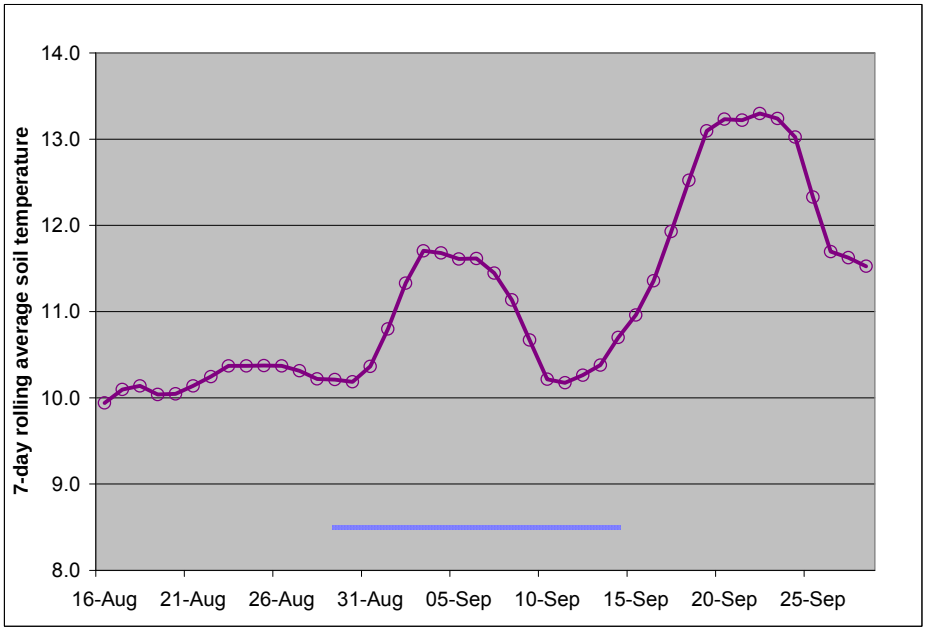


Figure 3-8 The 7-day rolling average soil temperatures (°C) for August and September in relation to the period in 2006 when pupation of arrested larvae changed from 3 to 42% of the total recovered life cycle stages (blue bar), (A) 2005 and (B) 2006

3.4.3 Rainfall & Soil Moisture

In 2005, the total rainfall at the experimental site was 603 mm. A total of 514 mm of rain fell at the nearest Bureau of Meteorology station in Lismore, 30 km west of the experimental site. This was 18% below the long-term average of 625 mm (Table 2-1).

Drought conditions prevailed over most of south-eastern Australia in 2006. The rainfall at the experimental site was 392 mm. In Lismore, a total of 381 mm of rain fell in 2006, which was only 61% of the long-term average.

The daily rainfall patterns from autumn to mid spring are shown in Figure 3-9. The weather was drier in late autumn in 2005 compared with 2006, which had more raindays and more rainfall per month. April and May received less than 20 mm rain in 2005, whereas in 2006, the monthly rainfall exceeded 50 mm in April and May (Table 3-8). However, there was considerably more rainfall in late winter and early spring in 2005 than in 2006. The total rainfall from July to October was 288 mm in 2005 compared to only 144 mm in 2006. During this time, there were 15 days in 2005 and 10 days in 2006 with a daily rainfall exceeding 5 mm/day. The heaviest rainfall was recorded on 31 August in 2005 and on 25 August in 2006, receiving 40.8 mm and 18 mm, respectively.

The total rainfall for the week following each deposit date is listed in Table 3-9. The most rain fell after the deposit made on 5 October 2005, when 51.6 mm was recorded. Several deposits had less than 5 mm rain for the week following deposition of larvae, including deposits on 16 March, 6 April and 26 October 2005, and 1 March 2006, 22 March, 30 May, 18 July and 12 October 2006.

The monthly means of daily soil moisture, measured at a depth of 10 cm at the experimental site for both years of the study, are shown in Table 3-8. In 2005, the soil remained dry until June, and then consistently held a moderate amount of moisture until mid-October. In 2006, good autumn rainfall increased the average soil moisture in early May. This then gradually declined until an increase in mid-July, after which it again decreased, with small peaks following some rainfall in late August and September. The soil at the site remained dry from mid-September onwards.

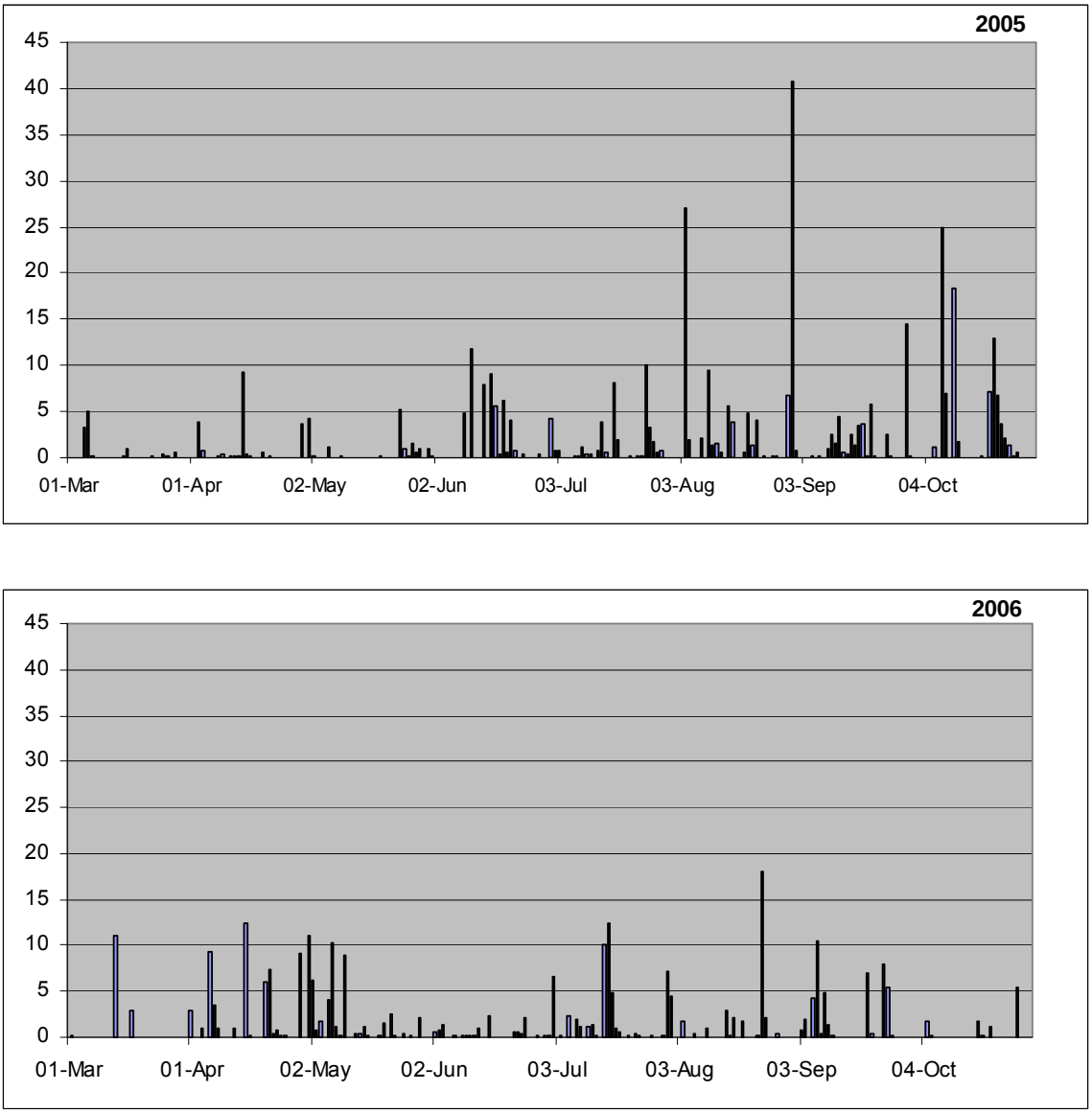


Figure 3-9 Daily rainfall (mm) measured at the experimental site between 16 March and 31 October in 2005 and 2006

Table 3-8 Monthly rainfall (mm) and monthly means of the daily average soil moisture (percentage saturation by volume) measured at the experimental site in 2005 and 2006

Month	Total rainfall		Soil moisture	
	2005	2006	2005	2006
Jan	21.0	49.6	0.2	0.2
Feb	108.4	46.6	4.1	0.2
Mar	11.0	14.2	0.2	0.1
Apr	20.0	55.6	0.1	0.5
May	16.6	54.0	0.1	8.6
Jun	52.2	11.4	5.7	1.5
Jul	40.6	52.6	8.7	5.9
Aug	112.6	35.6	15.7	3.6
Sep	46.4	45.2	11.6	1.6
Oct	88.6	10.6	10.1	0.1
Nov	46.0	16.8	0.3	0.1
Dec	39.2	1.0	0.2	0.1

Table 3-9 The average daily minimum, mean and maximum soil temperatures (°C), total rainfall (mm), and average soil moisture (percentage saturation by volume) for the week following each deposit of larvae in 2005 and 2006

Year	Date of deposit	Minimum	Mean temperature	Maximum	Rainfall	Soil moisture
2005	16-Mar	15.2	18.1	22.2	1.2	0.154
	06-Apr	15.8	18.6	22.9	1	0.152
	26-Apr	12.1	14.7	18.0	8	0.145
	18-May	11.5	13.2	16.1	5.4	0.142
	08-Jun	10.6	11.9	13.5	24.6	0.676
	12-Jul	7.2	8.7	10.7	15.4	7.979
	10-Aug	7.0	8.3	10.4	22.4	19.233
	19-Sep	11.0	12.5	14.7	9	10.456
	05-Oct	10.9	12.8	15.0	51.6	10.661
	26-Oct	14.1	16.1	18.9	0.8	4.891
	30-Nov	15.5	18.8	23.0	8.2	0.166
2006	10-Jan	18.2	22.0	27.9	10.6	0.159
	24-Jan	21.1	24.3	28.9	13.8	0.166
	07-Feb	16.4	19.8	24.8	14.4	0.148
	01-Mar	19.0	22.1	26.8	0.2	0.152
	22-Mar	18.0	21.0	25.3	0	0.148
	11-Apr	12.2	14.2	17.1	13.6	0.133
	19-Apr	10.8	12.3	14.5	15.0	1.234
	01-May	11.2	12.4	13.4	34.0	9.670
	09-May	10.4	11.5	12.5	11.2	12.345
	30-May	8.4	9.6	11.0	2.8	3.947
	18-Jul	7.3	8.4	9.6	2.4	13.099
	22-Aug	9.1	10.2	11.5	20.8	4.521
	19-Sep	10.6	12.3	14.1	20.8	0.376
	12-Oct	14.0	16.5	19.3	1.8	0.129

CHAPTER 4

DISCUSSION – FIELD STUDY

Results from the field study provide a good description of the over-wintering phase of the life cycle of *L.cuprina* and the pattern of emergence of flies in spring for south-eastern Australia. Such data is essential for the formulation of practical and effective measures to control flystrike in this region

Over all times of deposition, with the exception of two times larvae put into the field pots were viable, as emergence of flies from laboratory pots exceeded 90%. Two deposits, on 6 April 2005 and 19 September 2006, respectively, showed a reduced viability, with only 49 and 75% of the larvae in the laboratory pots developing to flies. These larvae were visibly smaller, with those of 19 September being 20% lighter than those deposited at other times. Thermal stress during the feeding stage of these larvae may account for their reduced viability and smaller size because the temperature in the rearing laboratory dropped below the optimum for several days.

There was rapid development and a compact emergence of flies when postfeeding larvae entered the ground during the warmer parts of the year, from late spring through to early autumn. A transitional phase of larval development was observed during mid-autumn of both years (11-26 April). Some larvae deposited at these times pupated immediately and emerged as flies in late autumn, whereas others entered an arrested development and pupated and emerged as flies the following spring. The proportion of larvae developing immediately was higher when deposition took place early in the transitional phase, whereas towards the end of this period most larvae were arrested in their development.

Arrested development of larvae has been previously described for the Canberra region, where the transitional phase occurred from late March to early April, somewhat earlier than observed in the present study (Dallwitz and Wardhaugh 1984). Comparison of soil temperatures between the two studies showed that the minimum soil temperatures for Canberra were consistently 1-5^{o}C cooler than at Rokewood from late March onwards (Table A-1.1).

Previous studies on blowfly species have indicated that temperature and photoperiod experienced by both the parental and current generations play a role in the induction of arrested development or diapause of postfeeding larvae (Dallwitz and Wardhaugh 1984; Ring 1967a; Tachibana and Numata 2004b; Vinogradova 1974; Vinogradova and Zinovjeva 1972). However, the exact contribution of each factor is not well understood for all species. A study on *L.sericata* revealed that short days and low temperatures in the current and the parental generation favour the induction of diapause (Tachibana and Numata 2004b). However, flies and larvae used in the present study were reared in the laboratory under 24 hour light conditions and at 27°C, so it would appear that cooler temperatures, experienced by the postfeeding larvae, alone are sufficient to induce arrested development in *L.cuprina*.

A period of sustained low temperatures seems a more likely factor for inducing an arrested development, rather than a particular temperature threshold amongst the daily fluctuations that typically occur during autumn. Thus, the patterns of the 7-day rolling average, minimum and maximum soil temperatures in the week following the deposits exhibiting a split emergence (26 April 2005, 11 and 19 April 2006), and the deposits immediately following these deposits (18 May 2005 and 1 May 2006) were compared (Figure 3-7).

The 7-day rolling average and minimum soil temperatures were similar for the 18 May 2005 and 1 May 2006 deposits, being around 13 and 11.5°C, respectively. However, the 7-day rolling maximum soil temperatures in the week following the 18 May 2005 were around 16.2°C, more than 1.5°C higher than those after the 1 May 2006 deposit. In 2005, the 7-day rolling average, minimum and maximum soil temperatures after the 18 May deposit were well below those after the 26 April deposit. None of the larvae of the 18 May 2005 deposit emerged and so the differences in soil temperatures between the last deposit exhibiting a split emergence and the next deposit could only be examined in 2006.

The 7-day rolling average soil temperatures for the week following the 19 April 2006 deposit were similar to those of the next deposit on 1 May. The 7-day rolling minimum soil temperatures for the week following the 19 April deposit were slightly lower than those of the 1 May deposit, whereas the 7-day rolling maximum soil temperatures for the week following the 19 April deposit were slightly higher than those following the 1 May deposit. However, some larvae in the 19 April deposit pupated and emerged as flies in

late autumn, whereas larvae in the 1 May deposit did not develop until the following spring.

Foster et al. (1975) stated that temperatures below 10°C inhibit pupation. In Canberra, the average maximum and minimum temperatures experienced by the postfeeding larvae during the transitional phase were 23 and 12°C (Dallwitz and Wardhaugh 1984). The average maximum soil temperatures during the transitional phase were lower in this study than in Canberra, around 17-18°C, whereas the minimum soil temperatures in this and the Canberra study were similar, around 12°C.

Two explanations for the differences in timing of pupation, between the depositions made on 19 April and 1 May, are feasible. Firstly, the maximum temperatures experienced by the larvae may be more important for the initiation of their pupation in autumn because maximum soil temperatures were slightly higher in the week following the 19 April deposit. Secondly, the temperatures experienced for up to 15 days, instead of 7 days after deposition, may be more important. Dallwitz (1984) stated that pupation during the transitional phase occurred either within 15 days or was delayed until spring. In the second week after deposition of larvae, the 7-day rolling minimum soil temperatures dropped below 10°C for the 1 May deposit, whereas they stayed above 10.5°C for the 19 April deposit. Although, none of the larvae from the 18 May 2005 deposit emerged, a similar trend in the 7-day rolling minimum soil temperatures was seen as in 2005 between this deposit and the previous deposit, which exhibited a split emergence. This aspect of the biology of *L.cuprina* needs further investigation to clarify the thresholds and/or the duration of temperatures that induce arrested development. This information is needed to refine the models for the development of *L.cuprina*.

During the over-wintering period, mortality was high and quite variable, both from year to year and between pots, which were deposited at the same time. The high biological variance, around the mean number of flies emerging from pots that were set up at different times from late autumn to winter, was also seen in over-wintering data reported by McKenzie (1990) (Figure A-2.1).

In 2005, the percentage of larvae emerging as flies in spring, from deposits made from May to mid-July averaged 5%, compared to 32% in 2006. The mortality in 2005 was similar to previous studies in Canberra and Heidelberg, Victoria, where the proportion of flies developing from larvae, deposited during May and June, ranged from 1 to 12.7%

(McKenzie 1990; 1994; Whitten et al. 1976). In contrast, in 2006, the proportion of larvae reaching adulthood was in excess of 20% for deposits made from 9 May onwards.

The soil temperatures from late autumn to mid-winter in 2005 were slightly higher than those in 2006. This may explain the higher mortality of over-wintering larvae that was seen in 2005. Higher winter temperatures will increase the metabolic rate, thereby depleting the metabolic reserves that are required for pupation and fly metamorphosis. This hypothesis, which suggests an increase of over-wintering mortality when winter temperatures are mild, has been previously described for larvae of the goldenrod gall fly, *Eurosta solidaginis* (Irwin and Lee 2000) and for larvae of *L.sericata* (Pitts and Wall 2005).

The higher mortality of the over-wintering larvae in 2005 may have also resulted from the 40.8 mm of rainfall that occurred on 31 August 2005, just before the resumption of the development of larvae. However, it is understood that larvae can seek drier places when the soil becomes too wet (Foster et al. 1975), although the use of pots that constrained the larvae may have prevented them from doing this.

In both years, the proportion of flies developing from larvae deposited from May to August increased when deposits were made closer to spring. These findings are consistent with studies done by Whitten et al. (1976) and McKenzie (1990; 1994). In these studies, an association was found between mortality of larvae during the over-wintering period and time spent in the ground. The hypothesis of depleting energy reserves may also apply here. Metabolic reserves of larvae will decrease with time spent in the ground, resulting in lower numbers of larvae successfully pupating and developing as flies. In contrast, McLeod (1997) did not find a correlation between mortality of larvae and time spent in the ground. However, larvae only entered an arrested development in one of the three winters in this study at Fowlers Gap in western New South Wales. During the other two winters, larvae developed throughout winter and so did not exhaust their energy reserves. Consequently, it may be that the association between mortality and time spent in the ground only applies when arrested development occurs. Once again, it would appear that more studies are needed to explore this hypothesis.

None of the larvae deposited on 18 May 2005 emerged as flies in the field. The larvae were viable at the time of deposition, judged by the successful development of 93% of the larvae in the laboratory pots. The weather data, in the 2-week period after the deposition of larvae, were not unusual and therefore, unlikely to be the cause of the

uniform mortality of larvae. Five percent of larvae deposited 3 weeks earlier emerged as flies in spring after experiencing the same weather conditions as larvae deposited on 18 May. This finding makes the hypothesis of depleting the energy reserves of larvae less likely. Furthermore, the recovery of larvae and pupae the following summer did not provide more insights. More than 80% of the larvae were recovered as pupae, from which 16% appeared opened in a way similar to those that allow the young fly to emerge. The pupae did not show any macroscopic abnormalities, and deaths from pathogens and predators were unlikely to be responsible for the 100% mortality. Other field pots, deposited at different times, were nearby, and so experienced similar environmental conditions, but had a far lower mortality. Consequently, other unidentified factors appear to have been responsible for the extreme mortality of larvae in this particular deposit.

Resumption of development in spring may be associated with increased mortality, possibly from a depletion of energy reserves. Data from the sequential study supports this hypothesis with a 15% increase in mortality of incubated larvae recovered at the start of the resumption of development compared to that observed 2 weeks earlier (Table 3-6).

The serial sampling study revealed that the resumption of development of over-wintering larvae was around early to mid-September in 2006. It is not clear what initiates the resumption of development of larvae, although a specific pattern in temperature changes, including a particular rise and/or a sustained period of temperatures above a certain threshold in late winter or early spring, is assumed to start this process. In both years, the 7-day rolling average soil temperature increased 1.5 to 1.9°C over a 4-day period around the time the development of larvae resumed and remained above 11°C for at least 1 week (Figure 3-8).

In 2005, the first flies from larvae deposited from mid-April to August emerged in early October, whereas in 2006 it was a little earlier, in late September. Larvae deposited from mid-autumn to winter emerged as flies over a similar period the following spring, indicating the existence of a synchronous resumption of development of larvae that were in an arrested development during winter. The dates when the median number of flies emerged were consistent between all the autumn and winter deposits within each year, ranging from 13 to 27 October in 2005 and from 7 to 24 October in 2006. A similar, but later, synchronous emergence of flies from over-wintering larvae has been described in Canberra (Dallwitz and Wardhaugh 1984). Although, emergence of the median number

of flies was confined to a 2 week interval, the distribution of emergence was more spread than at other times of the year. In both years, the interval, between the first and last fly of the first generation of flies in spring, exceeded 50 days.

In both years, the first flies after winter were detected in the Lucitraps® at the experimental site about two weeks after the first flies were found in the field pots. This coincided with the start of the median emergence interval of flies emerging from the field pots. The number of flies in the traps at the experimental site followed a bimodal pattern, with a large peak in late November and a smaller one in early March (Figure 3-6). Fly numbers were low from mid-January to mid-February. This is a similar pattern to that described for the Canberra region (Fuller 1934; Gilmour et al. 1946; Tillyard and Seddon 1933).

The high mortality amongst larvae deposited on 10 January 2006 coincided with low fly abundance in the field. In contrast, the percentage emergence of flies from larvae, deposited 2 and 4 weeks later, exceeded 50%, whereas fly numbers in the field remained low. The number of flies developing from deposited larvae reflects the influence of weather conditions during summer on larval survival, whereas fly abundance in the field is also influenced by fly activity (Fuller 1934; Gilmour et al. 1946) and availability of susceptible sheep (Kitching 1977; Whitten et al. 1976).

Excessive heat is thought to make a substantial contribution to the decline of *L.cuprina* in mid-summer (Dallwitz 1984; Norris 1959). In the current study, the average daily maximum soil temperature for the week following the deposit on 10 January was 27.9°C. The maximum soil temperature exceeded 30°C for 5 and 7 hours on 21 and 22 January, respectively. These dates closely corresponded to the emergence of the median number of flies. Dallwitz (Dallwitz 1984) found that pupae were more sensitive to high temperatures in the late stages of their development. However, in the same study it was shown that survival of pupae, exposed daily for 7 hours at 36°C, was 98%. Consequently, the high soil temperatures might not have been the primary cause of the high mortality in larvae deposited on 10 January. However, soil temperatures at the experimental site were measured at a depth of 5 cm under pasture, whereas the field pots were uncovered and so the temperatures in the pots may have been higher than those recorded.

Subtle differences between this and similar previous studies are most likely related to differing soil temperatures, 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 number of laboratory generations was kept to a minimum. In contrast, flies used in previous studies were maintained in the laboratory for an extended period of time (Dallwitz, 1984; McKenzie, 1990; 1994). However, Dallwitz (1984) compared larvae derived from both laboratory stock and wild trapped flies and found that the pattern of emergence of flies was not significantly different between these two sources. To determine if there are strain differences between 'Victorian' flies and 'Canberra' flies, a study could be undertaken in which over-wintering larvae of 'Victorian' *L.cuprina* are deposited in Canberra and vice versa. Subsequently, the patterns of emergence of local and imported flies could be compared to determine if strain differences exist.

The existence of an arrested development in larvae during winter is a critical period in the life cycle of *L.cuprina* in south-eastern Australia. The high mortality of larvae that occurs during this time is a 'weak link' in the life cycle of this pest. It presents an opportunity for the strategic application of insecticides to sheep in early spring, before the first generation of flies emerge from over-wintering larvae. This removes favourable breeding sites for the relatively small number of flies comprising the first generation of flies in spring and has the potential to significantly reduce the numbers of flies and the prevalence of flystrike for the whole season (McKenzie and Anderson 1990).

An alternative proposal, which has not been examined in the field, is to treat sheep in autumn. This could dramatically reduce the number of larvae that enter the over-wintering phase. As previously discussed, a high mortality of larvae occurs during this over-wintering period (McKenzie 1990; 1994; Whitten et al. 1976). Consequently, the number of flies that emerge as the first generation in spring could be dramatically reduced.

The two strategic treatment options discussed above rely on four assumptions. Firstly, that sheep are the principal breeding ground for *L.cuprina*. This is generally true (Anderson et al. 1988; Waterhouse 1947; Waterhouse and Paramonov 1950), although other oviposition sites might exist (Wardhaugh 2001). Secondly, that *L.cuprina* is the main species responsible for flystrike in sheep. This is a well established and uncontested fact in south-eastern Australia (Barton 1982; Dallwitz et al. 1984; Mackerras and Fuller 1937; Waterhouse and Paramonov 1950; Watts et al. 1976). Thirdly, that the strike rate is correlated with the number of available blowflies. The literature is rather conflicting on this matter. In Canberra and the southern tablelands of New South Wales, the incidence

of flystrike was noted to increase with an increase in fly density and fly activity (Mackerras et al. 1936; Wardhaugh and Morton 1990). In contrast, studies in Victoria showed that prevalence of flystrike peaked when fly numbers were rather low and vice versa (Barton 1982). Finally, the success of strategic treatments could be compromised if there was substantial immigration of flies from other areas after the treatment period. Nevertheless, preliminary trials showed that a more timely application of insecticides in early spring could significantly reduce fly numbers, as well as the prevalence of flystrike, over the whole season (McKenzie and Anderson 1990). In contrast, farmers often treat their sheep when significant numbers of strike occur in a mob, usually during times of high fly abundance (Lottkowitz et al. 1984).

Simulation models of the dynamics of populations of *L.sericata* in England showed that in addition to the timing of insecticide treatment, the mortality achieved is critical (Wall et al. 1993b). High mortality of eggs and larvae, ideally above 99%, is desirable because the fly population will increase exponentially as soon as favourable weather conditions and increased prevalence of breeding sites returns. In particular, covert strikes around the pizzle and breech, early in the fly season, can make a substantial contribution to subsequent fly populations because they persist for long periods (Wardhaugh and Dallwitz 1984).

In south-eastern Australia, timing for implementation of strategic treatments is not clear-cut. In this region, spring lambing, from mid-August to mid-October, is a recommended management practice because it allows increased stocking rates and improved profitability (Lean et al. 1997; Morley 1994; White 1975). Despite this, management practices vary greatly between enterprises, although there is a strong preference for shearing to be undertaken in either November-December or February-March (Campbell 2006; Reeve and Thompson 2005). Possible treatment times of ewes in spring lambing flocks are either 2 to 4 weeks before lambing or at marking time, about 6 weeks after lambing. The question of which chemical is the most appropriate depends on the timing of treatment and shearing. It is recommended to use a persistent insecticide, such as dicyclanil or cyromazine, when ewes are treated before lambing. These provide protection against blowfly strike for up to 24 and 14 weeks, respectively (eMIMS 2006).

Treatment at marking time should take place before the peak abundance of flies in November. The choice of insecticide at marking time must also take account of the time of shearing and the withholding period for the wool. Spinosad is the only product with

no withholding period for wool and therefore may be the product of choice for a December shearing time.

Alternatively, insecticides could be applied before mid-autumn to prevent late season strikes, which may deposit large numbers of larvae, many of which will enter an arrested development. This strategy is probably more appropriate when shearing takes place in December. Treatment could be done 'off shears' with dicyclanil or 6 weeks after shearing with cyromazine or spinosad.

To maximise the potential of a strategic application of insecticides, all ewes should be treated on the breech and all wethers on the pizzle and the breech. Lambs should be treated on the breech at marking time, and depending on the weather conditions, on the body at weaning time. However, these recommendations are preliminary and field trials are necessary to demonstrate the benefits of more timely insecticide treatments.

In conclusion, this study confirmed the existence of a transitional phase of larval development during mid-autumn in south-eastern Australia. Some of the larvae deposited during this period pupated immediately and emerged as flies in late autumn, whereas others entered an arrested development and resumed their development the following spring. A period of sustained low temperatures ($\leq 10^{o}C$) was associated with the induction of this arrested development. The development of over-wintering larvae resumed in late winter-early spring after a pattern of rising soil temperatures and/or after a sustained period when soil temperatures remained above $11^{o}C$. The subsequent emergence of the first generation of flies in spring was synchronous for all deposits made from mid-autumn to winter within each year. In 2005, the first flies from over-wintering larvae emerged in early October, whereas in 2006 it was a little earlier, in late September. Further, this study showed that, in this region, mortality during the over-wintering period is high, although can be quite variable. For deposits made from May to mid-July the average mortality in over-wintering larvae was 95% in 2005, compared to 68% in 2006. Finally, this study found that the abundance of flies throughout the season followed a bimodal pattern with a large peak in late November and a smaller peak in early March. The data obtained provides information that is helpful for the formulation of strategic programs for the control of blowfly strike in this region.

CHAPTER 5

ASSESSING TEMPERATURE-DEPENDENT DEVELOPMENT MODELS OF *L.CUPRINA*

5.1 Introduction

The rate of development in insects (the reciprocal of the time taken for a specific stage to develop) is primarily determined by the temperature to which they are exposed. Development tends to be faster with increasing temperatures and occurs within a definite temperature range. A plot of insect development times against temperature appears as a backward "J", whereas when development rates are plotted against temperature the outcome is a typical sigmoid curve ("S"-shape), with a linear region through the mid-temperature range (Beck 1983; Sharpe and DeMichele 1977; Wagner et al. 1984). Extrapolation of this linear portion is commonly used to determine the theoretical threshold or base temperature under which no development occurs (Arnold 1959).

The association between insect growth and temperature has allowed entomologists to develop functions and models to describe this relationship. Insect development times can be estimated by either a heat summation approach or a rate summation approach. One of the oldest and most widely applied heat summation methods is the day degree method, which assumes that development rate is a linear function of temperature. Therefore, the number of day degrees necessary to complete development is presumed constant in the mid-temperature range and is calculated as the difference between the threshold temperature and the ambient temperature multiplied by the time required for 50% of the individuals to complete development (Collier and Finch 1985; Wagner et al. 1985). Linear models perform well in the mid-temperature range but fall short at either low or high temperatures (Gage and Mukerji 1976; Hilbert and Logan 1983; Howe 1967; Lactin et al. 1995; Sharpe and DeMichele 1977; Wang 1960).

The limitations of linear models have stimulated researchers to develop more complex, nonlinear models, using either a heat summation or a rate summation approach (Harcourt and Yee 1982; Hilbert and Logan 1983; Lactin et al. 1995; Logan et al. 1976;

Sharpe and DeMichele 1977; Stinner et al. 1974). These nonlinear functions often fit the data better, especially at the extremes of the temperature range. However, as for the linear day degree models, they can be biologically unrealistic and do not always perform well under field conditions, especially at high and low temperatures (Hilbert and Logan 1983; Lactin et al. 1995). Nonlinear models are generally harder to work with, and so their use is limited to people who have experience in nonlinear parameter estimation.

Deterministic models assume all individuals develop simultaneously. They predict either the mean or the median time needed for insect development. However, the variation in development times amongst individuals exposed to the same environmental conditions is quite high and the distribution of development times is typically skewed to longer development times (Stinner et al. 1975; Wagner et al. 1985). Therefore, the preferred approach is the prediction of the median development time because this measure is less affected by extreme values. In contrast, stochastic models incorporate variability into the model. Rather than generating a single value for the predicted development time, they produce a probability distribution of the estimates (Fenton et al. 1997; Stinner et al. 1975).

Models can usually forecast the development of insects quite accurately during the optimal growth period. However, it is more difficult to forecast when dormancy ends and insect development resumes (Pruess 1983). Models assume that all individuals respond equally to environmental changes. However, from the current study (Chapter 3), and the study of Dallwitz (1984) in Canberra, it has been shown that a transitional phase of larval development in *L.cuprina* occurs in mid-autumn, with some larvae pupating and emerging as flies in late autumn, whereas others enter a state of developmental arrest and resume their development the following spring.

Most models only use temperature to predict insect development, but it is unreasonable to expect temperature to explain all the variation in a biological response when it is only one of several important environmental factors. The temperature data, used to calculate development, must reflect the conditions experienced during the insect's life cycle. However, available meteorological data are often limited to daily minimum and maximum air temperatures, which, in Australia, have been standardised by measurements at 9am and 3pm, respectively. Air temperatures do not always predict development times accurately for insects living in soil (Collier and Finch 1985). Nevertheless, the majority of models simply require the input of daily minimum and maximum air temperatures.

Regression methods have been used to estimate daily or hourly soil temperatures from the observed air temperatures. However, soil temperatures are also influenced by factors other than air temperatures, such as solar radiation, soil water content, soil depth, and presence and type of vegetation and pasture litter (Paul et al. 2004). Therefore, many regression methods will only be relevant for a specific location, or a specific type of soil and soil depth (Langholz 1989). Where possible, hourly temperature data should be used, because this provides a better estimate of development times than mathematical functions fitted to daily minimum and maximum temperatures (Raworth 1994). On the other hand, some models require very detailed microclimatic data. This is often hard to obtain and can limit the validation and use of such models over a broader area.

Several models have been developed to describe the temperature dependent development of *L.cuprina*. FlyAlert is one of these models, which estimates the dates of spring emergence of *L.cuprina* flies from over-wintering larvae. FlyAlert is a FORTRAN program written by Geoff Foster at CSIRO, Canberra in 1989-1991. A data set of over-wintering larvae in the Canberra region (Dallwitz and Wardhaugh 1984) was used to develop this model (Wardhaugh 2001). The model estimates soil temperatures at a depth of 5 cm under pasture from daily maximum and minimum air temperatures. Subsequently, the program forecasts the dates in spring for pupation, emergence, mating and oviposition of flies from over-wintering larvae. The model requires the 5-day rolling average soil temperature to exceed 15°C before pupal development commences. Unfortunately, it appears that the model has never been validated. In addition, it has been revised several times, but these changes have not been published and so are not in the public domain.

Vogt and Bedo modified the FlyAlert model by improving the formula which computes hourly soil temperatures from daily maximum and minimum air temperatures (Wardhaugh 2001). The model was also able to calculate development using observed hourly soil temperatures. A nonlinear rate function calculates development in arbitrary development units (adu) for all the immature stages. Two factors could delay the start of pupation. Firstly, the model requires the minimum soil temperature to stay above 16°C until the prepupae have accumulated 16 adu necessary for their pupation. Each time the temperature drops below 16°C, the accrued development is set to zero. Secondly, all accrued development is deducted when rainfall exceeds 12 mm over the preceding 24h. From here on, this model will be referred to as the 'Vogt model'.

The Temsum model uses daily maximum and minimum air temperatures to estimate the dates of emergence of flies. It is not clear which development data was used to develop the model. The model generates hourly soil temperatures for different soil depths, either for bare soil or for soil covered with pasture, using the same formulae as in the FlyAlert model. The start date for calculations, as well as the threshold temperature for development, and the total day degrees needed to complete development from postfeeding larvae to the emergence of flies are arbitrary, and so need to be entered before each simulation. In contrast, to the FlyAlert and Vogt models, Temsum does not calculate the date of pupation.

Unfortunately, the data used to develop the three previous models is not accessible. In the case of the FlyAlert and Vogt models, the data was published as a figure, not the actual data, whereas for Temsum the data is not known. Therefore, it can only be assumed that the models predict median development times, rather than either the mean, or the time to development of the first individual of each life cycle stage of *L.cuprina*.

More recently, McLeod (1997; 2001) compared the predictions of a linear day degree and two nonlinear models: the second modified Logan model (Lactin et al. 1995) and the matched-asymptotic model, also known as "equation 6" (Hilbert and Logan 1983), with the observed spring emergence dates of *L.cuprina* flies at Fowlers Gap in western New South Wales. Expected times of emergence of flies were calculated, using the actual hourly soil temperatures. The three equations were derived using the development data for *L.sericata* postfeeding larvae under a range of constant temperatures (Wall et al. 1992) and the development data for *L.cuprina* pupae under constant temperatures (Dallwitz 1984) (see Figure 5-1 and Figure 5-2). The theoretical base temperature was calculated by extrapolating the linear portion of the rate of development versus temperature plot, over the mid-temperature range, to the abscissa (X-intercept method) (Arnold 1959). The threshold for pupal and postfeeding larval development was calculated to be 10.65°C and 11.03°C, respectively. The three models predicted the mean developmental times, including standard deviations, by using Monte Carlo simulation methods to add variance to each of the parameters of the models. In McLeod's (1997; 2001) study, the estimated times of the emergence of flies from over-wintering larvae, by each of the three models, were significantly different from the observed field data.

The parameters of each of the three models, presented by McLeod (1997) are summarized in Table 5-1, whereas a summary of the features of each of the six models validated is shown in Table 5-2.

This study used the data of the 2-year field study at Rokewood to validate each of the six models in south-eastern Australia.

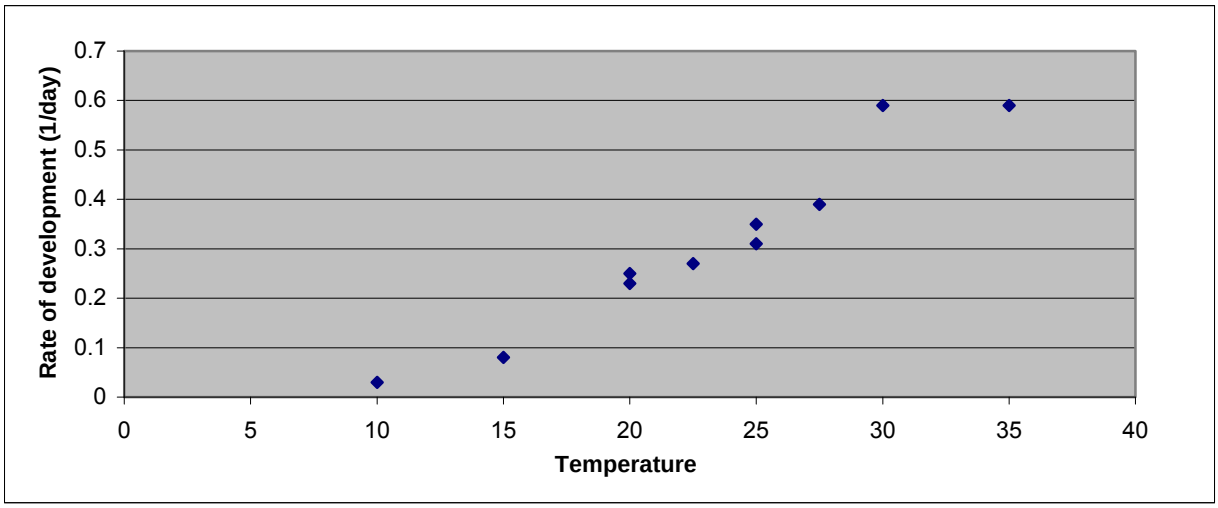


Figure 5-1 Rates of development for postfeeding larvae of *Lucilia sericata* measured at a range of constant temperatures (data from Wall et al. 1992)

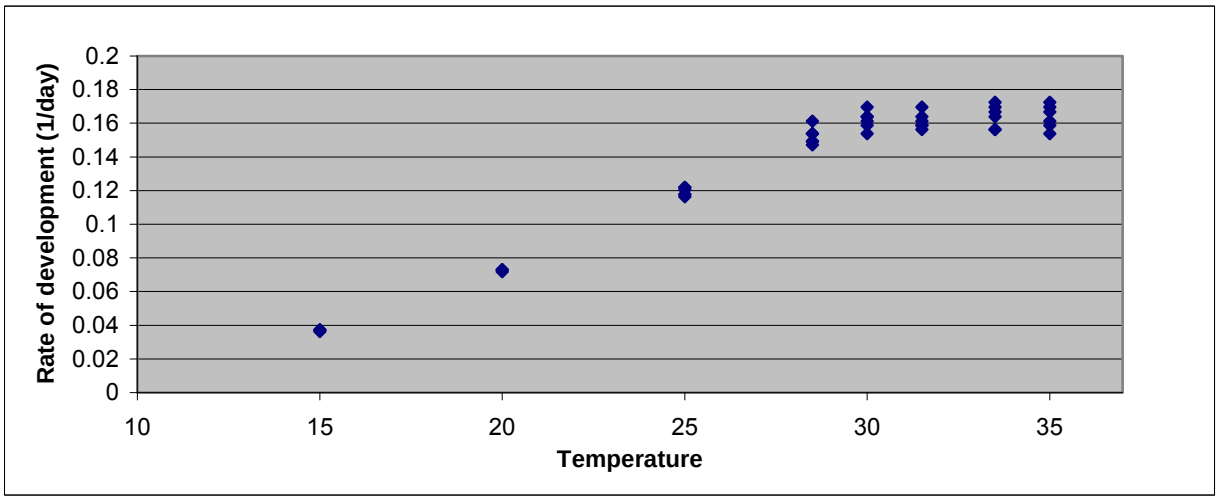


Figure 5-2 Rates of development for pupae of *Lucilia cuprina* measured at a range of constant temperatures (data from Dallwitz, 1984)

Table 5-1 A summary of the parameters calculated for each of the three models presented by McLeod (1997) for the development rates of postfeeding larvae and pupae

Model	Parameters for: Postfeeding larvae	Pupae
Linear day degree model		
$D^o=\sum(N^o-t^o) \times T$ N^o = measured temp* t^o = base temp T = time taken to complete development stage at N^o (* $t^o < N^o < 30^OC$; if $N^o < t^o$, $D^o = 0$)	r(T) = 0.026x-0.277	r(T) = 0.009x-0.095
Modified Logan model		
$r(T) = e^{\rho T}-e^{\{\rho Tmax-(Tmax-T/\Delta\}}+\lambda$ r(T) = development rate at temperature T (r(T) ≥ 0)	P = 0.018 Tmax = 36.1 Δ = 0.347 λ = -1.21	P = 0.008 Tmax = 47.2 Δ = 3.98 λ = -1.09
Matched-asymptotic model 'Equation 6'		
$r(T) = \Psi[T^2/(T^2+D^2)-e^{-(Tm-T)/\Delta T}]$ $T = T_0-T_b$ Ψ = development rate at base temp (T_b) T_0 = air temperature Tm = lethal maximum temperature threshold ΔT = width of high temp boundary area D = fitted parameter	$T = T_0$-10.63 Ψ = 1.12 D = 20.7 Tm = 25.0 ΔT = 0.204	$T = T_0$-11.03 Ψ = 0.236 D = 13.1 Tm = 29.9 ΔT = 2.32

Table 5-2 Type, input and predicted outcomes of the FlyAlert, Temsum, Vogt, linear day degree, modified Logan and matched-asymptotic models

Model	Type	Input data			Outcome	
		Temperature	Threshold	Start date	Pupation predicted	Fly emergence
FlyAlert	Nonlinear	Daily max & min air T	5-d soil T>15°C [a]	1 July	Yes	Median [c]
Temsum	Linear	Daily max & min air T	10°C	1 July	No	Median [c]
Vogt	Nonlinear	Daily max & min air T or hourly soil T	16°C [a]	1 July	Yes	Median [c]
Linear day degree	Linear	Hourly soil T	10.65°C [b] 11.03°C [a]	Deposit date	Yes	Median [cd]
Modified Logan	Nonlinear	Hourly soil T	-	Deposit date	Yes	Median [cd]
Matched-asymptotic	Nonlinear	Hourly soil T	10.65°C [b] 11.03°C [a]	Deposit date	Yes	Median [cd]

[a] pupation threshold; [b] threshold for postfeeding larvae development; [c] model predicts median development times; [d] model predicts mean development times if Monte Carlo simulations are used

5.2 Materials & Methods

5.2.1 Weather Data

The weather station and data logger (Monitor Sensors Aust Pty Ltd.) located at the experimental site near Rokewood recorded weather data continuously during 2005 and 2006. The details of these recordings are given on p 34-35 (Chapter 2).

The estimated daily average, minimum and maximum soil temperatures were derived by linear regression, using the air temperatures measured at Rokewood.

5.2.2 Models

This study evaluated the results of six models that predicted the emergence of *L.cuprina* flies in spring from over-wintering larvae, using the data from a 2-year trial carried out at Rokewood (Chapter 3). The dates predicted by each of the models were compared with the actual dates of fly emergence.

All models calculated the expected dates of fly emergence from the observed temperatures, measured at the experimental site. The models were first run in their original version with their specific air-soil temperature relationship and subsequently modified to allow the entry of actual hourly soil temperatures.

The models used by McLeod (1997) gave a mean and a standard deviation for the predicted development times using Monte Carlo simulations. Unfortunately, the code for calculating the distribution of emergence times was not available, and so these computations could not be undertaken.

For the purpose of this study, it has been assumed that all models predict the date of median emergence of flies. The exact data used for the development of the FlyAlert, Temsum and Vogt models have never been published, and so it is not clear what each model exactly predicts. The outputs from the three models presented by McLeod, when the Monte Carlo simulations are not used, are also not explicitly defined. However, one of the three models is a linear day degree model, and it is known that this predicts the time necessary for 50% of individuals to complete development (Collier and Finch 1985).

5.2.2.1 FlyAlert

During the development of the original FlyAlert model, from 1989-1991, a series of modifications was made. Consequently, the "May 1990 version" was used in this study. In this model, the start date for the calculations of the spring emergence date was arbitrarily chosen as 1 July. Expected times of fly emergence were computed using three different sets of temperature data for each year of the study. Firstly, the daily minimum and maximum air temperatures were entered, allowing hourly soil temperatures to be calculated by the model. Secondly, the program was modified to enable the actual daily minimum and maximum soil temperatures, measured at the experimental site, to be used. Finally, the model used the observed hourly soil temperatures to predict fly emergence times.

5.2.2.2 Temsum

The Temsum model predicted the date of fly emergence, but without first computing the date of pupation. The model estimated hourly soil temperatures at a depth of 5 cm under pasture from daily minimum and maximum air temperatures, using the same formulae as the FlyAlert model. The low temperature threshold was set at 10°C and a total of 100 day degrees was needed to complete development from postfeeding larvae to fly emergence. Again, the start date for all over-wintering deposits was arbitrarily selected as 1 July.

In addition, a modified Excel version of the Temsum model was created. This version allowed the entry of the observed hourly soil temperatures, but the threshold temperature and the number of day degrees needed to complete development remained unchanged. This modified version was able to estimate the timing of fly emergence for each of the 26 deposit dates.

5.2.2.3 Vogt Model

The FlyAlert model was modified by Vogt and Bedo (Wardhaugh 2001) in an attempt to predict the spring emergence of *L.cuprina* more accurately. The Vogt model used observed hourly soil temperatures and 1 July as start date to estimate the timing of emergence of flies in spring. The model required the minimum soil temperature to remain above 16°C until the prepupae accumulate the 16 adu needed for pupation. However, this model predicted no emergence of flies from any autumn or winter deposit in both years of the study. Consequently, the effect of changing this threshold was

explored by varying it in 1°C increments, within the range of 5 to 16°C. The effect of using either the maximum or 5-day rolling average temperature as a threshold was also investigated by varying these in 1°C increments between 5 and 16°C. The predicted dates of fly emergence were then compared to the actual dates and a decision on the most appropriate threshold temperature (or temperatures) for pupation was made.

5.2.2.4 McLeod Models

Three models presented by McLeod (1997) were also validated: a linear day degree model, and two nonlinear functions known as the second modified Logan model (Lactin et al. 1995) and the matched-asymptotic model, also labelled 'Equation 6' (Hilbert and Logan 1983). The equations and parameters for each of these models are given in Table 5-1. The timing of fly emergence was estimated using observed hourly soil temperatures and the actual deposit dates as the start dates for each model.

5.2.2.5 Predicted Versus Observed Emergence Times of Flies

The number of days for flies to emerge was taken from the date that larvae were deposited. The dates of emergence of flies predicted by each model were then compared with the actual dates, when either the first or the median number of flies was observed.

The difference between the observed and predicted times of emergence of flies was calculated as the difference in number of days (observed – predicted). A difference of less or equal than ± 7 days was considered a good fit. Subsequently, the median absolute deviation (MAD) for each model was calculated to compare the predictions generated by each of the models. This is one of several methods to compare the output of the models. However, this method was chosen because, from a practical point of view, it is important to know the difference between the predicted and the actual date of emergence of flies in number of days, rather than a difference expressed as a percentage or proportion of time. For this reason, the residual standard deviation (RSD) was considered as a method to compare the output of the models, but rejected.

All models predicted the emergence of flies as a single date. Therefore, when calculating the MAD for each model, the predicted emergence dates for deposits exhibiting a split emergence between autumn and spring were compared only with the observed spring emergence dates of these deposits.

5.3 Results

5.3.1 Calculation of Soil Temperatures from Air Temperatures

The minimum, maximum and average daily soil temperatures were estimated from the minimum, maximum and average daily air temperatures recorded at the experimental site, using linear regression analysis (Figure A-3.1). The estimated soil temperatures were compared with the direct readings of soil temperature at the experimental site at a depth of 5 cm. The equations used for these calculations are set out in Appendix A3.1. Regression analysis showed that the calculated values accounted for 60, 78 and 74% of the variation in the minimum, maximum and average daily soil temperatures, respectively.

Two modifications were made to the previously used equations in order to improve the estimation of soil temperatures from air temperatures. Firstly, the daily soil temperatures were calculated from a combination of the current daily air temperatures and the daily air temperatures measured 48 or 72 hours earlier. The equations for the estimated minimum, maximum and average daily soil temperatures are shown in Appendix A3.2. Regression analysis using the current daily air temperature and the air temperature recorded 48 hours earlier accounted for 69, 85 and 84% of the variation in the minimum, maximum and average daily soil temperatures, about a 10% improvement in the initial predicted values. However, there was no improvement in the predictions between the equations using the current daily air temperature and the daily air temperature recorded either 48 or 72 hours earlier.

The second modification to estimate soil temperatures was based on a recommendation made by Horton (pers comm, 2007), using a combination of current daily air temperatures and average air temperatures over the previous 10 days. The equations for the estimated minimum, maximum and average daily soil temperatures are shown in Appendix A3.3. Regression analysis showed that estimates for minimum, maximum and average daily soil temperatures accounted for 77% of the variation in minimum daily soil temperatures and 91% of the variation in both the maximum and average daily soil temperatures.

5.3.2 Validation of Models

5.3.2.1 FlyAlert

The FlyAlert model estimated the times of pupation and emergence of flies from over-wintering larvae, using 1 July as the start date for all over-wintering deposits. Three separate simulations were performed using either observed or calculated hourly soil temperatures. Calculated hourly soil temperatures were either from the daily minimum and maximum air temperatures, using the equations shown in Appendix A3.4, or from the observed daily minimum and maximum soil temperatures. The predicted dates for each of these simulations are given in Table 5-3.

Table 5-3 Dates of pupation and emergence of flies from over-wintering larvae predicted by FlyAlert using 1 July as start date

	Start	Predicted spring pupation date			Predicted spring emergence date		
year	date	A	B	C	A	B	C
2005	1 Jul	24 Jul	18 Oct	18 Oct	7 Sep	6 Nov	6 Nov
2006	1 Jul	15 Aug	11 Oct	11 Oct	19 Sep	2 Nov	2 Nov

[A] Simulation, using hourly soil temperatures calculated from daily minimum and maximum air temperatures, using equations in appendix A3.4
[B] Simulation, using hourly soil temperatures calculated from daily minimum and maximum soil temperatures
[C] Simulation, using hourly soil temperatures measured at the experimental site

To compare the estimates of the minimum and maximum soil temperatures, the output of FlyAlert, using equations 1.4.1 and 1.4.2 (Appendix A3.4), and the estimates derived from data collected at the experimental site, using equations 1.1.1 and 1.1.2 (Appendix A3.1), were calculated and are presented in Figure 5-3 and Figure 5-4. The soil temperatures predicted by FlyAlert were much higher than those derived from the Rokewood data. In particular, the algorithms in FlyAlert over-predicted the maximum soil temperatures at all times.

A comparison was also made between the estimated and observed hourly soil temperatures for each hour of the day during July, August, September and October 2005 and 2006 (see Figure 5-5). It is clear that the over-prediction mainly occured during the daylight hours, between 7 am and 3 pm.

If the differences between the predicted and observed values were small, the line in Figure 5-5 would be close to a horizontal line through zero. However, it can be seen that the average values from FlyAlert are up to 1.5°C higher than the observed values between 7am and 3pm in the winter and early spring in 2005, and between 1 to 2°C higher in 2006.

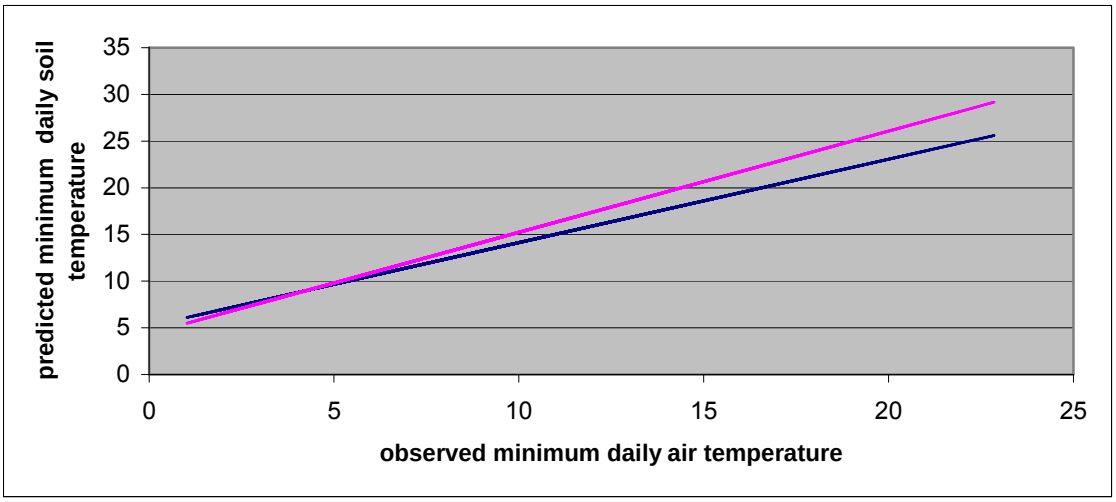


Figure 5-3 Estimates of the minimum daily soil temperatures from FlyAlert (pink line) and from data collected at the experimental site (blue line) using equations 1.4.1 and 1.1.1 in Appendix 3

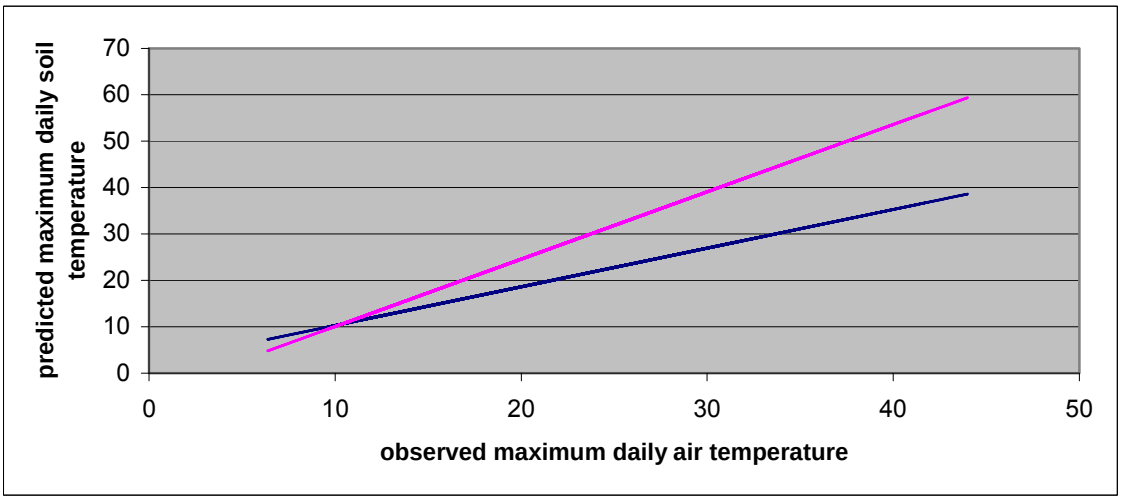
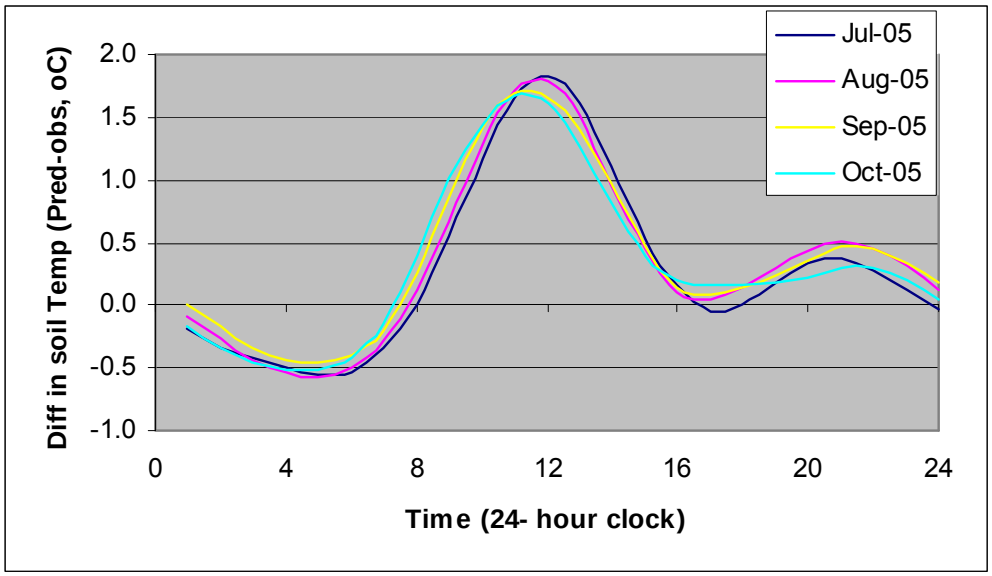


Figure 5-4 Estimates of the maximum daily soil temperatures from FlyAlert (pink line) and from data collected at the experimental site (blue line) using equations, 1.4.2 and 1.1.2 in Appendix 3

A)



B)

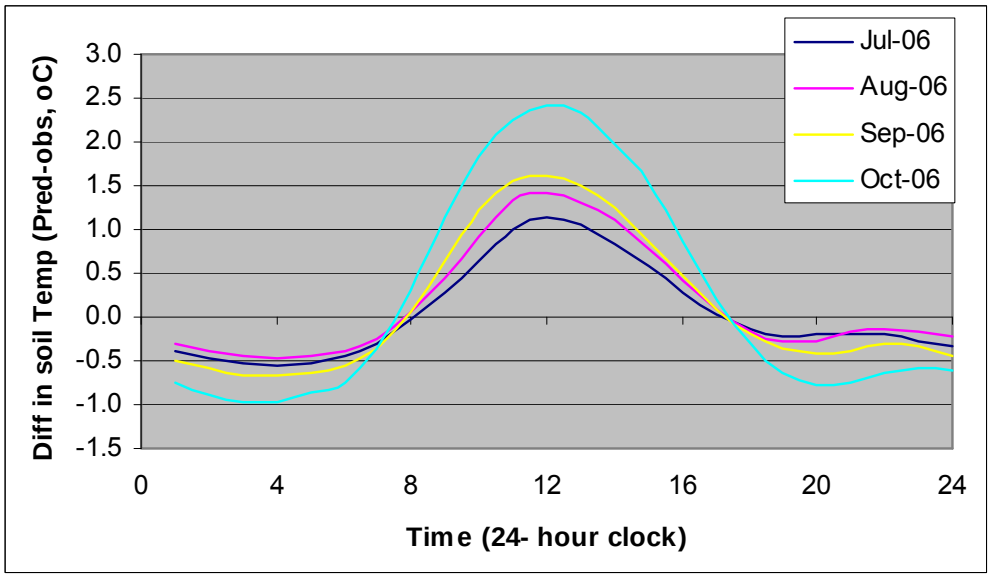


Figure 5-5 The difference between the average hourly soil temperatures predicted by FlyAlert and the soil temperatures measured at the experimental site for July to October in 2005 (A) and 2006 (B)

5.3.2.2 Temsum

The estimated dates of emergence of flies from over-wintering larvae, using the original Temsum model with 1 July as start date, were 1 August in 2005 and 12 August in 2006. Soil temperatures were estimated from air temperatures using the same equations as in the FlyAlert model (see Appendix A3.4).

Subsequently, the Temsum model was converted to Excel to facilitate the entry of the observed hourly soil temperatures. The predicted dates of emergence of flies from over-wintering larvae, using this version of the Temsum model, with 1 July as start date, were 4 and 6 October, in 2005 and 2006, respectively.

In addition, the Excel version of Temsum was used to calculate emergence times for each deposit of larvae in 2005 and 2006, using the observed hourly soil temperatures. The results are given in Table 5-4, together with the observed dates of emergence of the median number of flies for each deposit date.

The predicted dates of emergence of flies from larvae deposited during the warmer parts of the year (September-March) were generally earlier than the observed dates, although all were within 10 days of the observed dates. The predicted dates of emergence of flies from larvae deposited during May, July and August were also earlier than those observed, ranging from 2-27 days before the observed dates. The predicted date of fly emergence for the 26 April 2005 and 11 April 2006 deposits were much earlier than the observed dates of fly emergence in autumn, whereas the predicted emergence date for the 19 April 2006 deposit was not close to the observed date of fly emergence in either autumn or spring.

Table 5-4 Dates of emergence of the median number of flies predicted by the Excel version of Temsum for each deposit of larvae in 2005 and 2006

Year	Date of deposit	Predicted date fly emergence (A)	Observed date median fly emergence (B)	Difference (days, A-B)[a]
2005	16-Mar	30-Mar	4-Apr	-5
	6-Apr	21-Apr	25-Apr	-4
	26-Apr*	23-May	6-Jun	-14
	26-Apr	23-May	23-Oct	-153
	8-Jun	30-Sep	27-Oct	-27
	1-Jul[b]	4-Oct		
	12-Jul	5-Oct	13-Oct	-8
	10-Aug	9-Oct	19-Oct	-10
	19-Sep	20-Oct	21-Oct	-1
	5-Oct	28-Oct	4-Nov	-7
	26-Oct	8-Nov	13-Nov	-5
	30-Nov	12-Dec	14-Dec	-2
2006	10-Jan	19-Jan	21-Jan	-2
	24-Jan	31-Jan	3-Feb	-3
	7-Feb	17-Feb	19-Feb	-2
	1-Mar	10-Mar	12-Mar	-2
	22-Mar	1-Apr	10-Apr	-9
	11-Apr*	29-Apr	28-May	-29
	11-Apr	29-Apr	07-Oct	-161
	19-Apr*	3-Sep	13-Jun	+82
	19-Apr	3-Sep	13-Oct	-40
	1-May	21-Sep	13-Oct	-22
	9-May	30-Sep	9-Oct	-9
	30-May	6-Oct	10-Oct	-4
	1-Jul[b]	6-Oct		
	18-Jul	8-Oct	10-Oct	-2
	22-Aug	10-Oct	24-Oct	-14
	19-Sep	16-Oct	13-Oct	+3
	12-Oct	30-Oct	2-Nov	-3

[a] between the predicted and actual dates of the emergence of the median number of flies; a -ve sign indicates the predicted date was earlier, a +ve sign indicates the predicted date was later than the observed date

[b] 1 July used as arbitrary start date for over-wintering larvae

* Indicates deposits that exhibited a split emergence of flies between autumn and spring.

5.3.2.3 Vogt Model

The Vogt model, as described by Wardhaugh (2001), predicted that no flies would emerge from over-wintering larvae in either 2005 or 2006. This is due to the requirement that the minimum soil temperature needed to remain above 16°C until the larvae accumulated 16 adu necessary for their pupation and this did not occur until early November in both years of the study.

To overcome this problem, the threshold temperature, nominated as either the minimum, maximum or 5 day rolling average temperature, was lowered in steps of 1 degree within the range of 5 to 16°C, as described in section 5.2.2.3. The predicted dates of emergence of flies, using these thresholds for pupation and a start date of 1 July, are shown in Table 5-5. The dates within the shaded cells most closely approach the observed dates of the emergence of the median number of flies. These cells correspond to a threshold temperature of a minimum of 8°C, a maximum of 10°C, and a 5-day rolling average temperature of 9 or 10°C.

Subsequently, the modified Vogt model, using a threshold for pupation of a maximum soil temperature of 10°C, was run for each deposit of larvae in 2005 and 2006, using the observed hourly soil temperatures. The results are given in Table 5-6, together with the observed dates of emergence of the median number of flies for each deposit.

Overall, the modified Vogt model predicted the timing of emergence of flies later than observed in the field, but predominantly stayed within 15 days of the actual dates. The predicted dates of emergence of flies for deposits, exhibiting a split emergence between autumn and spring, did not approximate the observed dates of fly emergence in either autumn or spring.

Table 5-5 Dates of emergence of the median number of flies predicted by the modified Vogt model, using 1 July as start date. The threshold temperature needed to accumulate the 16 adu [a] necessary for pupation was set in increments of 1°C, from 5 to 16°C, for each threshold shown. The shaded cells indicate the best match with the actual dates of the emergence of the median number of flies

Threshold temp (°C)	Minimum		5-day average		Maximum	
	2005	2006	2005	2006	2005	2006
5	1-Oct	2-Oct	1-Oct	2-Oct	1-Oct	2-Oct
6	19-Oct	9-Oct	1-Oct	2-Oct	1-Oct	2-Oct
7	23-Oct	9-Oct	1-Oct	2-Oct	1-Oct	2-Oct
8	2-Nov	14-Oct	1-Oct	2-Oct	1-Oct	2-Oct
9	2-Nov	4-Nov	19-Oct	12-Oct	1-Oct	9-Oct
10	2-Nov	4-Nov	20-Oct	16-Oct	19-Oct	12-Oct
11	14-Nov	11-Nov	1-Nov	25-Oct	20-Oct	26-Oct
12	15-Nov	25-Nov	2-Nov	3-Nov	22-Oct	3-Nov
13	17-Nov	26-Nov	2-Nov	4-Nov	1-Nov	3-Nov
14	24-Nov	7-Dec	12-Nov	9-Nov	3-Nov	5-Nov
15	14-Dec	7-Dec	14-Nov	10-Nov	12-Nov	5-Nov
16	> 31 Dec	11-Dec	23-Nov	25-Nov	12-Nov	11-Nov

[a] arbitrary development units, see section 5.1

Table 5-6 Dates of pupation of the median number of larvae and emergence of the median number of flies, predicted by the modified Vogt model for each deposit in 2005 and 2006, using a threshold for pupation of a maximum soil temperature of 10°C [1]

Year	Date of deposit	Predicted date for		Observed date of median fly emergence (B)	Difference (days, A-B)[a]
		Pupation	Fly emergence (A)		
2005	16-Mar	21-Mar	8-Apr	4-Apr	+4
	6-Apr	10-Apr	9-May	25-Apr	+14
	26-Apr*	5-May	1-Jul	6-Jun	+25
	26-Apr	5-May	1-Jul	23-Oct	-114
	8-Jun	1-Jul	17-Sep	27-Oct	-40
	1-Jul[b]	5-Sep	19-Oct		
	12-Jul	5-Sep	19-Oct	13-Oct	+6
	10-Aug	5-Sep	19-Oct	19-Oct	0
	19-Sep	2-Oct	1-Nov	21-Oct	+11
	5-Oct	17-Oct	8-Nov	4-Nov	+4
	26-Oct	2-Nov	22-Nov	13-Nov	+9
	30-Nov	5-Dec	20-Dec	14-Dec	+6
2006	10-Jan	13-Jan	22-Jan	21-Jan	+1
	24-Jan	26-Jan	7-Feb	3-Feb	+4
	7-Feb	11-Feb	23-Feb	19-Feb	+4
	1-Mar	04-Mar	16-Mar	12-Mar	+4
	22-Mar	25-Mar	20-Apr	10-Apr	+10
	11-Apr*	22-Apr	30-Jun	28-May	+33
	11-Apr	22-Apr	30-Jun	7-Oct	-99
	19-Apr*	2-May	22-Jul	13-Jun	+39
	19-Apr	2-May	22-Jul	13-Oct	-83
	1-May	17-May	15-Aug	13-Oct	-59
	9-May	27-May	27-Aug	9-Oct	-43
	30-May	28-Aug	12-Oct	10-Oct	+2
	1-Jul[b]	28-Aug	12-Oct		
	18-Jul	28-Aug	12-Oct	10-Oct	+2
	22-Aug	11-Sep	18-Oct	24-Oct	-6
	19-Sep	3-Oct	31-Oct	13-Oct	+18
	12-Oct	19-Oct	12-Nov	2-Nov	+10

[1] if the maximum soil temperature fell below 10°C, the larvae had to restart their development until they accumulated 16 adu needed for their pupation

[a] between the predicted and actual dates of the emergence of the median number of flies; a -ve sign indicates the predicted date was earlier, a +ve sign indicates the predicted date was later than the actual date

[b] 1 July used as arbitrary start date for over-wintering larvae

* Indicates deposits that exhibited a split emergence of flies between autumn and spring

5.3.2.4 McLeod Models

5.3.2.4.1 Linear Day Degree Model

The dates for pupation and emergence of flies, as predicted by the linear day degree model (McLeod 1997), are summarised in Table 5-7, together with the observed dates of the emergence of the median number of flies.

The linear day degree model generally predicted the fly emergence dates later than those observed in the field, but the predictions stayed mostly within 14 days of the observed dates. In 2005, the predicted date of emergence of flies for the deposit exhibiting a split emergence between autumn and spring (26 April) was not close to the observed median emergence date in either autumn or spring. However, in 2006, the predicted dates for the two deposits that exhibited a split emergence (11 and 19 April) were close to the observed median emergence dates in spring. The predicted pupation dates for these deposits were 26 April and 11 May, respectively. These dates are inconsistent with the results of the sequential study in 2006, which indicated that the start of pupation most likely took place between 29 August and 14 September.

In addition, the actual deposit dates were nominated as the start dates for pupal development (i.e. assuming no development of postfeeding larvae took place). The results of this simulation are given in Table 5-8, together with the observed dates of the emergence of the median number of flies. In this simulation, the predicted fly emergence dates were very close to the observed dates, with 20 of the 24 predicted dates being within 7 days of the observed dates. The predicted fly emergence date for the 26 April 2005 deposit, which exhibited a split emergence between autumn and spring, corresponded very closely to the observed autumn emergence date. In contrast, the predicted emergence dates for the 2 deposits exhibiting a split emergence in 2006 (11 and 19 April) were closer to the observed spring emergence dates.

5.3.2.4.2 Second Modified Logan Model

The predicted dates of the emergence of the median number of flies, using the second modified Logan model as presented by McLeod (1997), are given in Table 5-9, alongside the observed dates of the emergence of the median number of flies. The estimated dates of fly emergence were mostly after the observed dates, ranging from 3 to 17 days later.

The predicted dates of fly emergence for the deposits exhibiting a split emergence of flies between autumn and spring (26 April 2005, 11 and 19 April 2006) were close to the observed spring emergence dates, especially in 2006.

5.3.2.4.3 Matched-asymptotic Model

The estimated dates of the emergence of flies, using the matched-asymptotic model as presented by McLeod (1997), are given in Table 5-10, together with the observed median emergence dates. For deposits made from November through to mid-March, this model predicted emergence dates within 7 days of the actual dates. The predicted fly emergence dates for deposits exhibiting a split emergence of flies between autumn and spring were estimated within 17 days of the observed spring emergence dates. However, the predicted fly emergence dates for the remaining deposits made from late March to October were much later than the observed dates, ranging from 9 to 70 days too late.

Table 5-7 Predicted dates for pupation (A) and emergence of flies (derived from A) from each deposit in 2005 and 2006, using the linear day degree model presented by McLeod (1997), and the difference between the predicted and observed dates of the emergence of the median number of flies

Year	Date of deposit	Predicted date for		Observed date of median fly emergence (C)	Difference (days, B-C)[b]
		Pupation (A)	Fly emergence [a] (B)		
2005	16-Mar	21-Mar	5-Apr	4-Apr	+1
	6-Apr	11-Apr	3-May	25-Apr	+8
	26-Apr*	6-May	15-Sep	6-Jun	+101
	26-Apr	6-May	15-Sep	23-Oct	-38
	18-May	14-Jun	17-Oct	No emergence	
	8-Jun	10-Sep	23-Oct	27-Oct	-4
	1-Jul[c]	22-Sep	25-Oct		
	12-Jul	22-Sep	25-Oct	13-Oct	+12
	10-Aug	24-Sep	26-Oct	19-Oct	+7
	19-Sep	5-Oct	1-Nov	21-Oct	+11
	5-Oct	19-Oct	6-Nov	4-Nov	+2
	26-Oct	2-Nov	19-Nov	13-Nov	+6
	30-Nov	5-Dec	17-Dec	14-Dec	+3
2006	10-Jan	14-Jan	22-Jan	21-Jan	+1
	24-Jan	27-Jan	6-Feb	3-Feb	+3
	7-Feb	11-Feb	21-Feb	19-Feb	+2
	1-Mar	4-Mar	13-Mar	12-Mar	+1
	22-Mar	25-Mar	13-Apr	10-Apr	+3
	11-Apr*	26-Apr	4-Oct	28-May	+129
	11-Apr	26-Apr	4-Oct	7-Oct	-3
	19-Apr*	11-May	12-Oct	13-Jun	+121
	19-Apr	11-May	12-Oct	13-Oct	-1
	1-May	2-Sep	16-Oct	13-Oct	+3
	9-May	16-Sep	18-Oct	9-Oct	+9
	30-May	22-Sep	23-Oct	10-Oct	+13
	1-Jul[c]	22-Sep	23-Oct		
	18-Jul	23-Sep	23-Oct	10-Oct	+13
	22-Aug	24-Sep	24-Oct	24-Oct	0
	19-Sep	6-Oct	29-Oct	13-Oct	+16
	12-Oct	19-Oct	9-Nov	2-Nov	+7

[a] using A as start date for pupal development

[b] between the predicted and actual dates of the emergence of the median number of flies; a -ve sign indicates the predicted date was earlier, a +ve sign indicates the predicted date was later than the observed date

[c] 1 July used as arbitrary start date for over-wintering larvae

* Indicates deposits that exhibited a split emergence of flies between autumn and spring.

Table 5-8 Predicted dates for the emergence of flies from each deposit in 2005 and 2006, using the linear day degree model presented by McLeod (1997) accruing pupal development from the deposit date, and the difference between the predicted and observed dates of the emergence of the median number of flies

Year	Date of deposit	Predicted date for fly emergence (A)	Observed date of median fly emergence (B)	Difference (days, A-B)[a]
2005	16-Mar	31-Mar	4-Apr	-4
	6-Apr	24-Apr	25-Apr	-1
	26-Apr*	11-Jun	6-Jun	+5
	26-Apr	11-Jun	23-Oct	-134
	18-May	3-Oct	No emergence	
	8-Jun	14-Oct	27-Oct	-13
	1-Jul[b]	18-Oct		
	12-Jul	18-Oct	13-Oct	+5
	10-Aug	19-Oct	19-Oct	0
	19-Sep	24-Oct	21-Oct	+3
	5-Oct	1-Nov	4-Nov	-3
	26-Oct	12-Nov	13-Nov	-1
	30-Nov	13-Dec	14-Dec	-1
2006	10-Jan	19-Jan	21-Jan	-2
	24-Jan	2-Feb	3-Feb	-1
	7-Feb	18-Feb	19-Feb	-1
	1-Mar	11-Mar	12-Mar	-1
	22-Mar	4-Apr	10-Apr	-6
	11-Apr*	17-Sep	28-May	+112
	11-Apr	17-Sep	7-Oct	-20
	19-Apr*	1-Oct	13-Jun	+110
	19-Apr	1-Oct	13-Oct	-12
	1-May	8-Oct	13-Oct	-5
	9-May	11-Oct	9-Oct	+2
	30-May	14-Oct	10-Oct	+4
	1-Jul[b]	14-Oct		
	18-Jul	14-Oct	10-Oct	+4
	22-Aug	15-Oct	24-Oct	-9
	19-Sep	20-Oct	13-Oct	+7
	12-Oct	3-Nov	2-Nov	+1

[a] between the predicted and actual dates of the emergence of the median number of flies; a -ve sign indicates the predicted date was earlier, a +ve sign indicates the predicted date was later than the observed date

[b] 1 July used as arbitrary start date for over-wintering larvae

* Indicates deposits that exhibited a split emergence of flies between autumn and spring

Table 5-9 Predicted dates for pupation (A) and emergence of flies (derived from A) from each deposit in 2005 and 2006, using the second modified Logan model, presented by McLeod (1997), and the difference between the predicted and observed dates of the emergence of the median number of flies

Year	Date of deposit	Predicted date for		Observed date of median fly emergence (C)	Difference (days, B-C)[b]
		Pupation (A)	Fly emergence [a] (B)		
2005	16-Mar	22-Mar	7-Apr	4-Apr	+3
	6-Apr	11-Apr	6-May	25-Apr	+11
	26-Apr*	8-May	29-Sep	6-Jun	+115
	26-Apr	8-May	29-Sep	23-Oct	-24
	18-May	30-Jul	21-Oct	No emergence	
	8-Jun	14-Sep	26-Oct	27-Oct	-1
	1-Jul[c]	24-Sep	29-Oct		
	12-Jul	24-Sep	29-Oct	13-Oct	+16
	10-Aug	26-Sep	29-Oct	19-Oct	+10
	19-Sep	6-Oct	2-Nov	21-Oct	+12
	5-Oct	20-Oct	7-Nov	4-Nov	+3
	26-Oct	2-Nov	19-Nov	13-Nov	+6
	30-Nov	5-Dec	18-Dec	14-Dec	+4
2006	10-Jan	14-Jan	22-Jan	21-Jan	+1
	24-Jan	27-Jan	6-Feb	3-Feb	+3
	7-Feb	12-Feb	22-Feb	19-Feb	+3
	1-Mar	5-Mar	15-Mar	12-Mar	+3
	22-Mar	26-Mar	18-Apr	10-Apr	+8
	11-Apr*	28-Apr	11-Oct	28-May	+136
	11-Apr	28-Apr	11-Oct	7-Oct	+4
	19-Apr*	16-May	15-Oct	13-Jun	+124
	19-Apr	16-May	15-Oct	13-Oct	+2
	1-May	8-Sep	19-Oct	13-Oct	+6
	9-May	18-Sep	22-Oct	9-Oct	+13
	30-May	25-Sep	25-Oct	10-Oct	+15
	1-Jul[c]	27-Sep	25-Oct		
	18-Jul	27-Sep	25-Oct	10-Oct	+15
	22-Aug	29-Sep	26-Oct	24-Oct	+2
	19-Sep	7-Oct	1-Nov	13-Oct	+19
	12-Oct	19-Oct	11-Nov	2-Nov	+9

[a] using A as start date for pupal development

[b] between the predicted and actual dates of the emergence of the median number of flies; a -ve sign indicates the predicted date was earlier, a +ve sign indicates the predicted date was later than the observed date

[c] 1 July used as arbitrary start date for over-wintering larvae

* Indicates deposits that exhibited a split emergence of flies between autumn and spring

Table 5-10 Predicted dates for pupation (A) and emergence of flies (derived from A) from each deposit in 2005 and 2006, using the matched-asymptotic model, presented McLeod (1997), and the difference between the predicted and observed dates of the emergence of the median number of flies

Year	Date of deposit	Predicted date for		Observed date of median fly emergence (C)	Difference (days, B-C)[b]
		Pupation (A)	Fly emergence [a] (B)		
2005	16-Mar	23-Mar	10-Apr	4-Apr	+6
	6-Apr	12-Apr	29-Jun	25-Apr	+65
	26-Apr*	30-May	20-Oct	6-Jun	+136
	26-Apr	30-May	20-Oct	23-Oct	-3
	18-May	16-Aug	3-Nov	No emergence	
	8-Jun	10-Sep	5-Nov	27-Oct	+9
	1-Jul[c]	2-Oct	7-Nov		
	12-Jul	4-Oct	8-Nov	13-Oct	+26
	10-Aug	16-Oct	10-Nov	19-Oct	+22
	19-Sep	22-Oct	16-Nov	21-Oct	+26
	5-Oct	27-Oct	19-Nov	4-Nov	+15
	26-Oct	4-Nov	25-Nov	13-Nov	+12
	30-Nov	6-Dec	21-Dec	14-Dec	+7
2006	10-Jan	14-Jan	23-Jan	21-Jan	+2
	24-Jan	27-Jan	7-Feb	3-Feb	+4
	07-Feb	12-Feb	23-Feb	19-Feb	+4
	1-Mar	5-Mar	17-Mar	12-Mar	+5
	22-Mar	26-Mar	19-Jun	10-Apr	+70
	11-Apr*	3-Jul	24-Oct	28-May	+149
	11-Apr	3-Jul	24-Oct	7-Oct	+17
	19-Apr*	9-Aug	1-Nov	13-Jun	+141
	19-Apr	9-Aug	1-Nov	13-Oct	+19
	1-May	17-Sep	4-Nov	13-Oct	+22
	9-May	19-Sep	4-Nov	9-Oct	+26
	30-May	25-Sep	5-Nov	10-Oct	+26
	1-Jul[c]	7-Oct	9-Nov		
	18-Jul	10-Oct	10-Nov	10-Oct	+31
	22-Aug	13-Oct	12-Nov	24-Oct	+19
	19-Sep	15-Oct	13-Nov	13-Oct	+31
	12-Oct	25-Oct	21-Nov	2-Nov	+19

[a] using A as start date for pupal development

[b] between the predicted and actual dates of the emergence of the median number of flies; a -ve sign indicates the predicted date was earlier, a +ve sign indicates the predicted date was later than the observed date

[c] 1 July used as arbitrary start date for over-wintering larvae

* Indicates deposits that exhibited a split emergence of flies between autumn and spring

5.3.2.5 Predicted Versus Observed Emergence Times of Flies

The dates of emergence of the median number of flies, predicted by each of the models, were compared with the actual dates, when either the first or the median number of flies were observed. The number of days needed for flies to emerge, as calculated by each of the models, is shown in Figure 5-6 and Figure 5-7. The solid line in both figures represents the perfect fit between the predicted and the observed dates. If data points are above the solid line, the predicted date of emergence was later than the observed date. Conversely, if data points are below the solid line, the predicted date of emergence was earlier than the observed emergence date.

In these figures, the linear day degree, modified Logan, matched-asymptotic, modified Vogt and Temsum models have a data point for each deposit date. However, FlyAlert and Temsum(A) use 1 July as start date for all over-wintering deposits and their results are compared to the average date when the first and median number of flies emerged in spring. Consequently, there are only two data points for these models, one for each year.

The data points in the bottom left corner are from deposits made from spring through to early autumn. During this time, postfeeding larvae and pupae developed more rapidly. All models, except Temsum(B), predicted the emergence of flies from these deposits later than was observed.

The red, green and purple markers represent the three deposits exhibiting a split emergence of flies between autumn and spring (26 April 2005, 11 April 2006 and 19 April 2006).

The comparison between the predicted and observed times of emergence of flies is given in more detail in Table 5-12 and 5-13. Table 5-12 compares the times of emergence of flies, predicted by each of the models, using 1 July as start date, with the average date when the first and median number of flies emerged from over-wintering larvae. The mean date of the emergence of the first fly from over-wintering larvae was 4 October and 1 October in 2005 and 2006, respectively. Further, the mean date of the emergence of the median number of flies was 21 October and 12 October in 2005 and 2006, respectively. The linear day degree, modified Vogt and Temsum models predicted the spring emergence dates of flies closest to the observed median emergence dates, in both 2005 and 2006.

Table 5-13 compares the emergence of flies predicted by each of the models, using the actual deposit dates as start dates, with the dates when the first and median number of flies emerged in the field.

Temsum and the linear day degree model gave the best results during the warmer parts of the year, whereas the matched-asymptotic model was the least accurate during this time. All models predicted the emergence of flies from larvae deposited in summer within 5 days of the observed median emergence dates.

None of the models was able to predict a split emergence of flies between autumn and spring. For the deposits exhibiting a split emergence between autumn and spring, the modified Vogt and Temsum models predicted emergence closer to the observed dates in autumn, whereas the other models predicted emergence times closer to the observed emergence times in spring. For deposits made during late autumn and winter, Temsum and the linear day degree model predicted the dates of emergence the most accurately.

The median absolute deviation (MAD) for each of the models was first calculated over all deposit dates, then subsequently calculated using only deposits made from mid-April to August (Table 5-11). For those deposits, exhibiting a split emergence between autumn and spring, only the spring emergence dates were used when estimating the MAD for each model.

The linear day degree model had the lowest MAD when comparing the predicted emergence dates with the observed median emergence dates. This model had also the lowest MAD when only the deposits made from mid-April to August were compared. In addition, the Temsum model had a very low MAD when comparing the predicted dates of emergence with the observed date of emergence of the first fly for deposits made from mid-April to August.

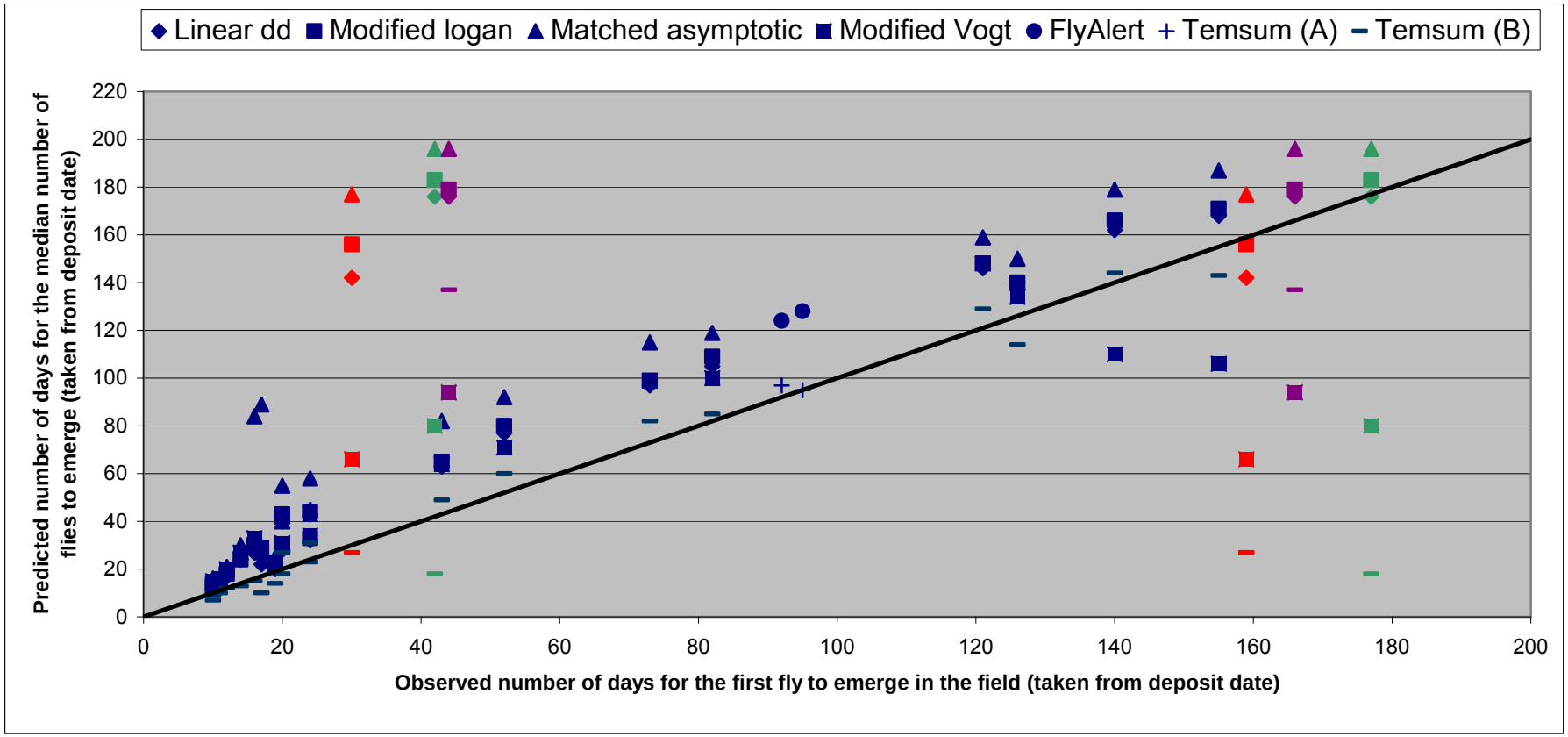


Figure 5-6 The observed number of days (taken from the deposit date) for the first fly to emerge in the field versus the number of days predicted by each of the models

[1] Using actual soil temperatures for each deposit.
[2] The red, green and purple markers indicate deposits which exhibited a split emergence (26 April 2005, 11 April 2006 and 19 April 2006, respectively).
[3] The solid line denotes the perfect fit of the predicted and observed dates.
[4] FlyAlert and Temsum (A) use 1 July as the start date with the results compared to the mean date of emergence of the first flies in spring.

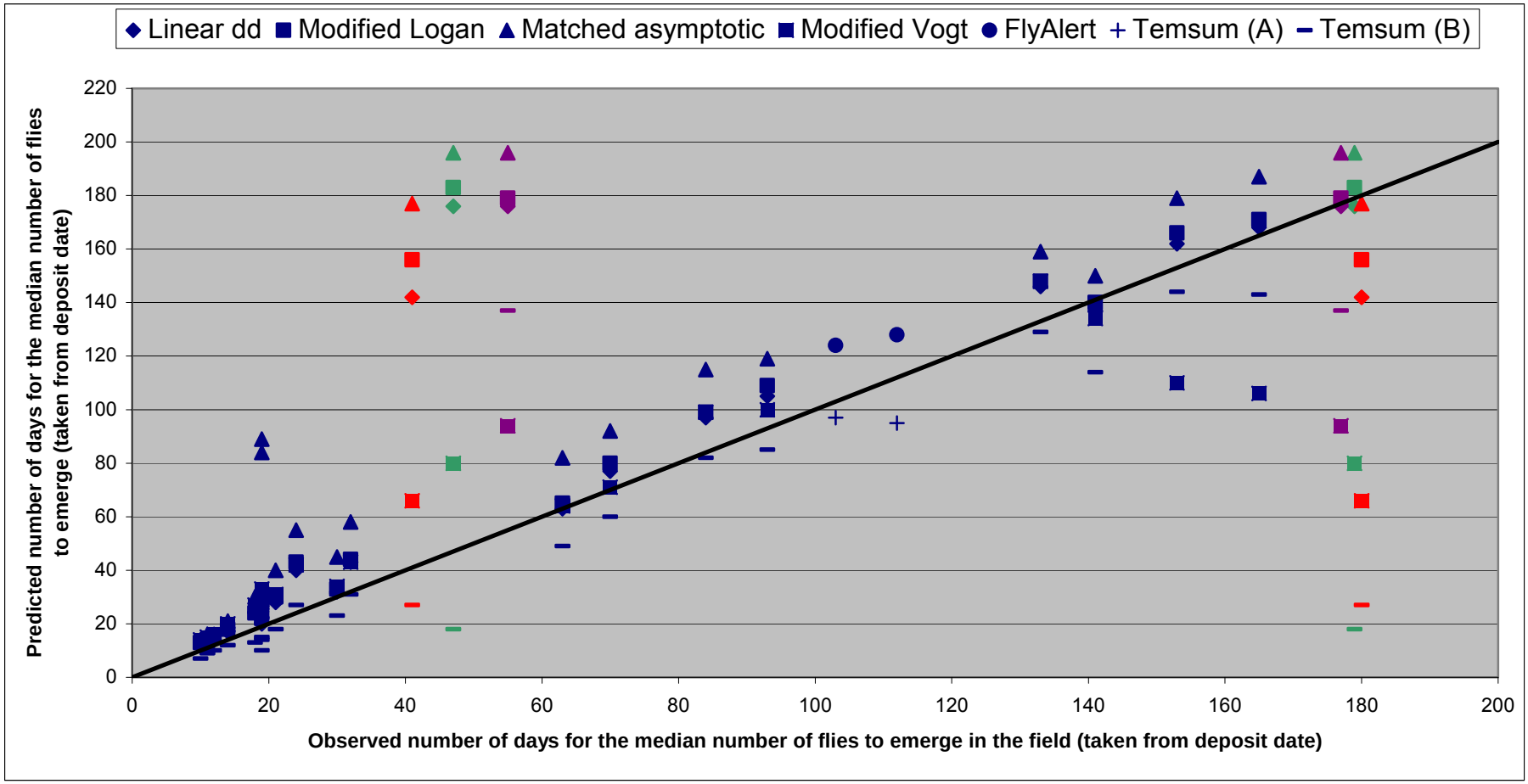


Figure 5-7 The observed number of days (taken from the deposit date) for the median number of flies to emerge in the field versus the number of days predicted by of the each models

[1] Using actual soil temperatures for each deposit.
[2] The red, green and purple markers indicate deposits which exhibited a split emergence (26 April 2005, 11 April 2006 and 19 April 2006, respectively).
[3] The solid line denotes the perfect fit of the predicted and observed dates.
[4] FlyAlert and Temsum (A) use 1 July as the start date with the results compared to the mean date of emergence of the median number of flies in spring.

Table 5-11 The median absolute deviation (MAD) for the linear day degree, modified Logan, matched-asymptotic, modified Vogt and Temsum models, when predicted emergence dates were compared to the observed dates of the first and the median emergence of flies

	Linear		Logan		Matched asympt		Mod. Vogt[c]		Temsum	
	First	Med	First	Med	First	Med	First	Med	First	Med
MAD[a]	10.5	3.5	11.5	6	27	19	14	7.5	5.5	5
MAD[b]	20	7	22	10	37	22	25	40	9	14

[a] including all deposit dates; for deposits exhibiting a split emergence between autumn and spring, only the observed spring emergence dates were compared
[b] including only deposits made from mid April though to August; for deposits exhibiting a split emergence between autumn and spring, only the observed spring emergence dates were compared
[c] Vogt model, using a maximum temperature of 10^{o}C as the threshold temperature for pupation

Table 5-12 The number of days [a] between the predicted dates of emergence of flies and the observed mean date, when the first and median number of flies emerged in spring from over-wintering larvae in 2005 [b] and 2006 [c], using 1 July as start for each model

Year	Start date	Linear		Logan		Matched-asympt.		Modified vogt[d]		FlyAlert		Temsum	
		First	Med	First	Med	First	Med	First	Med	First	Med	First	Med
2005	1-Jul	21	4	25	8	34	17	15	-2	33	16	0	-17
2006	1-Jul	22	11	24	13	39	28	11	0	32	21	5	-6

[a] a -ve sign indicates the predicted date was earlier than the observed date, a +ve sign indicates the predicted date was later than the observed date
[b] mean date, when the first and median number of flies emerged from over-wintering larvae was 4 and 21 October in 2005
[c] mean date, when the first and median number of flies emerged from over-wintering larvae was 1 and 12 October in 2006
[d] Vogt model, using a maximum temperature of 10^{o}C as the threshold temperature for pupation

Table 5-13 The number of days between the predicted dates of emergence of flies and the observed dates, when the first and median number of flies emerged [a]. The numbers highlighted in yellow indicate that the predicted times of emergence were within 7 days of the observed emergence times

Year	Deposit date	Linear		Logan		Matched-asympt.		Modified vogt[b]		Temsum	
		First	Med	First	Med	First	Med	First	Med	First	Med
2005	16-Mar	1	1	3	3	6	6	4	4	-5	-5
	6-Apr	11	8	14	11	68	65	17	14	-1	-4
	26-Apr*	112	101	126	115	147	136	36	25	-3	-14
	26-Apr	-17	-38	-3	-24	18	-3	-93	-114	-132	-153
	18-May										
	8-Jun	11	-4	14	-1	24	9	-25	-40	-12	-27
	12-Jul	23	12	27	16	37	26	17	6	3	-8
	10-Aug	25	7	28	10	40	22	18	0	8	-10
	19-Sep	19	11	20	12	34	26	19	11	7	-1
	5-Oct	8	2	9	3	21	15	10	4	-1	-7
	26-Oct	10	6	10	6	16	12	13	9	-1	-5
	30-Nov	5	3	6	4	9	7	8	6	0	-2
2006	10-Jan	2	1	2	1	3	2	2	1	-1	-2
	24-Jan	3	3	3	3	4	4	4	4	-3	-3
	7-Feb	3	2	4	3	5	4	5	4	-1	-2
	1-Mar	2	1	4	3	6	5	5	4	-1	-2
	22-Mar	5	3	10	8	72	70	12	10	-7	-9
	11-Apr*	134	129	141	136	154	149	38	33	-24	-29
	11-Apr	-1	-3	6	4	19	17	-97	-99	-159	-161
	19-Apr*	132	121	135	124	152	141	50	39	93	82
	19-Apr	10	-1	13	2	30	19	-72	-83	-29	-40
	1-May	13	3	16	6	32	22	-49	-59	-12	-22
	9-May	22	9	26	13	39	26	-30	-43	4	-9
	30-May	25	13	27	15	38	26	14	2	8	-4
	18-Jul	24	13	26	15	42	31	13	2	9	-2
	22-Aug	20	0	22	2	39	19	14	-6	6	-14
	19-Sep	20	16	23	19	35	31	22	18	7	3
	12-Oct	8	7	10	9	20	19	11	10	-2	-3

[a] a -ve sign indicates the predicted date was earlier than the observed date, a +ve sign indicates the predicted date was later than the observed date

[b] Vogt model, using a maximum temperature of 10°C as threshold temperature for pupation

* Indicates deposits that exhibited a split emergence of flies between autumn and spring

5.4 Discussion

The ability to better predict the emergence of flies in different parts of south-eastern Australia could support the more precise timing of strategic treatments. These treatments are applied to reduce the susceptibility of sheep to flystrike, which is the main factor leading to flystrike. At the moment, only one option for strategic treatment has been investigated in any detail in this region, namely treatment in early spring (McKenzie and Anderson 1990). Treatment of sheep at this time will prevent development of eggs and larvae derived from the first generation of flies that emerge from over-wintering larvae. However, the success of this strategy depends greatly on the correct timing of treatment and achieving a high percentage kill of the target population (Wall et al. 1993b).

The current study compared several models for the development of *L.cuprina*. These were used to predict the emergence of the median number of flies from larvae deposited at different times throughout the year ('median emergence'). From a practical point of view, a model needs to forecast the median emergence of the last generation of flies in autumn, or the first generation of flies in spring, within about 7 days of the actual dates. The argument for this is as follows; this study showed that the time between the first and median emergence of flies from over-wintering larvae ranged from 2 to 21 days (mean: 13.1 days; 95% CI: 9.5, 16.7). It is also known, that females require 57 day degrees to mature their first batch of eggs. This amounts to about 8 days in spring, assuming an average air temperature of 15ºC (Vogt et al. 1985c). Therefore, the optimum time for treatment is at least 2 weeks before the predicted median fly emergence date because the first flies that appear will be preparing to oviposit at this time.

The models evaluated in this chapter could use either an arbitrary start date of 1 July, or the actual dates that larvae were deposited. Initially, each of the models was run using 1 July as the start date. The predicted emergence was compared with the mean date of the emergence of the median number of flies from deposits made from mid-April to August, namely 21 October 2005 and 12 October 2006. The linear day degree, modified Vogt and Temsum models all gave a satisfactory prediction of the actual dates (Table 5-12). The results from the modified Vogt and Temsum models were better, but for slightly different reasons. The modified Vogt model predicted the emergence date within 2 days of the actual average median emergence date for both years, whereas Temsum predicted

the emergence date 17 days too early in 2005 and 6 days too early in 2006. Although the results for Temsum seem rather inaccurate, to optimise strategic treatment it is better to predict fly emergence 2 weeks too early than 1 week too late. More importantly, Temsum was the only model that accurately forecasted the mean date of the emergence of the first fly from over-wintering larvae, with its prediction being within 5 days of the actual dates for both years of the study. Therefore, Temsum appears to provide the most useful overall prediction of the timing of spring emergence of flies. However, one objection to this model may be that it lacks a sound theoretical framework for its predictions. Firstly, it does not calculate a date of pupation, and secondly, the arbitrary choice of threshold temperature might not reflect the true threshold for immature stages of *L.cuprina*.

Following these initial runs, the outputs of the models, using the actual deposit dates as start dates, were compared with the actual dates of median fly emergence for deposits made from mid-April to August (Table 5-11 and Table 5-13). For these iterations, the linear and modified Logan models provided the best fit with the actual dates, with one third of the dates, predicted by both models, being within 7 days of the actual emergence dates. However, a valid criticism of both these models is that neither incorporated a lag phase, representing the arrested development of larvae during winter. The inaccuracy this produces is demonstrated by the poor fit between the pupation dates predicted by each model and the results from the sequential study (Table 3-5). The predictions of Temsum and the modified Vogt model also provided a reasonably good fit for deposits made in July and August, but not for deposits made from mid-April to June. This is probably of little practical benefit, as flies are not active in July and August and larvae would not be entering the soil at this time of the year.

An alternative timing for strategic treatment in south-eastern Australia is in autumn, to reduce the number of larvae that enter an arrested development and over-winter. However, none of the models could accurately predict the autumn emergence of flies from larvae entering the ground during the transitional phase in mid-April, and so would be currently of little benefit in refining the timing of this treatment option.

The predicted fly emergence dates for all models, with the exception of the matched-asymptotic model, were quite close to the actual dates during the warmer parts of the year, namely from October to early April. This demonstrates that all except the matched-asymptotic model simulated the development of immature stages well when soil temperatures remained above the minimum threshold temperatures for development of

postfeeding larvae and pupae. In contrast to the current findings, the matched-asymptotic gave the best fit in a study at Fowlers Gap, located in a warm, arid region in northwest New South Wales (McLeod 1997). One explanation for the difference in fit of the model between the two studies is that the parameters in this model are better suited to the warmer, arid region of New South Wales, although the model is generally considered to perform well in both high and low temperature ranges (Hilbert and Logan 1983).

This study highlighted several inadequacies and biological errors in the evaluated models. For example, none of the models was able to predict the split emergence of flies, or accurately predict the emergence of flies in autumn from larvae entering the ground during the transitional phase in mid-April. This may be related to the lack of any sophisticated rules for a 'lag phase', representing the period of arrested development of postfeeding larvae, in any of the models. This reflects that detailed information on this part of the life cycle of *L.cuprina*, namely the development of postfeeding larvae at low temperatures, is not available.

Nevertheless, the FlyAlert and modified Vogt models did incorporate some rules for pupation of over-wintering larvae. Although, these were not a simple low-temperature threshold, they were essentially similar, with both models requiring soil temperatures to remain above an explicit threshold for a certain time before pupation occurred. The biological inaccuracy produced by these rules was demonstrated clearly by the sequential study of larval development and pupation during late winter of 2006. The date of pupation predicted by FlyAlert was more than 4 weeks after the actual date. However, the modified Vogt model, using 1 July as start date, predicted pupation quite accurately. None of the four remaining models incorporated any special requirements for pupation, with Temsum being the only model that did not calculate a pupation date.

In summary, when the models predicted emergence of flies after the actual date of emergence, they were generally accumulating development too slowly. On the other hand, when the models predicted the emergence of flies too early, they did not account for an arrested development in larvae and so calculated an incorrect pupation date.

As briefly mentioned above, these findings underline the urgent need for more information on the development of immature stages of *L.cuprina*. The three models, presented by McLeod, and evaluated in this study, use development data for postfeeding

larvae of *L.sericata* because no corresponding data is available for *L.cuprina* (McLeod 1997). Although these are closely related species, they do have quite important differences in their life cycle and ecology (Stevens and Wall 1997; Wall et al. 1993a). Consequently, this could have caused inaccuracy in these three models. In addition, the limited development data for both postfeeding larvae and pupae, especially at low temperatures is probably a significant source of error (Figure 5-1 and Figure 5-2). For the development of postfeeding larvae, the development data for *L.sericata* from a study of Wall (1992) were used, which had only 10 data points between 10°C and 35°C. For the development of pupae, the three models used the data from a study of Dallwitz (1984), which described development under 8 constant temperature regimens, from 15°C to 35°C. Therefore, the low-temperature thresholds for *L.cuprina* larvae and pupae were calculated from this limited data. In addition, there are several reports that accumulation of development in many insects can be different under constant and fluctuating temperatures, with greater development accrued under fluctuating low temperatures than predicted from constant temperature studies (Gullan and Cranston 2005; Liu and Meng 2000; Son and Lewis 2005).

Further, the exact data used to develop the Flyalert and modified Vogt models is not accessible as it is published as a figure, rather than the actual data (Dallwitz and Wardhaugh 1984). The source of the development data for the immature stages that has been used in Temsum is also unclear. Consequently, modification or interpretation of the underlying biology of these models is made much more difficult.

Another important deficiency in FlyAlert and Temsum were the algorithms used to estimate soil temperatures from measured air temperatures. FlyAlert and Temsum consistently over-estimated the soil temperatures (Figure 5-3 and Figure 5-4). Consequently, these functions appear unsuitable for estimating the development of immature stages of *L.cuprina*, at least in this region. This is highlighted by the significant improvement in the outcomes of Temsum when using observed soil temperatures rather than soil temperatures estimated from air temperatures. In the initial simulations using this model, the predicted emergence dates were up to 8 weeks earlier when using estimated soil temperatures instead of actual soil temperatures, whereas the predicted dates were quite close to the actual emergence dates when using the observed hourly soil temperatures.

Subsequently, the relationship between soil and air temperatures at the experimental site was explored. The daily minimum, average and maximum soil temperatures were accurately estimated from a combination of the current daily air temperatures and the average of the daily air temperatures over the previous 10 days. However, before a firm recommendation can be made as to the most appropriate equation(s) for deriving soil temperatures from observed air temperatures in south-eastern Australia, more data from a range of soil types and localities within this region is needed. Thus for any studies on development models of the immature stages of *L.cuprina*, it is probably advisable to use observed soil temperatures rather than estimated soil temperatures.

In this study, all but one of the evaluated models use only temperature data to calculate development times of larvae and pupae. However, it is suggested that other factors, such as excessive rain can cause a delay in development (Wardhaugh 2001). The exception was the modified Vogt model, which discounted all accrued development if rainfall in the preceding 24 hours exceeded 12 mm (Wardhaugh 2001). To simplify calculations and comparison between models, this rule was not applied in the present study.

The results of the field study (Chapter 3) indicate that the temperature experienced by the postfeeding larvae as they drop off the sheep and burrow into the soil is probably the single most important factor that controls the induction of the arrested development in autumn. However, the role of other environmental cues, such as declining maternal photoperiod in autumn, was not investigated. As discussed in chapter 1 and 4, this plays a role in the induction of the arrested development or diapause of other blowfly species, including the related species *L.caesar* (Ring 1967a) and *L.sericata* (Tachibana and Numata 2004b). The possibility of maternal photoperiod also having a role in the induction of the arrested development of *L.cuprina* larvae was raised by Dallwitz and Wardhaugh (1984), but to date it appears that no studies have been undertaken to investigate this hypothesis. McLeod (1997) used *L.cuprina* larvae from 'laboratory-bred' (12:12 light regimen) and 'field-bred' (natural light regimen) flies in her over-wintering experiments, but was unable to determine any differences in over-wintering behaviour between the two types of flies. One explanation for the inability of this study to test the hypothesis of Dallwitz and Wardaugh (1984) was that the laboratory flies might not have kept long enough under the 12:12 light regimen.

A consistent transitional phase in mid-April was exhibited in the current study by flies, which were exposed to a 24-hour light regimen (Figure 3-2). Thus, it is likely that any

effect of the maternal photoperiod on the induction of the arrested development of postfeeding *L.cuprina* larvae is secondary to the effect of temperature experienced by the postfeeding larvae themselves. This aspect of the biology of *L.cuprina* deserves attention and could contribute to improve the development models of this species.

As discussed in chapter 4, information about the exact nature of temperature changes that stimulate the resumption of development of arrested larvae in spring, or other as yet undefined environmental cues, is lacking. Again, studies of this aspect of the biology of *L.cuprina* would contribute greatly to attempts to more accurately model this aspect of the life cycle of this important pest. For example, a pattern for late winter/ early spring soil temperatures, including an initial rise of more than 1.5°C, followed by a sustained period above 11°C, was seen around the estimated time of pupation in each year of this study (Figure 3-8). This observation, and other patterns, should be explored at a number of other sites where soil temperatures have been recorded for several years.

In conclusion, an ideal model is biologically sound and gives accurate predictions well in advance. It must be able to predict a split emergence for larvae entering the ground during the transitional phase, as well as calculate the mortality during the over-wintering period. It is likely to be most feasible to have two models, one to predict the last generation of flies in autumn and one to predict the first generation of flies in spring. In addition, it is important that these models are applicable in different areas of south-eastern Australia.

Temsum, using 1 July as start date and actual hourly soil temperatures, seems currently the best model available for south-eastern Australia to predict the timing of spring emergence well in advance. None of the current models was able to predict the last generation of flies in autumn, the time when larvae entered arrested development, or the occurrence of a split emergence. To improve the current models and develop new models, more developmental data of the immature stages of *L.cuprina,* especially at low temperatures, are necessary and a better understanding is needed of the exact cues controlling the induction of arrested development of larvae in autumn and the resumption of their development in late winter.

CHAPTER 6

GENERAL DISCUSSION & CONCLUSION

6.1 Introduction

Flystrike is a major problem for the Australian sheep industry, estimated to cost $280 million annually (Sackett et al. 2006). In the high rainfall areas, a large proportion (86%) of these losses are increased costs expended by producers as part of existing treatment and control programs, whilst the remaining 14% accrues from reduced income, such as decreased wool production and deaths from flystrike. In high rainfall areas, the losses from body and breech strike in high risk areas are estimated at $1.28 and $1.60 per head, respectively (Sackett et al. 2006). The losses from pizzle strike, an important form of covert strike, that can amplify fly numbers early in the season (Wardhaugh and Dallwitz 1984), are estimated to be $0.68 per head.

A review was conducted of the literature on blowfly strike and the ecology of the primary sheep blowfly, *Lucilia cuprina*, concentrating on studies performed in, or of relevance to, south-eastern Australia. This review revealed that there were still some key aspects of the biology of *L.cuprina* that were either not investigated in great detail, or these investigations had not been published. The review also found that there were very few recent studies of direct relevance to south-eastern Australia. In particular, there is little published information about the over-wintering ecology of *L.cuprina* in western Victoria, a high winter rainfall area with one of the highest concentrations of Merino sheep in Australia.

Following the literature review, a 2-year field experiment was designed to investigate the over-wintering ecology of *L.cuprina* in more detail. This experiment, conducted during 2005 and 2006 at a farm in Rokewood, in central western Victoria, was based broadly on the 2-year study of Dallwitz and Wardhaugh (1984) at Canberra. However, it incorporated more detailed observations on the weather and soil conditions experienced by the deposited larvae.

Following the collection of data on the emergence of flies from the field study at Rokewood, a review of models that could predict the development of *L.cuprina* in this region identified six models that were of potential use. However, none of these models had been validated in western Victoria, and so the data from the emergence of flies from each deposit date, and air and soil temperatures, were fitted to each model. In addition, modifications were made to some of the models in an attempt to make them more accurately predict the observed dates of fly emergence.

6.2 Results from the Field Study

The field study confirmed that there is a transitional phase of larval development in mid-April in south-eastern Australia, with some larvae pupating this time, but increasing numbers entering an arrested development and emerging as flies the following spring. A period of sustained low temperatures in mid-to late autumn, most likely below 10°C, resulted in the inhibition of pupation in late autumn, whereas a particular pattern of rising soil temperatures, and/or a sustained period when temperatures were above 11°C, was associated with the resumption of larval development in late winter or early spring.

Consistent with previous studies in Victoria (McKenzie 1990; 1994), there was a high mortality during the over-wintering period. However, this mortality did vary considerably between deposits within each year, and between years (Figure 3-5). For deposits made from May until mid-July, the average mortality was 95% in 2005 (range 91-100%) and 68% (range 50-89%) in 2006. This high mortality during the over-wintering phase is a 'weak link' in the life cycle of *L.cuprina* that creates an opportunity for better control of blowfly populations through a more timely strategic treatment.

The serial sampling study in 2006 suggested that resumption of development of over-wintering larvae took place around early to mid-September. The first flies from larvae deposited from mid-April to August were found in the field pots in early October in 2005 and in late September in 2006. The emergence of the first generation of flies in spring was synchronous (Figure 3-2), although there was a large interval between the emergence of the first and last fly, which was more than 50 days in both years.

Trapping of free-ranging flies at the experimental site found that the number of flies trapped per day followed a bimodal pattern, with a large peak in November and a smaller

peak in early March (Figure 3-6). In 2006, the last flies before winter were trapped in late April.

6.3 Validation of Models

The discussion in Chapter 4 identified that accurately predicting the last generation of flies in autumn, and the first generation of flies in spring, is essential when optimising the timing of strategic treatment programs to control blowfly strike in south-eastern Australia.

Validation of 6 models, using the data from the field study, showed that none of the models was able to predict the last generation of flies in autumn, the time when larvae entered an arrested development around mid-autumn, or the occurrence of a split emergence of flies between autumn and spring.

The Temsum model, using 1 July as start date and the actual hourly soil temperatures, was found the best model to predict the emergence of the first generation of flies in spring. However, this model was biologically unrealistic as it did not incorporate a lag phase, simulating a period of arrested development, and it did not predict a date of pupation.

Comparison of the soil temperatures derived by Temsum and FlyAlert from observed air temperatures found that the derived soil temperatures were continuously over-estimating the actual soil temperatures (Figure 5-3 and Figure 5-4), resulting in inaccurate predictions of the emergence dates. It is therefore advisable to use observed soil temperatures in the predictive models rather than estimated soil temperatures.

6.4 Integrated Control Programs

Currently, management practices to prevent flystrike are principally based on making sheep less attractive to blowflies. The measures targeting breech strike include tail docking and Mulesing, crutching and shearing, optimal worm control, and jetting of the breech with insecticides. More recently, there has been an increased interest in the selection of sheep with fewer wrinkles around the breech (James 2006) and decreased scouring (Larsen et al. 1999; Larsen et al. 1995). Body strike is typically associated with

wet, humid weather, which favours the development of fleece rot (Raadsma 1987). Prevention of body strike relies primarily on the use of insecticides when the weather suggests a possible fly wave may occur (Morley 1994), although selection against fleece rot can also reduce the susceptibility of Merino sheep (Atkins and McGuirk 1979; McGuirk et al. 1978; Raadsma 1987). In addition, an essential part of an integrated program to control flystrike is regular inspection of the flock, to find and treat any struck sheep (Armstrong et al. 2001).

In contrast to control programs for internal parasites, the use of insecticides to control blowflies is more based around management practices, such as time of shearing, lambing, and cropping than on any specific knowledge of the blowfly life cycle. Consequently, chemicals are often applied once sufficient strike occurs in a mob, rather than to control the blowfly population and reduce the risk of flystrike (Lottkowitz et al. 1984).

An Integrated Parasite Management (IPM) approach has the potential to control blowflies more effectively, and potentially reduce the use of insecticides on some farms. A thorough understanding of the ecology and population dynamics of *L.cuprina* is required to formulate successful IPM strategies for blowfly control, as well as a detailed knowledge of the influences of various management practices and seasonal weather conditions on the susceptibility of sheep to flystrike.

The seasonal variation in abundance of *L.cuprina* and risk of flystrike, in south-eastern Australia, creates the opportunity to use insecticides in a more strategic manner, as part of an IPM strategy to control blowfly strike. Results from one study in Victoria showed that treatment of sheep before blowflies emerge in spring can lower the number of *L.cuprina* flies throughout the season as well as the prevalence of flystrike (McKenzie and Anderson 1990). This is in contrast to the way the majority of farmers currently use insecticides, namely at times when fly abundance is maximal. An alternative strategy, which has not been examined in the field, is to treat sheep in autumn to reduce the number of larvae that enter arrested development.

At the moment, there are two highly effective IGR pesticides, cyromazine and dicyclanil, which have long residual activity (up to 14 and 24 weeks, respectively), and to which no resistance has been detected in field populations of *L.cuprina*. Cyromazine, in particular, is an extremely valuable compound, having been used for more than 25 years without the

appearance of resistant blowflies. Therefore, it is important that IPM strategies should aim to maximise the effectiveness of these insecticides whenever they are used.

6.5 Suggestions for Future Work

A number of aspects of the biology of *L.cuprina* were identified that deserve further study. In particular, the limited data regarding the development of immature stages of *L.cuprina*, especially at low temperatures, and a lack of understanding of the key factors which induce and terminate the arrested development of larvae, are significant deficiencies. Better knowledge of development at low boundary temperatures, including comparison between strains of *L.cuprina* from cold, temperate and sub-tropical areas of Australia, would help optimise IPM strategies for the control of blowfly strike. It would also provide data to either improve existing models of blowfly development, or help develop new models.

This study indicated that temperature was the major factor controlling the induction and termination of arrested development of larvae. However, Dallwitz and Wardhaugh (1984) have suggested the possible involvement of maternal photoperiod in the induction of arrested development in *L.cuprina*. Such a maternal influence has been confirmed in related *Lucilia spp* (Ring 1967a; Tachibana and Numata 2004a). Consequently, a study similar to that recently undertaken for *L.sericata* by Tachibana and Numata (2004b) would address the role of parental and direct effects of photoperiod and temperature on *L.cuprina* larvae in the onset of arrested development.

Future challenges, such as the phase-out of Mulesing by 2010 and climate change, will require changes to the current blowfly control programs. However, they also provide an opportunity to integrate new strategies with existing control measures. For example, breeding sheep less susceptible to flystrike, or using genetically altered flies to reduce the fly population, are ultimate long-term solutions. If needed, they could be integrated with more timely application of insecticides to produce very effective control programs. The genome blowfly project may assist in finding potential new strains of flies suitable for genetic control, as well as identify new targets for insecticides, vaccines or biological control agents (Dorrian 2006a; Lee et al. 2007).

How these strategies can be most effectively used will probably be best assessed by a combination of modelling and field studies. Development models of the immature stages of *L.cuprina* will be an important component of this process. Simulation models of the seasonal abundance of *L.cuprina* could support the integration of new strategies into an overall control program by estimating the effect of a new strategy, or a combination of strategies, on blowfly populations. For example, simulation of the population dynamics of *L.sericata* in Britain (Wall et al. 1993b) has predicted that seasonal suppression of blowfly populations could be achieved by targeting either the larvae of the first generation of flies in early spring or the second generation of adult flies after winter. One field trial in Victoria has investigated the first approach and found that treatment of sheep in early spring had significant effects on lowering the fly numbers throughout the season as well as the prevalence of flystrike (McKenzie and Anderson 1990). The second approach may be achieved by trapping flies and/or genetic control of *L.cuprina* flies. More work is obviously needed to validate these strategies against *L.cuprina*. However, field studies could already be investigating the effectiveness of certain elements of strategic control that are currently feasible, such as the more timely application of insecticide treatments in either autumn or spring, or a combination of both.

Climate change resulting from global warming could have a major impact on the ecology of *L.cuprina* and the prevalence of flystrike in south-eastern Australia. For example, relatively small increases in soil temperatures in autumn, winter or spring could lengthen the fly season considerably (Sutherst 2001). This would mean that arrested development of larvae in autumn might be delayed compared to what was observed in this study, and that resumption of development in late winter would be earlier than observed in this study. The net effect of this is that there will be a shorter period in winter when adult flies are absent, and in some localities flies may become present all year round, as already occurs in Queensland (O'Sullivan et al. 1983). In addition, shorter and milder winters will also influence the mortality of larvae during the over-wintering period. This study indicated that mortality decreased as the time larvae spent in the ground was reduced, but increased when winter temperatures were higher. The hypothesis of depleting energy reserves may form the basis of these findings, but a relative simple and cheap series of laboratory studies could more fully explain this.

Climate change will also influence the susceptibility of sheep to flystrike. It is expected that with increasing temperatures, the usually dry summers in the south-eastern Australia

will become wetter, with extreme daily rainfall events, such as thunderstorms, becoming more frequent (Anonymous 2007). This could increase the prevalence of fleece rot and scouring in summer, resulting in more body and breech strike. At the same time, increased withholding periods and decreased tolerance of insecticide residues will mean that emergency treatments close to shearing, with the possible exception of spinosad, will not be possible. Consequently, breeding sheep with decreased susceptibility to flystrike, by selecting for resistance to fleece rot, plain breeches and less scouring, and the more timely application of insecticides, will become increasingly important parts of an IPM strategy to control flystrike.

6.6 Conclusions

The field study conducted at a farm in Rokewood confirmed three fundamental features of the biology of *L.cuprina* in western Victoria. First, it identified a transitional phase of larval development in mid-autumn. Some larvae pupated immediately, whereas others went into an arrested development and emerged as flies the following spring. Secondly, the time of emergence of the first generation of flies in spring was similar for all larvae deposited in late autumn and winter, regardless of their deposit date. This synchronous emergence was slightly earlier than in a previous study in Canberra, with the first flies emerging on 26 September and 1 October in 2005 and 2006, respectively. Finally, it also confirmed the occurrence of a high, but variable mortality during the over-wintering phase of the life cycle of *L.cuprina*.

None of the 6 validated models for the development of *L.cuprina* could predict the last generation of flies in autumn, the time when larvae entered arrested development or the occurrence of a split emergence. However, a simple linear model (Temsum), using actual hourly soil temperatures and 1 July as a start date for all deposits, was able to accurately predict the emergence of the first generation of flies in spring for both years of the study.

Despite this, considerably more work is needed to refine the models assessed, or develop new models, so that they become applicable over a wider area and produce reliable simulations for a range of seasonal conditions. These development models could then be used as one part of a more complex simulation model to assess the impact of various strategic treatment options, and the impact of other emerging technologies such as genetic or biological control, on blowfly populations.

In addition to a number of simple, yet essential studies on the biology of the sheep blowfly that were identified, collaboration with the group involved in modelling the population dynamics of *L.sericata* in Britain could be an effective way of achieving the goal of improved modelling of *L.cuprina* for this region of Australia.

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APPENDICES

APPENDIX 1

SOIL TEMPERATURE DATA AT ROKEWOOD (2005-2006) & CANBERRA (1978-1979)

Table A-1.1 Comparison of average weekly minimum and maximum soil temperatures between the current study at Rokewood (2005-2006), and a study done at Canberra (Dallwitz and Wardhaugh, 1984)

Date	Rokewood				Canberra			
	2005		2006		1978		1979	
	Min	Max	Min	Max	Min	Max	Min	Max
21-27 Mar	14.4	21.8	17.9	25.5	15	24	14	27
28 Mar - 3 Apr	16.2	23.3	15.3	20.5	11	21	11	21
4-10 Apr	15.4	22.4	12.2	16.3	12	23	9	20
11-17 Apr	13.1	20.0	12.2	17.1	10	20	11	18
18-24 Apr	13.0	19.3	11.0	14.5	10	22	11	18
25 Apr - 1 May	12.8	18.7	11.6	15.4	5	18	11	18
2-8 May	11.2	16.1	10.6	12.9	7	21	9	17

APPENDIX 2

GRAPHIC REPRESENTATION OF THE DATA IN THE STUDY OF MCKENZIE (1990)

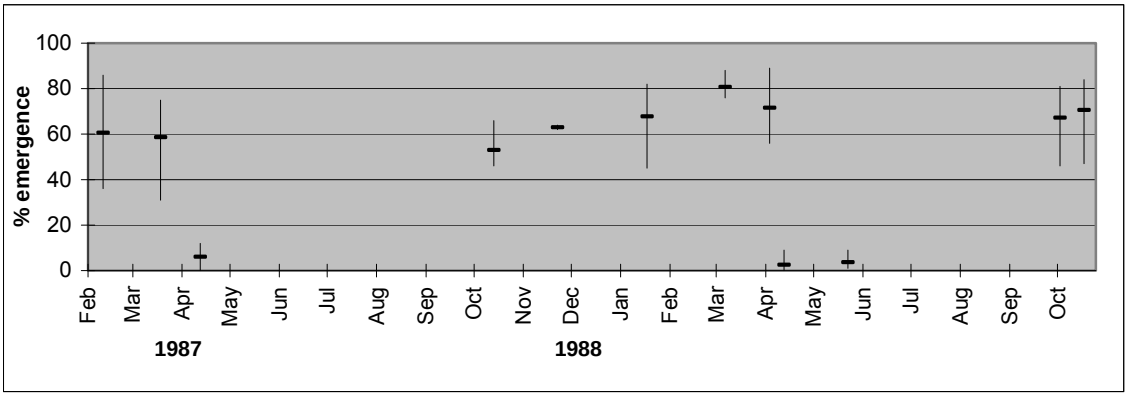

Figure A-2.1 The percentage of flies that emerged from larvae at each deposit in 1987 and 1988 in Heidelberg (data from McKenzie, 1990). The vertical line indicates the date of deposit, the bottom end of each line is the replicate with the lowest percentage, the crossbar is the average percentage of all replicates and the top end of each line is the replicate with the highest percentage of flies that emerged from the deposit

APPENDIX 3

ESTIMATED SOIL TEMPERATURES FROM AIR TEMPERATURES

A3.1Daily Soil Temperatures Estimated from Daily Air Temperatures

The minimum daily soil temperatures were calculated from the minimum daily air temperatures as

$$T_{sm} = 5.1986 + 0.893 \times T_{am} \quad (1.1.1)$$

where T_{sm} = estimated minimum daily soil temperature

T_{am} = observed minimum daily air temperature

The maximum daily soil temperatures were estimated from the maximum daily air temperatures, using the relationship

$$T_{sM} = 1.9561 + 0.8328 \times T_{aM} \quad (1.1.2)$$

where T_{sM} = estimated maximum daily soil temperature

T_{aM} = observed maximum daily air temperature

The average daily soil temperatures were computed from the average daily air temperatures as

$$T_{sa} = 3.04 + 0.887 \times T_{aa} \quad (1.1.3)$$

where Tsa = estimated average daily soil temperature

Taa = observed average daily air temperature

The estimated minimum, maximum and average daily soil temperatures from minimum, maximum and average daily air temperatures are shown in Figure A-3.1.

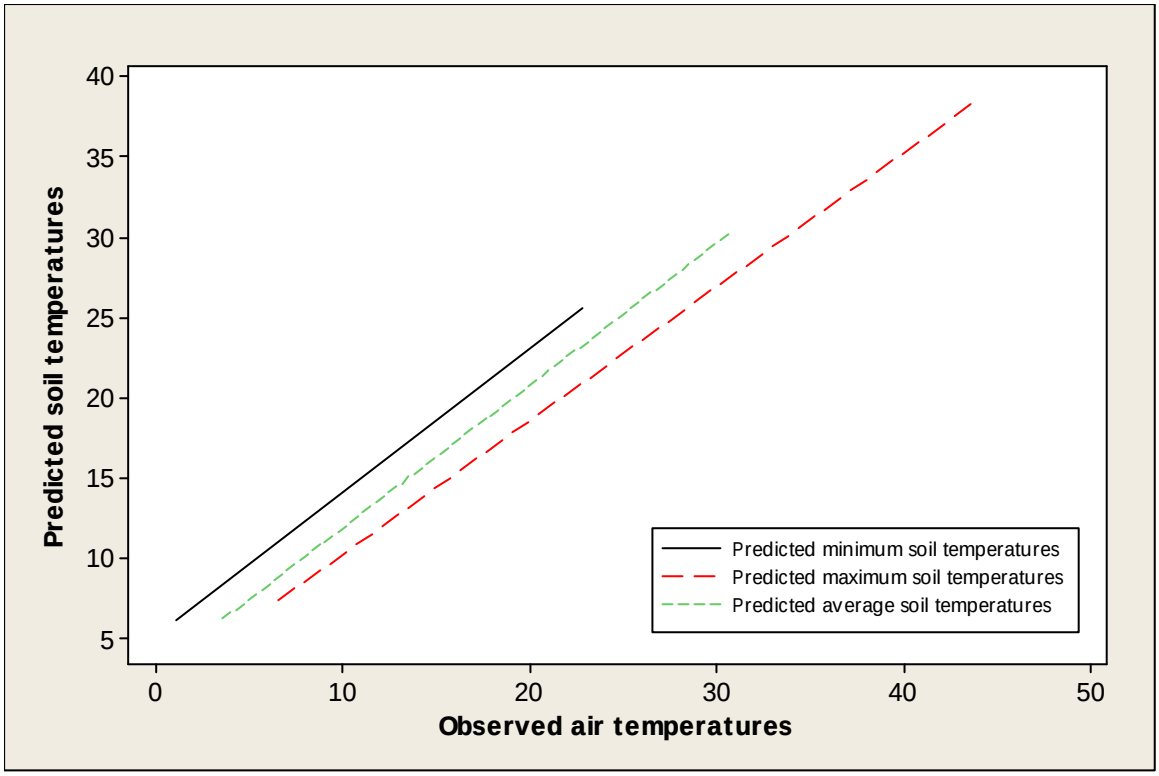

Figure A-3.1 Estimated minimum, maximum and average daily soil temperatures from daily minimum, maximum, and average daily air temperatures (°C) using the 2-year recordings from the weather station at the experimental site

A3.2 Daily Soil Temperatures Estimated from Daily Air Temperatures & Air Temperatures Measured 48 or 72 hours earlier

The minimum daily soil temperatures were computed from the current minimum daily air temperatures and the minimum daily air temperatures measured 48 or 72 hours earlier as

$$T_{sm} = 3.31 + 0.722 \times T_{am} + 0.390 \text{ x } T_{am48h} \quad (1.2.1.1)$$

$$T_{sm} = 2.96 + 0.773 \times T_{am} + 0.380 \text{ x } T_{am72h} \quad (1.2.1.2)$$

where T_{sm} = estimated minimum daily soil temperature

T_{am} = current minimum daily air temperature

T_{am48h} = minimum daily air temperature 48 hours previously

T_{am72h} = minimum daily air temperature 72 hours previously

The maximum daily soil temperatures were estimated from the current maximum daily air temperatures and the maximum daily air temperatures measured 48 or 72 hours earlier, using the relationship

$$T_{sM} = -0.419 + 0.650 \times T_{aM} + 0.304 \text{ x } T_{aM48h} \quad (1.2.2.1)$$

$$T_{sM} = -0.647 + 0.662 \times T_{aM} + 0.303 \text{ x } T_{aM72h} \quad (1.2.2.2)$$

where T_{sM} = estimated maximum daily soil temperature

T_{aM} = current maximum daily air temperature

T_{aM48h} = maximum daily air temperature 48 hours previously

T_{aM72h} = maximum daily air temperature 72 hours previously

The average daily soil temperatures were calculated from the current average daily air temperatures and the average daily air temperature measured 48 or 72 hours earlier, as

$$T_{sa} = 0.647 + 0.666 \times T_{aa} + 0.397 \text{ x } T_{aa48h} \quad (1.2.3.1)$$

$$T_{sa} = 0.419 + 0.704 \times T_{aa} + 0.374 \text{ x } T_{aa72h} \quad (1.2.3.2)$$

where T_{sa} = estimated average daily soil temperature

T_{aa} = current average daily air temperature

T_{aa48h} = average daily air temperature 48 hours previously

T_{aa72h} = average daily air temperature 72 hours previously

A3.3 Daily Soil Temperatures Estimated from Daily Air Temperatures & Average Daily Air Temperature over the previous 10 days

The minimum daily soil temperatures were computed from the current minimum daily air temperatures and the average of the minimum daily air temperatures over the previous 10 days as

$$T_{sm} = 0.941 + 0.479 \times T_{am} + 0.909 \text{ x } T_{am10d} \quad (1.3.1)$$

where T_{sm} = estimated minimum daily soil temperature

T_{am} = current minimum daily air temperature

T_{am10d} = 10 average of minimum daily air temperatures

The maximum daily soil temperatures were estimated from the current maximum daily air temperatures and the average of the maximum daily air temperatures over the previous 10 days, using the relationship

$$T_{sM} = -3.01 + 0.408 \times T_{aM} + 0.678 \text{ x } T_{aM10d} \quad (1.3.2)$$

where T_{sM} = estimated maximum daily soil temperature

T_{aM} = current maximum daily air temperature

T_{aM10d} = 10 day average of maximum daily air temperatures

The average daily soil temperatures were calculated from the current average daily air temperatures and the average daily air temperatures over the previous 10 days as

$$T_{sa} = -1.76 + 0.413 \times T_{aa} + 0.825 \text{ x } T_{aa10d} \quad (1.3.3)$$

where T_{sa} = estimated average daily soil temperature

T_{aa} = current average daily air temperature

T_{aa10d} = 10 day average of average daily air temperatures

A3.4 Minimum & Maximum Soil Temperatures Estimated from Observed Minimum & Maximum Air Temperatures by FlyAlert

The minimum daily soil temperatures were calculated from the minimum daily air temperatures as

$$T_{sm} = 4.3831 + 1.0846 \times T_{am} \quad (1.4.1)$$

where T_{sm} = estimated minimum daily soil temperature

T_{am} = observed minimum daily air temperature

The maximum daily soil temperatures were estimated from the maximum daily air temperatures, using the relationship

$$T_{sM} = -4.4446 + 1.4507 \times T_{aM} \quad (1.4.2)$$

where T_{sM} = estimated maximum daily soil temperature

T_{aM} = observed maximum daily air temperature